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Research Article
Detection of Milk Fat Adulteration in Commercial Butter and Sour Cream

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Abstract

Background and Objective: Adulteration of dairy products by substitution of milk fat by vegetable oil is common in the Eurasian Economic Union. The objective of the paper is to investigate the potential adulteration of the fat and to test the more convenient methods of detection, i.e., determination of fatty acids or sterols profiles in commercial butter and cream. **Materials and Methods:** Ten samples of commercial butter and 8 samples of commercial sour cream were collected on the national market of Kazakhstan. The analyses involved the original sour cream and butter without any modification (deep-freezing) and were achieved within the shelf-life period. The fatty acid composition was analyzed by GS-FID and Sterol fractions were analyzed by GS-MS. Statistical analysis was achieved by principal components analysis (PCA), Pearson types, Kruskal-Wallis test. **Results:** Sixty percent of the butter samples contained traces of phytosterols and one sample contained up to 78% β-sitosterol. In sour cream samples, only three contained 100% cholesterol while two contained more than 60% sitosterol. The detection of fat adulteration by analyzing the fatty acids patterns is convenient in case of massive substitution of milk fat, but a discrete substitution does not modify the fatty acids profiles leading to misinterpretation. **Conclusion:** The results exhort to give preference to sterol profile determination as an official method to detect fat adulteration in dairy products. This is even more important as the current standard used in Central Asia based on some fatty acids ratios can lead to incorrect conclusions.

Key words: Milkfat, adulteration, butter, sour cream, fatty acids, sterols, gas chromatography

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The fatty acids composition of the dairy products is partially modifiable by the diet[1-3] and could vary depending on the season[4] and the breed of animals both in cattle[5] and in camel[6]. In Kazakhstan, more than 80% of cow milk processed in the dairy plant is collected in small-scale farms. Dairy cattle feeding in those farms are not often reasoned and well standardized. Moreover, poor management of the reproduction season leads to a shortage of milk in the winter season. Face to such an irregular milk supply, the dairy plants used milk powder in complement. Consequently, fatty acids profiles in milk are highly variable and sometimes atypical.

Adulteration of cow milk and dairy products is a common feature in different countries of the world[7,8]. The substitution of milk fat by vegetable oil in dairy products processed by the dairy industry is usual for a long time but generally indicated on the packaging. However, last few years, the substitution of milk fat appeared more often without indications, especially in products as butter and cream with the mention "natural". Because vegetable oils are available on the local market at lower prices[9], such substitutions are more profitable for the stakeholders of the milk sector. However, such surreptitious substitution is not acceptable not only because it is not mentioned on the packaging, but also because the source and purity of vegetable fat are unknown and could have a health effect on consumers as it was reported in milk fat replaced by technical palm oil[10]. Notably, it has been shown that with a melting point at 33-39°C, certain fractions of palm oil could not be suitable for human digestion, the intestinal temperature being 35.6°C[11].

Both fatty acids and/or sterols profiles are the usual analytical methods to detect milk fat adulteration in dairy products including butter[12-14]. However, in the context of Kazakhstan, the method based on fatty acid profiles appeared longer and more costly than the method based on sterol determination.

The present paper had three main objectives: (i) To assess the relative importance of fat adulteration in the commercial dairy products widely consumed in the country; (ii) To test the convenience of the two methods (rapidity, reliability) to detect the fat adulteration and (iii) To give practical recommendations for the laboratories in charge of the official controls.

MATERIALS AND METHODS

Study area: All the analyses were performed at the private laboratory ANTIGEN LPP, Almaty (Kazakhstan) in March, 2019. This research project was conducted from 03/2017-03/2020.

Table 1: Characteristics of the 8 sour cream samples from the Almaty (Kazakhstan) market

Number	Fat (%)	Origin (country)
1	15	Kazakhstan
2	15	Kazakhstan
3	15	Russia
4	15	Kazakhstan
5	15	Kazakhstan
6	15	Belarus
7	15	Kazakhstan
8	15	Belarus

Table 2: Characteristics of the 10 butter samples from the Almaty (Kazakhstan) market

Number	Fat (%)	Origin (country)
1	72.5	Belarus
2	72.5	Russia
3	72.5	Kazakhstan
4	72.5	Kazakhstan
5	72.5	Kazakhstan
6	72.5	Russia
7	72.5	Kazakhstan
8	72.5	Russia
9	72.5	Kazakhstan
10	72.5	Kazakhstan

Sampling procedure: The study was performed on eight and ten commercial sour cream and butter samples, respectively. All samples had "natural butter" and "natural cream" labeling on the packaging and these samples represented all commercial butter and sour cream products available on the local market. Samples of different trademarks were bought from Almaty (Kazakhstan) market. Those sour cream samples originated from Russia (1), Belarus (2) and Kazakhstan (5), butter samples originated from Russia (3), Belarus (1) and different regions of Kazakhstan (6 samples).

The fat characteristics of the sour cream and butter samples were reported in Table 1 and 2, respectively.

The analyses involved the original sour cream and butter without any modification (deep-freezing) and were achieved within the shelf-life period.

Laboratory analysis: Two types of analysis were performed on all samples: (i) Fatty acids and (ii) Sterols composition. Sample preparation for each sample was carried out twice and analyzed by GC in duplicate. Thus, the data of all 4 analyzes was taken as the mean value.

