

THE EFFECT OF SEASONS ON THE PROTEIN CONTENT IN EWE'S AND COW'S LUMP CHEESE

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ABSTRACT

Cheese is one of the most consumable dairy products in the world. There is a substantial number of cheeses known. However, the production of each type is somewhat specific. Cheese provides significant nutritional benefits in terms of replenishing nutrients, such as protein, fat, minerals, vitamins, and more. In this written piece, the focus is on ewe's and cow's lump cheese, which is produced from ewe's and cow's milk. The ewes and cows provide milk as a nutritious raw material for traditional cheese products, in selected cases marked with the Traditional Specialty logo of the European Union. The physicochemical and microbiological composition of ewe's milk is dependent, as in cows and other mammals, on factors such as breed, stage of lactation, feeding, management, and milking systems. The study aimed to establish whether the seasonal factor can significantly affect the composition of traditional ewe's and cow's lump cheese. For this purpose, during the year of production, 46 samples of ewe's lump cheese and 46 samples of cow's lump cheese were subject to analysis according to ISO standards aimed at determining the total content of proteins, casein, and γ 3- and γ 2-casein using isoelectric focusing. The mean values of the content of individual samples in ewe's lump cheese were as follows: protein 18.33 g/100 g, casein 17.88 g/100 g, γ 3-casein 0.71 mg/100 g, and γ 2-casein 0.82 mg/100 g. The mean values of the content of individual samples in cow's lump cheese were as follows: protein 19.05 g/100 g, casein 18.35 g/100 g, γ 3-casein 0.72 mg/100 g, and γ 2-casein 0.83 mg/100 g. Considering all the assessed aspects of such a product, traditional ewe's lump cheese and cow's lump cheese represent relatively stable products in terms of content throughout the year.

Keywords: ewe's lump cheese, cow's lump cheese, protein, casein, γ 2-casein, γ 3-casein

INTRODUCTION

The authenticity of dairy products has become an important issue, drawing interest from researchers, producers, consumers, and policymakers. Certain practices, such as the partial substitution of fat or proteins, blending milk from different species, incorporating inexpensive dairy by-products (especially whey derivatives), or mislabeling products with protected designations of origin, are strictly prohibited in milk and dairy products. One of the common fraudulent practices involves the adulteration of ewe's milk with cow's milk, which occurs due to seasonal variations in ewe's milk availability, its higher market price compared to cow's milk, and the opportunity to utilize excess cow's milk. This type of adulteration also affects bryndza cheese, where ewe's lump cheese is sometimes replaced with more affordable cow's milk cheese without disclosure (Suhaj *et al.*, 2010).

Several factors, including housing conditions, regional characteristics, seasonal changes, nutrition, physiological shifts, the timing of lambing and calving, and the stage of lactation, directly impact the composition of milk and subsequently influence cheese quality (Micari *et al.*, 2007; Albenzio & Santillo, 2011; Sanz Sampelayo *et al.*, 2007; Coulon *et al.*, 2004; Zaitoun *et al.*, 2005; Zervas & Tsiplakou, 2011). Seasonal fluctuations significantly affect the chemical composition and functional attributes of milk (Hayes *et al.*, 2023), leading to changes in its levels of fat, protein, lactose, and salts (Li *et al.*, 2019). These compositional alterations have a direct impact on raw milk quality, modifying its functional properties and influencing the production of dairy products (Priyashantha *et al.*, 2021). As one of the earliest foods consumed by humans for nutritional sustenance (Thakore & Jain, 2018), milk holds significant value in the dairy industry due to its physical and chemical properties (Li *et al.*, 2019). Multiple factors, including genetics, lactation period, diet, age, udder health, and seasonal differences, contribute to variations in milk composition and yield (Ozrenk & Inci, 2008).

Climatic conditions, particularly temperature fluctuations, have a direct effect on milk production. While temperatures between 5°C and 25°C do not significantly impact milk yield, extreme summer heat reduces feed intake, leading to lower milk production. Additionally, during the warmer months, increased water consumption results in a decline in milk fat content and total solids. Conversely, autumn and winter provide more favorable environmental conditions and digestible feed, resulting in higher milk yields and an increased concentration of fat and total solids (Ivanov *et al.*, 2017).

Due to the seasonal nature of milk production, output rises notably in spring and declines between July and November. As a result, most ewe's milk cheeses in Southern Europe are manufactured between December and June (Medina & Nuñez, 2017). Ewe's milk is primarily used for cheese production, valued for its distinctive taste, aroma, and texture. Many European cheeses made from ewe's milk are protected by European Union regulations and bear specific labels such as PDO, PGI, and TSG. Some traditional cheeses incorporate a blend of ewe's and cow's milk (DOOR, 2014) to enhance their sensory appeal and meet consumer preferences. From a compositional standpoint, ewe's milk contains 11.5% non-fatty solids, 9% ash, 7.1% fat, 5.7% protein, and 4.8% lactose (Treacher & Caja, 2022). In contrast, cow's milk comprises approximately 87% water, with the remaining 13% consisting of solids such as proteins (3.3%), fat (3.4%), carbohydrates (4.9%), minerals (0.7%), and vitamins (Wangdi *et al.*, 2016).

This study focused on the analysis of ewe's lump cheese, known as "Ovčí hrudkový syr," and cow's lump cheese, known as "Kravský hrudkový syr." These cheeses are made using fresh milk and are recognized for their distinct sensory properties, as well as their unique physical and chemical characteristics. Typically, they have a spherical shape, with dry matter constituting more than 40% of their total weight, a fat content of at least 50% in the dry matter, and an acidity level ranging from pH 5.2 to 4.9 (Publication of an application for registration pursuant to Article 8(2) of Council Regulation (EC) No 509/2006). The primary objective of this study was to determine whether seasonal variations significantly influence the total protein content, casein levels, and γ -casein composition in ewe's lump cheese. Furthermore, the analysis of γ -casein fractions provides a valuable approach for authenticating traditional Slovak cheeses, allowing for the detection of adulteration, such as the addition of cow's milk cheese to ewe's milk products. These findings will contribute to refining the analytical methodology previously described by Zajác *et al.* (2021), enhancing its practical application in cheese authenticity verification.

