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ORIGINAL PAPER

Rapid Profiling of Animal-Derived Fatty Acids Using Fast GC × GC Coupled to Time-of-Flight Mass Spectrometry

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Abstract Rapid profiling of fatty acid methyl esters (FAME) from five different animal sources was examined in this study using fast comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (fast GC × GC–TOFMS). The result showed that GC × GC analysis combining two different microbore columns (SLB-5 ms, 10 m × 0.10 mm × 0.10 μm and DBWax, 0.5 m × 0.10 mm × 0.10 μm) allowed three-fold faster analysis times than those observed for conventional GC × GC analysis. The modulation ratio (M_R) of the system was defined at the value of 2.38 by optimizing the modulation period and offset temperature for precise mass spectral identification and highest effluent resolution in the analysis. In accordance with the normalized FAME level obtained from various animal fats, namely lard (LA), chicken fat (CF), beef tallow (BF), mutton tallow (MF) and cod liver oil (CLO), a clear discrimination of LA from the other species by principal components analysis (PCA) was observed. This was attributed to several FAME constituents involving methyl 6,9,12,15-heneicosatetraenoate (C21:4n-6), methyl 11,14-eicosadienoate (C20:2n-6), *trans*-9,12-methyl octadecadienoate (C18:2n-6t), *trans*-9-methyl octadecenoate (C18:1n-9t) and methyl hexadecanoate (C16:0).

Keywords GC × GC · TOFMS · Fast GC · FAME · PCA · Animal fats · Lard

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Introduction

For dietary and therapeutic reasons, the role of fatty acids as the major building blocks of food lipids has drawn increasing concern over aspects of their composition and functionality. Analysis of fatty acids commonly involves derivatization of fatty acids (FA) to methyl esters through a saponification process prior to individual analyte separation with gas chromatography [1, 2]. In conjunction with several detection methods, various stationary phases of different polarities have been used in order to verify the fatty acid methyl esters (FAME) composition from various foods [3, 4]. More importantly, such FAME profiles are an efficient method for discriminating lipids from a specific source. This is crucial for the growing health-consciousness of consumers, as well as religious commitments of some ethnic groups. For instance, usage of glycerides that were derived from, or consist of, lard is absolutely prohibited in the production of Kosher and Halal food, as dictated in Biblical Hebrew and Quranic guidance [5]. Nevertheless, the determination of the FAME profile, especially in animal glycerides, remains a great challenge, mainly because of their complex structural attributes that are rarely fully resolved in chromatography.

The GC × GC technique was introduced in the last decade to comprehensively unravel all the individual relevant components, which are barely resolved in the conventional single dimensional GC [6–9]. Recent advancement in

GC × GC has offered significant improvements in the aspects of resolution, capacity and sensitivity of the chromatography analysis for extremely complex samples. This technique has been successfully applied in diverse fields, namely petrochemicals, fragrances, flavor and environmental analysis [8–11]. Unlike the heart-cut multidimensional GC technique, GC × GC incorporates serial coupling of two columns with orthogonal retention behavior that allows all sample portions emerging from the first dimensional column to enter the second and be analyzed sequentially without losing any solute data. Moreover, the increase in sensitivity is due to the incremental increase of response height based on the refocusing of a zone of a peak eluting from the first chromatographic column prior to the modulating interface and its release to the fast second column as narrow modulated peaks. In fact, reliability of the acquired GC × GC data demands adequate peak modulation, which is mainly depicted by the modulated peak pattern as well as the modulation ratio [9–13]. Marriott and co-workers [12, 13] have stated that a minimum M_R value of 1.5 is required to ensure data precision during a semi-quantitative analysis. The modulated peak pattern (peak shape and magnitude) is governed by the release efficiency of each cryo-trapped solute particle remobilizing to the second column, which involves the offset heating operation, in the case where a thermal modulator is used. Specifically, a few GC × GC studies have been conducted on the FAME derived from marine oil using a column 30-m in length as the primary dimension; disclosing that substantial separation of FAME corresponded to their grouping on an equal-double-bond-number basic along the contour plot [11, 14]. However, the long analysis time for a conventional GC × GC analysis makes it an unfavorable technique, due to the increasing demands for high-throughput and fast-turnaround analytical approaches made by industry and laboratories today. In working to develop a fast GC technique, a microbore capillary column was evaluated recently for creating a rapid FAME profiling analysis in a one-dimensional GC application. Minor losses of resolution and peak capacity were reported as the main drawbacks [15]. Such a microbore column was also employed in chiral GC × GC work that showed a two-fold decrease in overall analysis time, while still providing the desired resolution of the majority enantiomer compounds [16]. Thus, it is desired that an analyte's separation on the secondary column be performed rapidly, within a millisecond period. An adequate data acquisition rate and modulation condition is crucial in the development of a fast GC × GC method.

Multivariate analysis is a mathematical procedure for resolving sets of data into orthogonal components whose linear combinations approximate the original data to any desired degree of accuracy. Principal component analysis (PCA) is one of the most commonly applied techniques in multivariate data analysis. PCA is an unsupervised clustering method that does not require any knowledge of the data set, which reduces the dimensionality of data, detects the number of components, and visualizes the outliers [17]. This statistical analysis has been applied widely to understand the relationships expressed in terms of similarity or dissimilarity among groups of multivariate data that are acquired from different instrumental analysis, such as GC, NMR, FTIR, and so on [18–20]. Attempting to establish a complete FAME profile with enhanced speed, the present work evaluated the FAME composition derived from animal fats, including cod liver oil, lard, beef tallow, mutton tallow and chicken fat using fast GC × GC analysis. The data obtained was further characterized using multivariate statistical analysis.

