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DETERMINATION OF MILK FAT ADULTERATION IN SOUR CREAM WITH VEGETABLE OILS BY GC-FID METHOD

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Abstract

Food adulteration appears in all areas of the food industry, most often to reduce production costs and to earn extra money. In the dairy industry, the adulteration of milk fat with vegetable oils is an act of intentionally reducing their qualities. Milk fat in milk and dairy products can be partially or completely substituted with vegetable oils. The most often adulterants of vegetable oils are palm oil and coconut oil. The aim of this study is determination of vegetable oils in sour cream by GC-FID method.

A total of fifty-five samples from the local market were analyzed in this study. Thirty-six samples were sour cream and nineteen samples were sour cream with peppers. Sample preparation was according to modified AOAC Official Method 996.06, 2005. The determination of fatty acids was carried out on a gas chromatograph with flame ionization detector (GC-FID). Butter milk fat, palm oil and coconut oil were used as reference standards. GC-FID analysis of fatty acids composition of sour cream in combination with multivariate statistical data processing, which included reference standards of fatty acids composition of milk fat, palm oil and coconut oil, were used for determination of vegetable fat.

The vegetable fats were detected in four samples (7.27%). In two samples were detected palm oil, in one sample was detected coconut oil, while in one sample was detected palm and coconut oil. Also, from the all positive samples of vegetable oils, in three samples the milk fat was partially substituted, while in one sample the milk fat was completely substituted.

This study provides a basic data for milk fat adulteration in sour cream with vegetable oils. The frequent monitoring of milk and dairy products, for the detection of vegetable fats, can prevent adulteration and protect the consumers from fraud.

***Key words**: Sour cream, Adulteration, GC-FID, Fatty acid.*

1. Introduction

Milk and dairy products in their natural form are good source of fats, proteins, carbohydrates, vitamins, minerals and thus have a high nutritive value. Milk fat is one of the most easily digested fats, while milk proteins contain amino acids needed for the growth of infants and children. According to this claim, milk and dairy products frequently are included in healthy diet consumed by large segments of population including infants, children, pregnant women and elderlies and they have enormous commercial importance in the food industry [1, 2, and 3].

Food adulteration appears in all areas of the food industry, most often to reduce production costs and to earn extra money. In addition to economic advantages the adulteration of food should be a health risk for consumers. The adulteration of milk and dairy products is old and illegal practice which in recent years has assumed a very serious dimension. Adulteration of milk and dairy products leads to reduction of their quality by removal of some valuable ingredient or substitution of inferior substances [1, 4, and 5].

From the various studies it can be concluded that the common milk adulterants are water, urea, detergent, skim milk, non-dairy proteins, melamine and oil (vegetable or animal), from which the water is the most commonly added adulterant in milk. Some of adulterants such as melamine and urea are a major risk to human health [1, 6].

The price of milk and dairy products in many countries around the world depends on the fat content. Because of this reason the adulteration of milk fat with vegetable oils is very popular and some manufacturers remove milk fat and compensate it by adding non-milk fat. The substitution of milk fat with vegetable oils can be partially or completely. The most often adulterants of vegetable oils are palm oil and coconut oil [6, 7].

Milk fat is one of the most complex fats due to the content of fatty acids and triacylglycerols. Moreover, milk fat is playing an important role for flavor in dairy products. The milk fat contains from 30 to 40 major fatty acids and a total of 400 fatty acids. The number of carbon atoms in milk fatty acids varying between 4 and 26. The content of saturated fatty acids is around 70%, the content of monounsaturated fatty acids is around 25% and the content of polyunsaturated fatty acids is around 5% [8, 9]. Palm oil has almost equal amounts of saturated and unsaturated fatty acids. Palmitic fatty acid (C16:0) and oleic fatty acid (C18:1n9c) are predominant fatty acids in palm oil and they are ranging from 39 to 45% and from 37 to 44%, respectively (6). The content of saturated fatty acids in coconut oil is ≥ 91%, and the main fatty acids is lauric fatty acid (C12:0) ranging around 48% [10 - 12].