MATERIAL AND METHODOLOGY

Design of the Experiment

A small food business operator Agrosúča, a.s., produced ewe's lump cheese in Dolná Súča, Slovakia, European Union (Coordinates: 48°58'N 17°59'E, Elevation: 429 – 751 m) from 29/3/2022 to 6/9/2022.

Animals and farming conditions:

- Ewes: 30 % Tsigai, 70 % Lacaune breeds. Since April, all livestock have been housed in a free-range barn with straw bedding, with access to outside stabled.
- Cows: 100 % Slovenské Strakaté. Since April, all livestock have been housed in a free-range barn with straw bedding, with access to outside stabled.

Feeding conditions:

- During winter months, ewes and cows have been stall-fed, with feed composing of haylage and silage. Since April, the ewes and cows attended pasture. In autumn, the grazing has been followed by stall-feeding, same as during the winter.

Milking conditions:

- Ewe's and cow's milk was collected in a round parlor, chilled to 14 °C, and then transported to a farm dairy.

Cheese Production Process

The production of ewe's and cow's lump cheese began with raw milk, which was processed within two hours of milking. Initially, the milk was heated to a temperature of 30–32 °C. A microbial rennet solution, containing 40 ml per 100 liters of milk, was gradually introduced under constant stirring. This rennet, derived from the salt-stabilized fungus *Rhizomucor miehei*, facilitated curd formation. The curds were then stirred and cut using a curd harp until they reached a grain size of approximately 0.5–1 cm. Next, previously boiled drinking water, cooled to 65 °C, was directly added to the curds to enhance whey separation. The curds were stirred once more and allowed to settle. Following this, they were manually compressed for around ten minutes using cheesecloth to form a solid lump. This lump was then suspended in cheesecloth and left to drain for roughly two hours. After the draining process, the cheese was transferred to a ripening room, where fermentation commenced. The temperature in this storage area was maintained at a minimum of 18–22 °C. Initially, the cheese remained wrapped in cheesecloth and was suspended from a hook, but later, it was placed on a specially designed shelf that facilitated whey drainage. The entire fermentation process lasted between two to three days (**Publication of an application for registration pursuant to Article 8(2) of Council Regulation (EC) No 509/2006**).

Cheese Sampling

Cheese sampling followed the guidelines outlined in ISO 707 (**ISO 707:2008, 2008**). Each sample was vacuum-sealed and stored at a temperature of -18°C to maintain its integrity. Monthly, two samples from each production batch were gathered from the producer and transported to the laboratory for analysis. In total, the study examined 46 samples of ewe's lump cheese and 46 samples of cow's lump cheese to ensure comprehensive evaluation

Methods for Cheese Proteins Composition Determination

Protein content: According to the method defined by ISO 8968-1:2014 - intended for milk and milk products using the Kjeldahl principle and crude protein calculation according to nitrogen content presence (**ISO 8968-1:2014, 2014**).

Casein content: Total protein content (TP%) was analysed according to *ISO 8968-1:2014 (ISO 8968-1:2014, 2014)*, and non-casein nitrogen (NCN%) content was determined using the Kjeldahl method (**ISO 17997-1:2004, 2004**). Casein content was calculated as the difference between TP and NCN content.

γ-caseins content: The content analysis followed the methodology described by **Zajác et al. (2021)**. However, for this study, commercially available Servalyte

Focus Gel 3-10 24 S (Serva) was utilized. This precast polyacrylamide gel, with a thickness of 0.65 mm, was bonded to a GEL-FIX™ support film for isoelectric focusing. The gels were formulated to be non-toxic, as any residual catalysts or unpolymerized compounds, such as acrylamide monomers, had been removed from the matrix. Additionally, they were enhanced with a SERVALYT™ cocktail to create an optimal pH gradient. Unlike conventional systems, this method did not require separate electrode solutions or strips, as the electrodes were placed directly on the gel surface.

Statistical analysis

All data were initially tested for normality using the Shapiro–Wilk test. As most variables did not follow a normal distribution, non-parametric Mann–Whitney U tests were applied to compare protein, casein, γ2-casein, and γ3-casein concentrations across seasons, specifically between summer and the combined spring and autumn periods. Statistical significance was set at $p < 0.05$. Results are reported as mean ± standard deviation (SD), and coefficients of variation (CV) were used to evaluate relative variability. All statistical analyses were performed using XLSTAT software, version 2021.4.1 (Addinsoft, France), software was used to statistically treat obtained results.

RESULTS AND DISCUSSION

Results of ewe's lump cheese

Table 1 summarizes the protein composition of ewe's lump cheese. The reported mean values are based on two independent analyses of duplicate samples (n = 46) originating from a single production batch. The average total protein content was determined to be 18.33 g/100 g, of which casein comprised 17.88 g/100 g.

Table 1 Ewe's lump cheese protein composition

Analyzed parameter	Mean	SD	CV (%)	Min	Max	S
Protein	18.33	1.27	6.93	15.68	20.15	$p > .05$
Casein	17.88	1.27	7.8	15.22	19.76	$p > .05$

Legend: Mean, SD – standard deviation, min, max (g/100 g), CV – Coefficient of variation (%). The mean represents the value calculated from all results found during the production season for two duplicate samples (n = 46), S – significance

The γ-casein profile of ewe's lump cheese is presented in Table 2. The reported mean values are based on two replicate analyses of duplicate samples collected from the same production batch (n = 46). The analysis showed that γ3-casein was present at an average concentration of 0.71 mg/100g, whereas γ2-casein averaged 0.82 mg/100 g.

