2026 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 16, Issue 4: 217-224; July 30, 2026

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2026.26

VITAMIN COMPOSITION OF ALPINE AND YOGHURT CRAFT HARD CHEESES MADE FROM UNPASTEURIZED GOAT MILK AFTER RIPENING WITH THE PARTICIPATION OF *Acarus siro* MITES

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Supporting Information

RESEARCH ARTICLE
PII: S222877012600026-16
Received: January 07, 2026
Revised: July 16, 2026
Accepted: July 18, 2026

ABSTRACT: Craft goat cheeses made from unpasteurized milk are considered elite food products with high nutritional and biological value for humans, which contributes to growing market availability and necessitates the assessment of their quality, authenticity and biological value. This study used hard craft cheeses, Alpine and Yoghurt, which ripened with the activity of *Acarus siro* mites and produced from unpasteurized goat milk on a local organic farm. The contents of retinol, tocopherol and riboflavin were determined in the cheeses after 7 days (young cheese), 6 months (mature cheese), and at the final stage of ripening (12 months for Alpine and 18 months for Yoghurt). In Alpine cheese during 12 months of ripening, the retinol content ranged from 5.04 to 5.30 mg/kg, the tocopherol content from 2.00 to 2.34 mg/kg and the riboflavin content from 2.19 to 2.48 mg/kg. The ripening period did not significantly affect the retinol and riboflavin content but increased the tocopherol content by 15.42 - 16.42%. In Yoghurt cheese, the retinol content ranged from 4.60 to 5.24 mg/kg, while the tocopherol content ranged from 2.18 to 2.33 mg/kg, and was not affected by the ripening period. The riboflavin content increased from 1.78 mg/kg to 2.58 mg/kg during ripening. The retinol content in the dry matter of both cheeses decreased with increasing ripening time, whereas the tocopherol content decreased only in Yoghurt cheese. To statistically assess the effect of ripening time on the vitamin content of the hard craft cheeses, one-way analysis of variance (ANOVA) was used. This analysis showed that the retinol concentration in the dry matter of both cheeses decreased with increasing ripening time, while the tocopherol concentration increased only in Yoghurt cheese. The results of the study indicate the high biological value of hard cheeses made from unpasteurized goat milk as sources of vitamins A, E, and B_2. Moreover, the use of dispersion analysis in combination with microbiological and physicochemical characteristics allows evaluating and confirming their quality, authenticity, and biological value.

Keywords: Alpine cheese, Maturation, Retinol, Riboflavin, Tocopherol, Yoghurt cheese.

INTRODUCTION

Cheese is a food product that simultaneously provides healthy microflora, nutrients (protein, fat, amino acids, fatty acids, mineral components), and a number of biologically active substances, including fat- and water-soluble vitamins (Iakubchak et al., 2024; Kukhtyn et al., 2024; Savchenko et al., 2024). Hard cheeses made from unpasteurized goat milk are of particular value, as they are characterized not only by valuable nutritional components but also by distinctive flavour characteristics, for which they are highly valued by consumers (Awad and Abdou, 2024).

Craft hard cheeses are becoming increasingly popular among goat cheeses. They are made using proprietary technologies and have a unique taste and aroma. Such properties are conferred by various biologically active compounds, in particular volatile fatty acids, organic acids, peptides, amino acids and vitamins. A significant amount of these compounds enters the cheese with the milk, but during ripening, they are also formed through lipolysis, proteolysis and glycolysis. It is known that cheese proteins can be hydrolyzed to form peptides and amino acids, and cheese lipids can serve as precursors of conjugated linoleic acid as well as certain fat-soluble vitamins (Vázquez-García and Martín del Campo, 2023; Sadvari et al., 2025).

