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Gas Chromatographic Method for Analysis of Fatty Acids in Milk Fat with a Single Injection

- Research Note -

Keum Taek Hwang† and Min Kyeong Shin

Department of Food Science and Human Nutrition, and Center for Healthcare Technology Development, Chonbuk National University, Jeonbuk 561-756, Korea

Abstract

The purpose of this study was to develop a gas chromatographic (GC) method to analyze fatty acids in milk fat with a single injection. The single-injection GC method we developed for analyzing fatty acid composition can separate a wide range of fatty acid methyl esters from butyric acid to docosahexaenoic acid. It separated 6 isomers of 18:1 (*cis*-6, *cis*-9, *cis*-11, *trans*-6, *trans*-9 and *trans*-11), 4 isomers of 18:2 (*cis*-9-*cis*-12, *trans*-9-*trans*-12, *cis*-9-*trans*-12 and *trans*-9-*cis*-12), and 4 isomers of conjugated 18:2 (*cis*-9-*trans*-11, *trans*-9-*cis*-11, *cis*-10-*trans*-12 and *trans*-10-*cis*-12).

Key words: conjugated linoleic acid, fatty acids, GC, milk fat, *trans*-fatty acid

INTRODUCTION

Milk fat is composed of diverse fatty acids such as short-chain fatty acids, *trans*-fatty acids and conjugated linoleic acids as well as regular long-chain fatty acids. The diversity of fatty acids in milk fat causes some difficulties in analyzing the whole composition at once, although methods for fatty acid composition analysis have been developed with special emphasis on octadecenoic acid isomers (1-3) and conjugated linoleic acids (4-7).

The objective of the study was to develop a gas chromatographic (GC) method to analyze a wide range of fatty acids in milk fat including short-chain fatty acids, octadecenoic acid isomers and conjugated linoleic acids as well as long-chain fatty acids with a single injection.

MATERIALS AND METHODS

Fatty acid methyl ester (FAME) standards

The FAMEs standards for GC analysis were as follows: mixture of 4:0, 6:0, 8:0, 10:0, 11:0, 12:0, 13:0, 14:0, 14:1 (*cis*-9), 15:0, 15:1 (*cis*-10), 16:0, 16:1 (*cis*-9), 17:0, 17:1 (*cis*-10), 18:0, 18:1 (*cis*-9), 18:1 (*trans*-9), 18:2 (*cis*-9, *cis*-12), 18:2 (*trans*-9, *trans*-12), 18:3 (*cis*-6,9,12), 18:3 (*cis*-9,12,15), 20:0, 20:1 (*cis*-11), 20:2 (*cis*-11,14), 20:3 (*cis*-8,11,14), 20:3 (*cis*-11,14,17), 20:4 (*cis*-5,8,11, 14), 20:5 (*cis*-5,8,11,14,17), 21:0, 22:0, 22:1 (*cis*-13), 22:2 (*cis*-13,16), 22:6 (*cis*-4,7,10,13,16,19), 23:0, 24:0 and 24:1 (*cis*-15) (Restek Co., Belleforonte, PA, USA); mixture of 18:0, 18:1 (*cis*-6), 18:1 (*cis*-9), 18:1 (*cis*-11), 18:1 (*trans*-6), 18:1 (*trans*-9), 18:1 (*trans*-11) and 18:2 (*cis*-9, cis-12) (Restek Co.); mixture of 18:0, 19:0, 20:0, 21:0 and 22:0 (Nu-Chek-Prep, Inc., Elysian, MN, USA); mixture of 16:1 (*cis*-9), 18:1 (*cis*-9), 20:1 (*cis*-11), 22:1 (*cis*-13) and 24:1 (*cis*-15) (Nu-Chek-Prep, Inc.); mixture of 18:0, 19:0, 20:0, 21:0 and 22:0 (Nu-Chek-Prep, Inc.); mixture of 14:0, 14:1 (*cis*-9), 16:0, 16:1 (*cis*-9), 18:1 (*cis*-9), 18:1 (*cis*-11), 18:2 (*cis*-9, *cis*-12), 18:3 (*cis*-9,12, 15), 20:0, 20:1 (*cis*-11), 20:2 (*cis*-11,14), 20:3 (*cis*-11,14, 17), 20:4 (*cis*-5,8,11,14), 20:5 (*cis*-5,8,11,14,17), 22:0, 22:1 (*cis*-13), 22:6 (*cis*-4,7,10,13,16), 23:0, 24:0 and 24:1 (*cis*-15) (Nu-Chek-Prep, Inc.); mixture of 16:1 (*cis*-9), 18:1 (*cis*-9), 20:1 (*cis*-11), 22:1 (*cis*-13) and 24:1 (*cis*-15) (Nu-Chek-Prep, Inc.); mixture of 14:0, 16:0, 16:1 (*cis*-9), 18:0, 18:1 (*cis*-9), 18:2 (*cis*-9, *cis*-12) and 18:3 (*cis*-9,12, 15) (Nu-Chek-Prep, Inc.); mixture of 18:2 (*cis*-9, *cis*-12), 18:3 (*cis*-9,12,15), 20:4 (*cis*-9) and 22:6 (*cis*-4,7,10,13, 16) (Nu-Chek-Prep, Inc.); mixture of 18:2 (*trans*-9, *trans*-12), 18:2 (*cis*-9, *trans*-12), 18:2 (*trans*-9, *cis*-12) and 18:2(*cis*-9, *cis*-12) (Supelco Co., Bellefonte, PA, USA); mixture of 18:2 (*cis*-9, *cis*-11), 18:2 (*cis*-9, *trans*-11), 18:2 (*trans*-9, *cis*-11), 18:2 (*trans*-9, *trans*-11), 18:2 (*cis*-9, *cis*-12), 18:2 (*cis*-10, *cis*-12), 18:2 (*trans*-10, *cis*-12) and 18:2 (*trans*-10, *trans*-12) (Nu-Chek-Prep, Inc.); individual 14:1 (*trans*-9); 16:1 (*trans*-9); 18:1 (*cis*-9); 18:2 (*cis*-9, *trans*-11) and 18:2 (*trans*-10, *cis*-12) (Nu-Chek-Prep, Inc.).