Table 2 Ewe's lump cheese γ-caseins composition

Analyzed parameter	Mean	SD	CV (%)	Min	Max	S
γ3-casein	0.71	0.10	13.68	0.53	0.88	$p > .05$
γ2-casein	0.82	0.11	13.01	0.67	1.04	$p > .05$

Legend: Mean, SD – standard deviation, min, max (mg/100 g), CV – Coefficient of variation (%). The mean represents the value calculated from all results found during the production season for two duplicate samples (n = 46), S – significance

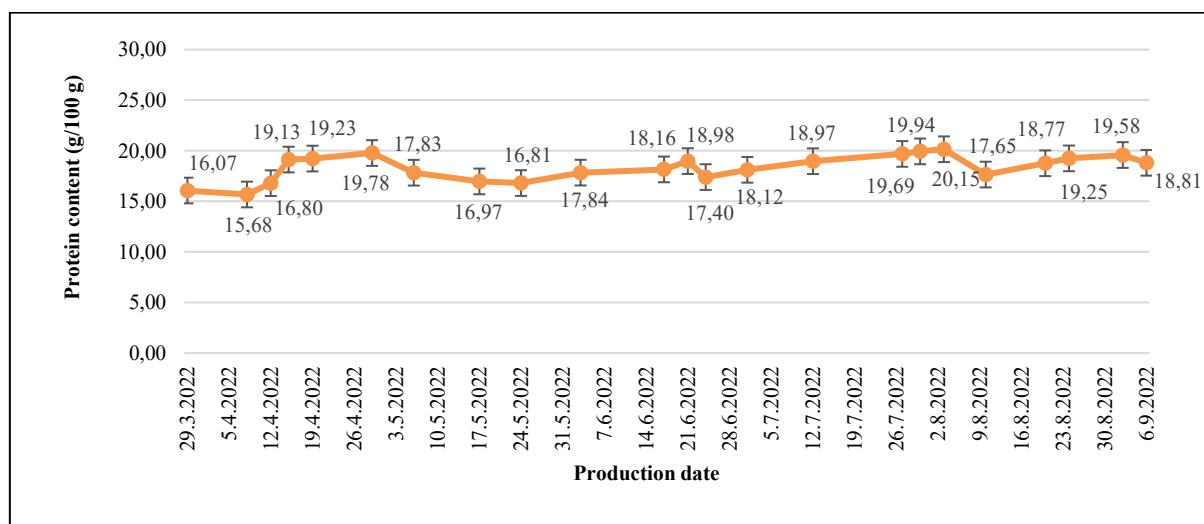


Figure 1 Seasonal trend in protein content of the ewe's lump cheese. Values are means of two replicates ± standard deviation (SD).

Relatively low seasonal fluctuations in protein content were observed throughout the production period. Initial measurements in March showed relatively low values (16.07 and 15.68 g/100 g). The protein concentration peaked at 19.78 g/100 g in early May, followed by a decrease to 16.81 g/100 g by the end of the month. A progressive increase was recorded during June, reaching 18.98 g/100 g in the latter half, before a slight decline to 17.40 g/100 g at the month's end. In the subsequent weeks, a gradual upward trend culminated in the highest recorded

value of 20.15 g/100 g in early August. This was followed by a marked drop to 17.65 g/100 g, although a partial recovery occurred later in the same month. The final measurement, taken in early September, indicated a protein content of 18.81 g/100 g. Statistical comparison between summer and spring + autumn revealed no significant difference ($p = 0.1661$), indicating that the variation remained within the expected biological range.

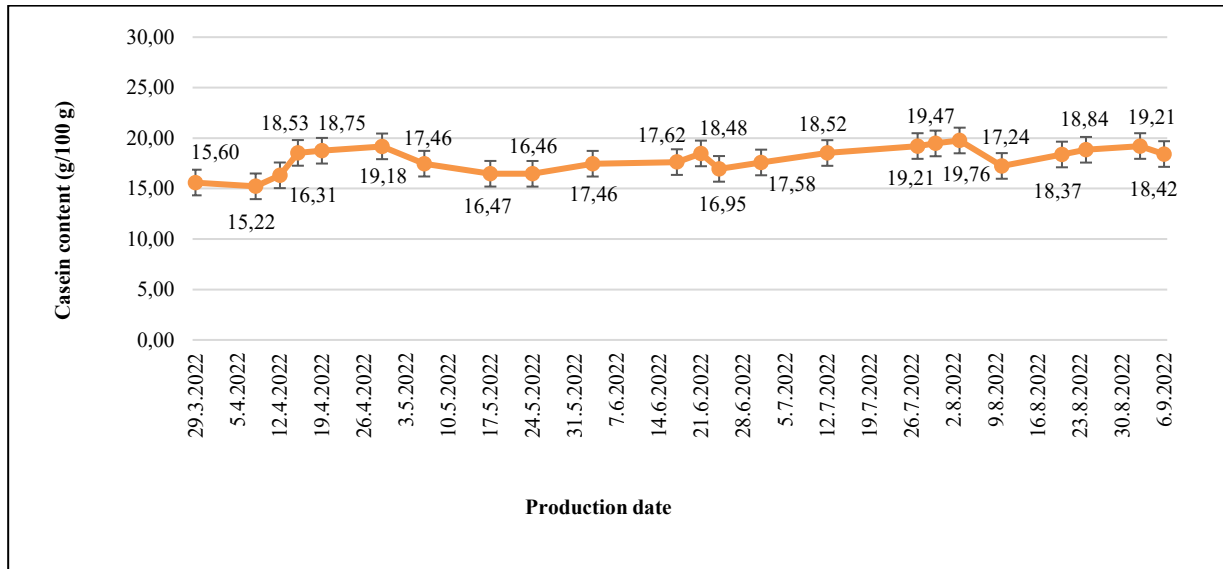


Figure 2 Seasonal trend in the casein content of the ewe's lump cheese. Values are means of two replicates ± standard deviation (SD).

The seasonal dynamics in casein concentration mirrored that of total protein, as illustrated in Figure 2. Initial casein concentrations at the start of the production season were 15.60 and 15.22 g/100 g. The maximum concentration of 19.18 g/100 g was observed in early May, while the minimum value of 16.47 g/100 g occurred at the end of May. Throughout June, casein levels steadily increased, reaching a peak of 18.48 g/100 g in the latter half of the month, followed by a slight decline to 16.95 g/100 g by month-end. This was succeeded by a consistent upward trend culminating in the highest recorded concentration of 19.76 g/100 g in early August.

