Determination of *cis* Unsaturation in Oils by Near Infrared Spectroscopy[1]

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ULTRAVIOLET AND INFRARED METHODS of analysis have wide application in the field of fat and oil chemistry. The ultraviolet method for polyunsaturation and the determination of *trans* double bonds by infrared are well-established analytical techniques.

A direct instrumental method for *cis* unsaturation is also attractive. The difference between iodine value and infrared *trans* analyses indicates the *cis* unsaturation, but a direct method would be more rapid and less costly because only a single measurement is required. The most favorable spectral region for this analysis is in the near infrared. Neither the ultraviolet nor visible spectral regions contain bands specific for *cis* unsaturation. In the infrared spectra of fatty acids and esters several bands caused by vibrations involving *cis* double bonds are found. None of these bands however is well suited to quantitative measurement.

The near infrared region of the spectrum is defined here as the wavelength interval from 0.7 to 2.6 μ. In this region are found many absorption bands resulting from overtone and combination bands associated with hydrogen atoms. For example, the first overtones of the —NH and —OH hydrogen stretching vibrations are found near 1.5 and 1.4 μ, respectively, while combination bands resulting from the C—H stretching and deformation vibrations of alkyl groups are observed between 2.2 and 2.6 μ.

The absorption bands in the near infrared are well suited to analytical applications because of their sharpness and relative freedom from interference. Analytical procedures based on near infrared spectroscopy have dealt with the determination of aromatic and aliphatic alcohols (1, 3) and amines (7), terminal unsaturation (2), and terminal epoxides (4).

Holman and Edmondson (6) have published several near infrared spectra of fatty acids and esters. Bands at 2.19, 2.14, and 1.18 μ were attributed to *cis* unsaturation. Goddu (2) gave the molar absorptivities at 2.14 μ of several compounds containing isolated *cis* double bonds and remarked on the similarity in the values obtained. He concluded that at 2.14 μ little interference from absorption because of *trans* unsaturation is to be expected and suggested that the *cis* content of mixtures of isomers be determined at that wavelength.

Recently Holman, Ener, and Edmondson (5) reported the determination of *cis* unsaturation in lipid components and hydrogenated oils by near infrared spectroscopy. They measured the absorbance of the band at 2.15 μ and related this to the iodine value of the sample corrected for *trans* content. They reported that *trans* unsaturation does not interfere at the analytical wavelength.

For the work to be reported here the absorption band at 2.14 μ was employed. This band is superimposed on a relatively strong C—H combination band (Figure 1) so that quantitative measurements are difficult on account of the steepness of the background. To circumvent this difficulty a solution containing a suitable saturated compound (usually the palmitic acid derivative) was placed in the reference beam of the instrument.

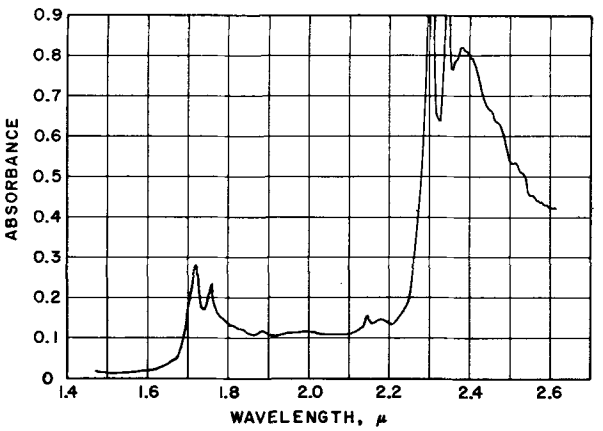

FIG. 1. Near infrared spectrum of 1% oleic acid in CCl_4 between 2.6 and 1.4 μ. *Cis* unsaturation band occurs at 2.14 μ.

Experimental

A Cary Model 14 Spectrophotometer was used throughout this investigation. The instrument conditions were chosen so that absorbances obtained were reproducible to the accuracy inherent in reading the chart paper ($\pm$ 0.001 unit). These conditions were: slit control, 25; scan speed, 10 A/sec. (10 mμ/scale division); scan range 2.20 to 2.10 μ, and PbS detector. The slit control resulted in a spectral slit width of 6A at 2.1 μ. The instrument was swept with dry nitrogen to remove any interference because of water vapor. Ten-centimeter path-length matched quartz or glass cells were used.

