Methods of Analysis of Mixtures of Oleic, Linoleic and Saturated Esters and Their Application to Highly Purified Methyl Oleate and Methyl Linoleate‡

By R. W. RIEMENSCHNEIDER* and D. H. WHEELER**

IN connection with a general investigation on glyceride synthesis and structure, highly purified specimens of methyl oleate and methyl linoleate were prepared as starting materials. An attempt to determine the degree of purity of these preparations resolved itself into a study of a number of analytical methods widely used in the determination of the composition of oils and fats.

Iodine numbers, thiocyanogen numbers, and determinations of saturated acids were considered as probably the more important chemical measurements for the purpose. The Hanus and Wijs methods for iodine numbers, the modified Kaufmann (1) method for thiocyanogen numbers, and the Bertram (2) procedure for saturated acids were employed in this investigation.

It was believed that careful application of these methods would not only give a reliable estimate of the degree of purity of methyl oleate and methyl linoleate but would also yield information concerning the precision and limitations of the analytical methods. The results of the study form the nucleus of this paper.

Experimental

Methyl oleate was prepared from the methyl esters of the mixed acids of olive oil by a procedure essentially the same as described in another paper by Wheeler and Riemenschneider (3).

Methyl linoleate was obtained by debromination of crystalline tetrabromostearic acid (m.p. 115.3°) by means of zinc and methyl alcohol as described in a previous publication (4), in which the probable identity of α, β, and natural linoleic acid is discussed.

Iodine numbers: Hanus and Wijs iodine solutions were made in accordance with the official (A. O. A. C.) directions. Approximately a 200-percent excess of iodine solution was used in all determinations reported.

The thiosulfate solution was standarized against potassium iodate, potassium biiodate, and iodine. These reagents were purified according to directions given by Kolthoff and Furman (5). The values for the normality of the solution in each case differed by less than 0.2 percent from the mean value.

The iodine numbers of the specimens of methyl oleate and methyl linoleate by the Hanus and Wijs methods for ½ hour and 1 hour are listed in Table I. Values very close to theory for methyl oleate were obtained by both methods.

However, the Hanus iodine numbers for methyl linoleate are significantly lower than theory, whereas the Wijs values are almost theoretical. This is in agreement with the general observations that the Hanus iodine numbers of semi-drying oils are somewhat lower than those obtained by the Wijs method.

Since it is possible to have a mixture of methyl oleate with equal amounts of saturated esters and methyl linoleate such as to give an iodine value equivalent to theory for methyl oleate, additional information was necessary to establish the purity of the preparation.

Determination of saturated acids: Of the various methods which have been developed for the

TABLE I

IODINE NUMBERS OF METHYL OLEATE AND METHYL LINOLEATE BY WIJS AND HANUS METHODS

	WIJS ½ HR.		WIJS 1 HR.		HANUS ½ HR.		HANUS 1 HR.		THEORY
METHYL OLEATE	85.7 85.8	85.8	86.0 85.9	86.0	85.6 85.6	85.6	85.5 85.7	85.6	85.7
METHYL LINOLEATE	172.3 173.0	172.7	172.6 172.7	172.7	166.5 167.2	166.9	168.9 168.8	168.9	172.5

TABLE II

DETERMINATION OF SMALL AMOUNTS OF SATURATED ACIDS BY THE BERTRAM PROCEDURE

	SATURATED ACID FOUND. PERCENT	SATURATED ACID FOUND MINUS STEARIC ADDED. PERCENT
A. IMPURE METHYL OLEATE (IODINE Nº 85.0)	0.86 *	0.86 *
A. + 0.47 % STEARIC ACID	1.27	0.80 *
B. METHYL LINOLEATE	0.00	0.00
B. + 0.48 % STEARIC ACID	0.47	-0.01
C. METHYL OLEATE (IODINE Nº 85.8)	0.18 *	0.18 *

* CALCULATED TO METHYL ESTER, THESE VALUES WOULD BE APPROXIMATELY 0.90 % AND 0.19 %, FOR A. AND C., RESPECTIVELY.

