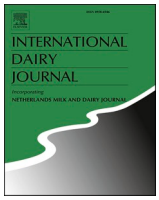
International Dairy Journal 166 (2025) 106239



Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj



Research paper



Reducing sodium in Kashar cheese by partial substitution of NaCl with KCl: Proteolysis, texture, meltability and sensory characteristics

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ARTICLE INFO

Keywords:
Salt uptake
Hypertension
Sodium
Consumer acceptance
Potassium
Salt reducing

ABSTRACT

In this study, the effects of partially substituting NaCl with KCl in Kashar cheese to formulate a reduced-sodium cheese was investigated. Four different salt substitute cheese were produced [N8 (control), 8% NaCl; N4, 4% NaCl; N4K4, 4% NaCl + 4% KCl; N2K6, 2% NaCl + 6% KCl] and their chemical composition, proteolysis, hardness, meltability and sensory profiles were monitored over a 60 d of ripening. The results showed that the replacement of NaCl had no significant effect on the pH, moisture or fat contents of the cheese. However, salt, protein and ash contents were significantly affected ($P < 0.01$). Proteolysis level was significantly higher in NaCl-substituted (N2K6) and reduced-sodium (N4) cheeses. Urea-PAGE electrophoresis revealed different degrees of hydrolysis in the casein fractions. The total FAA concentration increased significantly in the cheese samples during 60 days of ripening, with the highest levels observed in the cheese with the highest KCl content (N2K6). The study also found that cheese with reduced sodium content had lower hardness and higher meltability compared to control cheese ($P < 0.01$). Sensory analysis revealed that while panelists preferred the flavour of the control cheese, they most appreciated the texture of the reduced-sodium variety, indicating potential consumer acceptance of reduced-sodium cheese through the partial substitution of NaCl by KCl. Sensory analysis revealed that while participants preferred the taste of the full sodium (control) cheese, they liked the texture of the low sodium variety better, suggesting that the partial replacement of NaCl with KCl influences the potential consumer acceptability of the low sodium cheese.

1. Introduction

Turkey is a country known for its wide variety of cheeses, including such important varieties as Beyaz peynir, Tulum, Kashar, Van Otlu, Kelle, Dil, Mihaliç, Çökelek, Çerkez, Lor and Civil (Ozsoy & Yasar, 2022). Kashar cheese, a variety of pasta-filata cheese, is the second most consumed cheese in Türkiye. The traditional production process involves stretching the curd in hot brine (75–80 °C, 3–5 g NaCl/100 g, w/v) and then shaping the plasticized mass (Topcu et al., 2020). While fresh consumption is more common sometimes, ripened Kashar cheese develops a unique flavor during the ripening process (Erkaya-Kotan et al., 2023).

Salt (NaCl) is considered the "king of flavors" and is the most commonly used flavoring agent in foods. Sodium chloride has been used for thousands of years as a food additive and preservative to improve the quality of food by enhancing texture, safety and flavor (Wang et al., 2023). Hypertension in humans, a disease resulting from a multifactorial combination of genetic and environmental factors, provides clear evidence of a causal relationship between high salt intake and an increased risk of hypertension and stroke (Zonoubi & Goli, 2021). Increased salt consumption is associated with an increased risk of cardiovascular disease, kidney disease and stomach cancer. Reducing salt consumption has been shown to be an effective means of improving public health, which can be achieved by making prudent food choices or reducing added salt (Rysová & Šmídová, 2021). WHO (Word Health Organization) members have made a joint commitment to reduce salt consumption by 30% by 2025. This agreement is considered one of the most cost-effective and important preventive measures to protect human health (WHO, 2021).

Due to the increasing consumption of cheese worldwide, dairy research is increasingly trying to reduce the fat and salt contents in various types of cheese (Loffi et al., 2021). Sodium chloride (NaCl) plays a diverse role in cheese production and influences microbiological, physico-chemical, biochemical, rheological and sensory properties. When reducing the NaCl content in cheese, numerous factors that affect

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https://doi.org/10.1016/j.idairyj.2025.106239
Received 27 November 2024; Received in revised form 28 February 2025; Accepted 5 March 2025
Available online 7 March 2025
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cheese production and product properties must therefore be taken into account (Hoffmann et al., 2020). Over the past two decades, research into reducing sodium levels in cheese has intensified, taking a multidisciplinary approach to reducing sodium levels without compromising cheese quality and safety. One such approach involves the partial or complete replacement of NaCl with alternative salts such as KCl, $CaCl_2$ or $MgCl_2$ (Dugat-Bony et al., 2019; Bansal and Mishra, 2020; Sandhu et al., 2024; Ferroukhi et al., 2024).

Total or partial substitution of NaCl by KCl has already been studied before in other type cheeses e.g., Mozzarella (Ayyash & Shah, 2011), Prato (Baptista et al., 2017), Cheddar (Ganesan et al., 2014), Feta (Katsiari et al., 2000) and Serbian white-brined (Miocinovic et al., 2022) cheeses. However, the reduction of the normal sodium content of Kashar cheese and the use of KCl instead of NaCl have not yet been investigated. Therefore, the aim of this study was to evaluate the composition, physicochemical properties, proteolysis, hardness, melting behavior and other properties of Kashar cheese produced using 50% and 75% KCl as a substitute for NaCl compared to cheese produced using 100% NaCl (control).

2. Material and methods

2.1. Kashar cheese production

Cow's milk (BV, Holstein breed) from the dairy of Çanakkale Onsekiz Mart University Biga Vocational High School (Biga, Çanakkale, Türkiye) was used to produce Kashar cheese according to the procedures described by Yasar and Guzeler (2011). Each production batch consisted of 120 kg of cow's milk. The milk was heat treated at 68 °C for 10 min, then cooled to 35 °C and cheese starter cultures (a mixture of *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris:* Ezal Rhodia Food, France) and $CaCl_2$ were added. The milk was coagulated with REN-NA® Calf Rennet (1/16 000 MCU/mL, Mayasan, Istanbul, Türkiye). The quantity used for cheese production was calculated on the basis of the relative milk coagulation activity determined by the supplier for coagulation within 45 min. The curd was cut into approximately 0.5 cm^3 pieces and the whey was removed. The curd was fermented at 25 °C until a pH of 5.05 was reached and divided into four equal parts. It was then stretched for 3 min at 80 °C in brine solution (NaCl) with different amounts of salt, including Paşa salt (supplied from the local market, Bursa, Türkiye) and potassium chloride (KCl) (Sigma Aldrich, Germany, food grade). Four different solutions with boiling water were used for salting:

N8: 8% NaCl, 20 L of water (1600 g NaCl)
N4: 4% NaCl, 20 L of water (800 g NaCl)
N4K4: 4% NaCl + 4% KCl, 20 L of water (800 g NaCl + 800 g KCl)
N2K6: 2% NaCl + 6% KCl, 20 L of water (400 g NaCl + 1200 g KCl)

The cheese production was carried out in two replicates in a week interval. The Kashar cheese samples were labelled as N8, N4, N4K4 and N2K6, corresponding to the amounts of the salt mentioned above. The produced Kashar cheeses were ripened at 4 °C for 60 days and the analyses were performed after the 1st, 30th, and 60th days.

