Fatty acid composition of Kashkaval cheese with reduced sodium chloride content

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Abstract. The aim of the present study was to investigate the changes in the fatty acid profile and acid value of milkfat during ripening of Kashkaval cheese with reduced sodium chloride content. Three batches of Kashkaval cheese (with low 0.7%; medium 1.5% and standard 3.1% NaCl content) were produced. Analyses were carried out on days 1 and 45 of ripening. Based on the results obtained, it was established that lipolysis in Kashkaval cheese during ripening was significantly delayed ($p<0.05$) in all samples. A slight increase in Kashkaval fatty acid value during ripening was established. Not statistically significant changes in the fatty acid composition of the tested samples during the 45-day ripening period were found. The average values for saturated fatty acids (SFA) were 81.08 mg/100g and for the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were 11.03 and 1.04 mg/100g between control group (KSs with 3.1% NaCl content) and experimental groups (KMs with reduced NaCl content of 1.5% and KLs with a low NaCl content of 0.7 %) respectively. The predominant fatty acids (FA) were palmitic (C16, 37.56±1.13 mg/100g), myristic (C_{14}, 16.98±0.51 mg/100g), stearic (C_{18}, 15.35±0.46 mg/100g) and oleic acid ($C_{18:1}$, 10.47±0.31 mg/100g). At the end of the ripening period, Kashkaval cheese with low content of NaCl had a slightly higher acid value of 3.03±0.58 mg KOH/g in comparison with cheese samples with medium and standard NaCl content of 1.72±0.01 and 1.11±0.01 mg KOH/g respectively.

1 Introduction

Lipolysis is the biochemical process through which milk fat triglycerides are hydrolyzed into free fatty acids and glycerol [1]. The resulting fatty acids are considered the main precursor in flavor and aroma formation in mature cheeses [2]. While appropriate concentrations of free fatty acids have been shown to contribute to aroma formation, elevated contents can produce flavor defects in cheeses [3]. According to [4], excessive amounts of short-chain fatty acids can impart a rancid and soapy off-flavor to cheese.

In cheese manufacture, salting serves an important role by regulating the microbiological and biochemical processes, and markedly contributes to the sensory characteristics of the final product. Formation of diverse flavors and aromas in cheeses is directly related to the NaCl content, through which the processes of activation or inhibition of biochemical reactions during ripening and storage are controlled [5]. According to [6] who studied the effect of 1.9%, 1.2% or 0.9% salt concentrations and 33.0, 21.0 and 16.0% fat contents on lipolytic processes in cheeses, found that samples with a fat content of 33.0% by weight and salt contents of 1.9% and 0.9% had higher short-chain fatty acid levels compared to the other samples. Also [7] investigated the effect of reducing the amount of sodium chloride on lipolyic processes in Cheddar cheese. The authors found that in cheeses with lower sodium chloride content the concentrations of free fatty acid increased during the ripening period. Their findings are consistent with data obtained by [8], who observed a similar trend of lipolysis in low-sodium Cheddar cheese. In a study by [9], organoleptic defects and ketone formation were reported in low-sodium Cheddar cheese. In same time [10] evaluated the impact of sodium chloride reduction on the change in the lipid fraction in Parmesan cheese. Cheese with regular NaCl content was found to have higher concentrations of fatty acids ($C_{8:0}$ and $C_{18:0}$), while samples with low salt contents had higher fatty acid values $C_{10:0}$, $C_{14:1c}$ and $C_{16:0}$.

The aim of this work was to study the lipolytic changes in milk fat composition of NaCl-reduced Kashkaval cheese during ripening.

2 Materials and methods

2.1 Technology used in manufacturing cheese with reduced NaCl content

Kashkaval cheese samples with reduced NaCl content were produced for the purposes of this study in the education and production facilities of the Department of Milk and Dairy Products Technology, University of Food Technologies, Plovdiv.

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Raw cow's milk used in the experimental research complied with current regulatory requirements, according to Regulation 853/2004 [11] and Ordinance No 5/2023 [12]. Milk was adjusted to 0.75 casein: fat ratio (C/F) and subsequently thermized at a temperature of 63-65°C and cooled to 34±1°C. 20 ml of 50% $CaCl_2$ solution, DVS starter culture (consisting of *S. thermophilus*, *L. bulgaricus* and *L. helveticus* strains) and milk coagulation enzyme (Chr. Hansen's CHY-MAX®M) were added to the heat-treated and cooled milk. The next stage involved milk coagulation, curd processing and cooking of the curds at a temperature of 42°C. The cooked curd mass was drained and pressed, then cut into pieces and subjected to cheddaring. The cheddaring process lasted 1.5-2 h, pH of the curd reaching 5.20-5.25 upon its completion. The cheddared curds were then cut into thin slices, divided into three batches, which were thereafter manually heated, stretched and salted in a salt solution with 16.0%, 8.0% and 4.0% NaCl concentrations. Kashkaval cheese loaves were packed into molds of 1.0 kg. Fresh Kashkaval cheese loaves were left to set overnight at a temperature of 8-10°C, then taken out of the molds, air dried for several days and vacuum-sealed in foil. Ripening took place at a temperature of 9±1°Cand relative humidity of 75-80% for 45 days.