Fatty acid composition of sour cream and butter: The fatty acid composition was analyzed by gas chromatography with flame ionization detection (GC-FID, Shimazu GC2010, Japan) strictly according to the procedure described by ISO 12966-2:2011 "Animal and vegetable fats and oils preparation of methyl esters of fatty acids". The column used was HP-88 (p/n: 112-88A7, Agilent technology), 100 m×0.250 mm×

0.20 µm. The chromatography parameters were: 260°C for injector temperature, 100:1 for split mode, 104 mL min^{-1} for total flow, 1 mL min^{-1} for column flow, 250°C for detector temperature (FID); column temperature program started from 140°C for 5 min, then increased up to 240°C (4°C min^{-1}); after 5 min at 240°C, the temperature increased up to 250°C (2°C min^{-1}) and kept at this temperature for 7 min. The total time of analysis was 47 min. The analytical standard FAME Mix C4-C24, 100 mg Neat (catalog n°18919-1AMP, Sigma-Aldrich Co, LLC) was used. Identification of 37 fatty acid methyl-esters was achieved by comparing retention times of the analytical standard and samples. The quantitative analysis was carried out by the normalizing method of peak areas (the whole content of the sample was considered as 100%).

Sterols: Sterol fractions were analyzed by Gas Chromatography with Mass Spectrometric detection (GC7890B/MS 5977B, Agilent, USA). Chromatography was performed using an HP-5MS capillary column 30 m length, 0.25 mm inner diameter and 0.25 µm film thicknesses. The carrier gas (helium grade "A") was maintained at a constant rate of 1.0 mL min^{-1}. The temperature of the thermostat was programmed from 115°C (hold 1 min) to 260°C with a heating rate of 13°C min^{-1}, up to 290°C at a rate of 5°C min^{-1} (hold 6 min). The total time of chromatography was 24.15 min. The temperatures of the interface, quadrupole and source of MSD ions were 290, 150 and 230°C, respectively. Mass spectrometric detection was carried out in the Selected Ions Mode (SIM) (Table 2). The standards of cholesterol, β-sitosterol, brassicasterol, stigmasterol and campesterol were purchased from Sigma-Aldrich, USA. Interstate standard was 33490-2015: "detection of vegetable oils and plant-based fat by gas-liquid chromatography with mass-spectrometric detection".

Data processing included determination of retention times and peak areas, processing of spectral information. To decrypt the mass spectra, the 10th edition Wiley library (total number of spectra in the library-over 550 th.) was used. As for fatty acids, the content of phytosterols was determined by the normalizing method of peak areas.

Index of atherogenicity: Some saturated fatty acids are known for their risk of causing coronary heart disease. The Index of Atherogenicity (IA) is a value that determines the ability of a particular ingested food substance to cause atherosclerosis. The calculation of this index is based on the ratio of some saturated FA on polyunsaturated ones. In the present study, we used the formula reported by Ulbricht and Southgate[15] and modified by Konuspayeva[6] i.e.:

$$IA = \frac{(C12:0+(4*C14:0)+C16:0}{(C10:1+C14:1+C16:1+C17:1+C18:1+C18:2+C18:3)}$$

Besides, the values of some other specific ratios of fatty acids (C16:0/C12:0, C18:0/C12:0, C18:1/C14:0, C18:2/C14:0, (C18:1+C18:2)/(C12:0+C14:0+C16:0+C18:0)) were compared to the ranges of the values reported in official standards of Eurasian Economic Union (GOST), those ratio being used in Kazakhstan to check the authenticity of dairy products as butter.

Statistical analysis: The objectives of the statistical analysis were (i) To achieve a typology of fatty acids profile in the sour cream and butter samples and (ii) To assess the relationships between the types of FA profiles and the sterols profiles.

To achieve those objectives, the data tables "Fatty acids" of sour milk (8 samples*37 acids) and butter (10 samples*37 acids) were analyzed by Principal Components Analysis (PCA), Pearson type[16] using the sterols data table (for sour cream and butter respectively) as supplementary quantitative variables. To compare FA profiles according to sterols' patterns, the sour cream and butter samples were classified according to their level of cholesterol. Then, a Kruskal-Wallis test was achieved to identify the relationships between FA profiles and the level of cholesterol. The test was followed by a bilateral test (multiple pair comparison test) according to Dunn procedure[17] to identify the cholesterol level responsible for the significance at $p<0.05$.

Besides, Pearson correlation was calculated between sterols percentage and atherogenicity index. The software used was XLstat 2017 (Addinsoft ©).

RESULTS

The results of fatty acids and sterols composition are given in the form of a description of sour cream, butter data and their Atherogenicity index.

