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ARTICLE

Detection for Non-Milk Fat in Dairy Product by Gas Chromatography

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Abstract

The aim of this study was to evaluate the potential use of fatty acids, triacylglycerols, and cholesterol in the detection of adulterated milk fat. The fatty acid, triacylglycerol, and cholesterol profiles of the mixtures of milk and non-milk fat (adulteration ratios of 10%, 30%, 50%, 70%, and 90%) were analyzed by gas chromatography. The results showed that concentrations of the fatty acids with oleic acid (C18:1n9c) and linoleic acid (C18:2n6c), triglycerides with C52 and C54, and cholesterol detected are proportional to the adulteration ratios remarkably. Oleic acid (C18:1n9c), linoleic acid (C18:2n6c), C52, and C54 were lower in pure milk fat than in adulterated mixtures. In contrast, pure milk has a higher cholesterol concentration than all adulterated mixtures (adulteration concentration in the range 10-90%). Thus, we suggest that oleic acid (C18:1n9c), linoleic acid (C18:2n6c), C52, C54, and cholesterol are suitable indicators and can be used as biomarkers to rapidly detect adulterated milk fat by gas chromatography. This study is expected to provide basic data for adulteration and material usage. Moreover, this new approach can detect the presence of foreign oils and fats in the milk fat of cheese and can find application in related studies.

Keywords: adulteration, milk fat, cheese, gas chromatography, foreign fat

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Introduction

Milk and milk-derived products are commonly considered balanced and nutritive foods, and they are frequently included as important components of a healthy diet (Pereira, 2014). In particular, milk fat contains useful quantities of vitamins and a large variety of lipids and fatty acids (FA). Milk fat is responsible of the smooth taste of dairy products and is one of the most easily digested fats (IDF, 2008). It consists mainly of triacylglycerols, which are composed of a glycerol molecule (glycerol-3-phosphate) esterified with three fatty acids (Hocquette and Gigil, 2005).

Dairy products represent an important group of food commodities that are extensively consumed by large segments of population, including children, pregnant women, and elderlies (Miller *et al*., 2006). Cheese produced from cow's milk dominates the market in most countries, whereas another share of the market consists of cheese produced from milk of other species (Hurley *et al*., 2006). Because of the increasing demand, milk fat has been a target of fraudulent practices such as replacement with cheaper or more readily available non-milk fats of plant and animal origin (Ntakatsane *et al*., 2013). Even partial replacing of the highly priced milk fat by low-priced vegetable oils, animal fats, or margarine has economic advantages when the product is not labeled accordingly (Lipp, 1996; Marekov *et al*., 2011). Adulteration of milk used in cheese manufacture will result in a final product inferior to that expected by the consumer (Hurley *et al*., 2006). Moreover, replacement of milk fat with fat from other sources is not only an economic fraud but may also pose a risk to human health (Ntakatsane *et al*., 2013). The addition of non-milk fats such as vegetable oils to milk and dairy products is an old and illegal practice (Gutiérrez *et al*., 2009; Molkentin, 2007), and has become increasingly common and complex. Therefore, for legal reasons and for consumer protection and confidence, dairy products should be authentic and correctly labeled, particularly for genuine traditional products such as cheese (Hurley *et al*., 2004; Moatsou and Anifantakis, 2003). Fatty acid composition of milk fat has long been used as a criterion to detect adulteration with vegetable oils, mainly because milk fat is characterized by short-chain fatty acids, whereas vegetable oils have medium- to long-chain fatty acids (Molkentin and Precht, 1998; Ntakatsane *et al*., 2013). Based on this knowledge, studies related to the detection and quantification of non-milk fats in milk and dairy products

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are being constantly developed and represent prominent research areas in many centers, with high economic significance in national and international commerce (Fontecha *et al*., 2006; Parodi, 1971; Pinto *et al*., 2002). Destaillats *et al*. (2006) reported that foreign fats in milk fat can be detected by analyzing triacylglycerol (TAG) by gas–liquid chromatography using the standardized methodology. TAG determination has also been proposed as a means to detect mixtures of foreign fats in milk fat and has been considered by the European Community as an official method for assessing cow milk fat purity (Goudjil *et al*., 2003). However, adulteration of milk still represents a critical problem in Korea.

Therefore, the objective of this study was to develop and validate a method for the detection of non-milk fats in cheese produced in Korea. The study is based on the investigation of fatty acid, triacylglycerol, and cholesterol concentrations by gas chromatography (GC) analysis combined with a statistical technique.

Materials and Methods

Samples collection

Milk fat samples were chosen from dairy companies that are most popular in Korea as assessed by market research (Table 1). Soybean oil, corn oil, pork lard, and beef tallow were purchased from a local market as well as from a manufacturers, and were stored at 4°C (dairy product) and 25°C (non-dairy products).

In order to detect adulteration in milk fat, experiments were performed on the following samples: milk fat 90% + non-milk fat 10%, milk fat 70% + non-milk fat 30%, milk fat 50% + non-milk fat 50%, milk fat 30% + non-milk fat 70%, and milk fat 10% + non-milk fat 90%. The samples were melted, if necessary, and mixed together.

