Talanta 85 (2011) 252–258

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of elaidic and vaccenic acids in foods using GC × GC-FID and GC × GC-TOFMS

Jaeho Ha*, Dongwon Seo, Dongbin Shin

Food Analysis Center, Korea Food Research Institute, 516, Baekhyeon, Bundang, Seongnam, Gyeonggi, 463-746, Republic of Korea

ARTICLE INFO

Article history:
Received 18 October 2010
Received in revised form 14 March 2011
Accepted 23 March 2011
Available online 6 April 2011

Keywords:
Trans fat
Elaidic acid
Vaccenic acid
GC × GC
TOFMS

ABSTRACT

Trans fatty acids (TFAs) are present in meat and dairy products as m ruminant animals and in vegetable fats due to partial hydrogenation. This study aimed to discriminate between natural (N-TFA) and hydrogenated *trans* fatty (H-TFA) acids by GC × GC-flame ionization detection (GC × GC-FID) and comprehensive GC × GC-time-of-flight mass spectrometry (GC × GC-TOFMS). The separation of two kinds of *trans* fats, vaccenic acid (18:1 *trans*-11) and elaidic acid (18:1 *trans*-9), was performed using GC × GC-FID and GC × GC-TOFMS. A 100 m × 0.25 mm I.D. × 0.2 μm (film thickness) SP-2560 (bis-cyanopropyl polysiloxane) fused capillary column (first separation dimension, 1D) was coupled to a 1.5 m × 0.18 mm I.D. × 0.18 μm (film thickness) RTX-5 (5% diphenyl/95% dimethyl polysiloxane) fused capillary column (second separation dimension, 2D). The RSD of the intra-day repeatability by both GC × GC-FID and GC × GC-TOFMS for elaidic and vaccenic acids was ≤9.56% and ≤9.97%, and the RSD of the inter-day repeatability was ≤8.49 and ≤9.06%, respectively. It was found that the V/E value (vaccenic acid to elaidic acid ratio) could be used to distinguish H-TFA from N-TFA and to evaluate the quality of the fatty foods.

1. Introduction

Trans fatty acids (TFAs) are unsaturated fatty acids containing one or more double-bonds in the *trans* configuration. TFAs originate in our foods mainly from industrial hydrogenation, deodorization and natural ruminant sources (N-TFAs) [1–6]. The natural fats and oils that are present in the human diet contain only small amounts of TFAs. Animal sources of fats such as milk, butter, cheese and beef, usually contain 2–9% of their total fat as TFAs, which are generated during rumination and subsequently absorbed and stored in animal tissues [7–10]. In the latter, *trans*-vaccenic acid (11t-C18:1) accounts for over 60% of the total TFAs; whereas, a broad mixture of TFAs in the hydrogenated *trans* fatty acids (H-TFAs) is formed with elaidic acid (9t-C18:1) as the main source [2,3,11,12].

Most of the *trans* fats that humans consume come from partially hydrogenated vegetable oils, which are produced from liquid oils by industrial processing to create a firm fat [2,3,5,13,14]. Many reports on the harmful effects of *trans* fatty acids to human health have been made during last few decades, and many countries have adopted the regulation of the limitation and/or recommended the deduction of *trans* fatty acid intake [5,15–17].

In recent reports, obese rats that were fed a diet enriched with vaccenic acid, a naturally occurring *trans* fat found in milk and yogurt, had significant reductions in the total cholesterol, LDL (or "bad") cholesterol and triglycerides. The researchers reported that a key benefit of vaccenic acid (VA) was its ability to reduce the production of chylomicrons, small particles of fat, protein and cholesterol formed in the gut, all of which transport fats to various tissues of the body [18,19]. The study is similar to other reports that natural *trans* fats have different effects on the body than industrially created fats [7,20–23].

Evaluation of individual *trans* fats on human health has been carried out by many nutritionists, who have reported that a diet with enriched levels of *trans*-vaccenic acid, a natural animal fat found in dairy and beef products, can reduce risk factors associated with heart disease, diabetes and obesity [13,22,24]. Since then, many nutritionists have been interested in the ratio of elaidic acid (EA) to vaccenic acid (VA) in food with enriched *trans* fats including margarine, milk, cheese, and butter [11,18,19,21,22,25].

From the viewpoint of the nutritional value of octadecenoic acid isomers, determination of *trans* fatty acid content, separation of *trans* fatty acids from *cis* fatty acids and separation of individual isomers have been conducted by a few separation scientists [26–29]. Gas chromatography is by far the most widely used method for the analysis of *trans* fatty acids [30]. Flexible fused silica columns coated with highly polar cyanoakyl polysiloxane stationary phases have been recommended for analyses of milk fats that contain complex mixtures of geometric and positional isomers of monounsaturated fatty acids [29–31]. Kramer et al. [27] described several temperature programs coupled with different carrier gases, such as hydrogen and helium, that achieved remarkable separation of C18:1 isomers using a 100 m CP-Sil 88 and 60 m Supelcowax 10

* Corresponding author. Tel.: +82 31 780 9127; fax: +82 31 780 9280.
E-mail address: jhkfri@kfri.re.kr (J. Ha).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.03.053

columns. However, even with such successful chromatography, the complete separation of C18:1 isomers still remains a challenge [28–32].