Fat extraction for analyzing fatty acid composition

Fat was extracted from milk, butter and ice cream us-

†Corresponding author. E-mail: keum@chonbuk.ac.kr
Phone: +82-63-270-3857, Fax: +82-63-270-3854

ing the Röse-Gottlieb method (8) with minor modification: Samples of milk, butter or ice cream weighing about 0.3 g were placed into 250 mL graduated cylinders. Six mL ammonia solution (28%; Junsei Chemical Co., Tokyo, Japan) was added to each sample while swirling, followed by standing for 2 min. Thirty mL ethanol (95%; Junsei Chemical Co.) was then added while swirling. Seventy five mL ethyl ether (Acros Organics Co., NJ, USA) was added and the contents were vigorously mixed in the stoppered cylinder for 30 sec. Seventy five mL petroleum ether (Acros Organics Co.) was added and the contents were vigorously mixed for 30 sec in the stoppered cylinder. After settling, the clear upper layer was collected into a 250 mL round-bottomed flask. Solvent was removed using a vacuum rotary evaporator (N-N; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 36°C to collect fat. After flushing with nitrogen, the fat was stored at about -40°C until use.

Methylation of fat

FAME were prepared using 0.2 g fat according to the American Oil Chemists' Society method (9). Hexane containing FAMEs was transferred into a vial with a Teflon cap, flushed with nitrogen and stored at -40°C until analyzed by GC.

GC analysis of FAME

The FAMEs were analyzed on a Hewlett-Packard 6890 Series gas chromatography (Hewlett-Packard Co., Wilmington, DE, USA), equipped with a flame-ionization detector (FID). The column was an RT-2560 (100 m, 250 μm, 0.2 μm; Restek Co.). The detector temperature was 250°C. The injector was set at 250°C in a split ratio of 100:1. Helium was used as the carrier gas with a flow rate of 1.2 mL/min. One μL of each sample was injected. Column temperature was programmed as follows: Initial temperature of 60°C was held for 0.5 min and then increased to 100°C at 30°C/min. The temperature was then increased to 160°C at 20°C/min with a hold for 10 min, to 180°C at 1°C/min with a hold for 5 min, to 200°C at 3°C/min with a hold for 4 min, to 240°C at 4°C/min with a hold for 2 min, and to 245°C at 25°C/min with a final hold for 7 min.