Methodology

Materials

Different batches of subcutaneous tissues from four animal species, namely pigs, cows, sheep and poultry, as well as cod liver oil were collected from the local market in Selangor, Malaysia. Animal fat specimens were then extracted from the tissues using the microwave procedure described in a previous work [21]. FAME standards were purchased from Sigma–Aldrich Chemicals (Deisenhofen, Germany) and diluted to the range of 50–150 mg L^{-1}. Chromatography-grade methanol and hexane were purchased from Fisher Scientific (UK), while sodium hydroxide pellets was purchased from Merck Chemicals (Darmstadt, Germany).

Derivatization of FAME Compounds

The conversion of extracted fat to FAME was performed according to our previous report [22]. A 50-mg fat sample was dissolved in 800 μL of hexane prior to the addition of 200 μL of 1N methanolic sodium solution. The mixture underwent both hydrolysis and derivatization reactions under 1 min of vortex mixing. The resulting FAME extract was collected from the upper hexane layer and then transferred into a 2-mL vial for GC × GC analysis.

GC × GC Analysis

An Agilent 6,890N GC × GC that was connected serially with a Leco dual-stage thermal modulator was coupled to a Leco Pegasus 4D time-of-flight mass spectrometer (MI, USA) for FAME profiling analysis. The sample was injected into the GC injector at 250 °C. Helium gas with 99.9999% purity was used as the carrier gas, while configurations for conventional and fast GC × GC analysis

Table 1 Parameters of GC × GC operation

Configuration	Parameter 1 (conventional GC × GC)	Parameter 2 (fast GC × GC))
Primary column	DB5 ms; 30 m × 0.25 mm i.d. × 0.25 μm d_f[a]	SLB5 ms; 10 m × 0.10 mm i.d. × 0.10 μm d_f[b]
Secondary column	DBwax; 1 m × 0.10 mm i.d. × 0.10 μm d_f[c]	DBwax; 0.5 m × 0.10 mm i.d. × 0.10 μm d_f
Injection mode	1.0 μL; split ratio 100:1	0.1 μL; split ratio 100:1
Primary oven ramping	Initial 40 °C hold for 3 min, ramp to 160 °C at 15 °C min^{-1}, ramp to 250 °C at 2 °C min^{-1}, hold 5 min	Initial 40 °C hold 1 min, ramp to 160 °C at 70 °C min^{-1}, ramp to 250 °C at 8 °C min^{-1}, hold 3 min
Secondary oven ramping	45 °C hold for 3 min, ramp to 165 °C at 15 °C min^{-1}, ramp to 255 °C at 2 °C min^{-1}, hold 5 min	45 °C hold for 1 min, ramp to 165 °C at 70 °C min^{-1} ramp to 255 °C at 8 °C min^{-1}, hold 3 min
Modulation	4 s frequency, 1 s hot pulse	2 s frequency, 0.5 s hot pulse

[a] 5% Phenyldimethylsiloxane stationary phase (J&W, US)

[b] 5% Phenyldimethylsiloxane stationary phase (Supelco, US)

[c] Polyethylene glycol stationary phase (J&W, US)

were performed as stated in Table 1. The transfer line that connected the GC and TOF-MS was maintained at 240 °C. The TOF-MS generated 70 eV electron impacts at 220 °C with 1,500 V of detector gain voltage, and a mass scan ranging from 35 to 450 μg. Data points were collected using 100 and 150 spectra per second acquisition rates for conventional and fast GC × GC, respectively, prior to the integration of a deconvoluted apexing peak using the ChromaTOF software, version 3.34. The data was screened with a threshold of 100 for the signal-to-noise ratio prior to identification of FAME compounds from the specimens by matching their mass spectral data with FAME standards, NIST library version 2.0d as well as published data [23]. The normalized percentage of FAME was obtained and then evaluated using Minitab statistical software, version 14. Analysis of variance (ANOVA) without intercept and principal component analysis (PCA) with correlation matrix were conducted. At least three replicates were conducted for the entire experiment on each sample type.

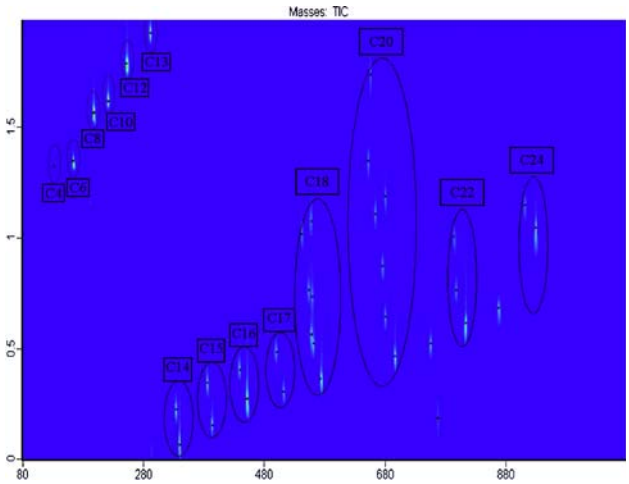

Fig. 1 Separation of 37 FAME compounds in contour plot acquired by fast GC × GC method