For the purposes of the study, three batches of Kashkaval cheese with different NaCl contents were produced: control group of samples KSs with 3.1% NaCl content; experimental group of samples KMs with reduced NaCl content of 1.5 %, and experimental group of samples KLs with a low NaCl content of 0.7 %. In this way the effect of reduced NaCl content on the process of lipolysis was evaluated. Changes occurring during ripening in the fatty acid composition and acid value of samples with different NaCl contents were compared.

3 Methods

3.1 Determination of fatty acid profile in Kashkaval cheese samples

Extraction of polar metabolites and fatty acids: 500.0 µl methanol was added to 100.0 mg of lyophilized, powdered material. Internal standards were added: 50.0 µl ribitol and 50.0 µl nonadecanoic acid (each in 10 mg/ml concentration); stirred by vortex for 10 s and incubation (30 min/70°C/300 rpm); cool to room temperature and add 300.0 µl distilled water +500.0 µl chloroform; after that again stirred by vortex for 10 sec. and centrifuge (10 min/22°C/13000 rpm).

Fatty acid fraction: 300.0 µl of the lower phase (a polar phase) was evaporated to dryness under vacuum. 1.0 ml (1M) sulfuric acid in methanol solution (transmethylation) was added to the dry residue followed by incubation at 96°Cfor 90 min; the cooled solution was extracted with hexane (3 × 500 ml); combined organic layers were evaporated to dryness under vacuum; 50 µl BSTFA (silylation reagent) and 50 µl pyridine were added followed by incubation at 70°C for 30 min.

Chromatographic conditions: 1.0 µl of the sample was injected into a system consisting of Agilent GC 7890A gas chromatograph and Agilent MD 5975C mass spectral detector. An HP-5MS column with the following parameters was used: length 52 m, diameter 0.32 mm and film coating thickness 0.25 µm at oven temperature program (temperature 70.0°C, hold 1 min, increase to 300.0°C at 5.0°C/min, hold 10 min); injector and detector temperatures 250.0 °C; the carrier gas used in this experiment is helium, with a flow rate of 1.0 ml/min. The mass detector is set to scan a range of m/z=50-550. The sample is injected with a volume of 1.0 µl, using a split flow ratio of 20:1.

3.2 Determination of acid value and acidity

Determination of acid value and acidity was made according to ISO 660:2020 [13]. The content of free fatty acids was expressed as

Acidity was calculated conventionally. The samples were dissolved in a mixture of solvents and the free fatty acids present were titrated using an ethanolic solution of potassium hydroxide.

Acidity as a percentage by weight is equivalent to:

$$V.c.M : 1000.100/W = (V.c.M):(10.W) \quad (1)$$

Where:
V = the volume of titrated potassium hydroxide solution used, in milliliters;
c = the exact concentration in moles per liter of the titrated solution of potassium hydroxide used;
M = the molar weight in grams per mole of the acid used to express the result (oleic acid = 282);
W = the weight in grams of the sample

3.3 Statistical analysis

The study's objectives required a comparison of the mean values of the triplicates. Therefore, one-way ANOVA was used with α=0.05 significance level. Tukey's multiple comparison test was performed with significance level of α=0.05. Results presented below include the corresponding p-values. In a given comparison, $P \leq 0.05$ indicated a statistically significant difference between the mean values of the studied quantities [14].

4 Results and discussion

Ripening is known to be a dynamic biochemical process that involves lactose hydrolysis, protein breakdown and possibly milk fat hydrolysis [15]. The fatty acid composition of milk fat in Kashkaval cheese samples with reduced NaCl content is presented in Table 1. Within the studied period, minor changes were observed and no statistically significant differences ($p>0.05$) were found in the fatty acid compositions of the samples. The minor changes found in the fatty acid composition at the end of ripening related to the heat treatment of the raw milk, leading to the inhibition of the better part of indigenous enzymes produced by bacteria present in the raw milk microflora. After thermization, the enzyme lipase known to be heat-resistant but highly sensitive to the salt content in the finished product remained active in milk. It is also known that the starter culture used in the manufacture of

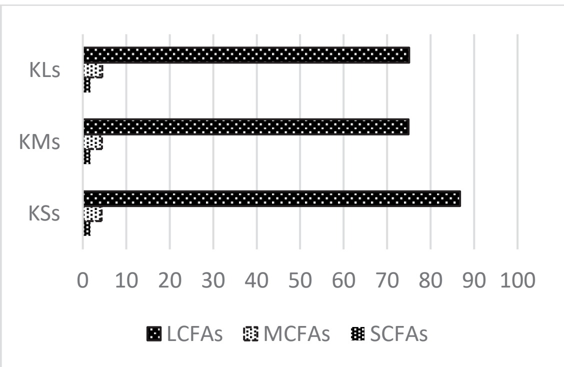
Kashkaval cheese does not have a high lipolytic activity. The predominant fatty acids in the milk fat in all of analyzed Kashkaval cheese samples from the three baches with different salt content were palmitic, myristic, stearic and oleic acids.