Sour cream: In the studied samples were analyzed 25 types of fatty acids, but only 14 of them were normalized according to the GOST 2012 (Table 3). The results in sour cream samples on the acid composition showed that palmitic (C16:0), oleic (C18:1n9 cis), myristic (C14:0) and stearic acids (C18:0) were, on average, the most abundant fatty acids in sour cream samples (Table 3). However, 3 samples contained a high proportion of linoleic acid (C18:2n6 cis). By the composition of sterols, Cholesterol was the only sterol (100%) in 3 samples while 2 samples

Table 3: Fatty acids and sterols composition of the eight commercial sour cream samples in Kazakhstan's market and standard values in Kazakhstan (GOST, 2012)

N° sour cream samples	Fatty acids (%)								
	1	2	3	4	5	6	7	8	Standards
C4:0	1.2	2.4	2.7	0.0	2.1	0.0	2.2	2.4	2.0-4.2
C6:0	0.9	1.8	1.9	0.0	1.5	0.0	1.6	1.8	1.5-3.0
C8:0	0.6	1.2	1.3	0.0	1.0	0.0	1.1	1.2	1.0-2.0
C10:0	1.3	2.9	2.7	0.0	2.2	0.0	2.5	2.9	2.0-3.5
C11:0	0.1	0.3	0.2	0.0	0.2	0.0	0.2	0.3	
C12:0	1.6	3.5	3.0	0.2	2.6	0.2	2.9	3.4	2.0-4.0
C 13:0	0.1	0.2	0.2	0.0	0.1	0.0	0.1	0.1	
C14:0	5.4	11.8	10.9	1.1	9.3	1.0	9.9	11.1	8.0-13.0
C14:1	0.3	0.8	0.7	0.0	0.7	0.0	0.6	0.8	0.6-1.5
C15:0	0.5	1.3	1.5	0.0	1.1	0.0	1.2	1.1	
C15:1	0.2	0.4	0.5	0.0	0.3	0.0	0.3	0.3	
C16:0	33.3	34.5	31.8	39.6	32.1	39.0	25.8	27.8	22.0-33.0
C16:1	0.6	1.6	1.6	0.0	1.2	0.2	0.9	1.2	1.5-2.0
C17:0	0.3	0.7	0.9	0.0	0.6	0.0	0.7	0.5	
C17:1	0.1	0.3	0.4	0.0	0.3	0.0	0.3	0.2	
C18:0	9.7	10.0	11.1	5.3	11.1	5.1	14.5	13.2	9.0-14.0
C18:1n9t	2.4	1.8	2.1	0.7	3.0	0.8	4.9	2.8	22.0-33.0
C18:1n9c	31.5	21.0	22.6	37.3	25.2	38.3	24.7	24.2	
C18:2n6t	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	2.0-4.5
C18:2n6c	8.7	2.1	2.0	14.6	4.0	14.8	2.9	2.6	
C20:0	0.3	0.2	0.3	0.9	0.3	0.4	0.6	0.2	<0.3
C20:1	0.4	0.7	0.8	0.0	0.6	0.2	0.9	0.8	
C22:0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.6	<0.1
C18:3n3	0.3	0.4	0.6	0.0	0.5	0.0	0.6	0.3	
C22:6n3	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	
	Sterols (%)								
Cholesterol	87.9	99.3	100.0	15.5	98.1	14.0	100.0	100.0	
Brassicasterol	0.9	0.7	0.0	0.0	0.8	16.3	0.0	0.0	
Campesterine	2.1	0.0	0.0	14.7	0.0	0.0	0.0	0.0	
Stigmasterol	1.0	0.0	0.0	6.3	0.0	7.2	0.0	0.0	
β-sitosterol	8.0	0.0	0.0	63.5	1.1	60.9	0.0	0.0	

were characterized by a high proportion (more than 60%) of β-sitosterol (Table 3). For further analyses, 3 groups of sterols' profiles were used: (1) those containing only cholesterol named as Full Cholesterol (FC), i.e., 100%, (2) Medium Cholesterol (MC), i.e., 80-99% and (3) Low Cholesterol (LC), i.e., less than 20%.

The two main factors of the PCA explained 90% of the total variance with a total predominance of the first factor explaining 78%. The first factor was explained by the opposition between the short chain and saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C13:0, C14:0) and some monounsaturated ones (C14:1, C15:1, C16:1, C17:1) from one side to polyunsaturated fatty acids (oleic C18:1n9 cis and linoleic acid C18:2n6 cis) on the other side. All these acids are highly correlated to the first factor (r^2>0.950; p<0.01). The supplementary variables are highly correlated to this first factor with a clear opposition between cholesterol, closed to short-chain and saturated fatty acids and phytosterols closed to polyunsaturated fatty acids (Fig. 1). Samples 2, 3, 5, 7 and 8 (belonging to groups FC and MC) were projected close to the group of saturated and monounsaturated/cholesterol variables while samples 6 and 4 (LC) were projected close to other sterols. The sample n°1 (MC-87.9% cholesterol) was close to the center of gravity showing its proximity with the mean profile.

The Kruskal-Wallis test confirmed the significant relationships between the short-chain fatty acids (butyric C4:0, caproic C6:0, caprylic C8:0, capric C10:0, pentadecanoic C15:0 and stearic C18:0) acids and sterols profiles marked by 100% cholesterol (FC) while the profile LC was characterized by a significant higher proportion of palmitic (C16:0) and linoleic (C18:2n6 cis and trans) acids (Table 4).