Results and Discussion

Configuration of Fast GC × GC Settings

According to the revised GC × GC development model [24], aspects of resolution, sensitivity and structure have to be compromised when speed of analysis is being emphasized. While use of the microbore GC column allows sufficient resolution under rapid-oven-ramping operation, the amount of the sample being introduced in the fast GC analysis (0.1 μL) was considerably less than that in the conventional GC analysis (1 μL) in order to reduce column overloading error. Complete separation of all 37 FAME compounds was obtained by fast GC × GC and a good distribution of these compounds was illustrated along the contour plots (Fig. 1) under these orthogonal systems. A three-fold shorter analysis time was possible, as compared to that needed for the conventional GC × GC approach [25].

During the set up of GC × GC, the modulation configuration is crucial for improving the resolution, sensitivity and peak structure. Evaluation of the C-18 cluster separation (Fig. 2) showed that the modulation period (P_M) was restricted below 2 s in order to acquire precise peak measurement, while the shorter second column with 0.5 m length was compromised. This revealed that a minimum value for the modulation ratio, M_R around 2.38, was required for the fast GC × GC analysis. Such a value was relatively higher than the recommended 1.5 M_R value for the conventional GC × GC analysis stated previously [12].

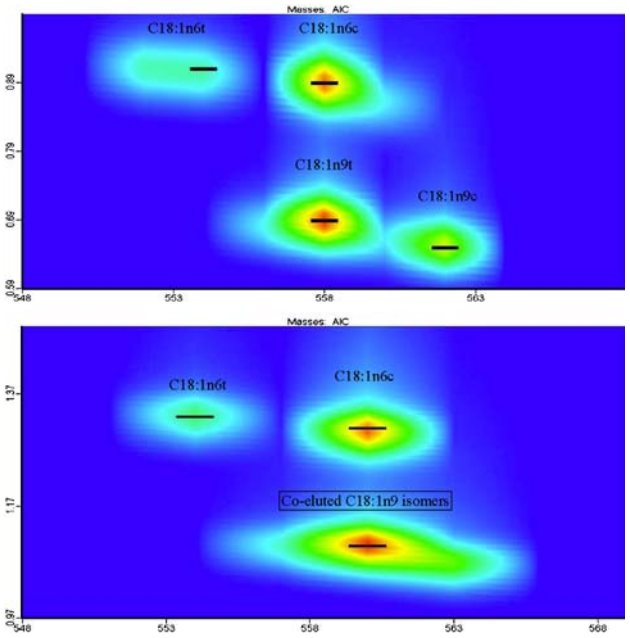

Fig. 2 Separation of C18 cluster in GC × GC contour plot acquired with modulation period of 2-s (*upper figure*) and 3-s (*lower figure*), respectively

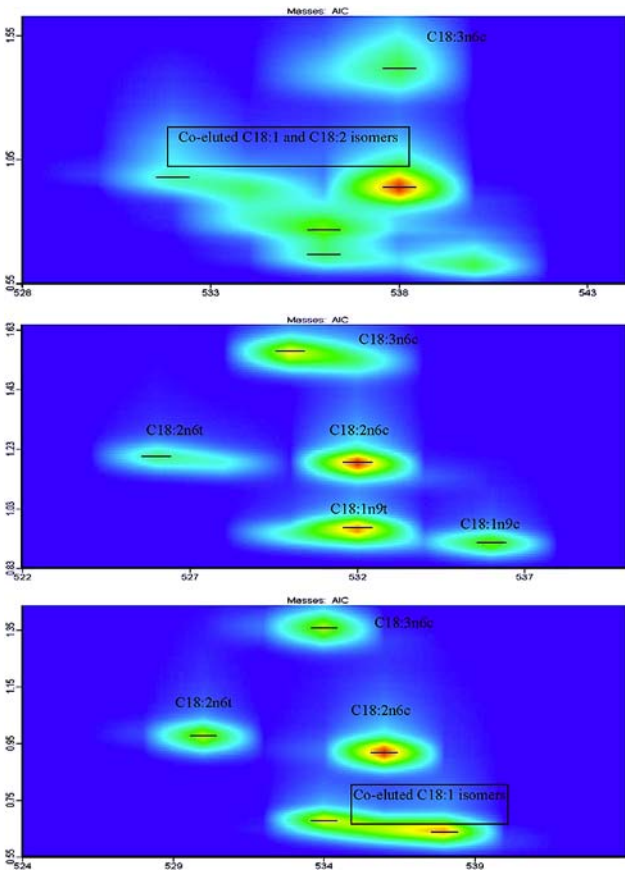

Fig. 3 GC × GC contour plot of C18 cluster acquired with modulation offset temperature at 35 °C (*upper figure*), 40 °C (*middle figure*), and 45 °C (*lower figure*), respectively

