報 文

Determination of Fatty Acid Composition of Food
Containing Short Chain Fatty Acids

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Because of the high volatility and water solubility of short chain fatty acids and their methyl esters, analysis of the fatty acid composition of food lipids in which they are present is more difficult than that of lipids containing only long chain fatty acids. The GLC analysis of such lipids of small amount (about 100 mg) was established in the present study as follows: a lipid was methylated with diazomethane and Na-methylate, and the produced fatty acid methyl esters were extracted in *n*-hexane. The short chain (4~13 and 16:0) fatty acid methyl esters were measured by temperature programmed GLC on 15% OV-275 column, and long chain (14~24) fatty acid methyl esters by isothermal GLC on a suitable column for the analysis of fatty acid methyl ester such as DEGS. To determine the complete fatty acid (4~24) composition, these data of fatty acid methyl esters contents were combined by mediation of methyl palmitate amount, since palmitic acid is the major fatty acids in many food lipids. The fatty acid composition of butter fat, margarine (containing milk fat) fat and coconut oil was determined, and the recovery of lipid as total fatty acid methyl esters was 97.6% butter fat, 98.5% margarine fat and 97.9% coconut oil, respectively.

1 Introduction

The importance of the fatty acid composition of the food lipid in the determination of its nutritive value is proved by many biochemical studies on lipids. In recent years, many processed foods containing butter or coconut oil have appeared on the market, and their lipids contain considerable amount of short chain fatty acids. However, there are comparatively few data available on the fatty acid composition of foods containing short chain fatty acids. Many years ago, the volatile and water soluble short chain fatty acids were analysed by alkaline titration after steam distillation to assess the milk fat quality, using the Reichert-Meissl¹⁾ or butyric acid values²⁾, for example. However, these methods cannot measure the content of each fatty acid, and these values vary according to the lipid amount analyzed.

In recent years, gas liquid chromatography (GLC) has been applied to the determination of short chain fatty acid methyl esters. However, there are some difficulties in GLC analysis of short chain fatty acid methyl ester compared

with that of long chain fatty acid methyl ester. Because of the high volatility of the short chain fatty acids and their methyl esters, the condensation of their solution by heating or evaporation must be avoided, and due to their water solubility, the recovery of short chain fatty acid methyl esters by extraction in organic solvent is considered to be somewhat low. As different from long chain fatty acid methyl esters, the sensitivity of the GLC peak area of short chain fatty acid methyl esters decreases with shortness of their carbon chain, so correction of the sensitivity of their peak area is necessary for GLC analysis. Furthermore, some organic solvents such as methanol, benzene or chloroform, interfere with GLC peak of methyl butyrate.

Christopherson *et al.*³⁾ established the analytical method of fatty acid composition of milk fat by GLC as follows: a stoichiometric amount of 2N methanolic alkali (Na-methylate or KOH-methanol) was added to the petroleum ether solution of lipid, and the obtained petroleum ether solution of fatty acid methyl esters was analysed by the temperature programmed

GLC on 15% DEGS column. Their method prevented the interference of methanol with methyl butyrate analysis and the loss of short chain fatty acids or their methyl esters. At the joint meeting of IDF, ISO and AOAC in 1975, the analytical method⁽⁴⁾ of short chain fatty acid methyl esters with GLC was discussed for the purpose of detecting foreign fat in milk fat: a lipid was methylated with methanolic-KOH as with Christopherson's method⁽³⁾ and analysed by temperature programmed GLC on 5% PEGA column. But even now, this method is not adopted as the standard method of IDF, ISO or AOAC. Iwaida *et al.*⁽⁵⁾ reported an improvement of this method by way of isothermal GLC analysis of short chain fatty acid methyl esters, so as to analyse many samples in a short time. Their method was adopted under the Standard Methods of Analysis for Hygienic Chemists (Pharm. Soc. Jap.) under the item of the GLC analysis of short chain fatty acid⁽⁶⁾.

These methods present some problems, for quick operations are required for analysis, because the fatty acid methyl ester is treated as an intermediate material of lipid hydrolysis reaction. Also, the stoichiometric measurement of lipid to methanol is considered difficult, and much lipid is involved (about 1 g). Further, the commercially obtained petroleum ether often contains materials which interfere with GLC peaks of methyl butyrate and methyl hexanoate, so the petroleum ether to be used must be refined by distillation.

The present study describes an analytical method to determine the fatty acid composition of food containing short chain fatty acids, using a small amount of lipid (about 100 mg). Thus, stable fatty acid methyl esters were obtained from lipid by addition of diazomethane ethyl ether solution and Na-methylate methanol solution, and extracted in *n*-hexane after addition of 3 N HCl solution. The residual methanol in *n*-hexane solution was removed by washing with water. Standard solutions of short chain fatty acid methyl esters (of different concentration) were also treated as sample solutions, for the correction of their recovery by treatment. The short chain (4~13 and 16:0) fatty acid methyl esters, and the long chain

(14~24) fatty acid methyl esters of the sample were separately measured by GLC in different conditions. The complete fatty acid (4~24) composition of the sample was obtained by combining these data of fatty acid methyl esters contents by mediation of methyl palmitate amount, since palmitic acid is the major fatty acids in many food lipids. As an example, the fatty acid composition of butter, margarine (containing milk fat) and coconut oil was determined.