Chemicals

The standard FA used in the analyses included C4:0 (butyric acid), C6:0 (caproic acid), C8:0 (caprylic acid), C10:0 (capric acid), C11:0 (undecanoic acid), C12:0 (lauric acid), C13:0 (tridecanoic acid), C14:0 (myristic acid), C14:1 (tetradecenoic acid), C15:0 (pentadecanoic acid), C15:1 (pentadecenoic acid), C16:0 (palmitic acid), C16:1 (hexadecenoic acid), C17:0 (margaric acid), C17:1 (margaroleic acid), C18:0 (stearic acid), C18:1 (octadecenoic acid), C18:2 (octadecadienoic acid), C18:3 (linolenic acid), C20:0 (arachidic acid), C20:1 (eicosenoic acid), C20:2 (eicosadienoic acid), C20:3 (eicosatrienoic acid), C20:4 (arachidonic acid), C20:5 (eicosapentaenoic acid), C21:0 (heneicosanoic acid), C22:0 (behenic acid), C22:1 (docosanoic acid), C22:2 (docosadienoic acid), C22:3 (docosatrienoic acid), C22:4 (docosatetraenoic acid), C22:5 (docosapentaenoic acid), C22:6 (docosahexaenoic acid), C23:0 (tricosanoic acid), C24:0 (lignoceric acid), and C24:1 (nervonic acid). Standard TAGs used have identical number of acyl-C atoms (C24-C54).

Sample preparation

The fatty acid was extracted using a modified version of Folch's method (Challinor, 1996). Each sample (2.5 g) was added to 25 mL of a chloroform-methanol solvent mixture (2:1, v/v). The mixture was homogenized at 2,500 rpm for 30 min, ultrasonicated for 20 min, and then added with 10 mL of a saturated NaCl solution. The suspension was then centrifuged at 4,000 rpm for 20 min at -4°C. The chloroform phase was recovered and transferred into a 25 mL round-bottomed flask. Next, each fat extract was dried via a rotary evaporator at 45°C under vacuum (Kim *et al*., 2013). After evaporation, approximately 0.5 g of oil was extracted from each sample and was treated with 8 mL of a 0.5 N NaOH methanolic solution and heated at 85°C for 10 min in a water bath. Next, 9 mL of 14% boron trifluoride (BF_3) was added over 2 min, the mixture was cooled in cold water, and 4 mL of hexane were then added under slow stirring. Subsequently, added mass up saponification flask using saturated solution of NaCl and stay for 3 min. After adding 1 g of anhydrous sodium sulfate, the mixture was allowed to stay for 10 min and FA were extracted with hexane (MFDS, 2014).

For TAG and cholesterol, 5 mL of hexane was added to 0.1 g of the extracted fats and the samples were subjected to GC analysis (Park *et al*., 2013).

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

Part	Sample name	Ingredients
Milk fat (MK)	Cheese (Maeil Dairies Co., Ltd.)	Raw milk 98.63%, salt, etc.
Vegetable oil (VG)	Soybean oil (CJ CheilJedang Corporation)	Soybean 100%
	Corn oil (CJ CheilJedang Corporation)	Corn germ 100%
Pork lard (PL)	Pork lard (Samyang Co., Ltd.)	Lard 100%
Beef tallow (BT)	Beef tallow (Ottogi Co., Ltd.)	Tallow 100%

Chromatographic analysis

Fatty acids

Separation and identification of FA were performed using an Agilent model 7890 gas chromatograph (Agilent, USA) equipped with a Supelco-24056, SP™-2560 capillary GC column (100 m × 0.25 mm id, df = 0.20 μm; Sigma-Aldrich Co., USA) and a flame ionization detector (FID). A run time of 67 min was used for each sample. GC-FID analysis was carried out under the following instrumental conditions: injection volume of 1 μL and nitrogen carrier gas flow rate of 1.0 mL/min with a split ratio of 50:1 and a constant flow control. The injector and detector temperatures were set at 250°C. The oven program was maintained at 180°C for 40 min and then gradually increased to 230°C at a rate of 3°C/min for 10 min (Table 2). An aliquot of the supernatant was transferred into an auto-sampler vial for GC-FID analysis.

Triacylglycerols

Separation and identification of TAG were also performed using an Agilent model 7890 gas chromatograph (Agilent, USA) equipped with a Supelco 25003, HT-5 aluminum clad capillary column (25 m × 0.32 mm id, df = 0.10 μm; Sigma-Aldrich Co., USA) and an FID. A run time of 20 min was used for each sample. GC-FID analysis was carried out under the following instrumental conditions: injection volume of 2 μL and nitrogen carrier gas flow rate of 1.8 mL/min with a split ratio of 50:1 and a constant flow control. The injector and detector temperatures were set at 380°C. The oven program was maintained at 340°C for 1 min and then gradually increased to 370°C at a rate of 5°C/min for 13 min (Table 2). An aliquot of the supernatant was transferred into an auto-sampler vial for GC-FID analysis.

Cholesterol

Separation and identification of cholesterol were performed using the same equipment with an Agilent 19091J-413 HP-5 column containing 5% phenyl methyl siloxane (30 m × 0.32 mm id, df = 0.25 μm; Agilent, USA) and an FID. A run time of 38 min was used for each sample. GC-FID analysis was carried out under the following instrumental conditions: injection volume of 1 μL and nitrogen carrier gas flow rate of 1.0 mL/min with a split ratio of 5:1 and a constant flow control. The injector and detector temperatures were set at 280°C and 290°C, respectively. The oven program was maintained at 250°C for 25 min and increased to 290°C at a rate of 10°C/min for 9 min (Table 2). An aliquot of the supernatant was transferred into an auto-sampler vial for GC-FID analysis.

Statistical analysis

Statistical analysis, carried out using the SPSS program (version 11.0; SPSS Inc., USA) for Windows 7 software, showed clear differences between fatty acids (oleic acid (C18:1n9c) and linoleic acid (C18:2n6c), triacylglycerols (C52 and C54), and cholesterol. The one-way ANOVA was used to determine the significance of differences between the sample means. Statistical significance was accepted at $p<0.05$.

