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Study on Hydrolysis Method For Extremely Small Amount of Lipids by Organic Basic Solution, Tetramethylammonium Hydroxide/Methanol and Capillary Gas Chromatographic Analysis of Fatty Acid Composition Depending on Derivatization Methods

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Abstract: Owing to the destruction and loss of fatty acid in the extraction and hydrolysis process of extremely small lipid sample with inorganic strong basic solution, KOH or NaOH/ethanol, there is a great necessity for a hydrolysis reagent to defend the destruction and enhance the extraction of hydrolyzed fatty acid during the hydrolysis and extraction steps. Tetramethylammonium hydroxide(TMAH)/methanol was employed as alternative. Standard triglycerides were hydrolyzed with 1*M* KOH/ethanol and 1 *M* TMAH/methanol, respectively, and fatty acid recoveries were determined in three different derivatives, methyl ester, trimethylsilyl(TMS) and N(O)-tert-butyldimethylsilyl(tBDMSi) derivatives. The recoveries of fatty acids from standard triglycerides with TMAH hydrolysis were higher from 1.3 to 65 times than those of KOH hydrolysis. Fatty acid recoveries from the sample lipids, beef tallow, corn oil and tuna eyeball oil, also showed same results as standard triglycerides. The fatty acid compositions of the sample lipids determined by TMAH hydrolysis showed close values with the values from the A.O.A.C.method regardless of derivatization methods. But in the case of KOH hydrolysis the fatty acid compositions showed significantly different values among the derivatives and from those of the A.O.A.C. method. In the KOH hydrolysis, the precision of the data obtained from recoveries or compositions of fatty acid were very poor compared to TMAH hydrolysis. In the extraction steps of hydrolyzed fatty acids on the case of KOH hydrolysis, substantial amount of fatty acids were simultaneously removed by the extraction with diethyl ether for removal of nonsaponifiable materials. When the hydrolyzed fatty acids after the extraction of nonsuponifiable materials were extracted with hexane, significantly small amount were extracted compared to TMAH hydrolysis. The total amounts of fatty acids recovered from the residues of each layer in the extraction steps, such as diethyl ether layer for the removal of nonsaponifiable materials, hexane layer extracted fatty acids and the water layer remained after extraction of hydrolyzed fatty acids, were significantly higher in the case of TMAH hydrolysis than those of KOH hydrolysis.

Key Words: Fatty Acids, Hydrolysis, Tetramethylammonium Hydroxide, N(O)-Tert.-Butyldimethylsilyl Derivatives, Gas Chromatography

Introduction

The inorganic basic solution, KOH or NaOH/alcohol, has been used with the main hydrolysis reagent for the determination of fatty acid composition on the lipid samples. There are not any problems in the case of using the large amount of lipid samples as in the A.O.A.C. method, but in the case of extremely small amount of samples, the losses of fatty acids by the destruction of very small amounts that may be arisen from the attack of the strong base and by the extraction steps with organic solvents in the process of recovery on the hydrolyzed fatty acids can be in serious problem. The development of a reagent to prevent the destruction of fatty acids from the attack of strong basic ion even though the amounts of destruction may be estimated at very small and to minimize the accompanied removal of fatty acids from the extraction of nonsaponifiable materials by the diethyl ether and to enhance the extraction of fatty acids by the hexane from the hydrolyzed sample solution is a essential work for extremely small amount of lipid samples.

Tetramethylammonium hydroxide(TMAH) were successfully used to saponification of lipids and the hydrolyzed fatty acids could be directly esterified to methyl ester with N,N-dimethylformamide and methyl iodide without any extraction steps but recovery of polyunsaturated fatty acids, linoleic and arachidonic acid, were depended on saponification temperature (Haan *et al.*, 1979). In this report the nonsaponifiable materials that were open to question on the interfering peaks were not removed. Because the interfering peaks are able to be existed according to the kind of samples and it is a prohibited thing to decide that there is not any interfering substances in the extracted lipids from samples, the procedure for removing nonsaponifiable materials is necessary to more accurate determination, especially for a diagnostic

analysis. But the loss of hydrolyzed fatty acids in the removal process of nonsaponifable materials must be estimated compared to conventional methods. Because the addition of water to the hydrolyzed sample solution must be done to remove the nonsaponifiable materials, the processes of acidification on the hydrolyzed fatty acids with HCl and extraction of acidified fatty acids with hexane must be followed. In these processes the loss of hydrolyzed fatty acids must be minimized. In this study we examined direct esterification after alkaline hydrolysis on the standard triglycerides, but recoveries were low compared to the case carried out all extraction step. It is probably because esterification reaction were not completely arisen owing to the existence of small amount of water produced between alkaline catalyst and alcohol. For the extremely small amount of lipid samples, we need a reagent that simultaneously accomplishes hydrolysis of sample lipids and minimizing of the losses of fatty acids from all of the hydrolysis and extraction steps, because there are many reports that polyunsaturated fatty acids, especially arachidonic acid, were lost during the processes of saponification with strong KOH and esterification with large excess of acidified methanol(dry HCl)(1-5). There is a report that 0.5 *M* KOH/methanol hydrolysis of lipids followed esterification with aqueous HCl/methanol was showed no differences in the relative percentage composition of the fatty acids (Jham *et al.*, 1982). But this report is only on the main fatty acids and not on the recovery of fatty acids. Even though fatty acid compositions were similar among the methods, it is not excellent method if the recovery of fatty acids were low because it is very difficult to obtain the pure lipids according to samples and there is a dangerous to make a mistake on the determination of fatty acid compositions, especially on the extremely very small sample.

In the previous report (Woo and Kim, 1999), we adapted an organic basic reagent, tetramethylammonium hydroxide(TMAH) which has same basic strength compared to the inorganic basic reagents for these purpose and it was very successful, on the other hand inorganic basic solution was impossible to use for these purpose and the obtained data had not entirely statistic reliability.

TMAH was used for methyl esterification of fatty acids. Tetramethylammonium salts of unesterified fatty acids in aqueous solution could be pyrolyzed to form methyl esters in the injection port of gas chromatography at 330-360°C, but most analysts have reported difficulties with this reagent (Christie, 1992),

The selection of this reagent was authenticated in facts as follows: (a) tetramethylammonium cation (TMA^+) constituting the fatty acid salt could prevent the destruction of fatty acid from the attack of strong basic ion by the steric hindering effect, and (b) could reduce the accompanied removal of fatty acid anion solved in water from the extraction with diethyl ether for the removal of the nonsaponifiable materials because of the property of the strong water retaining power of TMA^+ and (c) could enhance the extraction of fatty acid by the hexane from acidified water layer after extraction of nonsaponifiable materials, which was based on the fact that the existence of relatively hydrophobic counter-ions such as TMA^+ or tetrabutylanmmonium cation compared to Na^+ or K^+ ion effectively enhance the extraction of organic anion formed the salts with those hydrophobic counter cations by the organic solvents such as hexane (Harris, 1991).

In this study, we will improve the bases of the above theoretical assumptions by the determination of the amounts of fatty acids existing in the each solvent layer, such as diethyl ether layer for removal of nonsafonifiable materials, hexane layer for the extraction of hydrolyzed fatty acids and water layer remaining after extraction of fatty acids, which are generated through the experimental steps. Simultaneously we will examine on the resolution, identification and determination of fatty acid composition about three different fatty acid derivatives, methyl esters, N(O)-tert-butyldimethylsily (tBDMSi), and trimethylsily (TMS) derivatives, on the nonpolar capillary column by gas chromatogram.

Materials and Methods

Standard fatty acids and standard triglycerides were obtained from Sigma (St. Louis, MO. USA). N-methyl-N-(tert.-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), triethylamine, octadecane, BF_3-methanol, hexamethyldisilazane (HMDS), trifluoroacetic acid (TFA) and tetramethylammonium hydroxide (TMAH) were from Aldrich (Milwaukee, WI. USA). The other reagents were of analytical grade. The purified corn oil was purchased from commercial market, which was produced from professional oil company. Beef tallow and tuna eyeball oil were extracted from a lump of beef lipid and tuna eyeball by the Folch method (Folch *et al.*, 1957), but were not purified. They were used for sample oils.