2 Methods

2.1 Materials

Butter fat and margarine (containing 28% milk fat in lipid) fat were obtained by extraction into ethyl ether from commercially available butter or margarine, and reagent coconut oil was used.

2.2 Reagents

About 200 mg of each pure (99%+) short chain fatty acid methyl ester (4:0, 6:0, 8:0, 10:0, 12:0) and 400 mg methyl palmitate (99%+) were dissolved in 50 ml *n*-hexane to make fatty acid methyl ester undiluted standard solution. Methyl tridecanoate (99%+) *n*-hexane solution (2.5 mg/ml) was used as an internal standard for GLC analysis. As methylation reagents, diazomethane ethyl ether solution (from *N*-methyl-*N*-nitroso urea) and Na-methylate methanol solution (from small pieces of sodium metal and methanol) were made.

2.3 Gas Liquid Chromatography (GLC)

GLC was performed by Shimadzu 9A type gas liquid chromatograph equipped with FID detector and C-R 2A integrator. Glass columns (i.d. 3 mm×2 m) packed with the following materials were used: 5% PEGA coated on 80/100 mesh Chromosorb W; 20% DEGS coated on 60/80 mesh Celite 545; Diasolid ZF 80/100 mesh; 5% Advance DS coated on 80/100 mesh Chromosorb W; 10% FFAP coated on 100/120 mesh Gaschrom Q; and 15% OV-275 coated on 80/100 mesh Chromosorb P.

2.4 Experimental Methods

2.4.1 Measurement of Base Line Drift on GLC

In the same GLC conditions except for the packing materials in the column, the base line of temperature programmed GLC by single

column method was drawn (GLC conditions: column temperature 80~220°C, program rate 10°C/min, injection temperature 230°C, N₂ flow rate 50 ml/min, range 10² MΩ, recorder attenuation 32 mV). The base line drift was measured by the difference (cm) of base line level at column temperature 80°C and 220°C.

2.4.2 Measurement of Methanol

Residual methanol in solution was measured by GLC on 20% DEGS column (GLC conditions: column temperature 65°C, injection temperature 150°C, N₂ flow rate 50 ml/min).

2.4.3 Methylation of Lipid and Measurement of Fatty Acid Methyl Esters

Lipids (50~100 mg) were methylated by the method presented in **Chart-1**. A set of fatty acid methyl esters standard solutions (each short chain fatty acid methyl ester 0.2~20 mg/5 ml) was made from undiluted standard solution, and treated as indicated in **Chart-1**. The short chain (4~13 and 16:0) fatty acid methyl esters of samples and standard solutions were analysed by temperature programmed GLC on 15% OV-275 column (GLC conditions: column temperature 80~220°C, program rate 10°C/min, injection temperature 250°C, N₂ flow rate 50 ml/min), and their concentration of sample was measured by the log-log calibration plots

with treated standard solutions. The long chain (14~24) fatty acid methyl esters were analysed by isothermal GLC on 20% DEGS column (GLC conditions: column temperature 190°C, injection temperature 230°C, N₂ flow rate 50 ml/min). The quantitation was made by the peak area percentage method. The ratios of each fatty acid methyl ester amount to methyl palmitate were calculated for both short chain and long chain groups. The complete fatty acid (carbon number, 4~24) composition of the sample was obtained from these ratios.

3 Results and Discussion

3.1 GLC Column for the Analysis of Short Chain Fatty Acid Methyl Esters

The separation properties and the base line drift were examined by the temperature programmed GLC on several columns packed with different materials (5% PEGA, 20% DEGS, Diasolid ZF, 5% Advance DC, 10% FFAP and 15% OV-275; usually used for the analysis of fatty acid methyl esters). According to the results, the 15% OV-275 column was used in present study for the analysis of the short chain fatty acid methyl esters, because the base line drift was the smallest among them and its GLC separation property was desirable for their analysis.

3.2 Residual Methanol in Sample Solution

The treatment as shown in **Chart-1** was performed except for the addition of lipid or standard, and the residual methanol in *n*-hexane phase was measured before or after washing. The residual methanol in petroleum ether solution of Christopherson's method³⁾ (no addition of lipid) was also measured. These data are shown in **Table-1**. From the results of present method, a small amount of methanol was extracted into *n*-hexane phase and the residual methanol in *n*-hexane phase could be removed by washing, so that the GLC analysis of methyl butyrate was not hindered by methanol. In actual sample solution of Christopherson's method³⁾, by which the stoichiometric amount of lipid to methanol is added, the residual methanol will be less

Chart-1 Operation procedure.

Table-1 Residual methanol in sample solution.

