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Nutritional and microbiological characterization of semi-hard Caciotta-type cheese produced in the Khorezm region of Uzbekistan

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ABSTRACT

This study investigated the nutritional composition, microbiological safety, and potential functional properties of semi-hard Caciotta cheese produced from cow milk sourced in the Khorezm region of Uzbekistan, an area affected by moderate to high soil salinity. The research was conducted as a controlled experimental study under standardized cheese-production conditions. Proximate analysis revealed that the cheese contained 48.2% moisture, 24.8% fat, 3.1% ash, and 24.5% crude protein, values typical for semi-hard cheese varieties. Amino acid profiling was performed using high-performance liquid chromatography with diode-array detection (HPLC-DAD) after phenylthiocarbamyl (PTC) derivatization. The total quantified amino acid content was 121.142 mg/g of cheese (approximately 494 mg/g of protein). Lysine (20.0 mg/g), glutamic acid (18.3 mg/g), proline (16.1 mg/g), histidine (12.0 mg/g), and aspartic acid (9.75 mg/g) were the predominant amino acids. Branched-chain amino acids accounted for 8.43 mg/g, while sulfur-containing amino acids reached 9.30 mg/g, indicating important nutritional contributions to protein metabolism and antioxidant-related processes. Microbiological analyses confirmed the absence of coliform bacteria, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*, demonstrating compliance with food safety requirements under refrigerated and frozen storage conditions. Additionally, in silico analysis of bovine β-casein peptides using the AnOxPePred platform predicted several peptides with potential antioxidant and ion-chelating properties. Molecular docking analysis suggested moderate interactions under simplified computational conditions and does not represent real cheese systems. Overall, the results demonstrate that Caciotta cheese produced from cow milk in the Khorezm region has high nutritional quality and is microbiologically safe. The study also suggests potential bioactive properties of β-casein-derived peptides; however, these predictions require experimental validation. These findings provide baseline data on a regionally produced dairy product and highlight the need for further comparative studies to better understand the possible influence of environmental factors, such as soil salinity, on milk and cheese composition.

Keywords: Caciotta cheese, amino acid profile, molecular docking, β-casein peptide, microbiological safety

INTRODUCTION

Cheese is an important component of human nutrition and a valuable source of high-quality proteins, essential amino acids, lipids, vitamins, and bioactive compounds that support muscle protein synthesis, antioxidant defense, and overall health **[1], [2]**. Among different cheese varieties, semi-hard cheeses occupy a significant place in global dairy production due to their balanced texture, flavor, and nutritional composition. Caciotta is a traditional

Italian semi-hard cheese typically produced from cow, sheep, or mixed milk and characterized by a relatively short ripening period, mild flavor, and soft-to-firm texture resulting from controlled proteolysis during maturation **[3]**. Recent studies have shown that factors such as milk protein genetics, starter cultures, and technological conditions may significantly influence proteolysis, free amino acid release, and the formation of bioactive compounds in Caciotta and similar cheeses **[1]**, **[3]**, **[4]**.

Milk proteins, particularly caseins, are important precursors of bioactive peptides formed during cheese ripening. β-Casein constitutes approximately 30–36% of bovine casein and plays a key role in generating peptides with potential biological activities. Proteolysis of β-casein during cheese maturation may release peptides exhibiting antioxidant, antimicrobial, and metal-chelating properties. Previous research on semi-hard cheeses such as Gouda and Cheddar has demonstrated that β-casein-derived peptides containing proline-rich sequences and amino acids such as histidine and branched-chain residues can contribute to free radical scavenging activity and oxidative stability **[5]**. Recent advances in bioinformatics tools allow the prediction of such bioactive peptides using in silico approaches, including platforms such as AnOxPePred, which evaluate potential antioxidant activity based on peptide sequence characteristics **[6]**. Such peptides may contribute to oxidative stability and could mitigate environmental stress-induced alterations in milk composition.

Environmental conditions may also influence the composition and functional properties of milk and dairy products. In arid and semi-arid regions, including Central Asia, soil salinization represents a major environmental challenge affecting agricultural productivity and forage quality. In Uzbekistan, particularly in the Khorezm region located along the Amu Darya River basin, soil salinity represents an important environmental characteristic of the agricultural ecosystem, with reported levels ranging from 4 to 8 dS/m in irrigated lands **[7]**, **[8]**. While salinity may influence soil fertility and forage quality, its direct impact on the biochemical composition of cow milk and dairy products remains insufficiently studied and requires further investigation. Salinity may influence mineral balances and forage nutrient profiles and could potentially be associated with changes in milk composition or fatty acid composition **[8]**. Although several studies have examined the effects of salinity on the composition of camel and sheep milk, the impact of saline environmental conditions on cow milk and cheese produced from such milk remains insufficiently investigated **[9]**, **[10]**. Despite increasing interest in functional dairy products, there remains limited information on the detailed amino acid composition and bioactive peptide potential of cheeses produced from milk from salinity-affected environments. This represents an important knowledge gap, particularly in regions where dairy production plays a key role in food security and rural development **[11]**. Understanding the nutritional composition, microbiological safety, and potential functional properties of locally produced cheeses may contribute to the development of sustainable dairy systems under environmental stress conditions.