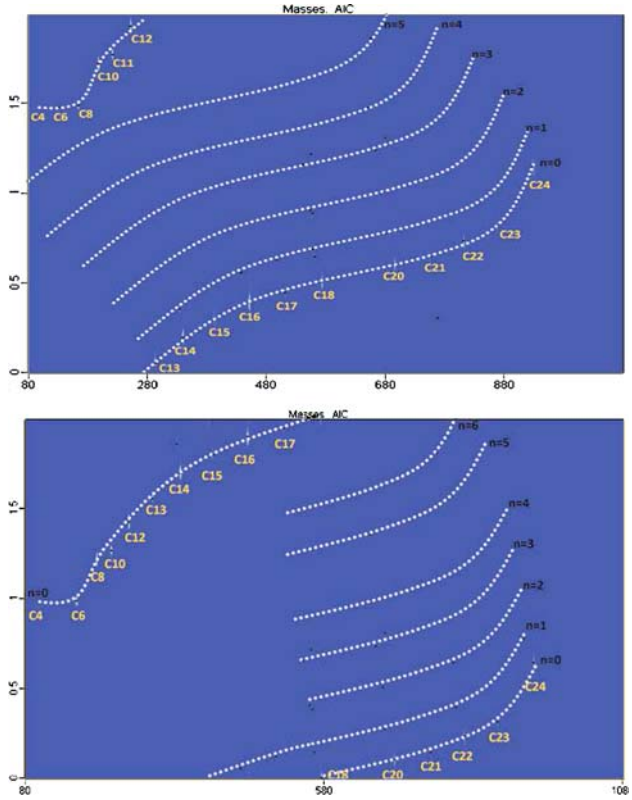

Fig. 4 Distribution of FAME compounds on GC × GC contour plot with modulation offsets shifted at 0 s (*upper figure*) and 0.5 s (*lower figure*), respectively

Error in peak identification was noticed at a value of 1.58 M_R when a longer P_M of 3 s was used, likely associated with the effect of phase shifting [13]. Further reduction of the modulation period, however, was undesirable since identification of FAME compounds corresponded to their distribution in the GC × GC contour plot became very difficult.

Furthermore, the modulation offset temperature was being maintained at 40 °C so that the release of the cold-trapped effluent could be achieved efficiently without degradation. Figure 3 shows that the peak shape of the modulated C-18 cluster became unacceptable under the lower offset temperature of 35 °C, whereas resolution was reduced at the higher offset temperature of 45 °C. This was also encountered in previous work [26]. Resolution of the C18:1 isomers in the critical region along the three-dimensional surface plot was measured on four consecutive days according to the peak-to-valley concept, as $R_s = [-1/2 \ln (1 - V)/2]^{1/2}$ where V is the valley-to-peak ratio [27]. By allocating the valley point between both isomers, resolution values acquired using fast GC × GC analysis ranged from 1.62 to 2.65, indicating sufficient separation for regioisomers in the profiling of FAME compounds.

