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New hydrolysis method for extremely small amount of lipids and capillary gas chromatographic analysis as *N*(*O*)-*tert*.-butyldimethylsilyl fatty acid derivatives compared with methyl ester derivatives

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Abstract

The organic basic solution, 1 *M* tetramethylammonium hydroxide (TMAH) in methanol, was employed for the hydrolysis of extremely small amounts of lipids compared to the classical inorganic basic solution, 1 *M* KOH in ethanol. The hydrolysed fatty acids were derivatized as *N*(*O*)-*tert*.-butyldimethylsilyl (tBDMSi) esters with *N*-methyl-*N*-(*tert*.-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and compared with the classical derivatives, the methyl esters, made by the BF_3–methanol method. Recoveries of fatty acids determined on the standard fatty acids and soybean oil hydrolysed with TMAH were high: about 1.1–2.1- and 2.0–5.4-times, respectively, in all fatty acids compared with the hydrolysis by KOH regardless of derivatization method. The relative standard deviations (RSDs) on the recoveries of standard fatty acids were less than 5% when hydrolysed with TMAH, regardless of derivatives, but when hydrolysed with KOH, RSDs were more than 5% for most fatty acids, especially for long-chain fatty acids. The RSDs on the recoveries of fatty acids on the soybean oil were also very high in the KOH hydrolysis. Fatty acid compositions of soybean oil were similar in the main fatty acids regardless of hydrolysis methods, but showed slightly different values, depending on the methods of derivatization. RSDs were also very high in the KOH hydrolysis. In view of these results, precision of analysis by KOH hydrolysis was very poor, so we could not rely on the data. On the other hand, the reliability of data by TMAH hydrolysis method was very high, so it is a useful new hydrolysis method for extremely small amounts of lipid samples. Both derivatives of 35 standard fatty acids were successfully separated on a HP-1 nonpolar capillary column. tBDMSi derivatives were completely resolved in 70 min by 295°C. In the methyl ester derivatives it took about 80 min to get satisfying resolution, but these derivatives were completely resolved by 250°C. The sensitivity of tBDMSi derivatives was about 1.5–6.3-times higher than that with methyl ester derivatives. The stability of tBDMSi derivatives was constant for about 144 h except arachidic, docosahexanoic, behenic and heneicosanoic acids, which were stable for only 86 h.

Keywords: Derivatisation, GC; Lipids; Fatty acids; Tetramethylammonium hydroxide

1. Introduction

For analysis of the fatty acid composition of lipids by gas chromatography (GC), the hydrolysis of

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lipids and the derivatisation of hydrolysed fatty acids to more volatile derivatives must be carried out first. The hydrolysis method by inorganic basic solution, KOH or NaOH in alcohol, that has been traditionally used cannot avoid some thermal oxidative destruction of polyunsaturated fatty acids, owing to the exposure to the strong base for about 1 h by a reflux distillation, which is necessary for the complete hydrolysis of lipids and for the separate recovery of the free fatty acids and the nonsaponifiable components [1]. To recover the free fatty acids, the hydrolysed solution must be acidified and extracted with hexane following the diethyl ether extraction of nonsaponifiable components. When short-chain fatty acids are present in the lipids (C_{12} or less), it is necessary to extract the acidified solution much more exhaustively, and even then it may be almost impossible to recover very short-chain fatty acids. Some losses through the extraction steps are unavoidable. Epoxy groups and cyclopropene rings in fatty acids are disrupted by acid if the exposure to the acidic conditions is long [1]. There is a need to find a reagent to minimise the destruction of polyunsaturated fatty acids during the hydrolysis of lipids and to maximise the recovery of the hydrolysed fatty acids through the extraction step. For the effective extraction, relatively hydrophobic counter-ions such as tetrametylammonium or tetrabutylammonium cation can be used. The presence of such ions in the extracting solution increase the extraction rate of the relatively less hydrophobic counter-ions into the organic extraction solvent [2]. We think that as the K^+ or Na^+ in the fatty acid salts produced during hydrolysis cannot protect the polyunsaturated fatty acid chains from the attack of strong base, a considerable amount of destruction might occur. If we use an organic reagent that has the same basic strength as the inorganic base and that could produce a steric configuration of long-chain fatty acid salt instead of sodium or potassium during hydrolysis, the steric hindering effect of the long-chain salts could protect the fatty acids from attack by destructive chemicals. Under this assumption, we chose and examined an organic basic reagent, 1 *M* tetramethylammonium hydroxide (TMAH) in methanol, that has the similar basic strength as an inorganic strong base, and that can provide a relatively hydrophobic counter-ion compared to the inorganic cations.

