

## An Oxidation-Adsorption Method for the Analysis of Methyl Ester Fractions<sup>1</sup>

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DURING the course of a study which dealt with the composition of certain methyl ester mixtures resulting from the analysis of fatty oils, there arose the problem of the determination of their saturated components. Because repeated attempts at the application of chromatographic adsorption techniques to the unaltered mixtures showed that no sharp separations could be obtained by this procedure, the thought suggested itself that the alternative might lie in the conversion of the unsaturated components to short-chain acidic fragments by permanganate oxidation followed by recovery of the unaffected esters. Bertram (1) was probably the first one to apply this principle to the type of problem in question.

One of the principal criticisms of the several modifications which have been suggested since the inception of the Bertram method stems from the fact that a rather lengthy procedure is required for the isolation of the saturated fatty acids and the removal of all oxidation fragments. Furthermore, because of emulsion losses, this procedure is better suited for the analysis of large samples rather than small ones. In view of these limitations the need of a method for removing the saturated components from the oxidation reaction mixture resulting in those instances when only small amounts of original material are available becomes obvious.

Described in the following paragraphs, with a critique in terms of recovery experiments and consistency of results, is the procedure which has been devised as one applicable to the analysis of mixtures of saturated and unsaturated methyl esters that would ordinarily be obtained during the course of a fractional distillation. It represents a combination of a Hilditch-Lea (4) described oxidation technique and the use of a tinted basic adsorbent, the latter having been found by Sylvester *et al.* (10) to be a serviceable tool for the removal of free fatty acids from a glyceride oil.

### The Method

**Oxidation.** A half-gram sample of methyl ester mixture is weighed into a 50-ml. Erlenmeyer flask provided with a ground glass joint and a fitted reflux condenser. Ten ml. of dry acetone is then added, after which the solution is brought to boiling. The addition of four one-gram portions of potassium permanganate, introduced into the boiling solution over a half-hour period, follows. In cases where the

sample is highly unsaturated, additional permanganate is introduced until a purple color persists. This usually necessitates slightly longer oxidation periods. After the mixture has refluxed for one hour, it is cooled and transferred with water to a 250-ml. beaker. Approximately five grams of sodium bisulfite in 25 ml. of water is added to the oxidation mixture with stirring, following which enough sulfuric acid (30%) is slowly added to the solution to make it acid to Congo red. It is then allowed to stand until cool.

**Extraction.** The cooled solution is transferred to a 250-ml. separatory funnel. The oxidation flask and beaker are rinsed with 25 ml. of chloroform and the washings are added to the funnel. After shaking, with due care given to the frequent release of sulfur dioxide in the mixture, the chloroform layer is transferred to a 125-ml. flask. Extraction is repeated three more times with 20-ml. portions of chloroform. The combined chloroform extracts and washings are then washed once with 50 ml. of water in the separatory funnel. Stirring rods and transfer funnels are washed with chloroform, and these washings are added to the main chloroform solution in the separatory funnel. The combined chloroform extracts and washings are returned to the 125-ml. flask, after which, as a precautionary measure, enough anhydrous sodium sulfate is added to remove any water remaining in the chloroform solution. At this point the latter contains all of the unoxidized neutral material and water insoluble oxidation fragments.

**Removal of Oxidation Products.<sup>3</sup>** Tinted alumina (Aluminum Ore Co., grade F-20) is prepared in the manner described by Sylvester *et al.* (10). To that end a small quantity of the adsorbent is treated with an alcoholic solution of bromthymol blue until strongly colored and then is dried in the oven at 110°. A sufficient amount of this strongly colored material is diluted with ordinary alumina until the adsorbent assumes a preceptible blue tint.

A 2 x 10-cm. tinted alumina column is prepared, after which the dry chloroform extract is introduced. After this solution has passed into the column, additional chloroform, which has first been used to rinse out the flask containing the sodium sulfate, is added. A total volume of 200 ml. of chloroform in addition to the original solution is allowed to percolate through the column. Acidic components remain at the top of

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<sup>3</sup> That removal of the oxidation products by adsorption is practically complete was indicated by the following simple experiment. Several saturated ester fractions, which had been obtained by the above described procedure, were titrated with 0.1 N potassium hydroxide solution to a phenolphthalein end-point. A maximum titration of 0.4 ml. per 100 mg. was obtained.

the alumina column and can be detected by the indicator color change from blue to buff color. This band should not take up more than one-half the length of the column. A column as described will hold about one-half gram of oxidation products from methyl linolenate.

The solvent in the percolate is distilled off in an all-glass apparatus until approximately 10 ml. remain. This is transferred with three successive chloroform washings to a tared 50-ml. beaker and carefully evaporated to dryness. The residual chloroform is removed under vacuum until constant weight is obtained. From the weight of the residue the percentage of saturated esters in the original samples is calculated. If the iodine number of the saturated esters is greater than 0.2, a correction is made assuming the unsaturated ester to be methyl oleate.