Therefore, the present study aimed to provide a comprehensive characterization of semi-hard Caciotta cheese produced from cow milk in the salinity-affected Khorezm region of Uzbekistan. The study included determination of total protein content using the Kjeldahl method (N × 6.38), analysis of the amino acid profile by high-performance liquid chromatography with diode-array detection (HPLC-DAD) after acid hydrolysis and phenylthiocarbamyl derivatization, and evaluation of microbiological safety indicators, including coliform bacteria, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* according to relevant GOST standards. In addition, potential bioactive peptides derived from bovine β-casein were predicted using the AnOxPePred platform **[12]**. Considering the saline environmental conditions characteristic of the Khorezm region, the possible influence of salt ions on milk protein-derived peptides was further explored using an in silico molecular docking approach. In aqueous environments, sodium chloride dissociates into sodium (Na^+) and chloride (Cl^-) ions, which may interact with amino acid residues in peptides, particularly those containing proline and histidine, through electrostatic and coordination interactions. To investigate these potential interactions, docking simulations were performed between Na^+ and Cl^- ions and selected β-casein-derived peptides predicted to possess antioxidant activity. Peptide structures were generated using the Pep-Fold server, and docking analyses were conducted using the CB-Dock2 platform with the AutoDock Vina scoring function to estimate potential binding affinities.

Scientific Hypothesis

Environmental conditions, including soil salinity, may indirectly affect agricultural systems by altering forage quality and mineral availability. However, evidence of the direct influence of such factors on cow milk composition and cheese properties remains limited and inconsistent. It is hypothesized that semi-hard Caciotta cheese produced in this region contains a measurable amino acid profile and may serve as a source of β-casein–derived peptides with potential functional properties. These characteristics can be evaluated through chemical analysis, microbiological assessment, and in silico prediction approaches.

Objectives

The main objective of this study was to evaluate the nutritional composition and potential functional properties of semi-hard Caciotta cheese produced from cow milk in the Khorezm region of Uzbekistan. The specific objectives were:

- to determine the proximate chemical composition of Caciotta cheese,
  to analyze the amino acid profile using HPLC-DAD,
  to evaluate microbiological safety indicators of the cheese, and
  to predict potential antioxidant peptides derived from bovine β-casein using *in silico* analysis
- to explore potential bioactive peptide properties using in silico approaches, considering the environmental context of the production region

MATERIAL AND METHODS

Samples

Samples description: Milk was collected from 3 local farms in the Khorezm region. Farms were selected based on similar feeding practices and moderate-to-high soil salinity levels (EC 4–8 dS/m).

Samples collection: Milk (100 L per batch) was collected in sterilized containers and temporarily stored at 10 °C before processing.

Samples preparation: Milk was pasteurized at 65 °C for 30 min, cooled to 32–34 °C, inoculated with a mesophilic starter culture (1% v/v), coagulated with calf rennet (0.02–0.03 g/L), cut, stirred, molded, pressed, salted in 20% brine for 12 h, and ripened at 10–12 °C and 85–90% RH for 30 days.

Number of samples analysed: Three independent batches (n=3 biological replicates) were produced, and technical triplicates were analyzed for each batch.

Chemicals

Agar media: Plate Count Agar (PCA, Merck), Listeria Selective Agar (Oxoid), Salmonella Selective Agar (HiMedia), and Baird-Parker Agar (Oxoid).

Animals, Plants and Biological Materials

Animals: Cow milk from local farms in Khorezm, Uzbekistan.

Microorganisms: Starter cultures (*Streptococcus thermophilus* and *Lactococcus lactis subsp. lactis/cremoris,* Chr. Hansen).

Instruments

Agilent 1260 Infinity II HPLC system with diode-array detector (Agilent Technologies, USA), Zorbax Eclipse Plus C18 column (4.6 × 150 mm, 3.5 µm), Pep-Fold server for peptide modeling, CB-Dock2 platform for molecular docking, standard laboratory equipment (pH meter, centrifuge, homogenizer).

Laboratory Methods

Proximate analysis: Moisture, fat, and ash by AOAC methods 925.10, 920.152, and 935.42. Total nitrogen by Kjeldahl method (ISO 8968-1:2014). Crude protein = TN × 6.38.

