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Microbiome of Kraft soft cheeses made from raw goat milk during ripening

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Abstract. The orientation of consumers towards healthy food increases the demand for artisanal cheeses made from raw goat milk. The production of such cheeses in Ukraine is carried out on small eco-farms, ensuring the uniqueness of their microbiological composition. The aim of the study was to determine the microbiome of soft goat cheeses Feta and Chèvre as a criterion of quality and safety during the ripening process. The MALDI-TOF method was used for the identification of microorganisms. In brined Feta cheese, on the 7th day, 18th, and 30th months of ripening, no moulds were detected, and the total number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), as well as yeasts, showed little change. The number of dominant lactic acid bacteria species in Feta cheese, *Lactococcus lactis* and *Lactobacillus paracasei*, depended on the ripening period. Small amounts *of Staphylococcus simulans*, *Serratia liquefaciens*, *Kurthia gibsonii*, *Enterococcus faecalis*, *Enterococcus durans*, and *Bacillus cereus* were also isolated from this cheese. In white mould-ripened Chèvre cheese, the MAFAnM count did not change significantly on the 3rd, 20th, and 42nd days of ripening. The amount of mould in this cheese showed a strong direct correlation, while yeast counts had a strong inverse correlation with the ripening period. The main species of lactic acid bacteria in Chèvre cheese were represented by *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. During ripening, small amounts of *Staphylococcus simulans*, *Serratia liquefaciens*, *Kurthia gibsonii*, *Escherichia coli*, and *Enterococcus durans* were isolated from Chèvre cheese. The primary moulds in Chèvre cheese were *Galactomyces candidus*, *Galactomyces geotrichum*, and *Penicillium halotolerans*. The species and quantitative composition of microorganisms in Feta and Chèvre cheeses indicated their proper quality and safety and can serve as criteria for their authenticity. The research results provided new data on the microbiome dynamics of artisanal soft cheeses made from raw goat milk produced in Ukraine

Keywords: Feta cheese; Chèvre cheese; mesophilic aerobic and facultative anaerobic microorganisms; lactic acid bacteria; moulds; yeasts

Introduction

Cheese is a product of milk processing involving a complex microbiome, which includes bacteria, moulds, and yeasts that are either intentionally introduced as starter cultures or naturally present in the milk (Centeno & Carballo, 2023). Recently, artisanal cheeses made from raw milk of small ruminants, particularly goats, have gained significant value and popularity among consumers. With the development of farming in Ukraine, the production and consumption of goat cheeses have been increasing annually. These cheeses are perceived as homemade and natural products that meet the criteria for healthy nutrition. Moreover, such cheeses are characterised by unique and exceptional flavour richness, which drives consumer demand. The expansion of the assortment of artisanal cheeses made from raw goat milk is associated with the production of a significant number of soft cheeses, which differ in ripening time, texture, and sensory characteristics (Holiachuk *et al.*, 2023). The homeland of soft cheeses that mature with the involvement of noble moulds like *Geotrichum candidum* (teleomorph *Galactomyces candidus*), such as Chèvre, Brie, or Camembert, is France. Moulds play an important role in the ripening of soft cheeses due to their metabolism of proteins, lipids, and organic acids, enabling their growth on the cheese surface and enhancing the development of organoleptic properties (Perkins *et al.*, 2020). Brined cheeses, such as

Feta, originate from Greece, are designed for long-term storage, and can be consumed on their own or paired with other dishes.

Cheeses made from raw milk (especially goat milk) are believed to have a more pronounced and rich flavour compared to those made from pasteurised or microfiltered milk. According to A. Camargo *et al.* (2021), the natural microbiota of raw milk is responsible for the typical sensory properties and flavour development of such cheeses. The dominance of lactic acid bacteria is a characteristic feature of artisanal cheeses, providing them with desirable qualities. However, alongside beneficial microflora, such cheeses can also be contaminated with undesirable microorganisms, which may cause spoilage or even pathogens that pose a risk to consumer health (Margalho *et al.*, 2020). Soft cheeses can carry various microbial hazards, including *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* (Dos Santos Rosario *et al.*, 2021; Remizova *et al.*, 2024).