A sharp decrease to 17.24 g/100 g ensued, with a subsequent partial recovery observed in the following weeks. The final measurement, taken at the beginning of September, indicated a casein content of 18.42 g/100 g. Overall, casein levels in ewe's lump cheese demonstrated a modest yet structured seasonal pattern, with two distinct peaks aligned with mid- and late-lactation, likely influenced by pasture quality and physiological factors affecting milk composition.

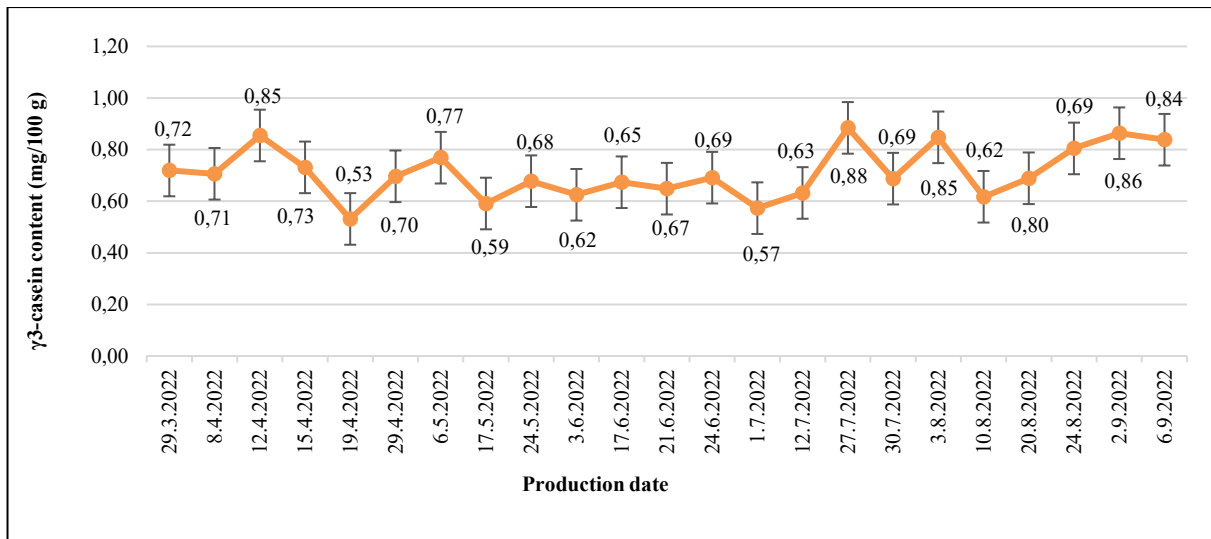


Figure 3 Seasonal variation in the γ3-casein content of ewe's lump cheese. Values are means of two replicates ± standard deviation (SD).

Seasonal changes in γ3-casein content were present but not pronounced, with values remaining within a relatively narrow range. The average γ3-casein concentration across all samples was 0.71 mg/100 g, with observed values ranging from 0.53 mg/100 g to 0.88 mg/100 g, reflecting moderate variability (CV ≈ 13.67%). The lowest concentration was recorded in April (19 April 2022; 0.53 mg/100 g), while the highest was observed in July (27 July 2022; 0.88 mg/100 g). Detailed analysis of the temporal trend reveals distinct periods: during the first half of the year (March to June), γ3-casein levels were generally lower, ranging between 0.6 mg/100 g and 0.7 mg/100 g, with the minimum value in April.

Conversely, from July to September, an increase in γ3-casein concentration was observed, peaking in July and August. This seasonal rise may be linked to improved ovine nutrition during summer and hormonal changes associated with the lactation peak, which contributes to more stable milk production and elevated casein content. Values in August and September remained elevated, with some samples reaching the maximum observed concentration of 0.88 mg/100 g. However, statistical comparison between summer and the spring + autumn periods revealed no significant difference ($p = 0.2815$), suggesting that while the seasonal trend was observable, it was not strong enough to reach statistical significance.

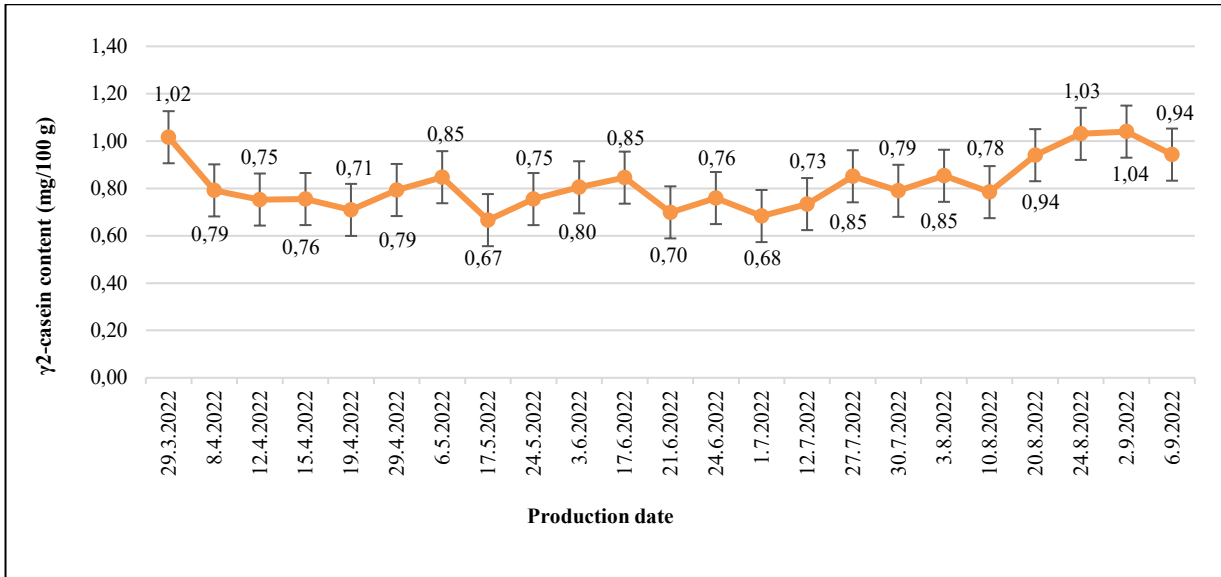


Figure 4 Seasonal trend in the γ 2-casein content of ewe's lump cheese. Values are means of two replicates \pm standard deviation (SD).