Amino acid analysis: Lyophilized samples (3 g) were hydrolyzed in 6 M HCl + 0.1% phenol at 110 °C for 24 h. Derivatized with PITC and analyzed by HPLC-DAD. Calibration curves were used for quantification; recovery was 92–105%, RSD <5%, LOD 0.1–0.5 nmol, and LOQ 0.3–1.5 nmol.

***In silico* prediction:** AnOxPePred predicted bovine β-casein peptides (FRS >0.6, CHEL >0.3).

Molecular docking: Selected peptides docked with Na^+ and Cl^- ions using CB-Dock2 (AutoDock Vina scoring).

Microbiological analysis: Coliforms, *L. monocytogenes*, *Salmonella spp.,* and *S. aureus* following GOST 31747-2012, 32031-2012, 31659-2012, and 31746-2012. Samples were tested under long-term (-8°C, 6 months) and short-term (+4–6°C) storage conditions. Microbiological analysis was performed after storage; however, time-dependent monitoring was not conducted.

Description of the Experiment

Study flow: Three independent batches were processed. First, milk samples were collected and processed for cheese production. Cheese was analyzed for chemical composition (moisture, fat, ash, and protein), amino acid profile, microbiological safety, and in silico peptide properties. Molecular docking assessed interactions between salt ions and β-casein-derived peptides. Data from all batches were statistically analyzed. No deviations from the protocol occurred.

Quality Assurance

Number of repeated analyses:3 technical replicates per batch.

Number of experiment replication: 3 independent batches.
Reference materials: Standard amino acid mixtures (Sigma-Aldrich) for HPLC calibration.
Calibration: All analytical instruments were calibrated prior to analysis according to manufacturer instructions and ISO 17025 laboratory guidelines. For amino acid quantification, external calibration was performed using certified amino acid standards (Sigma-Aldrich, USA). Calibration standards were prepared at six concentration levels (0.1, 1, 5, 10, 50, and 100 µmol/L) by serial dilution. Each calibration level was analyzed in triplicate. Calibration curves were constructed using linear regression analysis with the equation:

$$y = ax + b$$

where y represents peak area and x represents analyte concentration. The calibration curves demonstrated excellent linearity with correlation coefficients (R^2 = 0.998–0.9995), confirming analytical reliability. Method precision was evaluated through intra-day and inter-day repeatability tests, yielding relative standard deviation (RSD) values below 5%. Accuracy was assessed via recovery experiments by spiking known concentrations of amino acid standards into cheese matrix samples, resulting in recovery rates of 92–105%. Instrument performance was verified before each analytical run using quality control standards to ensure system stability.
Laboratory accreditation: Analyses were performed in accredited laboratories (ISO 17025).

Data Access

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) from three independent production batches (n = 3), each analyzed in technical triplicate. Differences among batches for each proximate parameter (moisture, fat, ash, total nitrogen, and crude protein) were evaluated separately using one-way analysis of variance (ANOVA). Tukey's post hoc test was applied for pairwise comparisons between Batch 1, Batch 2, and Batch 3. Statistical significance was defined at $p < 0.05$. All statistical analyses were performed using GraphPad Prism 8 software.

Reporting and transparency statement

Samples were randomly collected from farms; no blinding was applied. All three batches were included in the analysis. Each experiment included technical replicates, and no data were excluded.

RESULTS AND DISCUSSION

The proximate composition of semi-hard Caciotta cheese from three independent production batches (n = 3) is summarized in Table 1. Moisture content was 48.2 ± 1.3%, fat 24.8 ± 0.9%, ash 3.1 ± 0.4%, total nitrogen 3.85 ± 0.08%, and crude protein 24.5 ± 0.6%. Relative standard deviations (RSD) were ≤3.6%, indicating high reproducibility across batches. These values align with standard ranges reported for traditional semi-hard cheeses such as Italian Caciotta (protein 22–28%, moisture 45–52%, fat 22–30%) and Gouda (protein 23–27%, moisture 45–50%, fat 25–30%). No significant differences were observed among batches for proximate composition ($p > 0.05$, one-way ANOVA).

Table 1 Proximate composition and protein content of semi-hard Caciotta cheese (mean ± SD, n=3) Amino Acid Profile.