Hydrolysis of Standard Triglyceride and Sample Oils: The procedures were similar to previous report (Woo and Kim, 1999) The two 200μl (0.08mg triglyceride) of standard triglyceride solutions (0.4mg/1ml hexane) and 50μl (0.2 mg sample oil) and 200μl (0.8mg sample oil) of sample oil solutions (4.0mg/1ml hexane) were placed into each of 1ml conical vials, respectively and dried with nitrogen gas and then each of 200μl of 1 *M* TMAH/methanol and 1 *M* KOH/ethanol were added to standard triglyceride vials, separately. Each of 200μl of 1 *M* TMAH/methanol were added to three vials of sample oils (corn oil, beef tallow, tuna eyeball oil) dried 50μl of sample solutions and each of 200μl of 1 *M* KOH/ethanol were added to the three other vials of sample oils dried 200μl of sample solutions, respectively. The vials were tightly capped and heated for 1 h at 75°C, After cooling to room temperature, water(500μl) was added and nonsaponifiable components were removed by extraction four times with diethyl ether The diethyl ether extracts were washed three times with water, and the water washings were added to the aqueous layer, which were acidified with 6 *M* HCl below pH 2.0. The acidified fatty acids were immediately extracted

four times with 500μl of hexane. The extracts were combined and dried with nitrogen gas at room temperature. The dried fatty acids were derivatized to methyl ester, trimethylsilyl(TMS) and t-butyldimethylsilyl(tBDMSi) derivatives. We also examined the direct dervatization without any extraction steps and drying procedure on the hydrolyzed standard triglycerides solutions.

The reason of sampling to 4 times on the KOH hydrolysis compared to TMAH hydrolysis was the caused of the fact that we couldn't obtain the peaks of fatty acids which existed with very small amounts in the sample oils with the KOH hydrolysis unless the samples were taken to 4 times of the case of TMAH hydrolysis.

Recovery of Fatty Acids from Standard Triglyceride and Recoveries of Standard Fatty Acids in each Layer Through the Hydrolysis Steps: Recovery of fatty acids from standard triglyceride through the hydrolysis process was calculated as follow;

Recovery (%) = A/B × 100

A: The ratio between the peak area of fatty acid obtained from hydrolysis of standard triglyceride and peak area of internal standard.

B: The ratio between the peak area of fatty acid derivatized with the standard fatty acid of the amount corresponding to the theoretical amount of fatty acid obtainable from the standard triglyceride and the peak area of internal standard.

Recovery of fatty acid existing in each layer generating by the extraction process of hydrolyzed fatty acid were determined with the hydrolysis of standard fatty acids and with only tBDMSi derivatives after drying the solvent in each layer with nitrogen gas. The three layers were generated through the process of hydrolysis of sample and extraction of hydrolyzed fatty acids, such as the diethyl ether layer removed the nonsaponifiable materials, the hexane layer extracted the hydrolyzed fatty acids and the acidified water layer (pH was controlled below 2 just before extraction of hydrolyzed fatty acids with hexane) remaining after extraction of fatty acids. The comparison of destruction amounts of fatty acids by strong base during the experimental process were accomplished with determination of the total amounts of fatty acid in each layer according to the respective KOH and TMAH hydrolysis. This comparison of destruction amount was carried out with the standard fatty acids(0.02mg of each acid), which were passed through the procedures of the hydrolysis and extraction steps same as sample oils.

Derivatization: The methyl ester and tBDMSi derivatives were carried out same as previous report (Woo and Kim, 1999). *i.e.* For the methyl ester derivatization, 50μl of 0.5*M* NaOH/methanol and 200μl of 12.5%(w/v) BF_3-methanol were added to the vials containing the dried and hydrolyzed standard triglycerides and sample fatty acids. For direct derivatization, amount of two times of above reagents were directly added to the hydrolyzed standard triglyceride solutions. After tightly capping, the vials were heated for 30 min at 75°C. Saturated sodium chloride solutions(ca. 200μl) were added and after tightly capping, the vials were vigorously shaken and then 200μl hexane were added . Fatty acid methyl esters were extracted into the hexane layer. The hexane layer was transferred to a 1-ml conical vial. This procedure repeated four times. The combined hexane layer was dried with anhydrous nitrogen gas and then 280μl hexane, 20μl internal standard solution (1 mg octadecane/ml of hexane) and a small amount of Na_2SO_4 anhydrous were added to remove any water. The supernatant was injected into GC. For TMS derivatives, 200μl of hexane and 20 of internal standard solution, 75μl HMDS and 5μl TFA were added to the dried fatty acids in a 1 ml of conical vial and tightly capped and then derivatized for 30 min at 40°C. In the TMS derivatives the direct derivatization was not examined.

For tBDMSi derivatiztion dried fatty acids were dissolved with 200μl of hexane and then 20μl internal standard solution, 75μl of MTBSTFA and 5μl of triethylamine were added. After tightly capping, the contents were derivatized at 75°C for 30 min and injected into GC after cooling to room temperature. The direct derivatization was also carried out on the hydrolyzed standard triglyceride solution. Internal standard solutions were added same amount in all derivatives and at just before derivatization. The final volumes for derivatization were adjusted to 300 μl in all derivatives.

Fatty Acid Compositions: Fatty acid compositions on the sample oils hydrolyzed by the TMAH or KOH were determined with three different derivatives, tBDMSi, TMS and methyl esters, and compared to the fatty acid compositions determined after methyl esterification with A.O.A.C. method (Official Method of Analysis, 1995).

Gas Chromatography: The gas chromatography was also same as previous report (Woo and Kim, 1999) except TMS derivative. *i.e.* The GC system was a HP-5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with electron pressure control(EPC) and a flame ionization detector. The capillary column was a HP-1 (100% methylsiloxane) fused silica column (50 m × 0.2 mm I.D., 0.25μm film thickness, Hewlett-Packard) The temperature program was as follows; initial temperature held for 1 min at 40°C and then held for 2 min after increasing to 70°C with 60°C/min. After increasing to 205°C with 5°C/min, held for 25 min and then increased to 285°C with 5°C/min and held for 1 min. The injection port and detector temperature were at 285°C. The inlet pressure and carrier gas flow-rate (1.6 ml/min) were controlled with EPC. The inlet valve was also controlled with EPC, *i.e.* left OFF for 0.1 min after injection and then left ON for remaining time. For the TMS derivative, the carrier gas was 1.5 ml/min. Initial oven temperature held for 1 min at 40°C and then was increased to 110°C with 70°C/min and held for 1 min. After increasing to 190°C with 10°C/min and held for 13 min and then increased to 280°C with 4°C/min and held for 2 min. The injection port and detector temperature were at 280°C.

Results and Discussion

Chromatography of Standard Fatty Acid: chromatograms of standard fatty acid derivatized

to tBDMSi, TMS, methyl esters are shown compared to the chromatograms analyzed with A.O.A.C. method in Fig. 1. Thirty-five fatty acids are successfully resolved on a HP-1 nonpolar capillary column on the all of the derivatives. The resolved chromatograms were mutually similar in tBDMSi and TMS derivatives, and in methyl ester and A.O.A.C. method. In the tBDMSi and TMS derivatives, C_{18} group of fatty acids were not completely resolved. Nonadecanoic (19:0), arachidonic (20:4 n-6) and E.P.A (20:5 n-3) also were not completely resolved in the tBDMSi, but in the TMS derivative, oleic acid (18:1 n-9) and linolenic (18:3 n-6), nonadecanoic (19:0) and arachidonic acid (20:4 n-6), cis-11,14,17-eicosatrienoic (20:3) and cis-eicosenoic acid (20:1 n-9) also were not completely resolved. In the methyl ester and A.O.A.C. method, oleic acid that was not completely resolved with tBDMSi derivative was completely resolved from linolenic acid (18 : 3 n-6), but arachidonic (20:4 n-6) and E.P.A (20:5 n-3) also were not completely resolved. In the methyl ester, short chain fatty acids under C_6 have a possibility to get buried in the solvent peaks (Hammon, 1993).. In the short chain fatty acids under C_6, tBDMSi or TMS derivatives may be suitable. All of the derivatives were resolved according to the order of increasing of C number.

