CHAPTER 2

Monitoring of acarid mite populations in craft hard goat cheeses during ripening

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Abstract

The expansion of the assortment of artisanal hard cheeses ripened with the involvement of acarid mites on the food market necessitates the assessment of their safety. The use of mites contributes to the development of distinctive aroma and flavor profiles in mature and aged hard goat cheeses and requires the implementation of effective methods for regulating their population density. Four batches of cheeses produced from unpasteurized goat milk were manufactured at the Zhuravka Eco Farm (Kyiv region, Ukraine): Caciotta, Canestrato, Alpine, and Yogurt-type cheeses. Cheese heads at different levels of infestation with *Acarus siro* were selected for the study, and a methodology for monitoring mite density was developed. The most effective and practical method was the determination of mite density using a graduated grid and light microscopy, enabling the counting of mites at all developmental stages, including eggs. For unwashed cheeses, mite density is recommended to be determined in the surface powder accumulated on the rind and expressed per unit mass. For washed cheeses, mite numbers should be determined in rind scrapings and expressed per unit area. The number of *Acarus siro* mites in the "brown powder" accumulated on the surface of artisanal hard goat cheese heads ranged from 12.8 to 43.5 per 0.01 g. Insufficient control of mite population density during ripening resulted initially in superficial damage and subsequently in deep core deterioration, rendering the cheeses unsuitable for storage and consumption.

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Washing cheese heads with running water reduced mite density on the rind surface to 5.2–10.4/cm^2. Three months after washing, mite density reached 1.9–2.1/cm^2, while egg counts ranged from 9.9 to 11.7/cm^2 of rind. Treatment of hard goat cheese heads intended for sale with linseed oil resulted in complete elimination of *Acarus siro* mites and their eggs on the rind surface; however, it promoted secondary mold growth. Overall, the use of mites in the ripening of artisanal hard goat cheeses ensures the formation of distinctive sensory characteristics, which may serve as a criterion of product authenticity. Further research should focus on determining the aromatic composition of hard goat cheeses ripened with the participation of acarid mites.

Keywords

Collar pests, hard goat cheeses, craft production, method of counting mite density.

2.1 Introduction

One of the most common collar pests worldwide and in Ukraine is the acarid mite, in particular *Acarus siro* L. (Linnaeus, 1758), *genus Acarus* (Linnaeus, 1758), family *Acaridae* (Latreille, 1802). A feature of acarid mites is the ability of females to lay up to 800 eggs during their lifetime, and the development cycle lasts nine days. At the same time, the resistance of mites in the external environment is due to the ability of eggs to withstand a temperature of 0°C for several months. Mites belonging to the taxa *Acarina*: *Acaridae* are mainly characterized by small sizes and, when favorable conditions are created, which include optimal temperature and humidity, they multiply rapidly, which, without proper control of their population, leads to the appearance of various diseases in consumers and causes losses due to food losses.

Due to its physiological characteristics, *A. siro* is not picky about environmental conditions and is found in the temperate climate zone [1]. This species of mite often dominate other arthropod species in feed and food throughout the entire farm-to-fork chain. The spread of collar pests, such as *Acaridia* mites, to food products mainly occurs from the environment. They are found in warehouses, retail chains, ripening chambers for cheese and meat products, and in residential and non-residential premises. This mite often infests cereals, meat products, hard cheeses, various teas, dried fruits, and spices [2].

Damage caused by acarid mites to grain feeds often results in the loss of valuable seed material and feed suitability. Severe infestation of feeds and food products with mites causes unpleasant odors that consumers pay attention to.

Mites that breed in food products during storage can cause a variety of harm [3] in different ways:

– through direct contamination of various goods in the food chain;

– symbiotic relationships with microorganisms, including fungi and bacteria, which can increase the spoilage of a food product and lead to its loss of suitability for human consumption;

– production of allergens that may affect the health of the consumer.

