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Microbiological changes in craft hard cheeses from raw goat milk during ripening with the use of mites *Acarus siro*

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ABSTRACT

The nutritional and biological values of craft hard cheeses made from raw goat milk, in combination with their unique flavor characteristics, increase consumer demand and expand their range on the market. Production of such cheeses is concentrated on small farms and is becoming increasingly common in Ukraine. Ripening of such hard cheeses made from raw goat milk is provided by a significant species diversity of their biome, particularly bacteria, molds, yeasts, and mites, which requires a quality and safety assessment of such cheeses at different ripening periods. Microbiological indicators of Alpine and Yoghurt hard cheeses made from raw goat milk were determined during the study. A study was carried out using traditional methods of microbial analysis, as well as MALDI-TOF technology. Alpine cheese was characterized by relative stability in QMAFAnM at 7 days, 6, and 12 months. In Alpine cheese, the maximum amount of yeast was detected at 7 days of age, and the maximum amount of moulds was detected at 6 months of ripening. Lactic acid bacteria, in particular, Lactococcus lactis and Lactobacillus plantarum, formed the microbiome basis of Alpine cheese during all ripening periods. Staphylococcus simulans, S. equorum, Enterococcus faecalis, E. durans, Escherichia coli and Bacillus cereus were isolated in small amounts from Alpine cheese. The number of QMAFAnM in Yoghurt cheese increased throughout the ripening period, while the number of yeasts and moulds reached a maximum at 6 months. Lactic acid bacteria, in particular, Lactococcus lactis and Lactobacillus paracasei, dominated at all ripening periods of Yoghurt cheese (7 days, 6 and 18 months). Staphylococcus equorum, Enterococcus faecalis, Escherichia coli, Raoultella ornithinolytica, Providencia stuartii, and Kurthia gibsonii were isolated in small amounts from Yoghurt cheese. Alpine and Yoghurt hard cheeses made from raw goat milk are ripened with mycoid mites Acarus siro, which are involved in forming the cheese rind. The study results can be used as an element of the authenticity criterion for craft hard cheeses made from raw milk.

Keywords: Alpine, yoghurt bacteria, moulds, yeast, mites Acarus siro, hard cheese

INTRODUCTION

Cheese is one of the oldest food products made from animal milk, and today it is still one of the most important components of the human diet. An extraordinary variety of industrial cheeses represents the world market, but craft cheeses arouse ever-growing interest and generate consumer demand with every passing year [1]. Craft cheeses are typically produced in small-scale enterprises, often hand-made, mainly from raw cow, sheep, goat milk, or various blends thereof. It is commonly believed that craft cheeses made from raw milk have a higher



microbial diversity than cheeses made from pasteurized milk under production-line conditions. The unique microbiome of cheeses made from raw milk provides them with consummate taste, aroma, and texture. In contrast, industrial cheeses have a lower species diversity of the microbiota, which is related to pasteurization for product safety to be guaranteed.

Regarding taste characteristics, craft cheeses made from raw goat milk are among the most attractive to consumers worldwide. Goat cheese is an underappreciated food product with a high nutritional value and a promising potential as a human functional food [2]. Among the European Union countries, France, Spain, and Greece are in the top decile of the goat cheese producers, representing 17.3%, 7%, and 7.6% of global production in 2015-2017, respectively. In Ukraine, there is also an increase in the production and consumption of craft goat cheeses. Still, very few studies on their microbiome at different ripening periods would assess their quality and safety.

The microbiota of cheese made from raw milk plays an essential role in the ripening process and in forming its original taste qualities and safety. The dominant component of the microbial association of cheeses is lactic acid bacteria. In contrast, pathogenic and opportunistic pathogenic bacteria that cause cheese defects and pose a hazard to consumer health, such as *Escherichia coli, Staphylococcus spp.*, and *Pseudomonas spp.*, are absent or present in small amounts [3]. Among the lactic acid microorganisms used as a part of starter cultures, the genera *Lactococcus, Streptococcus, Lactobacillus,* and *Leuconostoc* are often used. Their role is to ensure rapid milk acidification during an initial fermentation, while the non-starter genera Lactobacillus, Pediococcus, Leuconostoc, and *Enterococcus* shape a development of organoleptic characteristics during the cheese ripening due to their metabolic activity [4], and [5].

When craft cheeses made from raw milk are ripened, changes in microbial associations occur, which are related to the ability of microbial populations to adapt to specific environmental conditions formed at different ripening periods of cheese [6]. The total number of microorganisms and their species ratio in cheese, depending on the ripening period, is due to their ability to withstand temperature and acidity and grow and tolerate a low water concentration and activity [7], which is typical for old-aged cheeses.

Considering the importance of the cheese microbiota in determining its quality, safety, and authenticity, it has been proposed to identify a microbial map responsible for unique sensory characteristics **[8]**.