2.2. Chemical composition and pH

Duplicate analysis were performed for all chemical analysis of the cheeses at the first day of ripening to determine fat, moisture, salt, titratable acidity (expressed as % of lactic acid) and total nitrogen contents. The analyzes were performed according to the protocols described by Ardö and Polychroniadou (1999). Salt content was determined by Mohr titration method, and it is expressed as sodium (molecular weight for NaCl: 58.5 g/mol) or potassium (molecular weight for KCl: 74.5 g/mol) salts as given in Table 1. To measure the pH, 10 g of cheese was homogenized in 10 mL of pure water. The pH of the resulting mixture was then determined using a digital pH meter (Mettler Toledo, Seven Compact, Switzerland).

Table 1
Chemical composition and pH of Kashar cheese made using partial substitution of NaCl by KCl.

Variable	Cheeses[a]			
	N8	N4	N4K4	N2K6
pH	5.56 ± 0.14[a]	5.50 ± 0.02[a]	5.64 ± 0.04[a]	5.56 ± 0.05[a]
Moisture (%)	48.40 ± 0.29[a]	48.75 ± 0.33[a]	48.00 ± 0.00[a]	46.95 ± 0.94[a]
Fat (%)	22.65 ± 0.21[a]	22.25 ± 1.06[a]	21.75 ± 0.35[a]	22.75 ± 1.06[a]
Salt (%)[b]	1.97 ± 0.04[a]	1.03 ± 0.02[d]	1.86 ± 0.02[b]	1.73 ± 0.41[c]
Total Protein (%)	27.24 ± 0.64[ab]	29.28 ± 1.15[b]	28.14 ± 0.19[ab]	26.09 ± 1.21[a]
Ash (%)	3.67 ± 0.07[a]	3.02 ± 0.02[d]	3.46 ± 0.07[b]	3.23 ± 0.13[c]

[a–d] Means ± SD within a column with no common superscript differ ($P < 0.05$).
[a] Salting system were: N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.
[b] The values given in the table are total salt. The levels of sodium salt for N8, N4, N4K4 and N2K6 were 1.97%, 1.03%, 0.82% and 0.36%, respectively. None of potassium in the samples N8 and N4; its levels in N4K4 and N2K6 are 0.83% and 1.37%, respectively.

2.3. Proteolysis

The proteolysis indicators, including water-soluble nitrogen (WSN) and 12% trichloroacetic acid-soluble nitrogen (TCA-SN), were evaluated together with urea-polyacrylamide gel electrophoresis (urea-PAGE) of the water-insoluble (freeze-dried) fractions and reversed-phase high-performance liquid chromatography (RP-HPLC) of the water-soluble (freeze-dried) fractions of the cheeses according to the methods described by Hayaloglu et al. (2021).

The concentration of free amino acids in cheese was measured according to the method described by Ayag et al. (2022). Amino acid standards (Sigma-Aldrich, St. Louis, USA) and cheese samples were prepared in a 0.1 N hydrochloric acid (HCl) solution and then injected into the same HPLC system. The separation of amino acids in cheese was performed using an Inertsil ODS-3 column (150 mm × 4.6 mm × 3 μm). Buffer A consisted of an acetonitrile solution adjusted to pH 6.55 with acetic acid, while buffer B was prepared from a combination of acetonitrile, deionized water and methanol in a volume ratio of 9:8:3. Both buffers were supplemented with 10 mg/L disodium ethylenediamine tetraacetate (Na_2EDTA). The sample mixture was injected into the column at a temperature of 45 °C and monitored at a wavelength of 254 nm, with the flow rate set to 1 mL/min. The results were expressed in mg/100 g of cheese.

2.4. Hardness

The tests were performed in triplicate using a texture analyzer (Lloyd, model LF plus, West Sussex, UK). Cheese specimens with a height of 10 mm and a diameter of 20 mm were prepared at 20 °C for the tests. An aluminum probe with a diameter of 25 mm was used at a test speed of 50 mm/min and compression set at 50%. Data acquisition and subsequent calculations were performed using Nexygen™ software in the apparatus.

2.5. Meltability

The melting behavior of the cheeses was evaluated after 1, 30 and 60 days of ripening using a modified version of the Schreiber method (Hayaloglu et al., 2021). The average of six diameter measurements was expressed in millimeters.

2.6. Sensory analysis

The cheese samples were evaluated by 15 semi-trained panelists (9

males and 6 females, aged between 22 and 50; they were familiar with Kashar cheese) for appearance, texture, flavour and overall acceptability of Kashar cheese on a 9-point hedonic scale (Sıçramaz et al., 2022). The study was conducted in accordance with the Declaration of Helsinki. All procedures involving human participants were carefully conducted in accordance with the university's ethical principles. The study protocol underwent a comprehensive review and received approval from the university. Informed consent was diligently obtained from all participants before their involvement in the study, ensuring their voluntary participation and a clear understanding of the research objectives.

2.7. Statistical analysis

An experimental design was drawn up with four types of cheese (corresponding to four different salt treatments) and three ripening periods, which were analyzed in two trials (4 treatments × 3 ripening time points × 2 trials). The GLM procedure of the SAS software (SAS, 2002) was performed. To facilitate pairwise comparisons of treatment means, Tukey's multiple comparison test with a significance level of 5% was applied to assess differences between treatments. In addition, principal component analysis (PCA) was used to compare the RP-HPLC data using the SPSS software package, version 9.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Chemical composition and pH

The pH, moisture and fat content of Kashar cheese were not affected by replacing sodium with potassium chloride ($P > 0.05$; Table 1). However, salt, protein and ash contents were statistically affected ($P < 0.01$). The mean salt content of N4 and N2K6 cheeses was 1.03% and 1.44%, respectively. The salt content of control (N8) and 50% NaCl-reduced cheese (N4K4) was 1.97% and 1.64%, respectively. The salt content of N4 and N2K6 cheese was reduced by 48% and 23%, respectively, compared to the control cheese. Therefore, N4 and N2K6 cheeses were highly beneficial with respect to the recommendation of WHO for a reduction in daily salt intake to less than 2 g sodium or 5 g salt (WHO, 2021). Similar results have been reported for other low-salt cheeses (Henneberry et al., 2015; Katsiari et al., 1998). In the study conducted by Costa et al., 2018, as in the present study, it was observed that an increase in potassium concentration was observed by reducing the sodium content (Table 1). The levels of sodium in the cheese samples were reduced by substituting of NaCl by KCl. In this context, the sodium salt contents for N8, N4, N4K4 and N2K6 were 1.97%, 1.03%, 0.82% and 0.36%, respectively. None of potassium in the samples N8 and N4; its levels in N4K4 and N2K6 are 0.83% and 1.37%, respectively.

