Effect of storage temperatures on Kashkaval texture

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Keywords:

Kashkaval
Storage
Temperature
Pasta-filata cheese
Texture

Article history:

Received 21.08.2020
Received in revised form 16.02.2021
Accepted 30.06.2021

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DOI: 10.24263/2304-974X-2021-10-2-3

Abstract

Introduction. The aim of the present study was to investigate the effect of storage temperature on the texture parameters of cow milk Kashkaval cheese.

Materials and methods. Kashkaval cheese samples were prepared according to a classic technology and stored at different temperatures (4.0±1.0 °C; 1.0±1.0 °C; 7.5±0.5 °C and -18.0±1.0 °C). Texture analysis was performed by StableMicroSystems TA-XT2i analyser equipped with a loading cell 50 kg.

Results and discussion. The obtained results showed a significant difference in the values of the hardness index between the cheese samples stored in a refrigerated state and those stored in a superchilled and frozen state. With increasing storage temperature of cheese, there was a tendency to decrease ($p<0.05$) the values of the cohesiveness indicator. The results obtained in the present study showed that the storage temperature had a decisive influence on the changes in the springiness of the cheese. Higher storage temperatures (4.0±1.0 °C) were accompanied by a significant decrease in the springiness of the cheese. The storage of the Kashkaval cheese in a refrigerated state was accompanied by a significant increase ($p<0.05$) in its adhesiveness. This trend intensified with the increase of the storage temperature. With the increase of the storage temperature of cheese, more significant decrease ($p<0.05$) of the values of the gumminess index was observed. In a comparative analysis of the changes in the hardness and gumminess of the experimental Kashkaval cheese samples, it was found that within the same temperature regime the decrease in the values of gumminess was greater than that of the hardness. As all three indicators declined in the process of refrigerated storage, it reflected significantly on the gumminess.

Conclusion. More intense changes in the texture of the studied Kashkaval cheese samples were observed with the increase of the storage temperature.

Introduction

The texture of foods, in particular cheeses, is a subject of mandatory research over the last decade (Bolhuis and Forde, 2020). The structural and mechanical characteristics of cheeses are essential and determine their sensory perception by the consumer (Foegeding and Drake, 2007).

Modern technologies, such as curd or cheese freezing, are applied during storage in order to achieve a longer cheese shelf life (Alinovi and Mucchetti, 2020; Marcuzzo et al., 2012). During the storage of the cheeses, changes in their texture occur. Many authors prove that the texture of the cheese is influenced by a number of factors, such as milk composition, water content, salt content, pH and degree of proteolysis during ripening (Hill and Kethireddipalli, 2013). The texture profile of the cheeses also changes during their storage, as the temperature and the storage time are determining factors for the degree of changes. Some authors suggest that the decrease in hardness and increase in the softness of fresh cheeses is directly related to the proteolytic activity of lactic acid bacteria, which breaks down proteins into peptides and subsequently into low molecular weight peptides and amino acids (Fox et al., 2017). This disrupts the integrity of the protein network in the cheeses during storage and gives them softness (Fox et al., 2017).

Some researchers suggest that cheeses with high protein and low fat content have higher values of hardness and elasticity (Borges et al., 2020). The same trend is observed for the stability of the gumminess indicator, which in turn is associated with the hardness and springiness of the cheese by other author teams (Diamantino et al., 2014). It is found that the decrease in pH resistance leads to a loss of colloidal maintenance of calcium phosphate by the casein submicel, as result of which small aggregates are formed. This in turn leads to softening of the cheese (Buriti et al., 2005).

According to Diefes et al. (1993), the dehydration of proteins and the formation of ice crystals in the cheese during freezing and its storage in the frozen state can cause the destruction of the structure of the proteins. This in turn allows the small oil balls to come into contact with each other and thus form granules. Kuo and Gunasekaran (2003) reported that prolonged storage of cheese in a frozen state could cause greater damage to its structure as a result of recrystallization of ice crystals.