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* Research Fellow, National Cottonseed Products Association in cooperation with the Food Research Division, U. S. D. A.

**Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

TABLE III

THIOCYANOGEN NUMBERS OF METHYL OLEATE AND METHYL LINOLEATE FOR VARIOUS ABSORBTION PERIODS

HOURS	1	2	3	4	6	17	24
METHYL OLEATE THEORY 85.7	82.5	85.2	85.4	85.4		85.3	85.7
	83.5	85.0	85.4	85.4		85.4	85.6
				85.4		85.2	85.2
				85.3		85.3	85.4
							85.6
							85.4
METHYL LINOLEATE THEORY 86.3	77.1	85.4	86.8	87.0	87.6	88.1	89.0
	77 4	85.3	86.9	87.0	87.6	88.2	89.0
			86.4	86.9		88.2	88.7
			86.5	86.9		88.2	88.9
						88.7	
						88.9	

determination of saturated acids, the Bertram (2) procedure, or a modification of it, is generally considered the most satisfactory, especially if the sample contains no saturated acids of lower molecular weight than that of myristic acid.

The method used in preparing methyl oleate precluded any probability of the presence of saturated esters lower than methyl palmitate and of any unsaponifiable material.

As a preliminary check on the precision of the method, a sample of somewhat impure methyl oleate known to contain a small amount of saturated esters and a sample of methyl linoleate, free of saturated esters, were subjected to the Bertram procedure[1]. As a control, weighed amounts of pure stearic acid were added to duplicate determinations of each. The results of these analyses, together with a determination on highly purified methyl oleate, are given in Table II.

These data indicate that on the most favorable type of samples the saturated acids can be determined with a precision somewhat greater than 0.1 unit-percent. The highly purified methyl oleate (C) contained about 0.2 percent of saturated esters. Therefore, to account for its iodine number (85.8), it is possible that about 0.2-0.3 percent of methyl linoleate may also be present as a contaminant.

Thiocyanogen numbers: The use of the thiocyanogen number in conjunction with the iodine number for determining the composition of a mixture of methyl oleate and methyl linoleate is based on the premise that quantitative addition of thiocyanogen takes place at only one double bond.

It was not expected that the small amount of methyl linoleate that may be present as an impurity in the specimen of methyl oleate could be accurately determined by the use of the thiocyanogen absorption method. Rather, from the data already presented, it was deemed that this sample and also the methyl linoleate were of sufficient purity to serve as a means of determining how quantitative the addition of thiocyanogen may be.

The solutions of thiocyanogen were made in accordance with the modified Kaufmann (1) procedure[2]. All values reported were obtained by using from 100 to 150 percent excess of fresh thiocyanogen solutions.

It was found that by using a 24-hour absorption time as called for in the procedure, appreciably higher values than theory for methyl linoleate were obtained. An effort was then made to ascertain whether a more favorable absorption period could be found. A series of thiocyanogen numbers were determined on this ester and also on methyl oleate for various absorption times.

The results, shown in Table III, indicate that an amount of thiocyanogen equivalent to one double bond of methyl linoleate is absorbed in about three hours, after which the absorption continues but at a much slower rate.

The values for methyl oleate, however, are nearly constant from the 3-hour to the 24-hour period and are close to theory but slightly low. These results are expressed graphically in Figure I, in which average values are used. The 3-hour values appear to be the best compromise with the theoretical.