Recently, a new separation technique has been used for the analysis of fatty acids and volatile oils; it relies on comprehensive two-dimensional gas chromatography (GC × GC or 2D GC), because such a system has higher peak capacity and potential resolving power than conventional one-dimensional gas chromatography (1D GC) [33,34]. Villegas et al. [35] has attempted separation using the two-dimensional GC × GC coupled with a flame ionization detector (FID), achieving optimal separations of positional and geometrical C18:1 fatty acid isomers. They have compared the use of two methods to achieve optimized separations of octadecenoic fatty acid isomers-comprehensive GC × GC, and silver ion high performance liquid chromatography interfaced to atomospheric pressure photoionization (APPI) mass spectrometry. The extra selectivity and reproducibility afforded by APPI-MS, together with the wide separation of *cis* and *trans* isomers by silver ion chromatography, resulted in a promising method for measurement of octadecenoic acid FAME [35–37].

In this paper, we have performed the separation of EA and VA in several foods, including margarine, butter, beef tallow, cheese and milk, using GC × GC coupled with a flame ionization detector (FID) and time-of-flight mass spectrometer (TOFMS). The purpose of our research lies in the separation and quantification of EA and VA to evaluate the distribution of these two important *trans* fat isomers in food.

2. Experimental

2.1. Materials and chemicals

Margarine, butter, beef tallow, cheese, and milk were obtained from a local grocery store. Individual reference fatty acid methyl ester (FAME) standards of *trans*-9-elaidic methyl ester (9t-C18:1, EA) and *trans*-11-vaccenic methyl ester (11t-C18:1, VA) were purchased from Nu-Chek Prep Inc. (Elysian, MN, USA). The solvents and reagents used in this work were all of analytical grade. A 14% boron trifluoride methanol solution (BF_3), sodium hydroxide and sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, normal hexane and methanol were purchased from J. T. Baker (Philipsburg, NJ, USA).

2.2. Sample preparation

For the TFA analysis, the lipids from the margarine, butter, beef tallow, cheese, and milk were extracted by a chloroform and methanol mixture (2:1, v/v) and evaporated to dryness [11,12]. The methyl ester of TFA was obtained by reaction in a Techne DB-3D heating block (Barloworld Scientific US Ltd., Burlington, NJ, USA) with BF_3 as a catalyst. A portion of a 20 mg oil sample was boiled with 2 mL of methanolic sodium hydroxide (0.5 M) at 100 °C for 5 min on a heating block. The BF_3 reagent (2 mL) was added and boiled for another 5 min. Next, 2 mL of hexane and a saturated sodium chloride solution was added and vortexed for 1 min. The hexane layer was transferred to a vial for subsequent GC × GC analysis.

2.3. Chromatographic system

The GC × GC system consists of an Agilent 6890N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an FID and cryogenic modulator (quadjet) cooled with liquid nitrogen. The data were processed using LECO Corp. ChromaTOF-GC software v 4.22 optimized for GC × GC. For GC × GC-TOFMS, a LECO Corporation Pegasus 4D instrument with an Agilent 6890N GC was used. The column set for GC × GC analysis consisted of two columns. The primary column had dimensions of 100 m × 0.25 mm I.D. × 0.2 μm film thickness SP-2560 (Supelco, Bellefonts, PA, USA), and the second column had dimensions of 1.5 m × 0.18 mm I.D. × 0.18 μm film thickness RTX-5 (Restek, Bellefonts, PA, USA). The injector temperature was 230 °C with a split ratio of 10:1, and the FID temperature was 270 °C. The primary oven temperature program was 45 °C for 4 min, 45–175 °C at a rate of 13 °C per minute and 175 °C for 27 min, 175–215 °C at a rate of 4 °C per minute and 215 °C for 10 min. The secondary oven temperature was increased 20 °C over the primary oven temperature; the modulator temperature offset was 40 °C; the second dimension separation time was 5 s; the hot pulse time was 1.0 s; and the cool time between stages was 1.5 s. Helium was used as the carrier gas with a constant flow rate of 1.0 mL/min and an injection volume of 1 μL. The mass spectrometer was operated at an acquisition rate of 100 spectra/s. No mass spectra were collected during the solvent delay for the first 15 min of each run. The transfer line temperature was 220 °C, and the ion source temperature was 220 °C. The detector voltage was 1650 V, and the electron energy was 70 V. Mass spectra were collected from *m*/*z* 35–400. Mass spectra were probability matched to the NIST Mass Spectral Search Program ver.2.0f using LECO Corp. Chroma TOF-GC software ver.4.22 optimized for PEGASUS 4D.

2.4. Statistic analysis

The experiment was carried out in triplicate and expressed as the mean ± standard deviation. The results obtained were subjected to statistical analysis using the program SPSS 13.0 for Window (LEAD TOOLS, LEAD Technologies, Inc., 2004). VA and EA compositional data for fatty acids, and comparisons of the fatty acid composition of fatty foods were analyzed as a one-way ANOVA.