Throughout the study period, γ 2-casein levels fluctuated within a moderate range, with an average value of 0.82 mg / 100 g and a minimum and maximum of 0.67 and 1.04 mg / 100 g, respectively. The lowest concentration was recorded in mid-May (17 May 2022; 0.67 mg/100 g), while the highest value was observed in early September (2 September 2022; 1.04 mg/100 g). Analysis of the temporal fluctuations in γ 2-casein concentrations reveals distinct phases. From March to June, concentrations ranged between 0.7 mg/100 g and 0.8 mg/100 g, remaining relatively stable with minor declines observed, particularly in April and May, where the lowest concentration was recorded. In contrast, during the summer months (July to September), γ 2-casein levels increased noticeably, reaching the maximum concentration in early September. This seasonal rise may be attributed to enhanced milk production in the later lactation stages and potentially improved nutritional conditions for the sheep during summer. Throughout July and August, γ 2-casein concentrations remained elevated, with several samples exceeding 0.85 mg/100 g. Despite the observable upward trend, statistical analysis revealed no significant difference between summer and spring + autumn periods ($p = 0.9755$), indicating that the fluctuations, while biologically plausible, were not statistically robust.

Results of cow's lump cheese

The protein composition of cow's lump cheese is summarized in Table 3. Mean values were calculated from two independent measurements of duplicate samples collected from the same production batch ($n = 46$). The analysis revealed an average total protein content of 19.05 g/100 g, with casein constituting 18.35 g/100 g.

Table 3 Cow's lump protein composition

Analyzed parameter	Mean	SD	CV (%)	Min	Max	S
Protein	19.05	1.67	8.79	16.16	22.54	$p < .01$
Casein	18.35	1.66	9.05	15.54	21.82	$p < .01$

Legend: Mean, SD – standard deviation, min, max (g/100 g), CV – Coefficient of variation (%). The mean represents the value calculated from all results found during the production season for two duplicate samples ($n = 46$), S – significance

The γ -casein composition of cow's lump cheese is presented in Table 4. Mean values were calculated from two independent determinations of duplicate samples obtained from the same production batch ($n = 46$). The results showed an average γ 3-casein content of 0.72 mg/100 g and a γ 2-casein content of 0.83 mg/100 g.

Table 4 Cow's lump γ -caseins composition

Analyzed parameter	Mean	SD	CV (%)	Min	Max	S
γ3-casein	0.72	0.07	9.14	0.57	0.86	$p > .05$
γ2-casein	0.83	0.13	15.88	0.60	1.10	$p > .05$

Legend: Mean, SD – standard deviation, min, max (mg/100 g), CV – Coefficient of variation (%). The mean represents the value calculated from all results found during the production season for two duplicate samples ($n = 46$), S – significance.

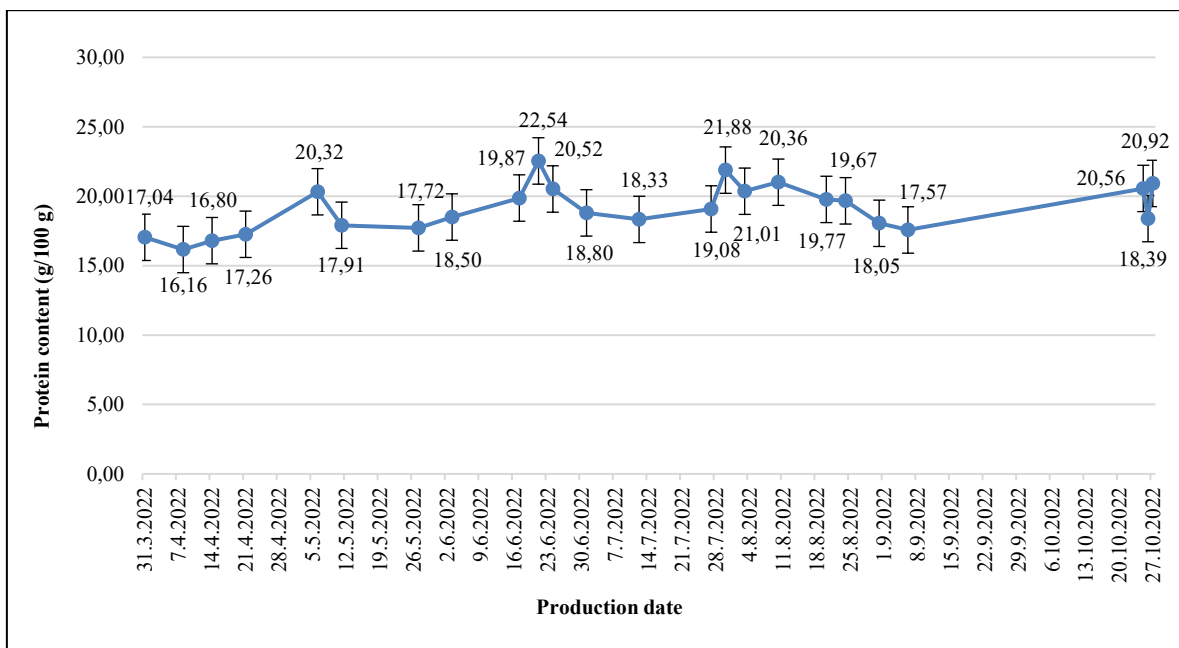


Figure 5 Seasonal trend in the protein content of the cow's lump cheese. Values are means of two replicates \pm standard deviation (SD).