Parameter	Mean ± SD	RSD (%)	Significance
Moisture (%)	48.2 ± 1.3	2.7	a
Fat (%)	24.8 ± 0.9	3.6	a
Ash (%)	3.1 ± 0.4	12.9	a
Total nitrogen (%)	3.85 ± 0.08	2.1	a
Crude protein (%)	24.5 ± 0.6	2.4	a

The total quantified amino acids after complete acid hydrolysis were 121.142 mg/g cheese (494 mg/g crude protein). Dominant amino acids were lysine (20.0 mg/g cheese; 82 mg/g protein), glutamic acid (18.308 mg/g cheese; 75 mg/g protein), and proline (16.106 mg/g cheese; 66 mg/g protein). Histidine content was 12.0 mg/g cheese (49 mg/g protein), BCAAs totaled 8.43 mg/g cheese (34 mg/g protein), and sulfur-containing amino acids (methionine + cysteine) summed to 9.30 mg/g cheese (38 mg/g protein) (Table 2). Relative standard deviations for major amino acids were <6%, confirming analytical precision.

Table 2 Amino acid composition of semi-hard Caciotta cheese (mg/g cheese).

Essential amino acids	Concentration (mg/g)	Non-essential amino acids	Concentration (mg/g)
Histidine	12.0	Aspartic acid	9.753
Isoleucine	1.315	Glutamic acid	18.308
Leucine	4.813	Serine	2.943
Lysine	20.1	Glycine	2.808
Methionine	7.108	Tyrosine	4.877
Phenylalanine	9.190	Cysteine	2.191
Threonine	2.287	Arginine	0.927
Valine	2.302	Alanine	4.114
		Proline	16.106
Total: 121.142			

Values are presented as mean ± standard deviation (technical triplicate HPLC analyses, n = 3).

The complete amino acid profile is presented in Table 2. RSD < 6% across technical triplicate HPLC injections, confirming analytical precision. HPLC-DAD chromatograms (Figure 1) exhibited well-resolved peaks for PTC-derivatized amino acids, with prominent signals in the polar (5–15 min) and hydrophobic (18–28 min) retention time ranges.

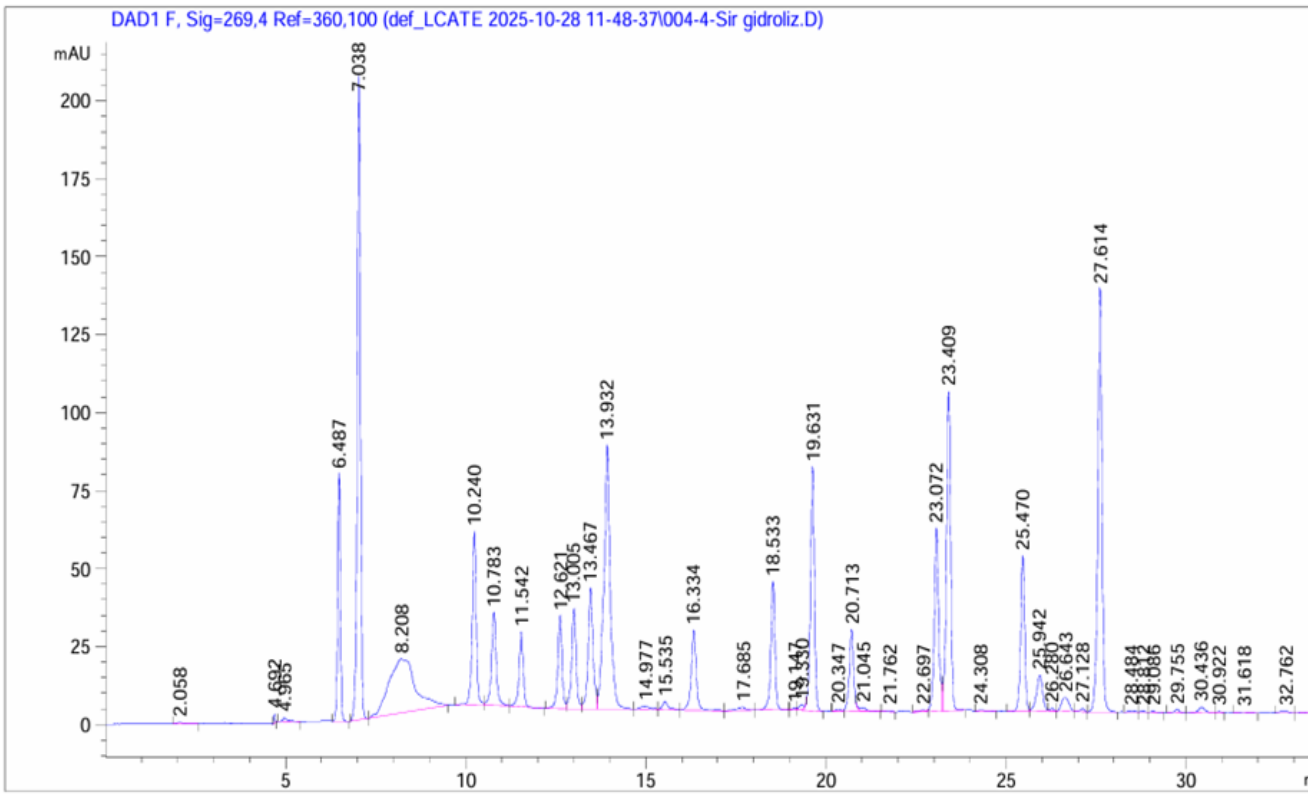

Figure 1 HPLC chromatogram of PTC-derivatized amino acids in semi-hard Caciotta cheese.