### Experimental

**Methyl oleate.** Methyl oleate was prepared from oleic acid which had been obtained from olive oil fatty acids by the use of low-temperature crystallization techniques from acetone solutions. A preliminary crystallization at  $-20^{\circ}$  removed the greater portion of the saturated acids, after which a crop of fairly pure oleic acid was obtained from the mother liquor cooled to  $-60^{\circ}$ . The latter was made up to a 10% solution, chilled to  $-60^{\circ}$ , and twice recrystallized at this temperature. Because the iodine number of the final product, 88.3, suggested contamination with small amount of saturated acids, the whole was recrystallized, but this time at  $-35^{\circ}$ . The resulting crystal crop, freed of acetone under vacuum and in the presence of carbon dioxide, was fractionally distilled at 1 mm., b.p. 186-187°. A colorless fraction of iodine number (Wijs) 90.2 (theoretical 89.9) and a sharp melting point  $12.8^{\circ}$  (reported  $13^{\circ}$ ) was converted to the ester for use in the chromatographic separations.

**Methyl linoleate.** This ester was prepared from the pure acid obtained by debrominating with zinc dust, in the presence of hydrogen chloride, a methanol solution of the tetrabromostearic acid which had been fractionally separated with petroleum ether from the mixed brominated acids of corn oil (3, 5). It was obtained as a pale yellow liquid. Distillation under reduced pressure through a short-path, all-glass apparatus gave a product having the following characteristics: b.p. 155-157° at 1.2 mm.,  $n_D^{25}$  1.4603, iodine number 173.9 (Wijs), (theoretical 172.5).

**Methyl linolenate.** Linseed oil fatty acids which had been recovered from the saponified oil by petroleum-ether extraction and decolorized by distillation under reduced pressure served as a source for the linolenic acid from which this ester was prepared. Bromination (9), followed by debromination (6) of the hexabromostearic acid with zinc in the presence of methanol, a supplementary esterification of the resulting reaction mixture of acid and ester after its recovery with petroleum ether, and, finally, distillation under reduced pressure through a short-path, all-glass apparatus, gave a product of b.p. 153-155° at 1 mm.,  $n_D^{25}$  1.9693, and iodine number (Wijs) 256 (theoretical 260.5).

**Esters of saturated acids.** With the exception of methyl caprate all the esters of this group were prepared from high-purity commercial acids (Neo-Fats Armour). They were purified by the method previously described (8). The caprate was obtained in

approximately 50% yield by fractionally distilling a brominated methyl ester mixture of elm seed oil fatty acids (7).

**Critical study of the method.** The potentialities of this oxidation-adsorption method were explored from several approaches, viz., a) recovery experiments on single esters representative of the saturated and the mono-, the di-, and the triethenoid types, respectively; b) recovery experiments on synthetic, two-component mixtures of esters of a saturated acid and one representative of each of three common types of unsaturated acids; c) a series of recovery experiments whose objective was a demonstration of the consistency of results which could be expected when a four-component mixture consisting of approximately equal parts of saturated and unsaturated esters is subjected to this treatment; d) application of the method to the analysis of methyl ester fractions obtained from natural sources.

### Results

In Table I are shown the results obtained by this procedure when applied to pure methyl esters. The lower saturated esters show a 2 to 4% loss because of either oxidation or water-solubility considerations or, perhaps, a combination of these causes. The unsaturated esters were almost completely oxidized after one hour, oxidation being less complete in the case of the lower unsaturated esters. Because of losses, particularly with methyl laurate, this method cannot be used satisfactorily if this ester or lower components are present in appreciable amount.

TABLE I  
Recovery Experiments on Methyl Esters

Ester	Oxidation period	Ester taken	Ester recovered	Saturated ester
	min.	mg.	mg.	
Laurate.....	60	516.6	499.2	96.6
Myristate.....	60	615.4	605.0	98.3
Palmitate.....	60	515.5	511.2	99.2
Stearate.....	60	517.4	520.0	100.5
Oleate.....	30	250.7	22.5	9.0
Oleate.....	60	251.0	9.5	3.8
Linoleate.....	30	275.7	13.0	4.7
Linoleate.....	60	247.1	3.1	1.3
Linolenate.....	30	253.4	6.2	2.5
Linolenate.....	60	263.3	6.0	2.3

The series of experiments (Table II) designed to test the quantitative reaction of the method when applied to binary mixtures of esters of a saturated acid and an unsaturated one demonstrated again that in its present form the procedure cannot be recommended in those cases where saturated acids of even carbon atom content lower than  $C_{14}$  are present. For want of material the behavior of the esters of acids beyond the triethenoid type was not studied. What may be expected of an ester of an acid of the tetraethenoid type is probably suggested by the behavior of the latter.

That the procedure is easily within control of the analyst and that duplicate determinations may be obtained in good agreement is revealed in the results of a series of six assays of a synthetic methyl ester mixture consisting of approximately equal amounts of the palmitate, the myristate, the oleate, and the linoleate. The difference found between the maximum and the minimum amounts of saturated esters recovered is 0.8% while the average deviation from the mean of 49.1% is  $\pm 0.3\%$ . These values agree well with the deviations shown in Table II.

