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ARTICLE

Determination of the Authenticity of Dairy Products on the Basis of Fatty Acids and Triacylglycerols Content using GC Analysis

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Abstract

Milk fat is an important food component, and plays a significant role in the economics, functional nutrition, and chemical properties of dairy products. Dairy products also contain nutritional resources and essential fatty acids (FAs). Because of the increasing demand for dairy products, milk fat is a common target in economic fraud. Specifically, milk fat is often replaced with cheaper or readily available vegetable oils or animal fats. In this study, a method for the discrimination of milk fat was developed, using FAs profiles, and triacylglycerols (TGs) profiles. A total of 11 samples were evaluated: four milk fats (MK), four vegetable oils (VG), two pork lards (PL), and one beef tallow (BT). Gas chromathgraphy analysis were performed, to monitor the FAs content and TGs composition in MK, VG, PL, and BT. The result showed that qualitative determination of the MK of samples adulterated with different vegetable oils and animal fats was possible by a visual comparision of FAs, using C14:0, C16:0, C18:1n9c, C18:0, and C18:2n6c, and of TGs, using C36, C38, C40, C50, C52, and C54 profiles. Overall, the objective of this study was to evaluate the potential of the use of FAs and TGs in the detection of adulterated milk fat, and accordingly characterize the samples by the adulterant oil source, and level of adulteration. Also, based on this preliminary investigation, the usefulness of this approach could be tested for other oils in the future.

Key words: dairy products, fatty acids, cheese, triacylglycerols, adulteration

Introduction

Milk fat, which is composed of 97 to 98% triacylglycerols (TGs), is a source of energy and nutrients and an important ingredient that provides desirable textural and flavor characteristics (Jensen, 2002). The fatty acids (FAs) composition of milk fat is typically 70% saturated fatty acids, 25% monounsaturated fatty acids, and 5% poly-unsaturated fatty acids (Grummer, 1991). Milk fat exists as fat globules in its natural state and is composed of a TGs core surrounded by a complex protein membrane, namely the milk fat globule membrane. Milk fat is characterized by diverse FAs and TGs compositions, which induces a wide melting transition. Milk products are widely consumed by humans, particularly during childhood, owing to their great nutritional relevance (Fox and McSweeney, 1998; Goudjil *et al*., 2003). Milk, cheese, butter, and other dairy products are consumed worldwide and have great commercial importance within the food industry. The composition of milk influences the characteristic flavor and sensory properties of cheese (Haza *et al*., 1999). Adulterated milk used in the manufacturing of cheese will result in an inferior final product than that expected by the consumer. The increased price and fluctuation in vegetable oils or animal fats make it a profitable practice for cheese or butter manufacturers to fraudulently adulterate expensive milk to reduce production costs and increase profit margins (Maudet and Taberlet, 2001). For legal reasons and consumer protection and confidence, cheese and butter should be authentic and correctly labeled (Hurley *et al*., 2004). Therefore, authentication issues exist in all areas of the food industry, and many different analytical approaches have been developed towards the detection of adulteration (Amigo *et al*., 1992; Dennis, 1998; Hurley *et al*., 2004, Levieux and Venien, 1994; Woolfe and Primrose, 2004). Globally, food safety and quality are considered significant issues in the food industry and are directly related to health and social improvement. Consumers increasingly search for trusted trademarks of food

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products and expect the producer and seller to serve high-quality products (Regattieri *et al*., 2007). Butter adulteration does not pose a threat to public health, but it is a severe breach of consumer trust. This can cause significant economic damage, thus prompting legislators to protect the joint interests of consumers and the industry. For example, margarine includes refined and preternaturally saturated vegetable oils, but butter is a natural food product containing partially saturated animal fat, and thus margarine is much cheaper than butter (De La Fuente and Juarez, 2005). Therefore, adulteration of butter with margarine is widespread in the food industry. The addition of vegetable oils or animal fats to milk and dairy products is an old but illegal practice in most countries (Gutierrez *et al*., 2009; Molkentin, 2007). Moreover, it has become increasingly common and sophisticated. Therefore, there is a need for sensitive methods that can detect milk adulteration. Generally, milk and its adulteration with non-milk fats are investigated using gas chromatography (GC) (Gutierrez *et al*., 2009). Fontecha *et al*. (2000) reported that the determination of TGs content by GC is effective for assessing the origin of milk to detect foreign fats. It is also used as an official method to evaluate the purity of cow milk fat in European Communities (Goudjil *et al*., 2003). Analytical techniques that have been used for food authentication include GC, chromatography combined with mass spectroscopy, isotopic ratio mass spectroscopy, nuclear magnetic resonance, and thermal analysis. TGs and FAs have been applied for the analysis of milk fat content, discrimination of animal fat, and determination of free FAs. Related studies have been developed for the discrimination of milk fat and foreign fats in milk fat (Fontecha *et al*., 2006; Gutierrez *et al*., 2009) and many methods can be used to detect FAs and TGs in milk fat.