It is believed that cheeses are characterized by an optional balance of vitamins for the human body. Lactic acid bacteria participate in vitamin synthesis. For example, *Limosilactobacillus reuteri* can synthesize riboflavin (vitamin B_2) in

Citation: Davydovych V, Shevchenko L, Shulyak S, Kudriashov A, Busol L, Ivaniuta A, Savchenko O, Nikolaenko M, Lialyk A, Beyko L. (2026). Vitamin composition of alpine and yoghurt craft hard cheeses made from unpasteurized goat milk after ripening with the participation of *Acarus siro* mites. *Online J. Anim. Feed Res.*, 16(4): 217-224. DOI: https://dx.doi.org/10.51227/ojafr.2026.26

an amount of 18.36 µg/ml when cultured in the presence of the fermentative bacterium *Streptococcus thermophilus,* which is used in the production of many cheeses (Spacova et al., 2022) and other food products (Rogoskii et al., 2020; Bal-Prylypko et al., 2024). A similar property has also been found in certain strains *of Lactococcus cremoris, Lactococcus lactis,* and *Limosilactobacillus fermentum* (Langa et al., 2024). The vitamins found in cheese include vitamins A, E, D, K, B_2, B_6, and B_{12} (Zhou et al., 2022).

The presence of such a complex composition of biologically active compounds in cheese contributes to its probiotic properties (Jena and Choudhury, 2023; Bustos et al., 2025), and its anti-carcinogenic, antioxidant and anti-inflammatory effects (Yerlikaya, 2023; Zhang et al., 2023). Thus, cheese consumption reduces the risk of cardiovascular, digestive and nervous system diseases and improves human quality of life (Saleem et al., 2024).

A limited number of studies have investigated the influence of the ripening process and period on the vitamin content of cheeses, and these have mainly focused on cheeses produced using industrial technologies, whereas craft cheeses have been scarcely studied in this respect. There is almost no information on the influence of ripening period on the vitamin content of hard cheeses made from unpasteurized goat milk and ripened with the participation of acarid mites. Therefore, the aim of this study was to determine the contents of retinol, tocopherol and riboflavin in hard craft cheeses made from unpasteurized goat milk during the ripening process with the participation of acarid mites.

MATERIALS AND METHODS

Animals

Unpasteurized goat's milk was used to produce hard cheeses. Milk was sourced from Anglo-Nubian goats at Eco-Farm Zhuravka in the Kyiv region.

Experimental Design

Research on the vitamin composition of hard craft cheeses made from unpasteurized goat milk and ripened with the participation of *Acarus siro* mites was conducted from May 2023 to January 2025. Alpine and Yoghurt cheeses were made from the milk of Anglo-Nubian goats at Eco-Farm "Zhuravka" in the Kyiv region, Ukraine. The technology for cheese production is described in our previous work (Davydovych et al., 2025a).

Sampling

To study the vitamin content, average samples of both types of cheese weighing 200 g were selected from 5 batches of each cheese type at different stages of ripening: 7 days (young cheese), 6 months (mature cheese), 12 months (old-mature cheese) for Alpine cheese, and 18 months (old-mature cheese) for Yoghurt cheese.

Sample analysis

Determination of vitamin A (retinol) content in cheeses was performed after saponification with an alcoholic KOH solution in the presence of the antioxidant pyrogallol. Following saponification, vitamin A was extracted from the cheese samples with diethyl ether, which was separated from the aqueous phase. The extract was then purified by passing it through anhydrous sodium sulfate to remove residual water. The next step involved concentration by evaporating the ether on a rotary evaporator, after which the residue was dissolved in chloroform. The resulting solution was used for the quantitative determination of vitamin A. Measurements were carried out using a KFK-3-01 photoelectric photometer at a wavelength of 620 nm against chloroform. A standard retinol solution (Vitamin A) 95% (Thermo Fisher Scientific, 1 g, USA) was used to construct the calibration curve in accordance with DSTU 4687:2006.

Determination of vitamin B_2 (riboflavin) in cheeses was based on the ability of riboflavin solutions to produce a yellow coloration and exhibit yellow-green fluorescence under ultraviolet light, the intensity of which depends on the concentration of riboflavin in the solution. For this purpose, a weighed sample was boiled in a hydrochloric acid solution, and the pH was adjusted to 6.0–6.5 using a saturated sodium carbonate solution. The filtered solution was transferred into the cuvette of a Fluorat-02-2M liquid analyzer, and fluorescence intensity was measured. In parallel, the fluorescence intensity of a standard working solution was measured, and sodium hydroxide solution was added to quench riboflavin fluorescence in accordance with DSTU 4687:2006.