It would be expected from these observations that the use of the 3-hour absorption time would lead to somewhat greater accuracy in determining the composition of mixtures of these esters, especially one with a high percentage of methyl linoleate. To test this experimentally, two known mixtures were made by direct weighing of the two purified esters. For comparison, Hanus and Wijs iodine numbers and 3- and 24-hour thiocyanogen numbers were determined and calculations of the composition were made using each of these values. The following equations based on the theoretical iodine and thiocyanogen numbers of methyl oleate and methyl linoleate

TABLE IV

ANALYSIS OF MIXTURES OF METHYL OLEATE AND METHYL LINOLEATE BY USE OF IODINE AND THIOCYANOGEN NUMBERS

MIXTURE	IODINE Nº HANUS ½ HR.	IODINE Nº WIJS ½ HR.	SCN. Nº 3 HR.	SCN. Nº 24 HR.	COMPOSITION						
					KNOWN BY WEIGHT	HANUS IOD. Nº		WIJS IOD. Nº		HANUS IODINE Nº ALONE	WIJS IODINE Nº ALONE
						SCN. Nº 3 HR.	SCN. Nº 24 HR.	SCN. Nº 3 HR.	SCN. Nº 24 HR.		
A	94.6	95.0	85.5	86.2	OLEATE % 89.4	89.2	90.8	88.7	90.3	89.7	89.3
					LINOL. % 10.6	10.5	9.7	11.0	10.2	10.3	10.7
					TOTAL % 100.0	99.7	100.5	99.7	100.5	100.0	100.0
B	127.7	130.0	86.0	87.6	OLEATE % 49.0	51.7	55.4	49.0	52.7	51.6	49.0
					LINOL. % 51.0	48.3	46.5	51.0	49.1	48.4	51.0
					TOTAL % 100.0	100.0	101.9	100.0	101.8	100.0	100.0

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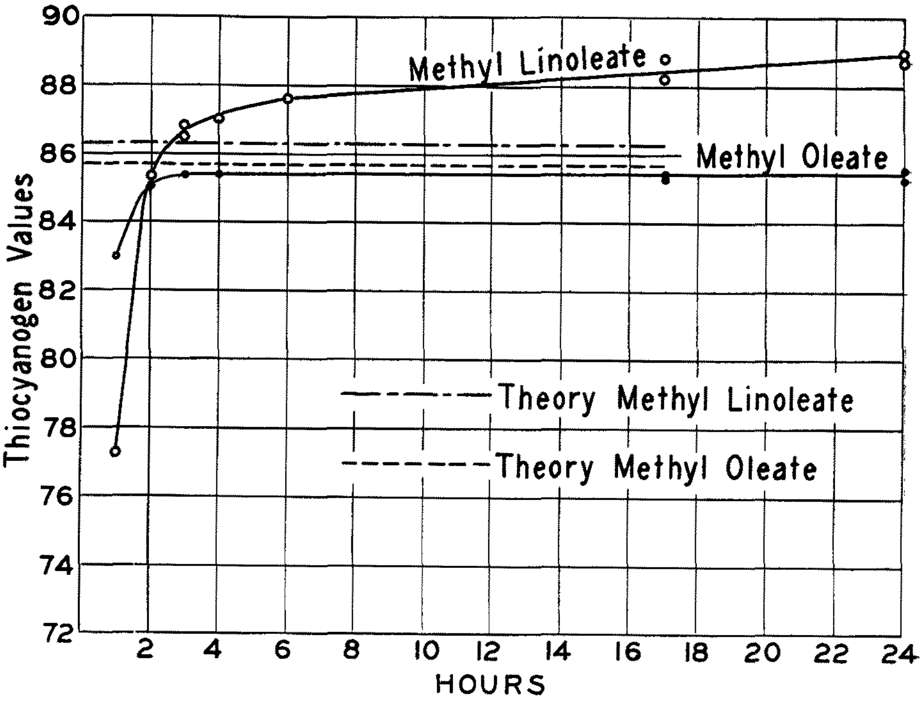
[1] The excess of permanganate was decolorized with SO_2 gas. The first precipitate of magnesium soaps was decomposed with hot dilute H_2SO_4 directly on the small Büchner funnel used in filtration. The liberated acids on the filter were dissolved by alternate additions of warm dilute NH_4OH and water. Similarly, the second precipitate of magnesium soaps was decomposed and the free acids were extracted from the filter by repeated additions of redistilled petroleum ether.