To evaluate the authenticity of milk fat in milk and dairy products are required adequate control and adequate analytical methods. Determination of fatty acids composition and determination of triacylglycerols profile by gas chromatography are the most effective analytical methods to detect of foreign fats in milk and dairy products [1, 4, 11, and 12].

Having all this in mind, the aim of this study was to investigate the adulteration of sour cream milk fat with palm oil and coconut oil (as vegetable oils) through fatty acid composition with GC-FID method.

2. Materials and Methods

2.1 Samples

In this study were analyzed a total of fifty-five samples purchased from the local market. From the total samples thirty-six samples were sour cream and nineteen samples were sour cream with peppers.

2.2 Reagents

Diethyl ether, petroleum ether, methanol and sodium sulphate anhydrous were purchased from Carlo Erba. HPLC water, ammonium hydroxide, pyrogallic acid, chloroform, ethanol, toluene, n-heptane, hydrochloric acid and boron trifluoride (14% BF_3 (w/w) in methanol) were purchased from Merck. From commercially available 14% BF_3 solution were prepared 7% BF_3 solution in methanol.

Fatty acids methyl ester mix (F.A.M.E. Mix), C4:0 - C24:0 certified reference material (CRM) was purchased from Merck. F.A.M.E. Mix CRM was prepared with 1 ml of n-heptane and was injected into GC-FID. F.A.M.E. Mix CRM was used for identification of individual fatty acids in the samples by comparing the retention times and peak area of individual fatty acids between CRM and real samples.

For determination and quantification of milk fat, palm oil and coconut oil in sour cream were used CRMs as follows Butter fat (CRM for milk fat, purchased from Sigma Aldrich), Palm oil (CRM for palm oil, purchased from Fluka) and Coconut oil (CRM for coconut oil, purchased from Sigma Aldrich). The CRMs were prepared with the same procedure as the samples.

2.3 Sample preparation

Sample preparation is performed in two steps: extraction of fat and methylation (preparation of methyl esters of fatty acids). Firstly, the sour cream samples were homogenized on ultraturax (T25 Basic, Ika Labore Technik, Germany). After that fat extraction and methylation were carried out under the conditions specified in the AOAC Official Method 996.06 (William and Latimer, 2005), [13]. In the next step, the samples were injected into GC-FID for detection and identification of fatty acids composition.

2.4 GC-FID analysis

The determination of fatty acids composition in sour cream were performed on a GC-FID (GC Agilent Technologies 7890 GC System, USA). For separation of fatty acids were used HP88 column (60m x 250mm x 0.2 µm, Agilent Technologies, USA). In the Table 1 are given the temperature conditions.

Table 1. Temperature conditions of the GC column

Parameters	Rate ^{0}C/ min.	Value ^{0}C	Hold Time min.	Run Time min.
Initial	/	70	1	1
Ramp 1	5	100	2	9
Ramp 2	10	175	2	18.5
Ramp 3	3	220	5	38.5

The temperature of injector was 250 ^{0}C, while the temperature of detector was 300 ^{0}C. Carrier gas was helium (flow rate: 1.4 mL and split ratio 200 : 1). Make up gas was nitrogen (flow rate: 23 mL/min). The flow rates for H2 and synthetic air were 30 mL/min. and 400 mL/min., respectively). Injection volume was 1 µL

[14]. Each sample was analyzed two times into GC-FID The calculation of results for fatty acids composition in sour cream was made with Chemstation software. The results were expressed as percentage (%) of total fatty acid.

3. Results and Discussion

The results for fatty acid composition of CRMs butter, palm oil, coconut oil and examined sour cream samples are given in Table 2.

From the total 55 samples the vegetable oils were detected in four samples (7.27%). In two samples (3.64%) palm oil was detected, in one sample was detected coconut oil (1.82%), while in one sample were detected palm and coconut oil (1.82%). Also, from the all-positive samples adulterated with vegetable oils, in three samples the milk fat was partially substituted, while in one sample the milk fat was completely substituted with palm oil.