As for allergens of mite origin in food products, they pose the greatest danger, since they induce IgE-mediated allergic reactions in humans, including anaphylactic manifestations. Such allergens are produced by four species of mites that infest food products. These include the following species: the American dust mite, *Dermatophagoides farinae* (Hughes) (*Acarina: Pyroglyphidae*), the scaly grain mite, *Suidasia sp. prob. pontifica* (Oudemans) (*Acarina: Suidasiidae*), the scaly mite of the genus *Saccharide*, *Thyreophagus entomophagus* (Portus & Gomez) (*Acarina: Acaridae*), and the mold mite, *Tyrophagus putrescentiae* (Schrank) (*Acarina: Acaridae*) [4].

These allergens can enter the human body not only directly – with food products infested with mites, but also indirectly through the respiratory system, by contact through the skin and mucous membranes, which provokes complications of allergic asthma, allergic dermatitis, intestinal and pulmonary allergies.

The scale of the spread of mites as grain pests and allergens is extremely large. Using data from the Czech Republic alone, where 514 grain silos were surveyed, 4 risk classes of grain mite allergy were identified, depending on the mite density: safe – 0 mites/g of grain; low – up to 1 mite/g of grain; high – 1–5 mites/g of grain; acute danger – more than 5 mites/g of grain.

At the same time, among the collar pests of grain crops, mites dominated, and their number reached 92%. Of these, 60% of mites belonged to species capable of producing allergens. The most common pests included *Acarus siro*, *A. faris*, *Tyrophagus putrescentiae* and *Lepidoglyphus destructor*. Accordingly, the obtained grain samples were classified according to allergic hazard as follows: safe – 37%, low – 53%, high – 6% and acute hazard – 4% [5].

As for the role of collar pests, in particular acarid mites, in the production of hard cheeses, two opposing aspects can be considered:

– harmful effects due to damage to cheeses during the ripening (storage) process;

– the beneficial role of mites in the ripening of hard cheeses is associated with the formation of exquisite sensory characteristics.

Regarding the harmful effects of mites, damage to cheeses can cause up to 25% weight loss in cheese wheels, especially in ripening chambers. Studies conducted in Spain have shown that damage by mites to Cabrales blue cheese, which ripens in natural Asturian caves, deteriorates its presentation. This is facilitated by the conditions in natural caves, characterized by temperature fluctuations of 10–15°C and relative humidity over 90%. This temperature-humidity regime ensures the ripening of the cheese and, at the same time, corresponds to environmental parameters favorable for the reproduction and development of mites.

Mite damage is often a feature of long-matured cheeses, and the deterioration of Pecorino cheese has prompted the search for and use of cheese treatments, including ozonation during the ripening process [6]. For the fumigation of food products aimed at reducing mite infestation, liquid smoke, xanthan gum [7], sulphuryl fluoride, and methyl bromide are also employed, although the latter is subject to restrictions under the Montreal Protocol [8].

It is worth emphasizing the useful function of mites, which is inherent in the ripening process of refined elite hard cheeses. This is primarily due to the presence of opisthonotal glands in *Astigmata* mites, which are capable of producing a number of sensory compounds, in particular monoterpenes, cyclic and aliphatic volatile nitrogen-containing compounds, which give such cheeses their uniqueness. Moreover, these substances act as pheromones and fungicides. These include the German Milbenkäse cheese and the French Mimolette, Artisou, Laguiole, Salers and Cantal vieux cheeses. Most of these cheeses were characterized by taste and aroma compositions inherent in the mites *Tyrolichus casei* and *Acarus siro* L. It is believed that the mites *T. casei* produce neral, a compound that is responsible for the lemon flavor of cheeses and is concentrated in the rind, which gives them authenticity. When eating the cheese rind, consumers can feel this taste and enjoy it [9].

