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by H. Seino, et al.

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Preparation of Methyl Esters from Fats and Oils Containing Short-Chain Fatty Acids Using Boron Trifluoride-Methanol Reagent

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Boron trifluoride(BF_3)-methanol complex is widely used for the preparation of methyl esters as the sample for gas chromatography. However, it is feared that short-chain fatty acids are lost, when esters are prepared from fats and oils containing these acids by the conventional BF_3-methanol method.

Collaborative studies were carried out for the purpose of establishing a standard method for the quantitative preparation of short-chain esters.

Soybean oils containing definite amounts of glycerol trihexanoate and glycerol trioctanoate were prepared and used as the samples for the collaborative studies. GC analysis of the esters prepared from these samples by the conventional method revealed the appreciable loss of hexanoic- and octanoic acids through the procedure. Partial evaporation of the short-chain esters and partial passage of them into aqueous sodium chloride layer were supposed to be responsible for the low recovery of these esters.

Further collaborative studies were performed to examine the conditions for the prevention of the loss of short-chain esters.

In conclusion, the collaborative studies suggested that the recovery of the esters of short-chain acids (C_6 and C_8) could be improved by the following modification of the conventional method.

a) Shaking the mixture after the addition of aqueous sodium chloride solution.
b) Reextraction of esters from the aqueous layer after the separation of hexane (or heptane) layer.
c) Immediate GC analysis after the esterification.

It was concluded that 7 ml of 7%-BF_3-methanol reagent was enough to esterify 300 mg of oil sample.

1. Foreword

The Gas chromatography Committee of the Sectional Committee on Experimental Methods for Fats and Oils and Their Products of the Japan Oil Chemists' Society has been studying the determination of fatty acids composition using elevated temperature gas chromatography. As their work[1)]

is nearly finished, it is expected that an experimental method will be established in near future.

By this method samples containing fatty acids ranging widely from low to high molecular weights can be analyzed by raising the column temperature during measurement. While fatty acids must be methylesterified for GC analysis, it is feared that esters of low molecular weight are lost when using the conventional method (Standard fats and oils analysis method 2.4. 20-71). This method, therefore, is not suitable for samples containig fatty acids under C_{10}. Evidently there is a pressing need to find a method of preparation of methyl esters from samples containing short-chain acids, in addition to establishing the above-mentioned fatty acid determination method using elevated temperature gas chromatography. p. 406

Consequently, in August 1975 a Subcommittee for the preparation method of fatty acid methyl esters (hereinafter referred to as the Subcommittee) within the Sectional Committee on Experimental Methods was established for the study.

Because methods in which fats and oils are saponified, decomposed and methylesterified once the fatty acids have been separated are not expedient in preventing losses of short-chain esters, the Subcommittee chose to investigate a convenient and quick method where Boron trifluoride-methanol complex (hereinafter abbreviated as BF_3-methanol) is used as reagent for esterification. A methylesterification method in which BF_3-methanol is used has been studied by Metcalfe et al[2),3)] and is at present adopted as an official standard method (A.O.C.S. method Ce 2-66) by A.O.C.S. The A.O.C.S., before adopting this method as an official standard method, carried out collaborative studies to investigate its basic problems and

concluded that it is a suitable method for preparation of methyl esters from glyceride.[4] Some questions concerning this method have been raised as evidenced in reports[5)-7)] which state that it is possible that (depending on reaction conditions) methoxyl derivatives of unsaturated fatty acids could be produced. However, the conclusion has been reached that if reaction conditions are carefully monitored this method could be well used for ordinary fats and oils. The A.O.C.S. method is said to be applicable to fatty acids up to C8 if solvents are not removed after esterification. The Subcommittee, therefore, carried out several collaborative studies to improve the method in order to extend the applicable range to fatty acids up to C6. As rather satisfactory results on fatty acids up to C6 were obtained, we report as follows.

