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

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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

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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