After thawing, proteins are not able to completely bind water. As a result, water remains free in the protein network, leading to the formation of a porous protein matrix of the cheeses, which are stored frozen for a longer time (Degner et al., 2014). Experimental studies with an electron microscope have shown that freezing and thawing cause the formation of larger cavities between the protein fibres in cheese samples due to the growth of ice crystals (Graiver et al., 2004).

Kuo and Gunasekaran (2009) investigated how freezing and frozen storage affect the microstructure of mozzarella cheese and pizza cheese. The authors observed visible differences in the microstructures of the two types of cheese. Freezing has a different effect on siren matrices. It causes the mozzarella protein matrix to break, while pizza cheese cracks and accumulates bacteria. The authors found that the protein matrix of pizza cheese was more susceptible to freezing due to freezing than that of mozzarella cheese, with tempering of the samples mitigating the negative effect of freezing. Disturbances in the structure of the cheese caused by freezing and storage in a frozen state can be minor with proper storage and tempering.

Although there are published data on the possibilities of using frozen curd in the production of Kashkaval cheese, no data examining the influence of the storage regime on its texture were found.

The *aim* of the present study was to investigate the effect of storage temperature on the texture parameters of cow milk Kashkaval cheese.

Materials and methods

Materials

The used raw cow milk corresponded to the national and European regulations (Regulation 853/2004). It was used in order to produce Kashkaval cheese samples stored at different temperatures with a known composition (Ivanov and Markova, 2020) and sensory profile (Ivanov et al., 2020).

Starter culture containing *Strepococcus thermophiles*, *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* was supplied by Lactina Ltd. Calcium dichloride solution (50%) as well as the rennet enzyme were purchased from Biokom Trendafilov Ltd.

Methods

Kashkaval preparation. Kashkaval samples were produced in a local dairy plant according to a classic applied technology (Kozhev, 2006). Cow milk was standardized in order to achieve casein to fat ratio equal to 0.69. It was further heat-treated at 65±1 °C for 15 s and cooled to temperature of incubation 33±1 °C. The prepared cow milk was inoculated with a thermophilic starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and calcium chloride 50% (diluted 1:10 with water, in amount 30 cm^3/100 L milk) and commercial animal rennet enzyme was also added (diluted 1:10 with water, in such amount that the first coagulation started 9-10 min after enzyme addition). When a firm coagulum was formed (after 30 min), the curd was cut and held in rest for 5 min. A stirring process of the curd started for 20 min. It was further heated at 38-40 °C for 40 min with continuous agitation. The formed whey was separated from the curd and it was collected in the form of a compact mass in order to assure the cheddaring process which took place at pH 5.3 (about 2 h). The curd submitted to cheddaring was cut, milled, salted and stretched in a concentrated salt solution (13%) at 72 °C. The fresh Kashkaval was moulded into 1 kg parallelepiped forms. It was dried for 15 h and was further vacuum-packaged in polyethylene bags. The fresh Kashkaval was subjected to ripening at 9.0±1.0 °C for 60 days. Storage of ripened Kashkaval was conducted at different temperatures (refrigerated at 4.0±1.0 °C; refrigerated at 1.0±1.0 °C; frozen at 7.5±0.5 °C and deep frozen at -18.0±1.0 °C) for 12 months. Different stages (1^{st}, 3^{rd}, 6^{th}, 9^{th} and 12^{th} month) of the storage period were investigated.

Texture Profile Analysis. Cheese cubes with dimensions 25·25·25 mm were prepared. A measurement of the sample sizes (length, width and height) by digital caliper and measurement of the sample weight by laboratory scales was done.

A texture analyzer StableMicroSystems TA-XT2i (Stable Micro System, Ltd, UK) equipped with loading cell 50 kg and specialized software „Texture Exponent 6.1 was used in order to determine their textural parameters. The applied software allowed calibrating the force and the sample height before the measurements. The deformation range for the instrument work was 0-500 mm (with resolution 0.001 mm) and the force range was 0-500 N (resolution: 0.001 N, trigger force was 0.05N, minimal measured firmness force was 1 N).