Table 2 FAME profiles derived from different species using fast GC × GC–TOFMS

FAME compound	Empiric formula	Normalized percentage				
		Lard	Chicken fat	Beef tallow	Mutton tallow	Cod liver oil
Methyl hexanoate	C6:0	nd	nd	trace	nd	nd
Methyl octanoate	C8:0	0.02 ± 0.01[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.03 ± 0.02[a]	0.87 ± 0.28[b]
Methyl decenoate	C10:1	nd	nd	nd	trace	nd
Methyl decanoate	C10:0	0.14 ± 0.01[a]	0.03 ± 0.02[a]	0.10 ± 0.04[a]	0.28 ± 0.12[b]	0.48 ± 0.13[c]
Methyl undecanoate	C11:0	trace	nd	nd	trace	trace
Methyl dodecanoate	C12:0	0.13 ± 0.05[a]	0.66 ± 0.66[a]	0.98 ± 1.04[a]	0.27 ± 0.18[a]	0.09 ± 0.07[a]
Methyl 10-methyldodecanoate	C13:0 ai	nd	nd	nd	trace	nd
Methyl tridecanoate	C13:0	nd	trace	trace	0.06 ± 0.06	nd
Methyl 12-methyltridecanoate	C14:0 ai	nd	nd	0.11 ± 0.04[b]	0.18 ± 0.06[b]	0.02 ± 0.03[a]
Methyl 11-tetradecenoate	C14:1	trace	0.14 ± 0.06[a]	1.71 ± 1.34[b]	0.04 ± 0.03[a]	0.08 ± 0.02[a]
Methyl tetradecanoate	C14:0	1.61 ± 0.35[a]	1.31 ± 0.17[a]	8.40 ± 5.10[b]	4.58 ± 0.26[ab]	6.55 ± 0.97[ab]
Methyl 4,8,12-trimethyltridecanoate	4,8,12 m-C15:0	nd	nd	nd	0.18 ± 0.17[a]	0.21 ± 0.02[a]
Methyl 13-methyltetradecanoate	c15:0 i	nd	nd	0.24 ± 0.20[a]	0.53 ± 0.08[b]	0.24 ± 0.04[a]
Methyl 12-methyltetradecanoate	c15:0 ai	nd	nd	0.39 ± 0.13[a]	0.66 ± 0.22[a]	0.03 ± 0.03[b]
Methyl pentadecenoate	c15:1	nd	nd	trace	trace	nd
Methyl pentadecanoate	C15:0	0.07 ± 0.02[a]	0.10 ± 0.05[a]	0.67 ± 0.23[bc]	1.17 ± 0.20[c]	0.39 ± 0.10[ab]
Methyl 14-methylpentadecanoate	C16:0 i	nd	nd	0.26 ± 0.09[a]	0.36 ± 0.07[a]	0.08 ± 0.02[b]
Methyl 7,10-hexadecadienoate	C16:2n-6	nd	0.06 ± 0.07[a]	nd	nd	0.01 ± 0.02[a]
Methyl 7,10,13-hexadecatrienoate	C16:3n-3	nd	nd	nd	nd	0.08 ± 0.04
Methyl trans-7-hexadecenoate	C16:1n-9t	1.52 ± 0.24[a]	4.81 ± 1.11[b]	3.87 ± 1.97[ab]	1.06 ± 0.65[a]	8.53 ± 0.34[c]
Methyl 9,12-hexadecadienoate	C16:2n-4	nd	nd	nd	nd	0.30 ± 0.27
Methyl cis-7-hexadecenoate	C16:1n-9c	nd	nd	0.21 ± 0.27[a]	0.58 ± 0.48[a]	0.21 ± 0.02[a]
Methyl hexadecanoate	C16:0	22.59 ± 3.48[a]	25.62 ± 8.96[a]	28.48 ± 10.30[a]	14.55 ± 4.42[a]	18.45 ± 2.03[a]
Methyl 2-methylhexadecanoate	2 m-C16:0	nd	nd	nd	0.03 ± 0.02	nd
Methyl cyclopentaneundecanoate	Cyclopenta-C11:0	nd	nd	nd	0.05 ± 0.03	nd
Methyl 7-methylhexadece-6-noate	7 m-16:1	nd	nd	nd	nd	0.44 ± 0.10
Methyl 2,6,10,14-tetramethylpentadecanoate	2,6,10,14 m-C15:0	nd	nd	nd	0.08 ± 0.05[a]	0.09 ± 0.02[a]
Methyl 15-methylhexadecanoate	C17:0 i	nd	nd	0.37 ± 0.17[a]	0.68 ± 0.10[b]	0.15 ± 0.03[a]
Methyl 14-methylhexadecanoate	C17:0ai	0.01 ± 0.01[a]	trace	0.71 ± 0.29[b]	1.01 ± 0.13[b]	0.08 ± 0.01[a]
Methyl heptadecenoate	C17:1	0.22 ± 0.06[ab]	0.03 ± 0.02[a]	0.65 ± 0.34[b]	0.32 ± 0.28[ab]	0.26 ± 0.07[ab]
Methyl heptadecanoate	C17:0	0.47 ± 0.09[a]	0.12 ± 0.01[a]	1.27 ± 0.52[b]	2.15 ± 0.09[c]	0.14 ± 0.05[a]
Methyl 16-methylheptadecanoate	C18:0 i	nd	nd	0.14 ± 0.08[b]	0.16 ± 0.03[b]	0.04 ± 0.02[a]
Methyl 3,7,11,15-tetramethylhexadecanoate	3,7,11,15 m-C16:0	nd	nd	0.03 ± 0.05[a]	0.16 ± 0.03[b]	0.17 ± 0.05[b]

