Influence of salt concentration on microbial growth in Kashkaval cheese

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Abstract. The aim of the present study was to evaluate the influence of NaCl concentration on the microflora in Kashkaval cheese produced from cow's milk. Three cheese samples were obtained - with low (0.7%), medium (1.5%), and standard (3.1%) content of NaCl. Microbiological analyses were performed on the 1st, 15th, 30th, and 45th d of cheese ripening. It was established that the NaCl content has a significant ($p < 0.05$) influence on the growth and activity of the microflora in studied samples. It has been observed that the total Lactic acid bacteria (LAB) increased up to 30 d during ripening, after which their concentration decreased. A higher LABs count of samples with 0.7% NaCl and 1.5% NaCl in comparison with those containing 3.1% NaCl was found. At the same time, the variations in the salt content do not have a significant ($p > 0.05$) impact on the growth of Psychrotrophic bacteria, while in samples with a low salt content, the growth of Yeast and Molds was more intense. The data obtained in the present study showed that the concentration of NaCl is important for the regulation of activity of microbiological processes during the ripening of the Kashkaval cheese samples.

1 Introduction

The salt content in foods has been subject to many discussions with experts in the fields of science, medicine, and food industry. Awareness of the benefits and harms of salt is of primary importance for consumer in choosing the correct food, making it possible to avoid the harmful effects of excessive use of salt.

Salting is a very important stage in the cheese making technological process, contributing to: 1) additional draining of the cheese due to the hygroscopicity of dry salt or the osmotic pressure of the brines; 2) regulation of the water activity in the cheese, affecting the development and activity of microflora during cheese ripening [1]; 3) regulation of the enzymatic processes involved in cheese ripening [2]; 4) formation of a thin rind in certain varieties of dry-salted hard and semi-hard cheeses; 5) cheese flavour formation [3], and 6) the presence of a certain salt content in the cheese [4].

A number of authors have found that the reduction of salt or its partial replacement with a different type of salt has an adverse effect on the course of physicochemical and biochemical processes in cheese, the autolysis rate of the lactic acid microflora, and organoleptic properties during ripening and storage [5-7]. According to Gueene [8] a 50% reduction of sodium chloride in cheese leads to a decrease in the viability, cell permeabilization and cell autolysis of the starter culture strains. This is consistent with the results of McSweeney and Fox [9]; McMahon et al. [10] and Santiago-Lopez et al. [11], who observed LAB to be the main mediators for flavour development in cheese during ripening. Lactic acid bacteria produce different extracellular and intracellular enzymes that hydrolyses the cheese ingredients, with important organic compounds being accumulated as a result of their metabolic activity.

The aim of the present study was to evaluate the influence of NaCl concentration on microflora development in cow's milk cheese.

2 Materials and methods

2.1 Sample collection

Samples of cow's milk cheese were produced using traditional cheese making technology at the study and production facilities of the Department of Milk and Dairy Products Technology, University of Food Technologies, Plovdiv. For the purposes of the experimental work, three batches of Kashkaval cheese were manufactured: *KSs* – with a standard NaCl content of 3.1%; *KMs* – with reduced salt content of 1.5%, and *KLs* – with a low salt content of 0.5%. The effect of the reduced salt content was thus evaluated on the quality characteristics of Kashkaval cheese from cow's milk during period of 45 d of ripening.

2.2 Cheese analysis

Changes in the dynamics of chemical parameters were followed during the ripening period (1, 15, 30, and 45 d): moisture and dry matter [12]; NaCl content [13]; aqueous phase salt, calculated by a formula according to Lawrence

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and Gilles [14]; total nitrogen by the Kjeldahl method [15], and then the protein content was calculated as the total nitrogen multiplied by 6.38; determination of fat content [16]; potentiometric pH; a_w - the water activity of the samples was determined using a Novasina AG CH-8853 a_w meter (Zurich, Switzerland) at a temperature of 20°C. Initially, the sample was homogenized and placed in the sample-holder (a small plastic petri dish), filled to a maximum of 2/3 of its volume.

