

DETECTION OF NON-MILK FAT ADDITION IN KAJMAK PRODUCTION

Mira Radovanovic, Marina Hovjecki, Sanja Djurdjevic, Andjelija Stanojevic, Jelena Miocinovic

¹University of Belgrade, Faculty of Agriculture, Department of Animal Source Food Technology, Nemanjina 6, 11080 Belgrade, Serbia
Correspondence author: Mira Radovanovic, m.radovanovic@agrif.bg.ac.rs
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Abstract: Kajmak is a very popular dairy product in Balkan countries, however, it is often a subject of adulteration. The aim of this study was to investigate the effects of kajmak adulteration on its yield and composition, and determine the possibility of detecting the adulteration using the iodine and acid values and sensory evaluation of kajmak quality. In this study, experimental kajmak samples were produced from milk with increased fat contents (by 18 % and 36 %), via the addition of refined palm oil, pig fat and margarine to milk. The experimental kajmak samples had increased yield (21-32 %), changed composition and sensory quality. The control had the lowest iodine value (35.60), while the experimental kajmak with pig fat had the highest value of this constant (49.95), so the chemical constant - iodine value can be useful for detecting of adulterated milk fat in kajmak with refined palm oil, pig fat and margarine. However, these adulterants did not lead to a significant change in the acid value of the kajmak samples. The adulterants - refined palm oil and margarine in experimental kajmak samples could not be detected by sensory analysis.

Key words: adulteration, milk fat, kajmak, iodine value, acid value

Introduction

Kajmak is a specific fat based dairy product which is produced in regions of Southeast Europe, and some Asian regions such as Turkey, Iran, Afghanistan and India. According to its composition and characteristics, kajmak is classified as somewhere between cheese and butter (*Pudja et al., 2008*). Due to the distinct diversity of production methods and conditions in different regions a very large variations in the composition, quality and properties of kajmak often are found (*Cakmakci and Hayaloglu, 2011; Akarca et al., 2014; Mirecki et al., 2017*). In Balkan countries, kajmak is highly valued product and consider as an

autochthonous dairy product, which is usually manufactured from cow milk by the traditional method (Dozet et al., 2017).

Kajmak production begins with heat treatment and pouring of the hot milk in shallow vessels followed by spontaneous cooling of milk by surrounding air for 12 or more hours (Dozet et al., 2004). The kajmak formation process can be split into two phases: hot and cold phase (Pudja et al., 2008). At the beginning of the hot phase, milk fat and proteins are concentrated on the top of the milk forming a kind of thin matrix layer. During the cold phase, the kajmak skin layer is growing up, due to the incorporation of milk components, mostly milk fat (Radovanovic et al., 2013). When the cold phase completes, a layer on top of milk, known as kajmak is collected. The kajmak formation process and quality are affected by several factors such as composition and heat treatment of milk; and ambient air parameters - temperature, relative humidity and air circulation dynamic (Pudja et al., 2008).

Milk products based on milk fat such as ghee and butter, are highly prone to milk fat adulteration with cheaper oils and fats (Derewiaka et al., 2011; Kala, 2013; Uysal et al., 2013; Gandhi et al., 2015; Nedeljkovic, 2019). Adulteration of milk fat with cheaper oils and fats has been often followed without labeling the product appropriately. According to Ntakatsane et al. (2013), such a situation poses a risk to human health and decreases functional value of the product.

The specific process of kajmak manufacture and the growing demand placed it in the premium price group of products, making it attractive for adulteration. Milk fat, as the paramount constituent of the kajmak, possesses good flavour, pleasant aroma, high caloric value, and represents a source of valuable nutrients such as fat-soluble vitamins and essential fatty acids (Truong et al., 2020). Cheaper vegetable oils and animal fats (ex. pig fat) are often used as admixtures to replace a part of the milk fat and reduce the cost of kajmak production. However, the legislation requires that this product not contain any oils or fats of non-milk origin (*Official Gazette of the Republic of Serbia No. 34/2014*).