Table 2 continued

FAME compound	Empiric formula	Normalized percentage				
		Lard	Chicken fat	Beef tallow	Mutton tallow	Cod liver oil
Methyl cis-9,12,15-octadecatrienoate	C18:3n-3c	nd	0.03 ± 0.01	nd	nd	trace
Methyl 6,9,12,15-octadecatetraenoate	C18:4n-3	nd	nd	nd	nd	1.91 ± 0.41
Methyl trans-9,12-octadecadienoate	C18:2n-6t	19.18 ± 2.41^{a}	20.59 ± 9.38^{a}	0.88 ± 0.53^{b}	1.09 ± 0.08^{b}	2.71 ± 1.99^{b}
Methyl 6,9,12-octadecatrienoate	C18:3n-6c	1.23 ± 0.68^{ab}	1.16 ± 0.64^{ab}	0.32 ± 0.55^{a}	2.26 ± 1.06^{b}	0.78 ± 0.42^{ab}
Methyl trans-9-octadecenoate	C18:1n-9t	35.42 ± 2.41^{a}	37.29 ± 4.12^{a}	25.52 ± 4.94^{b}	32.63 ± 5.01^{ab}	23.19 ± 0.06^{b}
Methyl cis-9-Octadecenoate	C18:1n-9c	0.02 ± 0.02^{a}	trace	1.82 ± 2.50^{a}	9.95 ± 4.46^{b}0.22 ± 0.02 ^{a}	
Methyl cis-9,12-octadecenoate	C18:2n-6c	nd	nd	trace	1.01 ± 0.29^{b}	0.09 ± 0.04^{a}
Methyl 11-octadecenoate	C18:1n-7	nd	nd	trace	trace	nd
Methyl octadecanoate	C18:0	15.31 ± 5.59^{bc}	7.60 ± 1.28^{ab}	21.47 ± 6.56^{c}	20.20 ± 6.31^{c}	2.31 ± 0.06^{a}
Methyl 7,8-octadecadienoate	C18:2n-10	nd	nd	0.46 ± 0.32	trace	nd
Methyl nonadecatrienoate	C19:3n-3	nd	nd	nd	0.80 ± 0.55	nd
Methyl nonadecatrienoate	C19:3n-6	nd	nd	nd	0.08 ± 0.03	nd
Methyl 17-methyloctadecanoate	C19:0i	nd	nd	nd	trace	nd
Methyl 16-methyloctadecanoate	C19:0ai	nd	nd	nd	trace	nd
Methyl 10-Nonadecenoate	C19:1	0.03 ± 0.03^{a}	nd	0.09 ± 0.08^{ab}	0.14 ± 0.06^{b}	trace
Methyl nonadecanoate	C19:0	0.02 ± 0.00^{a}	trace	0.08 ± 0.04^{a}	0.13 ± 0.12^{a}	nd
Methyl 5,8,11,14-eicosatetraenoate	C20:4n-6	0.15 ± 0.05^{ab}	0.07 ± 0.06^{a}	nd	nd	0.21 ± 0.08^{b}
Methyl 5,8,11,14-eicosapentaenoate	C20:5n-3	nd	nd	nd	nd	7.01 ± 0.94
Methyl 11,14,17-eicosatrienoate	C20:3n-3	0.05 ± 0.02^{a}	0.03 ± 0.02^{a}	nd	trace	trace
Methyl 8,11,14,17-eicosatetraenoate	C20:4n-3	nd	0.04 ± 0.03^{a}	nd	nd	0.40 ± 0.03^{b}
Methyl 11,14-eicosadienoate	C20:2n-6	0.62 ± 0.10^{a}	nd	nd	trace	0.13 ± 0.06^{b}
Methyl 11-eicosenoate	C20:1n-9	0.70 ± 0.21 ^{a}	0.21 ± 0.05 ^{a}	0.17 ± 0.17 ^{a}	0.14 ± 0.21 ^{a}	9.52 ± 2.00 ^{b}
Methyl 8,11,14-eicosatrienoate	C20:3n-6	0.07 ± 0.03^{a}	nd	nd	trace	0.03 ± 0.03^{a}
Methyl eicosanoate	C20:0	0.18 ± 0.05^{a}	0.05 ± 0.01^{a}	0.16 ± 0.13^{a}	0.51 ± 0.63^{a}	0.05 ± 0.02^{a}
Methyl 6,9,12,15,18-heneicosapentaenoate	C21:5n-3	nd	trace	nd	nd	0.08 ± 0.09
Methyl heneicosanoate	C21:0	trace	nd	nd	trace	nd
Methyl 4,7,10,13,16,19-docosahexaenoate	C22:6n-3	nd	trace	nd	trace	5.52 ± 2.49
Methyl 6,9,12,15-heneicosatetraenoate	C21:4n-6	0.05 ± 0.04	nd	nd	nd	nd
Methyl 7,10,13,16,19-docosapentaenoate	C22:5n-3	nd	nd	nd	nd	0.68 ± 0.06
Methyl cis-13,16-docosadienoate	C22:2n-6	trace	nd	nd	trace	nd
Methyl 13-docosenoate	C22:1n-9	trace	nd	trace	trace	6.87 ± 1.43
Methyl docosanoate	C22:0	trace	nd	trace	0.30 ± 0.50	trace

Table 2 continued

FAME compound	Empiric formula	Normalized percentage				
		Lard	Chicken fat	Beef tallow	Mutton tallow	Cod liver oil
Methyl tricosanoate	C23:0	trace	nd	nd	trace	trace
Methyl 15-tetracosenoate	C24:1	nd	nd	nd	nd	0.34 ± 0.15
Methyl tetracosanoate	C24:0	nd	nd	nd	0.02 ± 0.02	trace

nd not detected; *trace* average content below 0.005%

Mean values in the same row with different superscripts are significantly ($p < 0.05$) different

Profiling of FAME Derived from Various Animal Species

FAME components with different carbon-chain lengths and levels of unsaturated bonds were clustered orderly on the GC × GC contour plot by shifting the modulation offsets accordingly. Figure 4 illustrates the distribution of FAME compounds on the GC × GC contour plot with modulation offsets shifted at 0 and 0.5 s (n is the number of unsaturated bonds). This observation has greatly assisted in the recognition of the FAME compounds and eliminated the wrap-around effect. In general, the FAME fraction derived from the tested species was unraveled into a total of 68 compounds ranging from 6 to 24 carbon-chain lengths (Table 2). C16:0, C18:0 and C18:1n-9t compounds were found to be abundant in all the tested species, while levels of a few other components were noticeably high in specific species: C18:2n-6t in both lard and chicken fat; C14:0 in beef tallow; C18:1n-9c in mutton tallow; and C20:1n-9, C20:5n-3, C22:6n-3 and C22:1n-9 in cod liver oil.

The majority of individual FAME content was statistically varied ($p < 0.05$) among the tested species (Table 2). In a few previous studies, branch-chained fatty acids (BCFA) occurred as the characteristic FAs of various animals and bacteria [28, 29]. Our result also depicted that MU contained significantly higher amounts of C14:0ai, C15:0i, C15:0ai, C16:0i, C17:0i, C17:0ai and C18:0i. Levels of 4,8,12 m-C15:0, 2 m-C16:0, 2,6,10,14 m-C15:0.C18:1n-9c and C18:2n-3c components in MF were also markedly higher among the tested species ($p < 0.05$). Apart from the BCFAs of C14:0ai, C15:0ai, C16:0i, C17:0ai and C18:0i, high levels of C14:1, C14:0, C17:1 and C17:0 were observed in the BF.

trans-Positional unsaturated FAs were proposed previously as a characteristic of ruminants and animals with ruminant-like digestive systems [30]. In contradiction to this, CLO was characterized with significantly greater levels of several monounsaturated and long-chain polyunsaturated fatty acids (PUFA), including C16:1n-9t, C20:1n-9t, C20:5n-3, C22:1 and C22:6n-3. Besides containing lower level of BCFA and PUFA, both CF and LA also possessed distinctly greater amounts of C18:2n-9t, as compared to MF, BF and CLO samples. Nevertheless, the content of C16:1n-9t and C18:3n-3c in CF was significantly ($p < 0.05$) higher than LA, which consisted of elevated amounts of C20:2n-6 and C21:4n-6 compounds. The C21:4n-6 component was previously detected in the rat's liver [31] but has not been reported in lard fat to date.

