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[Received June 13, 1957]

## Observations on the Permanganate Oxidation of Unsaturated Esters<sup>1,2,3</sup>

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THE OXIDATION of fats with potassium permanganate to convert the unsaturated glycerides to azelaoglycerides was originally suggested by Hilditch and Lea (1). Kartha has suggested (2) that Hilditch's procedure (3) can be improved by conducting the oxidation in the presence of acetic acid. Kartha has pointed out that, during the permanganate oxidation of fats in acetone, alkaline products accumulate which may hydrolyze the ester groups of the fat. He believes that the ester linking the azelaic acid moiety to the glycerol is especially easily hydrolyzed under these conditions. Hilditch has expressed doubt that the hydrolysis which might occur during the permanganate oxidation is significant, especially when compared with that which might occur during subsequent alkaline extractions of the product. There have been exchanges of view-point in the literature (4, 5, 6).

Recently work has been started in this laboratory to try to improve the present methods of glyceride analysis. One approach that appeared feasible was to try to improve the methods of separating the azelaoglycerides and fully saturated glycerides obtained after permanganate oxidation of fat. However some of the products obtained by potassium permanganate oxidation according to Kartha's procedure were found to have an unexpectedly high proportion of ester groups. This led to a critical study of Kartha's procedure and other procedures on some known compounds. The results of this study are reported below.

### Materials and Methods

Undecylenic acid (Eastman practical) was converted to the methyl ester by refluxing for six hours with an excess of methanol and a sulfuric acid catalyst.<sup>4</sup> The methyl undecylenate was freed from acid by washing with 3% aqueous sodium carbonate and was distilled through a Vigreux column 25 cm. long at 1-mm. pressure. The fraction boiling between 80.2 and 80.5°C. amounted to about 80% of the charge.

It gave the following analysis:  $n_D^{20}$  1.4390, saponification value 283.4 (theory 282.9), iodine value 127.6 (theory 128.0),  $d_4^{20}$  0.88414 g./cc. When hydrogenated, the product had  $n_D^{20}$  1.4292 [calculated value 1.4291 (7)].

Triundecylenin was made by reacting 347 g. of the above methyl undecylenate with 127 g. of triacetin (Eastman), using 2.3 g. of sodium methylate as a catalyst. The reaction mixture was stirred with a magnetic stirrer, and nitrogen was bubbled through it. The pressure was reduced from 40 mm. to 5 mm. over several hours, and the mixture was left under 5 mm. of pressure over-night. Next day the pressure was reduced to 0.2 mm. for a few hours. The methyl acetate which distilled was caught in a dry ice trap and amounted to 93% of theory. The catalyst was destroyed with 3 ml. of acetic acid and 500 ml. of water. The product was washed free from acids with aqueous sodium carbonate. The product (349 g.) was crystallized from 1,750 ml. of ether at -65°C. The crystalline residue, which weighed 135 g., was crystallized from 1,350 ml. of methanol-ether (4:1) at -30°C. The product weighed 115 g. It gave the following analysis: saponification value 287.2 (theory 284.9), hydroxyl value zero, iodine value 128.1 (theory 128.9)  $n_D^{20}$  1.4650,  $d_4^{20}$  0.94416 g./cc., m.p. 8.0-8.3°C. When hydrogenated, the product had a m.p. 30.15-30.40°C. [reported for triundecanoin 30.50°C. (8)] and a  $n_D^{20}$  1.4541.

Methyl oleate was prepared as follows. A commercial oleic acid was purified with urea by the method of Swern and Parker (9). The oleic acid was then esterified with methanol, using a sulfuric acid catalyst, and the product was washed free of oleic acid with aqueous sodium carbonate solution. The methyl oleate was then distilled at 3 mm. through a three-foot column packed with glass helices. The fraction, whose boiling point indicated an 18-carbon chain, was collected and dissolved in 10 volumes of methanol. The portion crystallizing from the methanol at -30°C. was discarded. The methyl oleate recovered from the methanol filtrate gave the following analysis: saponification value 189.6 (theory 189.2), iodine value 85.2 (theory 85.6),  $n_D^{20}$  1.4528 [calculated 1.4520 (7)]. Alkali isomerization (10) and

<sup>1</sup> A report of this work was given at the American Dairy Science Association meetings, Stillwater, Okla., in June, 1957.