Studies related to the detection and quantification of foreign oils and fats in dairy products have been conducted for many decades. Detection of the adulterants in milk fat includes number of methods such as: Methods based on physical properties (melting point, opacity test, differential scanning calorimetry etc.); Spectroscopic methods (tests based on visible, infrared and Raman spectroscopy); Methods based on chemical properties (tests based on chemical constants - iodine value, Reichert-Meissl value, Polenske value; tests based on fatty acid profile using gas chromatography and tests based on the identification of DNA in milk fat) etc. (Sharma et al., 2020). Some of the methods require expensive equipment and trained staff, especially infrared and Raman spectroscopy, which have been widely used for years to characterize oils and fats. In contrast, the determination of chemical constants is fairly simple and relatively inexpensive tests are still used in practice. Furthermore, according to Dyminska et al. (2017), there is a good correlation between spectral parameters and the iodine value. The number of grams

of iodine absorbed by 100 g of fat under specified conditions represents the iodine value i.e. iodine number (*Sharma et al., 2020*). This constant is a measure of unsaturated linkages present in a fat. The iodine value for milk fat is lower in comparison to most of the other fats and oils except coconut oil (6-10) and palm kernel oil (10-18) (*Hazra et al., 2017*).

The acid value (acid number) is the quantity of base, expressed in milligrams of potassium hydroxide, which is required to neutralize all acidic constituents present in 1 g of sample (*ISO 660, 2009*). The acid value is a common parameter in the specification of fats and oils (*Koczon et al., 2008*). According to *Nielsen (2017)*, acid value of oils and fats are dependent on different factors such as free fatty acids (FFA), acid phosphate and conditions of the storage. The acid value of pure milk fat is 0.48 (*Park et al., 2007*). According to the *Codex Standard for edible fats and oils (1999)* and to the current Serbian legislation (*Official Gazette of the Republic of Serbia No. 43/2013*), the acid value of refined oils should be up to 0.6.

The aim of this study was to investigate the effects of the kajmak adulteration with non-milk fats (vegetable oil, animal fat and margarine), on its yield, composition, chemical constants (iodine and acid values) and sensory quality, as well as, to determine whether or not it is possible to detect the adulteration of kajmak using the chemical constants and sensory quality evaluation of the kajmak samples.

Material and Methods

Production of control and experimental kajmak samples

For each trial, 21 kg of raw milk (3.6% fat and 3.4% proteins, obtained from dairy farm “PIK Zemun”, Serbia) was portioned into seven 3 kg lots. Each lot was heat treated at 85°C for 10 min followed by 95°C for 10 min treatment – for production of both control and adulterated kajmak (experimental samples). The control sample was prepared without adding non-milk fats.

Six experimental samples were prepared by adding non-milk fats to milk at two different levels: 20 g and 40 g per lot, i.e. 6.7g/kg and 13.3 g/kg that represent an increase in fat content of 18% and 36% respectively (Tab. 1).

Table 1. Production of control and experimental kajmak samples

Kajmak	Non-milk fats	
	Amount added to milk, (g/kg)	
MM	Control sample	0.0
PU1	Palm refined oil	13.3
PU2		6.7
SM1	Pig fat	13.3
SM2		6.7
MG1	Margarine	13.3
MG2		6.7

The following non-milk fats were added: refined palm oil imported from Malaysia (d.o.o. "Uvita", Debeljaca, Serbia); pig fat ("Backa mast" Univerexport group Backa ad, Backa Palanka, Serbia); and margarine ("Vital", Vrbas, Serbia) (Tab. 2).

Table 2. Iodine and acid values of non-milk fats used for experimental kajmak production

Non-milk fats	Iodine value	Acid value
Palm refined oil ("Uvita")	55.82	0.11
Pig fat ("Backa mast")	62.99	1.91
Margarine ("Vital")	58.30	0.22

During the production of experimental samples - non-milk fats were added to milk at a temperature of 85°C in order to evenly melt and incorporate. After that, the milk was gently mixed for 3 minutes with a mixer and heated up to 95°C to complete the heating regime. The heat-treated milk samples were poured into round vessels where kajmak formation took place (dimensions 0.24 x 0.07 m) over 24 hours at room temperature imitating typical conditions in the traditional kajmak production, after which the kajmak was collected from the surface of the milk. The temperature of milk during collecting kajmak was 15°C. The kajmak samples were

weighed and analyzed 5 min after drainage on the grid. The experiments were carried out in triplicates.

Analytical analyses

Kajmak composition

Kajmak samples were analyzed for fat by butyrometric method (*FIL-IDF, 1986*), total nitrogen content was determined by Kjeldahl method (*AOAC, 1990*) on a Kjeltex System (Tecator 1002, Sweden), and total protein content was calculated by multiplying it with 6.38. Dry matter (DM) was determined by drying method at $102\pm 2^{\circ}\text{C}$ (*FIL-IDF, 1982*). Fat in dry matter (FDM), protein in dry matter (PDM), absolute amounts of fat and proteins in kajmak and the relative ratio of fat to protein ($\text{fat} \times 100 / (\text{fat} + \text{protein})$) were also calculated. All analysis were carried out at least in duplicate.