TABLE II  
Recovery of Saturated Methyl Esters from Synthetic Mixtures

Mixture components	Saturated esters		Deviation
	Calcd.	Found	
	%	%	%
Palmitate-oleate.....	48.2	49.0	+0.8
Palmitate-oleate.....	79.4	79.6	+0.2
Palmitate-linoleate.....	59.6	60.3	+0.7
Palmitate-linoleate.....	80.4	79.5	-0.9
Stearate-linolenate.....	58.7	58.2	-0.5
Palmitate-linolenate.....	60.8	60.1	-0.7
Palmitate-linolenate.....	88.8	88.0	-0.8
Myristate-linolenate.....	49.4	48.6	-0.8
Stearate-oleate.....	87.8	87.5	-0.3
Stearate-linoleate.....	9.0	9.3	+0.3
Palmitate-linoleate.....	39.2	39.4	+0.2
Myristate-linoleate.....	59.4	58.5	-0.9
Myristate-linolenate.....	51.3	51.2	-0.1
Stearate-linoleate.....	24.3	23.9	-0.4
Stearate-linolenate.....	34.6	35.0	+0.4
Mean error.....			±0.6

Pertinent to the application of the method to the analysis of the methyl esters which are obtained, alone or in binary mixture, as a result of a fractional distillation during the course of an investigation of a fatty oil, are the data in Table III. Seven such fractions, obtained from sabadilla and elm seed oils, were used as experimental materials. In calculating results, iodine numbers were used as a measure of the unsaturated esters, and the saturated ones were determined by the oxidation method. Because linolenic acid was not a constituent of the original oils, calculation of the oleate and linoleate content was simplified. It was made from iodine numbers alone. Corrections were made for unsaturated esters present in the saturated oxidation fractions from iodine numbers calculated as methyl oleate. The maximum iodine number obtained was 2.0.

For comparison, analyses of these samples were also made by the usual methods employing iodine numbers and thiocyanogen numbers or alkali isomerization by the method of Brice and Swain (2). These results appear in Table III.

TABLE III  
Application of the Oxidation-Adsorption Method to the Analysis of Methyl Ester Fractions from Natural Sources

Frac- tion	Iodine No. (Wijs)	Saturated calcd.	Esters found	Methyl oleate		Methyl linoleate	
				Calcd.	Found	Calcd.	Found
		%	%	%	%	%	%
1	85.0	37.2	35.4	26.9	30.4	35.9	34.2
2	97.2	25.3	25.3	36.4	36.5	38.3	38.3
3	98.5	20.3	19.9	45.9	45.7	34.3	34.4
4	60.8	47.7	47.5	33.8	34.9	18.5	17.6
5	71.6	38.1	35.7	40.5	41.6	21.4	20.9
6	81.2	29.5	30.0	46.5	45.6	24.0	24.4
7	82.7	29.6	30.6	44.5	42.6	25.9	26.8

Data for fractions 1 to 3 calculated from iodine and thiocyanogen numbers; those for fraction 4 to 7 from alkali-isomerization data.

In contrast to the generally applied methods, the proposed procedure for determining saturated components in a mixture can be carried out on a small sample of methyl esters with a considerable saving in time. This method employs the novel technique of removing acidic oxidation products by adsorption on a basic adsorbent. This has been shown to bring

about conveniently an almost quantitative removal of this material. The advantage of this over the alkaline extraction procedure which is employed in the usual oxidation methods lies in the fact that troublesome emulsions which have always been a source of error are avoided. The small sample required makes the method applicable to distillate fractions where generally only small amounts of material are available. This method may serve as a check to ascertain the accuracy of calculations from the usual analytical values since calculations of component saturated esters may be made from saponification data on the isolated saturated ester fraction.

The results which were obtained on duplicate samples agree within 1% of the known content of saturated esters and reproducibility of results has been shown to be of the order of 0.6%. In cases where oxidation is not complete, the iodine number of the saturated ester fraction can be utilized in applying a correction by calculating the unsaturated fraction as methyl oleate. In a series of determinations on mixtures of methyl esters the results obtained with the oxidation-adsorption method were found to be in good agreement with those calculated from iodine and thiocyanogen numbers or those obtained by alkali isomerization for the determination of the amount of unsaturated esters present.

### Summary

Conversion of the unsaturated components of the fractions obtained in the analytical distillation of fatty oil methyl esters to short-chain acidic fragments by permanganate oxidation, and their subsequent adsorption on alumina tinted with bromthymol blue is the basis for the method described. Recovery experiments show a) that the removal of these fragments is practically quantitative, provided acids lower than lauric are not present in the mixtures in appreciable amounts, b) that duplicate samples agree within 1% of the saturated ester content, and c) that reproducibility of results is of the order of 0.6%. In a series of determinations on mixtures of methyl esters the results obtained by the procedure described were found to be in good agreement with those obtained by calculation from iodine and thiocyanogen numbers or by alkali isomerization. Because only small samples are required the method is particularly applicable to the analysis of distillate fractions.

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