The reagent grade of carbon tetrachloride was used without further purification. The following compounds were obtained from the Hormel Foundation: oleic acid (IV = 89.8), linoleic acid (IV = 181.0), methyl oleate (IV = 85.6), methyl linoleate (IV = 172.5), and methyl palmitate (IV = 0). Tripalmitin and palmitic acid were obtained from Eastman.

Triolein and trilinolein were prepared in The Procter and Gamble Research Division. The triolein had an acid value of 0.07, hydroxyl value of 4.3, and an IV of 82.8. Gas chromatography of the methyl esters prepared from triolein showed 2.3% linoleic and 3.3% linolenic acids. The trilinolein had a hydroxyl value of 5.4, zero acid value, and an IV of 168. Gas chromatography of the methyl esters derived from trilinolein showed 5.5% oleic and 0.4% linolenic acids.

The methyl linoleate was prepared from acids isolated from linseed oil and contained less than 1% *trans* isomer. Gas chromatography results indicated

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the composition to be 94% methyl linolenate and 6% methyl linoleate.

The following analytical procedures were used in obtaining the data on oils.

Raw and Unhydrogenated Oils (I.R. *trans* <10%)

Calibration Curve. A series of five standard solutions were prepared by weighing quantities of triolein and tripalmitin of known purity and dissolving them in 50 ml. of CCl_4. The total sample weight in each case was 2.5 g.

The solution to be analyzed was put into a 10-cm. path-length cell, and the spectrum from 2.2 to 2.1 μ was scanned. The reference cell contained a solution of 2.5 g. of tripalmitin or trimyristin in 50 ml. of CCl_4.

The baseline was drawn tangent to the base of the 2.143 μ absorption band between 2.16 μ and 2.13 μ. The area under the band was calculated by measuring the half-band width in centimeters with vernier calipers and multiplying this value by the peak absorbance corrected for the baseline.

The calibration curve was constructed by plotting the area against the *cis* iodine value. The *cis* iodine value was calculated from the weights of triolein taken by the equation:

$$cis\ (\text{I.V.}) = \frac{\text{wt. triolein} \times \text{I.V. of triolein}}{2.5}$$

The calibration curve was extrapolated to permit handling of samples with an iodine value greater than that of triolein (86.0).

Unknowns

Solutions containing 2.5 g. oil/50 ml. of CCl_4 were prepared, the spectrum was scanned, the area was measured, and the *cis* (I.V.) was calculated from the calibration curve.

Hydrogenated Oils and Shortenings

Calibration Curve. Samples of hardened cottonseed oil of the following approximate composition were obtained, and 2.5 g. of each were dissolved in 50 ml. of CCl_4. Duplicate determinations of iodine value and % infrared *trans* acids were obtained on each of the original samples.

Sample No.	Iodine value	% I.R. *trans*
1	95–105	10 ± 3
2	85– 95	20 ± 3
3	80– 85	30 ± 3
4	75– 80	40 ± 3
5	70– 75	50 ± 3

The *cis* (I.V.) for each oil was calculated by the relationship: *cis* (I.V.) of oil = I.V. (of sample) − 0.860 (% I.R. *trans*). The constant 0.86 is the factor for converting % I.R. *trans* to iodine value.

The solutions were scanned from 2.20 to 2.10 μ in a 10-cm. cell against a tripalmitin or trimyristin blank. The peak absorbance of the band at 2.143 μ was measured and corrected for the absorbance at 2.157 μ. A calibration curve was prepared by plotting the corrected absorbance at 2.143 μ against the *cis* (I.V.).

Unknowns

A solution containing 2.5 g. of oil per 50 ml. of CCl_4 was put into a 10-cm. cell, the spectrum scanned, and the peak corrected absorbance determined. The equivalent *cis* (I.V.) was determined from the calibration curve for hydrogenated oils.

Results and Discussion

The molar absorptivities of several fatty acid derivatives of known purities were calculated from the slope of calibration curves. At least four different concentration levels of each compound were used. Each of the curves so obtained was linear and passed through the origin. The purities of these compounds were established from gas chromatographic, infrared, and chemical data.

The molar absorptivities thus obtained are summarized in Table I. The molar absorptivities of the polyene derivatives were found not to be integral multiples of the monoene oleate derivatives. An analysis scheme based only on absorbance measurements will yield results lower than the true value when applied to mixtures containing appreciable quantities of polyene *cis* unsaturation. If however the molar absorptivities are corrected for the differences in band widths, the corrected molar absorptivities for the polyene derivatives become integral multiples of the oleate values (Table I, column 2). The band-width corrections were made by measuring the band width at half the peak-absorbance value and multiplying this by the peak absorbance. This product is the approximate area under the absorption band and is so designated in Table I.