3. Results and discussion

3.1. Comparison of 1D GC and GC × GC

Many analyses of *trans* fats have been performed using gas chromatography with highly polar capillary columns that require a temperature program from 65 °C to 240 °C to elute all of the fatty acid methyl ester (FAME) from the short-chain fatty acid to long-chain poly-unsaturated fatty acid (PUFA) present in margarine and dairy products. As is often the practice, the long-chain FAME accumulates in the column and often causes interferences in subsequent analyses [27]. Although many attempts for the *trans* fatty acid analyses have been made using conventional gas chromatography (1D GC) with a highly polar column to avoid altering the relative elution sequence of several FAMEs, only a few attempts have been made to separate the C18:0 isomers using the GC × GC technique with a polar phase for the primary column and an intermediate polar phase for the secondary column, especially for the *trans* fatty acids in dairy products [33,34,37].

We have performed the separation of C18:1 isomers, especially aiming to successfully separate EA and VA as the quality factors for dairy products. Fig. 1 presents the partial gas chromatogram of standard EA and VA using 1D GC-FID, 2D GC-FID and 2D GC-TOFMS. In the 1D GC chromatogram as represented in Fig. 1(A), it can be seen that two major peaks dominate the chromatogram. The separation of EA and VA was partially achieved, but the complete base-line separation could not be achieved. Fig. 1(B) shows the chromatogram of EA and VA separated by GC × GC-FID and Fig. 1(C) shows the chromatogram by GC × GC-TOFMS using a 4 s modulation period for the cryogenic trap (represented as series of peak pulses). It was found that the separation by GC × GC-TOFMS required less time than by GC × GC-FID due to high vacuum of TOFMS, however, the better

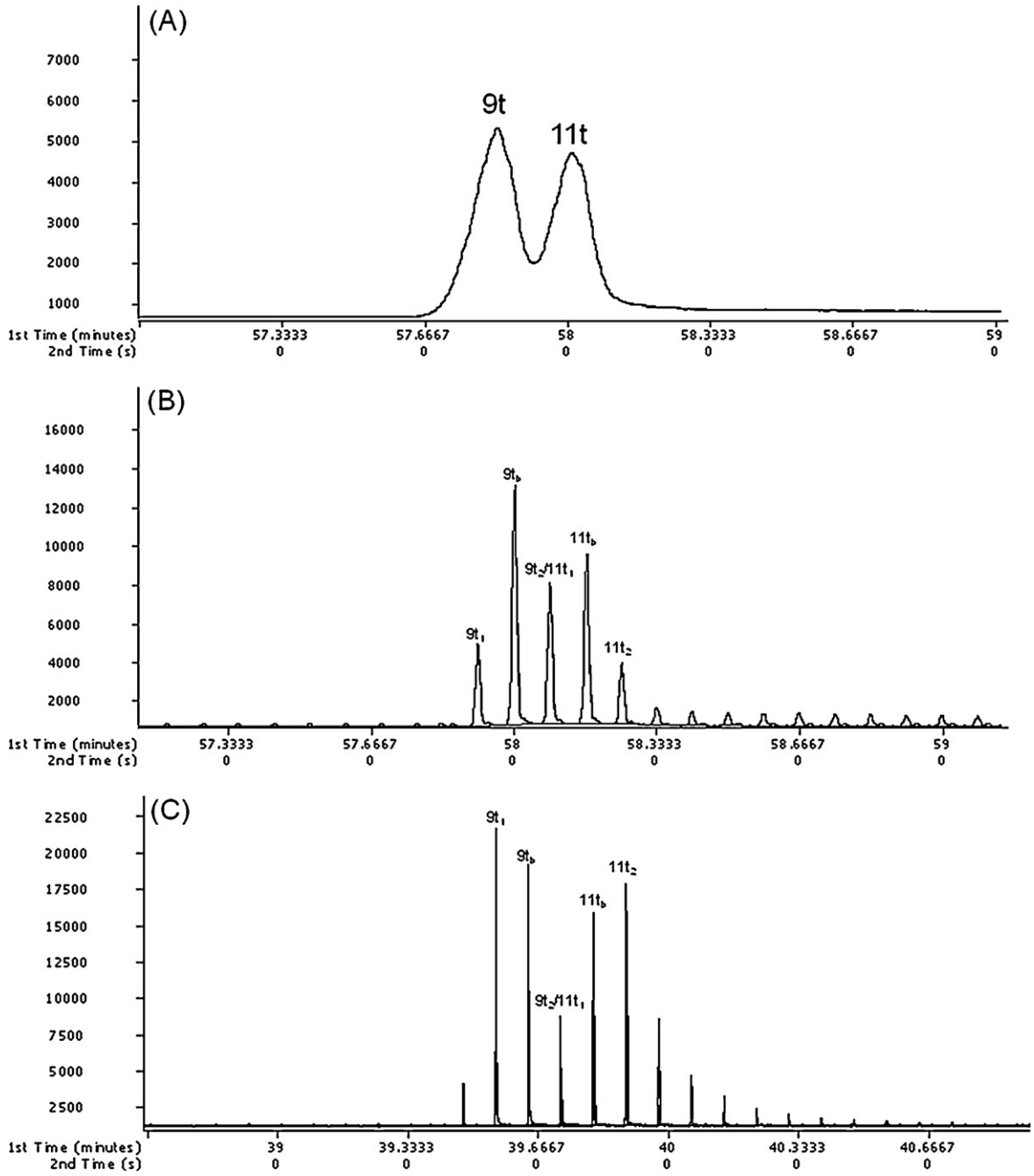

Fig. 1. Chromatogram of EA and VA standards by GC-FID (A), GC × GC-FID(B) and GC × GC-TOFMS (C). $9t_b$ and $11t_b$ are base peak for EA and VA. $9t_1$, and $9t_2$ are sub peaks for EA, and $11t_1$ and $11t_2$ for VA, respectively.

separation of individual isomers could be achieved by TOFMS. Using GC × GC-FID and TOFMS, EA and VA were separated into 3 peaks: $9t_1$, $9t_b$, $9t_2$ for EA and $11t_1$, $11t_b$, $11t_2$ for VA, respectively. Individual injection of each *trans* fat standard revealed that the $9t_2/11t_1$ peak from both EA and VA was not completely resolved (individual chromatogram was not shown) and came from both EA and VA.