In the derivatisation method of fatty acids for GC analysis, methyl esterification with BF_3–methanol has been widely used in spite of several defects such as the instability of the reagent, some loss of polyunsaturated and volatile fatty acids, complete destruction of epoxy, hydroepoxy, cyclopropenyl, cyclopropyl and hydroxy groups of fatty acids and nonderivatisation of unsaponifiable materials that may be contained in the lipids and may interfere with subsequent analyses [3–7]. Trimethylsilyl (TMS) derivatisation is known as a common method to correct the defects of methyl esterification [8–13]. But this method has some faults like thermal instability and possibility of hydrolysis of the derivatives even in the presence of very small amounts of water [1,14]. To overcome these defects, the *N*(*O*)-*tert*.-butyldimethylsilyl (tBDMSi) derivatisation method for GC analysis was developed [15–18]. In general, tBDMSi derivatives, compared to TMS derivatives, are known to have greater thermal and hydrolytic stability and inertness, these improve the separation of chromatographic analyses. Moreover, tBDMSi derivatives are also known to improve the sensitivity and selectivity for GC–mass spectrometry (MS) [14]. Nevertheless, we were unable to find reports in the literature that adopted the tBDMSi derivatives to the analysis of the fatty acid compositions of foods.

The aims of this study are: (a) to compare the recoveries of the standard fatty acids hydrolysed by both the inorganic basic reagent, KOH, and the organic basic reagent, TMAH, (b) to compare the fatty acid compositions of some food lipids hydrolysed with both basic reagents and analysed by both derivatives, and (c) to compare the analyses on the capillary nonpolar column by GC for the methyl esters and tBDMSi derivatives of the standard fatty acids occurring in foods.

2. Experimental

2.1. Materials

Standard fatty acids were obtained from Sigma (St. Louis, MO, USA), *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), triethylamine, octadecane, BF_3–methanol and TMAH were from Aldrich (Milwaukee, WI, USA). The other reagents were of analytical-reagent grade.

2.2. Sample preparation and lipid hydrolysis

Soybean oils were extracted by the Folch method [19]. The extracted lipid samples were dissolved in hexane. An aliquot containing 0.16 or 0.8 mg of lipid was each placed into a 1-ml conical vial and dried with nitrogen gas and then 200 μl of 1 *M* KOH in ethanol was added to the 0.8 mg of lipid and 1 *M* TMAH in methanol was added to the 0.16 mg of lipid. The vials were tightly capped and heated for 1 h at 75°C. After cooling to room temperature, water (500 μl) was added and nonsaponifiable components were removed by extraction three 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 50 μl of 6 *M* HCl. The acidified fatty acids were immediately extracted four times with 500 μl of hexane. The extracts were combined and dried with N_2 gas at room temperature. The dried fatty acids were derivatized to the methyl ester or tBDMSi derivatives. The recoveries of soybean oil fatty acids were estimated by the determination of the amount of hydrolysed and extracted fatty acids.

2.3. Derivatization

A 100-μl aliquot of standard fatty acids solution (0.1 mg/ml of hexane) was placed into a 1-ml conical vial and dried with N_2 gas at room temperature. For the tBDMSi derivatisation, the dried standard fatty acids and hydrolysed sample fatty acids were dissolved with 200 μl of hexane and then 20 μl of internal standard solution (1 mg octadecane/ml of hexane), 75 μl of MTBSTFA and 5 μl of triethylamine were added. After tightly capping, the contents were derivatized at 75°C for 30 min and injected into the GC system after cooling to room temperature. For the methyl ester derivatisation, 50 μl of 0.5 *M* NaOH in methanol and 200 μl of 12.5% (w/v) BF_3–methanol were added to the vials containing the dried fatty acids. After tightly capping, the vials were heated for 30 min at 75°C. Saturated sodium chloride solutions (ca. 100 μl) were added and after tightly capping, the vials were vigorously shaken and then 150 μl of hexane was added. Fatty acid methyl esters were extracted into the hexane layer. The hexane layer was transferred to a 1-ml conical vial. This procedure was repeated three times. The combined hexane layer was dried with anhydrous nitrogen gas and then 280 μl hexane, 20 μl internal standard and a small amount of Na_2SO_4 (anhydrous) were added to removed any water. The supernatant was injected into the gas chromatograph.

2.4. Recovery of standard fatty acids

The standard fatty acids solution (200 μl) was placed in a 1-ml conical vial and dried with N_2 gas at room temperature. The hydrolysis and extraction were the same as for the lipid hydrolysis procedure. The recoveries were estimated using the ratio of the relative molar response of the tBDMSi and methyl ester derivatives to the internal standard with or without the hydrolysis and extraction procedure.

2.5. Gas chromatography

The GC system was a HP-5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with electron pressure control (EPC) and a flame ionisation detector. The capillary column was a HP-1 (100% methylsiloxane) fused-silica column (50 m×0.2 mm I.D., 0.25 μm film thickness, Hewlett-Packard). The temperature programmes were 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 temperatures were at 300°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.