Determination of vitamin E (tocopherol) content in cheeses was carried out through the oxidation of tocopherols with ferric chloride and subsequent determination of the formed ferrous iron as a pink-red complex with 2,2′-dipyridyl. For the analysis, saponification was performed using an alcoholic KOH solution in the presence of pyrogallol. After saponification and extraction, the dry residue was dissolved in benzene and treated with the iron–dipyridyl reagent. Colorimetric measurements were performed using a KFK-3-01 photoelectric photometer at a wavelength of 520 nm against a reagent blank, in accordance with DSTU 4687:2006.

Statistical analysis

The results of the study were statistically processed using one-way analysis of variance, information technologies for data analysis, and modelling of ripening processes. Digital methods for collecting, processing and visualizing data were applied, which allowed for high accuracy and reproducibility of research. Correlation and regression analyses of Alpine and Yoghurt cheeses were also performed. A comparison of the indicators was carried out within each cheese type over the ripening period. The analyses were performed using Microsoft Excel 2016 and XLSTAT (Addinsoft, Paris - 2017). The data in the tables are presented as x ± SD (mean ± standard deviation). Differences between the indicators were considered significant at $P < 0.05$ using the Tukey's test with Bonferroni correction.

RESULTS AND DISCUSSION

The ripening period of hard Alpine cheese did not affect the content of retinol or riboflavin, whereas the content of tocopherol increased with age, reaching 2.32 mg/kg (by 15.42%) after 6 months and 2.34 mg/kg (by 16.42%) after 12 months compared with young cheese aged 7 days (Table 1). Analysis of the dry matter of Alpine cheese showed that the contents of tocopherol and riboflavin did not depend on the ripening period, whereas the retinol content decreased, in particular, to 7.29 mg/kg (by 22.03%) after 6 months, and to 7.46 mg/kg (by 20.21%) after 12 months compared with cheese aged 7 days (Table 2).

Correlation analysis showed a strong inverse relationship between the retinol content in the dry matter of Alpine cheese and the ripening period ($r = -0.775 \pm 0.119$, $P<0.01$). The regression line confirmed this relationship and was best described by a second-degree polynomial (Figure 1).

During the ripening of hard Yoghurt cheese, no changes in the concentration of retinol or tocopherol were observed. However, the riboflavin concentration after 18 months increased to 2.58 mg/kg, which exceeded the values in cheese aged 7 days by 44.94% and those in cheese aged 6 months by 35.08% (Table 3).

In the dry matter of Yoghurt cheese, specific patterns of vitamin accumulation were observed. In particular, the retinol content decreased to 7.24 mg/kg (by 26.72%) after 6 months of ripening and to 7.31 mg/kg (by 26.01%) after 18 months compared with young cheese aged 7 days (Table 4).

A similar pattern was observed for tocopherol, and its content in the dry matter of Yoghurt cheese decreased to 3.67 mg/kg (by 10.71%) after 6 months of ripening and to 3.14 mg/kg (by 23.60%) after 18 months compared with the 7-day-old cheese. In contrast, the riboflavin concentration in the dry matter of this cheese did not depend on the ripening period. To confirm the statistical significance of the detected changes in vitamin content during ripening, a one-way analysis of variance (ANOVA) was used, which allowed the establishment of a significant effect of ripening duration on tocopherol content ($P < 0.05$), whereas no significant effect was observed for riboflavin. Thus, a moderate inverse relationship was found between the retinol content in the dry matter and the ripening period of Yoghurt cheese ($r = -0.640 \pm 0.145$, $P < 0.01$), as well as a strong inverse relationship between the tocopherol content in the dry matter and the ripening period of Yoghurt cheese ($r = -0.890 \pm 0.086$, $P < 0.001$). In both cases, the regression line was best described by a second-degree polynomial (Figure 2).

Table 1 - Vitamin content in Alpine cheese during maturation, x ± SD, mg/kg.