2. Experiment and Results

2.1 Participating laboratories, materials and methods

2.1.1 Participating laboratories

The following 10 laboratories participated in the collaborative studies: Yokohama factory, Ajinomoto Co., Inc.; Research Laboratory, Asahi Electro Chemical Co., Ltd.; Home Products Research Laboratory, Kao Soap Co., Ltd.; Industrial Chemistry Laboratory, Faculty of Hygienic Sciences, Kitasato University; Faculty of Engineering, Kokushikan University; Laboratory of Industrial Chemistry, Kogakuin University; Division of Natural Organic Chemistry, Tokyo National Chemical Laboratory for Industry; Research Laboratory, Nisshin Oil Mills Ltd.; Japan Food and Oil Inspecting Association; Central Research Laboratory, The Lion Fat and Oil Co., Ltd.

2.1.2 Materials

(1) Glycerol trihexanoate and glycerol trioctanoate.

The following were placed into a four-mouthed flask equipped with a stirrer, a thermometer, a gas blower, a reflux condenser, and water measuring tube: 1.0 mol of glycerin, 3.3 mol hexanoic acids or octanoic acids (hereinafter abbreviated as C_6-acids or C_8-acids), 0.3 g of p-toluene-sulfonic acids, and 20 md xylene. The mixture was then heated under a nitrogen stream and stirred to maintain the temperature in the flask at a temperature where water produced by the reaction boiled together with xylene. When the water produced reached the theoretical amount, stirring was stopped. After cooling, the reaction mixture was dissolved into hexane of 2 to 3 times the amount of the mixture. The solution of hexane was washed 2 to 3 times with 1N-sodium hydroxide. The solution was then washed with water until neutralized and hexane was removed to obtain glycerol trihexanoate or glycerol trioctanoate. Before these glycerol triacyls were used, it was confirmed by GC that they did not contain glycerol monoacyl or glycerol diacyl.

(2) Materials for the collaborative studies.

The above-stated glycerol trihexanoate and glycerol trioctanoate were added to purified soy-bean oil and the following two kinds of samples were prepared for use in collaborative studies.

Sample A: Calculating from saponification values of soy-bean oil, a sample was prepared by adding glycerol trihexanoate and glycerol trioctanoate in such a way that composition of methyl esters become 5.0% each for methyl C_6-acids and methyl C_8-acids.

Sample B: In the same way a sample was prepared for methyl C_6-acids

and methyl C8-acids where each became 2.5% as above.

(3) BF_3-methanol reagent

Two kinds of BF_3-methanol complex (BF_3:13.5 to 14.5%) with different lot numbers were purchased in bulk from Wako Pure Chemical Industries Ltd. The lot numbers are shown below. And two ampuls of reagent from each lot were distributed to each participating laboratory for the collaborative studies.

reagents	lot number
No. 1	IEF 3618
No. 2	IEF 9233

2.1.3 Method

In the 1st and 2nd collaborative studies methylesterification was carried out in the following manner roughly following the A.O.C.S. method Ce 2-66.

About 300 mg of sample was placed in a 50 ml flask. To this 6 ml of 0.5 N-sodium hydroxide + methanol solution and boiling stones were added. Attaching a reflux condenser, the solution was then heated in a water bath until oil drops disappeared and the solution became homogeneous (about 5-10 min). Next, from the upper part of the condenser 7 ml BF_3-methanol reagent was added and boiled for 2 minutes. After heating was stopped the flask was removed from the condenser. Then saturated aqueous sodium chloride was added until the solution of methyl esters reached the neck of flask. About 1 ml heptane solution was transferred to a test tube; adding a small amount of sodium sulfate the solution was dried.

2.2 1st collaborative study

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In the 1st collaborative study the above-mentioned samples A, B

and BF_3-methanol reagent together with 4 brown ampuls were distributed to each participant. Each participating laboratory carried out the esterification twice for each of the A & B samples. After being dried with sodium sulfate, the ester solution was immediately packed into the ampuls and delivered on a specified date. The esters delivered were GC analyzed by different laboratories for each of the A & B samples.

Peak areas of each sample were obtained by digital integrator connected to gas chromatograph. Analytical values were corrected by a correction factor which had been previously obtained using standard samples. Apparatus and operating conditions used in analysis are shown in Table 1 and results in Tables 2 and 3.

Table-1 Operating conditions of gas chromatograph and digital integrator.