3. Results and discussion

3.1. Chromatography of standard fatty acids

Chromatograms of standard fatty acids analysed with tBDMSi and methyl ester derivatives are shown in Figs. 1 and 2. Thirty-five fatty acids were derivatized to tBDMSi and methyl ester derivatives and successfully resolved on an HP-1 capillary column. The tBDMSi and methyl ester derivatives of C_{18} unsaturated fatty acids, such as linoleic, linolenic,

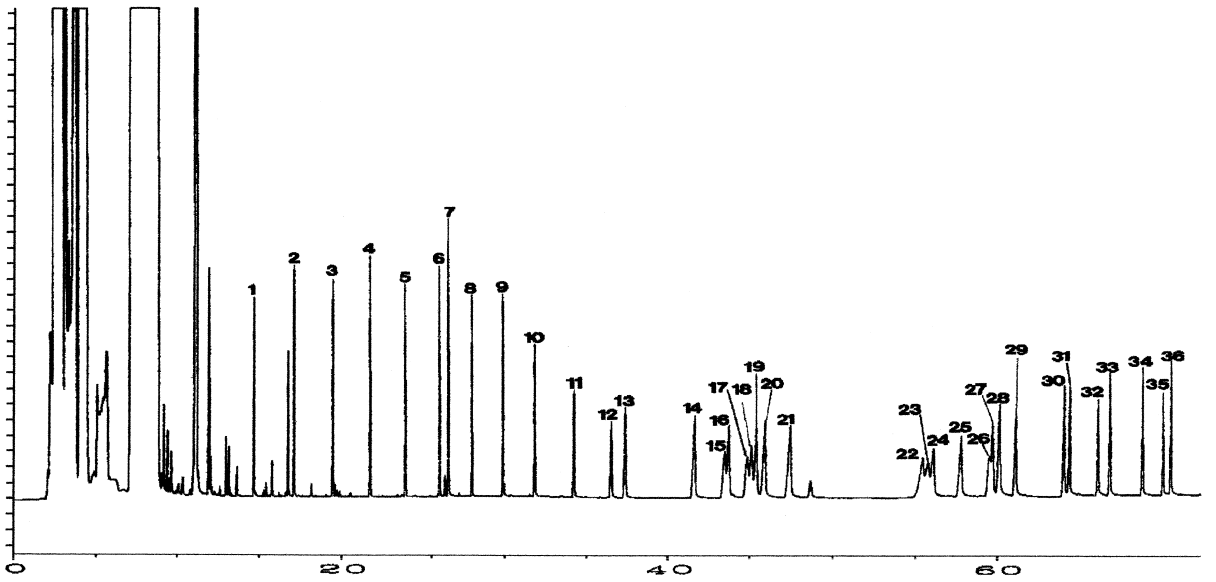

Fig. 1. Chromatogram of the standard fatty acids as *N*(*O*)-*tert*.-butyldimethylsily (tBDMSi) derivatives. 1=6:0, 2=7:0, 3=8:0, 4=9:0, 5=10:0, 6=11:0, 7=I.S., 8=12:0, 9=13:0, 10=14:0, 11=15:0, 12=16:1 *n*-7, 13=16:0, 14=17:0, 15=18:3, 16=18:4, 17=18:2 *n*-6, 18=18:3 *n*-3, 19=18:1 *n*-9, 20=18:1, 21=18:0, 22=19:0, 23=20:4 *n*-6, 24=20:5 *n*-3, 25=20:3 *n*-6, 26=20:3, 27=20:1 *n*-9, 28=20:1, 29=20:0, 30=21:0, 31=22:6 *n*-3, 32=22:1 *n*-9, 33=22:0, 34=23:0, 35=24:1 *n*-9, 36=24:0. Time scale in min.