TABLE I

Molar Absorptivities of Fatty Acid Derivatives at 2.14 μ

Compound	Molar absorptivity [a]	Area [b]	Area on total fatty acid basis
Oleic acid	0.132	0.119	0.119
Linoleic acid	0.222	0.235	0.235
Methyl oleate	0.145	0.127	0.121
Methyl linoleate	0.246	0.255	0.243
Methyl linolenate	0.345	0.369	0.351
Triolein	0.430	0.388	0.371
Trilinolein	0.741	0.773	0.740

[a] Molar absorptivity, $\epsilon = A/bc$, where A is the absorbance, b the cell path-length in cm. and c is moles per liter.

[b] Area = molar absorptivity, ϵ, multiplied by band width in cm. at half absorbance.

By measuring area rather than only absorbance, it is possible to analyze mixtures of *cis* derivatives without regard to the relative amounts of *cis* monoene and polyene components present. Only a single calibration curve is needed for the analysis of mixed fatty acids, methyl esters, or triglycerides because the areas for each of these derivatives may be related by the appropriate molecular-weight correction. The interrelation of areas is illustrated in Table I, column 3, where the areas are calculated on a total fatty acid basis.

The area calculation method was applied to several typical samples of refined cottonseed and soybean oils. The areas were compared to a calibration curve prepared from mixtures of triolein and tripalmitin of known purity, and the equivalent *cis* contents of the oils were calculated on an iodine value basis [*cis* (I.V.)]. The *cis* (I.V.) values obtained by near infrared measurements were compared with the results calculated from iodine value and infrared *trans* determinations.

Table II shows the results obtained from this comparison. The calculated values agreed well with the

near infrared *cis* method. The average absolute difference between the calculated *cis* (I.V.) and the infrared method was ± 0.8 I.V. units for refined soybean oils and ± 1.5 I.V. units for refined cottonseed oils.

TABLE II

Analysis of Refined and Bleached Soybean and Cottonseed Oils

Sample No.	Total I.V.	% I.R. *trans* as I.V.	Calc. *cis* (I.V.)	I.R. *cis* I.V.	Diff. (absol.)
Soybean Oils					
1	133.1	0.8	132.3	134.1	+ 1.8
2 [a]	125.8	9.0	116.8	117.4	+ 0.6
3	133.7	1.4	132.3	132.4	+ 0.1
4	131.8	1.4	130.4	131.9	+ 1.5
5	133.5	1.5	132.0	131.2	− 0.8
6	131.8	1.5	130.3	130.4	+ 0.1
7	133.1	1.1	132.0	131.1	− 0.9
					± 0.8
Cottonseed Oils					
8	114.5	1.5	113.0	113.3	+ 0.3
9 [a]	108.2	9.4	98.8	96.7	− 2.1
10	112.5	1.7	110.8	112.5	+ 1.7
11	112.1	1.5	110.6	112.2	+ 1.6
12	113.2	1.5	111.7	113.9	+ 2.1
				Av.	± 1.5

[a] Slightly hydrogenated oil.

The direct-area-measurement procedure, when applied to series of hydrogenated soybean and cottonseed oils, led to some error. It was found that the presence of more than 10% *trans* isomers in the hydrogenated oils had a marked effect on the slope of the baseline. Figure 2 illustrates the differences in slope observed. Results calculated from the calibration curve obtained from triolein-tripalmitin mixtures were low in approximately direct proportion to the amount of *trans* isomer present.

A new baseline was chosen so that this interference was minimized. The absorbance at 2.157 μ was used in place of the tangent baseline previously used. Even so, neither absorptivity nor area measurements

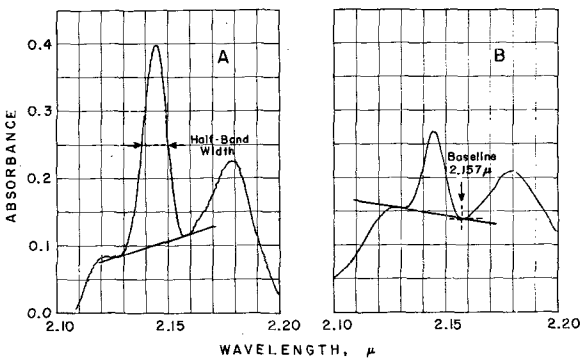

FIG. 2. Near infrared spectra of oils between 2.2 and 2.1 μ illustrating difference in baseline caused by *trans* isomers. A refined and B hydrogenated soybean oils.

were independent of the number of multiple double bonds present.