Butter: As for sour cream samples, palmitic (C16:0), myristic (C14:0), stearic (C18:0) and oleic acids (C18:1n9 cis) were predominant in butter (Table 5). Additionally, 2 samples contained a high proportion of linoleic acid (C18:2n6 cis). Six samples contained more than 95% cholesterol while one

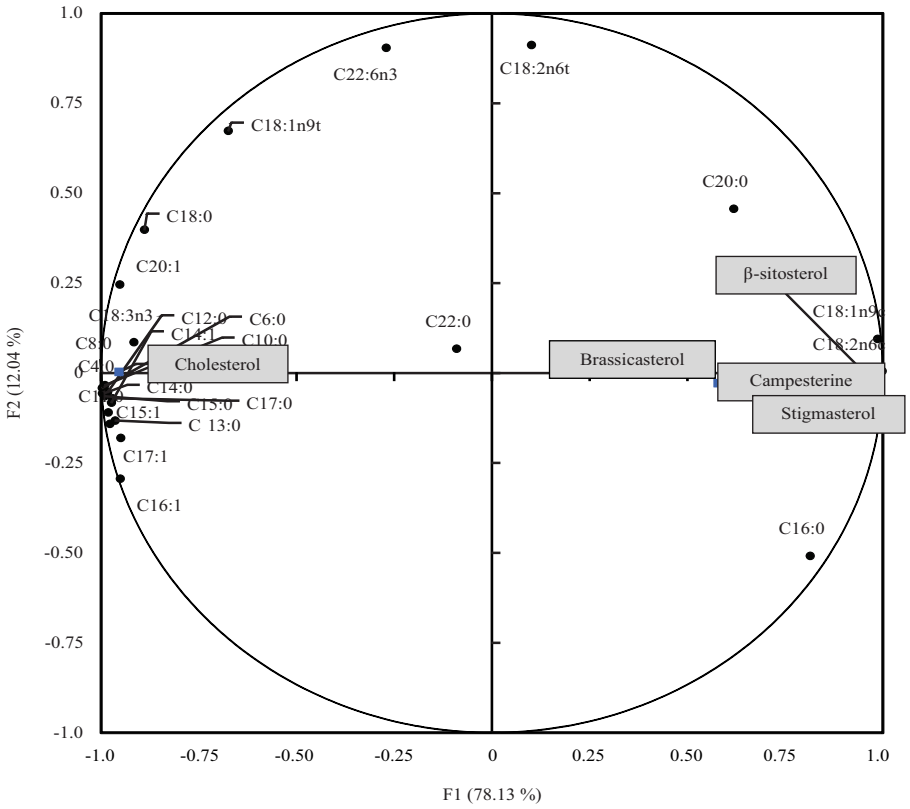

Fig. 1: Correlation circle of the PCA concerning sour cream fat composition and the projection of sterols

Table 4: Mean values of fatty acids in sour cream according to the cholesterol level (LC, MC and FC) and the p-value of the differences (Kruskal-Wallis test)

Fatty acids	FC	MC	LC	p-value
C4:0	2.41	1.90	0.00	0.045
C6:0	1.78	1.38	0.00	0.014
C8:0	1.19	0.93	0.00	0.014
C10:0	2.68	2.10	0.00	0.045
C11:0	0.23	0.19	0.00	0.075
C12:0	3.10	2.56	0.23	0.061
C 13:0	0.16	0.13	0.00	0.075
C14:0	10.65	8.84	1.04	0.061
C14:1	0.74	0.62	0.00	0.075
C15:0	1.27	0.97	0.00	0.045
C15:1	0.36	0.28	0.00	0.061
C16:0	28.46	33.27	39.30	0.005
C16:1	1.24	1.13	0.08	0.082
C17:0	0.72	0.56	0.00	0.061
C17:1	0.30	0.24	0.00	0.075
C18:0	12.94	10.25	5.22	0.005
C18:1n9t	3.25	2.37	0.74	0.061
C18:1n9c	23.86	25.90	37.82	0.061
C18:2n6t	0.24	0.18	0.23	0.037
C18:2n6c	2.48	4.94	14.68	0.046
C20:0	0.35	0.26	0.63	0.143
C20:1	0.82	0.56	0.11	0.005
C22:0	0.19	0.00	0.08	0.339
C18:3n3	0.48	0.43	0.00	0.061
C22:6n3	0.10	0.00	0.00	0.500

Significant higher values are in bold. LC: Low cholesterol, MC: Medium cholesterol, LC: Low cholesterol

contained 2.1% only. For further analyses, 4 groups were suggested: (1) Four samples of High Cholesterol (HC) with more than 98% cholesterol, (2) three samples of Medium Cholesterol (MC) with 90-97% cholesterol, (3) two samples of Low Cholesterol (LC) with 78-81% and (4) one sample Very Low Cholesterol (VLC), 2.1% only.

The two main factors of the PCA explained 74% of the total variance (respectively 56.1 and 17.8% for F1 and F2). The first factor was explained by similar opposition between the short chain and saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C13:0, C14:0) and some monounsaturated ones (C14:1, C15:1, C16:1, C17:1) from one side to polyunsaturated fatty acids (oleic C18:1n9 cis and linoleic acid C18:2n6 cis), behenic (C22:0) and nervonic acid (C24:1) on the other side. The correlation coefficients (r^2) of those acids with the first factor overpassed 0.950 ($p<0.01$) in all the cases. The supplementary variables are also highly correlated to this first factor with a clear opposition between cholesterol, close to short-chain and saturated fatty acids and phytosterols close to polyunsaturated fatty acids (Fig. 2) except brassicasterol which is correlated with the third factor mostly explained by linoleic (C18:2n6 trans), arachidic (C20:0) and docosahexaenoic (C22:6n3) acids with correlation coefficients

Table 5: Fatty acids and sterols composition of the ten commercial butter samples in Kazakhstan's market and standard values in Kazakhstan (GOST 32915-2014)