Vitamin name \ Cheese maturation period	7 days	6 months	12 months
Retinol	5.30 ± 0.22	5.04 ± 0.26	5.29 ± 0.08
Tocopherol	2.01 ± 0.16[b]	2.32 ± 0.07[a]	2.34 ± 0.11[a]
Riboflavin	2.23 ± 0.25	2.19 ± 0.23	2.48 ± 0.61

Note: different superscript letters indicate a significant difference between values in a row of the table ($P < 0.05$) using the Tukey test with Bonferroni correction

Table 2 - Vitamin content in dry matter of Alpine cheese during maturation, x ± SD, mg/kg

Vitamin name \ Cheese maturation period	7 days	6 months	12 months
Retinol	9.35 ± 0.48[a]	7.29 ± 0.35[b]	7.46 ± 0.09[b]
Tocopherol	3.53 ± 0.23	3.35 ± 0.10	3.31 ± 0.14
Riboflavin	3.93 ± 0.47	3.17 ± 0.36	3.52 ± 0.87

Note: different superscript letters indicate a significant difference between values in a row of the table ($P < 0.05$) using the Tukey test with Bonferroni correction

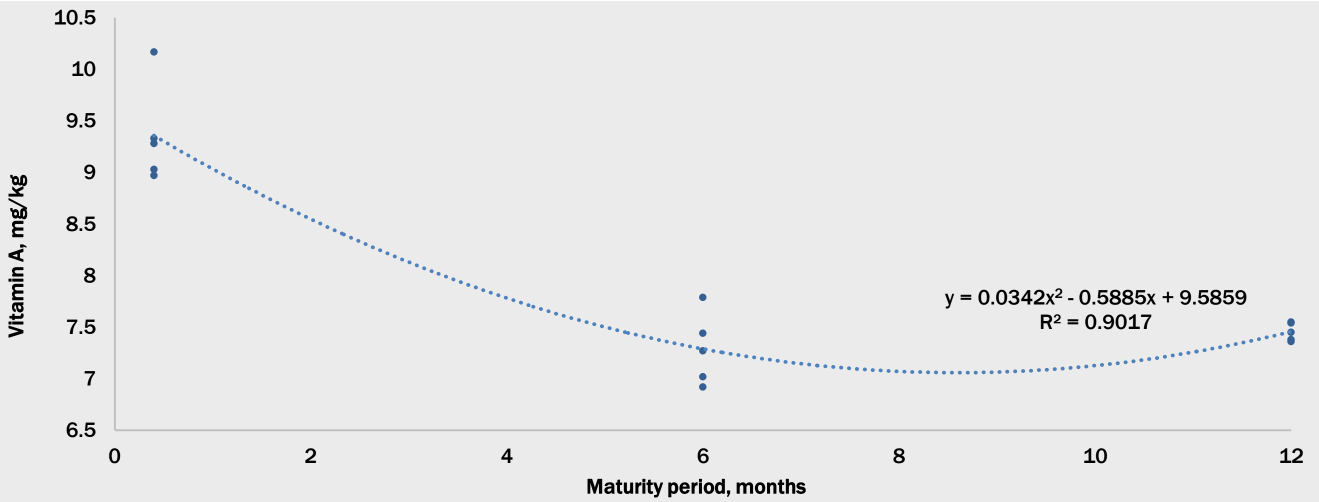

Figure 1 - Dependence of retinol content in dry matter of Alpine cheese on maturation period, n =15

Table 3 - Vitamin content in Yoghurt cheese during maturation, x ± SD, mg/kg

Vitamin name \ Cheese maturation period	7 days	6 months	12 months
Retinol	5.24 ± 0.26	4.62 ± 0.68	5.11 ± 0.23
Tocopherol	2.18 ± 0.15	2.33 ± 0.09	2.21 ± 0.13
Riboflavin	1.78 ± 0.08[b]	1.91 ± 0.36[b]	2.58 ± 0.19[a]

Note: different superscript letters indicate a significant difference between values in a row of the table ($P < 0.05$) using the Tukey test with Bonferroni correction (Bonferroni CE (1936))

Table 4 - Vitamin content in dry matter of Yoghurt cheese during maturation, x ± SD, mg/kg

Vitamin name \ Cheese maturation period	7 days	6 months	12 months
Retinol	9.88 ± 0. 75[a]	7.24 ± 1.02[b]	7.31 ± 0.36[b]
Tocopherol	4.11 ± 0.19[a]	3.67 ± 0.14[b]	3.14 ± 0.20[c]
Riboflavin	3.35 ± 0.17	3.01 ± 0.85	3.68 ± 0.32

Note: different superscript letters indicate a significant difference between values in a row of the table ($P < 0.05$) using the Tukey test with Bonferroni correction (Bonferroni CE (1936)).