This study aim to determine the FAs and TGs content in milk fat, vegetable oil, and animal oil samples by GC. The analytical method proposed here may also prove useful for the separation and analysis of FAs and TGs from other foreign fats including vegetable and animal fat.

Materials and Methods

Samples

Eleven varieties of fat were analyzed in this study. Samples were purchased in the market from a company (Table 1). Butter, cheese, soybean oil, corn oil, pork lard, and beef tallow were purchased from a local market as well as from a manufacturer and stored at 4°C.

Chemicals

Certified reference materials, FAs, and TGs in a frozen diet, were purchased from the National Institute for Standard and Technology (NIST; USA) and used for method validation. The FAs standards included Butyric acid (C4: 0), Caproic acid (C6:0), Caprylic acid (C8:0), Capric acid (C10:0), Undecanoic acid (C11:0), Lauric acid (C12:0), Tridecanoic acid (C13:0), Myristic acid (C14:0), Tetradecenoic acid (C14:0), Pentadecanoic acid (C15:0), Pentadecenoic acid (15:1), Palmitic acid (C16:0), Hexadecenoic acid (16:1), Margaric acid (C17: 0), Margaroleic acid (C17:1), Stearic acid (C18:0), Octadecenoic acid (C18:1), Octadecadienoic acid (C18:2), Linolenic acid (C18:3), Arachidic acid (C20:0), Eicosenoic acid (C20:1), Eicosadienoic acid (C20:2), Eicosatrienoic acid (C20:3), Arachidonic acid (C20:4), Eicosapentaenoic acid (C20:5), Heneicosanoic acid (C21:0), Behenic acid (C22:0), Docosanoic acid (C22:1), Docosadienoic acid (C22:2), Docosatrienoic acid (C22:3), Docosatetraenoic acid (C22:4), Docosapentaenoic acid (C22:5), Docosahexaenoic acid (C22:6), Tricosanoic acid (C23:0), Lignoceric acid (C24:0), and Nervonic acid (C24:1). TGs standards included: C24, C26, C28, C30, C32, C34, C36, C38, C40, C42, C44, C46, C48, C50, C52, and C54. Also, sodium hydroxide (NaOH), sodium chloride (NaCl) which were used in the

Table 1. Composition of milk, vegetable, pork lard, and beef tallow samples

Part	Sample name	Ingredients
Milk fat (MK)	Butter (Maeil Dairies Co., Ltd.)	Milk fat 100%
	Processing butter (Seoul Dairy Corporative)	Milk fat 99.61%, Elusion, etc.
	Cheese (Maeil Dairies Co., Ltd.)	Raw milk 98.63%, salt, control of acidity etc.
	Processing cheese (Seoul Dairy Corporative)	Natural cheese 80, etc.
Vegetable oil (VG)	Soybean oil (CJ CheilJedang Corporation)	Soybean 100%
	Soybean oil (Sajo Haepyo Co., Ltd.)	Soybean 100%
	Corn oil (CJ CheilJedang Corporation)	Corn germ 100%
	Corn oil (Sajo Haepyo Co., Ltd)	Corn germ 100%
Pork lard (PL)	Pork lard (Samyang Co., Ltd)	Lard 100%
	Pork lard (Pro Co., Ltd)	Lard 100%
Beef tallow (BT)	Beef tallow (Ottogi Co,, Ltd.)	Tallow 100%

testing, were purchased from Junsei Chemical (Tokyo, Japan), and boron trifluoride (BF_3) were purchased from Sigma (Sigma, St Louis, USA). Hexane, methanol and chloroform were purchased from Burdick and Jackson (Muskegon, MI, USA).

FAs and TGs extraction by saponification

For lipid extraction, 2.5 g of each sample was used as modified version of Folch's method (Christie, 1989) then, added to a 25 mL chloroform-methanol mixture (2:1, v/v). The mixture was homogenaised (2,500 rpm, 30 min), ultrasonicated for 20 min and 10 mL of saturated NaCl solution were added. The suspension was then centrifuged for 20 min at -4ºC (4,000 rpm). The chloroform phase was recovered and transferred into a round 25 mL flask, and each fat extract was dried via rotaty evaporator at 45ºC under vaccum (Kim *et al*., 2013). After evaporation, approximately 0.5 g of extracted oil from each sample was treated with 8 mL of methanolic solution of sodium hydroxide (NaOH, 0.5 N) at 85°C for 10 min. Then, 9 mL of BF_3 (14%) was added over 2 min in a cooled flask. Hexane (4 mL) was added to the cooled flask and agitated. Then, added mass up saponification flask using saturated solution of NaCl and stay for 3 min. FAs were extracted with hexane after adding 1 g of anhydrous sodium sulfate (MFDS, 2013). For TGs, 5 mL of hexane was added to 0.1 g of the extracted fat and the samples were subjected to GC analysis (Park *et al.,* 2013).