The texture profile analysis was made by a flat plate probe (P 50-Stainless steel cylinder probe, Ø=50 mm) with test speed 1 $mm.s^{-1}$, a strain of 50% and two bite time intervals of 5 s. The test was repeated on 3 cubes.

The textural characteristics of the cheese were determined as follow: Firmness force (F) was determined as the maximum force coordinate of 1^{st} peek of the curve; Adhesiveness was defined as the stress, necessary to pull up the measure probe after the 50% compression (negative integrated area, Nmm); Springiness was the distance of the detected height of the product on the second compression divided by the original compression distance where (Length 2 /Length 1); Cohesiveness was defined as the ratio between the 2nd and 1st loading area (A_2/A_1); Gumminess was computed like cohesiveness multiplied by the firmness; Chewiness was computed like cohesiveness multiplied by the firmness and the springiness (Zheng et al., 2016). The test was repeated on 3 cubes.

Statistical analysis. All statistical procedures were computed using the Microsoft Excel and Sigma Plot 2001 software. Statistical analysis was conducted by one-way ANOVA analysis (Donchev et al., 2002). Results were presented as mean of n=4 (2 batches with 2 repetitions) ± standard deviation (SD) and were considered as statistically different when $p<0.05$ (Petrova, 2002; Dilcheva and Kinova, 2008).

Results and discussion

The obtained results for the change of the hardness of the experimental samples of Kashkaval cheese in the process of refrigerated storage are presented in Figure 1.

There was a decrease in the values of the hardness index in all tested samples. The smallest decrease in hardness was found in Kaskaval cheese stored superchilled (-7.5±0.5 °C) and frozen (-18.0±1.0 °C). At the end of storage, the hardness values in these cheese samples were reduced to 61.4±1.5 N. In the present study, no statistically significant ($p<0.05$) differences in the hardness of Kashkaval cheese stored in superchilled and frozen state were found. This was probably due to similar changes in the microstructure of these samples due to the absence of intense proteolytic processes in the cheese matrix.

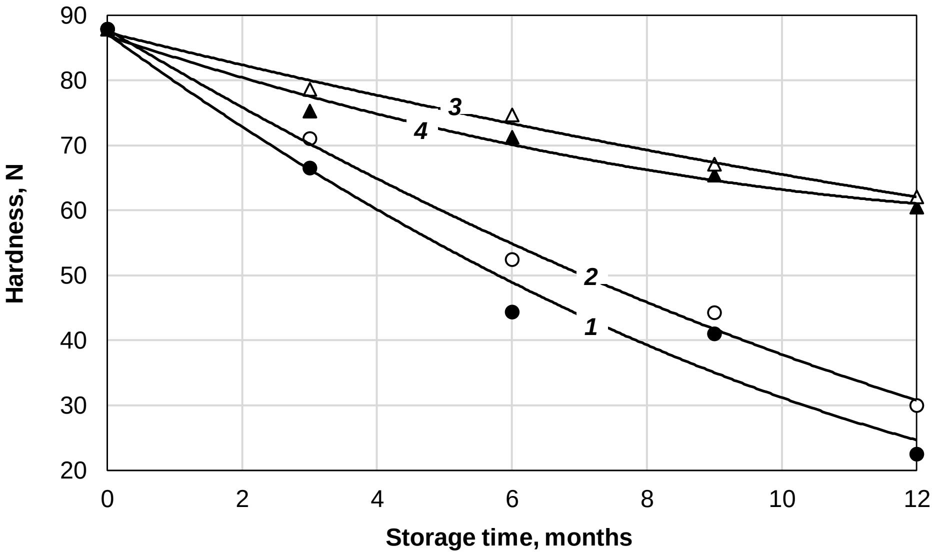

Figure 1. Changes in the hardness index of the analysed Kashkaval cheese samples from cow's milk stored in a refrigerated, superchilled and frozen state:
1 – Refrigerated Kashkaval (4±1 °C);
2 – Refrigerated Kashkaval (1±1 °C);
3 – Superchilled Kashkaval (-7±1 °C);
4 – Frozen Kashkaval (-18±1 °C).