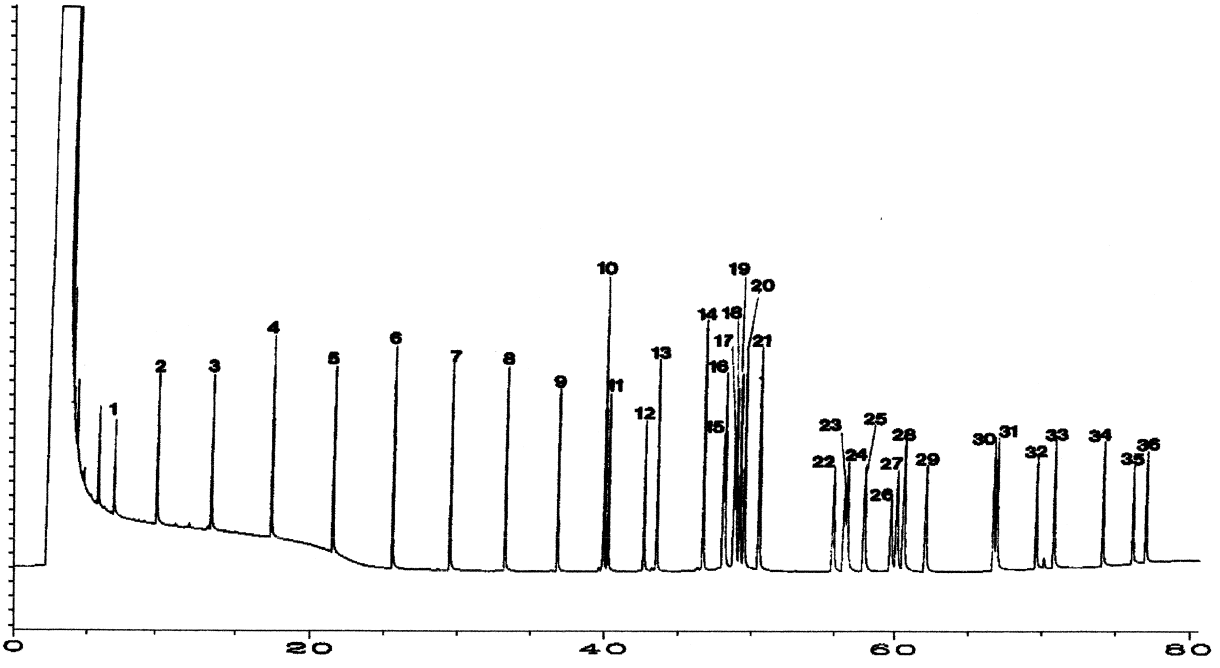

Fig. 2. Chromatogram of the standard fatty acids as methyl ester derivatives. 1=6:0, 2=7:0, 3=8:0, 4=9:0, 5=10:0, 6=11:0, 7=12:0 8=13:0, 9=14:0, 10=I.S., 11=15:0, 12=16:1 *n*-7, 13=16:0, 14=17:0, 15=18:3 *n*-6, 16=18:4, 17=18:2 *n*-6, 18=18:3 *n*-3, 19=18:1 *n*-9, 20=18:1, 21=18:0, 22=19:0, 23=20:4 *n*-6, 24=20:5 *n*-3, 25=20:3 *n*-6, 26=20:3, 27=20:1 *n*-9, 28=20:1, 29=20:0, 30=21:0, 31=22:6 *n*-3, 32=22:1 *n*-9, 33=22:0, 34=23:0, 35=24:1 *n*-9, 36=24:0. Time scale in min.

oleic and elaidic acids, were not completely separated. All of the tBDMSi derivatives were resolved in 70 min and the methyl ester derivatives were resolved in 80 min. But the methyl ester derivatives were completely eluted by 250°C compared to the tBDMSi derivatives that were completely eluted by 280°C. This phenomenon indicates that even though the boiling points of tBDMSi derivatives were higher than those of methyl ester derivatives, their chromatographic behaviours on the nonpolar column were superior than those of the methyl ester derivatives.

3.2. Recovery of standard fatty acids

The recoveries of standard fatty acids were 1.1–2.1-times higher in the TMAH hydrolysis compared to the KOH hydrolysis regardless of the derivatisation method (Table 1). This phenomenon was especially marked for the long-chain fatty acids. Re-

Table 1
Recovery as tBDMSi and methyl ester (ME) derivatives of standard fatty acids hydrolysed by 1 *M* TMAH in methanol and 1 *M* KOH in ethanol solution ($n=3$)