The structure of these compounds is very similar with the exception of one double-bond position; therefore, the complete separation of the two isomers could not be achieved. Instead, they clustered in a single peak labeled as $9t_2/11t_1$. Even though these two isomers are partially clustered and not fully separated, it was found that the base peaks ($9t_b$ and $11t_b$) were completely separated. Because the chromatographic conditions were essentially the same for the 1-D separation and the ^{1}D in GC × GC, it can be reasoned that extensive peak co-elution occurred in the first polar column in GC × GC, and the introduction of the second ^{2}D intermediate column resolved many of the C18:1 isomers co-eluted from the 1D column. For the quantitative analysis of individual *trans* fats, the two major peaks, $9t_1$ and $9t_b$ for EA and $11t_b$ and $11t_2$ for VA, obtained by GC × GC separation were selected.

3.2. Precision test of performance

An intra- and inter-day precision test (number of trials (n) = 6) was performed on EA and VA at three concentration levels for the GC × GC-FID and GC × GC-TOFMS methods. Table 1 shows intra- and inter-day precision data for the determination of the EA and VA concentrations, which indicates that intra- and inter-day precision

Table 1
Intra- and inter-day precision data[a] for elaidic acid and vaccenic acid analysis by GC × GC-FID and GC × GC-TOFMS.

Instruments	Analytes	Concentration (mg/kg)	Intra-day (RSD[b], %)	Inter-day (RSD, %)
GC × GC-FID	Elaidic	10	6.42	7.39
		50	8.53	9.01
		100	8.39	6.82
	Vaccenic	10	9.06	9.56
		50	8.85	7.66
		100	4.74	6.30
GC × GC-TOFMS	Elaidic	1	8.49	8.00
		5	6.24	4.86
		10	2.33	3.05
	Vaccenic	1	4.67	8.95
		5	6.49	9.97
		10	5.39	8.96

[a] $n = 6$ for all samples.
[b] RSD = Relative standard deviation.

Table 2
Repeatability data[a] of the V/E value[b] in foods by GC × GC-FID and GC × GC-TOFMS.

Items	GC × GC-FID	RSD, %	GC × GC-TOFMS	RSD, %
Margarine	1.27	2.37	1.35	1.48
Butter	2.38	0.42	3.13	0.32
Beef tallow	3.13	0.96	3.03	0.67
Cheese	5.56	0.18	6.67	0.15
Milk	3.13	0.96	2.94	0.68
Mean[c]	3.09		3.42	

[a] Values represent the average of six replicate analyses.
[b] Calculated from [peak areas ($11t_b + 11t_t$) of vaccenic acid divided by peak area ($9t_b + 9t_t$) of elaidic acid].
[c] Results are mean of whole items; t-test = 0.13 (2.13) at $P = 0.05$; Pearson correlation = 0.9708.

is acceptable in the range of 10 to 100 μg/g of EA and VA for GC × GC-FID and 1 to 10 μg/g of EA and VA for GC × GC-TOFMS. The RSD values for intra-day repeatability ranged from 6.42 to 8.53 for EA and 4.74 to 9.06 for VA by GC × GC-FID, while the RSD values for inter-day repeatability ranged from 7.39 to 9.01 for EA and 6.30 to9.56 for VA. The intra-day and inter-day repeatability data by the GC × GC-TOFMS are given in Table 2, showing similar trends in the GC × GC-FID results, even though the concentration of *trans* fats injected in the GC × GC-TOFMS was ten times lower than that for GC × GC-FID.

3.3. Repeatability data of V/E values in foods

The repeatability test (number of trials (n) = 6) was carried out to evaluate the accuracy of the analytical method for the determination of the V/E value by GC × GC-FID and GC × GC-TOFMS. The V/E value of five foods was determined and the results are shown in Table 2. The relative standard deviation (RSD) values of the repeatability by GC × GC-FID (injection concentration, 5 mg/kg) ranged from 0.18 to 2.37 and by GC × GC-TOFMS (injection concentration, 50 mg/kg) ranged from 0.15 to 1.48. These results reveal that the mean of V/E values obtained by GC × GC-TOFMS was slightly higher than that by GC × GC-FID. However, no significant difference was found from t-tests conducted to validate the two instruments. The reason for this slight difference in the V/E value between the two instruments is not clear, but the differences in the resolving power and injection concentration for each instrument may be possible reasons.