Instruments and experimental conditions

FAs

An Agilent model 7890 GC instrument equipped with a 100 m × 0.25 mm i.d. (df=0.2 μm) SP-2560 capillary GC column (Supelco 24056, USA) and an flame ionization detector (FID) was used to separate and detect FAs (park *et al*., 2013). A running time of 67 min was used for each sample solution. The following instrumental conditions were employed for GC-FID analysis: injection volume of 1 μL and nitrogen carrier gas flow rate of 1.0 mL/min at a split ratio of 50:1 with constant flow control. The injector and detector temperatures used were 250°C. The initial oven temperature was maintained at 180°C for 40 min and then gradually increased to 230°C at a rate of 3°C/min, after which it was maintained at 230°C for 10 min (Table 2).

TGs

An Agilent model 7890 GC instrument equipped with a 30 m × 0.32 mm i.d. (df=0.25 μm) HP-5 5% phenyl methyl siloxane (Agilent 19091J-413, USA) and an FID was used to separate and detect TGs. A running time of 140 min was used for each sample solution. The following instrumental conditions were employed for GC-FID analysis: injection volume of 1 μL and nitrogen carrier gas flow rate of 2.0 mL/min at a split ratio of 30:1 with constant flow control. The injector and detector temperatures used were 340 and 200°C, respectively. The initial oven temperature was maintained at 340°C for 140 min. An aliquot of the supernatant was transferred into an autosampler vial for GC-FID analysis (Table 2).

Statistical analysis

The statistical analysis was carried out using an SPSS 10.0 (SPSS Inc., USA). The T-test was used at $p<0.05$ to determine the level of significance of the differences between sample means.

Table 2. Operating condition for GC analysis on fatty acids content and triacylglycerols

Parameter	Condition	
	Fatty acids	Triacylglycerols
Instrument	Agilent 7890A GC	Agilent 7890A GC
Detector	FID (Flame ionization detector)	Flame ionization detector (FID)
Column	SP™-2560 Capillary GC column (Supelco 24056) (100 m × 0.25 mm × 0.2 μm)	HP-5 5% Phenyl methyl siloxane (Agilent 19091J-413) (30 m × 0.32 mm × 0.25 μm)
Inlet temperature	250ºC	340ºC
Injection Volume	1 μL	1 μL
Inlet split ratio	50 : 1	30:1
Carrier gas	N_2 (Nitrogen)	N_2 (Nitrogen)
Oven condition	180°C (40 min hold) → 3°C/min → 230°C (10 min hold) Oven condition	340°C (140 min hold)
Detector Temp.	250°C	200°C
Flow rate	1.0 mL/min.	2.0 mL/min
Run time	67 min	140 min

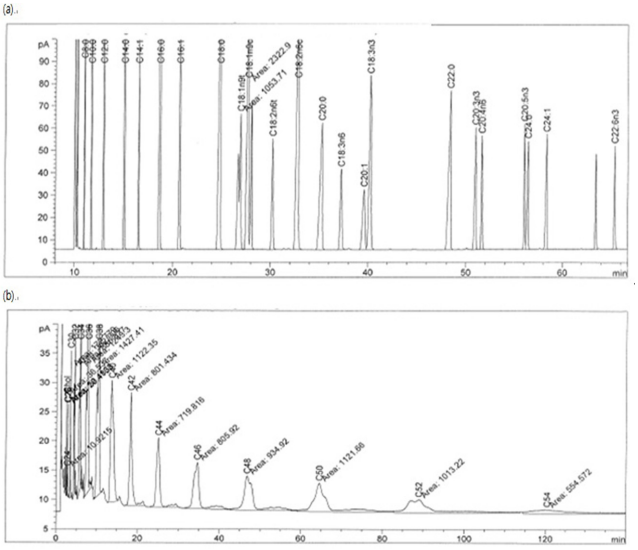

Fig. 1. GC-FID by gas chromatography with flame ionization detector. (a) Fatty acids on standard oil of CRM 2377, (b) Triacylglycerols on standard oil of CRM 632b.

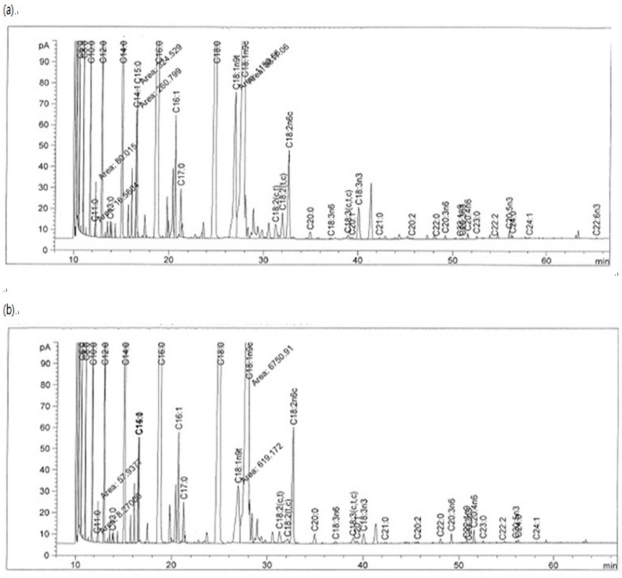

Fig. 2. Fatty acids on samples by gas chromatography with flame ionization detector. (a) butter, (b) cheese.

