Note

Determination of Butterfat in Mixed Fats (2)* Analysis of Triglyceride Compositions of Mixed Fats by Gas Chromatography and Mass Spectrometry Combined with Gas Chromatography

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Introduction

It is very difficult to detect a foreign fat in butterfat because of the mixed fat with have the similar fatty acid compositions and physical properties to butterfat. Usually, when the foreign fat in butterfat is of vegetable origin, an analysis of the sterol composition have been applied for the detection of the foreign fat in butterfat, and the adultrations are recognized from the characteristics of their fatty acid compositions. In general, the mixed butterfats are prepared by blending animal and vegetable fat, such as beef fat oleo oil, cacao fat, coconut oil, and palm kernel oil. To elucidate these adultrations, the fatty acid compositions of the mixed fats would be useful for the qualitative and quantitative determination of foreign fat. When the fat added is unknown, however, it would be impossible to detect the foreign fat in butterfat by the method based on the fatty acid compositions, since the characteristics of their fatty acid components is unknown.

The gas chromatographic analysis of total glyceride distribution of the fat can be used to detect the foreign fat in butterfat, An extensive study in this field has been undertaken by Kukis et al.[1] Kukis and McCarthy[2] separated triglyceride mixtures according to their carbon number by gas chromatography, with SE 30 as liquid phase, using a flame ionization detector and with temperature from 200 to 320°C. Moreover, the methods of triglyceride analysis by gas chromatography do not distinguish unsaturated glyceride from the saturated ones having the same acyl carbon numbers. In a previous paper[3] we described a technique of triglyceride fractionation by gas chromatography, with DEXSIL 300 as liquid phase, and found that the method based on differences in the distribution of triglyceride groups would be useful for the detection of foreign fat in butterfat, However, it is difficult to distinguish between coconut oil and palm kernel oil, or beef fat and cacao fat with triglyceride groups fractionated by gas chromatography, respectively. To overcome the difficulty, the mass spectrometric analysis of triglyceride is useful for the identification of the fatty acid distribution in the triglyceride.

Bezard et al.[4] have shown that triglyceride of coconut oil was fractionated by gas chromatography into 13 groups based on their carbon numbers of 28 to 52. However, it is difficult to calculate the fatty acid compositions of triglyceride by their method. Rhyhage and Stenhagen[5] have indicated the mass spectrum of one mixed triglyceride. Barber et al.[6] have shown that the mass spectra of mixed triglyceride are characterized by the peaks of M−RCO_2,RCO,RCO+74,and RCO+128.There are also reports on the determination of fatty acid compositions of mixed triglyceride by GC−MS.

This paper deals with the fractionation of triglyceride groups by gas chromatography, and qualitative determination of foreign fat in butterfat from the mass

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spectra of triglyceride C_{50} and C_{52}.

Experimental

Materials and Method

The butterfat used in this experiment was refined one donated by National Institute of Animal Industry. Cacao fat and beef fat were purchased from Wako Pure Chemical Industries Ltd., Gas chromatographic analysis were carried out on a Shimadzu GC 5APF gas chromatograph equipped with a flame ionization detector. The glass column of 30 cm ×3 mm was packed with80-100 mesh Chromosorb GAW coated with 2% DEXSIL 300. The injection port of gas chromatograph was set at 350℃;column oven temperature was programmed from 230 to 340 ℃ at 5 ℃/min; helium flow rate was 60ml/min.The mass spectrometric analysis was carried out on a Shimadzu-LKB 9000 connected with a gas chromatograph, The glass column of 30 cm ×3 mm was packed with 80 - 100 mesh Chromosorb GAW coated with 2 % DEXSIL 300.Column oven temperature was programmed from 230 to 330℃ at 5℃/min; helium flow rate was 30ml/min.The ionization voltage was 70 eV, accelerating voltage 3.5 KV, trap current was 60 μA, and separatory temperature was 300℃. Samples were diluted with chloroform to about 20%.The peak intensity and the magnetic field readings were determined by Shimadzu GC-MSPAC300.