Due to the lack of standardisation in artisanal production, data on the microbial parameters of soft cheeses made from raw goat milk remain scarce. Microbiological monitoring of the ripening process is particularly important for cheeses with a higher risk to consumers, i.e., those with a short ripening period. This is because artisanal cheeses are produced on small-scale farms, where the level of automation and mechanisation of production processes and environmental control is low. Under such conditions, there is a potential risk of microbial contamination of the milk (raw material) and cheeses during both production and ripening. This, in turn, leads to high variability in the total bacterial count and species diversity of lactic acid microorganisms between batches of soft cheeses, as noted by F. Pasquali *et al.* (2022). The microbiological status at each stage of cheese production can be useful for describing the entire food production chain and specifically assessing the variability of artisanal production criteria (Sadvari *et al.*, 2024). Currently, there are very few scientific studies worldwide devoted to the microbial quality of soft cheeses made from raw goat milk, especially in artisanal production (Silva *et al.*, 2021; Parente *et al.*, 2022). Thus, the aim of this research was to determine the species composition of the microbiota at different stages of ripening of artisanal soft Feta and Chèvre cheeses made from raw goat milk as criteria of quality and safety.

Materials and Methods

The study involved two batches of artisanal cheeses made from raw goat milk: brined Feta cheese and white mould-ripened Chèvre cheese. Under the conditions of the eco-farm “Zhuravka” in Kyiv region, five batches of Feta cheese weighing 5 kg each were produced, as well as 15 wheels of Chèvre cheese, each weighing 180-200 g. The study was conducted during 2022-2024. The milk used for cheese production was obtained from Anglo-Nubian goats raised on natural pastures. To produce Feta cheese, raw goat milk was heated to 33°C, and a mesophilic starter culture MA 11 (Danisco France SAS, France), containing *Lactococcus lactis* and *Lactococcus cremoris*, was added. The mixture was thoroughly stirred and left for 60 minutes. Liquid rennet Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine) was then added, mixed thoroughly for 30 seconds, and left to form a curd for 40 minutes. After the curd formed, it was cut into cubes measuring 1 × 5 cm, stirred gently for 20 minutes, and left to rest for 10 minutes to allow whey separation. The curd was transferred into moulds and left for 10-11 hours at a temperature of 20-22°C. Afterward, the cheese was removed from the moulds, cut into pieces weighing 200-300 g, placed into containers, and covered with whey in which 9% salt was dissolved. Care was taken to ensure there was no air between the container lid and the whey (Fig. 1a).

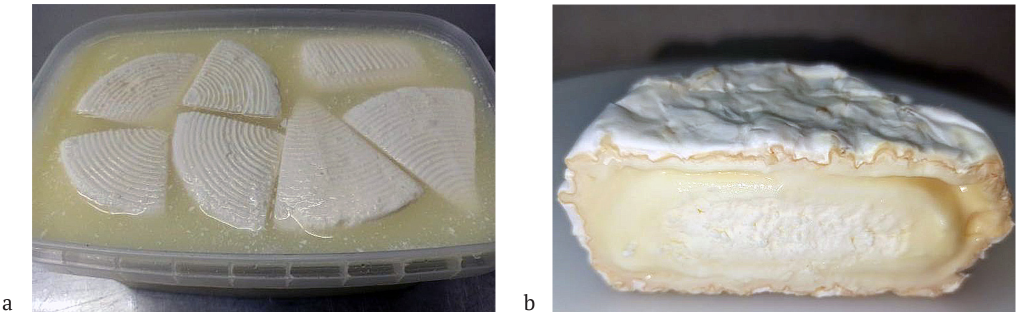

Figure 1. Feta cheese in brine aged 30 months (a), Chèvre cheese aged 40 days (b)
Source: created by the authors

The cheese was stored at 8-9°C for up to 30 months. To prepare Chèvre cheese, raw goat milk was slowly heated to 32°C while stirring constantly. A mesophilic starter culture MA 11 (Danisco France SAS, France), containing *Lactococcus lactis* and *Lactococcus cremoris*, was added to the milk. The mixture was thoroughly stirred, and mould cultures AGE M-GC (*Geotrichum candidum*) and AGE M-PC (*Penicillium candidum*) (IGEA Cultures, Italy) were introduced. Liquid rennet Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine) was then added, mixed thoroughly for 30 seconds, and left to coagulate for 9-11 hours at a temperature of 20-23°C. After coagulation, the whey was drained, and the dense curd was cut into cubes measuring 1 cm using a long knife. The curd mass was placed in a polyester bag and left for 10-12 hours to drain the whey. Salting of Chèvre cheese was done by adding salt at a rate of 20-25 g per kg of cheese. The salted mass was divided into equal portions weighing 180-200 g and shaped into firm cylinders with a thickness of 3 cm and a diameter of 8-9 cm. The cheese was placed on a drainage mat at a temperature of 20-23°C for drying and turned several times over 24 hours. The dried cheese was transferred to a ripening chamber at a temperature of 6-12°C for 40 days, with the wheels turned once daily (Fig. 1b).