Chromatograms for fatty acid composition are shown from Figure 1 to Figure 8. The chromatograms of fatty acid composition for CRMs butter, palm oil and coconut oil are given in Figure 1, Figure 2, and Figure 3, respectively. The chromatogram of fatty acid composition of sour cream are given in Figure 4, while from Figure 5 to Figure 8 are given the chromatograms of fatty acid composition of adulterated sour cream samples. Figure 5 is chromatogram of fatty acid composition of sour cream adulterated with palm oil, Figure 6 is chromatogram of fatty acid composition of sour cream completely adulterated with palm oil, Figure 7 is chromatogram of fatty acid composition of sour cream adulterated with coconut oil and Figure 8 is chromatogram of fatty acid composition of sour cream adulterated with palm and coconut oil.

Table 2. Fatty acid composition in CRMs and sour cream samples

Fatty acids	CRM			Sour cream samples (n = 55)				
				Sour cream (n = 51)	Positive samples (n=4)			
	Butter (%)	Palm oil (%)	Coconut oil (%)	Mean values (%)	Samples with palm oil (n = 1) (%)	Samples with palm oil-completely substituted (n = 1) (%)	Sample with coconut oil (n = 1) (%)	Sample with palm and coconut oil (n = 1) (%)
C4:0 (butyric)	2.84	nd[2]	nd	2.91	0.25	nd	1.57	nd
C6:0 (caproic)	1.98	nd	0.62	1.93	0.22	nd	1.32	0.13
C8:0 (caprilic)	1.26	nd	7.60	1.24	0.19	nd	2.42	1.56
C10:0 (capric)	2.85	0.17	5.81	2.96	0.34	nd	3.92	1.28
C11:0 (undecylic)	0.05	nd	nd	0.05	nd	nd	0.14	nd
C12:0 (lauric)	3.54	0.24	46.02	3.61	0.59	0.20	19.19	11.77
C13:0 (tridecylic)	0.08	nd	nd	0.10	nd	nd	nd	nd
C14:0 (myristic)	11.23	0.99	18.56	12.01	2.32	1.06	13.82	5.09
C14:1 (myristoleic)	1.54	nd	nd	1.55	0.16	nd	0.65	nd
C15:0 (pentadecanoic)	1.19	0.04	nd	1.19	0.23	nd	0.79	nd
C15:1 (cis-10 pentadecenoic)	0.27	nd	nd	0.22	nd	nd	nd	nd
C16:0 (palmitic)	30.19	40.02	9.57	31.02	39.87	40.61	22.66	30.97
C16:1 (palmitoleic)	1.32	0.23	nd	1.42	0.39	0.19	1.10	0.23
C17:0 (marganic)	0.63	0.08	nd	0.63	0.18	nd	0.43	nd
C17:1 (cis-10 heptadecenoic)	0.33	nd	nd	0.31	nd	nd	nd	nd
C18:0 (stearic)	11.67	4.11	2.73	10.99	5.55	4.09	9.40	4.87
C18:1n9t (elaidic)	2.39	nd	nd	1.76	0.24	nd	0.90	nd
C18:1n9c (oleic)	23.63	43.05	7.03	22.81	39.97	40.61	18.64	32.84
C18:2n6t (linolelaidic)	0.24	0.10	nd	0.18	nd	nd	nd	nd
C18:2n6c (α-linoleic)	1.16	10.26	1.94	1.85	8.70	8.97	2.99	10.46
C20:0 (arachidic)	0.17	0.29	nd	0.18	nd	0.23	nd	0.63
C18:3n3 (α-linolenic)	0.69	0.15	nd	0.47	nd	0.25	nd	0.19
SFA[1]	67.68	45.94	91.03	68.82	49.74	46.19	75.66	56.23

[1]Saturated fatty acids (SFA), [2]Not detected (nd).