Fatty acid	Recovery (%)							
	TMAH				KOH			
	tBDMSi	RSD (%)	ME	RSD (%)	tBDMSi	RSD (%)	ME	RSD (%)
6:0	74.04	2.2	73.05	2.9	35.44	14.7	36.50	11.3
7:0	90.28	4.1	89.65	3.6	66.65	5.1	64.20	5.9
8:0	97.58	1.9	98.45	2.0	83.38	5.0	81.39	5.5
9:0	99.85	2.3	98.54	3.6	91.78	4.8	90.87	3.4
10:0	101.57	1.4	99.25	4.5	96.04	3.8	95.64	2.1
11:0	101.44	1.1	103.55	2.4	95.54	2.2	91.54	2.7
12:0	102.50	1.8	97.43	1.6	92.33	0.8	90.21	0.7
13:0	97.73	2.0	95.35	1.9	85.11	2.0	84.57	3.2
14:0	98.75	2.1	97.56	1.1	76.07	2.9	74.01	1.4
15:0	93.31	2.1	94.45	3.7	65.04	10.1	63.24	8.5
16:1 *n*-7	93.27	2.6	94.35	3.7	66.93	11.4	69.83	10.2
16:0	109.37	3.2	95.27	2.7	77.33	3.3	74.21	4.6
17:0	92.62	2.5	90.30	1.8	56.61	15.7	57.58	6.8
18:3 *n*-6	94.61	3.8	92.65	4.6	66.07	9.4	68.01	4.6
18:4	91.63	2.5	90.25	3.4	77.58	4.2	70.57	2.4
18:2 *n*-6	95.39	2.8	96.21	2.5	61.58	12.1	60.47	14.3
18:3 *n*-3	91.68	1.9	90.15	2.6	67.03	7.1	67.09	8.1
18:1 *n*-9	91.69	3.8	90.28	1.4	57.31	13.1	50.39	9.6
18:1	86.77	3.7	83.29	3.2	53.21	13.8	51.20	11.3
18:0	109.10	3.2	89.30	3.5	73.44	7.4	71.45	5.8
19:0	82.89	5.0	80.35	2.5	61.91	12.2	60.99	9.0
20:4 *n*-6	85.07	3.9	82.35	5.0	51.31	11.2	52.35	8.9
20:5 *n*-3	85.50	4.6	82.65	5.0	45.54	10.8	42.87	7.6
20:3 *n*-6	89.57	3.7	87.27	4.9	52.46	13.9	54.15	15.8
20:3	85.24	4.0	82.37	2.6	49.59	12.0	47.27	20.4
20:1 *n*-9	87.47	2.5	84.56	4.9	49.19	13.5	48.13	11.3
20:1	85.09	4.2	87.01	3.7	45.69	10.9	41.38	14.9
20:0	88.32	0.7	86.32	3.8	48.49	13.7	47.11	13.2
21:0	72.49	2.7	70.39	2.8	49.45	9.3	47.35	11.0
22:6 *n*-3	86.00	4.8	85.27	4.8	43.14	11.2	42.10	4.8
22:1 *n*-9	82.18	4.9	80.97	5.0	41.76	11.8	40.80	4.8
22:0	82.85	4.8	83.25	3.0	42.03	11.3	44.47	12.2
23:0	80.84	4.9	78.24	1.6	40.60	13.0	42.16	16.8
24:1 *n*-9	79.98	4.7	77.25	2.4	37.71	11.5	36.70	10.8
24:0	77.78	3.8	70.34	5.0	39.32	14.9	35.79	15.5

coveries were decreased in the both of hydrolysis methods with increasing fatty acid chain length.

RSDs were less than 5% with the TMAH hydrolysis method, but in the KOH hydrolysis exceeded 5% for most of fatty acids. We think that this is for the following two reasons: (1) in the TMAH hydrolysis, the tetramethylammonium cation acted to protect the destruction of fatty acid anion during the hydrolysis process by the steric hinderance, and (2) in the fatty acid extraction step with hexane from hydrolysed solution, the extraction was enhanced by the existence of relatively less hydrophobic counterions, tetramethylammonium cations, compared to inorganic cations [2].

3.3. Recovery of fatty acids on soybean oil

In the soybean oil, we used up to five-times the amount (0.8 mg) in the KOH hydrolysis compared with TMAH hydrolysis because in the KOH hydrolysis we could not detect the very small amounts of fatty acids that we could determine with tBDMSi derivatives on the TMAH hydrolysis in the smaller amount than 0.8 mg under the given experimental conditions.

Twenty fatty acids were detected with the tBDMSi derivatives in both hydrolysis methods but with the methyl ester derivatives only 11 fatty acids were detected (Table 2). We think that this phenomenon was because of the higher detection sensitivity for the tBDMSi derivatives (Fig. 3).

In the soybean oil, the recoveries of fatty acids were markedly, higher 2.0–5.4-times with the TMAH hydrolysis. The recovery of total fatty acid was higher by about three-times using the TMAH hydrolysis method. RSDs were very high in the KOH method, exceeding 5%.

3.4. Fatty acid compositions of soybean oil

In main fatty acids, palmitic, linoleic, linolenic, oleic, elaidic and stearic acid, the compositions were

Table 2
Recovery of fatty acids determined with tBDMSi and methyl ester (ME) derivatives from soybean oil hydrolysed with 1 *M* TMAH in methanol and 1 *M* KOH in ethanol solution ($n=7$)

Fatty acid	Recovery[a] (%)							
	TMAH				KOH			
	tBDMSi	RSD (%)	ME	RSD (%)	tBDMSi	RSD (%)	ME	RSD (%)
6:0	0.068	4.4			0.014	50.0		
7:0	0.046	13.0			0.012	66.6		
8:0	0.530	8.1			0.100	38.0		
9:0	0.022	9.1			0.011	36.4		
10:0	0.062	8.1			0.030	3.3		
11:0	0.061	3.3			0.012	25.0		
12:0	0.204	13.2			0.048	6.3		
13:0	0.037	24.3			0.007	42.9		
14:0	0.281	6.4	0.350	8.6	0.070	1.4	0.065	15.4
15:0	0.086	7.0	0.252	15.1	0.016	50.0	0.045	24.4
16:1 *n*-7	0.096	7.3	0.138	5.8	0.028	10.7	0.033	33.3
16:0	6.747	1.5	6.351	4.7	2.284	49.9	1.293	29.9
18:2 *n*-6	33.806	3.2	28.513	7.2	11.903	23.0	6.095	32.1
18:3 *n*-3	5.383	6.2	6.600	7.6	1.798	33.3	1.465	25.5
18:1 *n*-9	11.927	7.1	10.388	1.9	3.790	47.6	2.170	33.4
18:1	0.744	8.5	1.225	1.8	0.230	33.5	0.313	30.7
18:0	2.600	2.6	2.363	1.5	0.858	4.7	0.443	37.9
20:0	0.238	15.5	0.125	0.8	0.063	31.7	0.029	27.6
22:0	0.228	3.1	0.146	4.1	0.053	54.7	0.025	40.0
24:0	0.258	1.2			0.040	12.5		
Total	63.419	2.2	56.424	3.8	21.359	16.5	11.976	18.1