Results and Discussion

Fatty acid analysis by GC

The forty-four fatty acids composition of the physical

Table 2. Operating conditions for GC analysis of fatty acids, triacylglycerols, and cholesterol content

Parameter	Condition		
	Fatty acids	Triacylglycerols	Cholesterol
Instrument	Agilent 7890A GC	Agilent 7890A GC	Agilent 7890A GC
Detector	Flame ionization detector (FID)	Flame ionization detector (FID)	Flame ionization detector (FID)
Column	SP™-2560 Capillary GC column (Supelco 24056) (100 m × 0.25 mm × 0.20 μm)	HT-5 (Aluminum clad) Capillary GC column (Supelco 25003) (25 m × 0.32 mm × 0.10 μm)	HP-5 5% Phenyl methyl siloxane (Agilent 19091J-413) (30 m × 0.32 mm × 0.25 μm)
Inlet temperature	250°C	380°C	280°C
Injection Volume	1 μL	2 μL	1 μL
Inlet split ratio	50 : 1	50 : 1	5 : 1
Carrier gas	N_2 (Nitrogen)	N_2 (Nitrogen)	N_2 (Nitrogen)
Oven condition	180°C for 40 min then 3°C/min to 230°C for 10 min	340°C for 1 min then 5°C/min to 370°C for 13 min	250°C for 25 min then 10°C/min to 290°C for 9min
Detector Temp.	250°C	380°C	290°C
Flow rate	1.0 mL/min.	1.8 mL/min	1.0 mL/min
Run time	67 min	20 min	38 min

mixtures of milk and non-milk fats (10:90, 30:70, 50:50, 70:30, and 90:10) was analyzed by GC using the experimental conditions described above. Clearly, as reported in Table 3 and 4, the concentrations of oleic acid (C18:1n9c) and linoleic acid (C18:2n6c) in the adulterated samples were significantly different ($p<0.05$) from those in milk fat.

In the case of adulteration with vegetable oil, an increase in the linoleic acid (C18:2n6c) concentration was observed as the concentration of adulteration increased. The linoleic acid (C18:2n6c) concentration in pure milk fat was measured to be 2.12 g/100 g. In samples adulterated with 10%, 30%, 50%, 70%, and 90% soybean oil, the linoleic acid (C18:2n6c) concentrations were 12.22, 20.17, 37.58, 45.84, and 48.84 g/100 g, respectively. In samples adulterated with 10%, 30%, 50%, 70%, and 90% corn oil, the linoleic acid (C18:2n6c) concentrations were 9.65, 25.79, 28.57, 47.05, and 50.36 g/100 g, respectively. In all groups of samples with different adulteration levels (10-90%), the concentration of linoleic acid (C18:2n6c) is clearly higher than that in pure milk fat. Thus, linoleic acid (C18:2n6c) concentration can be used as marker for the adulteration of pure milk fat with soybean or corn oil.

In the case of adulteration with animal fat, the concentration of oleic acid (C18:1n9c) increased with the increasing adulteration concentrations. The concentration of oleic acid (C18:1n9c) in pure milk fat was measured to be 24.13 g/100 g. In samples adulterated with 10%, 30%, 50%, 70%, and 90% pork lard, the oleic acid (C18:1n9c) concentrations were 26.91, 29.41, 34.61, 41.72, and 44.50 g/100 g, respectively. In mixtures containing 10%, 30%, 50%, 70%, and 90% beef tallow, the oleic acid (C18:1n9c) concentrations were 24.94, 25.40, 28.60, 33.70, and 37.55 g/100 g, respectively. Thus, it is clear that in all groups of samples with different adulteration concentrations (10-90%), the concentration of oleic acid is clearly higher than that in pure milk fat. Thus, oleic acid (C18:1n9c) concentration can be used as a marker to detect the adulteration of pure milk fat with pork lard or beef tallow.

We have demonstrated the utility of linoleic acid (C18:2n6c) and oleic acid (C18:1n9c) concentrations as detection tools for the adulteration of pure milk fat with vegetable and animal oils, respectively. In addition, these fatty acids are considered to be useful for the detection of animal fats and corn oil, respectively, in pure milk fat.

Triacylglycerol analysis by GC

The composition of fifteen triacylglycerols in the adulterated pure milk fat samples (containing 10%, 30%, 50%, 70%, and 90% non-milk fats) was analyzed by GC. As reported in Table 5, the adulterated samples show a significant difference ($p<0.05$) in the C52 and C54 concentrations compared with pure milk fat.

The result in the adulteration of vegetable oil, the C26 concentration could not be detected. In general, the concentration of TAGs with carbon numbers ranging from C24 to C50 decreased proportionally to the adulteration concentration, whereas TAGs with carbon numbers C52 and C54 increased. The C52 concentration in pure milk fat was measured to be 7.02 g/100 g. The groups of samples adulterated with 10%, 30%, 50%, 70%, and 90% soybean oil showed a C52 concentration of 19.44, 26.73, 28.80, 33.57, and 34.06 g/100 g, respectively. Similarly, the C54 concentration in pure milk fat was found to be 2.22 g/100 g, whereas the mixtures containing 10%, 30%, 50%, 70%, and 90% soybean oil showed C54 concentrations of 27.01, 40.74, 44.94, 53.55, and 55.19 g/100 g, respectively.

Similar TAGs profiles were observed for mixtures containing corn oil as the adulterant. Samples adulterated with 10%, 30%, 50%, 70%, and 90% corn oil showed, respectively, C52 concentrations of 22.29, 29.07, 34.27, 35.41, and 36.49 g/100 g, and C54 concentrations of 27.52, 41.07, 48.71, 50.76, and 52.19 g/100 g.

The result in the adulteration of pork lard, the concentration of TAGs, with the exception of C52 and C54, decreased with the increasing adulteration concentrations. Samples containing 10%, 30%, 50%, 70%, and 90% pork lard presented, respectively, C52 concentrations of 30.16, 51.04, 52.04, 56.25, and 58.96 g/100 g, and C54 concentrations of 7.71, 12.73, 13.01, 14.02, and 14.35 g/100 g.