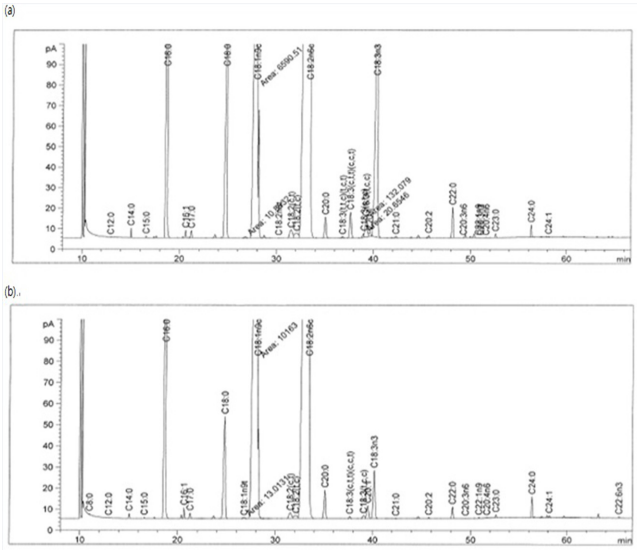

Fig. 3. Fatty acids on samples by gas chromatography with flame ionization detector. (a) soybean oil, (b) corn oil.

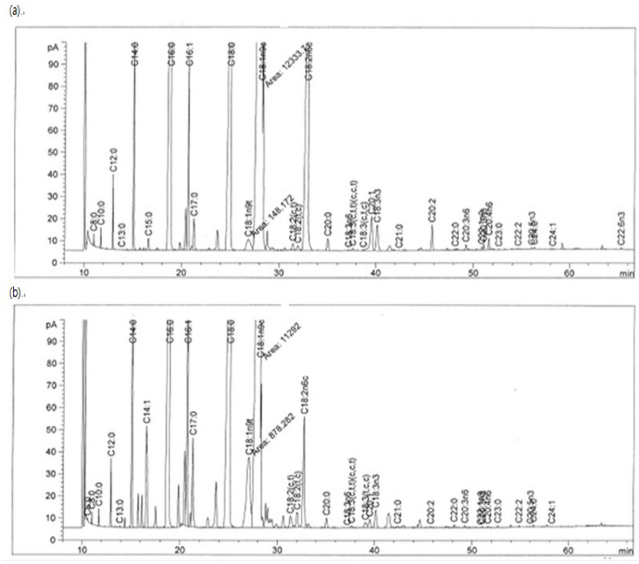

Fig. 4. Fatty acids on samples by gas chromatography with flame ionization detector. (a) pork lard, (b) beef tallow.

Results and Discussion

FAs profiles by Fatty Acids Methyl Esters (FAMEs)

Milk fat, vegetable oils, pork lard, and beef tallow (denoted as MK, VG, PL, and BT, respectively) were analyzed, in order to show the difference between MK and other common animal fats and VG.

GC chromatograms for FAs are shown in Fig. 1(a). using a Certified Reference Materials (CRM) qualitative standard as an internal standard. Each FAs in the test solution was successfully separated over a retention time (RT) and that from samples was separated with RT (Fig. 2-4). As based on Fig. 2-4, Table 3 shows the FAs composition (g/100 g) of the fat extracts from 11 commercial samples. According to result, biomarker to distinguish from MK, VG, PL and BT was followed: myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1n9c), stearic acid (C18:0), linoleic acid (C18:2n6c). Prandini *et al*. (2011) reported the oleic acid, vaccenic acid, and linoleic acid profiles for each cheese class were found to be consistent with those from other studies (Zhang *et al*.,