The presence of some minor FAME components such as C18:1n-5, C16:1n-7 and C21:1n-7, which have been reported previously in LA [32] was not observed in this work. This was likely due to the small injection volume as well as the rapid derivatization reaction during sample

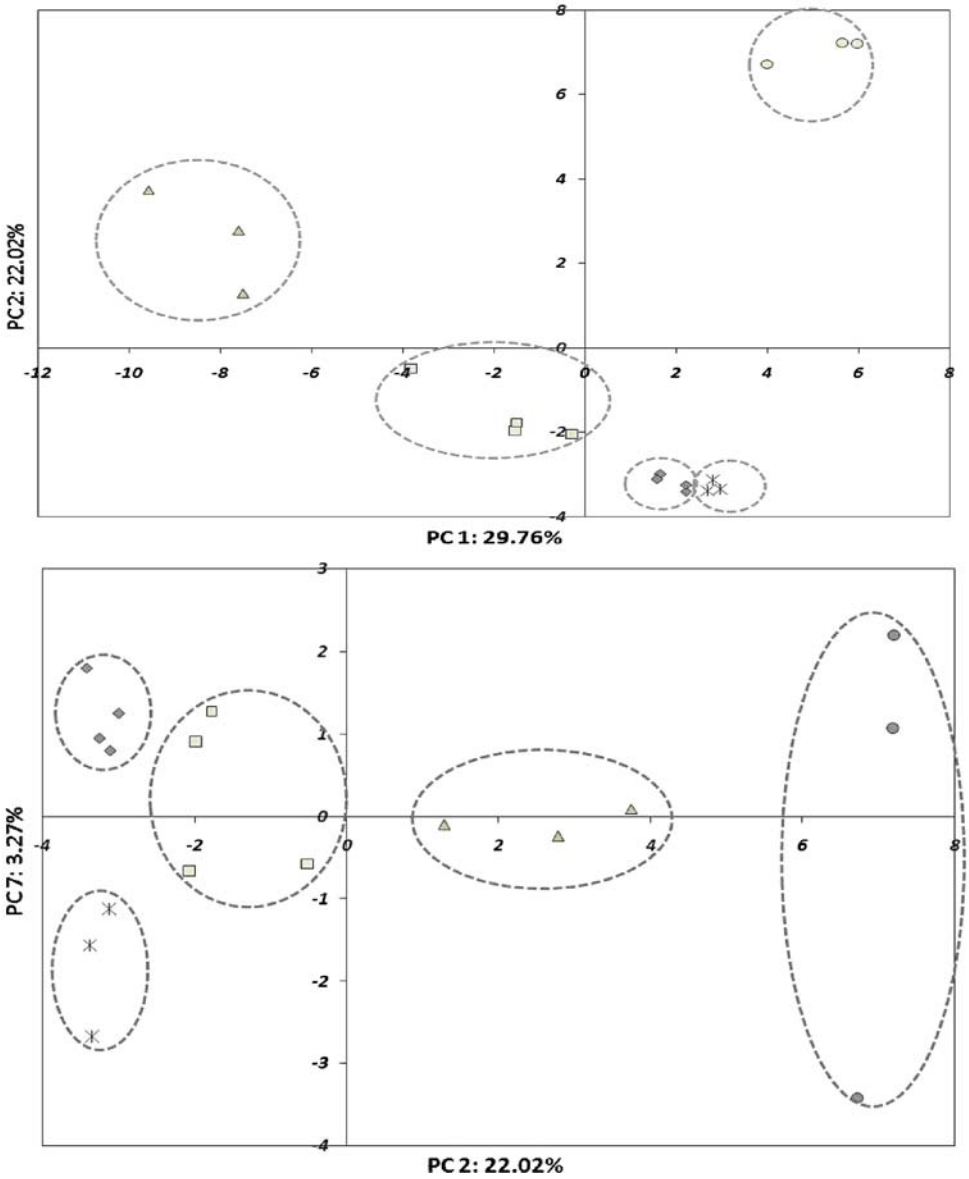

Fig. 5 PCA score plot projected by PC1 with PC2 (*upper figure*); and PC2 with PC7 (*lower figure*), respectively. *Filled diamonds* lard; *asterisks* chicken fat; *filled squares* beef tallow; *filled triangles* mutton tallow; *filled circles* cod liver oil

preparation. A saponification step could be utilized as an alternative for better recovery of unsaturated FAMEs as stated by Jua′reza [33]. Nevertheless, such derivatization methods have produced poorly reproducible results since the long and tedious preparation procedure has to be compromised. Different column combinations and a lower detection threshold could be utilized for complete profiling of FAME at trace levels, as demonstrated by *de* Koning et al. [34], who clearly distinguished the *cis*- and *trans*-regioisomers between C18:1n-9 and C18:1n-15 using a column combination of CP-WAX and VF-23 ms.