The complex microbiome of raw goat milk during the carbohydrate and protein metabolism facilitates the formation of substrates that can stimulate the growth of bacteria and molds [9] and arachnids, particularly mites. Mites, in particular, Acarus siro, propagate on the surface of the rind when hard cheeses have been ripening for more than 6 months and are considered contaminated with pantry pests that are dangerous to human health. Mites of the following families often propagate oneself in cheeses, air-cured and dried foods: Acaridae - Acarus siro (Linnaeus, 1758), A. farris (Oudemans, 1905), Tyrophagus putrescentiae (Schrank, 1781); Glycyphagidae -Lepidoglyphus destructor (Schrank, 1781), Glycyphagus domesticus (De Geer, 1778) and Gohieria fusca (Oudemans, 1902) and Chortoglyphidae - Chortoglyphus arcuatus (Troupeau, 1879) [10]. However, using certain mite types for cheese ripening is a long-standing tradition applied at cheese factories in France and Germany. The species of industrial interest include mites from the subclass Acari, order Astigmata of the family Acaridae, in particular, Tyrolichus casei (Oudemans, 1910), which is used for the production of Mimolette and Artisou cheeses (France), as well as Würchwitzer Milbenkäse cheese (Germany) [11]. Cheeses that are ripened with the use of mites have been studied little. The taste and aroma of cheeses, which are related to the secretion of the opisthonotal glands of mites Astigmata, have been mainly subjected to identification. The rind of such cheese acquires a lemon flavour due to mites [12]. In respect to studies on the microbiome of such cheeses, especially those made from raw goat milk during the ripening process, there is a small number of such studies, and they do not give a comprehensive answer to the quality and safety of these products.

Scientific Hypothesis

The first scientific hypothesis suggests that the microbiome of craft hard cheeses made from raw milk will allow consumers to assess their quality and safety. Determining the species composition of the microbiota of Alpine and Yoghurt hard cheeses made from raw goat milk, which are ripened with the use of mites Acarus siro, will allow consumers to determine the criteria of authenticity.

Objectives

To determine the microbiological indicators of Alpine and Yoghurt craft hard cheeses made from raw goat milk during ripening. To investigate the species composition of bacteria, yeasts, and fungi for young, mature, and old-aged cheeses. To identify mites, which are used in the ripening of hard cheeses.

Auxiliary objectives: to assess the quality and safety of craft hard cheeses made from raw goat milk. To identify indicators that can be the criteria of authenticity for craft hard cheeses.







MATERIAL AND METHODS

Samples

Samples description: The studies used the average samples of Alpine and Yoghurt craft hard cheeses made from raw goat milk.

Samples collection: The samples of cheeses were taken under sterile conditions, packed in vacuum bags, placed in a cooling bag at a temperature of 2°C, and sent to the laboratory for the study.

Samples preparation: The average samples of cheeses were taken in a weight of 200 g from 5 heads at each ripening period: for Alpine - laying for the ripening - 7 days, mature - 6 months and old-aged - 12 months, for Yoghurt - laying for the ripening - 7 days, mature - 6 months and old-aged - 18 months. The rind slices of old-aged cheeses were used to identify mites.

Number of samples analyzed: A total of 15 heads of Alpine cheese and 15 heads of Yoghurt cheese were used. The microbiological indicators of cheeses were compared within the same product in terms of age. Chemicals

Blood agar (BA) (BioMérieux, France), Buffered peptone water (BPW) (HiMedia, India), Baird parker agar (BPA) (HiMedia, India), Endo agar (Endo) (Pharmaktiv, Ukraine), Pseudomonas agar (Psedo) (HiMedia, India), Enterococcus agar (Pharmaktiv, Ukraine), Bacillus cereus agar (HiMedia, India), Bismuth sulfite agar (BSA) (HiMedia, India), Xylose lysine deoxycholate agar (XLD agar) (HiMedia, India), HCCA mass spectrometry matrix (art. No. 255344) (Bruker, Germany), Bacterial calibration standard (No. 255343) (Bruker, Germany) were used for microbiological studies.

Animals, Plants and Biological Materials

Alpine cheese and Yoghurt cheese (Eco Farm Zhuravka, Kyiv region, Ukraine), starter cultures for cheeses Alp D (Danisco France SAS, France), TOM V-02 (IGEA Cultures, Italy), YF-L 812 (Chr. Hansen, Denmark), Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine).

Instruments

MALDI-TOF MS mass spectrometer (Bruker Daltonics, Germany), 5050 ELV D-line vertical autoclave (Tuttnauer, Israel), ED115 drying chamber (Binder, Germany), Binder BD 115 thermostat (Binder, Germany), Leica DM500 LED binocular microscope (Leica, Germany), Micromed Evolution ES-4140 microscope with photomask (Ningbo Shenghen Optics & Electronics Co, Ltd, Bulgaria), refrigerating and freezing chamber (Liebherr, Switzerland).