Table 1. Fatty acid composition of milk fat in Kashkaval cheese samples with reduced NaCl content

Fatty acids mg/100g	**RI**	**Samples**		
		KSs 45 d	**KMs 45 d**	**KLs 45 d**
Butyric acid ($C_{4:0}$)	730	1.75±0.05[a]	1.77±0.05[a]	1.78±0.05[a]
Caproic acid ($C_{6:0}$)	927	1.19±0.03[a]	1.21±0.04[a]	1.24±0.04[a]
Caprylic acid ($C_{8:0}$)	1126	0.96±0.03[a]	0.98±0.03[a]	0.99±0.03[a]
Capric acid ($C_{10:0}$)	1332	2.18±0.06[a]	2.20±0.07[a]	2.21±0.07[a]
Lauric acid ($C_{12:0}$)	1517	2.94±0.09[a]	2.96±0.09[a]	3.00±0.09[a]
Myristic acid ($C_{14:0}$)	1726	16.96±0.51[a]	16.98±0.51[a]	17.02±0.51[a]
Pentadecylic acid ($C_{15:0}$)	1818	0.55±0.02[a]	0.58±0.02[a]	0.58±0.02[a]
Palmitoleic acid ($C_{16:1}$)	1907	0.52±0.01[a]	0.55±0.02[a]	0.56±0.02[a]
Palmitic acid ($C_{16:0}$)	1920	37.55±1.13[a]	37.58±1.13[a]	37.62±1.13[a]
Margaric acid ($C_{17:0}$)	2025	0.59±0.02[a]	0.61±0.02[a]	0.60±0.02[a]
Linoleic acid (ω–3)	2093	1.03±0.03[a]	1.04±0.03[a]	1.05±0.03[a]
Oleic acid ($C_{18:1}$)	2104	10.48±0.31[a]	10.49±0.31[a]	10.51±0.32[a]
Stearic acid ($C_{18:0}$)	2121	15.36±0.46[a]	15.39±0.46[a]	15.40±0.46[a]
Arachidonic acid ($C_{20:0}$)	2323	0.39±0.01[a]	0.41±0.01[a]	0.39±0.01[a]
Behenic acid ($C_{22:0}$)	2516	0.16±0.01[a]	0.17±0.01[a]	0.18±0.01[a]
Lignoceric acid ($C_{24:0}$)	2730	0.26±0.01[a]	0.27±0.01[a]	0.28±0.01[a]
Cholesterol	3178	5.88±0.18[a]	5.87±0.18[a]	5.79±0.18[a]
Cholinic acids	3255	0.87±0.03[a]	0.90±0.03[a]	0.76±0.03[a]
Total		**99.62±2.99[a]**	**99.69±2.99[a]**	**99.96±3.00[a]**

**a-b means that values within a row designated with the same symbols are not statistically different according to Tukey's test (p≤0.05); RI = relative index; Samples with NaCl content (KSs-3.1 %, KMs-1.5 % and KLs-0.7 %).*

The data presented in Fig. 1 revealed that short-chain fatty acids (SCFAs) constitute approximately 1.75mg/100g of the total amount. The percentages of SCFAs in the cheese samples KLS, KMs, and KLs are 1.75, 1.77, and 1.78 mg/100g, respectively (Table 1).



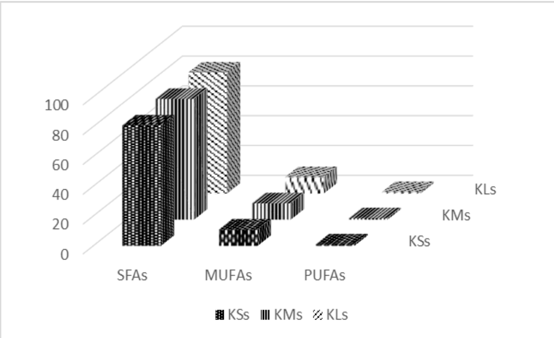
**Samples with NaCl content (KSs-3.1 %, KMs-1.5 % and KLs-0.7 %)*

Fig. 1. Quantitative distribution of fatty acids in Kashkaval cheese samples with reduced NaCl content

It is the short-chain fatty acids that contribute to flavor formation of Kashkaval cheese, since they act as flavor precursors for the production of various volatile compounds. Guinee and Surtherland [6] investigated the effect of different sodium concentrations in cheese on processes of lipolysis and found that short-chain fatty acid concentration increased in cheese samples.