Sensitivity: The sensitivity of each derivatives were determined with the peak area responses compared to internal standard peak area (Fig. 2). The sensitivity of tBDMSi derivative was higher about 1.1~10.8 times than those of TMS derivative and methyl ester ($P<0.01$). The TMS derivative and methyl esters had not significantly differences in the sensitivity.

The tBDMSi derivative have a higher heating stability than TMS derivative and more methyl radicals than TMS drivative or methyl esters (7, 13-17).

Recovery of Fatty Acid from Standard Triglyceride: The recoveries of fatty acids from standard triglyceride hydrolyzed with TMAH or KOH are shown in Table 1. In the KOH hydrolysis, all of the recoveries of fatty acids determined with three different derivatives were very low except only methyl ester of caprylic acid (75.9%) and relative standard deviations(RSDs) were very high, so the data had not entirely a statistical reliability.

In the TMAH hydrolysis, all of the recoveries of fatty acids were very significantly high (1.3-65 times)) compared to KOH hydrolysis ($P<0.01$). The RSDs values in the TMAH hydrolysis were less than 10% in most fatty acids. Only TMS derivative of caprylic acid was exceeded 10%. In the case of TMAH hydrolysis, some differences of recoveries were existed in the three derivatives, but there were not significant differences ($P<0.05$).

Recovery of fatty acids with direct esterification on the hydrolyzed standard triglycerides were low compared to the case passed through the extraction steps to obtain pure hydrolyzed fatty acids.

Recovery of Fatty Acids from Sample Oils: Table 2. 3. 4 show the recoveries of fatty acids from corn oil, beef tallow and tuna eyeball oil, respectively, determined with three different derivatives. These recoveries were expressed with the weight percentage of fatty acid recovered from the sample oil.

In all of the samples, as the standard triglyceride, the recoveries of fatty acid from the sample oils hydrolyzed with KOH were very low compared with the TMAH hydrolysis. In the case of KOH hydrolysis, the recoveries showed significant differences according to derivatization method, moreover the RSDs were very high in most of fatty acids.

In the case of TMAH hydrolysis, the recoveries were very similar to theoretical values which would be deduced from sample oils and the significant differences of recoveries among the three different derivatives were not existed in most of fatty acids. The total recoveries of fatty acids, in the case of TMAH hydrolysis, were high in order of corn oil, beef tallow and tuna eyeball oil. This order is the reverse of the content of nonsaponifiable materials. So we could assume that nonsaponifiable materials would be effectively removed by the extraction of diethyl ether following the TMAH hydrolysis.

Fatty Acid Composition of Sample Oils: Fatty acid compositions of corn oil, beef tallow and tuna eyeball oil are shown in Table 5. 6. 7, respectively. On the other hand the recovery of fatty acid, the compositions of fatty acids showed relatively similar values in the case of TMAH or KOH hydrolysis in the main fatty acids. Regardless of TMAH or KOH hydrolysis, the compositions of fatty acids among the three different derivatives rather showed some difference. These differences would be caused by the some differences of the number of fatty acid detected depending on the derivatives.

In the comparison with conventional A.O.A.C. method, the fatty acid composition determined from TMAH hydrolysis showed close values with the A.O.A.C. method in main fatty acids except some differences in linolenic, stearic, E.P.A and D.H.A in the tuna eyeball oil ($p<0.05$).

Recovery of fatty acids from the KOH hydrolysis were very low and the deviations were very large to the extent of that the data were worth little statistically. In spite of these facts, fatty acid compositions were similar in some degree with A.O.A.C. method. These phenomena could be assumed that the ratio of loss on each fatty acid by destruction or extraction steps were relatively constant even though the extent of loss were different in every experimental.

Recovery of Standard Fatty Acids from Each Lyer Through the Extraction Steps: Recovery of fatty acids on each layer through the extraction steps are shown in Table 8. In the diethyl ether layer extracted nonsaponifiable materials following the addition of water to the hydrolyzed sample solution, substantial amount (about 3 times) of fatty acids were extracted simultaneously with nonsaponifiable materials in the case of KOH hydrolysis compared to the TMAH hydrolysis ($P<0.01$). It is probably caused by the strong water absorbing ability of the TMA^+ ion. Fatty acid TMA salts would be protected by the water layer toughly surrounded the circumference of the salts. But in the case of KOH hydrolysis, the salting out effect by the K^+ ions would be caused for increasing the extraction of fatty acid by the diethyl ether.

The reagent of TMAH absorbed moisture in the

Table 1: Recoveries of Fatty Acids in the Standard Triglycerides as Three Different Derivatives after Hydrolysis with 1*M* KOH/ethanol and 1*M* Tetramethylammonium Hydroxide/Methanol (TMAH)

Triglycerides (Fatty acids)	tBDMSi				ME				TMS				(%)
	KOH	RSD	TMAH	RSD	KOH	RSD	TMAH	RSD	KOH	RSD	TMAH	RSD	
Tricaprylin (8:0)	47.6 (42.76	57.9 (56.8)	82.5 (80.3)	2.7 (5.7)	75.9 (60.0)	15.6 (23.8)	80.7 (76.9)	7.3 (8.1)	55.2	49.6	81.0	12.1	
Trilinolein (18 : 2 n-6)	6.0 (4.9)	124.8 (87.6)	96.5 (70.7)	7.5 (18.9)	3.1 (2.9)	52.7 (89.7)	103.4 (85.3)	3.3 (15.0)	1.5	19.8	104.7	2.0	
Trilinolenin (18 : 3 n-3)	8.66 (8.23)	106.8 (112.6)	89.8 (78.6)	3.78 (3.5)	4.7 (4.1)	18.7 (19.9)	89.6 (79.6)	3.6 (5.8)	4.3	14.2	82.6	6.8	
Triolein (18 : 1 n-9)	5.1 (5.0)	124.4 (135.8)	91.3 (85.8)	6.5 (7.8)	23.6 (18.6)	170.2 (57.9)	84.4 (70.5)	9.7 (9.7)	1.7	36.0	83.7	9.3	
Trielaidin (18:1)	4.0 (3.2)	134.3 (109.7)	81.4 (80.7)	8.2 (15.9)	1.7 (1.0)	75.88	88.6 (76.2)	9.2 (12.9)	0.9	23.5	79.8	9.7	

n=5
tBDMSi: N(0)-tert.-butyldimethylsilyl derivatives
TMS: Trimethylsilyl derivatives
ME: Methyl ester
RSD: Relative standard deviation
Recovery (%); refer to MATERIALS AND METHODS section in the text
The numbers in the parentheses are the estimated values with direct esterification

Table 2: The Comparisons of the Fatty Acid Recoveries Determined with Three Different Derivatives from Corn Oil Dependent on the Two Different Hydrolysis Methods, 1*M* KOH/ethanol and 1*M* Tetramethylammonium Hydroxide/methanol (TMAH)