Figure 2 Semi-hard Caciotta cheese produced from cow milk in the Khorezm region of Uzbekistan. Whole cheese and cut section showing the internal texture.

In Silico Prediction of Antioxidant Peptides

β-casein sequences were analyzed using the AnOxPePred platform to predict peptides with potential antioxidant and ion-chelating properties. These predictions are based solely on the theoretical amino acid sequence of bovine β-casein and do not confirm the actual presence of these peptides in the analyzed cheese matrix. Although some predicted peptides are rich in proline and histidine residues, this does not directly demonstrate their occurrence in the cheese, as no peptide identification (e.g., LC-MS/MS) was performed in this study, however, the overall amino acid composition of the cheese does not provide direct evidence for the presence of these specific peptide sequences.

Table 3 Selected top in silico predicted antioxidant peptides from β-casein.

FRS Score	CHEL Score	Peptide Sequence	Length	Key Features
0.715	-	PLLQSWMHQPHQPLPPTVM	19	Proline-rich, histidine (WMHQ)
0.714	-	PLLQSWMHQPHQPLPPTV	18	Histidine + glutamine cluster
0.703	-	LPLLQSWMHQPHQPLPPTVM	20	BCAAs + histidine-rich
-	0.379	SLTLTDVENLHLPLPLLQS	19	Histidine-rich (HLPL) for chelation

These in silico predictions suggest potential antioxidant and metal-chelating activity of casein-derived peptides, which may be influenced by salinity in the milk-producing environment. Experimental validation is needed to confirm functional properties.

Microbiological Indicators

Microbiological analyses were performed on cheese samples obtained from the three independent production batches. No coliform bacteria, Listeria monocytogenes, Salmonella spp., or coagulase-positive staphylococci (including Staphylococcus aureus) were detected in any sample within the detection limits specified by the respective GOST standards. These results demonstrate compliance with food safety requirements (GOST 31747-2012, 32031-2012, 31659-2012, and 31746-2012) and indicate effective pasteurization (65 °C for 30 min), hygienic processing conditions, and proper sanitation during production.

Table 4 Microbiological indicators of semi-hard Caciotta cheese (n = 3 samples).

Sample	Coliforms (BGKP) GOST 31747-2012	*L. monocytogenes* GOST 32031-2012	Pathogenic Salmonella GOST 31659-2012	*S. aureus* GOST 31746-2012
Standard	Not permitted in 0.001 g	Not permitted in 25 g	Not permitted in 25 g	Not permitted in 0.001 g
Batch 1	Not found in 0.001 g	Not found in 25 g	Not found in 25 g	Not found in 0.001 g
Batch 2	Not found in 0.001 g	Not found in 25 g	Not found in 25 g	Not found in 0.001 g
Batch 3	Not found in 0.001 g	Not found in 25 g	Not found in 25 g	Not found in 0.001 g

The results confirm the microbiological safety of the produced Caciotta cheese under the applied processing and storage conditions. However, a full shelf-life study with quantitative microbial monitoring over time was not conducted in the present work.

Molecular Docking Analysis

To further explore the potential influence of salinity-related ions on milk protein–derived peptides, molecular docking simulations were performed between Na^+ and Cl^- ions and the proline-rich histidine-containing β-casein peptide PLLQSWMHQPHQPLPPTVM.

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Table 5 Docking results of Na binding with proline-histidine and corresponding comments.

CurPoketID	Vina score	Cavity volume	Center	Docking size
C1	-5.2	19	28,35,29	23,23,23
C5	-4.8	1	38,28,33	23,23,23
C2	-4.7	6	34,32,24	23,23,23
C3	-4.2	3	33,27,29	23,23,23
C4	-4.1	1	27,26,31	23,23,23

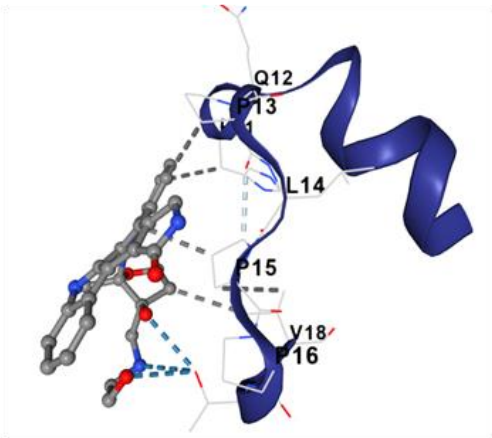

Figure 3 3D docking analysis of Na binding with proline-histidine pockets.