Determination of chemical constants (iodine and acid values)

Sample preparation

Approximately 100 g of kajmak was melted at 70°C and centrifuged at 2400 g/10 min (centrifuge model 5430; Eppendorf AG, Hamburg, Germany). Then the fat layer was removed and the residue was filtrated using cheesecloth. Finally, the cheesecloth was gently squeezed to extract every drop of liquid. The filtrate was reheated to 70°C and centrifuged at 2400 g/10 min. The clear fat supernatant was subjected to further examinations (iodine and acid values).

Determination of iodine value

Iodine value of the kajmak samples was determined by Wijs method (*ISO 3961, 2009*) and performed according to *Dyminska et al. (2017)*. The prepared kajmak sample was filtered through anhydrous Na_2SO_4 (Merck, Germany). The filtered kajmak sample was weighed (0.5500 – 0.6000g) into an erlenmeyer flask and 20 ml of reagent (cyclohexane: glacial acetic acid, 1:1) (Sigma-Aldrich, France) was added and the solution was mixed well. Then 25 ml of 0.1 mol/l Wijs-solution (Carl Roth, Germany) was added. Erlenmeyer was closed with a lid and placed in the dark for 60 minutes. After the reaction time, 20 ml of KJ (10% solution) was added. The sample was diluted with 150 ml of deionized water and titrated with 0.1 mol/l $\text{Na}_2\text{S}_2\text{O}_3$ until the solution became light orange. Then 10 drops of 2% starch solution were added and the titration was considered completed once the blue color disappeared. The Blank probe was prepared by the same way, but without the kajmak sample, and the blank value was determined as well.

Calculation:

Iodine value, (g/100g) = $((V_0 - V_1) \times C \times 12.69) / m$

Where:

- $(V_0 - V_1)$ is the difference between the volumes, in ml, of $\text{Na}_2\text{S}_2\text{O}_3$ required for the Blank probe and for the sample, respectively;
- C is the molarity of $\text{Na}_2\text{S}_2\text{O}_3$ solution, (mol/l);
- 12.69 is the conversion factor from mol $\text{Na}_2\text{S}_2\text{O}_3$ to grams of iodine (the molecular weight of iodine is 126.9 g/mol);
- m is the weight of the sample, (g).

Determination of acid value

Determination of acid value of kajmak was done with KOH ethanol solution and was performed according to method of *Koczon et al. (2008)*. The solvent (diethylether: ethanol absolute, 1:1) (Honeywell, Germany) was prepared by titrating it with 0.1 mol/l KOH in the presence of phenolphthalein as an indicator.

The sample of kajmak was weighed (about 10g) into an erlenmeyer flask and dissolved in 70 ml of the prepared solvent. After complete dissolution, the sample is titrated with 0.1 mol/l KOH. For the Blank probe, 70 ml of solvent was placed in an erlenmeyer flask and titrated with 0.1 mol/l KOH.

Calculation:

$$\text{Acid value (mg KOH/g)} = ((V_1 - V_0) \times 56.1 \times C) / m$$

Where:

- V_1 is volumes of KOH required for the sample titration, (ml);
- V_0 is volumes of KOH required for the Blank probe, (ml);
- C is the molarity of KOH solution, (mol/l);
- 56.1 is the conversion factor from 1 ml of solution to mol/l KOH (the molecular weight of KOH is 56.1 g/mol);
- m is the weight of the sample, (g).

Sensory quality evaluation

Sensory quality evaluation was performed according to the slightly modified method of *Koca and Metin (2004)* using a five-level quality scoring method as follows: excellent quality (quality score > 4.5); very good quality (3.5 <

score ≤ 4.5); good quality ($2.5 < \text{score} \leq 3.5$); poor/unsatisfactory quality ($1.5 < \text{score} \leq 2.5$); very poor quality ($0.5 \leq \text{score} \leq 1.5$); spoiled product/not for human nutrition ($0 \leq \text{mean score} < 0.5$). The five-level quality scoring was conducted by a sensory panel consisting of six members from the University of Belgrade, Faculty of Agriculture who were experienced in dairy product quality judging.

Overall sensory quality was assessed by evaluating four selected characteristics: appearance (including color), smell, flavor and texture. According to the individual impact on overall quality, the selected characteristics were assigned appropriate coefficients of importance: 3, 2, 6, and 9, respectively.