Results and Discussion

In a previous paper[3] we investigated a technique of triglyceride fractionation by gas chromatography and its application to triglyceride of mixed fat containing milk fat and vegetable fat. From the chromatogram of mixed fat triglyceride, it became clear that the foreign fat in butterfat was estimated from the composition of 15 peaks of triglyceride identified by their total acyl carbon numbers. The gas chromatographic elution patterns of the mixed fat triglyceride are shown in Fig.1 and 2. Each peak in gas chromatogram corresponds to a triglyceride group characterized by its acyl carbon numbers. A series of triglyceride peaks from C_{26} to C_{54} were observed for butterfat triglyceride. Comparable elution patterns were also recorded for beef and cacao fats triglyceride. in the chromatograms of beef

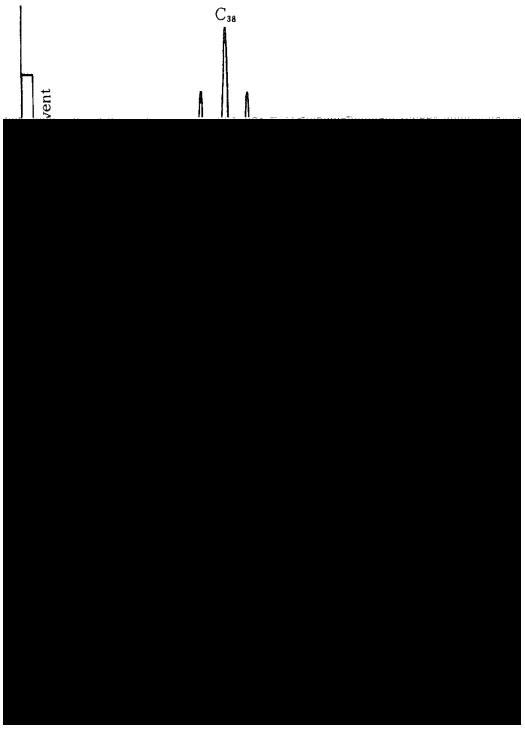

Fig.1 Gas chromatograms of single fats
(A):Butterfat
(B):Beef fat
(C):Cacao fat

and cacao fats, the peaks from C_{50} to C_{54} are major components in their triglyceride groups, and the peaks from C_{26} to C_{48} show a very weak intensity. The chromatographic elution patterns of beef and cacao fats differ from the butterfat. When either beef fat or cacao fat is added to butterfat, the peak intensities of C_{50} and C_{52} in the gas chromatogram increase in proportion to the contents of foreign fat added. The peak ratio of C_{50} and or C_{52} to C_{38} are presented in Table 1.From the results, the contributions of these added fat in butterfat to the C_{50} and C_{52} are readily recognized. However, it is difficult to clarify whether beef fat or cacao fat containing in butter fat with gas chromatographic ellution patterns, since these fats indicate the similar triglyceride groups in gas chromatogram.

Barber et al.[6] have reported the spectrum of mixed triglycerides. Lauer et al.[7] also have shown that major peaks of triglycerides are M - RCO_2,M - (RCO_2+CH_2),RCO+74,RCO+115,and RCO+128,and fatty acid

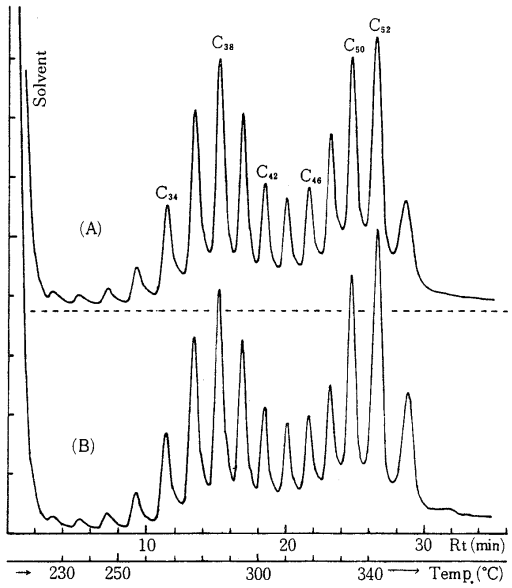

Fig.2 Gas chromatograms of mixed butterfats
(A):Contain 70% of butterfat and 30% of beef fat
(B):Contain 70% of butterfat and 30% of cacao fat

Table 1 Relation between foreign fat concentration and ratio of relative peak intensity of specific triglycerides in gas chromatogram

Foreign fat in butterfat	Foreign fat added (%)	Ratio of triglyceride groups	
		C_{50}/C_{38}	C_{52}/C_{38}
Beef fat	0	0.667	0.583
	5	0.795	0.814
	10	0.829	0.900
	20	1.045	1.236
	30	1.176	1.498
Cacao fat	0	0.667	0.586
	5	0.712	0.745
	10	0.791	0.840
	20	–	–
	30	1.032	1.397

moieties can be identified by these major peaks in the spectrum. Murata et al/[8] reported the method for the determination of fatty acid compositions using ion $M-RCO_2$,RCO+74,RCO+128,and RCO.