This peptide was selected due to its high predicted antioxidant score and the presence of residues known to participate in ion coordination. Docking analysis revealed that Na^+ exhibited a moderate binding affinity toward the peptide, with the best docking score reaching −5.2 kcal/mol in cavity C1.
Other potential binding pockets showed slightly weaker interactions, with Vina scores ranging from −5.0 to −4.4 kcal/mol. These results suggest that sodium ions may interact preferentially with residues within the peptide structure, possibly involving histidine and backbone carbonyl groups that can participate in electrostatic or coordination interactions. In contrast, docking simulations with Cl^- ions showed considerably weaker binding affinities. The best docking score observed for chloride was −1.2 kcal/mol across several cavities (C1, C3, and C5), while other cavities displayed even weaker interactions (−1.1 to −0.9 kcal/mol). Such low binding energies indicate minimal interaction between chloride ions and the peptide structure.

Table 6 Docking results of Cl^- binding with proline-histidine pockets.

CurPoketID	Vina score	Cavity volume	Center	Docking size
C1	-1.2	19	28,35,29	13,13,13
C3	-1.2	3	33,27,29	13,13,13
C5	-1.2	1	38,28,33	13,13,13
C2	-1.1	6	34,32,24	13,13,13
C4	-0.9	1	27,26,31	13,13,13

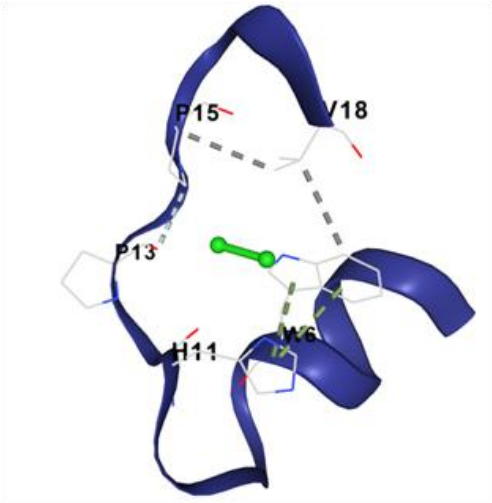

Figure 4 Molecular docking of Cl^- ions with the β-casein peptide.

Overall, the docking results suggest that sodium ions demonstrate stronger affinity toward proline-rich histidine-containing peptides compared to chloride ions. This difference may be attributed to the ability of Na^+ to interact with electron-rich functional groups present in peptide residues, whereas Cl^- ions primarily participate in weaker electrostatic interactions. Molecular docking simulations provide a theoretical indication of possible interactions between selected peptide sequences and ions. However, these results are based on simplified computational models and do not reflect the complexity of real cheese systems. No direct experimental validation

of these interactions was performed. Therefore, the in silico results should be interpreted as exploratory and hypothesis-generating rather than confirmatory.