With increasing storage temperature of Kashkaval cheese, more significant decrease ($p>0.05$) in the values of the hardness index was observed. During the 12-month storage period of the Kashkaval cheese samples at temperatures of 1.0±1.0 °C, their hardness decreased from 87.8±4.2 N to 29.9±3.3 N. For the cheese stored at the temperature regime of 4.0±1.0 °C, the decrease in the values of the hardness index was from 87.8±4.2 N to 22.5±2.8 N. The obtained results (Figure 1) indicated the direct dependence of the changes in the hardness of the cheese with the storage temperature. Alvarenga et al. (2011) investigated the effect of freezing on the rheology of sheep cheese samples. The authors found that the hardness index in frozen cheese samples was significantly higher than in chilled cheese samples. Diefes et al. (1993) suggested that this was due to the partial dehydration of the proteins in the cheese matrix of frozen cheeses. As a result, the cheeses acquire a firmer (harder) and elastic structure.

The obtained results showed that even the minimum temperature difference of 3 °C in the two storage modes of the refrigerated Kashkaval cheese samples (1.0±1.0 °C and 4.0±1.0 °C) led to significant differences in their hardness. The significant difference in the values of the hardness index between the cheese samples stored in a refrigerated state and those stored in a superchilled and frozen state was significant ($p<0.05$). As it was found during the storage of Kashkaval cheese in superchilled and frozen state, the proteolytic processes were almost completely inhibited (Ivanov and Markova, 2020). This favoured the preservation of its structure, which explained the higher hardness of these samples. Probably the phase transition of water in the process of superchilling and freezing accompanied by the formation of ice crystals destroyed to a lesser extent the structure of Kashkaval cheese compared to proteolysis. On the other hand, the cheese stored in a refrigerated state lacked a phase transition of water and the formation of ice crystals, but the ongoing proteolytic processes significantly weakened the paracasein matrix, which was accompanied by a decrease in its hardness.

The obtained results for the change of the indicator during storage are presented in Figure 2. In the present study, no statistically significant change in the values of this indicator was found in cheese stored in superchilled (-7.5±0.5 °C) and frozen (-18.0±1.0 °C) state. This indicator characterized the strength of the bonds in the paracasein matrix of Kashkaval cheese (Gunasekaran and Ak, 2004).

After 12 months of storage, the values of the cohesiveness index in the analysed cheese samples were maintained at the levels of 0.47±0.02. This showed that these two refrigeration regimes provided minimal changes in the structure of the cheese, which corresponded to the observed tendency for the change in the hardness of the analysed samples. The absence of intensive proteolytic processes in the cheese samples stored in superchilled and frozen state contributed to the preservation of their structure and their greater hardness compared to cheese stored in a refrigerated state.

With increasing storage temperature of cheese, there was a tendency to decrease ($p<0.05$) the values of the cohesiveness indicator. During the 12-month storage period of the cheese samples at temperatures of 1.0±1.0 °C, their cohesiveness decreased from 0.49±0.02 N to 0.43±0.01 N. These changes in the cohesivenes indicator were minimal, but statistically significant ($p<0.05$). A more significant decrease in the cohesiveness values was observed during the storage process at 4.0±1.0 °C. For the whole period of refrigerated storage (12 months) the decrease in the values of the cohesiveness indicator in these samples was from 0.49±0.02 to 0.24±0.02.