3.2. Proteolysis

The water-soluble nitrogen (WSN) serves as an indicator for the evaluation of the proteolysis process in cheese and provides information about the intermediate and smaller peptides and amino acids formed during this process (Kocak et al., 2020). As shown in Table 2, the replacement of NaCl with KCl and the duration of ripening had a significant effect on the WSN values of Kashar cheese ($P < 0.05$). During the entire ripening period, WSN values increased significantly in all cheese samples. The most significant increase was recorded in sample N8, which contained the highest amount of NaCl. Akan and Kinik (2018) reported a significant effect of replacing sodium chloride with various mineral salts in the production of white cheese on the WSN values of cheese during a 90-day ripening period, with values increasing during storage.

The replacement of NaCl with KCl and the ripening time significantly influenced the TCA-SN ($P < 0.05$). The TCA-SN values of all cheese samples significantly increased during the ripening period. The strongest increase was observed in sample N2K6, followed by sample N4 (Table 2). This increase is attributed to the increased proteolytic activity of bacterial peptidases (Ayyash & Shah, 2011) due to lower levels of sodium in these cheeses. Salt reduces the free water available in cheese, which consequently affects enzyme activity and growth of LAB (Ayyash & Shah, 2011). Several researchers have observed an increase in TCA-SN levels during the maturation process (Yilmaz & Dagdemir, 2012; Özdemir & Babacan, 2018). The TCA-SN defined non-protein nitrogen fractions consist of medium-sized peptides, amino acids and amines (Hayaloglu et al., 2021) and provide valuable information on the degree of maturity and proteolysis (Fox, 1989).

Table 2
Water-soluble (WSN) and 12% trichloroacetic acid-soluble nitrogen (TCA-SN) fractions (as % of total nitrogen, TN) of Kashar cheese during ripening made using partial substitution of NaCl by KCl.

Variable	Days	Cheeses[a]			
		N8	N4	N4K4	N2K6
WSN	1	$4.16 \pm 0.11^{b,A}$	$2.86 \pm 0.19^{a,A}$	$2.60 \pm 0.03^{a,A}$	$4.54 \pm 0.18^{b,A}$
	30	$12.51 \pm 0.01^{d,B}$	$9.84 \pm 0.25^{b,B}$	$6.93 \pm 0.38^{a,B}$	$11.11 \pm 0.74^{c,B}$
	60	$13.70 \pm 1.37^{a,B}$	$11.84 \pm 0.11^{a,C}$	$7.74 \pm 0.08^{b,B}$	$11.98 \pm 0.27^{a,B}$
TCA-SN	1	$1.53 \pm 0.21^{ab,A}$	$1.36 \pm 0.14^{ab,A}$	$1.17 \pm 0.20^{a,A}$	$1.73 \pm 0.01^{b,A}$
	30	$3.05 \pm 0.06^{c,B}$	$2.28 \pm 0.22^{b,B}$	$1.49 \pm 0.11^{a,A}$	$3.06 \pm 0.09^{c,B}$
	60	$4.19 \pm 0.40^{b,C}$	$5.71 \pm 0.03^{d,C}$	$2.99 \pm 0.00^{a,B}$	$5.00 \pm 0.01^{c,C}$

[a–d] Means ± SD within a column with no common superscript differ ($P < 0.05$).
[A–D] Means ± SD within a row with no common superscript differ ($P < 0.05$).
[a] Salting system: N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.

In the urea-PAGE electrophoretogram (Fig. 1), the first band (NaCN) represents Na-caseinate, while the subsequent bands represent the electrophoretic properties of the casein fractions after 1, 30 and 60 days of ripening. At the 30th and 60th day of ripening, the β-casein and α_{s1}-casein fractions of the N8 and N2K6 samples exhibited much more hydrolysis compared to the other samples. The band densities of β-casein to α_{s1}-casein decreased in all cheeses during ripening. Comparable results were reported by different researchers (Ayyash & Shah, 2011; Katsiari et al., 2000). Miocinovic et al. (2022) observed that the use of KCl instead of NaCl in Serbian feta cheese resulted in a decrease in the level of α_{s1}-casein during the ripening process, while the β-casein remained unchanged. Studies on the effect of salt on the degradation of β-casein have shown that it is related to many factors, such as the type/specificity of the coagulant, the degree of aggregation of casein, which is influenced by various parameters (e.g. pH, moisture content, calcium concentration, protein and fat content) and affects its susceptibility to hydrolysis (McCarthy et al., 2016). In cheese with reduced NaCl content, β-casein is more hydrolyzed, while in normal salted cheese, α_{s1}-casein is reduced the most (Bansal & Mishra, 2020). Similarly, reducing the salt content in Cheddar cheese below the critical level (<1.2% for salt) has been reported to result in an increase in the volume fraction and density of the casein network, a lower moisture-to-protein ratio and a lower α_{s1}-casein degradation rate (Fenelon & Guinee, 2000; McCarthy et al., 2016). In general, the effect of salt replacement on urea-PAGE electrophoretograms was found to be insignificant. This effect can be considered positive as the reduction of salt increases proteolytic activity and causes a bitter taste due to excessive formation or insufficient degradation of bitter peptides (Farkye & Guinee, 2017).

The peptide profiles of the soluble nitrogen fraction of Kashar cheese at pH 4.6 were determined by RP-HPLC for 60 days of ripening (Fig. 2a–c). In the chromatograms, hydrophilic peptides can be seen between 10 and 40 min and hydrophobic peptides between 40 and 75