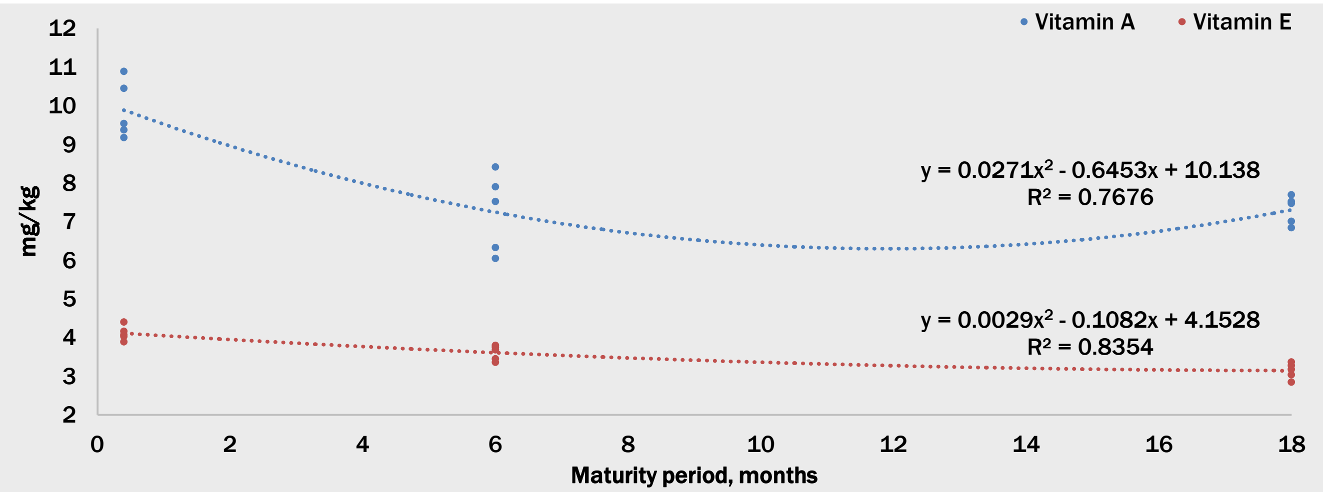

Figure 2 - Dependence of the content of retinol and tocopherol in the dry matter of Yoghurt cheese on the maturation period, n=15.

During the ripening of hard craft cheeses, their chemical components (Jakabová et al., 2023; Čapla et al., 2024), particularly protein and fat, play an important role, affecting cheese yield and its organoleptic characteristics (Davydovych et al., 2025b). In addition, all fat-soluble biologically active compounds, such as vitamins, are concentrated in fat. The content of vitamin A in the dry matter of Alpine and Yoghurt hard goat cheeses made from unpasteurized milk decreased during ripening, which may be due to the influence of physicochemical factors that contributed to oxidation processes during prolonged ripening.

One of the important factors that may have influenced the loss of vitamin A in the cheeses is the activity of *A. siro* mites. Thus, by feeding evenly on the rind of Alpine and Yoghurt hard cheeses, acarid mites could have increased the aeration not only of the surface but also of the deeper parts of the cheese core thereby increasing moisture loss and, consequently, affecting the solubility and distribution of both water-soluble and fat-soluble components with antioxidant properties (Davydovych et al., 2025a). The increased oxygen supply to the surface and deeper layers of the cheese matrix could, in turn, have intensified retinol oxidation. However, this assumption requires further verification.

It is practically impossible to compare the dynamics of retinol content in goat cheeses produced by other manufacturers because they are made using different technologies, including milk obtained from goats of different breeds and raised under different feeding systems. It is believed that the vitamin A content of goat milk cheeses is influenced by salting. According to the study by do Oriente et al. (2020), the vitamin A content was 3.87 ng/ml in salted cheeses compared with 1.27 ng/ml in cheeses produced without salt. However, these data were obtained from soft cheeses that were not subjected to a long ripening period, which does not allow a direct comparison with our results. It is more appropriate to compare our findings with published data on Italian hard and semi-hard cheeses. The retinol content was reported to be be 2.5–2.6 mg/kg in *Caciocavallo Palermitano* hard cheese made from raw cow's milk after 60 days of ripening, 2.5–2.7 mg/kg in *Pecorino siciliano* hard cheese made from raw sheep's milk after 210 days, 2.1–2.9 mg/kg in *Casizolu del Montiferru* hard cheese made from raw cow's milk after 180 days, and 2.9–4.1 mg/kg in *Caprino Nicastrese* semi-hard cheese made from raw goat milk after 20 days (Di Trana et al., 2022). In contrast, the retinol content in Alpine and Yoghurt hard cheeses made from unpasteurized goat milk exceeded these values throughout the ripening period, which may be attributed to regional and seasonal production conditions, as well as differences in breed, animal feeding regimen, and the botanical composition of pasture vegetation.