Rather than use triolein or trilinolein as standards, a series of cottonseed oils hydrogenated to different *trans* contents was employed. The calibration curve was prepared by plotting the absorbance difference between 2.143 μ and 2.157 μ against the *cis* (I.V.), calculated from iodine value and infrared *trans* acid data. Figure 3 illustrates the calibration curve obtained.

This method of calculation was applied with equal success to samples having *trans* contents greater than 10%. Among the samples examined were cottonseed and soybean oils hydrogenated under margarine conditions, cottonseed oils hydrogenated under shortening conditions, and several finished shortenings. The results are tabulated in Table III. The average relative difference (± 1.6%) between calculated and determined values for the *cis* (I.V.) is a little larger than that obtained with refined oils and undoubtedly reflects the statistical deviations inherent in the iodine value and *trans* determinations.

It would also be possible to use mixtures of triolein and trielaidin to prepare the calibration curve. Prepa-

TABLE III

Analysis of Hydrogenated Oils and Shortenings
Soybean Oils (Margarine Conditions)

Sample No.	Total I.V.	% I.R. *trans* as I.V.	Calc. *cis* (I.V)	I.R. *cis* (I.V.)	Diff. (abs.)	Diff. (rel.)
1	107.2	28.4	78.8	79.1	+ 0.3	+ 0.4
2	102.7	33.3	69.4	69.4	0	0
3	98.8	36.5	62.3	63.0	+ 0.7	+ 1.0
4	89.3	42.3	47.0	48.3	+ 1.3	+ 2.9
5	83.3	44.4	38.9	37.8	− 1.1	− 2.8
6	78.3	45.2	33.1	32.6	− 0.5	− 1.9
7	71.4	45.2	26.2	26.5	+ 0.3	+ 1.0
					Av. ± 0.6	± 1.6
Cottonseed Oils (Margarine Conditions)						
1 [a]	108.2	8.6	99.6	100.7	+ 1.1	+ 1.2
2	99.5	19.0	80.5	81.6	+ 1.1	+ 1.3
3	94.3	25.4	68.9	68.9	0	0
4	88.9	29.8	59.1	58.7	− 0.4	− 2.8
5	83.5	34.8	48.7	48.2	− 0.5	− 1.8
6	78.4	37.6	40.8	42.0	+ 1.2	+ 3.1
7	73.7	39.0	34.7	34.9	+ 0.2	+ 0.4
					Av. ± 0.6	± 1.6
Shortenings						
1	74.1	17.6	56.5	56.0	− 0.5	+ 2.1
2	76.2	28.6	47.6	48.2	+ 0.6	+ 0.9
3 [a]	62.9	8.1	54.8	54.5	− 0.3	− 0.5
4	78.0	26.5	51.5	51.1	− 0.4	+ 0.9
5 [a, b]	103.6	4.9	98.7	98.6	− 0.1	− 0.3
6 [b]	90.7	12.4	78.3	79.8	+ 0.5	+ 1.9

[a] Area calculation used because *trans* content below 10%.
[b] Hydrogenated CSO, shortening conditions.

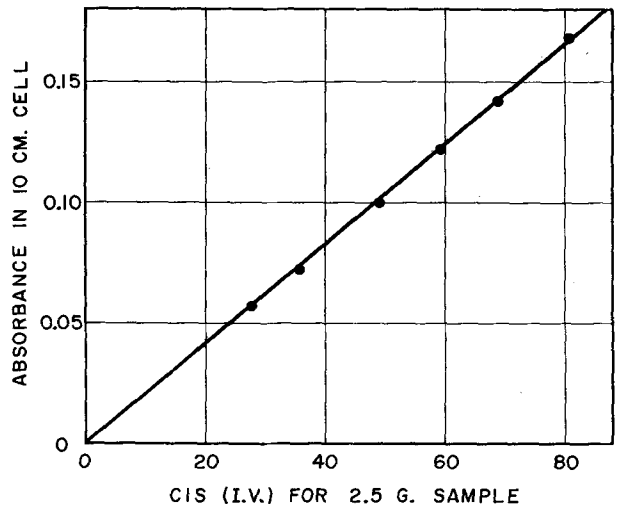

FIG. 3. Calibration curve prepared from hydrogenated cottonseed oils.

ration of a curve from hydrogenated oils is the more attractive because they more closely approximate the compositions of the unknowns to be analyzed.