In this study, the proximate composition of semi-hard Caciotta cheese produced from cow's milk under saline soil conditions (EC 4–8 dS/m) in the Khorezm region was investigated **[13]**. The values obtained for moisture (48.2 ± 1.3%), fat (24.8 ± 0.9%), ash (3.1 ± 0.4%), and total protein (24.5 ± 0.6%) were found to be within the typical ranges reported for traditional Italian Caciotta and other semi-hard cheese varieties **[14]**. Manuelian et al. (2023) reported analogous compositional ranges in their comparison of organic and conventional Italian cheeses, supporting the notion that the basic nutritional matrix of the cheese produced under local conditions is consistent with established production standards **[14]**. Di Cagno et al. also described similar compositional characteristics in their study of Caciotta-type cheeses manufactured with adjunct cultures of selected non-starter lactobacilli, further confirming the comparability of our findings with those reported in the literature **[15]**. However, the relatively high variability observed in ash content (RSD = 12.9%) requires special attention. The elevated variability in ash content may reflect fluctuations in the mineral composition of milk, which could be influenced by several interacting factors including feed composition, lactation stage, soil geochemistry, and the salting procedure applied during cheese-making. Although the study was conducted in a region characterized by moderate soil salinity (EC 4–8 dS/m), no direct measurements of milk mineral composition or specific salt ion concentrations were performed. Therefore, any potential influence of environmental salinity on milk or cheese composition remains speculative **[16]**. Turdaliev et al. documented the biogeochemical state of salinized irrigated soils in Central Asia, confirming that salinity is a significant environmental factor in the region; however, the absence of paired soil-plant-milk analytical data precludes direct attribution of cheese compositional variation to soil salinity alone **[16]**. The absence of a non-saline control group, the lack of detailed mineral composition analysis of milk, and insufficient control over confounding variables such as feeding regime, lactation stage, and the cheese salting process limit the strength of this interpretation. Aljumaah et al. demonstrated that salt supplementation directly alters milk composition, fatty acid profiles, and insulin response in lactating camels, highlighting the complexity of salt-milk interactions and the need for controlled experimental designs **[10]**. Cosentino et al. further demonstrated that even the addition of milk from different species, such as donkey milk, can significantly alter the compositional and sensory properties of Caciotta cheese, emphasizing the multifactorial nature of cheese composition **[17]**. Fox et al. similarly reported that the chemical composition of craft hard cheeses from raw goat milk undergoes substantial changes during ripening, demonstrating that animal source and processing conditions are critical determinants of final product composition **[18]**. Khan et al. noted that environmental stress, including salinity, may influence milk yield and composition; however, such effects are highly context-dependent and vary considerably across animal species, stress severity, exposure duration, and the animals' adaptive capacity **[19]**. Therefore, while soil salinity constitutes an important environmental characteristic of the study area, its specific role in shaping dairy product composition should be regarded as a hypothesis requiring rigorous future investigation rather than a confirmed causal effect. The total amino acid content of the cheese was determined to be 121.142 mg/g (approximately 494 mg/g protein), with lysine, glutamic acid, proline, and histidine identified as the dominant amino acids. Niro et al. demonstrated that the evolution of free amino acids during cheese ripening is strongly dependent on ripening time, with longer maturation periods leading to a significant increase in free amino acid concentrations due to progressive casein proteolysis **[20]**. In the present study, a relatively short ripening period of 30 days was employed, which may have limited proteolysis and the accumulation of free amino acids and bioactive peptides. Helal et al. monitored peptide evolution during cheese ripening and confirmed that the bioactive peptide profile undergoes substantial changes between 30 and 90 days of maturation, suggesting that the cheese's full functional potential may not have been reached within the ripening timeframe used in this study **[21]**. Vargas-Bello-Pérez et al. reviewed the implications of milk-derived bioactive peptides for human health, emphasizing that their release depends on the extent and specificity of proteolysis during cheese ripening **[11]**. Galli et al. further confirmed that proteolysis and peptide bioactivity in aged cheeses are positively correlated with ripening duration, supporting the interpretation that a 30-day ripening period may not be sufficient to generate a fully developed bioactive peptide profile **[22]**. Microbiological analyses confirmed the absence of major pathogens, indicating good hygienic manufacturing practices. Primavilla et al. assessed the microbiological safety and hygiene of raw and thermally treated milk cheeses marketed in Central Italy and reported similar trends in pathogen absence when appropriate manufacturing protocols were followed **[23]**. Calasso et al. studied the role of attenuated *Lactococcus lactis* and surface bacteria in improving the ripening of Caciotta cheese, demonstrating that bacterial dynamics play a critical role in both safety and flavor development **[24]**. Turchi et al. showed that the selection and use of autochthonous lactic acid bacteria in Caciotta della Garfagnana cheese can improve product safety and sensory characteristics **[25]**. Bancalari et al. confirmed that *Lactobacillus paracasei* 4341 can serve as an effective adjunct culture to enhance flavor in short-ripened Caciotta-type cheese **[26]**. However, the lack of time-dependent microbiological monitoring during storage in the present

study limits a full assessment of product stability and shelf-life. In silico analysis using the AnOxPePred platform predicted antioxidant potential in certain β-casein-derived peptides **[27]**. Olsen et al. developed the AnOxPePred platform using deep learning to predict the antioxidative properties of peptides, demonstrating the utility of computational approaches for screening bioactive peptide sequences **[12]**. Molecular docking simulations indicated moderate binding affinity with Na^+ ions. Iwaniak et al. applied an integrated approach to analyze antioxidative peptides derived from Gouda cheese with a modified β-casein content, confirming that computational methods can effectively predict peptide bioactivity **[5]**. Iwaniak et al. further characterized bioactive peptides from dairy products with particular emphasis on antioxidant and ACE-inhibitory properties, providing a comprehensive framework for interpreting in silico predictions **[27]**. Coscueta et al. screened novel bioactive peptides from goat casein using both computational and experimental approaches and reported similar predictive accuracy for antioxidant peptides **[28]**. However, these in silico results have important limitations that must be acknowledged. Baptista and Gigante emphasized that bioactive peptides from ripened cheeses require experimental validation using LC-MS/MS to confirm the actual presence and concentration of predicted peptide sequences **[29]**. In the present study, no peptides were experimentally identified in the cheese matrix, as the analysis was based solely on total amino acid composition, and docking simulations were performed under simplified conditions. Kurbanova et al. studied the role of *Lactobacillus* cultures in the development of bioactive peptides in Caciotta-type cheeses and demonstrated that starter and adjunct cultures significantly influence peptide release patterns—a factor not addressed in the present in silico approach **[1]**. Shafique et al. investigated proteolysis and peptide generation in Cheddar cheese and highlighted the importance of experimental peptide identification for validating computational predictions **[30]**. Kashung and his collegue confirmed the multifunctional bioactivity of β-casein peptides but also cautioned that simplified computational models may overestimate binding affinities and functional activities **[31]**. Dammak et al. reviewed the relationship between cheese bioactive peptides and fatty acid profiles, emphasizing that the cheese matrix exhibits complex interactions that are difficult to fully capture in computational simulations **[32]**.