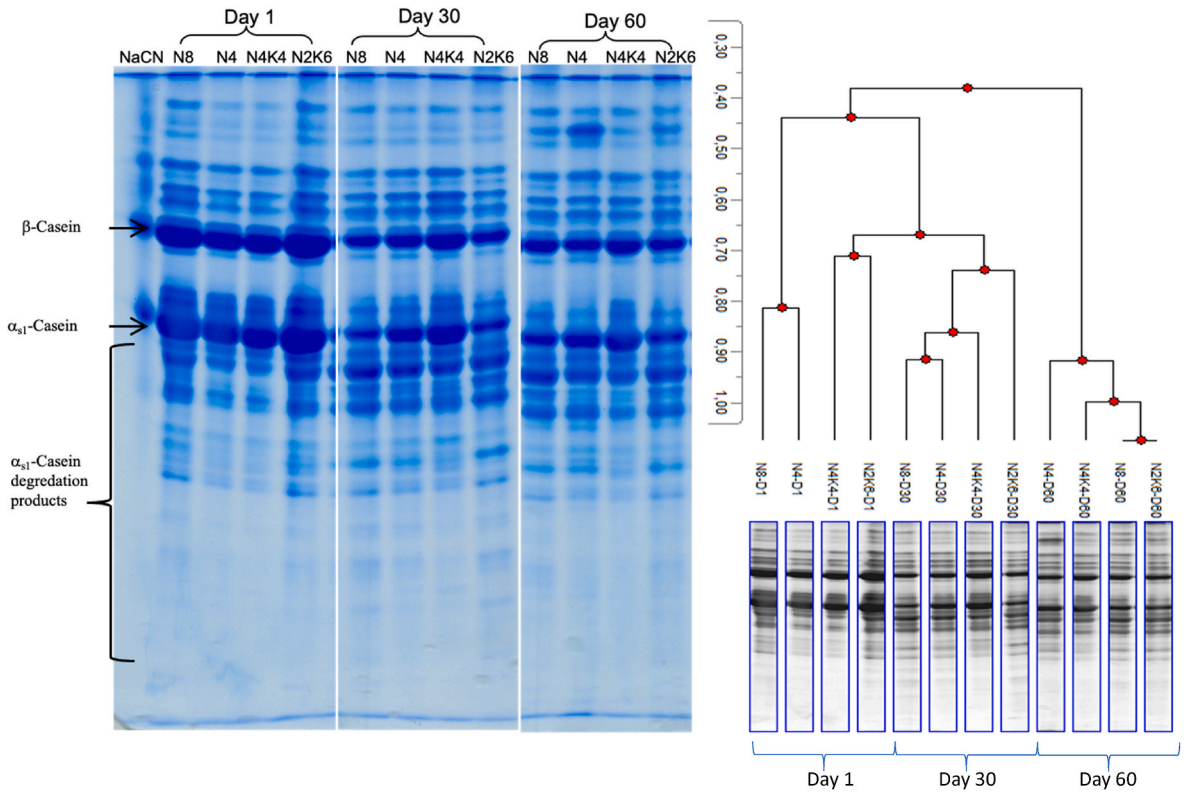


Fig. 1. Urea-polyacrylamide gel electroforetograms and dendrogram of Kashar cheeses after 1, 30 and 60 days of ripening. NaCN is sodium caseinate; N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.

min (Çakır et al., 2022). The peptide profiles of control (N8) and reduced sodium/KCl-substituted cheese (N2K6) exhibit a significant difference in both the number and intensity of peptides on day 30 of ripening. However, at day 60, a remarkable presence of intense peptides is observed in the N2K6 sample. As mentioned above, the salt content can affect the course of proteolysis by influencing water activity, enzyme and microbial activity and water binding capacity of caseins. However, when the NaCl content is replaced by KCl, thus lowering the sodium content, it is observed that the expected effects on proteolysis do not occur. This could indicate that the use of potassium instead of sodium makes a positive contribution to proteolysis.

Proteolysis in cheese leads to the formation of amino acids, which are crucial for its characteristic flavour (Sahingil et al., 2014). The amino acid content of Kashar cheese during the 60-day ripening period is shown in Table 3. During the 60-day ripening of Kashar cheese, the content of aspartic acid (Asp) increased, and the highest concentrations were observed in the N2K6 sample, indicating that KCl substitution enhances proteolysis. Glutamic acid (Glu) was also significantly increased in the N2K6 sample, suggesting that KCl favours its formation. Tryptophan (Trp) showed the most significant increase in the KCl-substituted samples (N4K4 and N2K6), reaching the highest levels at day 60. Other amino acids such as serine (Ser), glycine (Gly) and threonine (Thr) generally increased during ripening, especially in cheeses with higher KCl substitution. Proline (Pro), which is important for tissue, was also increased significantly, especially in the N2K6 sample. Valine (Val), methionine (Met) and cysteine (Cys) increased regularly, reflecting the favourable effect of KCl on the accumulation of free amino acids (FAA). Lysine (Lys) content increased during ripening but was more pronounced in cheeses with higher NaCl content, suggesting that NaCl may be a crucial factor for Lys accumulation. The total FAA content increased significantly in all samples, with the highest concentration (201.7 mg/kg) in sample N2K6. Overall, the results show that the partial substitution of NaCl with KCl enhanced proteolysis and amino acid accumulation and that KCl influenced significantly the flavour profile of the cheese.

PCA was used to analyze the 20 FAAs detected in four types of salt replacement cheese samples. The PCA score plot of the cheese during the ripening period is shown in Fig. 3. PC 1 and PC 2 accounted for 52 and 7.5 % of the total variance, respectively. The FAA content of the N2K6 and N4K4 samples was high, as shown by PC 1 and PC 2. Clustering for the ripening stages showed that the diversity and content of FAAs were similar in other cheeses.

The FAAs of the cheeses were analyzed based on the variance of the heat map clusters. As shown in Fig. 4, the samples were clustered horizontally during the ripening process, and the types of FAAs were clustered vertically. The content of all FAAs was color-coded in the heat map, corresponding to the content. The horizontal clustering showed that the samples were clustered in the same subclusters during the different ripening processes and showed a high correlation. The horizontal cluster was further divided into four subclusters due to the greater similarity of the samples. Subcluster 1 consisted of samples from day 1. subcluster 2 consisted of samples from day 30. subclusters 3 (N4, N8, N2K6) and 4 (N4K4, N2K6, N4) consisted of the corresponding samples. The results showed that the content of FAAs was lowest at the beginning of ripening and increased significantly with increasing ripening time.

Based on the horizontal clustering, the FAA types were categorized into five sections according to their content in Fig. 4. High content of FAAs (Trp, Ser, Thr, Asp and Val) was expressed by dark red, followed by light red and blue series with the decrease of FAAs content.

It was found that replacing NaCl with KCl had a significant effect on the free amino acid content of Kashar cheese ($P < 0.01$). Diana et al. (2014) pointed out that high salt concentration in cheese could impede bacterial growth, possibly affecting the degree of proteolysis and the formation of FAA. The most important ripening process, proteolysis, can be detected by an intentional increase in glutamic acid content or amino acid catabolism. The highest glutamic acid content was detected on the