However, the literature reports only a few studies on the production, quality control, and safety of hard cheeses ripened with mite involvement. On the other hand, the expansion of the market for craft hard goat cheeses ripened with the participation of mites and the increasing popularity of gastronomic tourism require the development of criteria for the authenticity of such cheeses, as well as control of the number of mites dur ing their ripening.

Therefore, the purpose of this work was to develop a method for controlling the number of acarid mites during the ripening of craft hard cheeses made from unpasteurized goat milk, which are produced in Ukraine.

2.2 Damage to hard cheeses by mites during ripening or storage

The attractiveness of food and feed for acarid mites has long been known. In addition, these mites can feed on the mycelium of mold fungi, which also infect food products, particularly cheeses. *Acarid* mites feed on a significant number of fungal species, including fungi used in the pharmaceutical industry and agriculture. Such fungi include many types of mold and yeast, in particular *Fusarium*, *Aspergillus*, *Candida*, *Hyphopichia*, *Penicillium*, *Rhizophus*, and *Trichophyton*. *Acarid* mites feed on spores and hyphae of fungi. This, in turn, ensures the spread of fungi by mites in the external environment. It has been proven that fungal spores are carried on the bodies of mites and are also excreted with their excrement.

Fungi, in turn, can cause cheese spoilage, creating both visible and invisible defects in the cheese wheels. The growth of fungi on the surface of the cheese rind causes the formation of metabolites that have unpleasant aroma, taste and texture, which is perceived by consumers as a deterioration in quality. Among the fungi that often contaminate hard cheeses and cause changes in their sensory properties, several genera are the most important, including *Penicillium*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor* and *Trichoderma*. In addition to cheese spoilage, molds can pose a danger to cheeses by producing and accumulating mycotoxins. The risk of mycotoxin accumulation in cheeses increases significantly when cheeses are contaminated with fungi of the genera *Aspergillus* and *Penicillium*. The main causes of fungal contamination of hard cheeses during ripening are the contact of the wheels with contaminated air, as well as the ripening chamber equipment [10].

The nutritional value of fungi for acarid mites lies in the presence of cell walls containing chitin, as well as intracellular components such as trehalose. When consuming fungal hyphae, the mite digestive system produces a specific enzyme, trehalase, which is able to break down the contents of the hyphae, ensuring the digestion and assimilation of fungi. It has been proven that different species of acarid mites have individual preferences for the species composition of mold fungi [11].

Thus, hard cheeses are characterized by a double attraction for mites:

– due to mold fungi that grow on them and represent a source of nutrition;

– due to the consumption of hard cheese itself.

In the production of craft hard cheeses, the rind is usually not covered with protective films or paraffin and is an important factor that determines the attractiveness for consumers. The rind of hard cheeses forms a special taste, and its appearance is responsible for the authenticity of the product. A complex multi-species microbiome is created on the surface of the rind of hard cheeses, which spontaneously gets onto the surface of the cheese from both raw materials and the air of the ripening chambers, as well as equipment and tools used in the cheesemaking process. It is believed that the rind microbiome is significantly different from the core of the cheese wheels, due to differences in physicochemical characteristics at the surface and inside the wheels.

A feature of the environment on the surface of the cheese rind is a sufficient amount of oxygen, which causes the growth and reproduction of mold fungi, which belong to strict aerobes [12]. The formation of the rind microbiome of hard cheeses is significantly influenced by physicochemical characteristics, in particular pH, humidity and air temperature, as well as the humidity of the cheese wheels, cheese-making technology, type, origin and blend of milk, and the quality and sanitary conditions of production.

Among the factors that determine the intensity of the spread, growth and reproduction of mold fungi on the surface of the rind of hard cheeses, moisture is in the first place. With a decrease in moisture in the environment, fungi belonging to the genus *Debaryomyces* grow better, as well as filamentous fungi of the genera *Aspergillus* and *Scopulariopsis.* They are the most common in cheeses with natural rind. They are characterized by resistance to the increase in the content of table salt, which is observed during the ripening of old hard cheeses [13].