<sup>2</sup> Journal Paper No. J-3245 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Ia., Project No. 1128.

<sup>3</sup> Supported in part by a grant from the American Dairy Association.

<sup>4</sup> During the preparation of this and the other unsaturated esters, the fatty acids were protected from autoxidation by oxygen-free nitrogen.

iodine value indicated it was 93.7% methyl oleate, 3.7% saturated esters, and 2.6% methyl linoleate.

Methyl linoleate was prepared from safflower oil by a procedure based on that used by the Hormel Institute, Austin, Minn. First, 600 g. of safflower oil fatty acids were dissolved in 2,410 ml. of methanol containing 953 g. of urea. Then the solution was allowed to cool to room temperature, and the crystalline precipitate was removed. The filtrate was evaporated down on a hot plate until it solidified, and this residue was broken up with water. The fatty acids recovered from the urea filtrate were distilled at 0.2 mm. in the Vigreux column used for the methyl undecylenate. Next, 250 g. of linoleic acid concentrate were recovered, which had an iodine value of 180.0 (theory 181.0) and were found to be 99.8% linoleic acid by alkali isomerization (10). These acids were esterified with methanol, using a sulfuric acid catalyst, and the product was washed free from acids with 3% aqueous sodium carbonate solution. The methyl esters were redistilled at 0.18 mm. The product gave the following analysis: saponification value 192.8 (theory 190.5), iodine value 170.3 (theory 172.4),  $n_D^{20}$  1.4621 [calculated 1.4614 (7)] Alkali isomerization indicated it was 99–100% methyl linoleate. After hydrogenation it melted at 38.4–38.8°C. [reported for methyl stearate 39.1°C. (11)].

The methanol and ether used in these experiments were reagent grade. Acetone was purified by refluxing it with potassium permanganate and potassium carbonate for two hours, then distilling.

Neutralization equivalents were obtained by dissolving about 100 mg. of sample in 25 ml. of ethanol. This was titrated to the phenolphthalein end-point with 0.1 N aqueous sodium hydroxide. The titration was corrected with a blank run on the solvent. Saponification values were run according to Chandlee *et al.* (12). Iodine values were run by a modified Wijs method (13).

The oxidation of the esters by Kartha's method (2) was carried out as follows. Five-gram samples were dissolved in 200 ml. of acetone and 12 ml. of acetic acid in a 500-ml. balloon flask, fitted with a condenser by means of a standard taper glass seal. A gram or two of potassium permanganate was added, and the reaction mixture heated with a heating mantle until the acetone began to boil. The heat was then turned off, and the reaction mixture was maintained at a boiling temperature by the addition of small quantities of potassium permanganate down the condenser until the reaction subsided. Then the reaction mixture was heated to keep the acetone boiling, and the addition of potassium permanganate continued until the color produced by about 1.5 g. of permanganate persisted for 25 to 30 min. It was impossible to get the permanganate color from 1 g. of permanganate to persist 45 min., as Kartha indicates, no matter how long the reaction was continued. For each 16 g. of permanganate added, 6 ml. of acetic acid were added. After the reaction was complete, the acetone was removed under reduced pressure. The manganese dioxide was dissolved by the addition of sodium metabisulfite and 10% sulfuric acid, and the product was extracted with ether. In the experiments with methyl esters the reduced reaction mixture was extracted continuously with ether over-night in order to get a complete recovery. Triundecylenin was extracted in a separatory funnel with ether, and the ether layer was washed free of mineral acid. The

ether layer was dried with sodium sulfate and evaporated to constant weight.

In order to compare the results by the other procedures with Kartha's procedure they were carried out as above except that the acetic acid was not added. Under these conditions it was possible to get the color produced by 1.5 g. of potassium permanganate to persist for more than an hour when the oxidation was complete.

The iodine values of the products obtained by these procedures have always been below four units and are below two units in most cases.