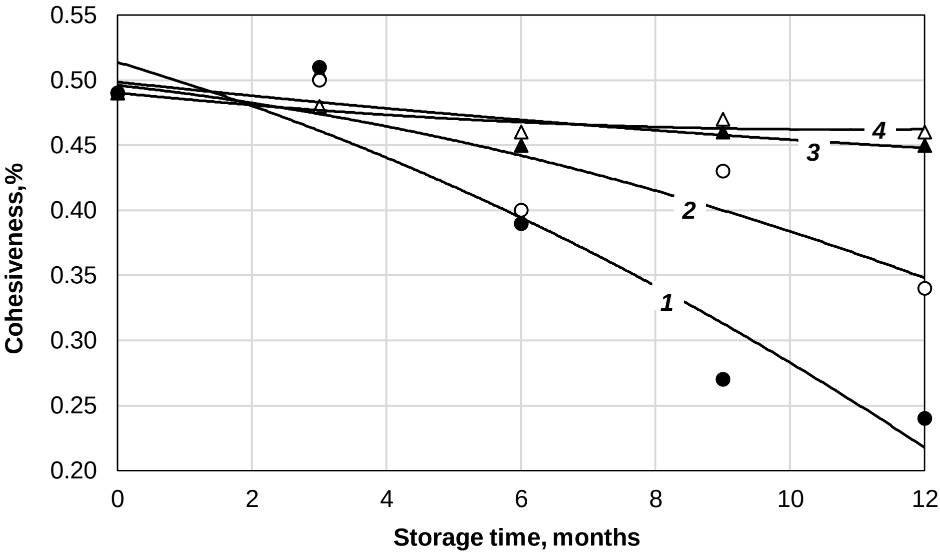

Figure 2. Changes in the cohesiveness index of the analysed Kashkaval cheese samples from cow's milk stored in a refrigerated, superchilled and frozen state:
1 – Refrigerated Kashkaval (4±1 °C);
2 – Refrigerated Kashkaval (1±1 °C);
3 – Superchilled Kashkaval (-7±1 °C);
4 – Frozen Kashkaval (-18±1 °C).

Everar et al. (2006) investigated the structural and mechanical properties of cheddar cheese left to mature for 9 months under refrigerated conditions. The indicators hardness, springiness, cohesiveness, adhesiveness and gumminess were studied. After the 9th month of storage, there was a decrease in the values of springiness, cohesiveness, adhesiveness and gumminess in the tested cheese samples whist was in correspondence of our results. Aday et al. (2010) examined white semi-hard cheese placed to mature at 2–4 °C for 12 months. After 9 months of cheese samples storage, the authors found that the cohesiveness decreased.

The obtained results in the present study (Figure 2) indicated a statistically significant ($p<0.05$) influence of the temperature regime of Kashkaval storage on the changes in the cohesiveness index. This effect was most noticeable when the cheese was stored in a refrigerated state, in which even the minimum temperature difference of 3 °C between the two experimental modes (1.0±1.0 °C and 4.0±1.0 °C) led to significant differences in the values of the cohesiveness indicator.

The data obtained in the present study on the change of the springiness index of the experimental samples of Kashkaval cheese in the process of refrigerated storage are presented in Figure 3. This indicator reflected the speed and extent to which a deformed object regained its original shape and size (Gwartney et al., 2004).