Fatty acids	KOH						TMAH						(%)
	tBDMSi	RSD	TMS	RSD	ME	RSD	tBDMSi	RSD	TMS	RSD	ME	RSD	
7 : 0	0.01	0.00	-	-	-	-	0.03	0.00	-	-	-	-	
8 : 0	0.02	150	-	-	-	-	0.15	15.33	-	-	-	-	
10 : 0	0.01	0.00	-	-	-	-	0.04	5.00	-	-	-	-	
12 : 0	0.01	0.00	-	-	0.07	0.00	0.05	2.00	-	-	0.08	12.50	
13 : 0	-	-	-	-	0.07	14.29	-	-	-	-	0.17	11.76	
14 : 0	0.02	0.00	0.07	42.86	0.13	23.08	0.36	10.56	0.41	4.88	0.31	2.58	
15 : 0	-	-	-	-	0.04	0.00	-	-	-	-	0.11	7.27	
16 : 1 (n-7)	0.02	0.00	-	-	0.08	25.00	0.11	9.09	-	-	0.12	0.00	
16 : 0	1.04	47.12	3.73	31.90	2.85	33.68	7.06	3.40	9.59	22.11	8.51	12.93	
17 : 0	-	-	-	-	0.29	27.59	-	-	-	-	0.17	9.41	
18 : 2 (n-6)	7.48	51.74	21.74	32.01	13.45	45.35	56.52	4.95	50.19	6.95	50.03	7.32	
18 : 1 (n-9)	2.66	53.38	8.56	33.06	5.20	49.42	21.45	4.61	20.34	7.03	19.94	7.37	
18 : 1	0.28	60.71	0.56	30.35	0.48	41.66	2.78	6.12	1.31	10.69	1.24	8.87	
18 : 0	0.18	44.44	0.91	62.64	0.95	37.89	1.83	21.31	4.18	6.04	2.75	4.58	
20 : 0	0.04	25.00	0.20	60.00	0.18	16.37	0.26	3.85	1.03	4.95	0.39	15.38	
Total	11.79	35.20	35.77	22.92	23.8	28.15	90.7	1.34	86.61	5.83	83.91	5.10	

n=5
tBDMSi: N(0)-tert.-butyldimethylsilyl derivatives
TMS: Trimethylsilyl derivatives
ME: Methyl ester
RSD: Relative standard deviation
Recovery %; (total weight of hydrolyzed fatty acids/sample weight) x 100

Table 3: The Comparisons of the Fatty Acid Recoveries Determined with Three Different Derivatives from Beef Tallow Dependent on the Two Different Hydrolysis Methods, 1*M*-KOH/Ethanol and 1*M* Tetramethylammonium hydroxide/methanol (TMAH)

(%)

Fatty Acids	KOH						TMAH					
	tBDMSi	RSD	TMS	RSD	ME	RSD	tBDMSi	RSD	TMS	RSD	ME	RSD
7 : 0	0.01	0.00	-	-	-	-	0.03	0.00	-	-	-	-
8 : 0	0.01	0.00	-	-	-	-	0.03	3.33	-	-	-	-
10 : 0	0.05	0.00	0.03	0.00	-	-	0.07	14.28	0.06	0	-	-
11 : 0	0.01	0.00	-	-	-	-	0.03	0.00	-	-	-	-
12 : 0	0.10	50.00	0.04	25.00	-	-	0.14	1.43	0.13	3.07	-	-
13 : 0	-	-	0.03	-	-	-	-	-	0.03	0.00	-	-
14 : 0	2.08	9.61	0.62	62.90	0.45	42.22	2.60	12.69	2.67	4.87	2.45	12.24
15 : 0	0.42	23.38	0.10	70.00	0.10	40.00	0.52	11.54	0.50	10.00	0.55	12.73
16 : 1 (n-7)	1.63	1.84	0.35	80.00	0.27	51.85	1.80	2.22	1.95	7.69	1.80	6.11
16 : 0	13.22	10.59	3.16	87.03	1.83	53.55	16.91	1.19	17.94	5.24	17.33	3.90
17 : 0	1.39	3.60	0.24	95.83	0.15	60.00	1.65	7.88	1.54	2.60	1.40	15.00
18 : 2 (n-6)	2.08	8.65	0.39	87.18	0.56	87.50	2.87	3.97	2.82	4.89	2.84	8.66
18 : 1 (n-9)	23.18	3.11	4.11	93.19	2.46	56.91	25.32	2.04	27.23	2.31	22.47	10.99
18 : 1	1.50	3.33	0.48	106.25	0.28	71.43	1.52	2.37	3.27	6.73	2.82	13.83
18 : 0	12.27	11.49	2.50	100.00	1.30	62.30	15.93	5.19	16.18	7.25	15.92	4.72
20 : 3 (n-6)	-	-	0.01	00.00	-	-	-	-	0.09	11.11	-	-
20 : 3	0.21	0.00	-	-	-	-	0.24	6.67	-	-	-	-
20 : 1 (n-9)	-	-	-	-	-	-	0.05	0.00	-	-	-	-
20 : 0	0.11	18.18	-	-	-	-	0.23	4.35	-	-	-	-
22 : 1 (n-9)	0.06	0.00	-	-	-	-	0.07	4.28	-	-	-	-
24 : 1 (n-9)	-	-	0.03	0	-	-	-	-	0.30	3.33	-	-
Total	58.40	3.64	12.09	53.02	7.45	26.58	70.08	3.27	74.75	2.21	67.63	4.58

n=5
tBDMSi: N(0)-tert.-butyldimethylsilyl derivatives
TMS: Trimethylsilyl derivatives
ME: Methyl ester
RSD: Relative standard deviation
Recovery %; (total weight of hydrolyzed fatty acids/sample weight) x 100

Table 4:The Comparisons of the Fatty Acid Recoveries Determined with Three Different Derivatives From Tuna Eyeball Oil Dependent on the Two Different Hydrolysis Methods, 1*M* KOH/Ethanol and 1*M* Tetramethylammonium hydroxide (TMAH)

(%)

Fatty acids	KOH						TMAH					
	tBDMSi	RSD	TMS	RSD	ME	RSD	tBDMSi	RSD	TMS	RSD	ME	RSD
7:0	0.01	0.00	-	-	-	-	0.03	0.00	-	-	-	-
8 : 0	0.06	66.66	-	-	-	-	0.19	14.74	-	-	-	-
10:0	0.01	0.00	-	-	-	-	0.07	14.28	-	-	-	-
12 : 0	0.03	33.33	0.02	0.00	-	-	0.07	4.28	0.10	4.00	-	-
13 : 0	0.01	0.00	0.01	0.00	-	-	0.03	0.00	0.04	0.00	-	-
14 : 0	0.43	46.51	0.26	34.61	0.49	69.39	2.41	2.49	2.27	9.25	2.62	5.34
15 : 0	0.10	60.00	0.05	40.00	0.16	75.00	0.81	1.23	0.72	11.11	0.98	7.14
16 : 1 (n-7)	0.49	61.22	0.23	52.17	0.60	83.33	3.86	3.11	3.76	10.37	4.21	8.07
16 : 0	1.3	70.77	0.64	60.94	1.80	85.55	11.42	13.4	11.86	11.30	12.66	9.00
17 : 0	0.07	71.43	0.02	50.00	0.14	78.57	0.79	2.53	0.71	5.49	0.94	9.57
18 : 4	0.07	71.43	-	-	-	-	0.34	5.88	-	-	-	-
18 : 2 (n-6)	0.12	50.00	0.04	75.00	0.17	88.23	1.37	9.71	1.11	4.32	2.85	6.32
18 : 3 (n-6)	-	-	-	-	-	-	-	-	-	-	0.57	5.61
18 : 1 (n-9)	0.72	73.61	0.29	79.31	1.18	93.22	8.17	4.04	8.13	12.42	10.66	12.19
18 : 1	0.12	75.00	0.04	100.00	0.22	109.09	1.39	5.75	1.37	1.90	2.04	8.82
18 : 0	0.36	80.55	0.15	66.67	0.53	66.04	3.67	4.33	2.99	5.38	3.62	10.22
20 : 4 (n-6)	0.08	62.50	0.02	50.00	-	-	0.94	10.64	0.82	8.54	0.80	12.50
20 : 5 (n-3)	0.31	64.51	0.08	75.00	0.43	95.35	2.59	2.35	3.03	6.27	2.71	6.60
20 : 3	0.07	57.14	-	-	-	-	0.73	6.85	-	-	-	-
20 : 1 (n-9)	-	-	-	-	-	-	-	-	-	-	0.92	11.95
20 : 0	0.02	50.00	-	-	-	-	0.25	2.00	-	-	-	-
22 : 6 (n-3)	0.69	79.71	0.20	95.00	1.28	90.62	9.87	5.80	10.88	7.35	8.35	6.70
24 : 1 (n-9)	-	-	0.07	28.57	-	-	-	-	0.28	4.64	-	-
Total	5.06	25.74	2.17	28.57	6.67	35.83	49.08	2.73	48.14	5.25	53.98	6.68

n=5
tBDMSi: N(0)-tert.-butyldimethylsilyl derivatives
TMS: Trimethylsilyl derivatives
ME: Methyl ester
RSD: Relative standard deviation
Recovery %; (total weight of hydrolyzed fatty acids/sample weight) x 100