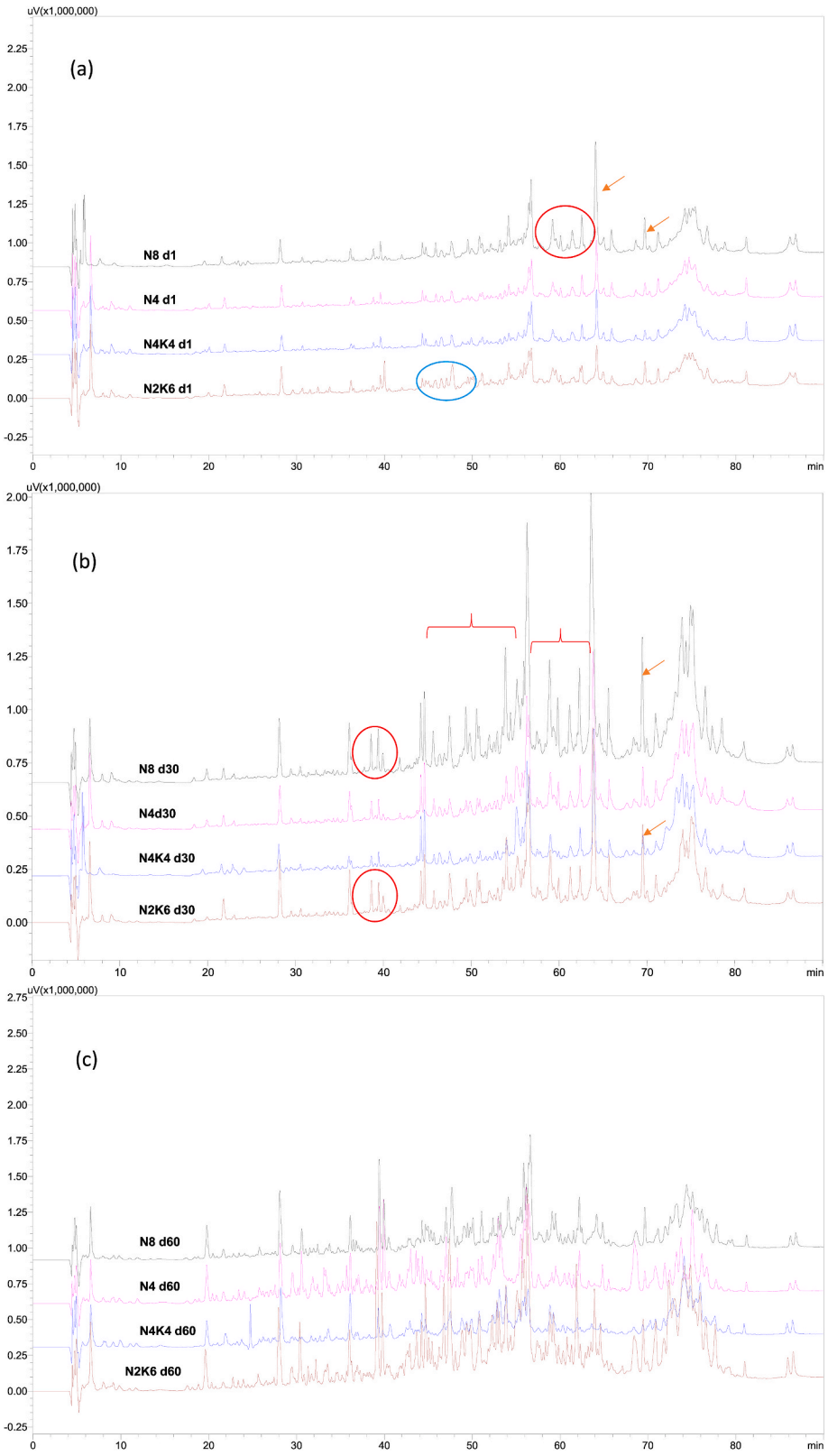


Fig. 2. Reversed-phase high performance liquid chromatography peptide profiles of pH 4.6-soluble fractions of Kashar cheeses after 1 (a), 30 (b) and 60 (c) days of ripening. N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.

60th day of ripening, especially in the N2K6 sample. Ayag et al. (2022) identified Glu, His, Tyr, Ile, Phe and Trp as FAA in Kashar cheese. The differences in FAA concentration between cheeses were closely related to the production methods (including the addition of starter cultures, pasteurization, salt content, etc.), ripening conditions and the extent of proteolysis. The free amino acid content of the samples increased during ripening. Numerous researchers have documented that the amino acid content of cheese tends to increase during ripening (Garbowska et al.,

Table 3
Individual free amino acid concentrations (mg/kg cheese) in Kashar cheese made using partial substitution of NaCl by KCl at 1, 30, 60 d of ripening.