Average samples of Feta cheese for analysis were collected on the 7th day, 18th month, and 30th month of storage, with 200 g taken from five containers. Samples of Chèvre cheese were collected on the 3rd, 20th, and 40th days of ripening, with five wheels sampled each time. Microbiological studies were conducted at the LLC "Expert Centre Biolights" (ISO/IEC 17025 (2017) accreditation), Ternopil, Ukraine. Sterile disposable plastic containers with a volume of 100 cm^3 were used for sampling. All cheese samples were placed in a cooling container at 2°C and delivered to the laboratory after collection. For analysis, 10-fold dilutions were prepared. All studies were performed in triplicate. The quantity of bacteria and fungi in the cheeses was determined in colony-forming units (CFU), and the data are presented in the tables as lg CFU/g. The identification of bacterial and fungal species in the cheeses was performed according to the operational instruction "Working with MALDI-TOF Bruker Biotyper". Microorganisms were isolated and identified using MALDI-TOF, compliant with ISO 16140-1:2016 (2016).

For bacterial enumeration in the cheeses, the following culture media were used: Plate Count Agar M091 (HiMedia, India), *Lactobacillus* MRS Agar M641 (HiMedia, India), M17 Agar Base (HiMedia, India), Blood Agar (BioMérieux, France), Buffered Peptone Water (BPW) (HiMedia, India), Baird-Parker Agar (BPA) (HiMedia, India), Endo Agar (Farmaktiv, Ukraine), *Pseudomonas* Agar (HiMedia, India), *Enterococcus* Agar (Farmaktiv, Ukraine), *Bacillus cereus* Agar

M833 (HiMedia, India), Bismuth Sulfite Agar (BSA) (HiMedia, India), Xylose Lysine Deoxycholate Agar (XLD Agar) (HiMedia, India), HCCA Matrix for mass spectrometry (art. 255344) (Bruker, Germany), and Bacterial Calibration Standard (art. 255343) (Bruker, Germany). For the isolation of yeasts and moulds, Sabouraud Agar (HiMedia, India) was used.

After cultivation on Petri dish media, an isolated colony of microorganisms with a volume of 1-2 μL was selected and applied to the surface of a chip well. After drying, 1 μL of matrix solution was applied to the sample. The chip was transferred to the MALDI-TOF device, and the isolated cultures were identified using MBT Compass MALDI Biotyper 3.1 Compass 1.4 for FLEX-Volume 1 and 2 Software and Manuals (Bruker Daltonik, Bremen, Germany). The study results included bacteria identified with a score value of 2.00 or higher. Statistical analysis of the obtained data was performed using one-way analysis of variance (ANOVA). The dynamics of the mycological indicators of the cheeses were analysed using regression and correlation analyses. Data processing was carried out using Microsoft Excel 2016 combined with XLSTAT software. Data in the tables are presented as x ± SD (mean ± standard deviation). Differences between variants were considered significant at $P < 0.05$ using Tukey's test with Bonferroni correction.

Results and Discussion

Microbiological characteristics of Feta cheese

The number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in brined Feta cheese was highest at the beginning of the ripening period. By the 18th month, it had decreased and remained at this level until the 30th month (Table 1).

Table 1. *Microbiological indicators of goat Feta cheese during ripening*

Maturation period	MAFAnM	Mould Fungi	Yeast
7 days	8.39 ± 0.14a	< 1a	6.84 ± 0.16a
18 months	6.96 ± 0.32b	< 1a	4.67 ± 0.17b
30 months	7.02 ± 0.29b	< 1a	6.67 ± 0.11a

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate values that differ significantly within the same column ($P < 0.05$) according to Tukey's test with Bonferroni correction
Source: created by the authors

Moulds were not detected in Feta cheese; however, yeasts were a consistent component of its microbiome throughout the ripening period, with a slight decrease observed by the 18th month. Unique properties of Feta cheese are associated with its sensory aspects and are determined by the geographical location of the production area, its diverse vegetation, the chemical composition of soils and water, the specifics of the production culture, and historical traditions (Tzora *et al.*, 2021). A distinctive feature of artisanal Feta cheese made from raw goat milk is the relative stability of the total count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) and yeasts, with the absence of mould fungi throughout the entire maturation period. In contrast, a study by A. Geronikou *et al.* (2023) found that the most dominant yeast species in white brined cheeses, such as Feta, were *Geotrichum candidum*, *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, and *Trichosporon* spp. According to A. Geronikou *et al.* (2023), the mycological characteristics of brined cheeses, which typically have a shelf life of 52 weeks, depend on storage temperature and duration. An increase in yeast counts was observed during the first 12-14 weeks of storage, stabilising afterward within the range of 4.19-7.08 log CFU/g. The dominant yeast species in white brined cheeses were *Candida zeylanoides* and *Debaryomyces hansenii*, while *Candida parapsilosis*, *Kluyveromyces lactis*, *Kazachstania bulderi*, *Pichia kudriavzevii*, *Pichia fermentans*, *Torulaspora delbrueckii*, *Rhodotorula mucilaginosa*, and *Wickerhamomyces anomalus* were detected less frequently. As expected, the bacterial composition of Feta cheese made from raw goat milk was primarily dominated by lactic acid bacteria (Table 2).