Changes in the starter microorganisms added with the starter culture were monitored during the period of ripening (Determination of total Lactic acid bacteria – cultivation on solid selective media M17 and MRS). Methodology followed the course described in [17, 18]. Samples were collected and prepared according to ISO [19]. The non-starter microflora was determined (psychrotrophic microorganisms [20]; moulds and yeasts [21]; coliform bacteria and *Escherichia coli* determined simultaneously by deep inoculation on Chromocult chromogenic coliform agar [22]; *Listeria monocytogenes* [23]; *Staphylococcus aureus* [24]; BDS EN ISO 6888-1:2022, and *Salmonella sp.* [25].

2.3 Statistical analysis

Computer processing of the results was performed by Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by LSD method. The results are presented as mean values ± SD (n=3). and were considered as statistically significant when $P < 0.05$.

3 Results and discussion

The composition of Kashkaval cheese samples with reduced NaCl content at the end of the ripening process is presented in Table 1.

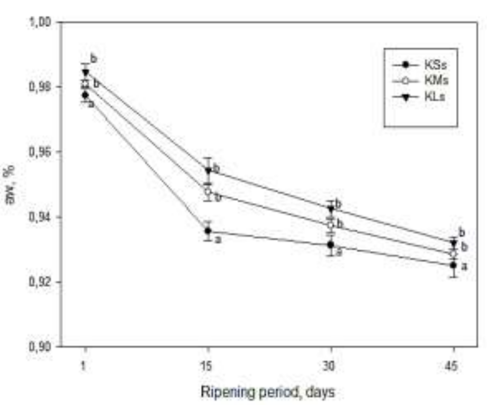
As evident, NaCl content was decreased 2.0 times for KMs and 4.0 times for KLs compared to the control sample KSs.

Table 1. Composition of Kashkaval cheese with different salt concentrations

Properties	**Kashkaval cheese samples**		
	KSs	**KMs**	**KLs**
Moisture, %	40.50±0.25a	40.80±0.22a	40.40±0.21a
Total solids (TS), %	59.50±0.23a	59.20±0.25a	59.60±0.23a
Fat content, %	26.50±0.50a	26.00±0.50a	27.00±0.50a
Proteins, %	28.7±0.50a	30.4±0.60b	30.6±0.40b
Salt content, %	3.10±0.05a	1.50±0.04b	0.70±0.03c
Salt-to-moisture ratio (S/M), %	7.70±0.14a	3.60±0.13b	1.70±0.05c

a-c Mean values designated with different letters within a row are significantly different (p < 0.05)
Data are presented as mean ± SD (standard deviation)

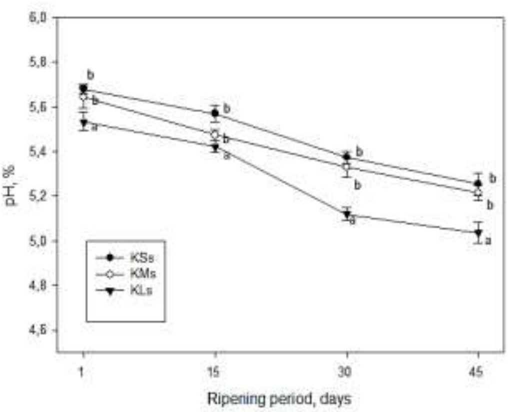
The salt content in the cheese aqueous phase is the indicator that largely determines the conditions for growth of lactic acid bacteria in the microenvironment. The recorded value of the S/M indicator was 7.70% for KSs, while for the rest of the samples it reciprocally decreased with the decrease in the amount of salt - 3.70% for KMs, and 1.70% for KLs. The trend observed in this study is consistent with that found by Khetra et al. [26], who reported a negative correlation between the S/M index of cheese and water activity. It has been demonstrated that there is an inverse relationship between the indicators of moisture content and salt [7, 27-29]. These authors found that the decrease in the amount of salt in cheese would increase its moisture content. In contrast, this dependence was not established in our study as it was focused on the production of cheese with three different amounts of salt in the total mass, while very close, almost identical values were reached for the dry matter and moisture indicators of the final product. It was found that during the experimental period, the samples of Kashkaval cheese had no statistically significant ($P > 0.05$) changes in the values of the chemical parameters such as total solids and fats content, probably due to the fact that ripening process is under vacuum conditions. The proteins content varies this can be explained by the higher losses of solids in the whey of these samples during processing. Changes of aw in Kashkaval cheese with different salt concentrations during the ripening period are given in Fig. 1.