Method		Methanol (%)
Present method*	before wash	0.63
	after wash	0.01
Christopherson's method ³⁾ *		2.50

* : except addition of lipid

than shown by the data in **Table-1**. However, the residual methanol may virtually make the GLC analysis of methyl butyrate impossible, when the lipid amount is less than the equivalent amount of methanol.

3.3 Recovery and Calibration Plots

The short chain fatty acid methyl esters were somewhat soluble in water, so their recovery by extraction into *n*-hexane was examined at various concentration of fatty acid methyl ester standard solution which was treated as shown in **Chart-1**. The recovery of the fatty acid methyl esters is shown in **Table-2**, and the log-log calibration plots in **Fig.-1**. The recovery of methyl butyrate (4:0) and methyl hexanoate (6:0) is somewhat low, depending on the concentration. However, their log-log calibration plots show a linear relationship over a wide concentration range of each fatty acid methyl ester, in spite of the lower sensitivity of their GLC peak area than that of methyl palmitate. Then these plots were used for

Table-2 Recovery of fatty acid methyl ester after treatment as **Chart-1**.

FAM*	Recovery (%)		
	FAM amount (mg)** in 5 ml standard solution		
	0.51 f	3.2 f	20 f
4:0	66	79	86
6:0	86	93	99
8:0	95	95	100
10:0	95	98	100
12:0	100	100	100
16:0	100	100	100

* FAM: fatty acid methyl ester

** f: factor of each fatty acid methyl ester in sample solution; 0.788 (for 4:0 FAM), 0.749 (for 6:0 FAM), 0.815 for 8:0 FAM), 0.757 (for 10:0 FAM), 0.920 (for 12:0 FAM), 1.883 (for 16:0 FAM)

Fig.-1 Log-log calibration plots of fatty acid methyl ester. **Fig.-2** Gas liquid chromatogram.

their calibration, which also covered the correction of their recovery. On the other hand, the log-log calibration plots of methyl octanoate (8:0), methyl decanoate (10:0), methyl laurate (12:0) and methyl palmitate (16:0) fitted

Table-3 Fatty acid composition
(% as methyl ester)

FAM*	Butter	Margarine**	Coconut oil
4:0	4.8	1.4	—
6:0	2.6	0.6	0.8
8:0	1.4	0.4	8.8
10:0	3.1	0.8	6.5
12:0	3.4	1.1	50.5
14:0	9.8	3.1	15.2
14:1	2.1	0.5	—
16:0	22.8	17.4	7.7
16:1	2.8	1.0	—
18:0	11.6	8.2	2.3
18:1	25.5	35.0	6.2
18:2	2.5	27.6	1.7
18:3	2.0	0.8	0.1
20:1	0.3	0.5	—
Other***	5.5	1.8	0.1

* FAM: fatty acid methyl ester

** Margarine: contain 28% milk fat in lipid

*** other: 10:1, 13:0, 15:0, 15:1, 16:2, 17:0

almost the same regression equation (slope $\approx$ 1). Thus, the sensitivity of the GLC peak of these fatty acid methyl esters was considered to be comparable.

3.4 Fatty Acid Composition of Sample Lipids

GLC chromatograms on OV-275 of short chain fatty acid methyl ester standard solution, butter fat and coconut oil are shown in **Fig.-2**. The fatty acid composition of butter fat, margarine (containing milk fat) fat and coconut oil were measured by the present method, and the results are shown in **Table-3**. The recovery of lipid as the total fatty acid methyl ester content was 97.6% for butter fat, 98.5% for margarine fat, and 97.9% for coconut oil. These fatty acid methyl esters were stable for several days, so quick operations were not necessary the present method. Only a slight amount of methyl tridecanoate was detected in sample lipids examined, so methyl tridecanoate is considered suitable as an internal standard

for GLC analysis of fatty acid methyl ester.

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低級脂肪酸を含む食品の 脂肪酸組成測定法

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低級脂肪酸またはそのメチルエステルの高い揮発性及び水溶性のため、このような脂肪酸を含む食品の脂肪酸組成の分析は、高級脂肪酸のみのものに比べて困難である。本報では、少量(約 100 mg)の脂質試料を用いて、低級脂肪酸を含む食品の脂肪酸組成分析法が以下のように確立された。すなわち、脂質をジアゾメタン及び Na-メチラートでメチル化し、生成した脂肪酸メチルエステルを *n*-ヘキサンで抽出する。低級脂肪酸メチルエステル(4~13 及び 16:0)を 15% OV-275 カラムによる昇温 GLC で、高級脂肪酸メチルエステル(14~24)を脂肪酸メチルエステルに適するカラム(例えば DEGS)による定温 GLC で測定し、これらのデータをパルミチン酸メチル量を仲介として、全脂肪酸組成(4~24)を測定する(パルミチン酸は多くの食品の主な脂肪酸のひとつである)。バター脂質、マーガリン(乳脂肪含有)脂質及びやし油の脂肪酸組成が測定された。全脂肪酸メチルエステル量としての脂質の回収率は、バター脂質(97.6%)、マーガリン脂質(98.5%)及びやし油(97.9%)であった。