Statistical analysis confirmed that variations among batches were not significant ($p > 0.05$), indicating good reproducibility of the production process. Although salinity is widespread in the Khorezm region, the absence of a control group makes it premature to directly attribute the observed compositional characteristics to salinity. Overall, this study provides baseline data confirming the nutritional composition and microbiological safety of Caciotta cheese produced under local conditions, while identifying several directions for future research, including extended ripening, experimental peptide identification, and controlled salinity exposure studies.

Limitations

Several limitations should be considered when interpreting the findings of this study. First, the number of samples was limited to three independent production batches, which may constrain the statistical power and generalizability of the results. Although technical replicates were included, future studies involving larger sample sizes across multiple farms and seasons would strengthen the robustness of the conclusions. Second, the absence of a clearly defined non-saline control group limits the ability to directly assess environmental effects. Without comparative samples from non-saline regions, it is not possible to isolate or quantify the specific influence of salinity on milk composition and cheese properties. Therefore, interpretations related to salinity should be considered with caution. Third, the ripening period of 30 days is relatively short for semi-hard cheeses. Extended maturation is known to enhance proteolysis and influence the development of free amino acids and bioactive peptides. As such, the present results may not be directly comparable to longer-ripened cheeses such as Cheddar or Gouda. Fourth, the use of acid hydrolysis for amino acid analysis may lead to partial degradation or transformation of certain amino acids, potentially affecting quantitative accuracy. This represents a recognized methodological limitation and should be taken into account when interpreting the data. Fifth, the in silico peptide prediction and molecular docking analyses provide only theoretical insights. These approaches do not confirm the actual presence, concentration, or biological activity of peptides within the cheese matrix. Experimental validation, such as LC–MS/MS-based peptidomics and functional assays, is required to support these predictions. Finally, environmental variability, including seasonal differences in feed composition and farm-specific management practices, was not fully controlled and may have contributed to variation in milk composition and derived cheese properties.

CONCLUSION

This study provides baseline data on the nutritional composition, amino acid profile, and microbiological safety of semi-hard Caciotta-type cheese produced from cow milk in the Khorezm region of Uzbekistan. The proximate composition (crude protein 24.5 ± 0.6%, moisture 48.2 ± 1.3%, fat 24.8 ± 0.9%) falls within the typical range reported for semi-hard cheeses, indicating that the applied cheesemaking process yielded a product of standard quality. Amino acid analysis demonstrated a balanced profile, with lysine, glutamic acid, proline, and histidine as predominant components. While the absolute concentrations of certain amino acids may be influenced by methodological limitations of acid hydrolysis, the overall composition is consistent with that of casein-based dairy products and supports the nutritional value of the cheese. Microbiological evaluation confirmed the absence of major foodborne pathogens in the analyzed samples, indicating compliance with relevant safety standards at the time of analysis. However, no comprehensive shelf-life study was conducted, and therefore conclusions regarding long-term storage stability cannot be drawn. In silico analysis predicted several β-casein-derived peptides with potential antioxidant and ion-chelating properties. It should be noted that these peptides were computationally predicted from protein sequences and were not experimentally identified in the analyzed cheese. Similarly, molecular docking results provide only theoretical insight into possible ion–peptide interactions and do not confirm their occurrence under real dairy processing conditions. Overall, the results characterize the studied cheese as a nutritionally valuable and microbiologically safe product. However, due to the absence of a non-saline control and direct mineral composition analysis, no definitive conclusions can be drawn regarding a possible influence of environmental salinity on milk or cheese composition. Future studies should include comparative sampling from non-saline regions and experimental validation of peptide bioactivity to better understand potential environmental effects.

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