Likewise, samples adulterated with beef tallow showed significantly different C52 and C54 profiles compared with that shown by pure milk fat. Mixtures containing 10%, 30%, 50%, 70%, and 90% beef tallow showed, respectively, C52 concentrations of 20.25, 34.28, 34.32, 35.02, and 36.74 g/100 g, and C54 concentrations of 7.11, 12.92, 13.15, 13.72, and 14.07 g/100 g. Thus, in this study, we have confirmed that C52 and C54 concentrations in adulterated pure milk fat are approximately three times higher than those in pure milk fat. These results prove the potential utility of C52 and C54 TAGs profiles in the detection of milk fat adulteration.

Cholesterol analysis by GC

GC analysis of the cholesterol profile in the mixtures of milk and non-milk fats (90:10, 70:30, 50:50, 30:70, and 10:90) showed that the concentration of cholesterol in the

Table 3. Gas chromatographic characterization of fatty acids in milk fat adulterated with vegetable oils (g/100g)

Fatty acid	Type	MK[1)]	MK:VG1[2)]					MK:VG2[3)]				
		100	90:10	70:30	50:50	30:70	10:90	90:10	70:30	50:50	30:70	10:90
C4:0	Saturated	0.00±0.00[4)]	00.00±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	1.62±0.00	1.08±0.00	0.80±0.00	0.41±0.01	0.17±0.00	0.05±0.01	0.58±0.00	0.89±0.00	0.65±0.01	0.21±0.01	0.06±0.01
C8:0	Saturated	0.98±0.00	0.96±0.00	0.72±0.01	0.30±0.00	0.11±0.01	0.04±0.00	0.79±0.01	0.65±0.00	0.54±0.00	0.14±0.00	0.06±0.01
C10:0	Saturated	2.18±0.00	2.01±0.01	1.59±0.00	0.60±0.00	0.23±0.01	0.08±0.00	2.07±0.01	1.36±0.00	1.20±0.00	0.27±0.00	0.10±0.00
C11:0	Saturated	0.03±0.00	0.35±0.00	0.27±0.00	0.09±0.00	0.03±0.01	0.02±0.00	0.27±0.00	0.19±0.00	0.18±0.00	0.04±0.00	0.02±0.01
C12:0	Saturated	3.72±0.00	3.22±0.03	2.59±0.00	1.03±0.00	0.37±0.01	0.12±0.01	3.48±0.00	2.17±0.00	1.99±0.02	0.43±0.01	0.16±0.00
C13:0	Saturated	0.06±0.00	0.12±0.00	0.10±0.00	0.05±0.01	0.01±0.00	0.00±0.00	0.07±0.01	0.07±0.00	0.06±0.00	0.01±0.01	0.01±0.01
C14:0	Saturated	10.50±0.01	9.16±0.00	7.44±0.00	3.03±0.01	1.12±0.00	0.43±0.01	9.94±0.00	6.23±0.00	5.65±0.00	1.25±0.00	0.49±0.00
C14:1	Unsaturated	0.80±0.01	0.90±0.02	0.72±0.00	0.16±0.01	0.05±0.00	0.02±0.01	0.73±0.01	0.57±0.01	0.38±0.00	0.06±0.00	0.02±0.00
C15:0	Saturated	0.75±0.03	0.60±0.00	0.46±0.01	0.46±0.01	0.17±0.02	0.07±0.00	0.85±0.03	0.40±0.00	0.52±0.00	0.19±0.00	0.08±0.01
C15:1	Unsaturated	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.01	0.02±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.01	0.02±0.03
C16:0	Saturated	29.41±0.01	25.53±0.02	22.69±0.00	15.08±0.01	11.80±0.03	10.68±0.01	27.05±0.00	21.48±0.01	20.56±0.00	13.50±0.01	12.23±0.00
C16:1	Unsaturated	1.14±0.00	1.03±0.01	0.82±0.00	0.38±0.01	0.20±0.01	0.13±0.00	1.15±0.00	0.70±0.00	0.68±0.00	0.23±0.01	0.15±0.00
C17:0	Saturated	0.45±0.00	0.55±0.00	0.46±0.00	0.24±0.00	0.14±0.01	0.10±0.00	0.56±0.00	0.37±0.00	0.28±0.00	0.13±0.01	0.10±0.01
C17:1	Unsaturated	0.00±0.00	0.17±0.01	0.16±0.00	0.09±0.00	0.06±0.00	0.06±0.01	0.21±0.00	0.13±0.03	0.19±0.01	0.06±0.00	0.06±0.00
C18:0	Saturated	14.29±0.00	11.44±0.01	10.13±0.00	6.52±0.01	4.97±0.01	4.38±0.00	12.02±0.00	8.16±0.00	7.33±0.00	3.26±0.00	2.49±0.00
C18:1n9t	Unsaturated	2.21±0.01	2.61±0.00	1.96±0.01	0.82±0.02	0.28±0.03	0.13±0.01	2.84±0.00	1.65±0.01	1.45±0.00	0.36±0.00	0.13±0.00
C18:1n9c	Unsaturated	24.13±0.05	24.66±0.01	24.87±0.01	25.73±0.03	26.75±0.00	26.99±0.01	25.66±0.00	26.50±0.00	26.96±0.00	30.15±0.01	30.98±0.03
C18:2n6t	Unsaturated	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	0.01±0.00	0.00±0.00