3.4. Determination of V/E value in dairy products

Foods have been characterized by their fatty acid profile. Some of these foods have high saturated fatty acid content while others have a high unsaturated fatty acid content. Margarine made by hydrogenation contains a high level of EA, while natural butter is rich in VA. Recently, it has been reported that VA has a different effect on human health than elaidic acid. The former can directly reduce the risk of chemically induced mammary carcinogenesis in rats, or it may be mediated via the conversion to *cis*-9, *trans*-11 conjugated linoleic acid (CLA) [18,19]. The ratio of vaccenic acid to eladic acid (V/E value) can be an important parameter to evaluate fatty food.

Fig. 2 shows the partial chromatogram of C18 isomers including *cis* and *trans* fats obtained by a conventional GC-FID and a comprehensive GC × GC-FID. It was found that C18:1 isomers were not completely separated in the 1D chromatogram, as represented in Fig. 2 (A) from margarine and Fig. 2(C) from butter. This result reveals that the peak profiles of margarine and butter in the region of C 18:1 *trans* fat could be discriminated by their shapes. The peak top of the TFA region of margarine, a typical H-TFA, showed the saw-tooth shape, while that of the butter, a typical N-TFA, showed a smooth shape. It seemed that the quantitative determination of individual TFAs by the 1D GC was difficult to achieve due to the relatively poor separation. However, the base line separation of EA and VA in margarine and butter could be achieved by GC × GC, as shown in Fig. 2(B) from margarine and Fig. 2(D) from butter. Because a standard of individual fatty acid could not obtained from a commercial company and the structures of C18:1 isomers were very similar, the perfect separation of individual C18:1 isomers could not be achieved using GC × GC-TOFMS. However, we could confirm the elution position of EA and VA by comparing the elution time, and we could determine the EA/VA value based on the major peaks.

The partial chromatograms of C18:1 isomers from five kinds of fatty foods (margarine, butter, beef tallow, cheese and milk) obtained by GC × GC-TOFMS are shown in Fig. 3. The intensity of the individual peaks was different due to the different concentration of fat to fatty acid ratio. It was found that the C18:1 *trans*

Table 3
Results of the V/E value determination in various foods by GC × GC-FID and GC × GC-TOFMS.

Items	GC × GC-FID V/E value range	Mean ± SD[a]	GC × GC-TOFMS V/E value range	Mean ± SD[a]
Margarines ($n = 6$)[b]	1.14–1.47	1.31 ± 0.12	0.96–1.46	1.09 ± 0.19
Butters ($n = 8$)	2.03–3.42	2.48 ± 0.46	2.45–3.55	2.94 ± 0.38
Beef tallow ($n = 9$)	2.49–3.54	3.00 ± 0.37	2.82–3.95	3.42 ± 0.40
Cheeses ($n = 10$)	2.70–5.98	3.89 ± 1.19	2.98–6.38	3.89 ± 1.32
Milks ($n = 10$)	2.20–3.69	2.58 ± 0.43	2.63–3.40	3.07 ± 0.27
F-test ($P < 0.05$)[c]	14.93 (2.61)		16.76 (2.62)	
t-test ($P < 0.05$)[c]		1.57 (2.13)		

[a] SD = Standard deviation.
[b] The number (n) of analyses is shown in parentheses.
[c] Tabulated values of the F-test at $P = 0.05$ among items and t-test at $P = 0.05$ between GC × GC-FID and GC × GC-TOFMS are shown in parentheses; Pearson correlation between GC × GC-FID and GC × GC-TOFMS = 0.9562.