Table 3. Chemical characterization in fatty acids content using GC analysis

	Type[1)]	RT[2)]	MK1[3)]	MK2	MK3	MK4	VG1[4)]	VG2	VG3	VG4	PL1[5)]	PL2	BT1[6)]
C4:0	Saturated	10.290	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C6:0	Saturated	10.561	1.57±0.00	1.851±0.01	1.62±0.00	1.80±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00
C8:0	Saturated	10.996	0.93±0.00	1.24±0.00	0.98±0.00	1.18±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.00	0.05±0.00	0.01±0.06	0.05±0.00
C10:0	Saturated	11.728	2.05±0.00	3.02±0.00	2.18±0.00	2.74±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.09±0.00	0.08±0.00	0.08±0.00
C11:0	Saturated	12.266	0.03±0.00	0.06±0.00	0.03±0.00	0.05±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C12:0	Saturated	12.965	3.55±0.00	4.27±0.03	3.72±0.00	3.45±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.40±0.00	0.19±0.00	0.36±0.00
C13:0	Saturated	13.874	0.06±0.00	0.10±0.00	0.06±0.00	0.09±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00
C14:0	Saturated	15.060	10.0±0.01	11.59±0.01	10.50±0.01	10.53±0.01	0.07±0.00	0.00±0.00	0.04±0.00	0.04±0.00	1.70±0.00	1.76±0.00	2.88±0.00
C14:1	Unsaturated	16.543	0.78±0.02	0.92±0.02	0.80±0.01	0.81±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.17±0.00	1.23±0.00
C15:0	Saturated	16.598	0.73±0.02	1.12±0.02	0.75±0.03	1.07±0.02	0.02±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.17±0.00	0.00±0.00	0.00±0.00
C15:1	Unsaturated	18.501	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C16:0	Saturated	18.621	29.2±0.02	28.39±0.03	29.41±0.01	28.54±0.02	10.69±0.00	10.70±0.00	11.95±0.00	11.98±0.00	26.59±0.01	23.76±0.01	25.66±0.00
C16:1	Unsaturated	20.654	1.11±0.01	1.25±0.00	1.14±0.00	1.50±0.01	0.09±0.00	0.09±0.00	0.12±0.00	0.11±0.00	1.97±0.00	2.35±0.00	2.87±0.01
C17:0	Saturated	21.235	0.43±0.00	0.53±0.00	0.45±0.00	0.63±0.00	0.10±0.00	0.09±0.00	0.08±0.00	0.07±0.00	0.39±0.00	0.41±0.00	1.10±0.00
C17:1	Unsaturated	23.823	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C18:0	Saturated	24.668	14.2±0.01	11.09±0.01	14.29±0.00	11.48±0.01	4.27±0.00	4.12±0.00	2.10±0.00	2.02±0.00	10.93±0.00	11.24±0.00	16.45±0.00
C18:1n9t	Unsaturated	26.617	2.54±0.02	4.05±0.04	2.21±0.01	3.38±0.01	0.02±0.00	0.04±0.00	0.04±0.00	0.03±0.00	0.50±0.01	0.50±0.01	2.94±0.02
C18:1n9c	Unsaturated	27.538	24.8±0.00	20.33±0.02	24.13±0.05	23.38±0.01	21.15±0.01	24.77±0.01	33.06±0.01	30.92±0.01	41.65±0.03	42.82±0.00	37.99±0.02
C18:2n6t	Unsaturated	30.191	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C18:2(c,t)	Unsaturated	31.389	0.29±0.01	0.34±0.00	0.28±0.00	0.30±0.00	0.28±0.00	0.30±0.00	0.19±0.00	0.18±0.00	0.14±0.00	0.11±0.00	0.26±0.00
C18:2(t,c)	Unsaturated	31.872	0.18±0.01	0.50±0.00	0.15±0.00	0.36±0.00	0.22±0.00	0.24±0.00	0.14±0.00	0.13±0.00	0.12±0.00	0.08±0.00	0.27±0.00
C18:2n6c	Unsaturated	32.09	2.22±0.00	1.61±0.01	2.12±0.00	1.41±0.00	53.31±0.01	51.22±0.01	49.43±0.02	51.75±0.01	11.05±0.00	11.75±0.00	1.91±0.00
C20:0	Saturated	35.004	0.19±0.00	0.13±0.00	0.19±0.00	0.14±0.00	0.38±0.00	0.38±0.00	0.50±0.00	0.46±0.00	0.22±0.00	0.18±0.00	0.18±0.00
C18:3(t,t,t)	Unsaturated	35.175	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C18:3 (t,t,c), (t,c,t)	Unsaturated	36.779	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.04±0.00	0.03±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C18:3n6	Unsaturated	37.207	0.05±0.00	0.02±0.00	0.05±0.00	0.03±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.02±0.00	0.00±0.00
C18:3 (c,t,t), (c,c,t)	Unsaturated	37.543	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.00	0.44±0.00	0.05±0.00	0.04±0.00	0.05±0.00	0.04±0.00	0.05±0.00
C18:3 (c,t,c)	Unsaturated	38.905	0.14±0.00	0.10±0.01	0.15±0.01	0.09±0.00	0.00±0.00	0.06±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.00±0.00
C18:3 (t,c,c)	Unsaturated	39.181	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.42±0.00	0.38±0.00	0.09±0.00	0.09±0.00	0.00±0.00	0.00±0.00	0.13±0.00
C20:1	Unsaturated	39.428	0.06±0.00	0.04±0.00	0.05±0.00	0.05±0.00	0.03±0.05	0.15±0.00	0.28±0.00	0.26±0.00	0.79±0.02	0.91±0.00	0.18±0.00
C18:3n3	Unsaturated	40.081	0.22±0.00	0.69±0.00	0.22±0.00	0.80±0.00	7.09±0.00	5.52±0.00	0.97±0.00	0.97±0.00	0.55±0.01	0.65±0.00	0.36±0.00
C21:0	Saturated	42.331	0.03±0.00	0.04±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.00±0.00	0.04±0.00
C20:2	unsaturatd-	45.741	0.03±0.00	0.02±0.01	0.03±0.00	0.02±0.00	0.04±0.00	0.04±0.00	0.02±0.00	0.02±0.00	0.37±0.00	0.45±0.00	0.02±0.00
C22:0	Saturatd	48.162	0.06±0.00	0.05±0.04	0.07±0.00	0.07±0.00	0.43±0.00	0.45±0.00	0.16±0.00	0.15±0.00	0.02±0.00	0.01±0.00	0.06±0.00