Laboratory Methods

Microbiological studies of cheeses were conducted at Expert Centre "Biolights" LLC, Ternopil (Ukraine). Microbiological studies of cheeses were conducted following the standard methods, and screening was carried out following the MALDI-TOF method. This method is based on detecting microorganisms in biological samples by accumulating and subsequently seeding on differential media with the identification by the MALDI-TOF [13]. Preparing nutritional media and seeding microorganisms were conducted following the applicable instructions. Grown colonies were identified with the use of the MALDI-TOF chip and MBT Compass MALDI Biotyper 3.1 Compass 1.4 for FLEX- Volume 1 and 2 Software and Manuals (Bruker Daltonik, Bremen, Germany). Samples of detected bacteria were considered positive if identified with a value of scope 2.00. The number of bacteria, moulds, and yeasts in cheeses was determined in colony-forming units (CFU), and the results were expressed in lg CFU/g.

Mites *Acarus siro* were detected and identified using 3-4 mm rind slices of Alpine and Yogurt cheeses [14], [15].

Description of the Experiment

Study flow: The experiment used two craft hard cheeses made from raw goat milk. The heads of Alpine and Yoghurt cheeses were produced with an average weight of 4.5-5.0 kg. Milk from Anglo-Nubian goats that grazed on natural grasslands was used to produce such cheeses.

Raw goat milk was heated to a temperature of 35°C, and the mesophilic-thermophilic starter culture Alp D (Danisco France SAS, France) was added to produce Alpine cheese. The specified starter culture consists of the following: *Lactococcus lactis subspecies lactis, Lactococcus lactis subspecies cremoris, Lactococcus lactis subspecies lactis subspecies lactis, Lactobacillus, Lactobacillus helveticus, Lactobacillus lactis.* After adding the specified starter culture, the milk was stirred and left for 30 minutes. After that, Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine) was added, evenly distributed over the entire volume with constant stirring, and left for 40-45 minutes for the milk to be coagulated. After coagulation, the milk coagulum was

vertically cut into equal cubes of 0.5-1.0 cm in size, left for 5 min, and then it was horizontally cut. The curd was gently stirred for 15 minutes, maintaining a temperature of 35°C. After that, 50% of the whey was drained off, and the curd was heated to a temperature of 48°C with constant stirring for 40 minutes. The cheese mould was filled under a whey layer and placed on a drainage mat. The cheese was pressed for 8-10 hours in 3-4 stages, gradually increasing the weight of the press with constant turning of the cheese head. After pressing, the cheese was removed from the mould and left at room temperature for 8-10 hours. At this time, a 20% salt water solution, which was cooled in a refrigerator for 8-10 hours, was prepared for the cheese to be salted. For this purpose, the cheese was turned over for the uniform salting of the entire mass. The salted cheese was removed from the brine, the heads were dried with a paper towel and placed in a ripening chamber with a humidity of 80-85% and a temperature of 8-11°C. For the first few days, the cheese was turned over 3 times a day, and after the rind was dried - once a day within the first month, and then 2-3 times a week. The cheese heads were stored for 12 months to provide the ripening.

Yoghurt cheese was made from raw goat milk according to the author's technology modification. The milk was heated up to a temperature of 37°C, and two starter cultures were added to its surface, mixed, and left for 55-60 minutes. The specified thermophilic starter cultures were: TOM V-02 (IGEA Cultures, Italy), which contains *Streptococcus thermophilus*, and YF-L 812 (Chr. Hansen, Denmark), which contains *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus*. Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine) was constantly stirred throughout the milk. After that, the milk was left for 55-60 minutes to coagulate, and the milk coagulum was cut into equal cubes of 1.5-2.0 cm in size. After cutting, the curd was slowly and thoroughly mixed for the next 25 minutes, maintaining a temperature of 37-39°C. The whey was drained off, and the curd was put into a mould, pressed, and turned over several times after 30 minutes. After pressing, the cheese heads were cooled to a temperature of 8-10°C. After 12 hours, the cheese heads were removed from a mould and immersed in a 25% table salt solution. The salting duration was about 5-6 hours per 500 g cheese. After salting, the cheese was removed from a mould and placed in a ripening chamber at an 8-10°C temperature and a humidity of 85-90% for 18 months.

Alpine and Yoghurt cheeses were washed with running water, which was obtained from an artesian well, starting from the 4-month ripening period, dependent on the growth intensity of mite *Acarus siro*, every 2-3 months.

Quality Assurance

Number of repeated analyses: Five samples were used in each study. Number of experiment replication: 1.

Reference materials: -

Calibration: Each instrument was calibrated before each experiment, and calibration checks were performed regularly to maintain measurement accuracy. Each instrument was calibrated before each experiment, and calibration checks were performed regularly to maintain measurement accuracy.