The average protein content across all samples was 19.13 g/100 g, with individual values ranging from 16.16 to 22.54 g/100 g. Given a coefficient of variability (CV) of $\approx 8.79\%$, the dataset shows relatively low variability in protein content over the season. The lowest concentration was recorded in early April (8 April 2022; 16.16 g/100 g), while the highest value was observed in late June (21 June 2022; 22.54 g/100 g). Analysis of protein concentration fluctuations throughout the year reveals several distinct phases. During the early season (March to May), protein levels ranged between 16.04 g/100 g and 20.32 g/100 g, exhibiting a steady increase and peaking in early May. This period was characterized by relative stability with minor variations, including a notable rise in late April (17.26 g/100 g) and early May. Throughout the summer months (June to August), protein content increased further, reaching a maximum in late June, followed by a

slight decline in early July (18.80 g/100 g). Protein concentrations remained elevated during this period, with multiple samples exceeding 20 g/100 g, likely reflecting optimal milk production and quality during the late lactation phase and improved nutritional conditions in summer. In the final months of the production season (September to October), protein concentrations fluctuated within a relatively narrow range, between 17.57 g/100 g to 20.92 g/100 g. Although slightly lower than summer values, protein levels stayed elevated compared to early-season measurements, indicating consistent production conditions during the transition to autumn. Statistical testing confirmed that summer protein levels were significantly higher than the combined spring + autumn values ($p = 0.0058$), underscoring a pronounced seasonal effect on total protein concentration.

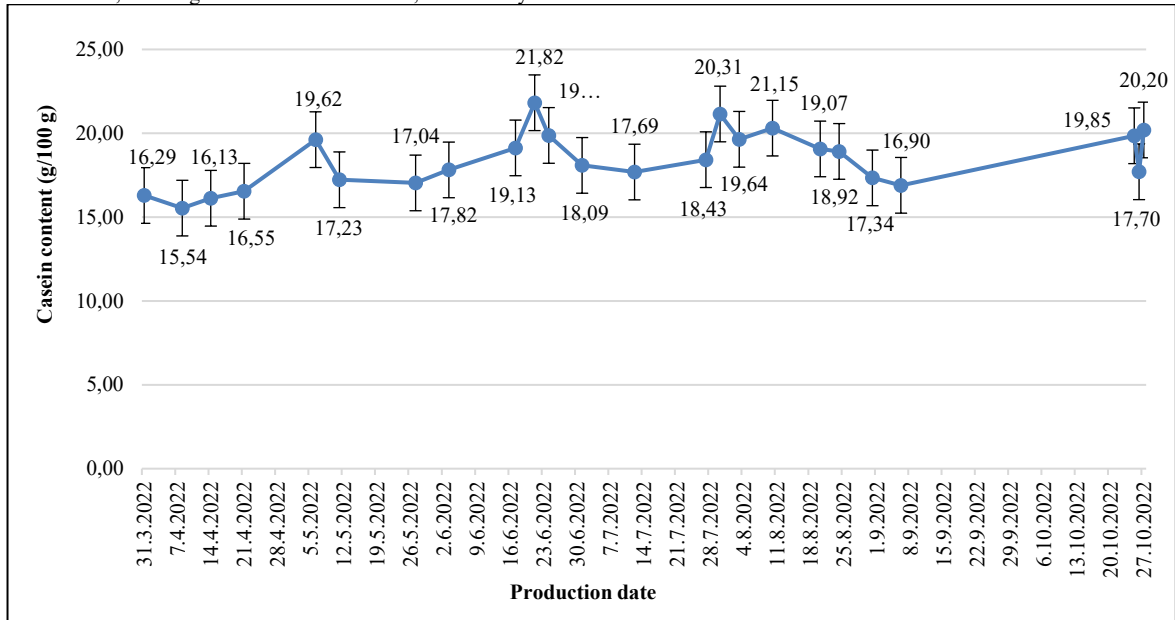


Figure 6 Seasonal trend in the casein content of the cow's lump cheese. Values are means of two replicates \pm standard deviation (SD).

Casein concentrations in cow's lump cheese ranged from 15.54 to 21.82 g/100 g, with a mean of 18.43 g/100 g and a coefficient of variation of approximately 9%, indicating relatively low seasonal variability. The lowest concentration was recorded in early April (8 April 2022; 15.54 g/100 g), while the highest value was observed in late June (21 June 2022; 21.82 g/100 g). Temporal analysis of casein concentrations throughout the year identified several distinct phases. From March to May, casein levels ranged between 15.54 g/100 g and 19.62 g/100 g, showing a gradual increase with a peak in early May, suggesting improving milk quality. A minor decline occurred in mid-April (16.13 g/100 g), followed by a rise towards late April and early May. During the summer months (June to August), casein concentrations peaked, reaching a maximum of 21.82 g/100 g in late June. This

increase may be associated with enhanced milk quality or dietary changes in the cows during this period. Throughout July and August, elevated casein levels persisted, with several samples exceeding 19 g/100 g and a notable peak in late July (21.15 g/100 g). In the final months of the season (September to October), casein content exhibited a slight decline but remained relatively stable, fluctuating between 16.90 g/100 g and 20.20 g/100 g, indicating consistent casein levels as environmental conditions transitioned to cooler temperatures. Statistical comparison confirmed that summer casein concentrations were significantly higher than those from spring and autumn combined ($p = 0.0048$), underscoring a clear seasonal effect on milk protein composition.