		Sample A	Sample B
GC	Instrument	Hitachi-063	Shimazu GC-4 BPTF
	Column	25%-DEGS/Chromosorb WAW, ϕ 3 mm × 2 m	20%-DEGS/Chromosorb WAW, ϕ 3 mm × 2 m
	Column temp.	80~200℃, 5℃/min.	80~200℃, 5℃/min.
	Injection temp.	230℃	250℃
	Detector	FID	FID
Integrator		Instrument: KENT Chromalog II Slope sampling rate: 1/2 s^{-1} Slope sensitivity: 5	Instrument: Shimazu ITG-4 A Noise filter: 3 Minimum peak count: 100 Peak detect: 30 μV/min. Threshold: ~ μV Delay: 0 s.

Table-2 Analysis of Sample A (The 1st collaborative study).

Laboratory	Fatty acid						
	C_6 (%)	C_8 (%)	C_{10} (%)	C_{12} (%)	$C_{18:1}$ (%)	$C_{18:2}$ (%)	$C_{18:3}$ (%)
A	3.68	3.42	8.32	3.62	17.42	54.06	9.48
	3.69	3.12	8.89	2.99	17.55	55.39	8.38
B	3.50	3.31	8.68	3.25	17.47	54.52	9.27
	2.60	3.27	8.81	3.60	18.01	55.63	8.08
C	3.29	3.55	8.86	3.01	17.36	55.47	8.47
	3.14	3.46	8.89	2.97	17.38	55.71	8.46
D*	9.38	6.52	7.52	2.01	15.55	47.30	11.72
	—	—	—	—	—	—	—
E	3.75	3.25	8.86	2.91	17.80	54.91	8.52
	3.65	3.12	8.92	2.98	17.53	55.01	8.78
F	4.46	3.92	8.88	2.88	18.03	54.52	7.30
	3.74	3.40	9.16	3.05	17.79	54.20	8.65
G	3.47	3.21	9.32	3.20	18.52	53.39	8.91
	3.17	3.01	9.36	3.15	18.33	53.91	9.06
H	4.27	3.39	8.93	3.20	17.93	53.36	8.92
	3.68	3.30	9.18	3.06	17.88	54.05	8.67
I	4.27	3.48	9.26	3.14	18.04	52.91	8.90
	2.84	3.09	9.14	3.16	18.47	53.88	9.42
J*	5.87	5.66	8.61	2.02	15.36	52.74	9.73
	7.50	7.61	8.46	1.98	14.82	50.55	9.08
Average	3.59	3.33	8.97	3.14	17.84	54.43	8.70
C.V. (%)	14.3	6.75	2.77	6.68	1.98	1.67	5.97
Recovery (%)	71.8	66.6					

* Analytical values of these laboratories were excluded in the calculation of average, C.V., and recovery.

Table-3 Analysis of Sample B (The 1st collaborative study).

Laboratory	Fatty acid						
	C_6 (%)	C_8 (%)	C_{10} (%)	C_{12} (%)	$C_{18:1}$ (%)	$C_{18:2}$ (%)	$C_{18:3}$ (%)
A	1.97	2.15	9.52	3.15	20.29	55.37	7.58
	1.73	1.98	9.64	3.24	20.58	55.29	7.54
B	1.61	1.95	9.56	3.43	20.06	55.98	7.42
	1.66	2.03	9.71	3.89	20.16	55.28	7.27
C	1.70	1.95	9.32	3.52	19.87	56.32	7.32
	1.95	2.13	9.46	3.37	19.93	55.78	7.38
D*	13.31	11.27	8.12	3.74	14.62	42.74	6.20
	9.71	10.13	8.00	3.86	16.46	45.25	6.59
E	1.68	1.97	9.45	3.26	20.28	55.91	7.44
	1.65	2.02	9.52	3.45	20.08	55.86	7.41
F	1.75	2.04	9.33	3.48	20.07	55.77	7.56
	1.58	1.96	9.35	3.59	20.35	55.82	7.36
G	1.63	2.07	9.60	3.39	20.55	55.41	7.35
	1.61	1.91	9.57	3.43	20.17	55.97	7.35
H	1.81	2.04	9.41	3.29	20.82	55.20	7.43
	1.89	2.19	9.48	3.54	20.14	55.25	7.50
I	1.75	2.13	9.38	3.55	20.31	55.56	7.33
	1.80	2.10	9.59	3.89	20.14	55.19	7.28
J*	5.96	6.55	8.23	3.02	16.55	51.98	7.72
	4.46	5.13	8.64	2.37	18.01	53.49	7.91
Average	1.73	2.04	9.50	3.47	20.24	55.62	7.41
C.V. (%)	6.88	3.97	1.22	5.89	1.22	0.60	1.32
Recovery (%)	69.2	81.6					