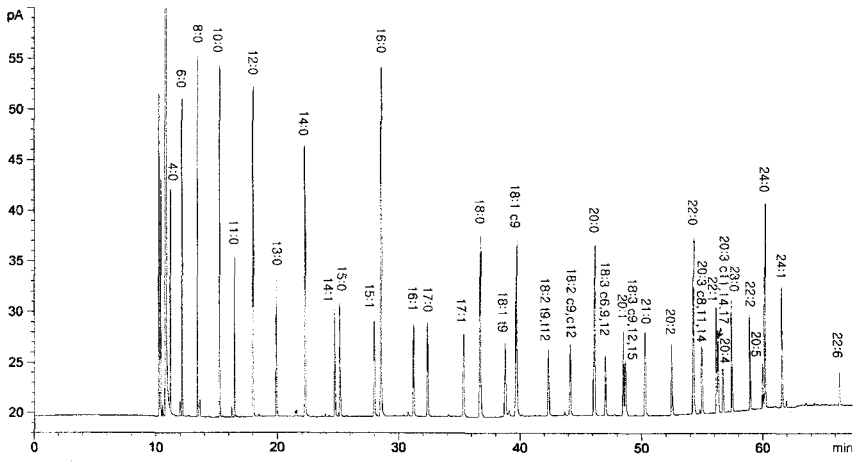

Fig. 1. Gas chromatogram of a wide range of fatty acid methyl esters.

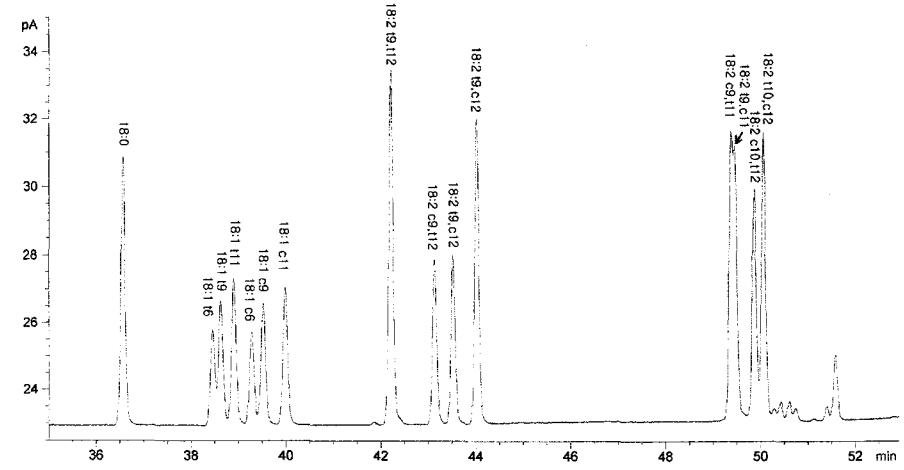

Fig. 2. Gas chromatogram of fatty acid isomers of C18:1 and C18:2.

RESULTS AND DISCUSSION

The single-injection GC method for fatty acid composition analysis described above could separate a wide range of fatty acid methyl esters from butyric acid to docosahexaenoic acid (Fig. 1). It separated 6 isomers of 18:1: *cis*-6, *cis*-9, *cis*-11, *trans*-6, *trans*-9 and *trans*-11; 4 isomers of 18:2: *cis*-9-*cis*-12, *trans*-9-*trans*-12, *cis*-9-*trans*-12 and *trans*-9-*cis*-12; and 4 isomers of conjugated 18:2: *cis*-9-*trans*-11, *trans*-9-*cis*-11, *cis*-10-*trans*-12 and *trans*-10-*cis*-12 (Fig. 2). One run of the GC analysis took 69.7 min. The retention times and response factors (19:0 as 1.00) of the FAME are shown in Table 1. The chromatograms of the FAME of fats from the milk analyzed by the GC method mentioned above are shown in Fig. 3. Palmitic acid was the most abundant fatty acid in the fats extracted from milk, butter and ice cream (Table 2), followed by oleic acid and stearic acid. Butyric acid, a typical fatty acid in milk fat, accounted for 2~3% of the fats in the milk and milk products. This method could detect 6 isomers of 18:1, *cis*-6, *cis*-9, *cis*-11, *trans*-6, *trans*-9 and *trans*-11, and a conjugated linoleic acid (*cis*-9, *trans*-11).