The mass spectra of triglyceride groups of C_{50} and C_{52} are shown in Fig.3,4. With the triglyceride group of C_{50} the ions RCO,RCO+74,RCO+128,and $M-RCO_2$ are observed. For example, in the case of butterfat a series of characteristic fragmentions at m/e 211(R_{14} Co),m/e 239(R_{16} Co), m/e 267,265,263(R_{18} Co), m/e 285(R_{14} Co+74), m/e 341,339,337(R_{18} Co+128), m/e 367(R_{16} CO + 128),and m/e 395,393(R_{18} Co+128)are observed. From these fragment ions it can be deduced that the fatty acid compositions of C_{50} triglyceride of butterfat are as follows:$C_{14}-C_{18}-C_{18}$,$C_{14}-C_{18}-C_{18:1}$,$C_{14}-C_{18:1}-C_{18:1}$,$C_{14}-C_{18}-C_{18:2}$,$C_{18}-C_{16}-C_{16}$,$C_{18:1}-C_{16}-C_{16}$,$C_{18:2}-C_{16}-C_{16}$,TheM$-RCO_2$ ions appear at m/e 551,549,547, m/e 579,577,575,and m/e 607,605,603.The fatty acids distribution of triglyceride can be determined from these ions if molecular ions are known. The mass spectra of C_{50} triglyceride of beef and cacao fats indicate similar fragment ions, and are not recorded the ions at m/e 211,285,603,605,and 607 originated from C_{14} fatty acid of C_{50} triglyceride. From the fact, it is noted that C_{50} triglyceride of beef and cacao fats do not contain C14 fatty acid. in the case of C_{52} triglyceride of butterfat, beef fat, and cacao fat, the ions $M-R_{18}Co_2$ are of high intensity, and the spectra are very similar to each other. With C_{52} triglyceride of cacao fat, the ratio of m/e 579/577 is stronger than that of butterfat and beef fat. Barber et al.[6] have reported that fatty acid compositions at 1 and 3 position of triglyceride can be identified from the ion $M-RCO_2+CH_2$,since the ion $M-(RCO_2+CH_2)$ resulted from loss of acyloxy group of 2 position of triglyceride is of low intensity and negligible. With C_{52} triglyceride, however, the $M-RCO_2CH_2$ ions due to 1 or 3 positions are also negligible. Thus, it is difficult to estimate the structure of triglyceride from the $M-(RCO_2+CH_2)$ ion.

The mass spectra of butterfat containing foreign fat are shown in Fig.5 and 6.In the case of mixed butterfat containing 10% to 30% of foreign fat(beef fat or cacao fat), the mass spectra of C_{50} triglycerides are similar to that of native butterfat.However,the fragment ion $R_{14}CO$ at m/e 211 resulted from the acyl ion of C_{14}

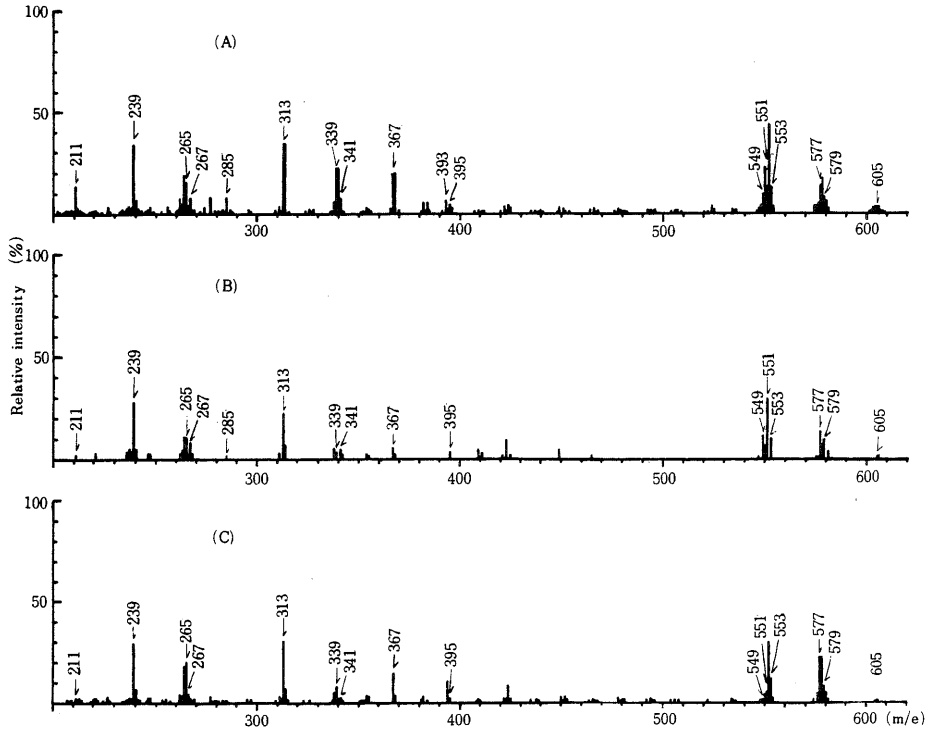

Fig. 3 Mass spectra of C_{50} triglycerides
(A) : Butterfat, (B) : Beef fat, (C) : Cacao fat