C18:2(c,t)	Unsaturated	0.28±0.00	0.38±0.00	0.34±0.01	0.38±0.00	0.37±0.00	0.38±0.01	0.31±0.00	0.26±0.01	0.26±0.01	0.21±0.01	0.20±0.00
C18:2(t,c)	Unsaturated	0.15±0.00	0.12±0.01	0.21±0.00	0.31±0.00	0.31±0.00	0.33±0.00	0.08±0.01	0.11±0.03	0.13±0.00	0.15±0.01	0.15±0.01
C18:2n6c	Unsaturated	2.12±0.00	12.22±0.01	20.17±0.01	37.58±0.00	45.84±0.00	48.84±0.01	9.65±0.00	25.79±0.01	28.57±0.00	47.05±0.00	50.36±0.00
C20:0	Saturated	0.19±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.03±0.00	0.19±0.00	0.11±0.00	0.00±0.00	0.02±0.00	0.00±0.00
C18:3 (t,t,t)	Unsaturated	0.00±0.00	0.30±0.00	0.30±0.00	0.34±0.00	0.37±0.00	0.39±0.01	0.27±0.00	0.58±0.00	0.34±0.00	0.45±0.00	0.47±0.01
C18:3(t,t,c),(t,c,t)	Unsaturated	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.00	0.04±0.00	0.05±0.00	0.00±0.00	0.07±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C18:3n6	Unsaturated	0.05±0.00	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
C18:3(c,t,t),(c,c,t)	Unsaturated	0.00±0.00	0.14±0.00	0.22±0.00	0.40±0.00	0.48±0.00	0.52±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.06±0.00	0.05±0.00
C18:3(c,t,c)	Unsaturated	0.15±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	0.15±0.03	0.00±0.00	0.00±0.00	0.01±0.00
C18:3(t,c,c)	Unsaturated	0.00±0.00	0.17±0.01	0.00±0.00	0.23±0.00	0.17±0.00	0.18±0.00	0.16±0.01	0.06±0.01	0.12±0.00	0.10±0.00	0.09±0.00
C20:1	Saturated	0.05±0.00	0.00±0.00	0.22±0.00	0.45±0.00	0.44±0.01	0.11±0.03	0.10±0.00	0.06±0.00	0.18±0.00	0.22±0.01	0.24±0.01
C18:3n3	Unsaturated	0.22±0.00	1.44±0.01	2.24±0.00	4.12±0.01	4.88±0.01	5.24±0.00	0.43±0.01	0.66±0.00	0.73±0.01	1.03±0.00	0.97±0.00
C21:0	Saturated	0.03±0.00	0.42±0.03	0.30±0.01	0.04±0.00	0.03±0.00	0.03±0.00	0.42±0.03	0.25±0.01	0.23±0.00	0.01±0.01	0.03±0.00
C20:2	Unsaturated	0.03±0.00	0.00±0.00	0.11±0.00	0.12±0.01	0.02±0.01	0.02±0.00	0.11±0.00	0.11±0.00	0.08±0.00	0.04±0.00	0.04±0.01
C22:0	Saturated	0.07±0.00	0.23±0.00	0.21±0.01	0.35±0.01	0.38±0.00	0.39±0.00	0.00±0.00	0.14±0.05	0.15±0.00	0.18±0.01	0.15±0.01
C20:3n6c	Unsaturated	0.13±0.00	0.15±0.01	0.27±0.00	0.06±0.00	0.02±0.00	0.02±0.00	0.00±0.00	0.10±0.00	0.09±0.00	0.01±0.01	0.02±0.01
C22:1n9	Unsaturated	0.04±0.00	0.15±0.00	0.00±0.00	0.08±0.03	0.05±0.00	0.06±0.01	0.00±0.00	0.05±0.01	0.45±0.00	0.09±0.00	0.04±0.00
C20:3n3	Unsaturated	0.00±0.00	0.16±0.00	0.20±0.00	0.05±0.00	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.06±0.00	0.03±0.00	0.02±0.01
C20:4n6	Unsaturated	0.17±0.00	0.52±0.02	0.09±0.00	0.10±0.00	0.03±0.00	0.02±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.00	0.01±0.00
C23:0	Saturated	0.03±0.01	0.20±0.01	0.23±0.01	0.07±0.01	0.07±0.00	0.08±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.01
C22:2	Unsaturated	0.00±0.00	0.00±0.00	0.11±0.01	0.04±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
C24:0	Saturated	0.04±0.00	0.00±0.00	0.00±0.00	0.12±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
C20:5n3	Unsaturated	0.03±0.00	0.00±0.00	0.00±0.00	0.11±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
C24:1	Unsaturated	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±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C22:6n3	Unsaturated	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
SUM		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1)]MK: 100% pure milk fat of cheese. [2)]MK:VG1: milk fat and soybean oil mixture. [3)]MK:VG2: milk fat and corn oil mixture. [4)]Values are mean±SD (*n*=3).

Table 4. Gas chromatographic characterization of fatty acids in milk fat adulterated with animal fats (g/100g)