Analysis of the abundance of molds and yeasts in craft hard cheeses made from unpasteurized goat milk showed their presence at all stages of ripening. Although some differences in their abundance and accumulation dynamics were noted between the ripening periods of Caciotta and Canestrato cheeses [14].

In young cheeses aged up to 3 months, intensive mite colonization of the wheels was not observed, which is probably due to incomplete colonization of the rind surface by mold fungi (**Fig. 2.1**).

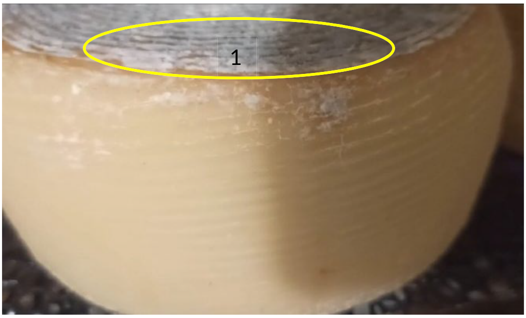

Fig. 2.1 Wheel of young hard goat cheese Caciotta: 1 – the upper surface is covered with the mycelium of mold fungi

No less important factor is the relationship between different types of micro- and macrobiota in the chambers for ripening hard cheeses. As a rule, in such chambers, the penetration of mites onto the surface of the cheese wheels occurs naturally.

The mites fall off the cheese wheels located on the upper shelves of the ripening chamber and thus fall onto the surface of the cheese wheels located on the lower shelves. At the same time, during the ripening of craft hard goat cheeses, colonization of the wheels by mites is observed only if there is a sufficient amount of fungal mycelium on the upper, lower and side surfaces.

Accordingly, acarid mites begin to appear on the surface of only those cheese wheels that are covered with continuous mycelium of mold fungi (**Fig. 2.2**).

Fig. 2.2 Wheel of Alpine hard goat cheese: 1 – area of the rind cleared of mold fungi following colonization by *A. siro* mites

During this period, they begin to consume the mushrooms themselves, cleaning the cheese rind from their mycelium. Outwardly, this looks like the appearance of separate areas on the rind of cheese wheels, free from the mycelium of mold fungi. Such neutralization of mold fungi on the surface of the rind of hard cheeses has an important meaning, which is as follows:

– no need to specifically clean cheese wheels to remove mold fungi;

– the participation of mites in creating different intensities of amber color of the peel and giving the wheels an attractive presentation;

– partial destruction of the cheese rind, which affects moisture loss from the wheels and ensures the uniqueness of the texture, taste, and aroma during the ripening process.

As the ripening period of hard cheeses increases, acarid mites spread over the entire surface of the cheese rind and their presence can be identified by the appearance of a "greyish powder", which is a mixture of cheese residues, dead and live mites and their excrement (**Fig. 2.3**).

In this case, mites primarily eat the mycelium of fungi growing on the upper surface of the rind of hard cheese wheels, and then on the side and bottom surfaces. Later, when the mites have consumed all the mycelium of fungi that has grown on the surface of the hard cheese wheel, they begin to consume the rind and the cheese itself, and a "brown powder" accumulates on its surface (**Fig. 2.4**). At this stage, fungal growth is practically not observed on the surface of the hard cheese wheels, and the entire wheel is covered with a continuous layer of "brown powder".

Fig. 2.3 Yoghurt cheese wheel with the upper surface completely populated by the *A. siro* mite:
1 – section of the lateral surface of the cheese wheel with remains of fungal mycelium;
2 – upper surface of the cheese wheel with "greyish powder"