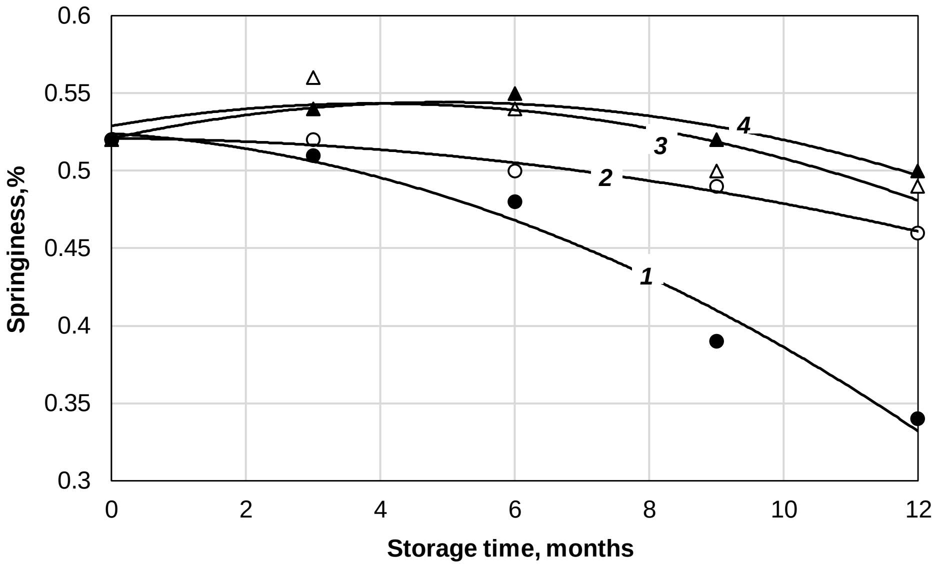

Figure 3. Changes in the springiness index of the analysed Kashkaval cheese samples from cow's milk stored in a refrigerated, superchilled and frozen state:
1 – Refrigerated Kashkaval (4±1 °C);
2 – Refrigerated Kashkaval (1±1 °C);
3 – Superchilled Kashkaval (-7±1 °C);
4 – Frozen Kashkaval (-18±1 °C).

In the present study, no statistically significant change in the springiness of the Kashkaval cheese samples stored in the superchilled (-7.5±0.5 °C) and frozen state (-18.0±1.0 °C) was found. At the end of storage, during these two refrigeration regimes, the elasticity of the cheese was preserved at about 0.49±0.02%. This trend corresponded to the lack of statistically significant changes in the indicators of hardness and cohesiveness of yellow cheese samples stored in superchilled and frozen conditions.

The storage of the cheese in a refrigerated state was accompanied by a decrease ($p<0.05$) in its springiness. This trend intensified with the increase of the storage temperature. During the 12-month storage period of the cheese samples at temperatures of 1.0±1.0 °C, their springiness decreased from 0.52±0.01% to 0.46±0.02%. Similar to the changes in the homogeneity of these samples, the observed decrease in the values of the elasticity index was minimal but statistically significant ($p<0.05$). A more significant decrease in the elasticity of the cheese was observed during storage at 4.0±1.0 °C. For the whole period of refrigerated storage (12 months) the decrease in the values of the elasticity index in these samples was from 0.52±0.02% to 0.34±0.02%. Zheng et al. (2016) examined the texture of cheddar cheese. The authors found that with increasing storage temperature, the springiness of the studied cheese samples decreased. The correlation between storage temperature and springiness index was negative.

The results obtained in the present study (Figure 3) showed that the storage temperature had a decisive influence on the changes in the springiness of the cheese. Higher storage temperatures (4.0±1.0 °C) were accompanied by a significant decrease in the springiness of the cheese, which was probably due to the ongoing proteolytic processes leading to weakening of the bonds in the paracasein matrix.

The data on the change of the adhesiveness index of the yellow cheese in the process of refrigerated storage are presented in Figure 4.