Conclusion

An analytical method has been developed capable of giving reproducible and accurate determinations of the *cis* unsaturation present in refined oils, hydrogenated oils, and finished shortenings. Molar absorptivity measurements indicate that similar methods are applicable to the analyses of mixed esters or fatty acids.

The method depends upon the measurement of a band in the near infrared at 2.143 μ that is caused by *cis* unsaturation. The following pertinent conclusions are drawn from data obtained in this investigation.

a) The areas under the absorption bands for *cis* polyenes are integral multiples of the areas under the bands of *cis* monoenes.

b) The *cis* content of oils containing less than 10% *trans* triglycerides can be determined accurately by measuring the area under the absorption band.

c) Accurate results can be obtained for samples containing quantities of *trans* fatty acids in excess of 10% if a point baseline is used and the peak absorbance measured.

REFERENCES

1. Crisler, R. O., and Burrill, A. M., paper presented at Pittsburgh Conference on "Applied Spectroscopy and Analytical Chemistry," March 1959.
2. Goddu, R. F., Anal. Chem., *29*, 1790 (1957).
3. Goddu, R. F., *ibid.*, *30*, 2009 (1958).
4. Goddu, R. F., and Delker, D. A., *ibid.*, *30*, 2013 (1958).
5. Holman, R. T., Ener, S., and Edmondson, P. R., Arch. Biochem. and Biophys., *80*, 72 (1959).
6. Holman, R. T., and Edmondson, P. R., Anal. Chem., *28*, 1533 (1956).
7. Whetsel, K., Robertson, W. E., and Krell, M. W., *ibid.*, *29*, 1006 (1957).

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Hydrogen Peroxide Variables in Increasing Epoxidation Efficiency[1]

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PRIOR STUDIES in this laboratory (1) have shown that the conventional technique for *in situ* epoxidation of olefins, using hydrogen peroxide, acetic acid, and sulfuric acid catalyst, can be modified advantageously. The modifications—use of partially preformed peracetic acid prepared by premixing the hydrogen peroxide, and the acetic and sulfuric acids, and control of agitation—result in reduced epoxide cleavage and give a product of higher epoxide content.

Previously reported work with the partially preformed peracetic acid epoxidation method involved use of 50% hydrogen peroxide and was based on employment of a 15 mole % excess of hydrogen peroxide over the reacting olefin.

The present paper extends study of the partially preformed peracetic acid epoxidation method to include variation of both the quantity and concentration of hydrogen peroxide used in the reaction. Quantity variations include use of hydrogen peroxide/olefin mole ratios equivalent to less than a 15 mole % excess of hydrogen peroxide, and the concentration variation includes use of 70% hydrogen peroxide. Soybean oil is used as a model olefin.

[1] Presented at the 32nd annual meeting, American Oil Chemists' Society, Chicago, Ill., October 20–22, 1958.

Part I. Hydrogen Peroxide/Olefin Mole Ratio

The object of varying the hydrogen peroxide/olefin mole ratio was twofold: a) to determine the minimum amount of hydrogen peroxide required to produce epoxidized soybean oil of high oxirane oxygen content and low iodine number for stabilizer-plasticizer use, and b) to determine how hydrogen peroxide could be best utilized to prepare epoxidized soybean oil for new polymer uses in which low iodine numbers may not be required.

Experimental

A series of soybean oil epoxidations with 50% hydrogen peroxide was carried out by using the partially preformed peracetic acid method (1). Each epoxidation was made with 400 g. of soybean oil (Iodine No. 133.2), using mole ratios of hydrogen peroxide to olefin in the range of 0.25 to 1.15. All the epoxidations were run at 57°C., using an acetic acid to hydrogen peroxide mole ratio of 0.5, a sulfuric acid concentration of 1.8% by weight of acetic acid and 50% hydrogen peroxide, and the previously reported optimum stirring rate of 140 r.p.m. Time for addition of the preformed peracetic acid solution was two hours except in the 0.25 and 0.50 hydrogen