Laboratory accreditation: Microbiological studies were conducted at Expert Centre "Biolights" LLC, Ternopil, Ukraine. The laboratory has been accredited in accordance with the requirements of DSTU EN ISO/IEC 17025:2019 (EN ISO/IEC 17025:2017, IDT; ISO/IEC) 17025:2017, IDT) and confirmed by the Accreditation certificate of the National Accreditation Agency of Ukraine.

Data Access

The data supporting the findings of this study are not publicly available.

Statistical Analysis

The study results were statistically processed with the use of one-factor dispersion analysis. A dynamics of the microbiological indicators of Alpine and Yoghurt cheeses during the ripening process was analyzed using regression and correlation analyses. Microsoft Excel 2016 software in combination with XLSTAT was used for this purpose. The tabulated data are presented as $x \pm SD$ (mean \pm standard deviation). The difference between the variants was considered significant at P < 0.05 using the Tukey test.

RESULTS AND DISCUSSION

Cheese production is based on the milk transformation process with the help of microorganisms, which play an essential role at all ripening periods and provide the uniqueness of organoleptic properties of finished products through a metabolic activity [16]. Today, cheeses are produced in various forms and flavors worldwide, making them a premium food product. Hard cheeses are particularly valuable from raw goat milk, which is characterized by their authentic microbiota at each ripening period. Alpine hard cheese was characterized by relative stability in terms of QMAFAnM at the beginning of the ripening period (7 days), at the age of 6 months, and 12 months



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(Table 1). At the same time, the number of moulds was substantially different in the cheese of different ages. Thus, at the age of 7 days no moulds were detected in Alpine cheese, and at the 6th month a peak in their number was found with a subsequent decrease of 1.56 lg CFU/g at the 12th month of the ripening. The correlation analysis revealed that the number of moulds in Alpine cheese directly connected moderate strength with its ripening period ($r = 0.593 \pm 0.152$, P < 0.01). The regression analysis confirmed a dependence between the number of moulds and the ripening period of Alpine cheese, which is expressed by a second-degree polynomial (Figure 1).

Table 1 Microbiological indicators of Alpine hard cheese during its ripening.

Ripening period of cheese	QMAFAnM	Moulds	Yeast
7 days	$7.79\pm0.28^{\rm a}$	< 1 ^a	$6.96\pm0.16^{\rm a}$
6 months	$7.31\pm0.23^{\mathrm{a}}$	$4.09\pm0.36^{\text{b}}$	$2.46\pm0.13^{\text{b}}$
12 months	$7.48\pm0.21^{\rm a}$	$2.53\pm0.36^{\rm c}$	$2.48\pm0.25^{\text{b}}$

Note: $x \pm SD$, n = 5, $\lg CFU/g$, different letters of superscript indicate values that differed significantly in the same table column (P < 0.05) according to the comparison results with the use of the Tukey test.

The number of yeasts in Alpine cheese reached its maximum level only at 7 days of age, and upon reaching its maturity at 6 months of age, it decreased by 4.5 lg CFU/g and remained so until 12 months of age. The goat milk for cheese production contains bacteria, moulds, and yeast, which can facilitate the fermentation and ripening of the cheese. Microorganisms, which are present on the teat skin, milking equipment, water, animal feed, soil, air, and other farm-related environments, can enter the milk during the milking process [17]. Immediately after the milking, the animal milk contains a mixture of bacteria, with lactobacilli as the main component. The genera Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, and Enterococcus are the most typical representatives of raw milk. The microbiome of Alpine cheese was based on lactic acid bacteria during all ripening periods. At the same time, their species composition was slightly different and depended on the ripening period of the cheese (Table 2). The most abundant species of lactic acid bacteria in Alpine cheese during the entire ripening period was Lactococcus lactis. Its origin can only be determined after strain identification, as it can be a starter and nonstarter component that is a part of the microbiome of raw goat milk. At the same time, the largest number of colonies of L. lactis was found in the cheese at 7 days of age, and with an increase of the ripening period, it decreased, reaching its minimum level at the 12th month. The correlation analysis revealed that there was a strong inverse dependence of L. lactis in Alpine cheese on its ripening period (r = -0.963 ± 0.067 , P < 0.001), and a regression line confirmed the presence of an inverse linear relationship (Figure 1).