For the quantitative analysis of changes in retinol content during the ripening period, univariate regression analysis was used. This analysis identified a statistically significant trend toward a decrease in retinol concentration in the dry matter of the cheese over time. The regression model was best described by a second-degree polynomial with significant coefficients ($P < 0.05$), indicating the nonlinear nature of these changes and allowing prediction of this parameter at later stages of ripening.

In addition to vitamin A, vitamin E is one of the most important fat-soluble vitamins in cheeses and also has antioxidant properties. The source of vitamin E in cheeses is animal milk, in which α-tocopherol is the predominant form. The tocopherol content of cheeses reflects their quality and biological value for humans. As an antioxidant, vitamin E plays an important role during cheese ripening by preventing the oxidation of other biologically active substances. A study of authentic Njeguški cheese showed that the content of α-tocopherol in cheese made from goat milk exceeded the corresponding values in the same cheese made from cow's and sheep's milk. At the end of the ripening period, the tocopherol content was 15.73 mg/kg in goat cheese, 10.39 mg/kg in cow cheese, and 11.52 mg/kg in sheep cheese (Bittante et al., 2022). Analysis of the tocopherol content indicates significant differences among hard and semi-hard Italian cheeses made from cow's, sheep's and goat's milk. It was found that hard Caciocavallo Palermitano hard cheese made from raw cow's milk contained 6.0–7.2 mg/kg of tocopherol after 60 days of ripening, *Pecorino Siciliano* hard cheese made from raw sheep's milk contained 3.6–5.8 mg/kg after 210 days of ripening, *Casizolu del Montiferru* hard cheese made from raw cow's milk contained 7.0–11.8 mg/kg after 180 days of ripening, and *Caprino Nicastrese* semi-hard cheese made from raw goat milk contained 7.2–15.0 mg/kg after 20 days of ripening (Di Trana et al., 2022). In another study, α-tocopherol levels in Pecorino and Casu Axedu cheeses were 2.0 and 3.0 mg/kg, respectively, on a natural moisture basis. At the same time, the researchers reported that sheep's milk contained more tocopherol than cow's or goat's milk. In our study, significantly lower tocopherol contents were obtained in Alpine and Yoghurt cheeses than in Njeguški cheese and the Italian cheeses described above. However, most of our results were similar to those reported for Pecorino and Casu Axedu cheeses. The decrease in tocopherol concentration in the dry matter of Yoghurt cheese was probably caused by oxidation, whereas no such trend was observed in Alpine cheese.

No decrease in tocopherol content was found in soft goat cheeses made from unpasteurized milk and ripened with the participation of noble white mold for 40 days, or in brined Feta cheese ripened for 30 months (Da-vydovych et al., 2026). Therefore, it can be assumed that the moisture content of cheese plays an important role in vitamin preservation. The rate of tocopherol oxidation in hard goat cheeses ripened with the participation of acarid mites may, to some extent, depend on the intensity of rind damage and the duration of ripening, as in the case of vitamin A.

A similar pattern was confirmed in a study of Pasta-filata cheese ripened for 30 or 60 days at a temperature of 14–16°C, in which a decrease in the levels of γ-tocopherol and β-carotene was observed. However, the concentration of α-tocopherol did not change (Marino and Schadt, 2016). This may indicate a significant influence of the cheese microbiome on vitamin E levels (Davydovych et al., 2025a). The results of another study indicated that the tocopherol content of ruminant milk used for cheese production depends on the feeding regimen, the presence or absence of grazing, the breed, and the season of production, thereby supporting our assumption (Martini et al., 2021).