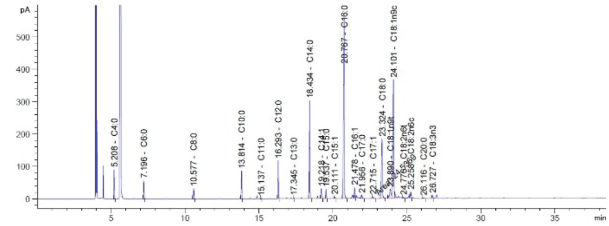

Figure 1. Chromatogram - fatty acid composition of CRM butter

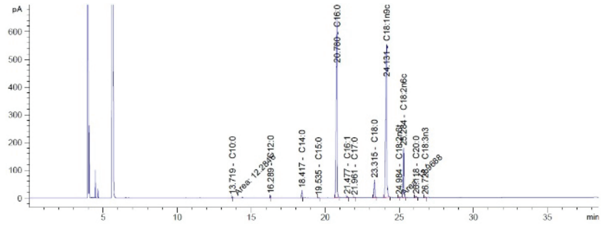

Figure 2. Chromatogram - fatty acid composition of CRM palm oil

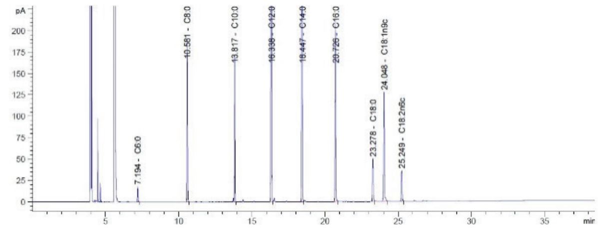

Figure 3. Chromatogram - fatty acid composition of CRM coconut oil

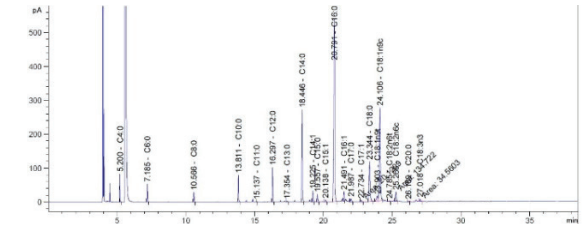

Figure 4. Chromatogram - fatty acid composition of sour cream

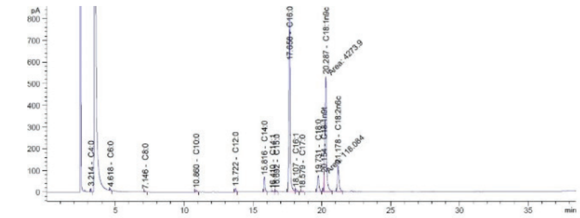

Figure 5. Chromatogram - fatty acid composition of sour cream adulterated with palm oil

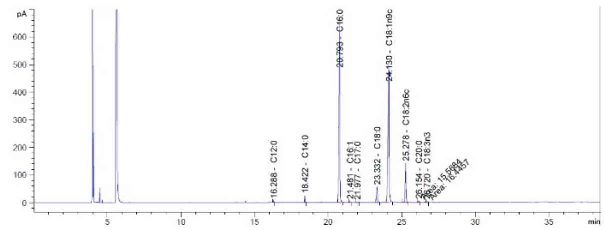

Figure 6. Chromatogram - fatty acid composition of sour cream adulterated with palm oil (completely substituted)

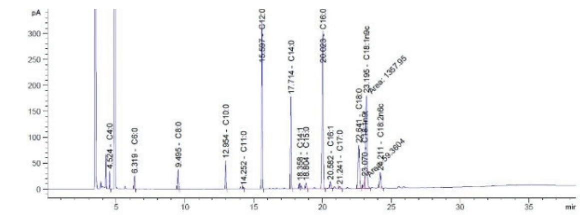

Figure 7. Chromatogram - fatty acid composition of sour cream adulterated with coconut oil

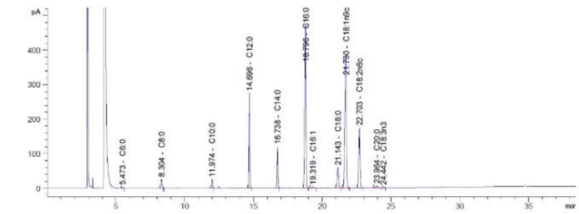

Figure 8. Chromatogram - fatty acid composition of sour cream adulterated with palm and coconut oil

The most common fatty acids in sour cream samples are myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:19c). Also, milk fat contains short chain fatty acid (C4:0-C12:0). The concentration of short chain fatty acids in sour cream is in agreement with the concentration of these fatty acids in CRM butter and the literature data [15, 16]. The concentration of SFA in sour cream samples is 68.82%. The concentration of SFA is in agreement with the concentration of SFA (67.68%) in the CRM butter. The contribution of individual fatty acids in sour cream samples found in this study was in accordance with the previously obtained results on the cow milk fat and with CRM for milk fat (butter) [15, 16].