* Analytical values of these laboratories were excluded in the calculation of average, C.V., and recovery.

Analytical values of these laboratories marked with asterisk (*) shown in Tables 2 and 3 were excluded in the calculation of average values and coefficient of variation, because they showed apparently abnormal values. As evident from recovery rates in the tables, large losses of C_6-and C_8-acids were observed in both A and B samples. Especially analytical values of C_6-acids of sample A showed remarkable changes. Evaporation during packing or opening of the ampuls may be responsible for the loss of these short-chain esters because of their high vapour pressures. Further, there were no significant differences observed in analytical values because of

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different lots of BF_3-methanol reagents.

Together with this collaborative study, a small group using camellia oil and preparing methyl esters with a similar method investigated the presence of trans-isomerization of double bond accompanying esterification by measuring IR spectra before and after esterification. Hardly any trans-isomerization was observed in their results.

2.3 2nd collaborative study

Using the same samples and reagents as used in the 1st study, 5 member laboratories of the Subcommittee participated and carried out the 2nd collaborative study.

Almost the same method was used as in the 1st study except hexane was used as solvent and GC analysis was performed at each laboratory immediately after esterification to avoid as much as possible evaporation of short-chain methyl after the reaction. GC operating conditions followed the 1st study. Table 4 shows only analytical results of C_6-and C_8-acids. The numbers in parentheses in the "average" and "recovery" columns in Table 4 are the values calculated excluding the analytical values of laboratory C which appeared to be abnormal.

Compared to the results of the 1st collaborative study, recovery rates of C_8-acids improved for both A and B samples showing over 90%. However, satisfactory recovery rates for C_6-acids could not be obtained. The reason for this probably lies in the passage of some short-chain methyl into the aqueous layer when the saturated aqueous sodium chloride was being added after the esterification reaction.

2.4 3rd collaborative study (Shift of short-chain methyl into aqueous layer).

In order to investigate to what extent short-chain methyl shifts

Table-4 Analytical values of C_6-and C_8-acids in the 2nd collaborative study.

Laboratory	Sample A		Sample B	
	C_6(%)	C_8(%)	C_6(%)	C_8(%)
A	4.7	5.1	2.2	2.4
	4.7	5.0	2.2	2.4
C	2.92	3.43	1.43	1.92
	2.34	3.08	1.38	1.84
G	3.08	4.20	1.35	1.93
	3.26	4.50	1.57	2.06
I	5.11	5.56	2.16	2.59
	4.55	5.17	2.21	2.59
J	4.13	4.89	2.82	2.52
	4.52	4.73	2.56	2.40
Average*	3.93 (4.26)	4.57 (4.90)	1.99 (2.13)	2.27 (2.36)
Recovery* (%)	78.6 (85.2)	91.4 (98.0)	79.5 (85.2)	90.6 (94.4)

* Values in the parentheses were calculated excluding the abnormal values of laboratory C.

Table-5 Passage of short-chain esters into aqueous sodium chloride layer (The 3rd collaborative study).

Laboratory	Recovery of methyl esters (%)		
	C_6	C_8	C_{18}
C	70.7	83.9	98.2
G	62.3	74.4	103.0
I	68.8	87.1	—
J	65.0	84.3	100.5
Average	66.7	82.4	100.6

into the aqueous layer in the group where fatty acid methyl, methanol, hexane, saturated aqueous sodium chloride exist, four Subcommittee member laboratories carried out collaborative studies using the following method.