Table 3. Chemical characterization in fatty acids content using GC analysis (continued)

	Type[1)]	RT[2)]	MK1[3)]	MK2	MK3	MK4	VG1[4)]	VG2	VG3	VG4	PL1[5)]	PL2	BT1[6)]
C20:3n6	unsaturated	49.253	0.13±0.00	0.05±0.00	0.13±0.00	0.06±0.00	0.01±0.00	0.00±0.00	0.02±0.01	0.02±0.00	0.08±0.00	0.10±0.00	0.03±0.00
C22:1n9	Unsaturated	50.859	0.05±0.00	0.01±0.00	0.04±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.04±0.00	0.04±0.00	0.01±0.00
C20:3n3	Unsaturated	51.073	0.01±0.00	0.01±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.07±0.00	0.08±0.00	0.01±0.00
C20:4n6	Unsaturated	51.609	0.16±0.00	0.07±0.00	0.17±0.00	0.08±0.00	0.01±0.00	0.01±0.00	0.01±0.01	0.00±0.00	0.14±0.00	0.18±0.00	0.02±0.00
C23:0	Saturated	52.585	0.03±0.00	0.04±0.00	0.03±0.01	0.04±0.00	0.05±0.00	0.06±0.01	0.04±0.01	0.05±0.01	0.01±0.00	0.01±0.00	0.03±0.00
C22:2	Unsaturated	52.543	0.01±0.00	0.01±0.00	0.00±0.00	0.07±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.01	0.01±0.00	0.02±0.00
C24:0	saturated	56.321	0.03±0.00	0.03±0.00	0.04±0.00	0.06±0.00	0.13±0.00	0.16±0.00	0.22±0.00	0.20±0.00	0.02±0.00	0.01±0.00	0.03±0.00
C20:5n3	Unsaturated	56.022	0.03±0.00	0.08±0.00	0.03±0.00	0.09±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.03±0.00	0.01±0.00
C24:1	Unsaturated	58.219	0.01±0.00	0.02±0.01	0.01±0.00	0.02±0.00	0.01±0.00	0.02±0.00	0.03±0.00	0.04±0.00	0.01±0.00	0.01±0.00	0.01±0.00
C22:6n3	unsaturated	65.367	0.00±0.00	0.01±0.00	0.00±0.00	0.02±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.03±0.00	0.00±0.00
Etc.			3.91	6.29	3.95	5.62	0.54	0.57	0.38	0.40	1.79	1.97	4.75
Sum			100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1)]RT, retention time (min); [2)]MK, milk fat sample 1-4; [3)]VG, vegetable oil sample 1-4; [4)]PL, pork lard sample 1-2; [5)]BT, beef tallow sample 1.

Table 4. Chemical characterization in triacylglycerols content using GC analysis

	RT[1)]	MK1[2)]	MK2	MK3	MK4	VG1[3)]	VG2	VG3	VG4	PL1[4)]	PL2	BT1[5)]
Chol	2.481	0.38±0.01	0.42±0.00	0.44±0.00	0.43±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.00	0.11±0.00	0.11±0.00
C24	2.645	0.27±0.03	0.42±0.05	0.25±0.03	0.25±0.14	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C26	2.713	0.35±0.01	0.48±0.00	0.41±0.02	0.47±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C28	2.741	0.26±0.01	0.37±0.01	0.31±0.01	0.30±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C30	3.426	1.20±0.00	1.73±0.01	1.31±0.02	1.54±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C32	4.404	2.94±0.00	4.01±0.03	3.16±0.00	3.54±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.40±0.00	0.19±0.00	0.36±0.00
C34	5.804	7.07±0.01	8.90±0.01	7.49±0.01	8.20±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.67±0.03	0.47±0.04	0.89±0.02
C36	8.286	13.36±0.02	12.99±0.01	13.77±0.02	13.01±0.02	1.76±0.06	1.91±0.02	1.90±0.36	1.85±0.30	4.07±0.08	4.08±0.16	3.11±0.06
C38	11.359	16.24±0.02	15.56±0.01	16.27±0.02	16.61±0.02	9.08±0.28	9.16±0.04	8.33±0.40	8.00±0.30	3.44±0.06	3.85±0.12	3.81±0.06
C40	13.616	11.57±0.02	12.04±0.01	11.16±0.01	12.63±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.71±0.01	0.00±0.00	0.57±0.03
C42	18.295	6.51±0.01	8.08±0.01	6.61±0.01	7.67±0.11	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.35±0.01	0.00±0.00	0.34±0.06
C44	25.018	5.87±0.00	7.21±0.01	6.06±0.01	6.46±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.52±0.04	0.29±0.02	0.82±0.03
C46	34.435	6.62±0.01	7.11±0.02	6.74±0.00	6.40±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.03±0.00	0.83±0.00	2.91±0.10
C48	46.753	7.97±0.02	7.27±0.03	7.92±0.01	6.89±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	4.38±0.01	3.36±0.01	11.10±0.03
C50	64.446	9.22±0.02	6.89±0.00	8.85±0.03	7.69±0.05	4.16±0.01	4.14±0.00	4.85±0.04	4.78±0.01	19.99±0.03	16.77±0.04	24.70±0.05
C52	87.091	7.65±0.04	4.08±0.02	7.02±0.04	5.97±0.02	31.26±0.08	30.96±0.03	33.29±0.34	33.45±0.25	51.58±0.10	55.75±0.40	36.95±0.07
C54	119.737	2.50±0.01	1.73±0.03	2.22±0.00	1.94±0.03	53.74±0.30	53.83±0.05	51.64±0.38	51.91±0.37	13.17±0.08	14.49±0.44	14.69±0.09
Sum		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1)]RT, retention time (min); [2)]MK, milk fat sample 1-4; [3)]VG, vegetable oil sample 1-4; [4)]PL, pork lard sample 1-2; [5)]BT, beef tallow sample 1.