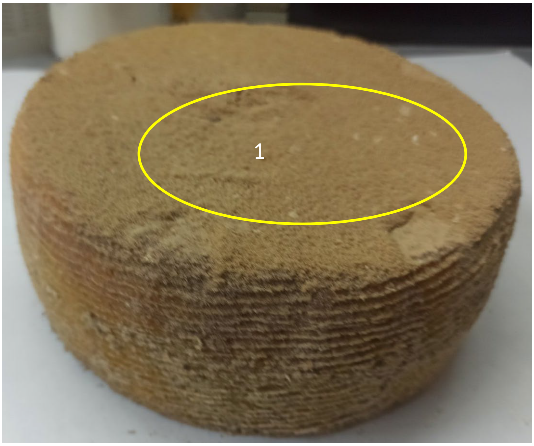

Fig. 2.4 The wheel of Yoghurt cheese is completely inhabited by the mite *A. siro*, upper and lateral surfaces: 1 – "brown powder" on the surface of the wheel of cheese

The largest layer of such powder is usually observed on the upper surface of the cheese wheels, while on the lower and side surfaces a brown, powdery mass crumbles and is clearly visible on the racks and drainage mats where the cheese wheels are located in the ripening chambers.

If at this stage methods are not used to reduce the number of mites on the surface of cheese wheels, their density increases, and they continue to eat the cheese, creating more pronounced defects on its surface.

This manifests itself in the form of damage to the peel of varying degrees (**Fig. 2.5**), and subsequently to the core, which causes the rejection of such wheels with subsequent disposal.

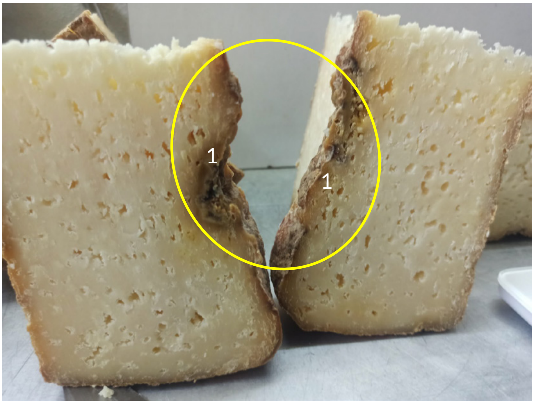

Fig. 2.5 A slice of hard mature Canestrato cheese affected by a mite: 1 – superficial damage to the wheel of hard cheese by the mite *A. siro*

The greatest damage to the wheels of hard cheeses by acarid mites, to which *A. siro* belongs, is observed on their upper surface, which is associated with the cylindrical shape of the wheels and the peculiarities of their placement in the ripening chamber.

Particularly significant damage caused by mites in hard cheeses is associated with their local penetration through the rind. Such penetration of mites into hard cheeses is visually characterized by defects and holes on the surface of the wheel and on its lateral areas.

These holes can be placed randomly on the entire surface of the cheese wheels, their number depends on the density of the mite population, and the size can range from a few mm to 2–3 cm or more (**Fig. 2.6**). The shape of the holes made by mites in the cheese rind can be round, oval, and most often irregular. Such holes can be of different depths:

– superficial (up to 2–5 mm);
– deep penetrating wheels.

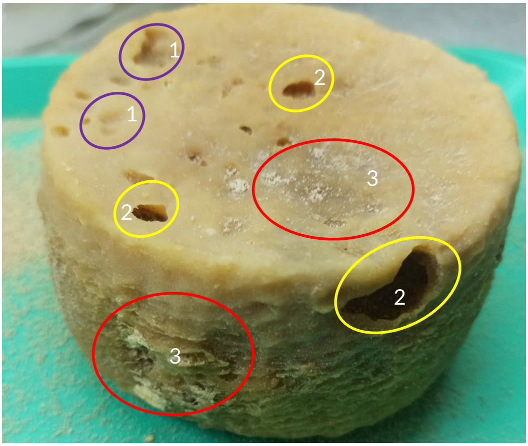

Fig. 2.6 Appearance of the wheel of hard goat Yoghurt cheese damaged by the *A. siro* mite: 1 – superficial; 2 – deep holes penetrating the core; 3 – deformation of the wheel surface

While the formation of surface holes and defects on the surface of the rind of hard cheese wheels is not harmful and even desirable, the penetration of mites into the core most often leads to spoilage of the wheels.