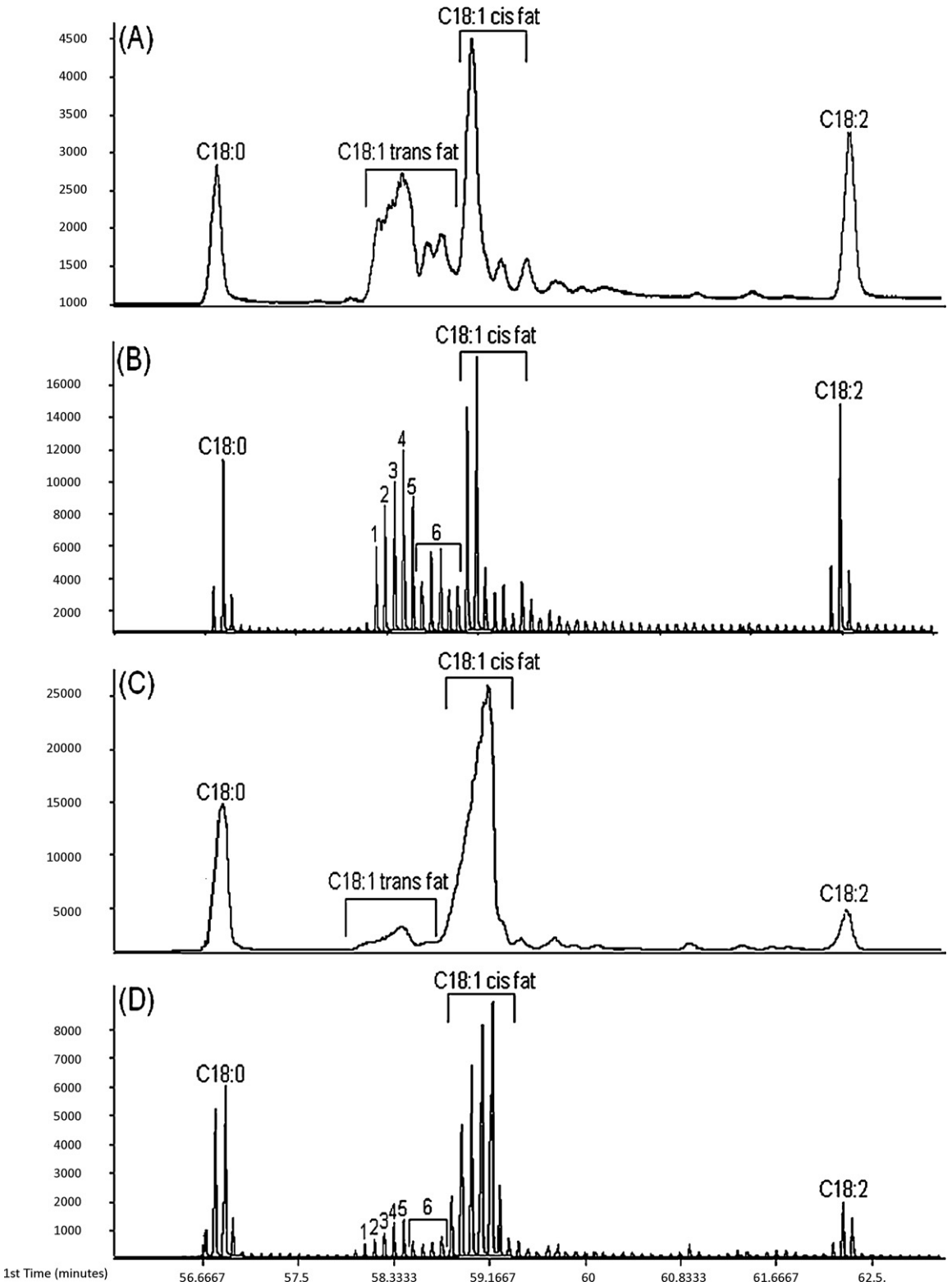

Fig. 2. Partial chromatogram of C18:1 *trans* and *cis* fats in foods. A: margarine by GC-FID, B: margarine by GC × GC-FID, C: butter by GC-FID, D: butter by GC × GC-FID. 1: $9t_1$, 2: $9t_b$, 3: $9t_2/11t_1$, 4: $11t_b$, 5: $11t_2$, 6: C18:1 *trans* fat isomers.

and *cis* fat isomers had been satisfactorily separated. Even though elaidic and vaccenic acids were separated, a tail of elaidic acid and the head of vaccenic acid were co-eluted and labeled as $9t_2/11t_1$. The individual chromatogram revealed that every food had a different chromatographic profile at the region of C18:1 *trans* fat isomers.

The V/E value of several foods has been determined by GC × GC-FID instead of GC × GC-TOFMS; these results are represented in Table 3. For the determination of the V/E value, the sum of the base peak ($9t_b$) and sub peak ($9t_1$) areas from EA was divided by that of the base peak ($11t_b$) and sub peak ($11t_1$) area from VA. The V/E values of margarines ranged from 1.14 to 1.47, while those of butters