Each laboratory prepared fatty acid methyl mixtures such as C_6, C_8, C_{16}, C_{18} and analyzed them by GC. Using the peak area of C_{16}-acid methyl as the standard, peak area ratios of each component were obtained. Next 300 mg of this mixture was transferred into 50 ml flask and 13 ml methanol and 5 ml hexane were added, dissolving esters. Afterwards saturated aqueous sodium chloride was added to bring the ester solution up to the neck of the flask. The ester solution which rose to the surface was analyzed by GC and each component's peak area ratio was obtained against the peak area of C_{16}-acid methyl. The recovery rate of each component was the percentage of the analytical value of the post aqueous sodium chloride treatment against the pre-treatment value. From the above experiments,

it was observed that a considerable amount of C_6-and C_8-acid methyl shifted into saturated aqueous sodium chloride as shown in Table 5.

2.5 Study on factors influencing shifts of short-chain esters into aqueous layer

It became evident that a fair amount of short-chain esters shift into saturated aqueous sodium chloride layer. Consequently, each Subcommittee member laboratory divided the work of investigating means of preventing this shift.

2.5.1 Effect of extraction of esters which shifted into aqueous layer.

Similarly to the 3rd collaborative study a mixture of fatty acid methyl was prepared. 300 ml of the mixture was placed into a flask and was dissolved by adding 13 ml methanol and 5 ml hexane. To this saturated aqueous sodium chloride was added. The hexane layer which rose to the surface was separated and analyzed by GC. Afterwards 5 ml hexane was added to aqueous layer and esters which had shifted were extracted. The upper layer was added to the above hexane solution and GC analyzed. Recovery rates of each component were calculated by dividing each GC analytical value by the mixed ratio of the samples. As shown in Table 6, it was observed that most of the shifted esters could be extracted. p. 409

Table-6 Effect of the reextraction of esters from aqueous sodium chloride layer.

Methyl ester	Mixed ratio (%)	1 st extract		Total extract	
		Analyticl value(%)	ecovery (%)	Analytical value(%)	Recovery (%)
C_6	13.85	12.68	92.9	13.58	98.1
C_8	14.68	14.12	96.2	14.83	101.0
C_{16}	32.55	33.67	103.4	32.36	99.9
C_{18}	13.73	13.75	100.1	13.76	100.2
$C_{18:1}$	25.19	25.59	101.6	26.08	103.5

2.5.2 Examination of other factors

The following studies were carried out on other factors which were thought to influence recovery rates of short-chain esters.

By the A.O.C.S. method, saturated aqueous sodium chloride is added after the conclusion of reaction and a portion of ester solution which rises to surface is collected. But in this study, after the addition of saturated aqueous sodium chloride the flask was stopped and shaken well. The solution was then allowed to stand before GC analysis of the upper layer. Results showed that recovery rates of short-chain esters increased in comparison to the method without shaking.

Next, various kinds of electrolytes other than sodium chloride were employed as salting-out electrolytes. Consequently it was observed that when ammonium sulfate was used instead of sodium chloride, the recovery rates of short-chain esters increased. In this case, however, as the fluctuation of GC analytical values was fairly large, it was considered impractical. Furthermore, esterification experiments were performed changing the amounts of hexane and saturated aqueous sodium chloride. When amounts of hexane relative to water increased the recovery rates of short-chain esters naturally improved. However, when the relative amounts of hexane increased too much, it became difficult to perform GC analysis. Yet when amounts of water decreased it was feared that surplus BF_3 could not be thoroughly removed. Consequently, further investigation was not carried out in this area.

2.6. 4th collaborative study.

As mentioned above, it was thought that short-chain esters could be fully recovered by shaking after addition of saturated aqueous sodium

chloride, and by reextraction of esters which had transferred into aqueous layer. Taking up these points the 4th collaborative study was carried out.

Participating laboratories were 5 Subcommittee members and A & B samples were used as in the 1st and 2nd studies.

BF_3-methanol is desirable at as low a concentration as possible in order to prevent any influence on unsaturated acids. Therefore each laboratory obtained the commercial product and diluted it to 7% concentration with methanol.