In the cases of adulteration of sour cream samples with palm oil and coconut oil there is a difference in the fatty acid composition. In the sour cream adulterated with palm oil the concentration of C16:0, C18:1n9c and C18:2n6c is very higher compared with concentration of this fatty acids in sour cream samples and CRM for milk fat (butter), while the concentration of C12:0, C14:0 and short chain fatty acid is very low. The concentration of SFA is 49.74% versus concentration of SFA in sour cream samples and CRM butter, where the concentration of SFA is 68.82% and 67.68%, respectively. In the sample with palm oil, where the milk fat is completely substituted, the short chain fatty acids weren't detected, as well as C14:1, C15:0, C15:1, C17:0 and C17:1. The concentration of C16:0, C18:1n9c and C18:2n6c is very similar with concentration of these fatty acids in CRM palm oil. Also, the concentration of SFA is very low, than the concentration of SFA in sour cream samples and CRM butter. On the other hand, concentration of SFA in this sample is very similar with concentration of SFA in CRM palm oil. Kesenkas *et al.*, [17], reported that the concentration of short-chain fatty acids (C4:0-C12:0) and medium-chain fatty acids (C14:0-C16:1) in the milk fat were higher than milk fat which contain vegetable oils. Same authors reported that the concentration of SFA in milk fat is higher than the samples with vegetable oils.

In the sample adulterated with coconut oil the concentration of lauric acid (C12:0) is higher than the milk fat. This fatty acid is characteristic for coconut oil. Also, in this sample the concentration of C14:0 and capric acid (C10:0) is higher than concentrations of these fatty acids in milk fat. On the other hand, the concentration of C16:0 and C18:1n9 is lower than the concentration of these fatty acids in milk fat.

The concentration of SFA in this sample is 75.66%, while the concentration of SFA in CRM for milk fat is 67.68%. The results are agreement with the previously obtained results from Salem et all. Salem et. all reported that C4:0, C17:1, C18:2nt and C18:3n3 were not found in coconut oil, while C8:0, C12:0 and C14:0 fatty acids were the most abundant fatty acid. Moreover, they reported that C10:0, C12:0 and C14:0 fatty acids in coconut oil were higher than in pure fat milk [8].

In the sample adulterated with palm oil and coconut oil can be noticed that the concentration of C12:0, which is characteristic fatty acid for coconut oil, is higher than milk fat, and concentration of C18:1n9c and C18:2n6c, which are characteristic fatty acid for palm oil, are higher than milk fat. Also, C4:0, C14:1, C15:0, C15:1, C17:0 and C17:1 fatty acids were not detected and the concentration of the C6:0 in the sample is lower than the concentration of C6:0 in milk fat. The obtained results are in agreement with the results published by Salem *et al.*, and Soha *et al.*, [8, 9].

In addition, a concentration of individual fatty acids in milk could be indicators for adulterated formulations. In the case with adulteration with palm oil and coconut oil, concentration of C4:0 and total saturated fatty acids is the main indicator for authenticity of milk fat, concentration of C16:0, C18:1n9c and 18:2n6c could be indicator for adulteration with palm oil, while the concentration of C12:0, C10:0 and C16:0 could be indicator for adulteration with coconut oil.

4. Conclusions

- From the present study it can conclude that the adulteration of milk fat with vegetable oils is real and the content of vegetable oils (palm oil and coconut oil) were confirmed in the sour cream samples.
-To prevent the adulteration, it is necessary to introduce permanent control and monitoring of quality of milk and dairy products.
- In addition, the contents and the concentration of individual fatty acids, as well as, concentration of SFA, could be used as a criteria for detecting adulteration in milk fat.

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