Indicator	Ripening period of cheese		
	7 days	6 months	12 months
Lactococcus lactis	$5.10\pm0.17^{\mathrm{a}}$	$4.47\pm0.14^{\text{b}}$	$4.01\pm0.16^{\rm c}$
Lactobacillus plantarum	< 1 ^a	$4.76\pm0.21^{\rm b}$	< 1 ^a
Staphylococcus equorum	< 1 ^a	< 1 ^a	$2.21\pm0.24^{\rm b}$
Staphylococcus simulans	< 1 ^a	$1.06\pm0.11^{\rm b}$	< 1 ^a
Enterococcus faecalis	< 1 ^a	$1.11\pm0.07^{\mathrm{b}}$	< 1 ^a
Enterococcus durans	< 1 ^a	$1.09\pm0.06^{\rm b}$	< 1ª
Escherichia coli	< 1 ^a	< 1ª	$1.12\pm0.12^{\rm b}$
Bacillus cereus	< 1 ^a	$1.26\pm0.15^{\text{b}}$	$1.19\pm0.09^{\rm b}$

Table 2 Species composition of bacteria isolated from Alpine hard cheese during its ripening period.

Note: $x \pm SD$, n = 5, $\lg CFU/g$, different letters of superscript indicate values that differed significantly in the same table column (P < 0.05) according to the comparison results with the use of the Tukey test.

In addition to *L. lactis*, the non-starter species *Lactobacillus plantarum*, which was isolated only at the age of 6 months, was found among the lactic acid bacteria in Alpine cheese. The cheeses' dominant lactic acid bacteria indicate their high resistance and antagonism toward other microbiota, making it possible to use them often as preservatives **[18]** or starter cultures for other food products **[19]**.

The species composition of staphylococci in Alpine cheese depended on the ripening period: *Staphylococcus simulans* were isolated at the age of 6 months, and *Staphylococcus equorum* was isolated at the age of 12 months. Regarding the presence of *S. equorum* in the cheeses, our data are consistent with the study results of the cheese brines from Danish factories, where these bacteria were also found [20], [21]; 53 isolates of *S. equorum* were isolated from the ripened samples of traditional blue-veined Cabrales cheese. Concerning *S. simulans*, it was isolated [22] from sheep and goat cheeses, which were also made from raw milk. Staphylococci are considered opportunistic pathogen microorganisms that can cause mastitis in animals, but they also have a certain positive

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role in cheese-making. Since staphylococci, together with yeast, are acid- and salt-resistant and can grow rapidly even in the first days of the cheese ripening, they can show antagonism to such pathogenic microorganisms as *L. monocytogenes*, which makes it possible to consider them promising in terms of the biocontrol of this bacterium [23].

In addition to staphylococci, the presence of enterococci in cheeses and dairy products in general also has a double meaning: on the one hand, it characterizes the hygiene of production (fecal contamination of raw materials), and on the other hand, it represents the achievement of the desired unique taste characteristics of cheeses [24]. In Alpine cheese made from raw goat milk, two types of enterococci were found in small amounts and only at 6 months - *Enterococcus faecalis* and *E. durans*. Similar data were obtained by [25], who isolated *Enterococcus faecium, E. faecalis,* and *E. durans* from traditional sheep and goat cheeses from Slovakia and Hungary's border area (Slanské vrchy region). They isolated 110 *Enterococcus sp.* from cheese samples, of which 52 strains were represented (*E. faecium* (12), *E. faecalis* (28) and *E. durans* (12)). It is believed that *Enterococcus species*, mainly *E. faecalis, E. faecium, E. italicus,* and *E. durans,* which are non-starter lactic acid bacteria, are usually detected at the beginning of the cheese ripening period. Mainly in Mediterranean cheese, enterococci facilitate the cheese ripening and taste development through proteolysis, lipolysis, and diacetyl production [17]. In addition, it has been established that they produce bacteriocins, which negatively affect the growth of pathogenic bacteria, particularly *Listeria monocytogenes, Staphylococcus aureus,* and *Clostridium perfringens* [26].

Based on studies conducted by several scientists, it may be concluded that there are neither absolutely safe nor hazardous species of enterococci for human health. Their pathogenicity depends on the number of CFU in the product and the ability to cause an infectious process in each organism. Thus, the beneficial strains demonstrate the desired metabolic profile and physiological properties suitable for producing dairy products, particularly cheeses of the highest sensory quality [27] and [28].

In addition to enterococci, a small number of *E. coli* colonies were isolated in Alpine cheese only at 12 months. It proves that the conditions for reproduction and growth of this bacterium are created in Alpine cheese, which is made from raw goat milk, during its ripening. Scientists even express concern over detecting *E. coli* colonies at all Ukrainian hard cheese production stages from raw cow milk. It of confirms a sufficiently high resistance of E. coli not only in raw milk but also in pasteurized milk and its processed products **[29]**.

Bacillus cereus is a bacterium that may cause food poisoning. It is widely distributed in the environment and is a part of the gut microbiome and ruminant milk, so its presence in cheeses is quite common. Moreover, milk pasteurization often plays the role of a germination activator of *B. cereus* spore with subsequent growth and reproduction in cheeses [30]. Therefore, the presence of this bacterium in Alpine cheese made from raw goat milk is not exceptional, especially in mature and old-aged ones. Similar results were obtained during the study of Mexican craft cheeses made from cow milk [31].