The study was carried out as follows: Using 7% BF_3-methanol reagent, it was allowed to react as in the 1st and 2nd studies. After the reaction, 50 ml saturated aqueous sodium chloride was added. The flask was stopped, shaken for 20 seconds and allowed to stand for 1 min. Afterwards the upper layer was separated and GC analyzed. 5 ml hexane was added into the lower layer and shaken again for 20 seconds and allowed to stand for 1 min. The upper layer was separated and GC analyzed together with hexane solution.

As above, experiments were carried out twice on each sample. Table 7 shows the analytical results on C_6-and C_8-acids.

As apparent in Table 7, by reextraction of esters in the aqueous layer after separation of hexane layer, more than 90% recovery rates were obtained for both C_6-and C_8-acids.

2.7 Investigation on amounts of sample collected and of BF_3-methanol used

In the 4th collaborative study, esterification was carried out using 7 ml of 7% BF_3-methanol reagent to 300 mg of sample. It is doubtful, however, whether methyl esters are quantitatively produced or not with this amount. In order to confirm this the following experiment was performed.

Table-7 Analytical values of C_6-and C_8-acids in the 4 th collaborative study.

Laboratory	Sample A				Sample B			
	C_6(%)		C_8(%)		C_6(%)		C_8(%)	
	1*	2**	1*	2**	1*	2**	1*	2**
A	4.0	4.8	4.5	4.5	2.3	2.6	2.8	2.7
	4.7	4.9	4.5	4.8	2.4	2.6	2.8	2.6
C	4.56	4.71	3.81	3.92	2.33	3.14	1.97	2.66
	5.09	5.08	4.09	4.28	2.23	3.48	1.98	3.25
G	4.28	4.57	4.85	5.10	2.21	2.33	2.65	2.69
	4.32	4.60	4.87	5.04	2.18	2.88	2.60	2.70
I	4.42	4.91	4.98	5.74	1.90	1.92	2.25	2.29
	3.87	4.17	4.54	4.84	1.90	1.91	2.24	2.24
J	4.77	4.89	4.87	5.11	2.27	2.48	2.32	2.58
	4.91	4.87	5.00	4.95	2.39	2.40	2.24	2.58
Average	4.49	4.75	4.60	4.83	2.21	2.52	2.39	2.63
Recovery (%)	89.8	95.0	92.0	96.6	88.4	100.0	95.6	105.2

* 1 First extract ** 2 Total extract

Taking 300 mg, 600 mg and 800 mg each of high grade soy-bean oil as samples, 6 ml of 0.5N-potassium hydroxide-ethanol solution and 7 ml of BF_3-methanol reagent were used; and esterification was carried out using same method as in the 4th collaborative study. But reextraction was not performed because short-chain acids were not contained. High-speed liquid chromatography was done on each reaction product under the following conditions. Results are shown in Fig. 1. p. 410

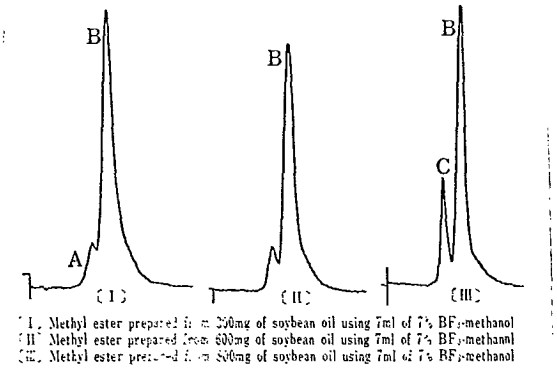
equipment: Hitachi 635 type high-speed liquid chromatograph.

column: Hitachi gel 3011, ϕ 4 mm x 500 mm.

solvents: Ethanol:hexane=60:40 (vol/vol), 2.1 ml/min.

detector: UV 221 nm, AUFS 0.1

[I], [II] and [III] in the figure are chromatograms of reaction products obtained by esterification using samples of 300 mg, 600 mg and 800 mg each. When identified using standard species, it was recognized that peaks



(I. Methyl ester prepared from 300mg of soybean oil using 7ml of 7% BF_3-methanol
(II. Methyl ester prepared from 600mg of soybean oil using 7ml of 7% BF_3-methanol
(III. Methyl ester prepared from 800mg of soybean oil using 7ml of 7% BF_3-methanol

Fig.-1 High-speed liquid chromatograms of methyl esters prepared from soybean oils using BF_3-methanol reagent.