[a] $\text{Recovery (\%)} = \frac{\text{Fatty acids mass}}{\text{Soybean oil mass}} \cdot 100$

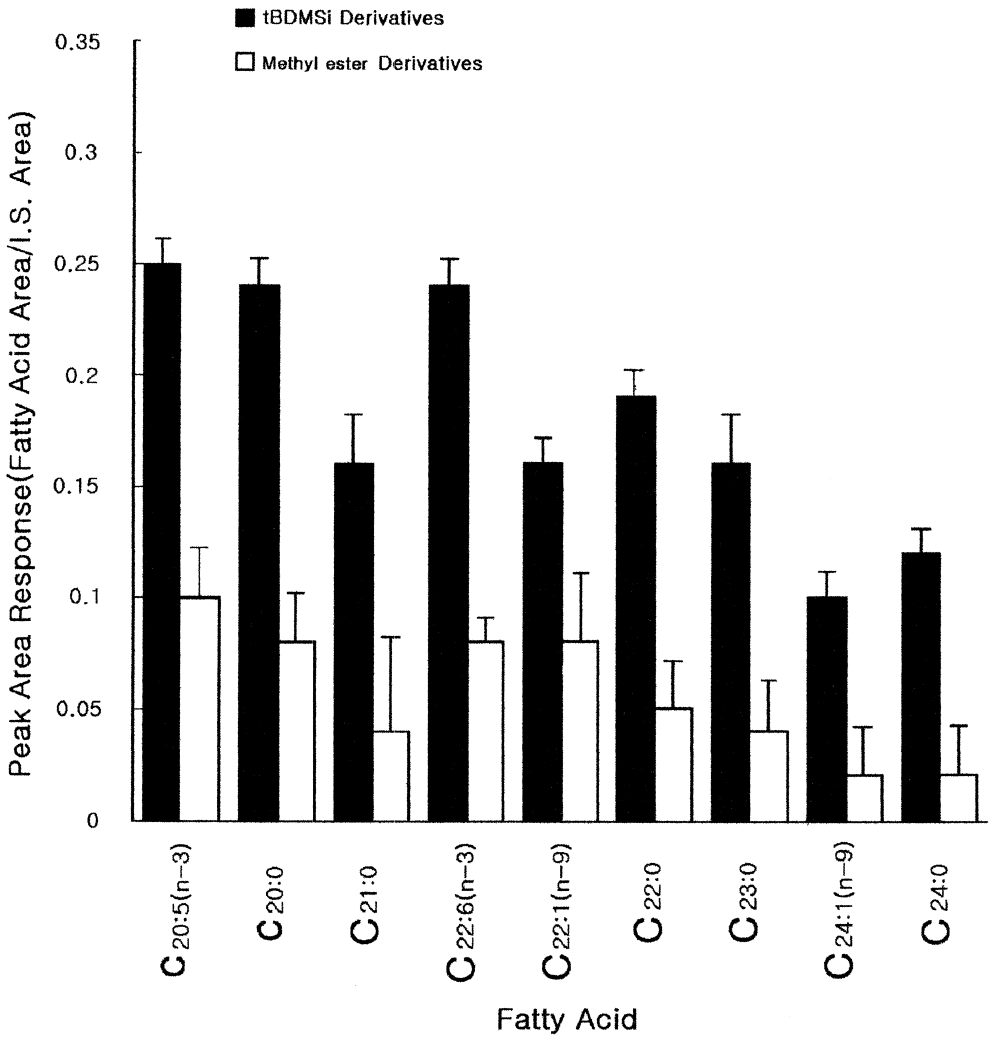

Fig. 3. Comparisons of peak response between tBDMSi derivatives and methyl ester derivatives fatty acids. Injection amount: fatty acid, 3.2 ng; I.S., 8.1 ng.

not significantly different according to hydrolysis methods when we consider only the mean values, but in view of statistics, the data of KOH hydrolysis method could not be used for experimental data because the RSDs were very high (Table 3). Significant differences existed for the derivatisation methods for linoleic, linolenic and elaidic acids using the TMAH hydrolysis method ($P<0.05$). These statistical differences were caused by the influence on the composition of the fatty acids existed with very small amount that was detected in the tBDMSi derivative. The other possible reason could be consider that the complete extraction of fatty acids derivatized to methyl ester with hexane in the procedure of the last step was impossible. But more serious studies will be needed to elucidate which kind of derivatisation methods will be the most accurate.