The GC method developed in this study can analyze a wide range of fatty acids in milk fat including short-chain fatty acids, octadecenoic acid isomers and conjugated linoleic acids as well as long-chain fatty acids with a single injection.

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Table 1. Retention times and response factors of gas chromatographic analysis for fatty acid methyl esters

	Fatty acid methyl ester	Retention time (min)	Response factor[1)]		Fatty acid methyl ester	Retention time (min)	Response factor[1)]
C4:0	Methyl butyrate	11.147	2.17	C20:0	Methyl arachidate	46.095	1.00
C6:0	Methyl caproate	12.103	1.38	C18:3	Methyl gamma linolenate (*cis*-6,9,12)	47.039	1.00
C8:0	Methyl caprylate	13.392	1.13				
C10:0	Methyl caprate	15.205	1.09	C20:1	Methyl eicosenoate (*cis*-11)	48.500	1.00
C11:0	Methyl undecanoate	16.452	1.09	C18:3	Methyl linolenate (*cis*-9,12,15)	48.629	1.00
C12:0	Methyl laurate	17.953	1.05	C18:2	Methyl octadecadienoate (*cis*-9, *trans*-11)	49.503	1.00
C13:0	Methyl tridecanoate	19.886	1.05				
C14:0	Methyl myristate	22.206	1.03	C18:2	Methyl octadecadienoate (*trans*-9, *cis*-11)	49.544	1.00
C14:1	Methyl myristelaidate (*trans*-9)	23.831	1.03				
C14:1	Methyl myristoleate (*cis*-9)	24.645	1.03	C18:2	Methyl octadecadienoate (*cis*-10, *trans*-12)	49.950	1.00
C15:0	Methyl pentadecanoate	25.115	1.02				
C15:1	Methyl pentadecenoate (*cis*-10)	27.985	1.02	C18:2	Methyl octadecadienoate (*trans*-10, *cis*-12)	50.180	1.00
C16:0	Methyl palmitate	28.499	1.02				
C16:1	Methyl palmitelaidate (*trans*-9)	30.349	1.01	C21:0	Methyl heneicosanoate	50.333	1.00
C16:1	Methyl palmitoleate (*cis*-9)	31.171	1.01	C20:2	Methyl eicosadienoate (*cis*-11,14)	52.449	1.00
C17:0	Methyl heptadecanoate	32.367	1.01				
C17:1	Methyl heptadecenoate (*cis*-10)	35.342	1.01	C22:0	Methyl behenate	54.213	1.00
C18:0	Methyl stearate	36.685	1.01	C20:3	Methyl eicosatrienoate (*cis*-8,11,14)	54.936	1.00
C18:1	Methyl petroselaidate (*trans*-6)	38.606	1.01				
C18:1	Methyl elaidate (*trans*-9)	39.000	1.01	C22:1	Methyl erucate (*cis*-13)	56.151	1.00
C18:1	Methyl transvaccenate (*trans*-11)	39.070	1.01	C20:3	Methyl eicosatrienoate (*cis*-11,14,17)	56.256	1.00
C18:1	Methyl petroselinate (*cis*-6)	39.436	1.01	C20:4	Methyl arachidonate (*cis*-5,8,11,14)	56.630	1.00
C18:1	Methyl oleate (*cis*-9)	39.659	1.01				
C18:1	Methyl vaccinate (*cis*-11)	40.104	1.01	C23:0	Methyl tricosanoate	57.457	1.00
C19:0	Methyl nonadecanoate	41.679	1.00	C22:2	Methyl docosadienoate (*cis*-13,16)	58.914	1.00
C18:2	Methyl octadecadienoate (*trans*-9, *trans*-12)	42.326	1.00				
C20:5	Methyl eicosapentaenoate (*cis*-5,8,11,14,17)	59.952	1.00				
C18:2	Methyl octadecadienoate (*cis*-9, *trans*-12)	43.221	1.00	C24:0	Methyl lignocerate	60.123	1.00
C24:1	Methyl nervonate (*cis*-15)	61.536	1.00				
C18:2	Methyl octadecadienoate (*trans*-9, *cis*-12)	43.610	1.00	C22:6	Methyl docosahexaenoate (*cis*-4,7,10,13,16,19)	66.280	1.00
C18:2	Methyl linoleate (*cis*-9, *cis*-12)	44.085	1.00				

[1)]based on 19:0 as 1.00.