### Results and Discussion

The results are recorded in Table I. The theoretical weight recovery was calculated on the assumption that the undecylenic acid esters were converted to the corresponding sebacic acid esters, methyl oleate was converted to monomethyl azelate and nonanoic acid, and methyl linoleate was converted to methyl azelate and hexanoic acid. In most cases the recovery was somewhat less than theory. The recovery of the triundecylenin product was less than that of the methyl ester products. This was probably because the extraction with the continuous extractor used for the methyl esters was more efficient than the simple extraction in a separatory funnel used for the triglycerides. In the case of the methyl esters the extraction is believed to be essentially complete. They were extracted in the continuous extractor for more than 15 hrs., at which time no more ether-soluble material could be recovered.

The low weight recovery is partially caused by chain degradation of the products by potassium permanganate. This effect has been noted before (14) and was shown to occur in the present experiments by the following procedure. The product from the oxidation of triundecylenin was saponified, and the dicarboxylic acids recovered were chromatographed on paper in the *iso*-propyl alcohol-ammonium carbonate buffer system of Kalbe (15). Considerable amounts of azelaic, suberic, and shorter chain dicarboxylic acids as well as the expected sebacic acid could be detected. The same results were obtained with all the oxidation procedures that were tried. In the case of methyl oleate and methyl linoleate the nonanoic and hexanoic acid resulting from the oxidation may be partially volatilized when the solvent is removed. The high yields when linoleate is oxidized by Kartha's method is probably caused by a side reaction, which will be discussed below.

The percentage of ester recovered was calculated as follows. The ester value in meq./gram was calculated by subtracting the neutralization value in meq./gram from the saponification value expressed in meq./gram. The ester value multiplied times the weight of oxidation product recovered yields the total milliequivalents of ester recovered from the oxidation. The millequivalents of ester recovered times 100, divided by the millequivalents of ester put into the reaction mixture, gives the percentage of ester recovered.

It can be seen that esters oxidized by Kartha's method (except for the triundecylenin where the recovery may have been poor) gave more than 100% ester recovery. Thus ester groups were actually produced during the oxidation. The ester recovery was only slightly high for methyl undecylenate, but for

TABLE I  
 Analyses of the Products Obtained by the Various Oxidation Procedures

Ester	Method of ox.	Weight <sup>a</sup> recovered	% of theory	Neut. eq. <sup>b</sup>	Ester value <sup>c</sup>	% ester recovered
		grams		meq./g.	meq./g.	
Methyl undecylenate.....	Kartha	5.30	97.2	4.76	4.86	102
Methyl undecylenate.....	Kartha	5.43	99.6	4.81	4.79	103
Triundecylenin.....	Kartha	5.07	92.9	4.76	4.90	98
Methyl oleate.....	Kartha	5.97	98.2	5.51	3.55	126
Methyl oleate.....	Kartha	5.85	96.2	4.94	3.65	127
Methyl oleate.....	Kartha	5.64	92.8	5.43	3.38	113
Methyl oleate <sup>d</sup> .....	Kartha	5.78	95.1	5.23	3.41	117
Methyl oleate <sup>d</sup> .....	Kartha	5.75	94.6	6.14	3.59	122
Methyl linoleate.....	Kartha	5.56	102.8	5.64	4.93	161
Methyl linoleate <sup>d</sup> .....	Kartha	5.21	96.3	6.32	4.42	136
Methyl linoleate <sup>d</sup> .....	Kartha	5.46	100.9	5.65	4.41	142
Methyl undecylenate.....	Hilditch	5.36	98.3	5.70	4.29	91
Triundecylenin.....	Hilditch	4.82	88.3	5.20	4.36	83
Methyl undecylenate.....	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	5.42	99.4	5.85	3.93	84
Triundecylenin.....	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	4.96	90.8	5.24	4.30	84
Triundecylenin.....	MgSO <sub>4</sub>	4.92	90.1	5.09	4.38	85
Methyl undecylenate.....	Cont. addn.	5.43	99.6	5.12	4.11	89
Methyl undecylenate.....	CO <sub>2</sub>	5.13	94.1	5.08	4.67	95
Methyl undecylenate.....	CO <sub>2</sub>	5.16	94.7	5.04	4.53	93
Methyl linoleate.....	CO <sub>2</sub>	4.75	88.0	6.80	3.30	92

<sup>a</sup> All samples were 5 g.