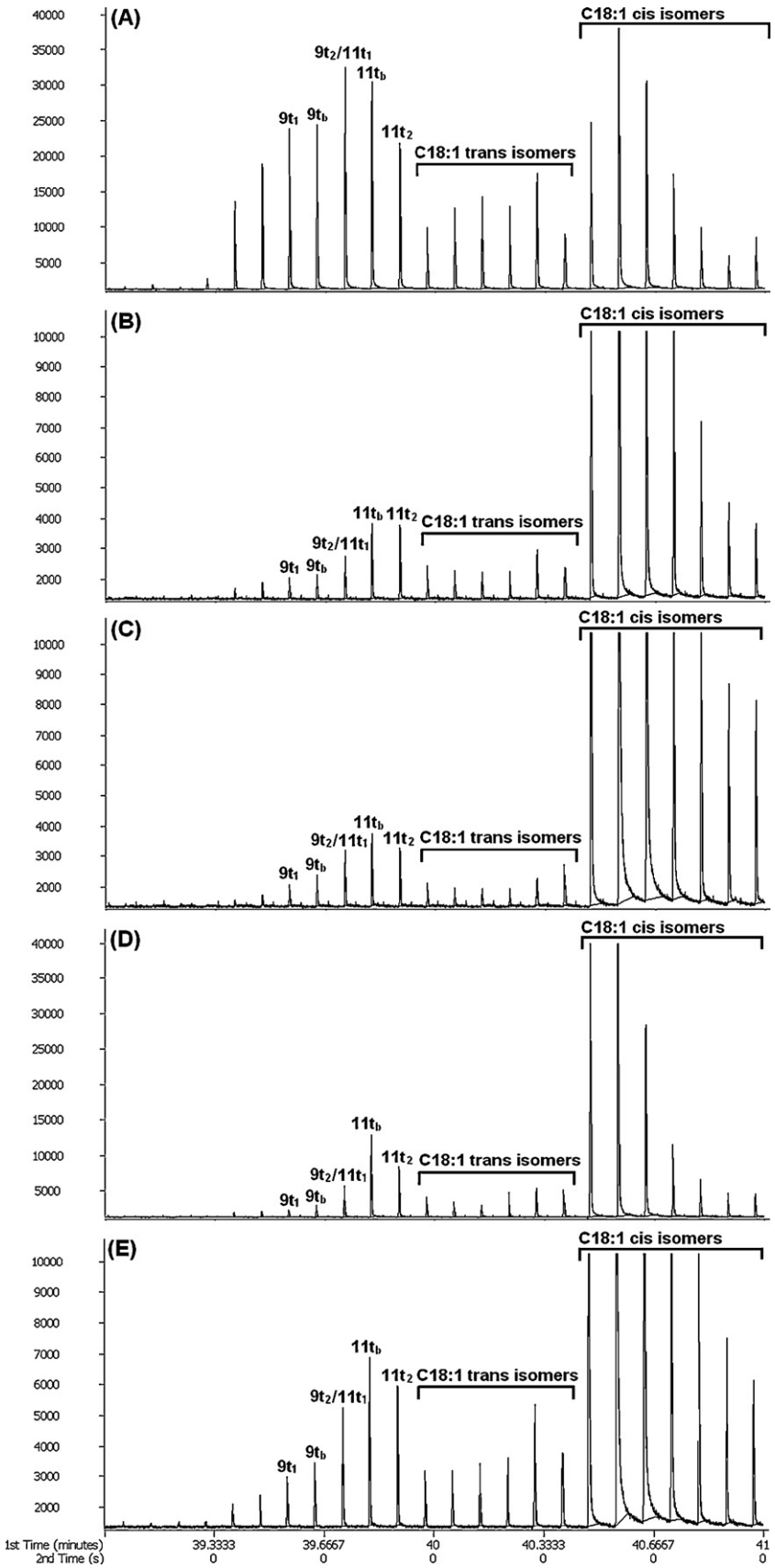

Fig. 3. Partial chromatogram of C18:1 *trans* fat (9t, 11t) and isomers in foods by GC × GC-TOFMS. A: margarine, B: butter, C: beef tallow, D: cheese, E: milk. $9t_b$ and $11t_b$ are base peak for EA and VA. $9t_1$, and $9t_2$ are sub peaks for EA, and $11t_1$ and $11t_2$ for VA, respectively.

ranged from 2.03 to 3.42. It is well-known that margarine is one of the typical fatty foods manufactured by the hydrogenated procedure and that butter is a typical naturally fatty food. The V/E values of natural fats were higher than 2.0, while those of margarine were lower than 1.5. As the results of ANOVA, the *F- ratio* of GC × GC-FID was 14.9 and *F*-critical value of GC × GC-FID was 2.61, and those of GC × GC-TOFMS, 16.76 and 2.62, respectively, which indicates that the significant difference of V/E values between the items was observed at a significance level of 0.05. In the meantime, there was no significant difference found from *t*-tests conducted to compare the GC × GC-FID with GC × GC-TOFMS.

It has been reported that vaccenic acid may provide *cis*-9, *trans*-11 CLA to the consumer through endogenous desaturation [18,19]. Therefore, the V/E value in the total TFA may play an important role to evaluate the quality of the fat source. From this point of view, the V/E value in *trans* fatty acids in several foods, including butter and beef tallow, can be considered and adapted as an important parameter to assess the quality control of daily products together with n-6/n-3 ratio [11]. It was also found that the V/E value could be used as an index of TFA to discriminate hydrogenated *trans* fat from natural *trans* fat. Furthermore, because the V/E value can be varied from different regions that produce the fat source, we suggest that an extensive survey on the V/E value of TFA in foods should be conducted to use the V/E value as a new quality parameter of fat.

4. Conclusion

Using GC × GC-FID and GC × GC-TOFMS, the separation of two kinds of *trans* fats, vaccenic acid(18:1 *trans*-11) which is a naturally occurring *trans* fat found in the fat of ruminants and in dairy products and elaidic acid (18:1 *trans*-9) which is the major *trans* fat found in hydrogenated vegetable oils was performed. The ratio of vaccenic acid to elaidic acid (V/E value) can be used to discriminate the natural *trans* fat (N-TFA) from hydrogenated *trans* fat (H-TFA).

It seems that the comprehensive GC × GC provided good information on the distribution of the double-bond position throughout the successful separation of EA and VA. The V/E value could be used to discriminate H-TFA and N-TFA and to evaluate the quality of fatty foods.

Acknowledgments

This research was supported by the Korea Food Research Institute (project no. E0104300) and the Ministry for Food, Agriculture, Forestry and Fisheries.