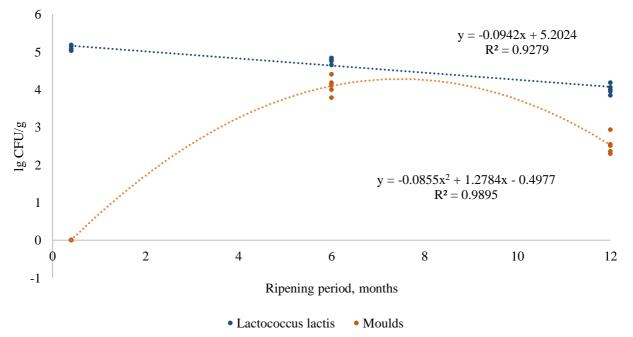


Figure 1 Dependence of the number of lactic acid bacteria and moulds on ripening period of Alpine hard cheese. Note: n = 15.



The microbiological indicators of Yoghurt hard cheese were substantially different from Alpine cheese, primarily in terms of the dynamics of QMAFAnM during the entire ripening period. The number of QMAFAnM in the cheese at the beginning of the ripening period (7 days) was the lowest, with a gradual increase of 1.18 lg CFU/g at the 6th month and 0.54 lg CFU/g at the 18th month of the ripening (Table 3). The number of QMAFAnM in Yoghurt cheese depended on its ripening period, and the regression line was expressed as a second-degree polynomial (Fig. 2).

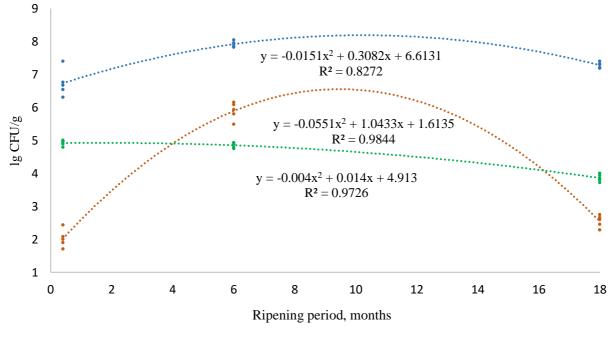
The amount of yeast in Yoghurt cheese also reached its maximum level at the age of 6 months, while at the beginning of the ripening period (7 days) and at the 18th month it was lower by 3.87 lg CFU/g and 3.35 lg CFU/g, respectively. Similarly to QMAFAnM, the number of yeasts in Yoghurt cheese during the ripening period depended on its ripening period. The regression line was expressed as a second-degree polynomial (Fig. 2). A significant number of yeasts belonging to the genera *Candida, Clavisporalus, Cryptococcus, Debaryomyces, Geotrichum, Issatchenkia, Kazachstania, Kluyveromyces, Kodemaea, Pichia, Rhodotorula, Saccharomyces, Saturnispora, Torulas pora, Trichosporon, Yarrowia and Zygosaccharomyces* have been isolated and identified from craft cheeses [32]. Moreover, yeast was an essential microbiome component throughout the entire ripening period in Alpine and Yogurt cheeses. It is believed that, depending on the name and origin of the cheese, the different types of yeast have an indispensable influence on the development of taste and structure, demonstrating the deoxidising, proteolytic and/or lipolytic activity [33].

No moulds were detected in Yoghurt cheese at the beginning (7 days) and at the 18th month of the ripening period, but their maximum amount was isolated from the samples of the mature cheese at the age of 6 months. This is due to the formation peculiarities of the cheese rind during this period. The appearance of moulds in cheeses is often caused by their presence in the composition of raw milk. The ripening chambers and the cheese factory's air environment are also considered important sources of moulds on the surface of the cheese rind. A study of the mycoflora of dairy products and dairy production environments showed 109 species of filamentous fungi. Cladosporium, Penicillium, Aspergillus, and Nigrospora are the most common genera in milk production environments [34].

Table 3 Microbiological indicators of Yoghurt hard cheese during its ripening period.

Ripening period of cheese	QMAFAnM	Moulds	Yeast
7 days	$6.74\pm0.21^{\mathrm{a}}$	< 1ª	$2.02\pm0.27^{\rm a}$
6 months	$7.92\pm0.19^{\text{b}}$	$2.41\pm0.24^{\rm b}$	$5.89\pm0.26^{\rm b}$
18 months	$7.28\pm0.13^{\circ}$	< 1ª	$2.54\pm0.18^{\rm c}$

Note: different letters of superscript indicate values that differed significantly in the same table column (P < 0.05) according to the comparison results with the use of the Tukey test. (x \pm SD), n = 5, lg CFU/g



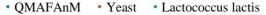


Figure 2 Dependence of the number of QMAFAnM, *Lactococcus lactis* and yeast on the ripening period of Yoghurt hard cheese. Note: n = 15



The conditions formed in freshly cooked (7 days) and old-aged cheese (18 months) are substantially different in terms of moisture content and salt distribution [35]. However, the ripening of Yogurt cheese has an equally adverse effect on the growth of moulds. A similar pattern was observed for Caciotta craft cheese made from raw goat milk [36].