Table 2. *Species composition of bacteria in goat Feta cheese during ripening*

Indicator	**Cheese maturation period**		
	7 days	**18 months**	**30 months**
Lactococcus lactis	5.04 ± 0.18a	3.61 ± 0.13b	< 1c
Lactobacillus paracasei	< 1a	3.59 ± 0.21b	4.11 ± 0.17c
Staphylococcus simulans	1.73 ± 0.26a	< 1b	< 1b
Enterococcus faecalis	< 1a	1.63 ± 0.20b	< 1a
Enterococcus durans	< 1a	2.04 ± 0.19b	< 1a
Serratia liquefaciens	1.69 ± 0.32a	< 1b	< 1b
Kurthia gibsonii	2.01 ± 0.19a	< 1b	< 1b
Bacillus cereus	< 1a	1.03 ± 0.16b	< 1a

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate significant differences within the same row of the table ($P < 0.05$) based on the results of Tukey test with Bonferroni correction
Source: created by the authors

In freshly prepared Feta cheese (7 days old), *Lactococcus lactis* was the dominant species. At 18 months of age, the cheese contained both *Lactococcus lactis* and *Lactobacillus paracasei*, while in 30-month-old cheese, only *Lactobacillus paracasei* was present. The species composition of lactic acid bacteria in Feta cheese varied with age, characterised by a decrease in *Lactococcus lactis* from the 7-day-old cheese to the 18-month-old cheese, with its complete absence in the 30-month-old cheese. At the same time, *Lactobacillus paracasei* was not detected in 7-day-old Feta but appeared at 18 months and increased in abundance by 30 months of storage.

The bacterial composition forming the microbiome of Feta cheese made from unpasteurised goat milk and stored for 30 months is quite dynamic, consistent with findings from analyses of similar cheese made from unpasteurised sheep milk (Tsigkrimani *et al.*, 2022). This indicates the presence of bacteria and yeasts in the cheese that are highly resistant to a 9% salt concentration, but not moulds. It is believed that most non-starter lactic acid bacteria are facultative anaerobes resistant to salt and acid, capable of reproducing and continuing their growth in cheese. The dominant bacteria in artisanal Feta cheese made from raw goat milk include two key species: *Lactococcus lactis* and *Lactobacillus paracasei*. The role of these bacteria and their proportions in forming the Feta cheese microbiome during ripening stages of 7 days, 18 months, and 30 months is crucial. Similar types of microorganisms were found in Feta cheese after 3 and 6 months of storage (Tzora *et al.*, 2021). It is indisputable that *Lactobacillus paracasei* contributes to the unique flavour of aged Feta cheese made from unpasteurised goat milk at 30 months of age. In addition, specific strains of *Lactobacillus paracasei*, due to their anti-listerial activity, are considered to have significant practical potential as starter cultures in cheesemaking.

The growth and development of microorganisms during cheese ripening are influenced by physicochemical parameters (water activity, pH, ripening chamber temperature, redox potential), chemical composition (salt content, organic acids, and their ratios), and bacteriocins produced by starter and non-starter cultures of microorganisms. Salt content in the cheese matrix often acts as a preservative with antimicrobial effects (György & Laslo, 2021). Brined cheeses are characterised by the growth of salt-resistant microorganisms, as observed in the microbiome analysis of Feta cheese made from raw goat milk. A small number of *Staphylococcus simulans*, *Serratia liquefaciens*, and *Kurthia gibsonii* were isolated from 7-day-old Feta cheese, but these microorganisms were absent as the ripening period increased. Two species of enterococci, *Enterococcus faecalis* and *Enterococcus durans*, as well as *Bacillus cereus*, were detected only at the 18-month ripening stage (Table 2). A distinctive feature of the microbiome in Feta cheese stored in brine for 30 months is the presence of an almost monocultural population of *Lactobacillus paracasei*. The presence of staphylococci and yeasts in Feta cheese is attributed to their entry from raw milk. In raw goat milk, R. Hoving-Bolink *et al.* (2023) identified dominant bacterial genera such as *Staphylococcus*, *Pseudomonas*, *Lactococcus*, *Microbacteria*, *Acinetobacteria*, and *Corynebacteria*. M. Akinyemi *et al.* (2023) in their studies determined the ratios in raw goat milk. In raw milk, the dominant bacterial genera include *Lactobacillus* (36%), *Streptococcus* (34%), and *Enterococcus* (12%), as well as fungi such as *Kluyveromyces* (28%), *Saccharomyces* (24%), and *Candida* (18%).