[2] The $Pb(SCN)_2$ was dried first over $CaCl_2$ in vacuo for several days and finally over P_2O_5 at atmospheric pressure for one week. The absorption of thiocyanogen took place at 20 — 24° C. with N/10 solutions not over three days old.



were used in the computations:

% methyl linoleate=1.159 × (iodine no. — SCN no.)

% methyl oleate=1.167 × (2 SCN no. — iodine no.)

The results of the analysis and the calculated compositions of the mixtures are given in Table IV.

For mixture A, which has only 10.6 percent of methyl linoleate, the calculated compositions show good agreement with the known, with the exception of that calculated from the Hanus iod. no. and 24-hour SCN no.

In the computed compositions of mixture B, which contained 51.0 percent of methyl linoleate, the discrepancies due to the 24-hour SCN. no., as well as to the Hanus iod. no., are quite evident. The composition calculated by using the 3-hour SCN. no. and Wijs iod. no. is practically identical with the known, and with that computed from the Wijs iodine number alone.

As a further indication of the reliability of the 3-hour absorption time, the percent of saturated esters in a distilled sample of methyl esters of 18 C atom acids of olive oil was computed from the Wijs iod. no. (98.5) and 3-hour SCN. no. (80.5). The calculated amount, 6.1 percent of saturated esters, agrees very well with 5.9 percent found by the Bertram procedure.

It should be noted that this investigation was limited to methyl esters. Until the study of thiocyanogen absorption is extended to include pure glycerides or mixtures of glycerides of known composition, the use of the 3-hour thiocyanogen absorption time directly on fats and oils may not be justified. Such an extension of the method is contemplated in future work.

Summary

Iodine numbers by the Hanus and Wijs methods and thiocyanogen numbers using various absorption periods, were determined on methyl oleate and methyl linoleate and on mixtures of these esters. It is concluded that iodine numbers by the Wijs method and three-hour thiocyanogen numbers are more satisfactory for methyl linoleate and for mixtures containing large amounts of this ester.

Small amounts of higher saturated acids were determined with a precision of about 0.1 unit-percent by means of the Bertram procedure. A purified specimen of methyl oleate was found to contain about 0.2 percent of saturated ester by this method.

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Variation in Free Fatty Acid Within a Seed Sample

By PROCTER THOMSON
THE PROCTER & GAMBLE CO., IVORYDALE, OHIO

THE A. O. C. S. seed samples of the 1938-39 season have been analyzed by Edeler's method as described in Oil & Soap, Volume XV, pages 291-2. The F. F. A. was determined on ten samples of ten seed each. This was done on each of the ten A. O. C. S. samples. The analytical work was done by Mr. Reuben Lambert of the Procter & Gamble Service Laboratory.

The results were as follows:

Extraction No.	Seed No. 1		Seed No. 2		Seed No. 3		Seed No. 4	
	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance	F.F.A.	Appearance
1	1.01	—	0.60	—	0.71	—	0.25	—
2	0.84	—	0.72	—	4.31	1 bad seed	0.27	—
3	6.87	—	0.68	—	0.65	—	0.42	—
4	2.15	—	0.82	—	0.61	—	0.61	1 br. spotted
5	0.67	—	0.67	—	0.46	—	0.31	—
6	0.74	—	0.54	—	0.51	—	1.33	1 bad seed
7	1.56	—	2.03	1 bad seed	0.53	—	0.26	—
8	0.89	—	0.71	—	0.61	—	0.34	—
9	0.70	—	0.77	—	0.76	—	0.36	—
10	0.59	—	1.98	—	0.98	—	0.48	1 translucent
Average	1.60		0.95		1.01		0.46	
Accepted Average by Seed Analysis Com.	1.1		0.8		0.5		0.5	