The quality of kajmak was expressed as a percentage of the total quality (from 0 to 100%) and was obtained by adding up the multiplied scores of every assessed characteristic of kajmak and appropriate coefficient of importance.

Statistical analysis

Data were analyzed in order to investigate the influence of palm oil, pig fat and margarine on the composition and yield of the produced experimental kajmak samples by using STATISTICA 6.0 (StatSoft, USA) data analysis software. Mean comparisons of the parameters were performed by LSD-test, with the levels of significance at 0.05.

Results and Discussion

Kajmak yield and composition

The increase in the content of fat in milk by adding non-milk fats (for 18% and 36%) led to an increase in the yield of kajmak (Tab. 3). Higher amounts of added non-milk fats (PU1, SM1, MG1) increased the fat content of milk by about 36%. These amounts of the fats resulted in an increase in the yield of kajmak by about 30% in the kajmak samples made with added pig fat and margarine ($P < 0.05$), while the addition of palm oil resulted in a slight increase in the yield of kajmak, which was not statistically significant ($P > 0.05$) (Tab. 3).

Lower amounts of added non-milk fats (PU2, SM2, MG2) increased the fat content of milk by about 18%. In these kajmak samples, the yield was statistically significantly increased ($P < 0.05$) in all samples with added the fats compared to the control. The yield increase was about 22% for all experimental samples – 21% with palm oil; 24% with margarine; 25% with pig fat (Tab. 3).

Table 3. Yield of kajmak samples

Kajmak	Yield, (g)	Kajmak	Yield, (g)
MM	136.79 ^a	MM	136.79 ^a
PU1	139.88 ^a	PU2	165.50 ^b
SM1	180.58 ^b	SM2	170.66 ^b
MG1	177.03 ^b	MG2	169.70 ^b

Values with different letters within the same column are significantly different ($P < 0.05$)

Milk fat was the dominant component in all kajmak samples. Increasing of fat content in milk (from 18% to 36%) contributed to the increase of % fat and % DM, as well as % FDM of kajmak samples. At same time, the increase in fat content in milk resulted in a slight decrease in % protein of the kajmak samples as well as % PDM (Tab. 4).

Table 4. Kajmak samples composition

Kajmak	Fat, (%)	Protein, (%)	DM, (%)	FDM, (%)	PDM, (%)
MM	57.50	3.93	63.97	89.26	6.10
PU1	64.00	3.89	73.87	90.91	5.53
PU2	60.75	4.03	71.25	89.72	5.95
SM1	65.00	3.75	71.77	93.30	5.38
SM2	57.00	3.90	63.08	86.42	5.91
MG1	60.50	3.95	67.79	91.23	5.96
MG2	59.00	3.92	67.27	89.84	5.97

The addition of the non-milk fats improved fat separation and increased kajmak yield. Experimental kajmak samples prepared with lower amounts of the added fats (PU2, SM2, MG2), had the percentages of fat and protein practically unchanged compared to the control sample. However, the absolute amounts of fat and protein were higher, so the yields were higher compared to the control sample of kajmak (Tab. 4, Fig. 1). The relative ratios of fat to protein were identical for all kajmak samples and ranged from 93 to 94 % for both percentage and absolute amounts (Fig. 1 C, D). With added higher amounts of the fats (PU1, SM1, MG1), this regularity was not established (Fig. 1 A, B).

The obtained kajmak samples were not compact, which was quite pronounced in kajmak produced with the added refined palm oil. In this case, during the kajmak collection, a considerable amount of the lower layer of kajmak was separated and lost, which affected the reduction of the fat content and the yield of the final kajmak. It is likely that a lower temperature when removing the kajmak from milk, would reduce these defects to a certain extent and contribute to a higher yield and compactness of the lower layer of the formed kajmak.