One of the reasons for the detection of enterococci in Feta cheese, such as *Enterococcus faecalis* and *E. durans* (which also belong to the group of lactic acid microorganisms), is their widespread presence in the environment and in the milk of small ruminants. It has been proven that the most frequently isolated species from raw sheep milk were *Enterococcus faecium*, *Lactiplantibacillus plantarum*, and *Pediococcus pentosaceus* (Tsigkrimani *et al.*, 2022). Another factor determining the ability of enterococci to persist for extended periods in brined cheese is their resistance to 10-15% NaCl in whey solutions. The role of enterococci in cheesemaking has been actively discussed by researchers. Alongside undesirable effects, M. Tsigkrimani *et al.* (2022) identified 189 strains of lactic acid bacteria isolated from Feta and Kefalograviera cheeses made from sheep milk, including *Enterococcus faecium*, *E. faecalis*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactiplantibacillus pentosus*, *Latilactobacillus curvatus*, *Lp. plantarum*, *Levilactobacillus brevis*, and *Weissella paramesenteroides*, which tolerate bile salts. This makes them potential candidates for use as starter or adjunct cultures.

The dynamic microbiome composition of Feta cheese observed during study over a long storage period aligns with data from M. Tsigkrimani *et al.* (2022) obtained during the microbiological analysis of Feta cheese made from sheep milk. In Feta cheese, *Lp. plantarum* and *E. faecium* dominated during the early ripening stage, while *Weissella paramesenteroides* prevailed in the late ripening stage. In Kefalograviera cheese, *Levilactobacillus brevis* and *E. faecium* dominated in the early stage, while *W. paramesenteroides* and *E. faecium* were prevalent in the late stage. Another advantage of enterococci over other cheese microorganisms is their ability to grow at temperatures ranging from 10°C to 45°C, in a 6.5% NaCl solution, in a 40% bile solution, and at pH levels from 4 to 9.6. Additionally, they can withstand heating to 60°C for 30 minutes (Graham *et al.*, 2020). In Portugal, Spain, Italy, and Greece, enterococci are highly valued and used in cheesemaking as components of starter cultures to enhance the flavour and aroma of cheese during ripening. Moreover, some enterococci can produce bacteriocins active against spoilage-causing and pathogenic microorganisms in food (such as *Listeria monocytogenes*) and exhibit probiotic properties, which are compelling arguments for their application in the production of fermented food products (Sioziou *et al.*, 2023).

Regarding the isolation of *Serratia liquefaciens* from Feta cheese made from unpasteurised goat milk during the ripening process, data align with the findings of P. Papadakis *et al.* (2021), who identified unpasteurised milk as a source of this bacterium. In Feta cheese made from unpasteurised goat milk at the 7-day ripening stage, *Kurthia gibsonii* was detected, which belongs to relatively new bacterial species. This bacterium was recently isolated from chickens by L. Lozica *et al.* (2022), as well as from wastewater, and is considered a conditionally pathogenic microorganism, like in the study by M. Romance *et al.* (2024). Considering that Feta cheese production takes place on an eco-farm where not only goats but also other animals, such as chickens, are kept, the latter can be regarded as a potential source of this bacterium. According to standards, Feta cheese must mature for at least two months, but it continues to mature throughout its storage period in whey brine. During this time, bacteria such as *Bacillus cereus* are detected after 18 months, which is uncharacteristic for young cheese and is supported by similar data obtained by A. Tzora *et al.* (2021) in their analysis of Feta cheese after six months of storage. Overall, the microbiome of Feta cheese, made from unpasteurised goat's milk, is dynamic and changes with the age of the cheese, adapting to storage conditions and contributing to the formation of its unique characteristics.

Microbiological characteristics of Chèvre cheese

Unlike Feta cheese, Chèvre belongs to cheeses with a short ripening period. The MAFAnM count in Chèvre cheese did not differ significantly on the 3rd, 20th, and 40th days of ripening. More pronounced changes in the microbiome of this cheese were related to moulds and yeasts. An increase in the number of moulds in Chèvre cheese was observed on the 20th day of ripening, with an increase of 1.73 lg CFU/g and 1.58 lg CFU/g compared to the 3rd day (Table 3).