Number of butter samples	Fatty acids (%) 1	2	3	4	5	6	7	8	9	10	Standard
C4:0	1.98	2.34	2.28	1.00	1.90	2.83	3.14	0.00	2.72	2.66	2.4-4.2
C6:0	1.52	1.72	1.77	0.66	1.45	2.15	2.31	0.00	1.84	1.92	1.5-3.0
C8:0	1.01	1.20	1.31	0.42	1.10	1.48	1.59	0.05	1.15	1.29	1.0-2.0
C10:0	2.37	2.90	3.17	0.88	2.37	3.27	3.59	0.15	2.41	3.01	2.0-3.8
C11:0	0.25	0.26	0.29	0.00	0.22	0.32	0.31	0.00	0.25	0.32	
C12:0	2.98	3.57	4.18	1.13	3.84	3.78	4.74	0.35	2.78	3.66	2.0-4.4
C13:0	0.00	0.16	0.00	0.00	0.13	0.15	0.13	0.00	0.19	0.00	
C14:0	10.18	11.87	12.34	4.38	9.01	11.99	11.85	1.42	9.89	12.12	8.0-13.0
C14:1	0.80	0.82	0.87	0.22	0.58	0.93	0.81	0.04	0.90	1.00	0.6-1.5
C15:0	1.02	1.37	1.36	0.54	0.99	1.26	1.23	0.09	1.25	1.41	
C15:1	0.24	0.37	0.35	0.00	0.30	0.37	0.32	0.00	0.36	0.33	
C16:0	36.25	33.80	35.93	33.83	22.18	33.06	28.57	35.04	26.44	34.47	21.0-33.0
C16:1	1.04	1.55	1.81	0.38	0.79	1.39	1.26	0.16	1.07	1.73	1.5-2.4
C17:0	0.50	0.72	0.64	0.38	0.51	0.58	0.60	0.11	0.67	0.69	
C17:1	0.27	0.36	0.28	0.00	0.23	0.26	0.33	0.01	0.23	0.34	
C18:0	9.83	10.74	9.17	8.52	13.89	10.96	10.45	4.79	13.83	10.16	8.0-13.5
C18:1n9t	0.00	1.20	1.08	2.18	11.24	1.94	3.76	0.15	5.13	1.45	22.0-32.0
C18:1n9c	21.48	21.14	18.80	33.00	25.46	20.21	20.49	32.71	23.58	19.84	
C18:2n6t	0.13	0.18	0.17	0.18	0.64	0.17	0.18	0.11	0.25	0.17	2.5-5.5
C18:2n6c	5.92	1.91	2.72	10.78	1.66	1.89	2.74	23.25	2.18	1.60	
C20:0	0.24	0.22	0.22	0.32	0.42	0.07	0.00	0.28	0.31	0.24	<0.3
C20:1	0.97	0.63	0.51	0.47	0.80	0.42	1.02	0.15	1.94	0.67	
C18:3n3	0.54	0.45	0.41	0.36	0.00	0.00	0.00	0.00	0.00	0.50	<1.5
C22:1n9	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	
C22:0	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.20	0.00	0.00	<0.1
C22:2	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.06	0.00	0.00	
C20:4n6	0.00	0.00	0.00	0.00	0.00	0.14	0.20	0.00	0.29	0.00	
C20:5n3	0.00	0.00	0.12	0.00	0.20	0.35	0.39	0.37	0.35	0.00	
C24:1	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.53	0.00	0.00	
C22:6n3	0.48	0.27	0.14	0.21	0.40	0.00	0.00	0.00	0.00	0.40	
	Sterols (%)										
Cholesterol	91.3	95.3	99.0	78.7	80.7	98.2	99.0	2.1	95.3	99.4	
Brassicasterol	0.5	0.7	0.7	0.9	0.6	0.7	0.0	0.0	0.8	0.6	
Campesterine	2.3	0.0	0.0	3.8	6.2	1.1	1.0	12.9	1.5	0.0	
Stigmasterol	0.7	0.0	0.0	1.6	0.5	0.0	0.0	6.9	0.0	0.0	
β-sitosterol	5.3	4.0	0.3	14.5	12.0	0.0	0.0	78.1	2.4	0.0	

(r^2) of 0.652, 0.774 and 0.840 respectively. Most of the samples (HC, MC and LC) were projected close to cholesterol variable and short-chain fatty acids. The VLC samples (n°4 and 8) were on the opposite side.

According to the Kruskal-Wallis test, butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), undecanoic (C11:0) acids as well as stearic (C14:0), myristoleic (C14:1) and palmitoleic (C16:1) acids were significantly in higher proportion in HC and MC groups. On the reverse, low cholesterol butter contained significantly more oleic (C18:1n9 cis), behenic (C22:0) and eicosanoic (C20:1) acids (Table 6).

Atherogenicity index: The atherogenicity index varied from 0.8-3.02 in sour cream samples and from 0.73-3.42 in butter samples (Table 7). Significant positive correlations were observed between this index and the percentage of cholesterol in butter ($r = 0.725$; $p<0.01$) and sour cream samples ($r = 0.832$; $p<0.01$). Negative significant correlations were also reported for campesterine ($r = -0.858$; $p<0.05$), stigmasterol ($r = -0.736$; $p<0.05$) and β-sitosterol ($r = -0.740$; $p<0.05$) in butter samples. In sour cream samples, only stigmasterol ($r = -0.832$; $p<0.01$) and β-sitosterol ($r = -0.830$; $p<0.01$) were significantly negatively correlated.