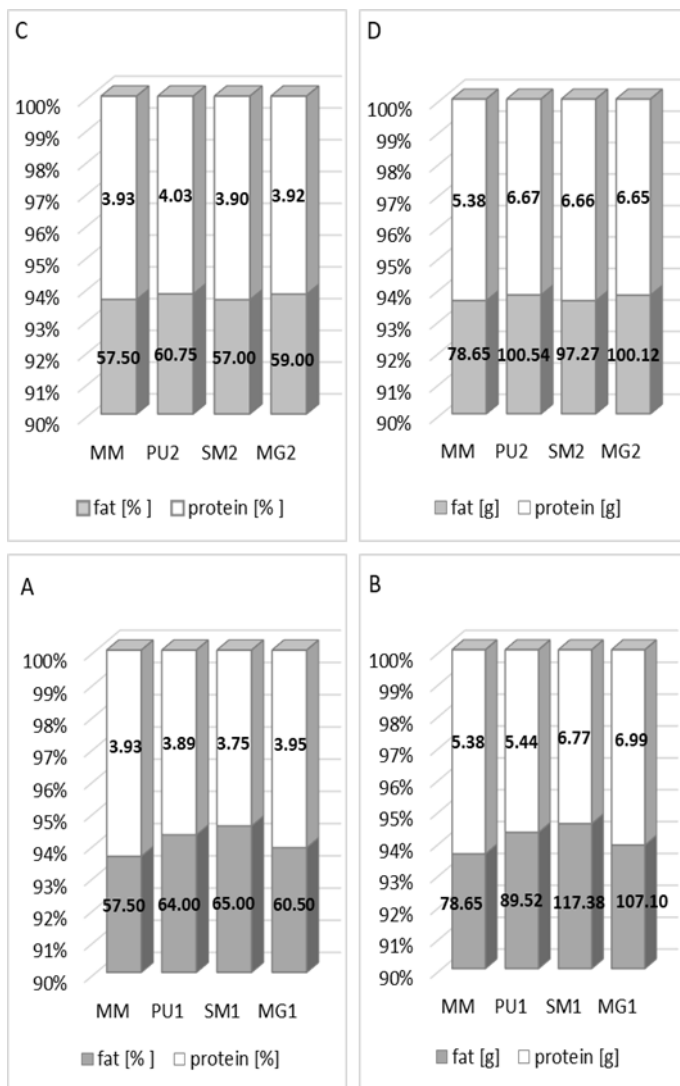


Figure 1. The relative ratios of fat to protein of kajmak samples

(The relative ratios of fat to protein for their percentage (A, C) and absolute amounts (B, D))

Iodine and acid values

The iodine value of milk fat in the control kajmak sample was 35.60, which is in accordance with the literature data for the iodine value of pure milk fat (*Gandhi et al., 2015; Salem et al., 2019; Sharma et al., 2020*). According to *Bolton (1999)* pure milk fat contains about 35% of total unsaturated acids, most of which consists of oleic acid. Because of that the iodine value for milk fat ranges from 26 to 35, which is lower in comparison to most of the other fats and oils (*Hazra et al., 2017*).

Animal body fats show slightly higher iodine values ranging from 36 to 49. However, for vegetable oils, the iodine value is very high (74-145), while for hydrogenated oil fats, it lies in the range of 70 to 79 (*Hazra et al., 2017*). According to *Tarmizi et al. (2008)* iodine value of refined palm oil is about 53, while for margarine it is about 56.88 (*Kahyaoglu and Cakmakci, 2016*). In accordance with our findings for obtained kajmak data in Table 6, it could be observed that the control kajmak sample had the lowest iodine value (35.60) among all the kajmak samples. On the other hand, the kajmak adulterated with pig fat had the highest iodine value (49.95) compared to the other kajmak samples.

Based on these results, it can be concluded that the iodine value is easily derived and can be successfully used as a screening test to detect the adulteration of milk fat in kajmak with refined palm oil, pig fat and margarine.

In agreement with our findings for kajmak, *Kahyaoglu and Cakmakci (2016)* reported that iodine value could be reliably used to discriminate pure butter from butter adulterated with the two margarine types in various mixing ratios. Also, according to *Metin (2008)* the iodine value of the milk fat increases with the addition of vegetable fats, except coconut oil (*Salem et al., 2019*). Additionally, it was found that iodine values in adulterated ghee are increasing proportionally to the level of added palm oil (*Kauser et al., 2022*).

According to *Salem et al. (2019)*, acid value of pure milk fat is 0.45, while for butter it is in interval from 0.45 to 0.70 (*Koczon et al., 2008*). Our result of acid value for milk fat in the control kajmak sample was 0.61. However, there were no significant differences in acid values between differently adulterated kajmak samples (Tab. 5), so the chemical constant - acid value is not suitable for determining adulterated milk fat in kajmak with oils and fat used in this study.

Table 5. Iodine and acid values of kajmak samples

Kajmak	Iodine value	Acid value
MM	35.60	0.61
PU1	40.76	0.64
SM1	49.95	0.61
MG1	45.49	0.57
PU2	38.16	0.54
SM2	42.67	0.69
MG2	40.07	0.64

Sensory quality evaluation

Samples of kajmak adulterated with palm oil and margarine (PU2, MG2) were as high evaluated as the control sample MM. On the other hand, the lowest sensory quality was assigned to the samples adulterated with pig fat for all quality parameters, due to the prevalence of the untypical smell of the pig fat (Tab. 6).