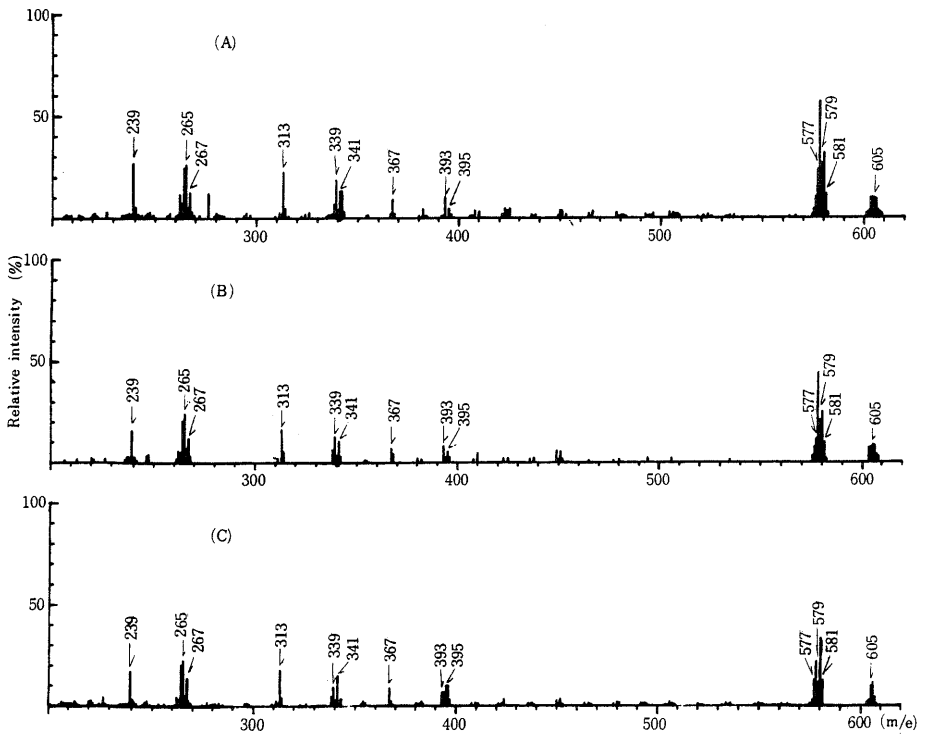

Fig.4 Mass spectra of C_{52} triglycerides
(A):Butterfat,(B):Beef fat,(C):Cacao fat