Table 5: The Comparisons of the Fatty Acid Composition in Corn Oil Determined with Each Derivative Depending on the Hydrolysis Methods by 1*M* KOH/Ethanol and 1*M* Tetramethylammonium Hydroxide(TMAH)

(%)

Fatty Acids	KOH						TMAH						A.O.A.C	RSD
	tBDMSi	RSD	TMS	RSD	ME	RSD	tBDMSi	RDS	TMS	RSD	ME	RSD		
(7:0)	0.14		-	-	-	-	0.03	0.00	-	-	-	-	0.36	5.56
		71.42												
(8:0)	0.23	86.96	-	-	-	-	0.17	14.76	-	-	-	-	0.75	4.00
(9:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.02	0.00
(10:0)	0.14	71.43	-	-	-	-	0.05	6.00	-	-	-	-	-	-
(12:0)	0.19	84.21	-	-	0.33	36.37	0.06	16.67	-	-	0.10	10.00	-	-
(13:0)	-	-	-	-	0.38	52.63	-	-	-	-	0.21	9.52	-	-
(14:0)	0.37	72.97	0.21	23.81	0.62	30.65	0.40	10.25	0.45	0.00	0.38	10.42	0.03	33.33
(15:0)	-	-	-	-	0.21	14.29	-	-	-	-	0.13	2.31	-	-
(16:1 n-7)	0.22	59.09	-	-	0.40	45.00	0.13	7.69	-	-	0.14	0.00	0.09	0.00
(16:0)	9.06	6.07	10.49	4.19	12.45	12.29	7.79	2.95	11.00	6.09	10.13	7.40	7.46	2.14
(17:0)	-	-	-	-	1.35	30.37	-	-	-	-	0.21	3.33	0.06	0.00
(18:4)	-	-	-	-	-	-	-	-	-	-	-	-	0.66	45.45
(18:2 n-6)	63.31	0.58	60.95	2.13	56.14	1.92	62.29	1.06	58.04	5.05	59.68	2.56	64.54	0.33
(18:1 n-9)	22.28	3.19	23.88	1.72	21.46	5.64	23.64	1.18	23.52	5.06	23.79	2.82	22.39	0.63
(18:1)	2.26	13.72	1.60	8.75	2.03	23.65	3.06	4.25	1.52	7.24	1.48	2.70	1.37	2.92
(18:0)	1.67	11.38	2.45	41.63	4.06	17.49	2.03	10.15	4.75	5.58	3.23	3.96	1.53	5.23
(20:1 n-9)	-	-	-	-	-	-	-	-	-	-	-	-	0.19	5.26
(20:0)	0.25	8.00	0.49	55.10	0.73	15.07	0.29	0.00	1.20	5.68	0.47	6.38	0.31	6.45
(22:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.08	25.00
(24:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.10	30.00
Total	100.12		100.07		100.16		99.94		100.48		99.95		99.94	

n=5
tBDMSi: N(0)-tert.-butyldimethylsilyl derivatives
TMS: Trimethylsilyl derivatives
ME: Methyl ester
A.O.A.C.;Methyl ester by A.O.A.C. method
RSD: Relative standard deviation

Table 6: The Comparisons of the Fatty Acid Composition in Beef Tallow Determined with Each Derivative Depending on the Hydrolysis Methods By 1*M* KOH/Ethanol and 1*M* Tetramethylammonium Hydroxide (TMAH)

(%)

Fatty acids	KOH						TMAH						A.O.A.C	RSD
	tBDMSi	RSD	TMS	RSD	ME	RSD	tBDMSi	RSD	TMS	RSD	ME	RSD		
(7:0)	0.02	0.00	-	-	-	-	0.04	2.50	-	-	-	-	0.59	3.39
(8:0)	0.03	0.00	-	-	-	-	0.05	2.20	-	-	-	-	-	-
(10:0)	0.09	11.11	0.77	116.89	-	-	0.10	0.00	0.08	0.00	-	-	-	-
(11:0)	0.02	0.00	-	-	-	-	0.04	0.00	-	-	-	-	-	-
(12:0)	0.17	47.06	0.67	105.97	-	-	0.20	15.00	0.17	23.53	-	-	-	-
(13:0)	-	-	0.07	28.57	-	-	-	-	0.04	0.00	-	-	-	-
(14:0)	3.57	10.36	6.79	46.83	6.49	14.18	3.72	4.03	3.57	3.92	3.73	4.75	2.83	2.12
(15:0)	0.73	1.37	1.00	33.00	1.49	18.79	0.75	12.00	0.67	11.94	0.84	14.29	0.24	8.33
(16:1 n-7)	2.80	4.29	3.23	17.96	3.81	9.459	2.55	1.57	2.61	9.20	2.78	9.78	2.11	1.42
(16:0)	22.59	4.91	26.78	4.14	24.73	7.20	24.28	11.29	23.99	3.83	25.08	12.68	25.21	0.99
(17:0)	2.38	2.10	1.94	8.25	2.05	13.66	2.37	10.13	2.06	1.94	2.11	12.32	1.42	2.11
(18:2 n-6)	3.58	12.85	3.77	42.71	8.18	76.41	4.05	3.58	3.77	14.85	4.27	11.48	3.58	11.73
(18:1 n-9)	39.75	4.35	32.27	12.98	32.67	3.58	35.85	1.43	36.44	3.35	34.22	4.96	39.90	0.43
(18:1)	2.57	4.67	3.36	31.55	3.49	34.38	2.15	4.42	4.37	6.64	4.30	8.37	1.60	1.88
(18:0)	20.97	5.72	18.65	19.25	17.04	13.15	22.92	1.63	21.63	5.22	22.63	9.93	21.91	1.10
(19:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.08	0.00
(20:3 n-6)	-	-	0.11	45.45	-	-	-	-	0.12	8.33	-	-	-	-
(20:3)	0.36	5.56	-	-	-	-	0.34	8.82	-	-	-	-	-	-
(20:1 n-9)	-	-	-	-	-	-	0.07	0.00	-	-	-	-	0.27	3.70
(20:0)	0.20	10.00	-	-	-	-	0.33	9.39	-	-	-	-	0.11	0.00
(22:1 n-9)	0.10	10.00	-	-	-	-	0.10	10.00	-	-	-	-	0.10	10.00
(24:1 n-9)	-	-	0.72	115.28	-	-	-	-	0.40	8.50	-	-	-	-
Total	99.93		100.13		99.95		99.91		99.92		99.96		99.95	

n=5
tBDMSi: N(0)-tert.-butyldimethylsilyl derivatives
TMS: Trimethylsilyl derivatives
ME: Methyl ester
A.O.A.C.; Methyl ester by A.O.A.C. method
RSD: Relative standard deviation

Table 7: The Comparisons of the Fatty Acid Composition in Tuna Eyeball Oil Determined with Each Derivative Depending on the Hydrolysis Methods by 1*M* KOH/Ethanol and 1*M* Tetramethylammonium Hydroxide (TMAH)