***Table 3.** Microbiological parameters of goat Chèvre cheese during ripening*

Maturation period, days	MAFAnM	Mould fungi	Yeast
3	8.26 ± 0.21a	4.16 ± 0.14a	7.02 ± 0.13a
20	7.97 ± 0.26a	5.89 ± 0.31b	5.63 ± 0.15b
40	8.04 ± 0.12a	5.74 ± 0.25b	5.31 ± 0.18b

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate statistically significant differences within a column ($P < 0.05$) based on Tukey test with Bonferroni correction

Source: created by the authors

At the same time, for yeast, an inverse relationship was identified: by day 20 of Chèvre cheese ripening, their quantity decreased by 1.39 lg CFU/g, and by day 40, it dropped by 1.71 lg CFU/g compared to 3-day-old cheese. Correlation analysis of the mycological parameters of Chèvre cheese showed that the number of moulds had a strong positive correlation with the ripening period ($r = 0.704 \pm 0.134$, $P < 0.01$), while the number of yeasts exhibited a strong inverse correlation with the ripening period ($r = -0.948 \pm 0.060$, $P < 0.001$). In both cases, the regression line was expressed as a second-degree polynomial (Fig. 2).

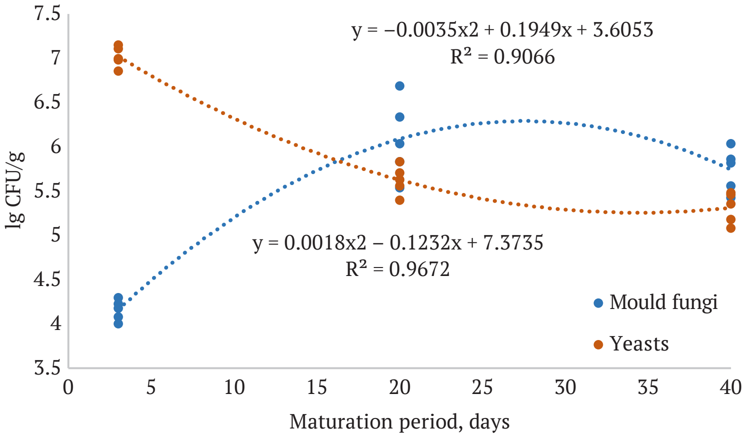

Figure 2. Dependence of mould fungi and yeast counts on the ripening period of Chèvre cheese, n = 15
Source: created by the authors

The species composition of lactic acid bacteria in Chèvre cheese was somewhat similar to that of Feta cheese, particularly regarding *Lactococcus lactis*, which dominated in young cheese and in cheese at the middle of the ripening period (Table 4). At the same time, on day 20 of ripening, three dominant species of lactic acid bacteria were identified in Chèvre cheese: *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. By day 40, only *Leuconostoc mesenteroides* remained dominant. Staphylococci were found only in 3-day-old Chèvre cheese, represented by *Staphylococcus simulans*. On day 20 of ripening, *Serratia liquefaciens* and *Kurthia gibsonii* were isolated from the cheese. By day 40, the bacterial diversity of Chèvre cheese slightly increased compared to the 3-day-old cheese, due to the presence of *Escherichia coli* and one enterococcus species, *Enterococcus durans* (Table 4).

Table 4. *Species composition of bacteria in goat cheese Chèvre during maturation*

Indicator	**Cheese maturation period (days)**		
	3	**20**	**40**
Lactococcus lactis	5.14 ± 0.22a	2.32 ± 0.19b	< 1c
Leuconostoc mesenteroides	< 1a	4.23 ± 0.42b	4.98 ± 0.21b
Lactobacillus plantarum	< 1a	5.08 ± 0.44b	< 1a
Staphylococcus simulans	2.03 ± 0.61a	< 1b	< 1b
Enterococcus durans	< 1a	< 1a	2.06 ± 0.11b
Escherichia coli	< 1a	< 1a	1.61 ± 0.23b
Serratia liquefaciens	< 1a	1.90 ± 0.38b	1.32 ± 0.33b
Kurthia gibsonii	< 1a	1.63 ± 0.22b	< 1a

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate values that significantly differed within a row of the table ($P < 0.05$) based on Tukey test with Bonferroni correction
Source: created by the authors

The ripening of Chèvre cheese is characterised by the formation of a rind involving moulds, which varied in species composition depending on the ripening period. On the 3rd day of ripening, three species of moulds were isolated from Chèvre cheese: one starter species

(*Galactomyces candidus*) and two non-starter species (*Galactomyces geotrichum* and *Penicillium halotolerans*) (Table 5). As the ripening period of Chèvre cheese increased, the species diversity of moulds decreased. Specifically, 20-day-old cheese contained two mould species: *Galactomyces candidus* and *Galactomyces geotrichum*, whereas only the starter culture species *Galactomyces candidus* was identified in 40-day-old cheese. In studies of Mexican artisanal cheeses, P. Cuevas-González *et al.* (2024) identified significantly greater diversity of lactic acid bacteria, including the genera *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Lactococcus*, *Weissella*, *Pediococcus*, *Carnobacterium*, *Aerococcus*, and *Tetragenococcus*.