Determination of authenticity and detection of adulteration of dairy products have been performed by a number of analytical methods previously, and sensory analysis is recognized as one of them (*Kamal and Karoui, 2015*). Several authors considered the possible detection of adulterated dairy products on the basis of their sensory properties. The results of a research conducted to detect and discriminate fresh cheeses adulterated with different ratios of vegetable fat using sensory analysis, showed that the power of discrimination was high ($P < 0.05$) in each panelist and panel level for all attributes evaluated (*Juarez-Barrientos et al., 2019*). In our study, the fact that samples adulterated with palm oil or margarine (PU2, MG2) were highly evaluated as the control sample suggests that these adulterants may not be detectable by consumers.

Table 6. Quality of kajmak samples

Kajmak	Quality, (%)	Description of kajmak samples
MM	87	Typical pale-yellow color; typical smell; typical structure with a little separated serum
PU1	55	Typical pale-yellow color; typical smell; the kajmak is not compact, it is disintegrated; a larger amount of serum is extracted compared to other kajmak samples
PU2	86	Typical pale-yellow color; typical smell; the kajmak is compact, a larger amount of serum is released, but less compared to PU1
SM1	57	The color is lighter compared to the other samples; the smell is not typical, it smells like pig fat; the kajmak is compact during extraction, a larger amount of serum is separated compared to the control sample
SM2	53	The color is lighter compared to the other samples; the smell is not typical, it smells like pig fat; the kajmak is compact during extraction, a typical amount of serum is separated
MG1	80	Pronounced yellowish color of the kajmak; typical smell; the kajmak is compact and firm; a small amount of serum is separated;
MG2	86	Pronounced yellowish color of the kajmak; typical smell; the kajmak is compact and firm; a small amount of serum is separated;

Conclusions

Kajmak samples were prepared by adding non-milk fats to milk at two different levels, representing an increase in milk fat content by 18% and 36%. The kajmak samples produced from milk with an increased fat content of 18% had an increased yield (21-25%) and an increased content of absolute amounts of fat and proteins while their relative ratios remained unchanged. The kajmak produced from milk with an increased fat content of 36% had an increased yield of max. 32%. It was demonstrated that the chemical constant - iodine value can be useful for the detection of the adulterated milk fat as a screening test in all kajmak samples.

The kajmak samples adulterated with palm oil and margarine were sensory highly scored indicating that these adulterants could not be detected by consumers. In the further research, it would be interesting to investigate the effects of the range of concentrations considering these adulterants to establish the sensory detectable sensitivity threshold.

Detekcija dodavanja ne-mlečnih masti u proizvodnji kajmaka

Mira Radovanovic, Marina Hovjecki, Sanja Djurdjevic, Andjelija Stanojevic, Jelena Miocinovic

Rezime

Kajmak je veoma popularan mlečni proizvod na području Balkana i Bliskog istoka, mada je često predmet falsifikovanja. Cilj ovog rada je bio da se ispita uticaj falsifikovanja mlečne masti kajmaka na randman i hemijski sastav kajmaka, kao i da se ispita mogućnost detekcije falsifikovanja pomoću jodnog i kiselinskog broja, kao i senzorne analize kvaliteta kajmaka. Eksperimentalni uzorci kajmaka su pripremljeni dodavanjem ne-mlečnih masti (rafinisano palmino ulje, svinjska mast i margarin) u mleko za proizvodnju kajmaka, u količinama od 18% i 36%. Rezultati su pokazali da su eksperimentalni uzorci kajmaka imali povećan prinos (21-32%), izmenjen sastav i senzorni kvalitet. Kontrolni uzorak kajmaka je imao najnižu vrednost jodnog broja (35,60), dok je eksperimentalni uzorak sa svinjskom mašću imao najvišu vrednost (49,95), te se može zaključiti da se jodni broj može koristiti za detekciju falsifikovanja mlečne masti kajmaka. Vrednosti kiselinskog broja ispitivanih uzoraka kajmaka nisu bile značajno različite. Senzornom analizom kvaliteta kajmaka nije se moglo ustanoviti falsifikovanje mlečne masti rafinisanim palminim uljem i margarinom.

Ključne reči: falsifikovanje, mlečna mast, kajmak, jodni broj, kiselinski broj

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