Cheeses[a]												
Amino acids	Day 1				Day 30				Day 60			
	N4	N8	N4K4	N2K6	N4	N8	N4K4	N2K6	N4	N8	N4K4	N2K6
Asp	4.27 ± 0.40^{a}	6.39 ± 0.80^{b}	5.65 ± 0.29^{b}	4.00 ± 0.33^{a}	4.06 ± 1.81^{a}	6.69 ± 0.11^{ab}	9.72 ± 0.31^{b}	5.04 ± 0.32^{ab}	5.71 ± 2.30^{a}	10.78 ± 0.61^{b}	15.53 ± 2.93^{c}	12.10 ± 0.23^{b}
Glu	5.19 ± 0.91d	3.03 ± 0.01^{b}	4.02 ± 0.14^{c}	0.41 ± 0.29^{a}	2.29 ± 1.25^{a}	6.68 ± 0.11^{b}	5.46 ± 0.00^{ab}	6.19 ± 0.65^{ab}	4.25 ± 0.37^{a}	8.80 ± 0.09^{ab}	6.22 ± 4.40^{a}	12.66 ± 0.71^{b}
Asn	1.88 ± 0.01^{a}	1.39 ± 0.05^{a}	1.38 ± 0.35^{a}	1.38 ± 0.13^{a}	4.47 ± 0.10^{b}	3.60 ± 0.68^{b}	1.73 ± 0.27^{a}	4.05 ± 0.15^{b}	4.68 ± 0.79^{a}	3.85 ± 0.06^{a}	6.22 ± 0.79^{b}	5.23 ± 1.13^{ab}
Ser	0.30 ± 0.01^{a}	0.18 ± 0.01^{b}	N.D.	0.20 ± 0.00^{b}	0.16 ± 0.11^{a}	N.D.	0.11 ± 0.07^{a}	3.81 ± 0.07^{b}	2.76 ± 0.84^{a}	5.07 ± 0.28^{ab}	10.78 ± 2.02^{b}	4.11 ± 0.66^{a}
Gln	1.07 ± 0.00^{a}	0.72 ± 0.03^{a}	1.15 ± 0.28^{a}	0.86 ± 0.17^{a}	2.47 ± 0.22^{ab}	2.77 ± 0.76^{ab}	1.41 ± 0.33^{a}	3.97 ± 0.13^{b}	4.47 ± 0.59^{a}	4.14 ± 0.03^{a}	6.20 ± 0.26^{a}	5.21 ± 0.88^{a}
Gly	1.47 ± 0.36^{a}	1.4 ± 0.09^{a}	1.41 ± 0.06^{a}	1.28 ± 0.05^{a}	2.43 ± 0.06^{b}	3.01 ± 0.19^{b}	1.68 ± 0.14^{a}	2.83 ± 0.04^{b}	3.17 ± 0.16^{a}	3.27 ± 0.00^{a}	4.30 ± 0.63^{a}	2.40 ± 0.48^{a}
His	2.54 ± 0.03^{c}	1.17 ± 0.12^{a}	1.28 ± 0.00^{a}	1.89 ± 0.08^{b}	6.63 ± 0.71^{c}	3.69 ± 0.06^{b}	2.85 ± 0.26^{ab}	1.93 ± 0.58^{a}	4.83 ± 0.39^{b}	2.13 ± 0.07^{a}	3.19 ± 0.28^{a}	5.23 ± 0.02^{b}
Arg	0.55 ± 0.40^{a}	0.93 ± 0.38^{a}	1.66 ± 0.82^{b}	1.81 ± 0.90^{b}	3.04 ± 1.53	3.48 ± 0.00	2.72 ± 0.91	1.39 ± 0.31	4.77 ± 2.43^{a}	3.60 ± 1.02^{a}	3.79 ± 1.14^{a}	5.51 ± 0.33^{a}
Thr	0.44 ± 0.03^{ab}	0.59 ± 0.09^{b}	0.22 ± 0.03^{a}	0.38 ± 0.07^{ab}	1.49 ± 0.31^{ab}	1.25 ± 0.33^{b}	0.77 ± 0.04^{a}	1.89 ± 0.43^{b}	1.76 ± 0.21^{a}	1.99 ± 0.21^{a}	3.59 ± 0.53^{a}	1.80 ± 0.58^{a}
Ala	3.71 ± 1.54^{ab}	3.36 ± 0.03^{ab}	3.07 ± 0.25^{a}	4.08 ± 0.15^{b}	6.58 ± 0.66^{ab}	8.51 ± 1.66^{ab}	5.12 ± 0.9^{a}	9.35 ± 0.23^{b}	12.41 ± 1.22^{a}	10.89 ± 0.23^{a}	13.72 ± 0.53^{a}	9.16 ± 1.08^{a}
Pro	3.92 ± 0.15^{ab}	3.71 ± 0.19^{ab}	3.42 ± 0.11^{a}	4.33 ± 0.08^{b}	5.30 ± 0.14^{a}	5.82 ± 0.06^{a}	5.05 ± 0.30^{a}	7.34 ± 0.04^{b}	12.01 ± 0.83^{a}	10.90 ± 0.25^{a}	11.97 ± 0.98^{a}	11.03 ± 1.34^{a}
Tyr	2.36 ± 0.61^{a}	2.22 ± 0.27^{a}	1.85 ± 0.15^{b}	2.51 ± 0.53^{a}	3.55 ± 0.00^{b}	2.27 ± 0.00^{a}	2.87 ± 0.00^{a}	4.81 ± 0.10^{c}	14.02 ± 1.13^{b}	11.59 ± 1.06^{ab}	13.32 ± 3.53^{b}	8.38 ± 4.14^{a}
Val	1.62 ± 0.05^{a}	1.73 ± 0.15^{a}	1.91 ± 0.59^{a}	1.83 ± 0.21^{a}	1.73 ± 0.44^{ab}	1.89 ± 0.36^{ab}	1.22 ± 0.37^{a}	3.40 ± 0.10^{b}	3.55 ± 1.02^{a}	2.13 ± 0.40^{a}	4.29 ± 0.17^{b}	5.33 ± 2.13^{b}
Met	2.34 ± 0.22^{a}	2.71 ± 0.00^{a}	4.96 ± 0.25^{b}	0.72 ± 0.13^{c}	3.62 ± 1.82^{a}	3.41 ± 2.41^{a}	5.12 ± 1.07^{ab}	7.76 ± 1.10^{b}	3.77 ± 0.06^{a}	2.84 ± 0.00^{a}	5.21 ± 0.61^{b}	5.15 ± 0.08^{b}
Cys	4.41 ± 0.26^{c}	0.78 ± 0.00^{a}	0.42 ± 0.29^{a}	3.08 ± 0.70^{b}	2.71 ± 0.08^{a}	3.22 ± 0.48^{ab}	3.09 ± 0.00^{ab}	5.27 ± 0.60^{b}	5.96 ± 0.14^{a}	5.21 ± 0.06^{a}	8.81 ± 2.39^{a}	7.30 ± 4.15^{a}
Ile	0.49 ± 0.00^{a}	0.40 ± 0.04^{a}	0.22 ± 0.05^{a}	0.76 ± 0.33^{a}	8.20 ± 5.15^{a}	7.33 ± 0.00^{a}	8.08 ± 0.26^{a}	21.31 ± 0.00^{b}	24.21 ± 0.94^{b}	10.91 ± 7.04^{a}	24.43 ± 5.69^{b}	24.66 ± 3.12^{b}
Leu	1.19 ± 0.00^{b}	0.60 ± 0.05^{a}	1.66 ± 0.25^{b}	1.63 ± 0.21^{b}	2.03 ± 0.04^{a}	5.59 ± 2.36^{b}	1.72 ± 0.09^{a}	2.57 ± 0.16^{a}	5.81 ± 2.33^{ab}	11.23± 7.40^{b}	2.51 ± 0.39^{a}	9.05 ± 5.33^{ab}
Phe	1.48 ± 0.63 ab	0.83 ± 0.10^{a}	0.96 ± 0.15^{a}	1.17 ± 0.22^{a}	1.79 ± 0.32^{a}	0.93 ± 0.05^{a}	1.27 ± 0.17^{a}	1.25 ± 0.36^{a}	1.85 ± 0.04^{a}	2.21 ± 0.00^{a}	2.39 ± 0.19^{a}	2.20 ± 0.19^{a}
Trp	1.08 ± 0.01^{a}	3.73 ± 1.35^{b}	8.12 ± 5.74^{d}	6.26 ± 1.54^{c}	15.28 ± 2.78^{a}	16.58 ± 1.40^{a}	35.06 ± 4.96^{c}	22.32 ± 3.51^{b}	21.39 ± 7.06^{a}	33.29 ± 5.36^{a}	52.36 ± 0.00^{b}	50.08 ± 6.68^{b}
Lys	4.60 ± 0.62^{a}	10.79 ± 3.18^{c}	7.12 ± 0.00^{b}	15.51 ± 4.14^{d}	15.84 ± 2.13^{c}	11.40 ± 2.67^{b}	8.63 ± 0.00^{a}	7.81 ± 1.69^{a}	21.92 ± 4.95^{b}	11.73 ± 1.41^{a}	11.28 ± 1.85a	15.11 ± 3.80^{ab}
Total	44.75	46.66	50.57	54.09	93.67	98.12	103.7	124.29	163.3	156.6	210.11	201.70

[a–d] Means ± SD within a row with no common superscript differ ($P < 0.05$).
[a] Salting system were: N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl. ND: Not determined.

2020; Singh et al., 2023; Xu et al., 2020).