Scientists and technologists are concerned about the presence of many mold species isolated from cheeses. There are two points of view in this regard: the first is the harmfulness of moulds as producers of dangerous mycotoxins [37], and the second is the benefit of molds as an essential component of the microbiome, which provides authentic taste characteristics and expands the range of cheeses [38].

The analysis of the species composition of Yoghurt cheese bacteria revealed that lactic acid bacteria were dominant at all ripening periods. The correlation and regression analyses of the microbiological indicators of Yoghurt cheese showed that the number of lactic acid bacteria had a strong inverse dependence on its ripening period ($r = -0.952 \pm 0.058$, P < 0.001). The regression line was expressed as a second-degree polynomial

(Figure 2). The dominant species of lactic acid bacteria in Yoghurt cheese was *Lactococcus lactis*, as in Alpine cheese, during the ripening period (Table 4). Only in this case was its origin in the cheese exclusively non-starter. At the beginning of the ripening period of Yoghurt cheese, the species diversity of lactic acid bacteria was more than in mature (6 months) and old-aged (18 months) cheese, as it included *Lactobacillus paracasei* in almost the same titer as *Lactococcus lactis*.

The isolation of non-starter lactic acid microorganisms from cheeses confirms their origin from the milk or the environment [39]. It is believed that unique strains of lactic acid microorganisms can provide a so-called microbial signature authentic to each craft cheese producer [40]. During the cheese ripening, the pH value decreases within the range of 4.8 to 5.4 units, which creates unfavourable conditions for several pathogenic bacteria. Still, the subsequent growth and reproduction of lactic acid bacteria increases the production of organic acids and bacteriocins. This eventually leads to the dominance of lactic acid microorganisms in cheese.

Indicator	Ripening period of cheese		
	7 days	6 months	18 months
Lactococcus lactis	$4.95\pm0.23^{\rm a}$	$4.85\pm0.18^{\rm a}$	$3.86\pm0.11^{\text{b}}$
Lactobacillus paracasei	$4.79\pm0.21^{\rm a}$	< 1 ^b	< 1 ^b
Staphylococcus equorum	< 1 ^a	< 1 ^a	$2.32\pm0.16^{\text{b}}$
Enterococcus faecalis	< 1 ^a	$2.44\pm0.31^{\rm b}$	$2.51\pm0.24^{\rm b}$
Escherichia coli	< 1 ^a	$1.61\pm0.17^{\rm b}$	< 1 ^a
Raoultella ornithinolytica	$1.32\pm0.09^{\rm a}$	< 1 ^b	< 1 ^b
Providencia stuartii	$1.07\pm0.16^{\rm a}$	< 1 ^b	< 1 ^b
Kurthia gibsonii	$1.21\pm0.25^{\rm a}$	< 1 ^b	< 1 ^b

Table 4 Species diversity of bacteria isolated from Yoghurt hard cheese during its ripening period.

Note: $x \pm SD$, n = 5, $\lg CFU/g$, different letters of superscript indicate values that differed significantly in the same table column (P < 0.05) according to the comparison results with the use of the Tukey test.

The microbiota of raw goat milk, which is used for cheese production, depends heavily on the hygiene and production technology. Among the main genera of microorganisms that are presented in raw goat milk are Staphylococcus, Pseudomonas, Lactococcus, Microbacteria, Acinetobacteria, and *Corinebacteria* [41]. Similar representatives of staphylococci and enterococci, in particular, *Staphylococcus equorum and Enterococcus faecalis, as well as E. coli, were isolated from the old-aged Yoghurt and Alpine cheeses, which indicate their dairy origin since raw milk from the same goats was used for both kinds of cheese to be produced. Moreover, three representatives of other bacteria species, which are not typical for Alpine cheese, were isolated from Yoghurt cheese: <i>Raoultella ornithinolytica, Providencia stuartii* and *Kurthia gibsonii* (Table 4). *Raoultella ornithinolytica* and *Providencia stuartii* belong to the family *Enterobacteriaceae* and are widely distributed in the environment, in particular, water, soil, and livestock facilities [42], they contaminate cooked meat products [43], milk and dairy products [44], their pathogenicity has been poorly studied [45].

Kurthia gibsonii is a bacterium that is quite resistant in the environment, belongs to psychrotrophic microorganisms, and even bactofugation of milk does not help to get rid of it [46]. As can be seen from the obtained data, this bacterium was isolated in Yoghurt cheese only at the early ripening period, which indicates the safety of this cheese at a mature and old age.