<sup>b</sup> Theoretical values for the products are methyl undecylenate 4.62, methyl oleate 5.55, methyl linoleate 6.28, triundecylenin 4.65.

<sup>c</sup> This value represents the difference in the millequivalents of acid per gram, determined by the saponification and neutralization procedures.

<sup>d</sup> In these samples the oxidation was continued several hours after it was considered complete.

methyl oleate and linoleate the values were quite high. This effect is only observed when the oxidation is carried out in the presence of acetic acid. The neutralization equivalents of the products oxidized by Kartha's procedure are slightly high for methyl undecylenate and below theory for all except one of the oleate and linoleate samples. The high values probably result from chain degradation and slight contamination with mineral or short chain organic acids. The low values in the methyl oleate and linoleate products are probably partly caused by loss of volatile nonanoic and hexanoic acid. However part of this low value probably is also caused by failure of the acids to cleave at the double bond. Prolonging the oxidation after it was considered complete did not greatly decrease the amount of excess ester that was formed. Treatment of the oxidation product with 3% aqueous sodium carbonate revealed that there was 6 to 15% neutral material in the methyl oleate and linoleate preparations. Moreover most of the excess ester groups were in the neutral fraction. Armstrong and Hilditch (16) reported a similar neutral product with high saponification equivalent when ethyl oleate was oxidized with potassium permanganate in glacial acetic acid.

These results are readily explained if one assumes that, during permanganate oxidation in the presence of acetic acid, some of the double bonds are not cleaved but are acetylated by the acetic acid. This would explain the high saponification values and low neutralization equivalents. It would also explain the high yields that are obtained in the oxidation of methyl linoleate. It may be that this reaction is similar to the addition of acetic acid to double bonds which occurs in the presence of certain catalysts (17), or it may result from a reaction of acetic acid with some intermediate formed during the permanganate attack on the double bond. It has not been possible materially to increase the yield of the neutral product with the additional ester groups by changing the reaction conditions, and it has not seemed profitable to examine its exact nature any further.

If the acetic acid is omitted from the reaction mixture, the procedure becomes essentially the same as Hilditch's procedure (3) except that the solution is more dilute. It can be seen from Table I that, when the reaction is carried out in this way, a low ester

recovery is obtained and the neutralization equivalent is high. This indicates that some saponification does occur during the oxidation with permanganate, as Kartha has contended (2). It does not seem likely that the low ester recovery is based on incomplete extraction of the ester in the reaction mixture as the ester recoveries are lower than the weight recoveries. Also the neutralization equivalent is raised, indicating additional free acid groups.

Since saponification seems to occur during the oxidation of esters by Hilditch's procedure and since acetic acid leads to undesirable side reactions, other possible means of buffering the reaction mixture were sought. Waters (18) has suggested that magnesium sulfate may be used to buffer the reaction mixture during permanganate oxidations. Yakubov (19) has advocated bubbling carbon dioxide through the reaction medium. Steger and van Loon (20) have advocated the slow addition of permanganate to the reaction mixture by a continuous extractor. In addition to these, ammonium sulfate was tried as a buffer. The ammonium sulfate and magnesium sulfate were added in 100 to 150% excess, and the reaction was carried out as usual. The carbon dioxide was added by running a tube down the condenser and bubbling the gas through it at a brisk rate. This caused considerable loss of acetone; therefore acetone was added at times to keep the reaction mixture up to volume. In the continuous addition procedure the permanganate was added slowly, and 24 hrs. were required for the oxidation to be completed. The results of these experiments are shown in Table I. It can be seen that none of them gives a quantitative ester recovery, and none of them seems to give much improvement over the unbuffered Hilditch procedure. Thus it appears that none of the methods for permanganate oxidation of fatty acids is very satisfactory.