3.3. Hardness and meltability

At all stages of ripening, sample N8 consistently showed the highest hardness (N), while sample N2K6 showed the lowest hardness. A general decrease in hardness was observed with increasing ripening time, which was particularly evident after 60 days (Table 4). The use of different types of salt and the variation in ripening time significantly influenced the hardness of the cheeses ($P < 0.01$).

The reduction in NaCl content led to significantly lower hardness values in the cheeses. The addition of NaCl facilitates the hydration of casein and improves protein interaction with lower electrostatic repulsive forces, may change the texture of the cheese. Conversely, reducing the salt content reduces the ability of the cheese structure to resist deformation, resulting in a softer and more brittle cheese (Carmi & Benjamin, 2017). The decrease in salt content can be partly attributed to the increase in moisture content (Watkinson et al., 2001). In Muenster cheese, a decrease in salt content was found to reduce casein swelling and firmness despite the increase in moisture content (Pastorino et al., 2003). The salt content itself has a concentration-dependent effect on the hydration of the para-casein and the volume fraction of the casein network; therefore, probably influences the cheese rheology (McCarthy et al., 2016).

Kashar cheese occupies an important place in Turkish cuisine and serves as an important ingredient in many foods such as toast, pita, pastries, pizza, meatballs with cheese and mushrooms with cheese. Given its widespread use, consumers value Kashar cheese above all for its excellent melting properties. When analyzing the melting properties, sample N4 showed the highest melting value (13.60 mm) on the first day of ripening. On the 60th day of ripening, sample N2K6 had the highest melting values (18.05 mm), while sample N8 had a lower melting capacity compared to the other samples (Table 4).

The different use of salt significantly influenced the melting properties of the cheeses, with statistical significance ($P < 0.01$). It was determined that the meltability of sample N2K6 (11.10 ± 0.14; 18.05 ± 0.35 mm) was 36% and 30% higher than that of sample N8 (8.15 ± 0.49; 13.90 ± 0.42 mm) on the 1st and 60th days of storage, respectively. A reduction in salt content, as observed in Mozzarella cheese, from 1.8% to 0.9%, resulted in increased meltability (Ganesan et al., 2014). Similar results were reported by Henneberry et al. (2015). Arboatti et al. (2014) observed that the melting temperature decreased with decreasing NaCl content, suggesting that Mozzarella cheese has lower melting temperatures when the NaCl content is reduced.

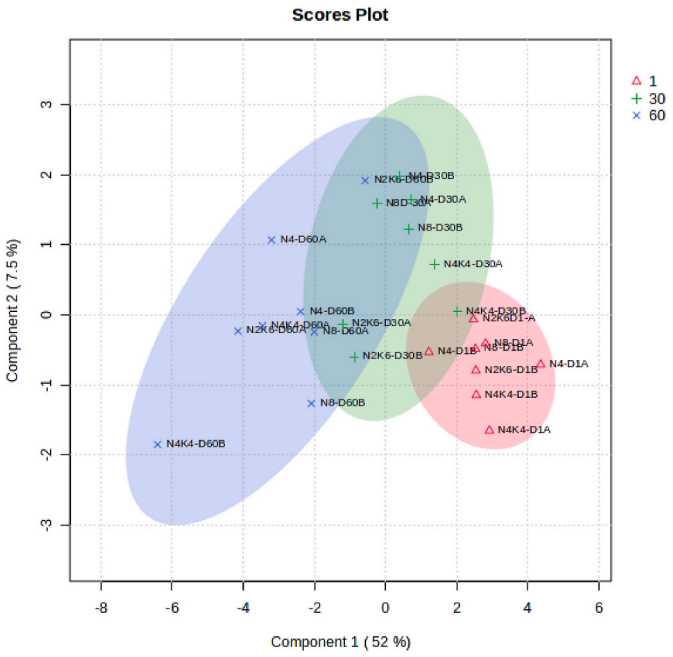


Fig. 3. Principal component analysis score plot of individual free amino acids in Kashar cheese after 1 (D1), 30 (D30) and 60 (D60) days of ripening. N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.

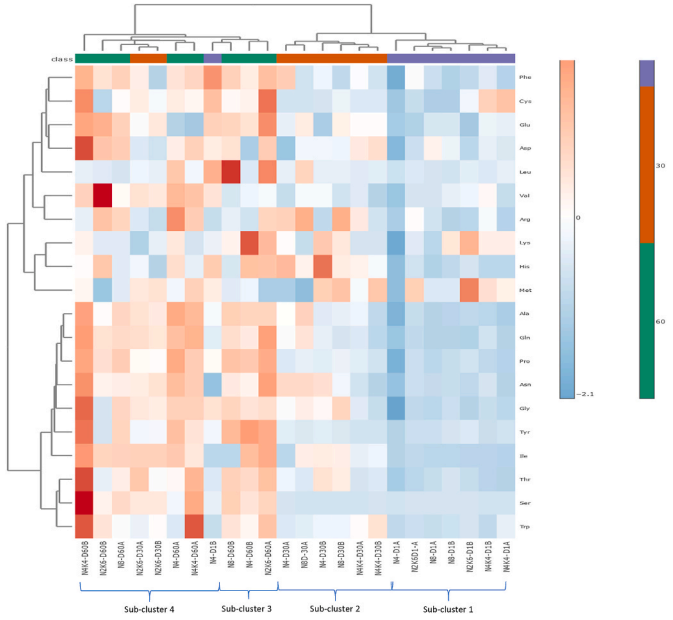


Fig. 4. Heatmap cluster analysis of individual free amino acids in Kashar cheese after 1 (D1), 30 (D30) and 60 (D60) of ripening. N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.

During the entire storage period, a clear increase in melting values was observed for all samples, which was statistically significant ($P < 0.01$) (Table 4). Sample N2K6 showed the highest increase, while sample N8 showed the lowest increase. The melting behavior of cheese is closely related to factors such as pH and water activity. An increased degree of proteolysis during storage can contribute to an increased tendency to melt. Proteolysis leads to structural changes in the cheese, which often result in an increase in pH due to the formation of NH_3 (Sahan et al., 2008), release of bound inorganic phosphate (Upreti & Metzger, 2007) and calcium solubility (Hassan et al., 2004). Hydrolysis

Table 4
Hardness (N) and meltability (mm) of Kashar cheese during ripening made using partial substitution of NaCl by KCl.