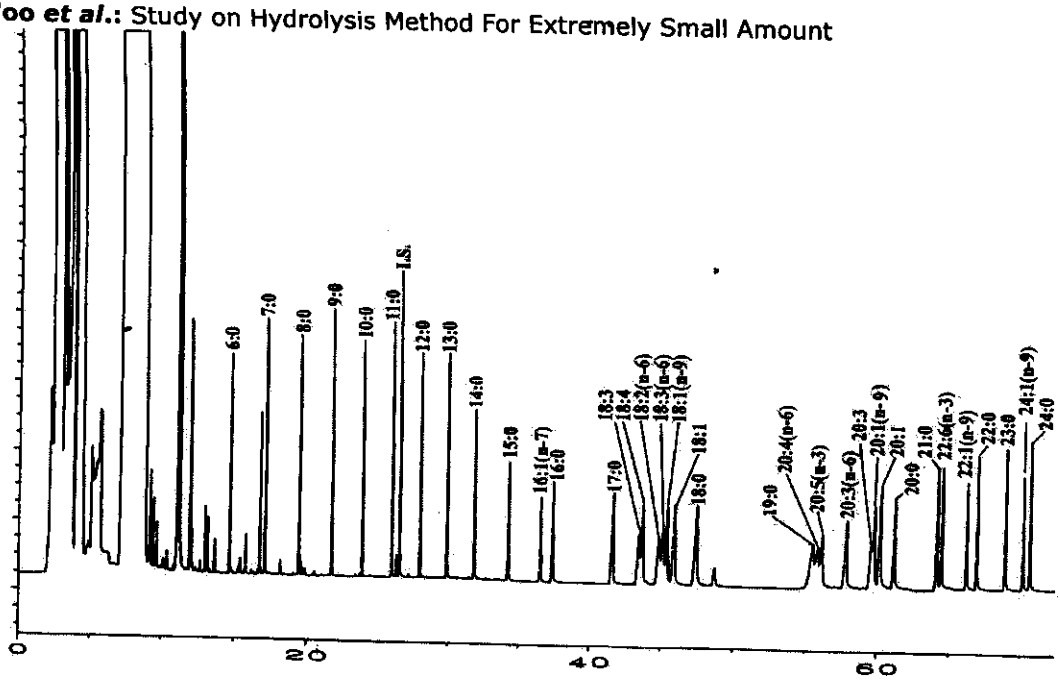
Fatty acids	KOH						TMAH						A.O.A.C	% RSD
	tBDMSi	RSD	TMS	RSD	ME	RSD	tBDMSi	RSD	TMS	RSD	ME	RSD		
(7:0)	0.40	77.50	-	-	-	-	0.06	0.00	-	-	-	-	0.93	5.384
(8:0)	1.50	78.00	-	-	-	-	0.39	14.10	-	-	-	-	-	-
(10:0)	0.38	52.63	-	-	-	-	0.15	20.00	-	-	-	-	-	-
(12:0)	0.80	56.25	1.49	38.26	-	-	0.15	20.00	0.22	4.91	-	-	-	-
(13:0)	0.19	26.32	0.57	35.09	-	-	0.07	0.00	0.08	0.00	-	-	-	-
(14:0)	9.80	24.39	13.56	19.47	9.50	32.42	4.92	2.24	4.73	2.75	4.89	9.41	3.50	2.29
(15:0)	2.18	13.76	2.81	13.88	3.05	31.48	1.66	3.01	1.50	4.00	1.83	10.38	0.83	7.23
(16:1 n-7)	10.11	10.58	11.26	8.35	9.85	21.62	7.87	4.83	7.82	1.92	7.83	8.17	6.05	2.15
(16:0)	25.08	7.38	29.51	2.91	28.85	10.43	23.24	11.27	24.62	1.50	23.59	9.75	24.74	2.91
(17:0)	1.43	13.99	1.14	7.89	1.67	14.97	1.61	2.48	1.48	5.41	1.74	7.47	0.22	45.45
(18:3)	-	-	-	-	-	-	-	-	-	-	-	-	0.24	33.33
(18:4)	1.59	35.85	-	-	-	-	0.69	8.70	-	-	-	-	0.93	3.23
(18:2 n-6)	2.68	35.45	2.14	17.29	2.84	23.59	2.80	18.93	2.29	16.16	5.05	5.69	0.55	23.64
(18:3 n-6)	-	-	-	-	-	-	-	-	-		1.03	4.78	0.60	6.67
(18:1 n-9)	14.02	7.20	12.46	15.57	17.14	5.72	16.66	4.80	16.87	3.08	19.76	3.95	15.83	2.02
(18:1)	2.28	12.28	1.66	27.71	2.70	34.81	2.83	7.42	2.83	10.95	3.79	5.80	1.73	4.62
(18:0)	6.96	29.60	7.01	20.40	10.49	38.23	7.43	4.79	6.19	4.85	6.79	16.79	4.39	1.14
(19:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.36	16.67
(20:4 n-6)	2.34	95.73	0.70	28.57	-	-	1.92	11.98	1.71	2.34	1.48	6.76	0.32	50.00
(20:5 n-3)	5.23	8.41	3.73	14.75	4.23	47.52	5.30	14.15	6.33	4.74	5.03	13.12	8.42	4.63
(20:3 n-6)	-	-	-	-	-	-	-	-	-	-	-	-	0.17	70.59
(20:3)	1.24	7.26	-	-	-	-	1.49	6.04	-	-	-	-	0.26	7.69
(20:1 n-9)	-	-	-	-	-	-	-	-	-	-	1.71	14.04	-	-
(20:1)	-	-	-	-	-	-	-	-	-	-	-	-	0.11	18.18
(20:0)	0.37	8.11	-	-	-	-	0.51	17.65	-	-	-	-	0.42	9.52
(22:6 n-3)	12.94	12.06	8.00	33.63	13.50	32.81	20.17	17.40	22.68	4.63	15.40	23.05	28.19	1.42
(22:1 n-9)	-	-	-	-	-	-	-	-	-	-	-	-	0.17	23.53
(22:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.28	78.57
(23:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.14	21.43
(24:1 n-9)	-	-	4.52	45.13	-	-	-	-	0.56	3.93	-	-	0.32	28.13
(24:0)	-	-	-	-	-	-	-	-	-	-	-	-	0.31	12.90
Total	101.52		100.56		103.82		99.92		99.91		99.92		100.01	

n=5
BDMSi: N(0)-tert.-butyldimethylsilyl derivatives
TMS: Trimethylsilyl derivatives
ME: Methyl ester
A.O.A.C.; Methyl ester by A.O.A.C. method. RSD; Relative standard deviation