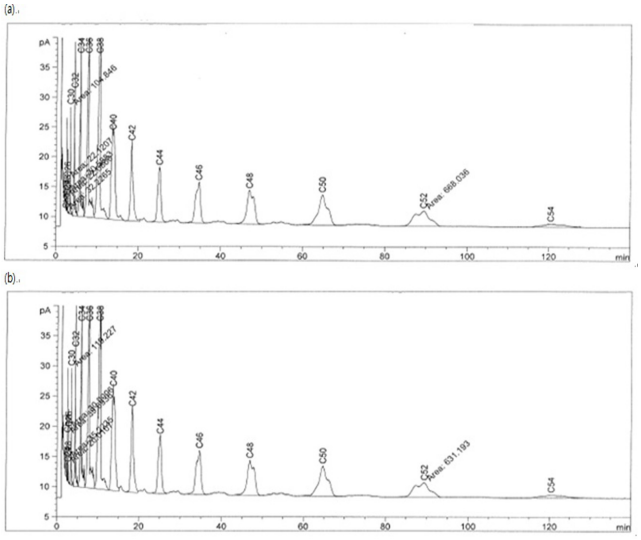

Fig. 5. Triacylglycerols on samples by gas chromatography with flame ionization detector. (a) butter, (b) cheese.

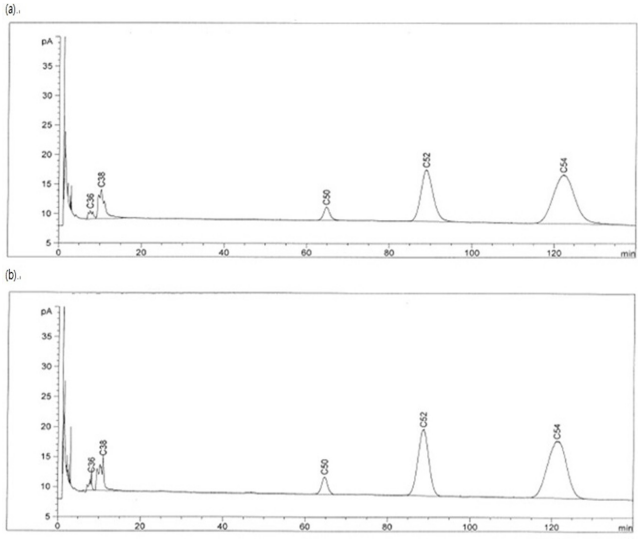

Fig. 6. Triacylglycerols on samples by gas chromatography with flame ionization detector. (a) soybean oil, (b) corn oil.

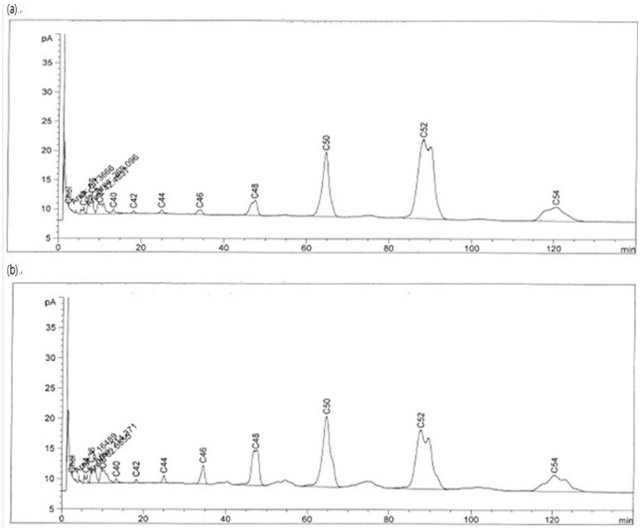

Fig. 7. Triacylglycerols on samples by gas chromatography with flame ionization detector. (a) pork lard, (b) beef tallow.