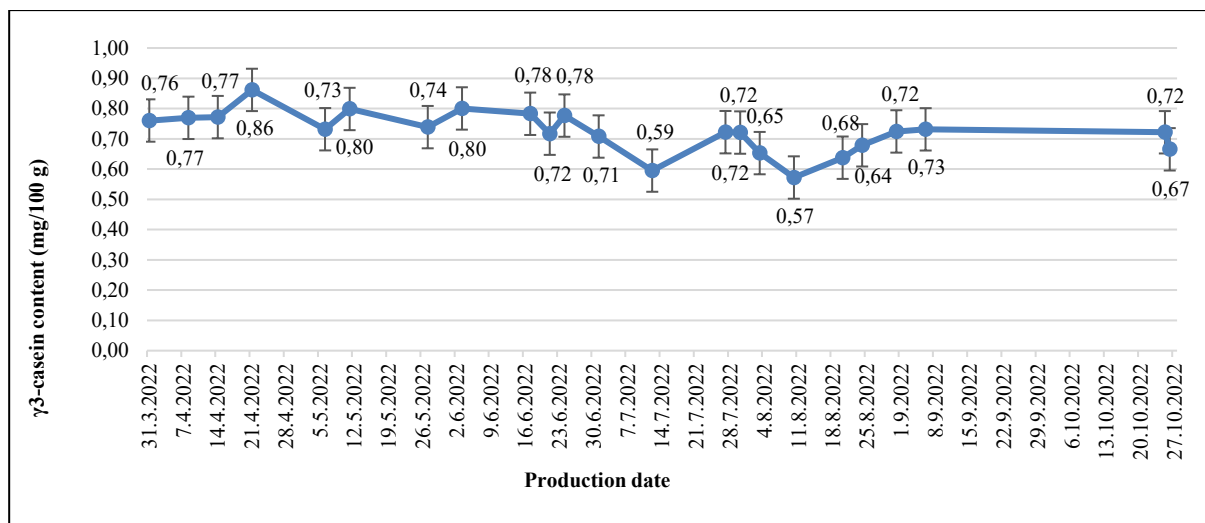


Figure 7 Seasonal trend in the γ_3 -casein content of cow's lump cheese. Values are means of two replicates \pm standard deviation (SD).

The average γ_3 -casein concentration across all samples was 0.72 mg/100 g, with a range from 0.57 to 0.86 mg/100 g, indicating relatively low variability. The lowest level was recorded in mid-July (12 July 2022; 0.60 mg/100 g), while the highest occurred in late April (21 April 2022; 0.86 mg/100 g). From March to June, γ_3 -casein concentrations remained relatively stable, typically ranging

between 0.70 and 0.80 mg/100 g, with minor fluctuations and a slight dip noted in April and May. During the summer months (July to August), a clear downward trend was observed, reaching the seasonal minimum in mid-July. This decline may be associated with heat stress, dietary shifts, or changes in milk quality due to environmental conditions. From late July through August, concentrations

gradually increased again, fluctuating between 0.57 and 0.72 mg/100 g, with a modest recovery trend by the end of summer. In September and October, γ_3 -casein levels stabilized between 0.67 and 0.73 mg/100 g, reflecting a return to more favourable production conditions. Notably, the seasonal difference between

summer and the rest of the production period approached statistical significance ($p = 0.0508$), suggesting a potentially biologically relevant seasonal effect.

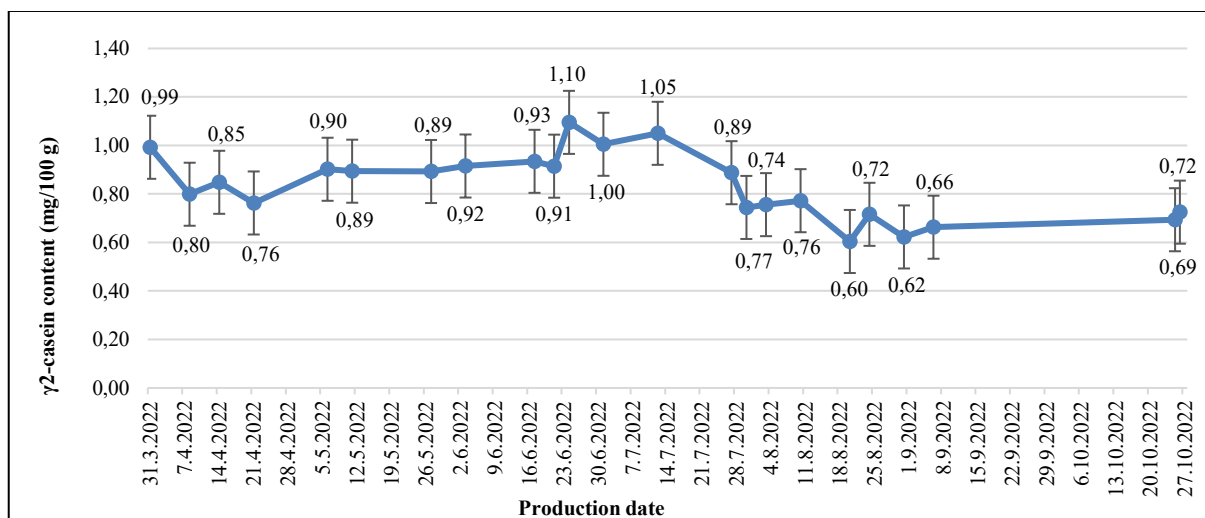


Figure 8 Seasonal trend in the γ_2 -casein content of cow's lump cheese. Values are means of two replicates \pm standard deviation (SD).

With a mean concentration of 0.83 mg / 100 g and a coefficient of variation near 15.7%, γ_2 -casein levels showed moderate seasonal variability across the production cycle. The minimum was recorded in late August (20 August 2022; 0.60 mg/100 g), whereas the maximum appeared in late June (24 June 2022; 1.10 mg/100 g). From March to June, γ_2 -casein levels remained relatively stable between 0.70 and 1.00 mg/100 g, with minor dips in April and early May and a transient peak in late March (0.99 mg/100 g). A marked increase occurred in early summer, culminating at the seasonal maximum in late June. This rise could reflect enhanced milk quality or dietary changes associated with peak pasture availability. Following this peak, concentrations declined sharply, reaching the annual minimum in mid-August; such a drop is plausibly linked to heat stress and reduced feed intake during the warmest period. In September and October, γ_2 -casein values recovered modestly, stabilising between 0.62 and 0.73 mg/100 g as temperatures moderated and feeding conditions improved. Statistically, the overall difference between summer and the combined spring + autumn periods was not significant ($p = 0.556$), indicating that the observed fluctuations fall within normal seasonal variability.