References

[1] R.G. Ackman, S.N. Hooper, D.L. Hooper, J. Am. Oil Chem. Soc. 51 (1974) 42–49.
[2] J.L. Beare-Rogers, L.M. Gray, R. Hollywood, Am. J. Clin. Nutr. 32 (1979) 1805–1809.
[3] T. Sommerfeld, Prog. Lipid Res. 22 (1983) 221–233.
[4] A. Grandgirard, J.L. Sebedio, J. Fleury, J. Am. Oil Chem. Soc. 61 (1984) 1563–1568.
[5] J.E. Hunter, T.H. Applewhite, Am. J. Clin. Nutr. 44 (1986) 707–717.
[6] S.F. O'Keefe, V.A. Wiley, D. Wright, J. Am. Oil Chem. Soc. 70 (1993) 915–917.
[7] I.A. Brouwer, A.J. Wanders, M.B. Katan, PLoS ONE 5 (2010) 1–10.
[8] F.T. Gillan, R.B. Johns, T.V. Verheyen, J.K. Volkman, H.J. Bavor, Appl. Environ. Microbiol. 41 (1981) 849–856.
[9] J.D. Bansal, J.M. deMan, J. Food Sci. 47 (1982) 1338–1344.
[10] Z.Y. Chen, G. Pelletier, R. Hollywood, W.M.N. Ratnayake, Lipids 30 (1995) 15–21.
[11] N. Aldai, M.E.R. Dugan, J.K.G. Kramer, J. Food Compos. Anal. 23 (2010) 326–332.
[12] J. Domagala, M. Sady, T. Grega, H. Pustkowiak, A. Florkiewicz, J. Food Compos. Anal. 23 (2010) 238–243.
[13] R.P. Mensink, M.B. Katan, N. Engl. J. Med. 323 (1990) 439–445.
[14] W.C. Willett, M.J. Stampfer, J.E. Manson, G.A. Colditz, F.E. Speizer, B.A. Rosner, L.A. Sampson, C.H. Hennekens, Lancet 341 (1993) 581–585.
[15] C. Bolton-Smith, M. Woodward, S. Fenton, M.K. McCluskey, C.A. Brown, Br. J. Nutr. 74 (1995) 661–670.
[16] R.M. Krauss, R.H. Eckel, B. Howard, L.J. Appel, S.R. Daniels, R.J. Deckelbaum, J.W. Erdman, P. Kris-Etherton, I.J. Goldberg, T.A. Kotchen, A.H. Lichtenstein, W.E. Mitch, R. Mullis, K. Robinson, J. Wylie-Rosett, S.S. Jeor, J. Suttie, D.L. Tribble, T.L. Bazzarre, Circulation 102 (2000) 2284–2299.
[17] J. Salmeron, F.B. Hu, J.E. Manson, M.J. Stampfer, G.A. Colditz, E.B. Rimm, W.C. Willett, Am. J. Clin. Nutr. 73 (2001) 1019–1029.
[18] A.L. Lock, C.A.M. Horne, D.E. Bauman, A.M. Salter, J. Nutr. 135 (2005) 1934–1939.
[19] E.E. Mosley, M.K. McGuire, J.E. Williams, M.A. McGuire, J. Nutr. 136 (2006) 2297–2301.
[20] W.J. Decker, W. Mertz, J. Nutr. 91 (1967) 324–330.
[21] A.L. Lock, B.A. Corl, D.M. Barbano, D.E. Bauman, C. Ip, J. Nutr. 134 (2004) 2698–2704.
[22] Y. Wang, M.M. Jacome-Sosa, M.R. Ruth, S.D. Goruk, M.J. Reaney, D.R. Glimm, D.C. Wright, D.F. Vine, C.J. Field, S.D. Proctor, J. Nutr. 139 (2009) 2049–2054.
[23] P.H. Yu, J. Mai, J.E. Kinsella, Am. J. Clin. Nutr. 33 (1980) 598–605.
[24] T. Ide, M. Watanabe, M. Sugano, I. Yamamoto, Lipids 22 (1987) 6–10.
[25] S.M. Innis, Atheroscler. Suppl. 7 (2006) 17–20.
[26] W.M.N. Ratnayake, R. Hollywood, E. O'Grady, J.L. Beare-Rogers, J. Am. Oil Chem. Soc. 67 (1990) 804–810.
[27] J.K.G. Kramer, C.B. Blackdar, J. Zhou, Lipids 37 (2002) 823–835.
[28] C. Cruz-Hernandez, Z. Deng, J. Zhou, A.R. Hill, M.P. Yurawecz, P. Delmonte, M.M. Mossoba, M.E.R. Dugan, J.K.G. Kramer, J. AOAC Int. 84 (2004) 545–562.
[29] M.M. Mossoba, A. Seiler, J.K.G. Kramer, V. Milosevic, M. Milosevic, H. Azizian, H. Steinhart, J. Am. Oil Chem. Soc. 86 (2009) 1037–1045.
[30] W. Horwitz (Ed.), Official Methods of Analysis of AOAC International, 18th ed., AOAC International, Maryland, USA, 2005, p. p. 41 (Chapter 41).
[31] W.M.N. Ratnayake, S.L. Hansen, M.P. Kennedy, J. Am. Oil Chem. Soc. 83 (2006) 475–488.
[32] K.H. Wagner, E. Plasser, C. Proell, S. Kanzler, Food Chem. 108 (2008) 1054–1060.
[33] X. Di, R.A. Shellie, P.J. Marriott, C.W. Huie, J. Sep. Sci. 27 (2004) 451–458.
[34] M. Adahchour, J. Beens, U.A. Th. Brinkman, J. Chromatogr. A 1186 (2008) 67–108.
[35] C. Villegas, Y. Zhao, J.M. Curtis, J. Chromatogr. A 1217 (2010) 775–784.
[36] C. von Muhlen, C.A. Zini, E.B. Caramao, P.J. Marriott, J. Chromatogr. A 1105 (2006) 39–50.
[37] Leco Corporation, Separation Science Application Note, Form No. 203-821-277, 2009,1–6.