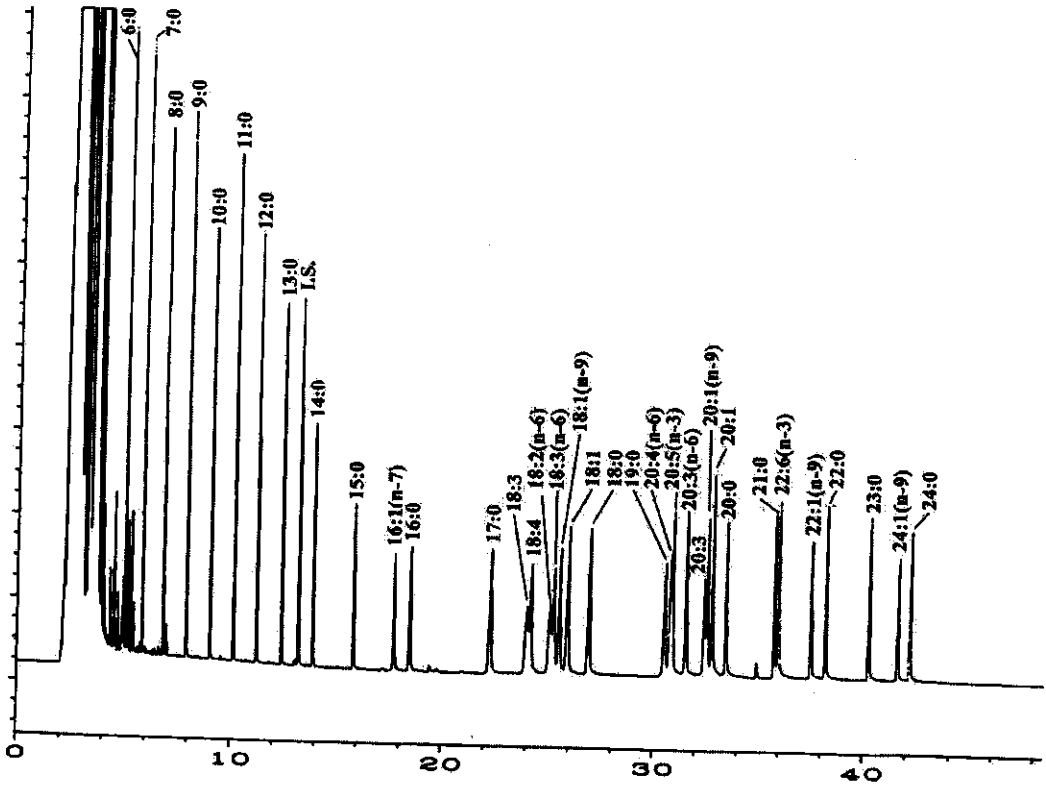
atmosphere as fast as that we couldn't measure the weight of the reagent on the oil paper in the room and the swelling volume of TMAH absorbed water was as large as that we couldn't make even up to 6 *M* concentration with aqueous solution.

We could assume that the structure of TMA^+ absorbed water have many vacant spaces which could exclude the uncharged materials and could include the fatty acid anion and so protect from the extraction of diethyl ether. Extraction with hexane of fatty acid uncharged by acidification of water layer (pH was below 2) were high about 1.3 times in the case of TMAH hydrolysis compared to KOH hydrolysis ($p<0.01$). We could explain this phenomenon with the two reason as follow; (Haan *et al.*, 1979) The fatty acids in the vacant space of TMA^+-water structure loss the anion charge by acidification with HCl and are excluded by Cl^- ions from the vacant space and more easily extracted by hexane. (Brown, 1930) There is a theory that the existence of more hydrophobic counter-ion (in this report, TMA^+) in the extracting solution enhance the extraction rate (7,12,). The TMA^+ ion is more hydrophobic counter-ion compared to K^+ ion, so the TMA^+ ion enhance the extraction of fatty acids compared to K^+ ion.

The remaining fatty acids in the water layer after extraction of fatty acids by hexane were slightly high in the case of TMAH hydrolysis compared with KOH hydrolysis, but there were not significant differences. The total fatty acids recovered from all of the layer were significantly high in the case of TMAH hydrolysis ($P<0.01$). This phenomenon indicate that the amount of fatty acids destroyed by OH^- ion during the hydrolysis step were more small because of the protection of hydrolyzed fatty acids by the steric hindering effect of TMA^+ ions. Because only three layers elucidated in the experimental section existed in this experimental process, so we could conclude that the difference of total amount of fatty acids in the three layers was produced with the difference of destruction rate by strong base. TMAH was very excellent organic base for the hydrolysis of lipids compared to inorganic bases, especially on the extremely small amount of samples. In the conventional method of fatty acid determination using relative large amount of sample, the destruction of fatty acid would be a negligible quantity, but there is no an obvious proof that fatty acids are not destroyed in any degree during the process of hydrolysis with strong base, and there are many report that the polyunsaturated fatty acids were lost during hydrolysis and esterification step(1-5), so we think that a substantial error caused by the destruction of fatty acid with strong base in the extremely small amount of sample have to be considered.

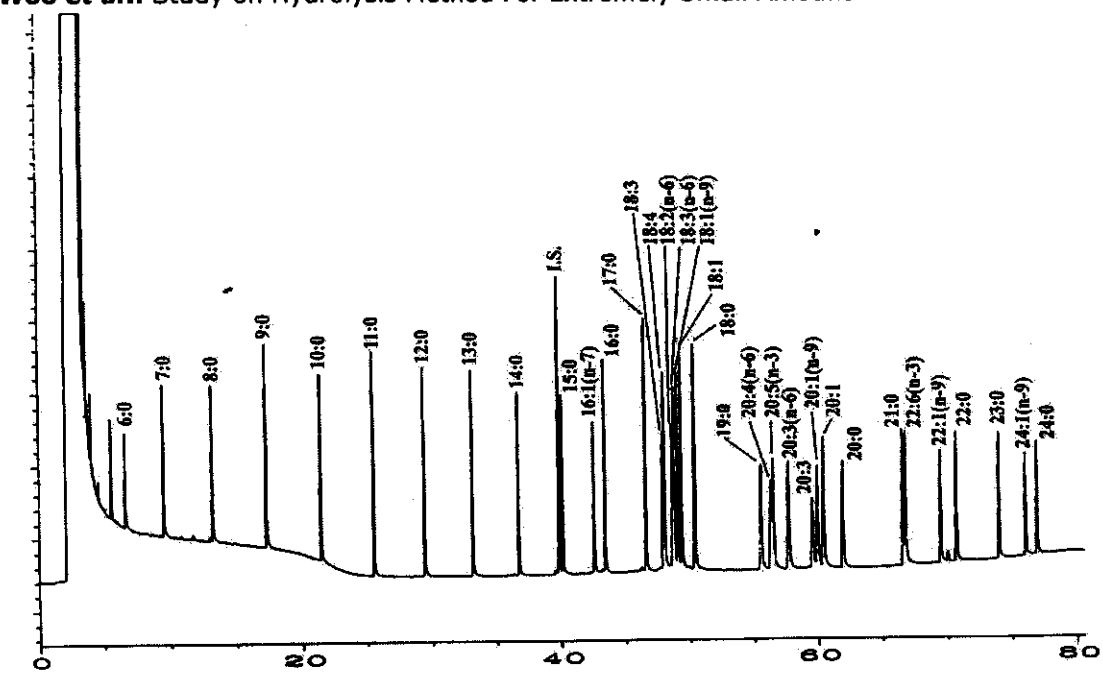


(A)

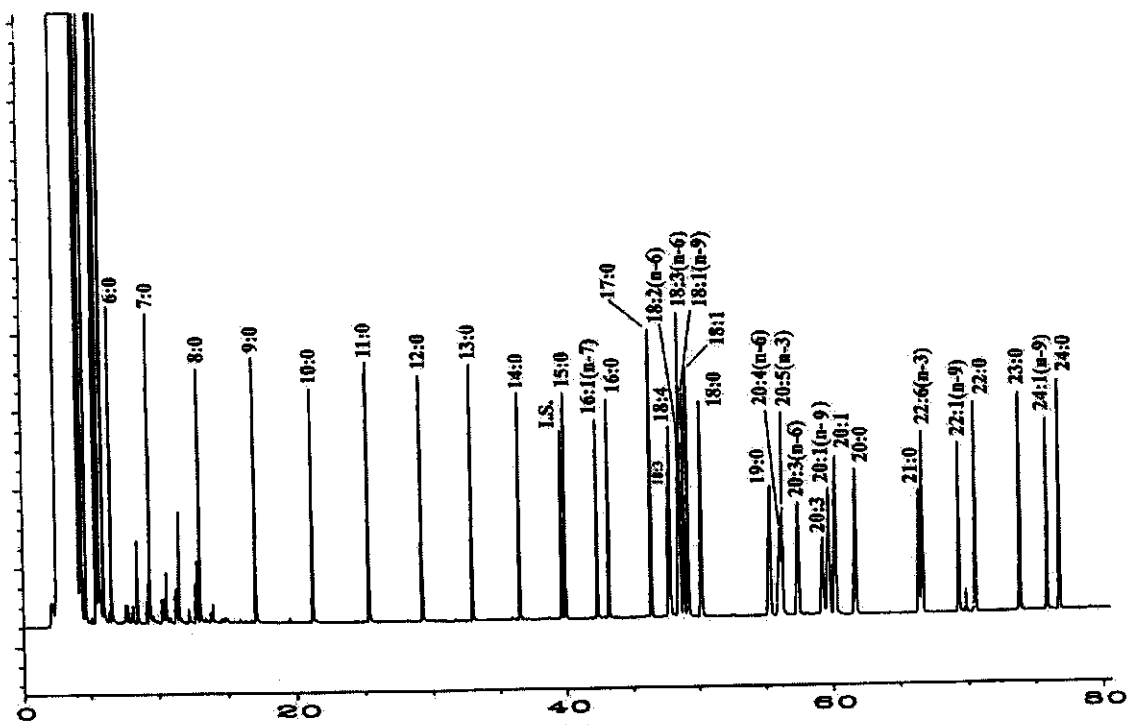


(B)