Many consumers prefer purchasing this type of cheese directly from the farm while it is still in its unfermented, "sweet" state. However, the health concerns related to these cheeses remain a topic of debate. To ensure safety, it is highly recommended that cheeses be consumed only after fermentation, which typically takes 2-3 days, resulting in a slightly sour flavour (Herian, 2015). Matured ewe's lump cheese or a blend of ewe's and cow's lump cheese is also used in the production of various traditional products, such as Oštiepok, Korbáčik, Smoked lump cheese, various types of steamed cheeses, and Bryndza (Semjon et al., 2018; Suhaj et al., 2010; Zajác et al., 2019; Zimanová et al., 2016).

The standards for lump cheese outline the precise production process and its key characteristics. It is made from fresh milk, with its distinct flavor developing during fermentation and influenced by its unique lump shape. The cheese typically takes on a spherical form, with a lump weight of up to 5 kg (Zajác et al., 2017; Herian, 2014).



Figure 9 Lump shape of ewe's lump cheese

This study quantified seasonal effects on the protein fractions of ewe's and cow's lump cheese. Protein and casein in cow's cheese displayed a clear summer surge, whereas the same analytes in ewe's cheese varied only modestly and did not reach statistical significance.

In ewe's lump cheese, the protein content varied from 15.68 to 20.15 g/100 g, with an average of 18.33 g/100 g. Casein content ranged between 15.22 and 19.76 g/100 g, averaging 17.88 g/100 g. These variations align with previous research indicating that factors such as breed, lactation stage, and diet influence milk composition and, consequently, cheese quality (Albenzio & Santillo, 2011; Coulon et al., 2004). The observed protein and casein fluctuations may be attributed to changes in feeding practices and environmental conditions throughout the year. For instance, seasonal variations in pasture quality and availability can significantly impact the nutritional intake of ewes, thereby affecting milk composition (Kljajevic et al., 2018).

In contrast, cow's lump cheese demonstrated a clear seasonal increase in protein and casein during summer. Protein content ranged from 16.16 to 22.54 g/100 g (mean = 19.13 g/100 g), and casein from 15.54 to 21.82 g/100 g (mean = 18.43 g/100 g), with both parameters showing statistically significant differences between seasons ($p = 0.0058$ for protein; $p = 0.0048$ for casein). These findings are consistent with studies highlighting the influence of seasonal factors on cow's milk composition (Hayes et al., 2023; Li et al., 2019). The increase in protein content during the summer months could be linked to improved pasture quality and longer grazing periods, enhancing nutrient intake and milk quality. Additionally, photoperiod, or the length of daylight, has been shown to influence milk yield and composition, with longer days potentially leading to increased milk production and altered protein content (Chen et al., 2014).

The study also examined γ_2 - and γ_3 -caseins, proteolytic fragments that contribute to cheese texture and ripening. Average γ_3 -casein content was 0.71 mg/100 g in ewe's cheese and 0.72 mg/100 g in cow's cheese; γ_2 -casein averaged 0.82 mg/100 g and 0.83 mg/100 g, respectively. While cow γ_3 -casein showed a near-significant seasonal difference ($p = 0.0508$), the other γ -casein values remained relatively stable across the year, with no statistically significant fluctuations ($p = 0.2815$ – 0.9755). These γ -caseins are proteolytic fragments resulting from casein breakdown during cheese ripening, contributing to texture and flavour development (Zajác et al., 2021). The relatively stable γ -casein levels suggest consistent proteolytic activity throughout the production season. Proteolytic activity is influenced by factors such as milk pH and enzyme activity, which can vary seasonally and affect cheese ripening processes (Frederiksen et al., 2011).

The observed seasonal variations in protein and casein content have practical implications for cheese producers. Understanding these fluctuations can aid in optimizing production processes to maintain consistent cheese quality. For instance, adjusting feeding strategies during periods of lower protein content could help stabilize milk composition. Studies have shown that incorporating by-products like grape pomace into ewe diets can modify milk's fatty acid profile without adversely affecting protein content, potentially offering a strategy to enhance milk quality (Bennato et al., 2022). Additionally, monitoring environmental factors and implementing management practices to mitigate their impact on milk quality can be beneficial. For example, managing heat stress during high-temperature periods is crucial, as elevated temperatures can negatively affect milk yield and composition (Bernabucci et al., 2015).

These insights are particularly relevant for small-scale producers striving to meet quality standards year-round. Despite the inherent variability in milk composition due to seasonal changes, the cheeses analysed met the required quality specifications. This underscores the potential for small producers to consistently produce high-quality traditional cheeses by adapting to seasonal influences. Implementing feeding practices that optimize the forage-to-concentrate ratio and

include nutrient-rich supplements can enhance milk yield and composition, thereby improving cheese quality (Paraffin et al., 2018).

In conclusion, seasonal variation had a significant impact on protein composition in cow's lump cheese, but only a minor, non-significant effect in ewe's cheese. γ -Caseins remained largely unaffected by season. By recognizing and addressing these variations, producers can enhance cheese quality and ensure product consistency throughout the year. Future research could explore specific management practices and dietary interventions to further mitigate the impact of seasonal changes on cheese composition. For instance, investigating the effects of different forage types and feeding schedules on milk protein content could provide valuable insights for optimizing cheese production (O'Callaghan et al., 2016).

CONCLUSION

The protein composition of both ewe's and cow's lump cheese exhibited seasonal trends; however, only cow's cheese showed statistically significant increases in protein and casein content during the summer months. In contrast, ewe's cheese remained relatively stable, with modest, non-significant variation. Importantly, all samples consistently met the established quality standards for traditional production. This highlights the capacity of even small-scale producers to maintain high cheese quality year-round, despite environmental variability. The measured seasonal variability in protein fractions—including γ 2- and γ 3-caseins—provides a realistic reference for interpreting natural fluctuations. This information is particularly relevant when assessing measurement uncertainty in authenticity control of traditional Slovak Bryndza cheese, especially in cases where cow's milk cheese adulteration is suspected. Finally, the results reinforce the value of isoelectric focusing of bovine and ovine γ 2- and γ 3-caseins as a sensitive and robust method for verifying cheese origin and composition, even in the face of seasonal variation.

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