Since Kartha's theory of glyceride structure is based on data obtained by the permanganate oxidation method, one may ask, do the data obtained here invalidate Kartha's theory? In his analysis of azelao-glycerides Kartha makes use of the Bertram separation to distinguish long chain saturated fatty acids from the oxidation products of the unsaturated fatty acids. If the side reaction which occurs during Kartha's oxidation resulted in some of the unsaturated fatty acids behaving like saturated fatty acids,

many of his observations would not be valid. To test this possibility olive oil was oxidized with potassium permanganate with and without the addition of acetic acid. The fatty acids were recovered and subjected to the Bertram procedure (21). No significant difference in the saturated acids determined by the two oxidation methods could be observed. Thus while Kartha's method is obviously of limited accuracy, it has not been shown that his conclusions are necessarily untrue.

### Summary

Various procedures for the permanganate oxidation of fats have been tried on purified model fatty acid esters. It was found that all the procedures tested except Kartha's procedure caused considerable loss of ester groups. Kartha's procedure, on the contrary, caused ester groups to be synthesized during the oxidation. It was concluded that none of the oxidation procedures that were tried is a very reliable method for the study of glyceride structure in fats. However the limitations which were discovered in Kartha's procedure could not be shown to invalidate Kartha's hypothesis of glyceride structure.

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[Received August 12, 1957]

## Correlation of Chromatographic Absolute Loss Determinations with the A. O. C. S. Cup Refining Method in Soybean Oil

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AMONG THREE METHODS (chromatographic, acetone-insoluble, and Wesson methods) available for determining refining efficiency, the chromatographic method is more and more the focus of interest because of its simplicity. While the acetone-insoluble determinations and the Wesson method are time-consuming and elaborate and require extreme skill on the part of the analyst, the chromatographic method can be run rapidly with relatively little experience. A series of two or more columns can be handled simultaneously, and a number of samples can be put through the adsorbent in succession.

The method is based on the relative difference in affinity of neutral oil and so-called minor constituents of crude oil toward the alumina adsorbent. Free fatty acids, phosphatides, moisture, volatile materials, certain pigments and impurities, such as meal and linters, having more affinity than neutral oil, are adsorbed by the column and cannot be eluted with ether. Neutral oil, on the other hand, can be recovered quantitatively with ether.

The technique was first developed by the International Chemical Union and published by Handshumaker and Linteris (1). The brief history of the method and its significance, compared to other methods for neutral oil determinations and the A.O.C.S. Cup Refining method, were presented at the A.O.C.S. Short Course in 1955 (2).

Previous studies in this laboratory and others have shown lack of correlation between the phosphorus and free fatty acid (FFA) content of degummed soybean oil and the A.O.C.S. Cup Loss method. It is also known that some degummed oils cannot be fairly val-

ued for trading by means of the A.O.C.S. Cup Loss method because the increased loss incurred in degumming is not always compensated by additional oil premium.

Since the Chromatographic Loss method is a simple and accurate index for unavoidable losses resulting from the nonneutral oil fraction of the oils, it offered good opportunity to study the behavior of the A.O.C.S. Cup Loss method.

### Experimental

The method used in our laboratory is similar to that used by the F.A.C. subcommittee for total neutral oil for collaborative work in 1956 and is called the "1954 Modified Chromatographic Method."

The 20 x 400-mm. chromatographic tubes with coarse fritted discs were ordered from Corning Catalogue L. P. 34, No. 38, 450. Activated alumina, Grade F-20, Mesh 80-200, was obtained from the Aluminum Ore Company, East St. Louis, Ill.

Instead of the ether-methanol solution however, one lot of U.S.P. ether was used and was found quite satisfactory for our study. Nevertheless it should be pointed out that, in the case of reproducing results between laboratories, U.S.P. ether is unsatisfactory because of its varying alcohol content. Hence the ether methanol solution was adopted by the F.A.C. subcommittee on neutral oil, which developed the chromatographic method referred to in this paper.

Two g. of sample were used for crude soybean oils, and four g. were used for degummed oils.

Special precaution was taken in pouring the dissolved oil-ether solution into the tube. A plastic