Table 5. *Species composition of moulds in goat Chèvre cheese during ripening*

Indicator	Cheese maturation period (days)		
	3	20	40
Galactomyces candidus (*Geotrichum candidum*)	3.57 ± 0.24a	4.98 ± 0.25b	5.74 ± 0.25c
Galactomyces geotrichum	2.19 ± 0.11a	2.61 ± 0.23b	< 1c
Penicillium halotolerans	3.91 ± 0.33a	< 1b	< 1b

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate values that significantly differed within a row of the table ($P < 0.05$) based on Tukey test with Bonferroni correction
Source: created by the authors

In freshly made Chèvre cheese from unpasteurised goat milk, the species diversity of bacteria is quite limited. In addition to lactic acid bacteria, staphylococci, including *Staphylococcus simulans*, were isolated from the cheese which was also characteristic of young Feta cheese. *S. simulans* in cheese can originate from milk, as well as from the mucous membranes and skin of goats (Výrostková *et al.*, 2021; Wesołowska & Szczuka, 2023; Aguiar *et al.*, 2024). Currently, the study of the ability of pathogenic and conditionally pathogenic bacteria to synthesise enterotoxins is particularly relevant. As shown in studies by P. Wiśniewski *et al.* (2024), the presence of staphylococci in milk and cheese does not always indicate a risk to human health. Moreover, staphylococci, including *S. simulans*, are capable of exhibiting anti-listerial activity in dairy products, including cheeses (Bockelmann, 2002; Ferrocino *et al.*, 2022).

Considering that both Chèvre and Feta cheese are produced from raw milk of the same goats, it is not surprising that despite differences in production technology and ripening periods, they share a similar species composition, including certain lactic acid bacteria, *Serratia liquefaciens*, *Kurthia gibsonii*, and *Enterococcus durans*. In Chèvre cheese, a small amount of *Escherichia coli* was also isolated on the 40th day of ripening. *E. coli* and coliforms serve as indicators of milk and cheese production hygiene, reflecting faecal contamination of the raw material and final product. The number of indicator microorganisms should not exceed 100 CFU/g, which is the threshold value in many countries (György & Laslo, 2021; Jaramillo-Bedoya *et al.*, 2021). In this case, their levels in the cheese did not exceed the recommended value, indicating proper hygiene practices in this artisanal production. In addition to bacteria, mould fungi play a key role in the ripening of Chèvre cheese, as they contribute to the formation of the rind and the unique sensory characteristics of this cheese. Although studies did not reveal a high species diversity of mould fungi in Chèvre cheese, other researchers have isolated *Galactomyces candidus*, *Candida parapsilosis*, *C. batistae*, *C. sake*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *K. lactis*, *Yarrowia lipolytica*, and *Pichia kudriavzevii* from such cheeses (Banjara *et al.*, 2015).

Artisanal cheeses have a heterogeneous microbiota characterised by dynamic population changes and are associated with the geographical location of the region. The soft Italian PDO (Protected Designation of Origin) cheese Robiola di Roccaverano is made from raw goat milk with natural starters. In samples of fresh and aged cheese, a significantly higher diversity of microorganisms was detected, including *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Leuconostoc pseudomesenteroides*, *Lactobacillus brevis*, *Leuconostoc citreum*, along with fungi such as (*Geotrichum candidum*, *Trichosporon coremiiforme*, *Saturnispora silvae*, *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Yarrowia lipolytica* (Biolcati *et al.*, 2021; Ferraz *et al.*, 2024; Lokes *et al.*, 2024). At various stages of ripening, one to three species of mould fungi were isolated from Chèvre cheese made from unpasteurised goat milk, including one starter species (*Galactomyces candidus*) and two non-starter species (*Galactomyces geotrichum* and *Penicillium halotolerans*). At the end of the ripening period, only one starter fungal species, *Galactomyces candidus*, dominated in Chèvre cheese, which could be attributed to the moisture content of the cheese (Zhang *et al.*, 2022) and its antagonistic effect against other representatives of its mycobiota. Thus, the microbiome of artisanal soft goat cheeses made from unpasteurised milk, such as brined Feta and white noble mould Chèvre, is characterised by a similar species composition of the predominant microorganisms, which may be one of the criteria for determining their quality, safety, age, and comprehensive assessment of authenticity.