The ratios of some fatty acids according to GOST 2014 were calculated for checking the authenticity of butter (Table 8). The ratio of fatty acids C16:0/C12:0 was representing the outgoing values for samples 4, 5 and 8. And for C18:2/C14:0 also in samples 1, 4, 5 and 8. For C18:0/C12:0 data corresponded for samples 4 and 8. The ratio C18:1/C14:0 corresponded for all samples from 4-9 and C18:1+C18:2/C12:0+C14:0+C16:0+C18:0 matched the data for samples 3, 4, 5 and 8.

Table 6: Mean values of fatty acids in butter according to the cholesterol level (VLC, LC, MC and HC) and p-value of the differences (Kruskal-Wallis test)

Fatty acids	HC	MC	LC	VLC	p-value
C4:0	2.73	2.35	1.45	0.00	0.045
C6:0	2.04	1.69	1.05	0.00	0.008
C8:0	1.42	1.12	0.76	0.05	0.005
C10:0	3.26	2.56	1.63	0.15	0.005
C11:0	0.31	0.25	0.11	0.00	0.002
C12:0	4.09	3.11	2.48	0.35	0.091
C13:0	0.07	0.12	0.06	0.00	0.596
C14:0	12.07	10.64	6.69	1.42	0.008
C14:1	0.90	0.84	0.40	0.04	0.045
C15:0	1.32	1.21	0.76	0.09	0.060
C15:1	0.34	0.32	0.15	0.00	0.160
C16:0	33.01	32.16	28.00	35.04	0.756
C16:1	1.55	1.22	0.59	0.16	0.027
C17:0	0.63	0.63	0.45	0.11	0.149
C17:1	0.30	0.29	0.12	0.01	0.086
C18:0	10.19	11.46	11.20	4.79	0.505
C18:1n9t	2.06	2.11	6.71	0.15	0.343
C18:1n9c	19.84	22.07	29.23	32.71	0.002
C18:2n6t	0.17	0.19	0.41	0.11	0.223
C18:2n6c	2.24	3.34	6.22	23.25	0.475
C20:0	0.13	0.26	0.37	0.28	0.016
C20:1	0.66	1.18	0.64	0.15	0.289
C18:3n3	0.23	0.33	0.18	0.00	0.682
C22:1n9	0.01	0.01	0.00	0.00	0.867
C22:0	0.00	0.00	0.09	0.20	0.044
C22:2	0.02	0.00	0.00	0.06	0.289
C20:4n6	0.09	0.10	0.00	0.00	0.800
C20:5n3	0.21	0.12	0.10	0.37	0.448
C24:1	0.00	0.08	0.00	0.53	0.078
C22:6n3	0.13	0.25	0.31	0.00	0.598

Significant higher values are in bold. VLC: Very low cholesterol, LC: Low cholesterol, MC: Medium cholesterol, HC: High cholesterol

Table 7: Values of atherogenicity index of the 10 butter and 7 sour-cream samples calculated according to the formula reported by Ulbricht and Southgate[15]

Butter		Sour-cream	
1	2.65	1	1.28
2	3.07	2	3.02
3	3.42	3	2.60
4	1.11	4	0.84
5	1.53	5	2.05
6	3.16	6	0.80
7	2.73	7	1.94
8	0.73	8	2.33
9	2.06		
10	3.25		

Table 8: Ratios of some fatty acids according to GOST 2014 to verify the authenticity of butter

Ratio	C16:0/C12:0	C18:0/C12:0	C18:1/C14:0	C18:2/C14:0	C18:1+C18:2/C12:0+ C14:0+C16:0+C18:0
GOST	5.80-14.5	1.90-5.90	1.60-3.60	0.10-0.50	0.40-0.70
1	12.17	3.30	2.11	0.71	0.46
2	9.46	3.01	2.98	0.34	0.41
3	8.59	2.19	2.61	0.39	0.37
4	29.94	7.54	9.71	2.64	0.96
5	5.78	3.62	14.06	0.82	0.80
6	8.75	2.90	3.63	0.32	0.40
7	6.03	2.21	5.49	0.42	0.49
8	101.51	13.88	23.11	16.43	1.35
9	9.51	4.97	7.51	0.47	0.59
10	9.42	2.78	3.09	0.31	0.38