Alpine and Yoghurt hard cheeses are ripened without covering the heads with a film. The formation of their rind is provided with the use of mycoid mites *Acarus siro* (Linnaeus, 1758) order Acariformes (Zachvatkin, 1952) suborder Sarcoptiformes (Reuter, 1909) infraorder Astigmata (G. Canestrini, 1891) superfamily Acaroidae (Latreille, 1802) family Acaridae (Latreille, 1802) genus *Acarus* (Linnaeus, 1758). These mites begin to colonise







the rind surface during the period when moulds have grown to the greatest possible extent, starting from 5-6 months of cheese age (Fig. 3). It is facilitated by high humidity at the level of 80-90% and temperature of 8-11°C, which are provided in the cheese ripening chamber. Subsequently, the growth rate of moulds on the rind surface decreases due to the moisture loss by the cheese head and the mycelium consumption by mites (Fig. 4). After that, the number of mites on the cheese rind surface is controlled by washing the heads with running artesian water, as in the absence of moulds and massive population growth, mites begin to eat the cheese and penetrate the inside of the heads, leading to the appearance of undesirable changes in the structure of the cheese dough.

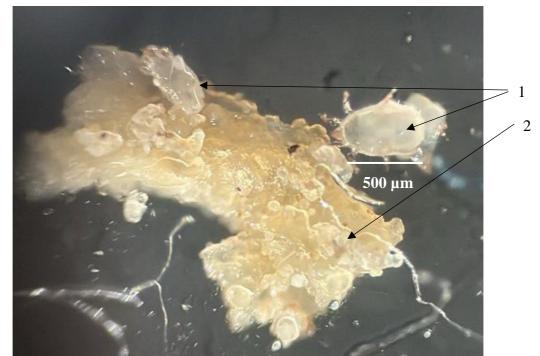


Figure 3 Slice of Alpine cheese rind aged 12 months: 1 – Acarus siro, 2 – cheese rind.



Figure 4 Colonization beginning of mite *A. siro* on the rind surface of Alpine cheese: 1 - area of mycelium consumption of moulds.







a b **Figure 5** Cheese heads a – Alpine aged 12 months, b - Yoghurt aged 18 months, which are fully colonized by population of mite *A. Siro.*



a b **Figure 6** Washed cheese heads: a – Alpine aged 12 months, b – Yoghurt aged 18 months.

The accumulation of fine crumbs, which are mites, their remains, and excrement on the entire surface of the rind is observed during the ripening process of the cheese heads with the use of mites *A. siro* (Fig. 5a, b). Thus, the washing procedure of the heads is aimed not only at reducing the number of mites but also at localizing them on the cheese rind surface (Fig. 6a, b).

The ripening process of cheeses with mites gives them specific taste characteristics. It may be taken into account as an independent criterion of authenticity and in combination with other indicators, for example, individual fractions of caseins for the origin of cheese to be determined **[47]**. The detection and identification of mites *A*. *siro* on the rind surface of hard cheeses in the future involve the development of a rapid determination method of their number for the population growth to be controlled **[48]**. One of the possible control solutions of the mite population number on the cheese head surface may be the development of a mathematical model that will make it possible to predict the interval and frequency of head washing for each cheese, with the temperature and humidity conditions of the ripening chamber taken into account **[49]**.





CONCLUSION

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Alpine and Yoghurt craft hard cheeses made from raw goat milk have individual characteristics in terms of the number of QMAFAnM, moulds, and yeasts, and they depend on their ripening period. The majority of microorganisms, which were isolated from both cheeses, are non-starter lactic acid bacteria: in Alpine cheese - *Lactococcus lactis* and *Lactobacillus plantarum*, in Yoghurt cheese - *Lactococcus lactis* and *Lactobacillus plantarum*, in Yoghurt cheese - *Lactococcus lactis* and *Lactobacillus paracasei*. During the ripening period, a small amount of staphylococci were isolated from Alpine cheese: *S. simulans, S. equorum*; enterococci: *E. faecalis and E. durans*, as well as *Escherichia coli* and *Bacillus cereus*. A small amount of staphylococci and enterococci, in particular, *S. equorum* and *E. faecalis*, as well as *E. coli*, *Raoultella ornithinolytica, Providencia stuarti* and *Kurthia gibsonii*, were detected in Yoghurt cheese at different ripening periods. From the age of 5-6 months, the rind surface of Alpine and Yoghurt craft hard cheeses is colonized by the mycoid mite *Acarus siro* (Linnaeus, 1758), which participates in their ripening and provides the original taste characteristics. The study results indicate the proper quality and safety of craft hard cheeses made from raw goat milk. They can be used as a criterion for their authenticity, as well as for development of the population control method of acarid mites at different ripening periods of hard cheeses.

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