Fatty acid	Type	MK[1]	MK:PL[2]					MK:BT[3]				
		100	90:10	70:30	50:50	30:70	10:90	90:10	70:30	50:50	30:70	10:90
C4:0	Saturated	0.00±0.00[4]	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	1.62±0.00	0.73±0.00	0.72±0.00	0.63±0.01	0.25±0.00	0.04±0.00	0.70±0.00	1.02±0.00	0.47±0.01	0.42±0.00	0.17±0.00
C8:0	Saturated	0.98±0.00	0.83±0.01	0.74±0.00	0.50±0.02	0.18±0.00	0.04±0.00	0.98±0.00	0.94±0.00	0.69±0.02	0.39±0.01	0.17±0.01
C10:0	Saturated	2.18±0.00	2.09±0.01	1.82±0.00	1.18±0.00	0.42±0.00	0.12±0.01	2.50±0.00	2.00±0.00	1.86±0.01	0.86±0.01	0.30±0.00
C11:0	Saturated	0.03±0.00	0.29±0.00	0.24±0.00	0.15±0.05	0.06±0.02	0.02±0.00	0.29±0.00	0.30±0.03	0.20±0.00	0.11±0.01	0.03±0.01
C12:0	Saturated	3.72±0.00	3.50±0.00	3.06±0.00	1.99±0.01	0.72±0.02	0.25±0.00	4.06±0.00	3.34±0.00	3.21±0.03	1.62±0.00	0.76±0.01
C13:0	Saturated	0.06±0.00	0.08±0.00	0.07±0.01	0.06±0.00	0.02±0.00	0.01±0.03	0.08±0.01	0.14±0.03	0.06±0.03	0.03±0.01	0.02±0.00
C14:0	Saturated	10.50±0.01	10.33±0.00	9.15±0.00	6.50±0.00	3.20±0.01	2.00±0.00	11.44±0.00	9.87±0.03	9.51±0.00	5.91±0.00	3.92±0.00
C14:1	Unsaturated	0.80±0.01	0.73±0.03	0.57±0.02	0.27±0.00	0.17±0.00	0.21±0.00	0.57±0.03	0.51±0.00	0.51±0.00	0.37±0.03	0.31±0.00
C15:0	Saturated	0.75±0.03	0.89±0.00	0.83±0.00	0.95±0.00	0.22±0.01	0.01±0.01	1.85±0.00	1.71±0.00	1.67±0.01	1.49±0.01	1.39±0.03
C15:1	Unsaturated	0.00±0.00	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
C16:0	Saturated	29.41±0.01	28.96±0.05	28.14±0.01	26.34±0.03	24.07±0.01	23.12±0.01	29.67±0.05	29.53±0.01	27.89±0.03	27.55±0.01	26.53±0.01
C16:1	Unsaturated	1.14±0.00	1.46±0.00	1.61±0.01	1.93±0.00	2.37±0.03	2.49±0.01	1.46±0.00	1.61±0.01	1.78±0.00	2.49±0.00	2.93±0.01
C17:0	Saturated	0.45±0.00	0.51±0.01	0.48±0.00	0.54±0.03	0.45±0.00	0.41±0.00	0.50±0.00	0.60±0.00	0.56±0.00	0.90±0.00	1.11±0.01
C17:1	Unsaturated	0.00±0.00	0.26±0.00	0.26±0.00	0.34±0.03	0.34±0.01	0.39±0.01	0.30±0.01	0.33±0.01	0.34±0.00	0.60±0.00	0.71±0.00
C18:0	Saturated	14.29±0.00	13.61±0.01	13.05±0.03	12.28±0.00	11.28±0.00	11.06±0.01	13.30±0.00	13.74±0.00	13.34±0.01	15.76±0.00	16.79±0.00
C18:1n9t	Unsaturated	2.21±0.01	3.01±0.01	2.54±0.03	1.93±0.00	0.88±0.01	0.58±0.01	3.26±0.00	3.21±0.01	3.37±0.03	3.43±0.00	3.36±0.00
C18:1n9c	Unsaturated	24.13±0.05	26.91±0.00	29.41±0.00	34.61±0.05	41.72±0.00	44.50±0.00	24.94±0.00	25.40±0.00	28.60±0.00	33.70±0.01	37.55±0.03
C18:2n6t	Unsaturated	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
C18:2(c,t)	Unsaturated	0.28±0.00	0.30±0.01	0.27±0.01	0.22±0.00	0.15±0.01	0.12±0.03	0.31±0.01	0.34±0.03	0.32±0.00	0.29±0.00	0.28±0.01
C18:2(t,c)	Unsaturated	0.15±0.00	0.09±0.01	0.18±0.03	0.08±0.00	0.13±0.01	0.09±0.01	0.17±0.00	0.29±0.00	0.88±0.00	0.25±0.01	0.27±0.00
C18:2n6c	Unsaturated	2.12±0.00	3.82±0.00	5.11±0.01	7.57±0.01	10.92±0.00	12.02±0.00	2.15±0.00	2.19±0.01	2.42±0.00	2.60±0.01	2.81±0.01
C20:0	Saturated	0.19±0.00	0.29±0.00	0.16±0.00	0.17±0.00	0.18±0.00	0.17±0.01	0.28±0.00	0.26±0.00	0.20±0.00	0.24±0.01	0.19±0.03
C18:3 (t,t,t)	Unsaturated	0.00±0.00	0.23±0.00	0.40±0.03	0.32±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.00	0.03±0.00
C18:3(t,t,c),(t,c,t)	Unsaturated	0.00±0.00	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
C18:3n6	Unsaturated	0.05±0.00	0.00±0.00	0.06±0.01	0.00±0.00	0.11±0.03	0.02±0.01	0.05±0.00	0.45±0.05	0.00±0.00	0.02±0.00	0.01±0.00
C18:3(c,t,t),(c,c,t)	Unsaturated	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.01	0.04±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.00	0.04±0.00
C18:3(c,t,c)	Unsaturated	0.15±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.15±0.01	0.22±0.01	0.15±0.01	0.15±0.01	0.14±0.01
C18:3(t,c,c)	Unsaturated	0.00±0.00	0.11±0.01	0.17±0.01	0.10±0.01	0.00±0.00	0.02±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C20:1	Saturated	0.05±0.00	0.14±0.00	0.28±0.00	0.46±0.03	0.85±0.01	0.92±0.00	0.08±0.00	0.14±0.00	0.10±0.00	0.16±0.01	0.20±0.01
C18:3n3	Unsaturated	0.22±0.00	0.40±0.01	0.40±0.01	0.49±0.01	0.62±0.01	0.66±0.03	0.29±0.03	0.37±0.00	0.33±0.00	0.36±0.01	0.37±0.05
C21:0	Saturated	0.03±0.00	0.45±0.03	0.06±0.01	0.05±0.05	0.02±0.00	0.02±0.00	0.04±0.03	0.50±0.00	0.49±0.00	0.05±0.01	0.04±0.01
C20:2	Unsaturated	0.03±0.00	0.00±0.00	0.25±0.03	0.34±0.05	0.46±0.03	0.47±0.03	0.05±0.00	0.00±0.00	0.04±0.00	0.09±0.03	0.03±0.01
C22:0	Saturated	0.07±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.01	0.04±0.01	0.12±0.00	0.12±0.00	0.28±0.00	0.11±0.00	0.05±0.00
C20:3n6c	Unsaturated	0.13±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.12±0.01	0.10±0.01	0.14±0.00	0.16±0.00	0.06±0.01	0.07±0.00	0.04±0.00
C22:1n9	Unsaturated	0.04±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.18±0.01	0.26±0.01	0.10±0.03	0.01±0.01
C20:3n3	Unsaturated	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	0.12±0.00	0.00±0.00	0.04±0.00	0.01±0.00
C20:4n6	Unsaturated	0.17±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	0.09±0.00	0.05±0.00
C23:0	Saturated	0.03±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	0.00±0.00	0.06±0.03	0.04±0.01
C22:2	Unsaturated	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	0.02±0.00
C24:0	Saturated	0.04±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	0.01±0.00
C20:5n3	Unsaturated	0.03±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	0.03±0.00
C24:1	Unsaturated	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±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C22:6n3	Unsaturated	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
SUM		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]MK: 100% pure milk fat of cheese. [2]MK:PL: milk fat and pork lard mixture. [3]MK:BT: milk fat and beef tallow mixture. [4]Values are mean ± SD (*n*=3).