Taking into account the content of B vitamins in cheeses is an important criterion not only for their biological value but also for their authenticity. Since B vitamins are water-soluble, a significant proportion is lost during whey separation from the curd in cheese production, although this process depends on the salt content of the cheese mass (Ferroukhi et al., 2022). It is believed that lactic acid bacteria of the genera Lactococcus and Streptococcus not only influence the chemical composition and ripening process of cheeses (Sadvari et al., 2024a, 2024b) but also synthesize vitamins B_2 and B_6 during ripening, which is consistent with previous findings (Ferroukhi et al., 2022). The recommended daily intake of vitamin B_2 for adults is 1.1–1.3 mg. Thus, hard cheeses can provide approximately 10% of the recommended daily intake of vitamin B_2, as well as 20% of vitamin B_6 and 40% of vitamin B_{12} (Rashidinejad et al., 2017; Sohail et al., 2024). The results of the riboflavin analysis in Alpine and Yoghurt cheeses are consistent with these findings. Unlike fat-soluble vitamins, B vitamins can be synthesized by microorganisms and their concentration therefore increases during the ripening of soft goat cheeses (Davydovych et al., 2026). However, in hard cheeses, the decrease in moisture content during ripening limits microbial biosynthesis. At the same time, riboflavin is probably less susceptible to degradation than the fat-soluble vitamins A and E, and its concentration in the dry matter of hard cheeses does not undergo significant fluctuations.

CONCLUSION

The ripening period significantly influenced the content of vitamins A, E and B_2 in Alpine and Yoghurt hard craft cheeses made from unpasteurized goat milk and ripened with the participation of mites. The tocopherol content was directly dependent on the age of Alpine cheese: its level increased to 2.32 mg/kg (by 15.42%) after 6 months and to 2.34 mg/kg (by 16.42%) after 12 months of ripening, whereas the levels of retinol and riboflavin remained stable. In the dry matter of Alpine cheese, the contents of tocopherol and riboflavin did not change, whereas the retinol content decreased to 7.29 mg/kg (by 22.03%) after 6 months and to 7.46 mg/kg (by 20.21%) after 12 months of ripening. During the ripening period of Yoghurt cheese, the riboflavin content increased by 35.08-44.94%, reaching a maximum of 2.58 mg/kg after 18 months, whereas the retinol and tocopherol contents remained unchanged. During the ripening of Yoghurt cheese, a decrease in the dry matter content of fat-soluble vitamins was observed. In particular, the retinol content decreased to 7.24 mg/kg (by 26.72%) after 6 months and to 7.31 mg/kg (by 26.01%) after 18 months. Likewise, the tocopherol content decreased to 3.67 mg/kg after 6 months and to 3.14 mg/kg after 18 months. Alpine and Yoghurt cheeses contain sufficient amounts of retinol, tocopherol, and riboflavin, which, together with microbiological and other physicochemical characteristics, indicate that they are biologically valuable products. The obtained results may serve as a basis for assessing the quality, nutritional value, and authenticity of craft hard goat cheeses made from unpasteurized milk. The research results were statistically analysed using one-way analysis of variance (ANOVA), implemented using information technology, to assess the significance of the effect of the ripening period on the contents of retinol, tocopherol, and riboflavin in the cheeses. This approach made it possible to identify statistically significant differences in vitamin composition according to the stage of cheese ripening and to confirm the biological dynamics of the formation or preservation of individual compounds.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Viktor Davydovych, Larysa Shevchenko: contribute to the research, data analysis, and manuscript writing.
Svitlana Shulyak: conceptualization, Investigation. Lesia Busol: data curation, writing – review & editing.
Mykola Nikolaenko: formal analysis, visualization. Anastasia Lialyk: funding acquisition.
Andrii Kudriashov: methodology, software. Anastasiia Ivaniuta: project administration, supervision.
Lyudmila Beyko: writing – review & editing, resources.
Oleksandr Savchenko: validation, writing – original draft.

Acknowledgments

We would like to thank you to Eco Farm Zhuravka, Kyiv region, Ukraine.

Ethical consideration

No experimental procedures or manipulations involving animals were performed in this study. Therefore, ethical approval for animal experimentation was not required.

Funding

This research was carried out within the framework of the Grant of the President of Ukraine for Young Scientists No. 2025.05/0050, "Scientific Foundations for the Development of a Complex of Specialized Food Technologies.

Competing interests

The authors have not declared any competing interests.

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