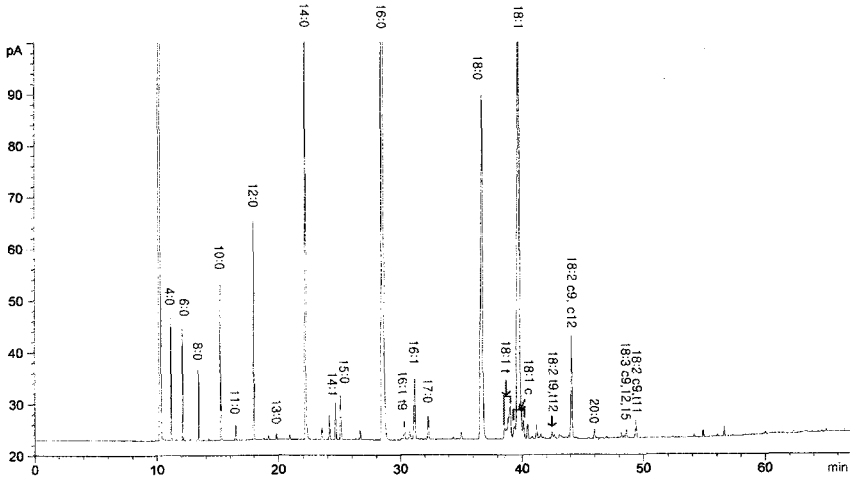

Fig. 3. Gas chromatogram of fatty acid methyl esters of milk fat.

Table 2. Fatty acid compositions of fats extracted from milk, butter and ice cream (%)

Fatty acid	Milk	Butter	Ice cream
C4:0	2.4±0.1[1)]	1.7±0.2	2.9±0.9
C6:0	1.5±0.1	1.2±0.0	1.3±0.1
C8:0	0.8±0.1	0.9±0.0	0.8±0.0
C10:0	2.0±0.2	2.3±0.0	2.0±0.1
C11:0	0.2±0.0	0.2±0.0	0.2±0.0
C12:0	3.3±0.4	3.5±0.0	3.0±0.1
C13:0	0.1±0.0	0.2±0.0	0.1±0.0
C14:0	10.8±1.6	10.7±0.0	9.7±0.2
C14:1 *cis*-9	0.7±0.2	0.8±0.0	0.7±0.0
C15:0	0.9±0.2	0.9±0.0	0.8±0.0
C16:0	34.7±5.5	31.1±0.2	30.0±0.2
C16:1 *cis*-9	1.9±0.2	1.7±0.0	1.7±0.0
C16:1 *trans*-9	0.4±0.0	0.4±0.0	0.4±0.0
C17:0	0.6±0.0	0.6±0.0	0.6±0.0
C18:0	15.7±2.3	14.0±0.1	14.3±0.1
C18:1 *cis*-6	0.6±0.0	0.5±0.0	0.4±0.0
C18:1 *cis*-9	15.7±0.5	22.3±0.1	23.7±0.0
C18:1 *cis*-11	0.6±0.1	0.5±0.1	0.7±0.0
C18:1 *trans*-6	0.4±0.0	0.4±0.0	0.3±0.0
C18:1 *trans*-9	0.4±0.3	0.9±0.2	0.2±0.1
C18:1 *trans*-11	1.6±0.8	1.1±0.0	1.5±0.0
C18:2 *cis*-9, *cis*-12	2.8±0.4	2.4±0.0	3.0±0.0
C18:2 *trans*-9, *trans*-12	0.2±0.0	0.2±0.1	0.2±0.0
C18:2 *cis*-9, *trans*-11	0.5±0.0	0.4±0.0	0.4±0.0
C18:3 *cis*-9,12,15	0.2±0.0	0.1±0.0	0.2±0.0
C20:0	0.2±0.0	0.2±0.0	0.2±0.0

[1)]Mean±standard deviation of 3 determinations.

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