Values in bold-italic characters correspond to out-values

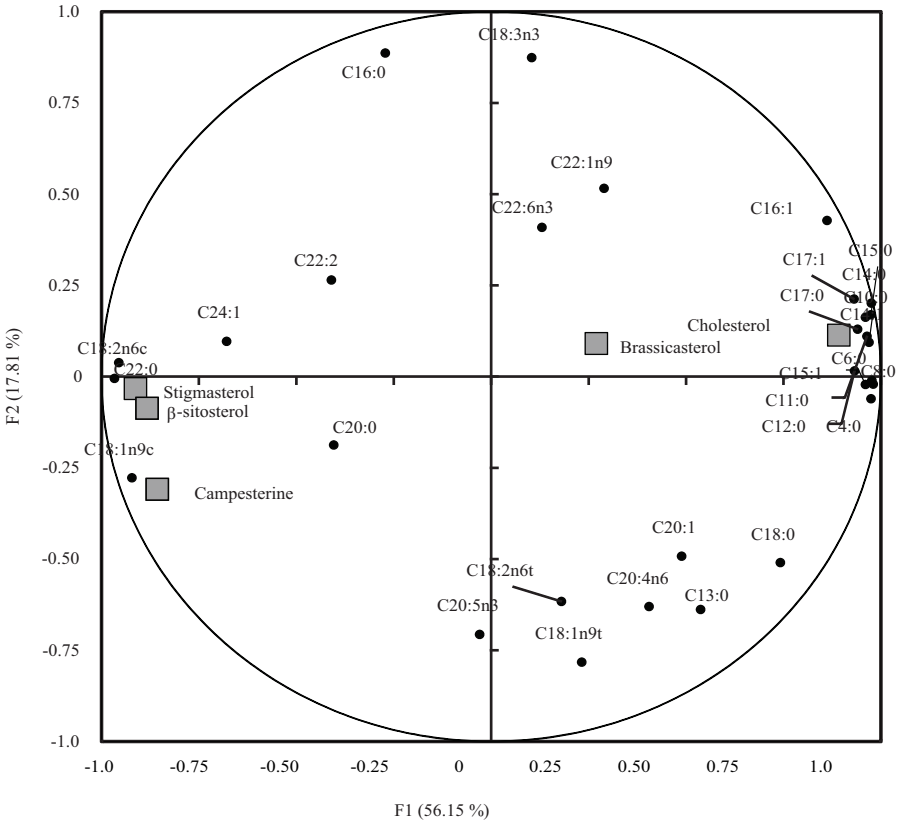

Fig. 2: Correlation circle of the PCA concerning butterfat composition and the projection of sterols

DISCUSSION

Six butter samples among the ten samples contained traces of phytosterols, including one containing up to 78% β-sitosterol. In sour cream samples, only three among eight contained 100% cholesterol while two contained more than 60% sitosterol. Thus, the substitution of milk fat by vegetable oil is common in dairy products commercialized in the country. To our knowledge, it is the first time that such an investigation was achieved in Kazakhstan. Few references are available in other countries. In Poland, only two samples of butter among sixteen contained phytosterols[4].

Most of the references regarding butter or cream adulteration discussed the equipment to be used. For detecting adulteration in the present study, gas chromatography with flame ionization detection was used. Other types of equipment were reported in the literature as Raman spectroscopy[18], differential scanning calorimetry[19,20], laser-induced breakdown spectroscopy[21], synchronous fluorescence spectroscopy[22], or photopyroelectric calorimetry[23]. The technique used in our study is globally accurate but costly and time-consuming. More rapid and low-cost techniques to detect adulteration are fluorescence spectroscopy[12] or FTIR-ATR spectroscopy[24]. However, those techniques were validated for fatty acids only, not for sterols and need to be standardized.

Regarding the methods used for describing milk fat, in Kazakhstan, two are currently applied by State control laboratory: National Standard (NS) n°31979-2012 "Milk and milk products. Detection method of vegetable fat in lipid phase by gas-liquid chromatography of sterols" focused on fatty acids profiles and NS n°32915-2014 "Milk and milk products. Determination of fatty acid content by Gas Chromatography method" focused on sterols profiles. However, for detecting milk fat adulteration, the first method only is officially used. Thus, the official detection of fat adulteration based on a certain standard of fatty profiles is imposed across the country despite the high variability observed[6]. Finally, the standard imposed by the legal authorities seems poorly corresponding to the reality of the field. Moreover, the methods used in Kazakhstan are moving from national to the Eurasian Economic Union (EEU) standards. Thus, a better adequation with field observations is essential, because of the risk of false-positive or negative responses in the national context. Such false responses could lead to small-scale farmers and dairy plants out of the law.

The official standard range for FA in Kazakhstan is calculated on 30 acids only, while in our samples 37 acids were determined. The percentages in sour cream and butter samples were recalculated based on 30 acids to allow a relevant comparison between our results and the official standard. Several studies regarding fat adulteration in dairy products were focused on the analysis of the fatty acids' profiles alone[25,26]. The partial substitution of milk fat by vegetable oils effectively changed the FA profiles. For example, in our sour cream samples, only those without traces of phytosterols contained a percentage of butyric acid (C4:0) within the official standard range of Kazakhstan (2.4-4.2%). On the reverse, samples containing a high proportion of phytosterols (for example sour-cream samples 4 and 6 or butter sample 8), had no butyric, caproic, caprylic, capric and undecanoic acids. However, in the case of slight substitution in butterfat (less than 10% vegetable oils), the changes in fatty acid profiles were reported as insufficient to determine the authenticity of milk fat[14]. Moreover, the FA profile alone did not allow assessing semi-quantitatively the level of fat substitution while sterol determination can detect the amount of fat substitution. In fact, according to a standard used in Kazakhstan for assessing fat adulteration, which includes 20 parameters (15 fatty acids and 5 FA ratios), all-butter samples should be adulterated. However, some pure butter samples containing 99% cholesterol (butter n°7 and 10) could be regarded adulterated just because of their fatty acid profile: indeed for sample 7, C12:0 was higher than the standard and C16:0 lower; for sample 10, C16:0 was higher (34.47 vs. 33.0), the sum C18:1n9t +C18:1n9c was lower (21.29 vs. 22.0) and C18:2n6t+C18:2n7c also lower (1.77 vs. 2.5 in official standard). Additionally, the FA profile could not detect a slight substitution of milk fat and reversely, some samples despite the lack of adulteration (100% cholesterol) did not correspond to the expected profile for pure dairy products. For example, butter samples n°3 or 7, despite having 99% cholesterol and being regarded as almost pure butter, had abnormal ratios of (C18:1+C18:2)/(C12:0+C14:0+C16:0+C18:0) (sample n°3) and C18:0/C14:0 (Sample n°7). Thus, regarding butter fat samples, 70% was substituted as shown by the presence of phytosterols (more than 1%) and 3 remaining samples were false positives when using the FA profiles proposed by the national standard. For sour cream, based on the presence of phytosterols, 25% of samples were highly adulterated while 37.5% were partially adulterated. Based solely on the fatty acid profiles, relative to the standard, two samples become false negatives. Finally, the assessment of fat adulteration in dairy products based on these indicators could be a source of misinterpretation.