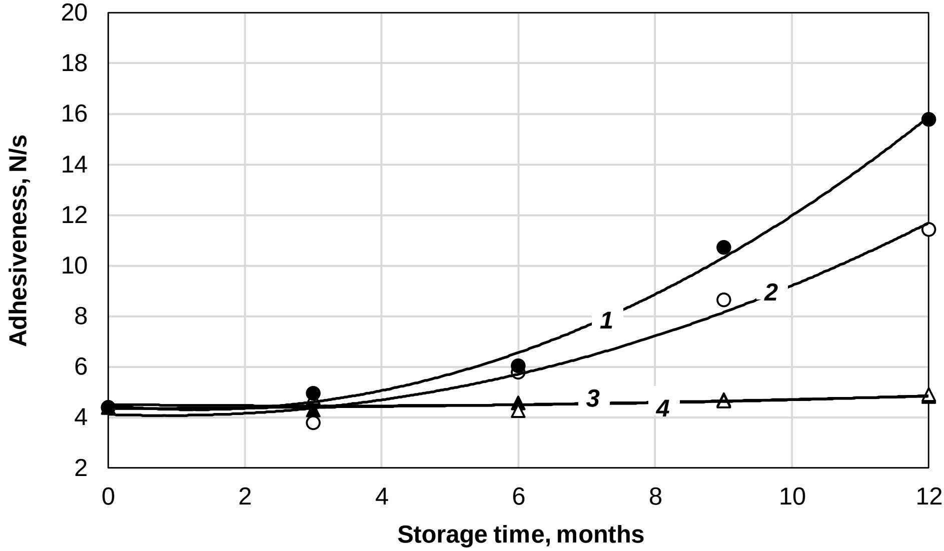

Figure 4. Changes in the adhesiveness index of the analysed Kashkaval cheese samples from cow's milk stored in a refrigerated, superchilled and frozen state:
1 – Refrigerated Kashkaval (4±1 °C);
2 – Refrigerated Kashkaval (1±1 °C);
3 – Superchilled Kashkaval (-7±1 °C);
4 – Frozen Kashkaval (-18±1 °C).

This indicator reflected the effort required to overcome the adhesion forces of the sample to the working tool of the apparatus (Tunick, 2000). Sensory, this was the degree of adhesion of the sample to the teeth of the consumer in the process of its chewing (Gwartney et al., 2004). In the present study there was a minimal but statistically significant increase in the values of the adhesiveness index in the process of storing cheese in superchilled (-7.5±0.5 °C) and frozen state (-18.0±1.0 °C). At the end of storage under these two refrigeration regimes, the adhesiveness of the yellow cheese reached values of 4.81±0.08 N.s. The observed increase in the values of this indicator was most likely due to the partial rupture of the bonds in the paracasein matrix of the cheese as a result of the formation of ice crystals during the refrigeration, superchilling and freezing treatments. However, the lack of intensive proteolytic processes in the cheese stored at these two temperatures did not allow a significant increase in its stickiness (Ivanov and Markova, 2020).

The storage of the Kashkaval cheese in a refrigerated state was accompanied by a significant increase ($p<0.05$) in its adhesiveness. This trend intensified with the increase of the storage temperature. During the 12-month storage period of cheese samples at temperatures of 1.0±1.0 °C, their adhesiveness increased from 4.39±0.07 N.s to 11.43±0.09 N.s. A more significant increase in the stickiness of the cheese was observed during storage at 4.0±1.0 °C. For the whole period of refrigerated storage (12 months) the increase in the values of the adhesiveness index in these samples was from 4.39±0.07 N.s to 15.77±0.08 N.s.

The results obtained in the present study (Figure 4) corresponded to the data on proteolysis in Kashkaval cheese stored at the same conditions (Ivanov and Markova, 2020). Higher storage temperatures (1.0±1.0 °C and 4.0±1.0 °C) were accompanied by a higher degree of hydrolysis of casein to low molecular weight compounds, the accumulation of which in Kashkaval led to an increase in its adhesiveness. Similar trends were found by other authors (Chevanan et al., 2006; El-Bakry et al., 2011). Zheng et al. (2016) investigated the influence of storage temperature on the physicochemical composition and structural and mechanical parameters of mozzarella cheese. The authors found that with increasing storage temperature of the studied cheese samples and the adhesiveness index increased. According to the authors of the study, the low protein and high fat content softened the cheese matrix, which in turn increased its stickiness.

The results obtained for the change of the gumminess of the experimental samples of Kashkaval in the process of refrigerated storage are presented in Figure 5.