Conclusions

The total number of MAFAnM in Chèvre cheese did not depend, and in Feta cheese it was characterised by an inverse dependence on the ripening period. The microbiological characteristics of artisanal soft cheeses made from unpasteurised goat milk, including brined Feta cheese and Chèvre cheese with noble white mould, indicate that the mesophilic aerobic and facultative anaerobic microorganisms throughout their ripening period are primarily composed of lactic acid bacteria. Their population size and species composition are dynamic. The dominant species of lactic acid bacteria in Feta cheese are *Lactococcus lactis* and *Lactobacillus paracasei*, while in Chèvre cheese, they are *Lactococcus lactis, Leuconostoc mesenteroides*, and *Lactobacillus plantarum*.

The ripening of Chèvre cheese involves three species of mould fungi: one starter species (*Galactomyces candidus*) and two non-starter species (*Galactomyces geotrichum* and *Penicillium halotolerans*). With increasing ripening time, the species diversity of mould fungi in Chèvre cheese decreases from three to one of the starter *Galactomyces candidus*. A feature of brine Feta cheese is the absence of mould fungi at all stages of ripening and a stable amount of yeast, while in Chèvre cheese, the number of mould fungi had a direct dependence, and yeast – an inverse dependence on its age. The species diversity of bacteria in Feta and Chèvre cheeses is similar in number of *Staphylococcus simulans*, *Enterococcus durans*, *Serratia liquefaciens* and *Kurthia gibsonii* and depends on the term of their ripening. In both cheeses, species of non-fermented microorganisms were found that are related to the formation of a rich, bright and unique taste and structure. For most sanitary-indicative microorganisms, artisanal cheeses made from unpasteurised goat milk (Feta and Chèvre), produced at an eco-farm in Ukraine, can be considered of good quality and safe for consumers at all stages of ripening. In the future, to assess the quality, safety, authenticity, and age of artisanal goat cheeses Feta and Chèvre, it is planned to study their chemical composition, as well as conduct microstructural analysis.

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Conflict of Interest

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Мікробіом м'яких крафтових сирів, виготовлених із сирого козячого молока, під час дозрівання

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Анотація. Орієнтація споживачів на здорову їжу збільшує попит на крафтові сири, виготовлені з непастеризованого козиного молока. Виробництво таких сирів в Україні здійснюється на невеликих екофермах, що забезпечує унікальність їх мікробіологічного складу. Метою дослідження було визначити мікробіом м’яких козиних сирів Фета і Шевр як критерій якості і безпечності у процесі дозрівання. Для ідентифікації мікроорганізмів використано метод MALDI-TOF. У розсільному сирі Фета на 7-му добу, 18-й і 30-й місяць дозрівання плісеневих грибів не виявляли, а загальна кількість мезофільних аеробних і факультативних анаеробних мікроорганізмів (МАФАМ), а також дріжджів мало змінювалась. Чисельність домінуючих видів молочнокислих бактерій у сирі Фета *Lactococcus lactis* і *Lactobacillus paracasei* залежала від терміну його дозрівання. В незначній кількості із цього сиру виділяли *Staphylococcus simulans*, *Serratia liquefaciens* і *Kurthia gibsonii Enterococcus faecalis* і *E. durans*, а також *Bacillus cereus*. У сирі з білою благородною плíснявою Шевр на 3-тю, 20-ту і 40-ву добу дозрівання чисельність МАФАМ суттєво не змінювалась. Кількість плісеневих грибів у цьому сирі мала пряму сильну залежність, а дріжджів – обернену сильну залежність від терміну його дозрівання. Основні види молочнокислих бактерій сиру Шевр представлені *Lactococcus lactis*, *Leuconostoc mesenteroides* і *Lactobacillus plantarum*. У процесі дозрівання з сиру Шевр виділяли незначну кількість *Staphylococcus simulans*, *Serratia liquefaciens*, *Kurthia gibsonii*, *Escherichia coli*, *Enterococcus durans*. Основу пліснevих грибів сиру Шевр складали *Galactomyces candidus*, *G. geotrichum* і *Penicillium halotolerans*. Видовий і кількісний склад мікроорганізмів сирів Фета і Шевр свідчить про належну їх якість і безпечність і може служити критерієм їх автентичності. Результати досліджень розкривають нові дані динаміки мікробіому крафтових м’яких сирів з непастеризованого козиного молока, вироблених в Україні

Ключові слова: сир Фета; сир Шевр; мезофільні аеробні і факультативні анаеробні мікроорганізми; молочнокислі бактерії; плісеневі гриби; дріжджі