Table 5. Gas chromatographic characterization of triacylglycerol and cholesterol content in adulterated milk fat samples

Sample	Chol (mg/100g)	Triacylglycerol (g/100g)															TG SUM
		C26	C28	C30	C32	C34	C36	C38	C40	C42	C44	C46	C48	C50	C52	C54	
MK[1)]	44.00±0.00[6)]	0.41±0.02	0.31±0.02	1.31±0.02	3.16±0.00	7.49±0.01	13.77±0.02	16.27±0.01	11.16±0.01	6.16±0.01	6.06±0.01	6.74±0.00	7.92±0.01	8.85±0.03	7.02±0.04	2.22±0.00	100.00
90:10[2)]	36.40±0.00	0.00±0.00	0.44±0.03	0.88±0.07	1.66±0.05	4.25±0.05	7.99±0.05	10.89±0.02	6.82±0.05	3.63±0.05	3.28±0.07	3.37±0.05	3.88±0.10	6.48±0.01	19.44±0.02	27.01±0.03	100.00
70:30	25.48±0.01	0.00±0.00	0.19±0.01	0.38±0.05	0.93±0.03	2.09±0.03	4.17±0.03	6.64±0.03	3.66±0.02	1.79±0.02	1.64±0.02	1.66±0.01	2.02±0.07	7.37±0.01	26.73±0.05	40.74±0.01	100.00
50:50	23.48±0.00	0.00±0.00	0.13±0.01	0.31±0.01	0.83±0.01	1.47±0.02	3.09±0.02	5.59±0.01	2.72±0.03	1.25±0.02	1.19±0.02	1.22±0.01	1.53±0.05	6.95±0.02	28.80±0.03	44.94±0.01	100.00
30:70	11.57±0.01	0.00±0.00	0.00±0.00	0.07±0.01	0.31±0.01	0.20±0.01	0.97±0.01	3.28±0.02	0.57±0.01	0.23±0.01	0.27±0.01	0.00±0.00	0.39±0.01	6.59±0.02	33.57±0.05	53.55±0.02	100.00
10:90	6.17±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.24±0.01	0.12±0.01	0.75±0.01	2.89±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.31±0.01	6.43±0.03	34.06±0.03	55.19±0.01	100.00
90:10[3)]	22.44±0.01	0.00±0.00	0.43±0.03	0.71±0.03	1.73±0.05	2.93±0.05	6.36±0.07	11.11±0.05	6.16±0.03	3.24±0.03	2.95±0.03	3.06±0.02	3.46±0.02	8.06±0.01	22.29±0.03	27.52±0.03	100.00
70:30	15.99±0.00	0.00±0.00	0.24±0.02	0.41±0.02	1.00±0.03	1.48±0.03	3.20±0.05	6.37±0.02	3.44±0.02	1.67±0.02	1.58±0.02	1.55±0.05	1.90±0.01	7.03±0.01	29.07±0.05	41.07±0.01	100.00
50:50	12.12±0.00	0.00±0.00	0.00±0.00	0.13±0.02	0.47±0.02	0.45±0.02	1.59±0.02	3.64±0.01	1.03±0.01	0.55±0.01	0.56±0.01	0.47±0.03	0.73±0.01	7.41±0.02	34.27±0.01	48.71±0.01	100.00
30:70	9.04±0.01	0.00±0.00	0.00±0.00	0.06±0.01	0.33±0.01	0.23±0.01	1.08±0.02	3.13±0.01	0.62±0.01	0.32±0.01	0.36±0.01	0.25±0.01	0.44±0.01	7.00±0.02	35.41±0.01	50.76±0.02	100.00
10:90	5.82±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.22±0.01	0.11±0.01	0.73±0.01	2.54±0.01	0.00±0.00	1.17±0.01	0.24±0.01	0.00±0.00	0.26±0.02	7.04±0.02	36.49±0.01	52.19±0.01	100.00
90:10[4)]	34.29±0.01	0.18±0.02	0.44±0.02	0.89±0.07	1.58±0.05	4.39±0.05	8.89±0.07	10.28±0.01	6.86±0.05	3.62±0.05	3.32±0.07	3.77±0.02	5.46±0.02	12.47±0.01	30.16±0.02	7.71±0.03	100.00
70:30	30.33±0.02	0.00±0.00	0.11±0.01	0.31±0.03	0.57±0.03	0.97±0.02	3.43±0.03	3.54±0.02	1.73±0.02	1.12±0.02	1.19±0.02	1.77±0.01	4.19±0.01	17.31±0.01	51.04±0.05	12.73±0.01	100.00
50:50	25.68±0.00	0.00±0.00	0.08±0.01	0.27±0.02	0.49±0.02	1.13±0.03	3.08±0.03	3.17±0.02	1.50±0.02	0.94±0.02	1.05±0.01	1.63±0.01	4.11±0.01	17.47±0.02	52.04±0.04	13.01±0.02	100.00
30:70	18.91±0.00	0.00±0.00	0.00±0.00	0.14±0.01	0.22±0.01	0.51±0.02	2.06±0.02	1.93±0.01	0.64±0.01	0.45±0.02	0.59±0.01	1.19±0.01	3.81±0.02	18.20±0.02	56.25±0.05	14.02±0.01	100.00
10:90	13.85±0.01	0.00±0.00	0.00±0.00	0.07±0.01	0.08±0.01	0.17±0.01	1.53±0.01	1.05±0.01	0.00±0.00	0.22±0.01	0.40±0.01	0.96±0.01	3.63±0.01	18.58±0.03	58.96±0.04	14.35±0.01	100.00
90:10[5)]	43.05±0.00	0.25±0.01	0.59±0.05	0.98±0.07	2.15±0.05	4.22±0.02	8.47±0.07	10.39±0.02	7.06±0.05	4.42±0.05	4.21±0.07	5.44±0.02	9.79±0.02	14.67±0.01	20.25±0.02	7.11±0.03	100.00
70:30	37.47±0.00	0.00±0.00	0.22±0.03	0.35±0.05	0.64±0.03	1.50±0.03	3.11±0.01	3.63±0.03	1.85±0.02	1.23±0.02	1.68±0.02	3.55±0.01	12.01±0.01	22.30±0.01	34.28±0.04	12.92±0.01	100.00
50:50	33.22±0.01	0.00±0.00	0.09±0.02	0.23±0.01	0.57±0.01	1.14±0.01	2.35±0.02	2.81±0.01	1.61±0.02	1.14±0.01	1.58±0.02	4.05±0.01	12.78±0.01	24.22±0.02	34.32±0.04	13.15±0.01	100.00
30:70	32.46±0.01	0.00±0.00	0.00±0.00	0.15±0.01	0.40±0.02	0.70±0.01	1.78±0.00	2.51±0.02	1.00±0.00	0.73±0.02	1.25±0.01	3.41±0.01	12.52±0.01	24.74±0.02	35.02±0.05	13.72±0.01	100.00
10:90	31.64±0.00	0.00±0.00	0.00±0.00	0.08±0.01	0.25±0.01	0.44±0.01	1.22±0.01	1.66±0.00	0.52±0.01	0.47±0.01	1.02±0.01	4.00±0.00	14.73±0.02	27.56±0.03	36.74±0.05	14.07±0.02	100.00