PCA Classification of FAME Species

Classification of the normalized data was evaluated using PCA. An accumulative eigenvalue of about 90% was obtained with seven principal components (PCs). The score plot projected by PC1 and PC2 explained 51% of the variance (Fig. 5), and this result revealed that MF and CLO groupings were well-separated along the PC1 axis while PC2 displaced the CLO and MF groupings along its positive side and CF and LA groupings along the negative side. However, it was not possible to distinguish LA from these groupings by projection of these PCs. Alternatively, the score plot projected by PC2 and PC7, with less variability of 25% (Fig. 5) illustrates a clear classification for each species, of which the LA and CF groupings were scattered at the positive and negative end of PC7, respectively.

As defined by the loading plot for the species classification (Fig. 6), C21:4n-6 and C20:2n-6 components corresponded to the scattered projection of the LA grouping from CF while C16:0, C18:2n-6t and C18:1n-9t were

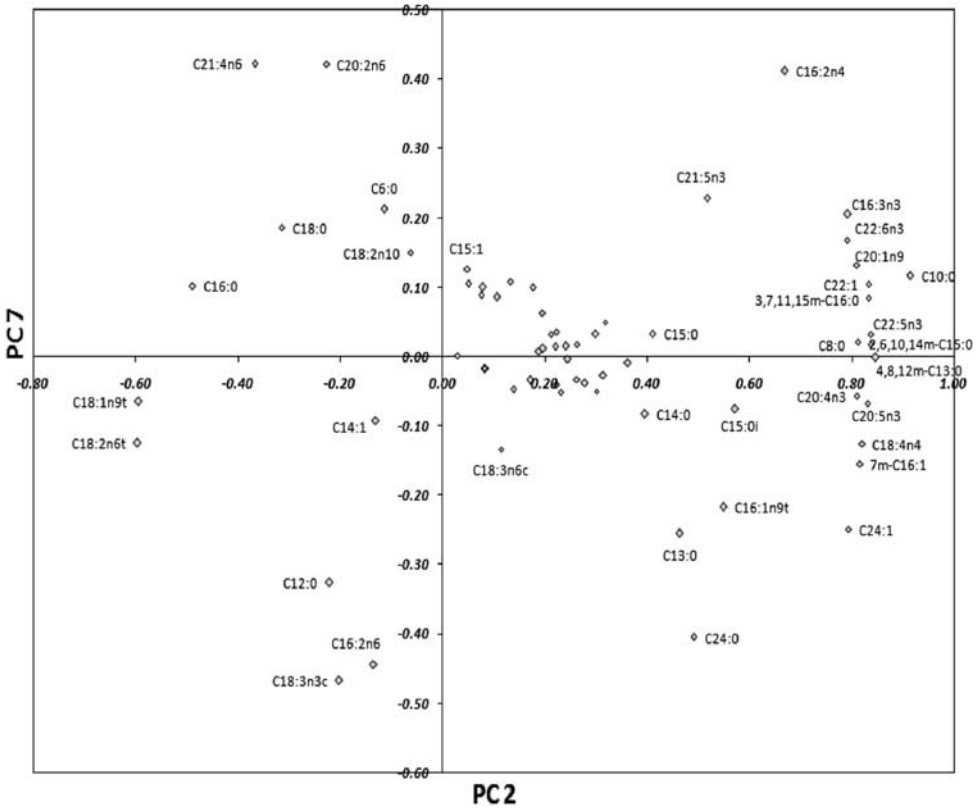

Fig. 6 PCA loading plot of PC2 versus PC7

attributed to the separation of LA from CLO, MF, and BF groupings. Despite the variation in FAME composition of the samples due to various factors including age, cuts and animal diets, the classification of different animal fats was performed using rapid analysis by the fast GC × GC approach with simple methylation and a high detection threshold. The detection of LA as an adulterant has gained considerable importance in many parts of the world. Previous techniques for LA detection were predominately based on the determination of saturated FA, specifically C16:0 at the sn-2 position of the triacylglycerols [22]. In this study, additional variables that involved C21:4n-3, C20:2n-6, C18:2n-6t and C18:1n-9t were used to distinguish LA from other fat samples. The use of C16:0 alone will not achieve the same result.

Conclusion

Although information is widely available on the FAME composition of different lipids, data comparison is difficult to perform, mainly due to the use of varied experimental designs and analytical methods. Rapid analysis of FAME using a fast GC × GC approach involving the coupling of two microbore GC columns was demonstrated in this study. The desired resolution was obtained by manipulating the modulation program under orthogonal column configurations. In addition, classification of various animal fats derived from five different animal species was successfully achieved using this approach. The fast GC × GC approach is definitely advantageous for the comprehensive determination of animal fat composition with exceptional speed and precision. Further studies are needed for the characterization of regioisomers and enantiomers of acylglycerol molecules from various sources, using the fast GC × GC approach to facilitate food authenticity studies. A discriminant study of LA adulteration in other edible oils utilizing the preceding unsaturated FAs should be investigated and validated further.

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