Medium-chain fatty acids (MCFAs) in the three beaches of Kashkaval cheese samples accounted for approximately 4.38 mg/100g of the total amount of fatty acids, with the largest proportion made up by myristic and palmitic acids (Table 1). The percentages of MCFAs in the cheese samples KLS, KMs, and KLs are 4.33, 4.39, and 4.44 mg/100g, respectively (Fig.1). The long-chain fatty acids (LCFAs) with the highest contents were stearic and oleic fatty acids (Table 1). As evidenced by the data in Fig. 1, these long-chain fatty acids constitute approximately 87.3 mg/100g. The percentages of LCFAs in the cheese samples KLS, KMs, and KLs are 86.79, 74.95, and 75.07 mg/100g, respectively. (Table 1).

The high level of saturated fatty acids in the studied Kashkaval cheese samples correlated with low values of unsaturated fatty acids (Fig. 2). It should be noted that the amounts of unsaturated fatty acids (USFAs) in milk fat depend on animal feed, and since they are not synthesized in the body of ruminants their lack can cause a number of biological disorders [16]. Monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids are known to be present in low amounts in milk fat, however, they have exhibited a protective effect against cardiovascular diseases [17]. In this study the percentage saturated fatty acids (SFAs) and in the cheese samples KLS, KMs, and KLs were 80.84, 81.11, and 81.29 mg/100g, respectively (Fig. 2).



**Samples with NaCl content (KSs-3.1 %, KMs-1.5 % and KLs-0.7 %)*

Fig. 2. Quantitative distribution of saturated and unsaturated fatty acids in milk fat in Kashkaval cheese samples with reduced NaCl content

The percentage of uunsaturated fatty acids (USFAs) between control group (KSs with 3.1% NaCl content) and experimental groups (KMs with reduced NaCl content of 1.5% and KLs with a low NaCl content of 0.7 %) were 12,03, 12,08 and 12.12 mg/100g respectively In the three beaches of Kashkaval cheese samples, the average amount of SFAs was 81.08, and 12.08 mg/100g for USFAs (Fig. 2).

In the Kashkaval cheese samples studied, monounsaturated fatty acids were represented by oleic acid (C18:1) and palmitoleic acids (C16:1), whose average content is 10.49 and 0.54 mg/100g, respectively. The only representative of polyunsaturated fatty acids was linolenic acid; its values remained relatively stable, varying within narrow limits between 1.03 and 1.05 mg/100g within the studied period. Polyunsaturated acid content in our study was lower than that found by Marinho et al. [18] in Coalho cheese, which is probably due to various factors related to the animals feeding practices, breed, lactation period, etc.

The process of lipolityc change in the fat in Kashkaval cheese samples with reduced NaCl content in this study was estimated by the change in the indicator of acid value. An upward change in this indicator caused deterioration in the organoleptic characteristics, nutritional and biological value of the samples.

Table 2. Acid value changes in Kashkaval cheese samples with reduced NaCl content

Indicators	**Samples**					
	KSs		**KMs**		**KLs**	
	1d	**45d**	**1d**	**45d**	**1d**	**45d**
Acid value, mgKOH/g	0.87±0.01^a	1.11±0.01^b	1.01±0.02^a	1.72±0.01^b	2.87±0.01^a	3.03±0.01^b

** a-b means that values within a row designated with the same symbols are not statistically different according toTukey's test (p≤0.05); Samples with NaCl content (KSs-3.1 %, KMs-1.5 % and KLs-0.7 %)*

As evidenced by the data in Table 2, the indicators of acid value in the Kashkaval cheese samples with reduced salt content changed throughout the between 1st and 45th day of studied period. The acid value in samples KLs KMs, KSs underwent statistically significant ($p<0.05$) changes between days 1 and 45 of ripening. Reducing the amount of NaCl below 1.5% enhanced lipolysis, which was accompanied by an increase in the acid value. Similar tendencies were also reported by [19]. Thus, the results of the present study show that even a minimal reduction in the NaCl content could influence the lipolytic processes taking place in Kashkaval cheese during ripening.

5 Conclusion

The present study found no statistically significant differences ($p>0.05$) in the fatty acid composition between the experimental and control Kashkaval cheese samples at the end of the ripening processes. An increase in the acid value was recorded throughout the entire period of ripening, which suggested lipolysis. It was established that lowering the NaCl content in the samples resulted in an increase in the acid value. These results indicated that reduced NaCl concentration had a significant effect on the intensity of lipolysis in Kashkaval cheese during ripening.

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