The formation of deep, penetrating holes in the rind of hard craft goat cheeses, in turn, causes deformation of its surface in various areas and ultimately leads to the loss of not only its presentation, but also its quality.

The surface of such a wheel of cheese resembles a "lunar landscape" and looks as if it has melted, which is explained by the varying intensity of mites eating the rind.

Sometimes there is local penetration of mites into the core of the cheese, and the wheel does not look damaged externally, but on the cut, it has characteristic traces of internal damage by the *A. siro* mite (**Fig. 2.7**).

In the early stages of mite penetration into the cheese core, changes in texture and color can be observed. Channels of various diameters made by mites in the cheese are visible, but the greyish powdery mass is not yet present (**Fig. 2.7**).

At a late stage of infestation of hard cheeses by mites, a crater-like area filled with greyish powder is clearly visible on the cut. This area is clearly demarcated from the texture of the undamaged core, which is evident in the example of old-ripened goat cheese, Caciotta (**Fig. 2.7**).

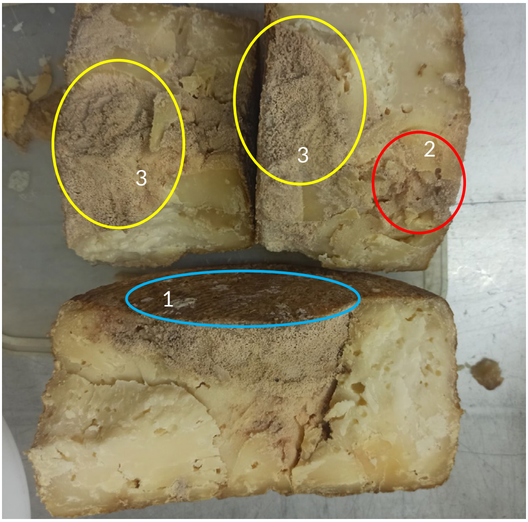

Fig. 2.7 Wheels of hard old-ripened goat cheese Caciotta in cross-section with varying degrees of damage to the core by the *A. siro* mite: 1 – internal damage to the core of hard cheese by the mite (the surface of the wheel has no visible defects on the outside); 2 – early stage of damage to the cheese core by the mite; 3 – late stage of damage to the cheese core by the mite

At the same time, in the core of such a wheel, a powdery mass of greyish color contains mites of various stages of development, their excrement and cheese residues.

When cut, such cheese has a specific odor, which is formed as a result of the vital activity of mites, as well as microbial processes accompanied by the breakdown of components of damaged cheese, excrement and dead mites. This odor often resembles ammonia and can vary in intensity depending on the degree of damage and the size of the hard cheese wheels.

Moreover, cheese wheels with such damage by core mites are not suitable for further storage or ripening and must be disposed of.

Many species of mites are common in Ukraine, which can infect food products and cheeses in particular. Analysis of the species affiliation of mites in individual regions showed that the dominant species of mites include *A. siro* and *T. putrescentiae*. Slightly less common are *Gl. burchanensis*, *Gl. destructor*, *Gl. domesticus*, *T. perniciosus*, *N. sokolovi*, *N. rhizoglyphoides*, *Ch. arcuatus*, *G. fusca*, *Ct. plumiger*, *T. casei*, *Al. ovatus* and others. This explains the fact that the most common mites are *A. siro* and *T. putrescentiae*, which are isolated from food and feed produced and stored at various enterprises in Ukraine. As for other species of mites, *Gl. burchanensis*, *N. sokolovi* and *Gl. destructor* are less common as collar pests [2].