-B which exist in common in [I], [II] and [III] were due to fatty acid methyl, and peak C which exists in [III] was due to fatty acids. Peak A which is observed in [I] is thought to be an un-identified peak somewhat different in retention volume from C, while the peak seen before B in [II] is thought to be due to overlapping of A and C. TLC results of each reaction products showed that free fatty acids were observed in considerable amounts in 800 mg sample, in very small amounts in 600 mg sample, and not at all in 300 mg sample.

From the above results it is thought that using 7 ml of 7%-BF_3-methanol reagent, esterification could proceed completely if a 300 mg sample were used. With larger samples, however, esterification will probably not proceed quantitatively. Therefore, it is necessary to increase amount of BF_3-methanol reagent in proportion to sample.

3. Conclusion

From the above experimental results the following conclusions were obtained.

1) A.O.C.S. Method Ce 2-66, in which BF_3-methanol complex is used as

a reagent for methyl esterification is a quick and convenient preparation method for fatty acid methyls. However, when fats and oils containing short-chain acids are starting materials, short-chain acids can not be recovered as methyl esters by this method. This method, therefore, is not applicable as is.

2) The following facts are thought to be responsible for the loss of short-chain acids: a portion of short-chain esters shifts into aqueous layer after the reaction when saturated aqueous sodium chloride is added to reaction mixture bringing the ester solution to the surface; and a portion of short-chain esters evaporate during the time before GC analysis after esterification.

3) In order to prevent the above problems, A.O.C.S. method was modified as follows so that the application range could be extended to fats and oils containing C_6 fatty acids.

After the reaction, saturated aqueous sodium chloride was added. The flask was stopped, shaken well and allowed to stand. In the cases where no short-chain acids were present the upper layer was immediately GC analyzed. In cases where short-chain acids were contained the upper layer was separated and 5 ml hexane (or heptane) was added to the lower layer and shaken again. After standing the upper layer was separated and immediately GC analyzed together with the above-mentioned upper layer.

4) When considering its influence on unsaturated acids, the lowest possible concentration degrees and amounts of BF_3-methanol reagent are preferable. 7 ml of 7%-BF_3-methanol to 300 mg of samples was enough to esterify. However, with samples in large amounts it is likely that esterification might not proceed quantitatively. It is, therefore, necessary to

increase amount of 7%-BF_3-methanol reagent in proportion to amount of sample.

In closing we wish to express of our sincere graditude to secretaries Shozo Asahara and Tetsutaro Hashimoto of the Sectional Committee on Experimental Methods for Fats and Oils and Their Products for their thorough guidance and encouragement during this study; and to each of those cooperating institutions and those who carried out the actual experiments in this collaborative experiment.

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References

1) 渡辺昭一郎ら, 油化学, **24**, 588 (1975)
2) L.D. Metcalfe, A.A. Schmitz, *Anal. Chem.*, **33**, 363 (1961)
3) L.D. Metcalfe, A.A. Schmitz, J.R. Pelka, *Anal. Chem.*, **38**, 514 (1966)
4) Report of the Instrumental Techniques Commitee, AOCS, 1966-1967, *J. Am. Oil Chem. Soc.*, **45**, 103 (1968)
5) A.K. Lough, *Biochem. J.*, **90**, 4 C (1964)
6) W.K. Fulk, M.S. Shone, *J. Lipid Res.*, **11**, 276 (1970)
7) 清野 肇, 千原直子, 渡辺昭一郎, 油化学, **24**, 59 (1975)

1) WATANABE Shoichiro et al, Yukagaku (Oil Chem. Soc.,) 24, 588 (1975)

7) SEINO Hajime, CHIHARA Naoko, WATANABE Shoichiro, Yukagaku (Oil Chem. Soc.,) 24, 59 (1975)