a-b Mean values designated with different letters within a row are significantly different (p < 0.05). Data are presented as mean ± SD (standard deviation)

Fig. 1. Change of aw in Kashkaval cheese with different salt concentration

At the beginning of the process, the aw values were measured at 0.975, 0.982 and 0.985 for KSs, KMs and KLs, respectively. A more intensive change in the index was observed during the first 15 d of ripening, when the values decreased more significantly by about 0.045 in samples KSs and KMs, and only by 0.035 in sample KLs. After the 15th d until the end of the ripening period, there was relatively little change in this indicator for the three samples. The highest aw value was reported for sample KLs-0.932, followed by KMs-0.927, and the lowest was for KSs-0.921. Over the entire period, the water activity in the samples decreased by approximately 0.055. Our results are consistent with those obtained by Rulikowska et al. [30] for Cheddar cheese, according to which the aw index increases with the decrease in the amount of NaCl in cheese. The change in the pH indicator during ripening of Kashkaval cheese samples with reduced salt is presented in Fig. 2.



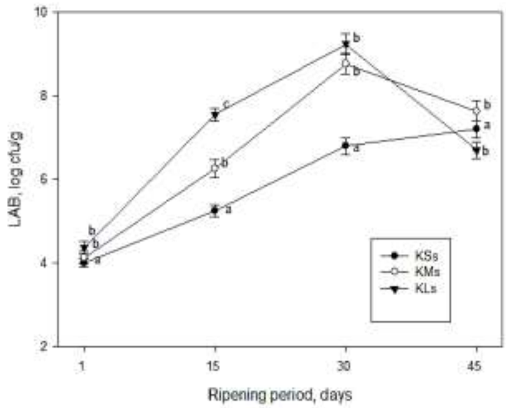
a-b Mean values designated with different letters within a row are significantly different (p < 0.05). Data are presented as mean ± SD (standard deviation)

Fig. 2. Change in pH in Kashkaval cheese with different salt concentrations

Dynamics in the development of the fermentation process in the samples directly reflected in changes in the pH values.

The fermentation process in the KSs samples occurred at a more gradual rate compared to the rest of the samples. Throughout the ripening period, the pH dropped by about 0.40 units. This trend was closely associated with the NaCl content of the samples, which also affected the growth and activity of the lactic acid microflora (Fig. 3). The lactic acid process was most intense in the KLs samples, where the pH values decreased more rapidly, regardless of the buffering capacity of the cheese. The results obtained for this indicator are evidence of activation of the fermentation process in samples KLs, compared to samples KMs and KSs. These results are consistent with the results of Ganesan et al. [31] and Arboatti et al. [32], who found no statistical differences between pH in Mozzarella cheese where the sodium content was reduced by 35% to 60%. This can be due to the higher a_w founded in our research.

For optimal ripening process, there should be proper conditions for growth of microflora (Fig. 3). The results herein show that at the beginning of the ripening process the total lactic acid bacteria count in the three samples was about 4 log cfu/g.



a-c Mean values designated with different letters within a row are significantly different (p < 0.05). Data are presented as mean ± SD (standard deviation)

Fig. 3. Change in LAB in Kashkaval cheese with different salt concentrations

In the course of ripening, their number increased rapidly. This tendency was more pronounced in the samples with lower NaCl content (KMs, KLs) compared to the control sample KSs. It is evident from the results that the development of lactic acid bacteria was slightly suppressed given the higher NaCl content, which reflected on the activity of microorganisms.

Between days 1 and 30, the lactic acid bacteria increased by 3.4 cfu/g in KSs to 4.6 log cfu/g in KMs, and by 5.0 log cfu/g in KLs. After that period, the number of lactic acid bacteria decreased and at the end of ripening LAB population was reduced by about 2.0-3.0 log cfu/g. The higher amount of bacterial biomass found for KLs and KMs can be explained by the more gradual change in pH in these samples. In the manufacture of Minas cheese, with partial replacement of sodium chloride by potassium (0%, 25% and 50%) and addition of arginine (1% w/w), Felicio et al. [33] observed similar trends.