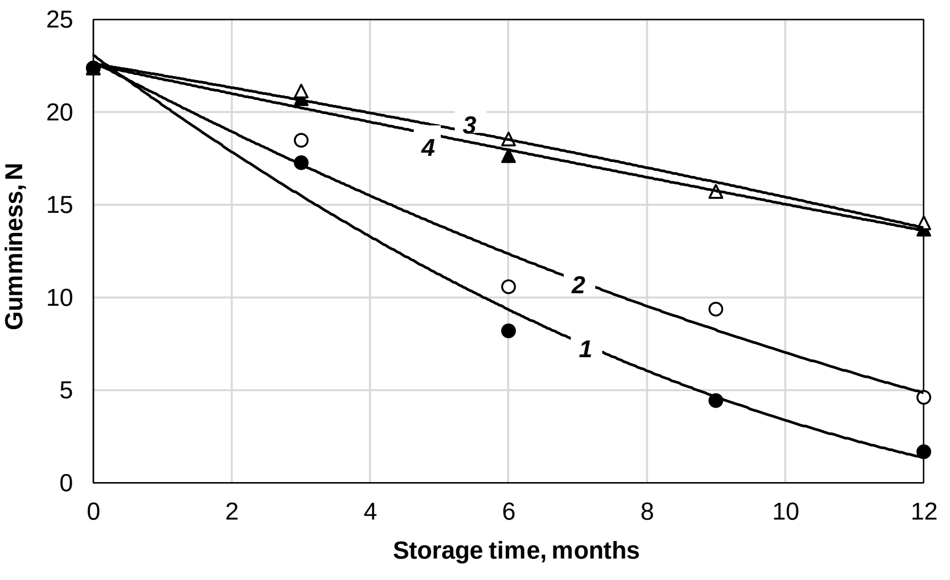

Figure 5. Changes in the gumminess index of the analysed Kashkaval cheese samples from cow's milk stored in a refrigerated, superchilled and frozen state:
1 – Refrigerated Kashkaval (4±1 °C);
2 – Refrigerated Kashkaval (1±1 °C);
3 – Superchilled Kashkaval (-7±1 °C);
4 – Frozen Kashkaval (-18±1 °C).

This indicator reflected the energy required to destroy the structure of the cheese to the extent which was absorbed by the consumer (Chevanan et al., 2011). As shown in the presented data (Figure 5), the gumminess of Kashkaval cheese decreased during storage for all four experimental refrigeration modes. Due to the direct relationship existed between the gumminess and hardness of cheese, the trends in the changes of these two indicators were similar.

In the present study, the smallest decrease in gumminess was found in cheese stored in superchilled (-7.5±0.5 °C) and frozen (-18.0±1.0 °C) state. At the end of storage, the values of the gumminess index in these samples of Kashkaval cheese decreased to 13.8±1.1 N. In the present study, no statistically significant ($p<0.05$) differences in the gumminess of cheese stored in the refrigerated and frozen state were found. This was probably due to the similar changes in the microstructure of these samples due to the ongoing crystal formation processes during their refrigeration processing and storage, as well as to the absence of intensive proteolytic processes in the cheese matrix.

With the increase of the storage temperature of cheese, more significant decrease ($p<0.05$) of the values of the gumminess index was observed. During the 12-month storage period of the cheese samples at temperatures of 1.0±1.0 °C, their gumminess decreased from 22.4±1.1 N to 4.7±0.8 N. For the cheese stored at 4±1 °C, the decrease in the values of the gumminess index was from 22.4±1.1 N to 1.8±0.5 N. Mushtaq et al. (2015) reported a decrease in the values of the gumminess index in buffalo cheese samples. The data from the conducted research showed that with the increase of the storage temperature the gumminess indicator decreased. The authors explained this with a change in pH values. The reduction of the active acidity in the cheese led to demineralization and destabilization of casein micelles. This in turn led to the formation of low molecular weight compounds and softening of the texture of the cheese (Buriti et al., 2005).

In a comparative analysis of the changes in the hardness and gumminess of the experimental Kashkaval cheese samples, it was found that within the same temperature regime the decrease in the values of gumminess was greater than that of the hardness. The reason for this was the complex nature of the gumminess indicator, taking into account the combined perception of hardness, cohesiveness and springiness of Kashkaval cheese. As all three indicators declined in the process of refrigerated storage, it reflected significantly on the gumminess.

Conclusions

The changes in the structural and mechanical parameters of Kashkaval cheese in the process of its refrigerated storage were directly dependent on the applied temperature regime. During Kashkaval storage in refrigerated, superchilled and frozen state, a decrease in the values of the indicators hardness, springiness, cohesiveness, adhesiveness and gumminess was established. The most insignificant changes were found in the texture of Kashkaval samples stored in superchilled and frozen state. More intense changes in the texture of the studied Kashkaval cheese samples were observed with the increase of the storage temperature.

Acknowledgements. The authors would like to thank Mr. Minev, director of the company "Bor Chvor" Ltd. for the provided production area for this study.

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