Fig. 1: Chromatograms of the Standard Fatty Acids as N(O)-tert.-Butyldimethylsilyl(tBDMSi) Derivative
Fig.1A: Trimethylsilyl Derivatives
Fig. 1B: Methyl ester by this Experimental Method



(C)



(D)

Fig. 1C: and Methyl ester by A.O.A.C Method

Fig. 1D: (The Standard Fatty Acids were Directly Derivatized Without any Hydrolysis Procedure)

Table 8: The Recoveries of Standard Fatty Acids as tDBMSI Derivatives from Each Layer in the Extraction Steps after Hydrolysis with 1*M* KOH/Ethanol and 1*M* Tetramethylammonium Hydroxide (TMAH)

(%)

Layers	Ethylether		Hexane		H_2O		Total			
Fatty acids	KOH	TMAH	KOH	TMAH	KOH	TMAH	KOH	RSD	TMAH	RSD
(6:0)	-	-	-	-	-	-	-	-	-	-
(7:0)	-	-	46.26	28.82	0.90	-	47.17	27.18	28.82	10.71
(8:0)	-	5.77	71.23	91.14	2.54	2.74	74.62	11.99	99.65	18.41
(9:0)	-	10.21	86.01	81.69	-	3.40	86.01	6.01	95.40	5.69
(10:0)	-	-	92.82	93.62	0.90	6.47	93.78	3.80	100.10	5.31
(11:0)	-	-	92.55	96.89	0.20		92.76	3.23	96.89	1.44
(12:0)	2.96	1.44	73.96	95.45	0.22	3.02	76.17	31.57	99.91	7.30
(13:0)	7.07	5.96	68.48	90.66	0.40	-	75.97	30.60	96.62	3.20
(14:0)	15.28	8.86	63.52	89.90	2.25	-	81.07	26.78	98.76	2.09
(15:0)	21.02	8.71	57.41	87.95	3.66	-	82.11	23.82	96.66	0.94
(16:1 n-7)	23.16	10.01	57.18	85.47	4.51	7.79	84.85	26.26	103.36	2.41
(16:0)	33.55	3.75	65.83	100.82	9.83	2.72	109.21	22.14	107.29	11.87
(17:0)	29.62	10.33	61.25	84.47	5.01	6.80	95.89	2.67	101.61	1.74
(18:3)	22.84	24.10	68.15	80.26	3.62	5.37	94.61	3.90	109.73	2.94
(18:4)	15.93	10.20	68.35	83.15	3.64	5.27	87.94	4.64	98.64	2.79
(18:2 n-6)	28.82	11.20	67.87	82.36	4.48	6.15	101.19	3.93	99.72	1.24
(18:3 n-6)	23.23	10.32	64.60	85.28	4.30	6.18	92.14	3.55	101.79	0.22
(18:1 n-9)	33.12	12.78	58.57	84.92	5.16	8.05	96.86	2.84	105.76	3.21
(18:1)	32.02	11.69	56.99	80.61	4.72	6.50	93.74	2.35	98.81	1.37
(18:0)	33.72	12.39	62.12	92.19	7.25	2.32	103.10	2.35	106.90	7.57
(19:0)	29.85	8.75	58.43	72.92	3.74	5.11	92.03	6.81	86.78	6.37
(20:4 n-6)	36.91	14.63	58.37	80.51	3.81	6.29	99.10	5.56	101.44	3.24
(20:5 n-3)	27.96	11.35	57.13	76.14	3.22	5.19	88.32	6.20	92.69	2.67
(20:3 n-6)	33.66	12.44	53.07	75.12	3.20	5.93	89.94	4.41	93.50	1.39
(20:3)	33.07	11.18	54.28	77.00	3.16	5.70	90.52	8.76	93.89	0.69
(20:1 n-9)	38.77	13.53	54.63	81.91	4.33	7.06	97.74	2.22	102.51	1.97
(20:1)	38.02	12.63	55.10	82.15	4.08	6.22	97.20	5.08	101.01	1.05
(20:0)	39.25	16.27	57.44	85.47	4.48	7.25	101.19	5.22	109.00	0.76
(21:0)	34.32	17.61	51.68	69.39	2.14	5.15	88.15	4.82	92.15	6.47
(22:6 n-3)	35.74	12.41	53.46	77.93	3.48	6.39	92.69	6.84	96.73	2.76
(22:1 n-9)	39.74	13.40	49.89	76.97	3.07	6.16	92.71	7.10	96.53	1.75
(22:0)	34.83	12.83	55.56	79.55	3.08	6.13	93.48	8.47	98.51	2.07
(23:0)	31.70	12.63	58.96	78.85	2.54	5.93	93.21	9.16	97.42	3.15
(24:1 n-9)	38.67	13.98	52.85	78.85	1.62	6.69	93.15	9.26	99.53	2.48
(24:0)	28.01	12.97	64.39	82.82	1.45	6.25	93.86	11.26	102.05	0.42

n=5
RSD: Relative standard deviatio
Recovery %; (Fatty acid weight in each layer/weight of total sampling fatty acids) x 100,
Sample; standard fatty acids

Conclusion

1*M* tetramethylammonium hydroxide (TMAH)/methanol solution was an excellent organic basic solution for the lipid hydrolysis compared to the conventional inorganic basic solution, 1 *M* KOH/ethanol, especially extremely small amount of lipid samples. Recovery of fatty acids from the hydrolyzed standard triglyceride was high about 1.3~65 times in the case of hydrolysis with 1 *M* TMAH/ methanol compared to the case of 1 *M* KOH/ethanol. Recovery of fatty acids directly derivatized without any extraction steps from hydrolyzed standard triglycerides was low compared to the case of passed through the all extraction steps. Recoveries of fatty acids from the sample oils, corn oil, beef tallow and tuna eyeball oil, were also very high in the 1 *M* TMAH/methanol hydrolysis. Fatty acid compositions of lipid samples were similar with the values obtained from A.O.A.C. method in the case of 1 *M* TMAH/methan hydrolysis compared to the 1 *M* KOH/ethan hydrolysis. The hydrolysis with TMAH/methanol was ver effective to minimize the extraction of fatty acic accompanied with the nonsaponifiable material i the ethyl ether extraction and to maximize th extraction of hydrolyzed fatty acids by the hexan from the acidified sample solution. The TMA^+ ion c the TMA salt of fatty acid produced from the hydrolys with TMAH/methanol was very effective to protec the destruction of fatty acid from the attack of th OH^- ion because of the steric hindering effect of TMA ion, which was impossible with the potassium sa of fatty acid in the hydrolysis with KOH/ethan solution. The tBDMSi derivative of fatty acid wa high in the sensitivity compared to the TMS c methyl ester. The tBDMSi, TMS and methyl esters c

fatty acids were successfully resolved on the HP-1 nonpolar capillary column. Fatty acid compositions determined with three different derivatives, tBDMSi, TMS and methyl esters, showed some differences according to the derivatization method regardless of the hydrolysis with the TMAH/methanol or KOH/ethanol.

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