It should be more convenient to use sterols profile as an arbitration method. Indeed, pure butter could not contain sterols other than cholesterol, except traces of isomer Δ7-cholesterol which usually is less than 1%[27]. A large substitution of milk fat by vegetable oils changed completely the sterols profiles. For example, with around 15% cholesterol only in our sour cream samples n°4 and 6 and 2.1% cholesterol only in butter sample n°8, a massive substitution occurred while in samples containing around 80-90% cholesterol, the substitution of milk fat was limited. Moreover, the advantage of sterol profile analysis could contribute to the determination of the possible origin of vegetable oil used for substitution contrary to the FA profile method. For example, tea-seed, peanut and sunflower oils contain more than 60% β-sitosterol, rapeseed oil is especially rich in brassicasterol and campesterol, while stigmasterol is in high proportion in soy-bean oil[28,29].

Therefore, the substitution of milk fat by vegetable oils constituted a fraud if it is not mentioned on the packaging, but unless the substituted oil is coming from technical products, the addition of oil of vegetable origin could be beneficial for health. The index of atherogenicity which expresses the coronary failure risk for consumers appeared highly correlated with cholesterol percentage in the butter and sour cream samples and reversely negatively correlated to some of the phytosterols. Effectively, the substitution of fat milk with vegetable oils increased the proportion of polyunsaturated fatty acids, which is a commercial argument for the agro-industry in carrying out such substitution. However, it has been indicated that consumption of industrial Trans Fatty Acids (TFA) produced via partial hydrogenation of vegetable oils increased the risk of coronary heart diseases. In reverse, ruminant TFA present in dairy products (cheese, butter and cream) as vaccenic (C18:1 trans-11) and rumenic acid (18:2 trans-11) may have beneficial health effects for consumers[30]. Unfortunately, those FA were not detected in the present study. Moreover, the presence of vegetable oil in dairy fat has not necessarily a "health effect" concerning the improvement of atherogenicity index if the oil used is "technical" rather than "alimentary". The health effect of vegetable oil is also discussed by recent research on the link between dietary cholesterol (exclusively animal origin) and cardiovascular risk[31]. Indeed, cholesterol is an essential component of cell membranes and a precursor of different biological molecules as bile acids, steroid hormones and vitamin D. Cholesterol is synthesized in the human body in case of low dietary intake while phytosterols are not. Contrary to phytosterols, poorly absorbed in the intestine and rapidly

excreted, the absorption of cholesterol is efficient. Moreover, plasma cholesterol concentration seems to be poorly influenced by dietary cholesterol[32]. Probably, genetic and nutritional factors are regulating more efficiently cholesterol absorption or synthesis. Finally, epidemiological studies do not confirm a link between dietary cholesterol and cardiovascular diseases[33].

In the case of dairy products fat adulteration by animal fat (lard, tallow), the sterols profiles analysis is not convenient. In those conditions, other indicators must be determined, such as 3,5 cholestadiene, which is the specific molecule of animal adipose tissue[34]. However, such substitution is not common because of its low economical interest as animal fat is relatively costly compared to vegetable oils.

Thus, three main recommendations could be suggested: (i) To inform consumers through explicit labels, (ii) To change the standard method for detecting milk product adulteration by using sterol profile and (iii) To evaluate the effective health benefit of the substitution of milk fat by vegetable oils.

CONCLUSION

The present study has determined the extent of fraud regarding the labeling of dairy products in the Kazakhstan market. Consumers should be able to differ between "authentic natural products" and "enriched or modified products" with vegetable oils. A clear indication of the origin of vegetable oils used as a fat substitute could be a commercial argument for health benefits. To better detect adulteration, it appeared that sterols profile determination was globally more efficient than FA profiles analysis, especially in the case of slight substitution. This is even more important as the current standard used in Central Asia based on some FA ratios can lead to incorrect conclusions.

SIGNIFICANCE STATEMENT

This study highlights the interest of favoring the analysis of sterol profiles rather than that of fatty acids to assess fat adulteration in butter and cream. This study will help the researcher to propose new legal standards to the lawmaker for the control of milk fat substitution by vegetable oils, in a national context where the occurrence of adulteration cases is high and can have a public health impact. Thus, a new theory on an assessment of fat adulteration by sterol profile can be primarily disseminated in the country.

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