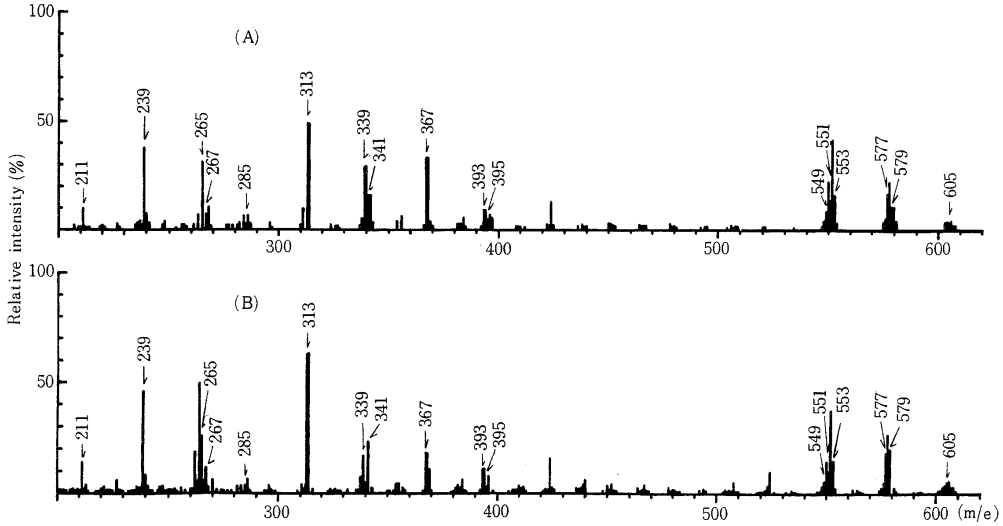

Fig.5 Mass spectra of C_{50} triglycerides

(A):Contain 70% of butterfat and 30% of beef fat

(B): Contain 70% of butterfat and 30% of cacao fat

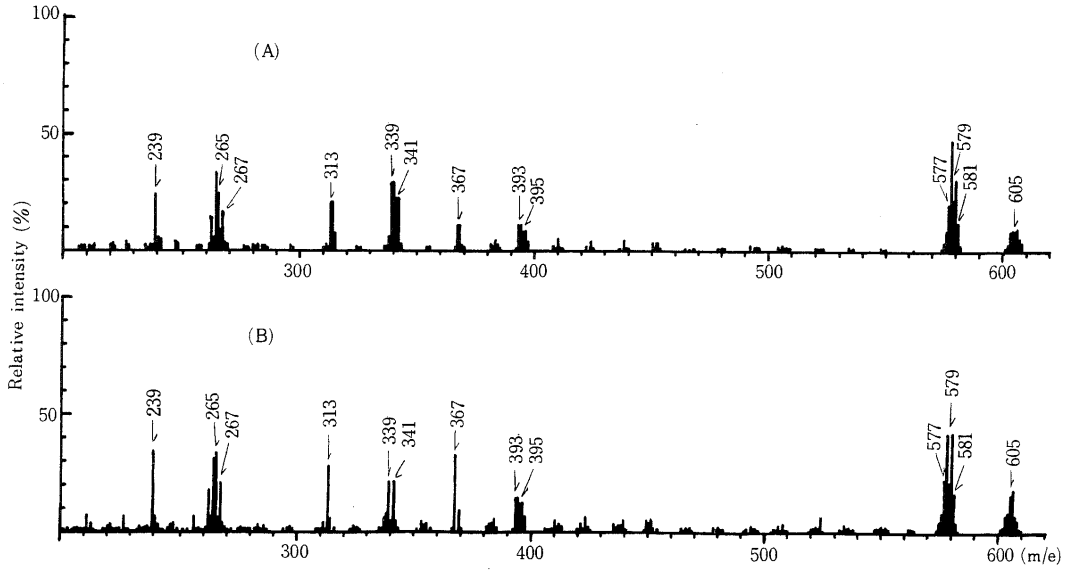

Fig.6 Mass spectra of C_{52} triglycerides

(A):Contain 70% of butterfat and 30% of beef fat

(B):Contain 70% of butterfat and 30% of cacao fat

fatty acid is of low intensity comparing to that of native butterfat. In the case of mixed butterfat containing 30% of cacao fat the ratio of m/e 579/577 of C_{52} triglyceride is lower than that of native butterfat, but in the case of mixed butterfat containing 30% of beef fat, the ratio is the same as butterfat.

Conclusion

The mixed butterfats containing beef fat or cacao fat were analyzed by GC and GC—MS. Major triglyceride peaks of beef and cacao fats were C_{50} to C_{54} in total acyl carbon number, and it was imposible to distinguish between beef and cacao fats from gas chromatographic elution pattern of triglyceride groups.

The fragment ions at m/e 211,285,603,605, and 607 originated from C_{14} fatty acid of C_{50} triglyceride of beef and cacao fats were not observed. The fragment ion $R_{14}Co$ at m/e 211 due to C_{14} fatty acid of triglyceride(C_{50}) of mixed butterfat containing 30% of foreign fat(beef fat or cacao fat) was of low intensity comparing to native butterfat

References

1)A. Kusis, M. J. McCarthy, and J. M. R. Beveridge : *J. Ame. Oil Chem. Soc.*, **41**, 201 (1964).
2)A. Kuksis and McCarthy : *Can. J. Biochem. Physiol.*, **40**, 679(1962).
3)M. Deki, T. Kato, and K. Kabaya : Reports of the Central Customs Laboratory, No. 12, 11(1972).
4)J. Bezard, M. Bugaut, and G. Clement : *J. Ame. Oil Chem. Soc.*, **48**, 134(1971).
5)R. Ryhage and E. Stenhagen : *J. Lipjd Res.*, 1, 11361(1960).
6)M. Barber, T. O. Merren, and W. Kelly : *Tetrahedron Letters*, **18**, 1063(1964).
7)W. M. Lauer, A. J. Aasen, G. Graff, and R. T. Holman : *Lipids*, **5**, 861(1970).
8)T. Murata and S. Takahashi; *Anal. Chem.*, **45**, 1816(1973).

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