2006). Especially, Kim *et al.* (2013) reported that fatty acids composition of cheese was common in palmitic acid, oleic acid, stearic acid, which consistent with our result. Butyric acid (C4:0), pentadecenoic acid (15:1), margaroleic acid (C17:1), linolelaidic acid (C18:2n6t), and C18:3 (t,t,t) were not detected in MK, VG, PL, or BT. Therefore, FAs can be distinguished by palmitic acid (C 16:0), oleic acid (C18:1n9c), stearic acid (C18:0), myristic acid (C14:0), and linoleic acid (C18:2n6c).

The proportion of palmitic acid (C16:0) in MK was similar to that of PL and BT, and was significantly more common in MK, PL, and BT than in VG. The proportion of palmitic acid was the lowest in VG. The proportion of stearic acid (C18:0) in MK was similar to that of PL and BT and was lower in MK than in BT but higher than in VG and PL. From the FAs profile, it was clear that more stearic acid was present in MK, PL, and BT than in the VG.

The proportion of oleic acid (C18:1n9c) in MK was similar to that in VG, and was significantly higher portion content in MK, PL, VG, and BT. The proportion of oleic acid in MK was the lower than in the others. From the FAs profile, it was evident that more less oleic acid was present in MK and VG than in PL and BT.

In summary, the FAs profiles of the samples are shown in Table 3. The commercial milk fat was richer in short- to medium-chain saturated FAs than the samples adulterated with vegetable oil or animal fat. Adulteration of reduced fat and nonfat milks with vegetable oil decreased the short-chain FAs content, almost saturated FAs except arachidic acid, C20:0, while the opposite was true for the unsaturated FAs content. Moreover, Kennelly (1996) indicated that milk fat contains significantly higher concentrations of short- and medium chain FAs and relatively lower concentrations of unsaturated FAs, compared to other sources of vegetable and animal fat. We also propose that adulterated milk fat can be discriminated using the total concentration of saturated FAs and unsaturated FAs as biomarker in myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1n9c), stearic acid (C18:0), and linoleic acid (C18:2n6c).

TGs analysis by GC

GC chromatograms for TGs are shown in Fig. 1(b) using a Certified Reference Materials (CRM) qualitative standard as an internal standard. Each TGs in the test solution was successfully separated over a retention time (RT) and that from samples was separated with RT (Fig. 5-7). As based on Fig. 5-7, the RT of 16 TGs from the sample extracts and the internal standard, which was used for quantitative purposes. Table 4 showed that the TGs content ranged from 0.38-0.44 g/100 g in MK, 0.10-0.11 g/100 g in PL, 0.11 g/100 g in BT, but the cholesterol content could not be quantified in the VG samples. C36, C38, and C40 were common in MK and ranged from 11.16-16.61 and were followed by C50 at 6.89-9.22 g/100 g. C54 was the least common in VG, PL, and BT. In VG, the highest C54 content (51.91-53.83 g/100 g) was found and C52 content was lower than C54, but higher than the others in VG. The C24, C26, C28, C30, C34, C40, C42, C44, C46, and C48 content could not be detected in VG. In PL, C52 was the most common (51.58-55.75 g/100 g). There was less C50 than C52 but more than the others. The C24, C26, C28, and C30 content could not be detected in BT.

Therefore, as a result, we were able to distinguish TGs using C36, C38, C40, C50, C52, and C54. The overall proportions of C36 and C38 in MK were higher those of the others. From the TGs profile, it was clear that C36 and C38 were more abundant in MK than in VG, PL, and BT. In MK, C40 was present in the greatest amount, while VG could not be detected in C40. Therefore, if C40 is not detected in milk fat, the sample is likely adulterated with another fat or oil. The proportion of C50 in MK was lower than that in PL and BT, but higher than that in VG. BT had a high content level at 24.70 g/100 g. The proportion of C52 in MK was the lowest while PL had the highest content at 51.58-55.75 g/100 g. Moreover, the proportion of C54 in MK was lower than the others, while VG had the highest content at 51.64-53.74 g/100 g. According to these results, we suggest a method distinguishes MK and VG. Park and Lee (2003) reported that in the case of adulterationed with cheaper oil including soy bean oil, the detection of adulterated oil was investigated by using TGs profile with the C54/C50 ratio rate.

In summary, we should suggest biomarker myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1n9c), stearic acid (C18:0), and linoleic acid (C18:2n6c) in FAs and C36, C38, C40, C50, C52, and C54 in TGs according to content and relative ratio. This study is expected to provide a basic date for monitoring adulteration and material usage. Moreover, industries and certified analysis institutions can use this method to monitor samples simultaneously.

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