We could draw the conclusion that the KOH hydrolysis method for the determination of the fatty acid compositions on extremely small amounts of oil samples was an unsuitable method.

3.5. Sensitivity of tBDMSi derivatives

Peak responses (fatty acid area/internal standard

Table 3
Fatty acid compositions determined with tBDMSi and methyl ester (ME) derivatives of soybean oil fatty acids hydrolysed by 1 *M* TMAH in methanol and 1 *M* KOH in ethanol solution ($n=7$)

Fatty acid	Composition (%)							
	TMAH				KOH			
	tBDMSi	RSD (%)	ME	RSD (%)	tBDMSi	RSD (%)	ME	RSD (%)
6:0	0.107	49.4			0.066	52.7		
7:0	0.073	13.2			0.056	68.7		
8:0	0.836	8.4			0.047	41.4		
9:0	0.035	9.3			0.052	39.9		
10:0	0.099	8.4			0.141	16.9		
11:0	0.096	39.6			0.056	30.0		
12:0	0.322	13.4			0.225	17.7		
13:0	0.058	24.5			0.033	45.9		
14:0	0.443	6.8	0.620	9.4	0.323	16.6	0.543	23.7
15:0	0.137	7.3	0.399	15.6	0.075	52.7	0.376	30.4
16:1 *n*-7	0.151	7.6	0.245	6.9	0.131	16.7	0.276	18.1
16:0	10.638	2.8	11.256	5.2	10.693	52.6	10.797	34.9
18:2 *n*-6	53.298	3.9	50.534	8.2	55.728	28.3	50.894	36.8
18:3 *n*-3	8.480	6.6	11.671	8.5	8.418	37.2	12.232	31.2
18:1 *n*-9	18.807	7.5	18.411	4.2	17.744	50.4	18.120	37.9
18:1	1.173	8.8	2.171	4.2	1.077	37.3	2.614	35.6
18:0	4.100	3.4	4.188	4.1	3.980	17.2	3.699	42.0
20:0	0.375	15.7	0.222	3.9	0.295	35.8	0.242	33.0
22:0	0.365	3.8	0.284	5.3	0.248	57.2	0.209	22.0
24:0	0.407	2.5			0.188	20.7		
Total	100.000	2.6	100.000	4.4	100.000	19.3	100.000	20.8

area) of tBDMSi derivatives compared with methyl ester derivatives of several long-chain standard fatty acids are shown in Fig. 3. In all detected fatty acids, the peak responses of tBDMSi derivatives were higher by about 1.5–6.3-times than for methyl ester derivatives. This phenomenon seem to be caused by the tBDMSi radical in the derivatives containing more methyl radicals than methyl ester derivatives with only one methyl radical. The other possible reason also seem to be incomplete extraction of fatty acid methyl esters, as indicated above.

The chromatogram demonstrating the sensitivity of tBDMSi derivatives of standard fatty acids is shown in Fig. 4. The derivatives were detected at 220 pg, but only several main fatty acids, linoleic, oleic, linolenic and palmitic acid, were detected at this level in methyl esters (not shown in the figure).

3.6. Stability of tBDMSi derivatives

The stability of tBDMSi derivatives of standard fatty acids, caprylic, linoleic and arachidonic acid, at room temperature is shown in Fig. 5. In all fatty acids except arachidic, docosahexanoic, behenic and heneicosanoic acid that showed stability for only 86 h, the stability was good for about 144 h.

4. Conclusion

The organic basic solution, 1 *M* TMAH in methanol, was found to be an excellent hydrolysis solution for the determination of the fatty acid composition and recovery, but the conventional reagent, 1 *M* KOH in ethanol solution, was found to be unsuitable for extremely small amounts of oil sample. The data for fatty acid compositions determined with 1 *M* KOH in ethanol was not statistically significant even though the sample size was increased five-times compared to the analysis with 1 *M* TMAH in methanol. The tBDMSi derivatisation of fatty acids was an excellent method for the determination of