Nonstarter microflora growth during ripening of Kashkaval cheese with reduced NaCl content is shown in Table 2. The analysis was conducted as control with the aim to prove the absence or presence, and the number of groups, genera or types of microorganisms with an adverse effect on the microbiological quality and safety of the product. The results did not reveal presence of pathogenic (*Listeria monocytogenes, Staphylococcus spp., Salmonella spp.*) or indicator (coliforms) organisms. Mould and yeast counts were < 10 cfu/g by day 15 for sample KLs, and by day 30 for samples KSs and KMs. With the progress of the ripening process the amount of mould and yeast increased to 1.2×10^2 cfu/g for KSs, followed by KMs with 3.7×10^2 cfu/g, and to 1.5×10^3 cfu/g for KLs. The trend observed was directly connected with several major factors related to the development of these microorganisms, namely: chemical composition of Kashkaval cheese (pH, aw, oxidation-reduction potential, nutrients, biological structure, sub-stances with antimicrobial activity), biological relationship between the microorganisms in the samples and proper technological processing of the raw material and final product. At the start of the ripening process, the amount of psychrotrophic microorganisms ranged from 1.0 to 1.2 × 10^2 cfu/g. During the studied period, their quantity increased and reached values from 1.1 to 1.6 × 10^5 cfu/g for the three samples, with the highest concentration in sample KSs.

Table 2. Survival of nonstarter microflora in Kashkaval cheese with different salt concentrations

Nonstarter microflora, cfu/g	**Kashkaval cheese samples**			
	KSs			
	1d	15d	30d	45d
Psychrotrophic bacteria	1.0 × 10^2	2.4 × 10^3	1.5 × 10^4	1.6 × 10^5
Yeast and mould	< 10	< 10	< 10	1.2 × 10^2
Coliforms	ND	ND	ND	ND
L. monocytogenes	ND	ND	ND	ND
Staphylococcus spp.	ND	ND	ND	ND
Salmonella spp.	ND	ND	ND	ND
	KMs			
Psychrotrophic bacteria	1.2 × 10^2	2.0 × 10^3	1.3 × 10^4	1.2 × 10^5
Yeast and mould	< 10	< 10	< 10	3.7 × 10^2
Coliforms	ND	ND	ND	ND
L. monocytogenes	ND	ND	ND	ND
Staphylococcus spp.	ND	ND	ND	ND
Salmonella spp.	ND	ND	ND	ND
	KLs			
Psychrotrophic bacteria	1.1 × 10^2	2.1 × 10^3	1.0 × 10^4	1.1 × 10^5
Yeast and mould	< 10	< 10	1.1 × 10^2	1.5 × 10^3
Coliforms	ND	ND	ND	ND
L. monocytogenes	ND	ND	ND	ND
Staphylococcus spp.	ND	ND	ND	ND
Salmonella spp.	ND	ND	ND	ND

ND-not detected

Similar conclusions about the nonstarter microflora of cow's milk Kashkaval cheese ripened at different temperatures were drawn by Ivanova et al. [34].

This could probably be due to the more gradual fermentation process, where a higher pH value was found in the medium (Fig. 2), a factor favoring the development of this particular microflora.

4. Conclusion

Reduction of the NaCl content in Kashkaval cheese samples has a significant impact on the activity and development of the cheese microflora. During the ripening process, samples with 0.7% and 1.5% NaCl had higher counts of lactic acid bacteria compared to those containing 3.1% NaCl. At the same time, variations in the salt content did not have a significant ($p > 0.05$) effect on the growth of *Psychrotrophic bacteria*, while in the low-salt samples yeast and mould growth was more intensive. The results obtained in this study indicate that the NaCl concentration has an important role in the regulation of microbiological processes, which necessitates further in-depth studies to find alternative approaches to the partial reduction of salt content or manufacture of salt-free cheeses that are, however, of unchanged composition and quality.

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