[1)]MK: 100% pure milk fat of cheese. [2)]90:10: milk fat and soybean oil mixture. [3)]90:10: milk fat and corn oil mixture. [4)]90:10: milk fat and pork lard mixture. [5)]90:10: milk fat and beef tallow mixture. [6)]Values are mean±SD (*n*=3).

adulterated samples is significantly different (p<0.05) from that in the pure milk fat, as depicted in Table 5.

The cholesterol concentration was 44.00 mg/100 g in pure milk fat. The groups of samples adulterated with 10%, 30%, 50%, 70%, and 90% soybean oil showed cholesterol concentrations of 36.40, 25.48, 23.48, 11.57, and 6.17 mg/100 g, respectively. When the adulterant was corn oil, the concentration of cholesterol decreased proportionally to the mixing ratio. Samples adulterated with 10%, 30%, 50%, 70%, and 90% corn oil presented cholesterol concentrations of 22.44, 15.99, 12.12, 9.04, and 5.82 mg/100 g, respectively. Sample adulterated with 10%, 30%, 50%, 70%, and 90% of pork lard contained 34.29, 30.33, 25.68, 18.91, and 13.85 mg/100 g of cholesterol, respectively. A similar profile was observed with generally increasing concentrations of beef tallow as the adulterant. Samples adulterated with 10%, 30%, 50%, 70%, and 90% of beef tallow showed cholesterol contents of 43.05, 37.47, 33.22, 32.46, and 31.64 mg/100 g, respectively.

The observed variation in the cholesterol concentration is proportional to the adulteration concentration. It noticeably shows a significant difference (p<0.05) in all the mixtures of adulterated samples. Thus, the analysis of cholesterol concentration can be used to detect milk fat adulteration.

In this study, a method to discriminate between pure milk fat and adulterated milk fat by gas chromatography, which aims at the detection of fatty acids, triglycerides, and cholesterol, was developed. As a result, the levels of oleic acid (C18:1n9c), linoleic acid (C18:2n6c), C52, C54, and cholesterol detected were proportional to the adulteration ratios; hence, these compounds can be used as biomarkers to detect adulterated milk fat in cheese. Our findings provide valuable information for future studies in this area.

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