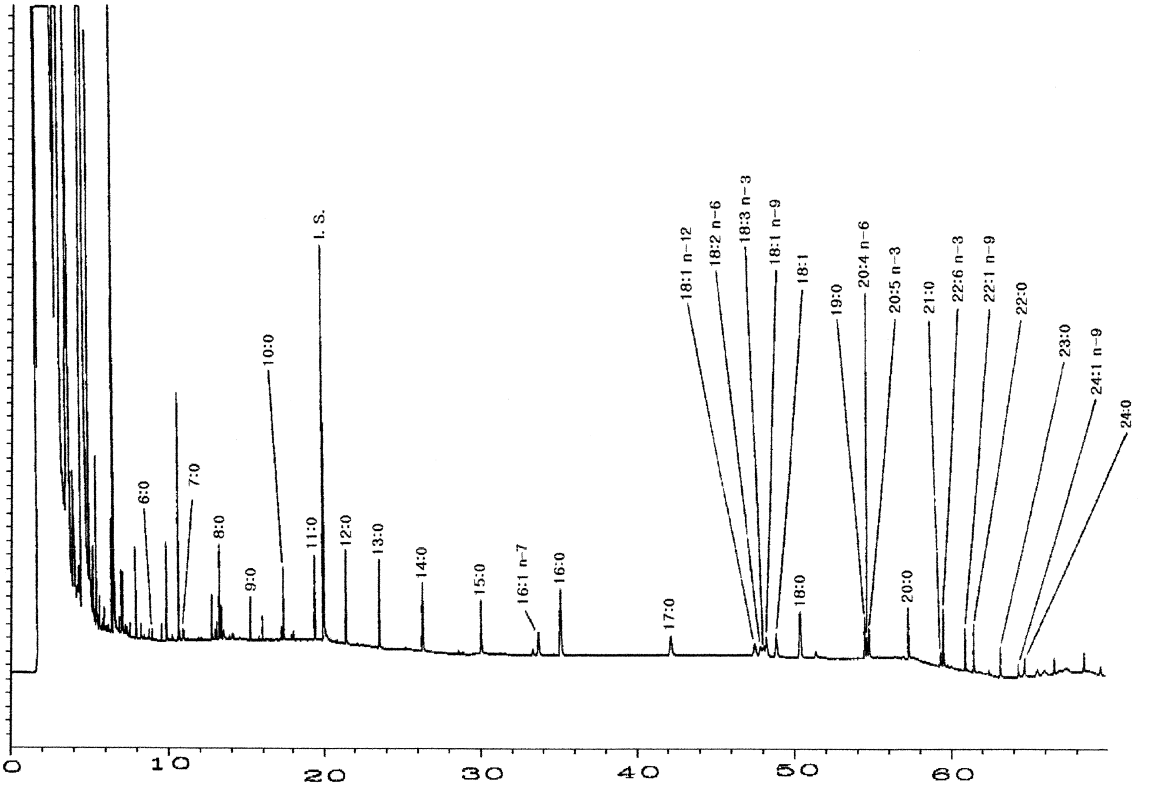

Fig. 4. Chromatogram of *N*(*O*)-*tert*.-butyldimethylsilyl (tBDMSi) derivatives of fatty acids showing the detection limit. Injection amount: fatty acids, 220 pg; I.S., 800 pg. Inlet valve, 0.5 min OFF; remaining time ON. Time scale in min.

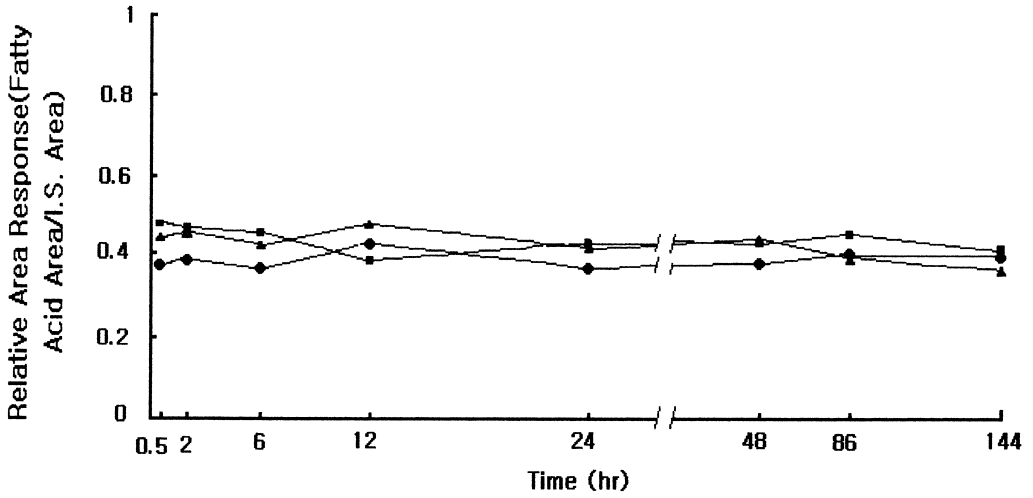

Fig. 5. Stability of *N*(*O*)-*tert*.-butyldimethylsilyl (tBDMSi) derivatives of fatty acids at room temperature. Each point is the mean value of three measurements. ■ = Caprylic acid (8:0), ● = linoleic acid (18:2 *n*-6), ▲ = arachidonic acid (20:4 *n*-6).

fatty acid composition. The fatty acids present in very small amounts that was could not be detected with methyl ester derivatives were detected with very high sensitivity using the tBDMSi derivatives. The tBDMSi derivatives were stable for 144 h at room temperature for most of fatty acids.

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