Variable	Days	Cheeses[a]			
		N8	N4	N4K4	N2K6
Hardness, N	1	1.99 ± 0.17^{a,A}	1.61 ± 0.01^{a,A}	1.75 ± 0.21^{a,A}	1.57 ± 0.23^{a,A}
	30	1.81 ± 0.09^{b,A}	1.39 ± 0.17^{a,B}	1.68 ± 0.14^{ab,A}	1.31 ± 0.08^{a,A}
	60	1.56 ± 0.21^{b,B}	1.14 ± 0.12^{ab,C}	1.29 ± 0.24^{ab,B}	0.89 ± 0.09^{a,B}
Meltability, mm	1	8.15 ± 0.49^{b,B}	13.60 ± 1.83^{a,A}	7.50 ± 0.84^{b,C}	11.10 ± 0.14^{a,B}
	30	13.35 ± 2.19^{a,A}	12.50 ± 0.01^{a,A}	14.05 ± 0.35^{a,B}	13.95 ± 2.19^{a,B}
	60	13.90 ± 0.42^{b,A}	15.35 ± 1.06^{ab,B}	15.95 ± 0.35^{ab,A}	18.05 ± 0.35^{a,A}

a–d Means ± SD within a row with no common superscript differ ($P < 0.05$).
$^{A–D}$ Means ± SD within a column with no common superscript differ ($P < 0.05$).
[a] Salting system were: N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.

of some casein fractions such as α_{s1}-casein can contribute to the weakening of the protein network, which ultimately leads to increased melting of the cheese (Costa et al., 2018). These results highlight the dynamic nature of cheese properties during storage, with proteolysis playing a crucial role in influencing both the structural changes and subsequent melting behavior of Kashar cheese samples.

3.4. Sensory analysis

The panelists found no significant differences in the appearance characteristics of Kashar cheese ($P > 0.01$) as shown in Table 5. Zonoubi and Goli, 2021 also reported no significant changes in the color

Table 5
Sensory evaluation of Kashar cheese during ripening made using partial substitution of NaCl by KCl.

Parameter	Days	Cheeses[a]			
		N8	N4	N4K4	N2K6
Appearance (1–9 point)	1	8.21 ± 0.70^{a,A}	8.35 ± 0.93^{a,A}	8.07 ± 0.99 ^{a,A}	7.92 ± 0.82 ^{a,A}
	30	8.01 ± 0.96^{a,A}	8.57 ± 0.51^{a,A}	8.06 ± 0.82 ^{a,A}	8.28 ± 0.83^{a,A}
	60	8.28 ± 0.61^{a,A}	8.02 ± 1.03^{a,A}	8.14 ± 0.86 ^{a,A}	7.92 ± 0.90 ^{a,A}
Texture (1–9 point)	1	7.64 ± 0.84 ^{a,A}	7.64 ± 1.2^{a,A}	7.28 ± 1.26 ^{a,A}	7.21 ± 1.05^{a,B}
	30	7.85 ± 0.86 ^{a,A}	7.64 ± 1.21^{a,A}	7.52 ± 1.01^{a,A}	7.42 ± 0.93^{a,AB}
	60	7.64 ± 1.08^{ab,A}	6.64 ± 1.15^{d,B}	7.14 ± 0.77^{bc,A}	8.02 ± 0.87 ^{a,A}
Flavour (1–9 point)	1	8.14 ± 0.77^{a,A}	5.00 ± 1.17^{c,A}	6.92 ± 0.16^{b,A}	5.14 ± 1.16^{c,A}
	30	8.13 ± 0.86^{a,A}	5.28 ± 0.99^{b,A}	6.78 ± 0.69^{b,A}	5.13 ± 1.09c^{AB}
	60	8.07 ± 0.47^{a,A}	4.85 ± 1.29^{b,A}	7.07 ± 1.02^{a,A}	4.28 ± 072b^{B}
Overall acceptability (1–9 point)	1	7.85 ± 1.02^{a,A}	4.14 ± 0.77^{d,A}	7.00 ± 1.11^{b,AB}	5.35 ± 1.27^{c,A}
	30	7.57 ± 1.01^{a,A}	4.71 ± 1.48^{b,A}	7.64 ± 0.92^{a,A}	5.35 ± 0.92^{b,A}
	60	8.42 ± 0.85^{ab,A}	4.21 ± 0.80^{c,A}	6.35 ± 1.00^{b,B}	3.78 ± 1.18^{c,B}

a–d Means ± SD within a row with no common superscript differ ($P < 0.05$).
$^{A–D}$ Means ± SD within a column with no common superscript differ ($P < 0.05$).
[a] Salting system were: N8: 8% NaCl; N4: 4% NaCl; N4K4: 4% NaCl +4% KCl; N2K6: 2% NaCl +6% KCl.

evaluation of Feta cheese between sodium and potassium brine treatments. However, significant differences in texture were found at the 60th day of ripening ($P < 0.01$). Panelists preferred the texture of sample N2K6, which had the lowest sodium content ($P < 0.01$). Interestingly, the N8 sample, which had the highest salt content, received the highest flavor scores, while the N2K6 sample with the lowest sodium content received the lowest rating ($P < 0.01$). Murtaza et al. (2014) found that cheese with low salt concentration had higher concentrations of volatile flavor components, but flavor scores were highest for cheese with 2.5% added salt and gradually decreased with decreasing salt content. Salt does indeed contribute to the aroma/taste score of foods by increasing the volatility of aroma compounds and/or by affecting the lubricity of saliva, thereby enhancing the sensation it leaves in the mouth. The decrease in aroma perception with reduced-salt cheese can be attributed to this factor (Hoppu et al., 2017; Ferroukhi et al., 2024). In terms of overall acceptability, panelists preferred the N8 sample with the highest salt content, followed by the N4K4 sample, while the N4 sample was the least popular. Baptista et al. (2017) found that the cheese (N4) with a 50% salt reduction was less acceptable than the control group in terms of flavor, texture and overall acceptability. These results highlight the intricate relationship between salt content, texture, flavor, and overall acceptability in the sensory perception of Kashar cheese.

4. Conclusion

In conclusion, changes in NaCl content (replacement or reduction) have an impact on the physicochemical, proteolytic and sensory analysis criteria of cheese. Cheeses with a lower salt content had a softer texture and better melting properties but received lower scores for flavor. Increased proteolysis, especially in potassium-replaced cheeses (N4K4 or N2K6), contributed to higher proteolytic activity. The results show that by using KCl instead of NaCl, sodium content can be effectively reduced while quality characteristics are largely preserved. However, careful management of the balance between flavor, texture and proteolysis is essential. Replacing NaCl with KCl offers a viable solution for the production of reduced sodium cheese, but care must be taken to maintain consumer appeal. The study showed that reducing the NaCl content or substituting NaCl with KCl in the production of Kashar cheese had no negative effect on the cheese during the 60-day ripening period. Future research could focus on optimizing the use of potassium chloride alone instead of sodium or investigating alternative minerals other than K, as well as investigating the combined effect of starter cultures or other ingredients to achieve health benefits in terms of reduced salt intake and improved palatability. In addition, it would be valuable to evaluate the sensory quality and consumer acceptance of Kashar cheese with reduced NaCl content, as these are key factors in overall product quality.

CRediT authorship contribution statement

Kurban Yaşar: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Didem Şahingil:** Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ali Adnan Hayaloğlu:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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