The entry of these and other types of mites into cheese ripening chambers and into the cheeses themselves is associated with their spread primarily in feed for ruminants, in particular goats. This is evidenced by analyses of feed samples taken from feeders and the floor of livestock premises. Such a spread of mites from livestock premises to the cheese manufacturing and ripening area is less typical for industrial production, since milk production and its processing are separated. However, for craft production, the spread of mites from feed and livestock premises to the cheese factory is quite possible, since farmers keep not only a herd of goats, sheep or other dairy animals, but also farm poultry. Mites can also be found in birdhouses where poultry are kept, fed, and watered. At the same time, milk production, its processing and sale of cheeses and other dairy products are concentrated on one farm, which significantly shortens the path of mites in the chain from "field to table".

Another factor in favor of the significant distribution of mites is their belonging to hygro- and thermophilic species, which allows them to tolerate adverse environmental conditions, survive, reproduce and spread. It has been established that such mites are able to form hypopuses in conditions of reduced temperature and relative humidity [2].

The prevalence of different mite species during cheese ripening has also been found in other studies, in particular, samples of Cabrales cheese ripened in caves contained representatives of *Acarus farris* (Oudemans) and *Tyrophagus neiswanderi* (Johnston and Bruce). This study notes that *A. farris* is not only the most common species, but also responsible for a significant part of the damage to Cabrales cheese [15]. Moreover, this mite can infect a wide range of other cheeses. It is believed that during cheese ripening, fungal growth occurs, which in turn stimulates an increase in the density of the mite population to a high level, which ultimately causes significant economic losses to producers. The prevalence of mite on Cabrales cheese causes a decrease in its marketability [16], which occurs due to the loss of weight of the cheese wheels, as well as an increase in the cost of workers involved in cleaning the wheels. The weight loss of cheese wheels can reach 2.5% and about 2% of additional costs are added to the cost of cheese due to the need to clean them from mites regularly. Before being sold to consumers, cheeses on which traces of mite activity are found require mandatory cleaning and control of remnants of collar pests. Such cheeses are recommended to be stored in refrigerators at a temperature of 2–4°C.

2.3 Beneficial effects of mites during the ripening of hard cheeses

In addition to the harmful effects of mites on cheeses, which are considered from the perspective of pests and the need to destroy them, there is a beneficial and desirable effect of certain species of mites during the ripening of hard cheeses. It has been found that mites in hard cheeses can give them specific and desirable sensory properties and thus make them unique and refined products. Cheeses that ripen with the participation of mites include quite well-known brands, in particular the French Mimolette and the German Milbenkäse [17].

Given that a large number of hard cheeses are produced worldwide and their range on the food market is constantly expanding, there is growing interest among scientists and producers in assessing their quality and safety, and in establishing criteria for authenticity. Some studies describe in considerable detail the production procedures for cheeses ripened with mite involvement [18].

The use of mites in cheese ripening has a history of over several hundred years. Two species of mites are used to make these cheeses, namely *Tyrolichus casei* (Oudemans) (*Tyrophagus casei* is a synonym of *Tyrolichus casei)*, which is specifically inoculated during the ripening of Würchwitzer Mühlenkäse cheese, and *Acarus siro* L., which is introduced into Mimolette cheese. The traditional Würchwitzer Mühlenkäse cheese, which has been certified as a "slow food" in Germany, has been produced since the Middle Ages, and its name comes from the region's ancient name.

This ancient technology of making cheeses ripened with mite involvement indicates that mites impart specific sensory properties that appeal to consumers who appreciate original cheeses. As a result of the analysis, it was concluded that ripening cheeses with these mites gives them a lemon flavor, which is formed due to the secretion of cheese mites [17].

The modern method of producing such cheeses is quite simple. Fresh sour milk cheese is made from cow's milk by fermentation with lactic acid bacteria. After that, caraway seeds and elderflowers are added to the cheese, elongated cylindrical loaves are formed and placed in a box with a colony of mites, where it ripens [19]. Such cheeses ripened with the participation of mites are unique and are in demand among gourmets.

Despite the fact that legislative documents are almost not developed for such products, the risks of mites and their waste products entering the body of consumers should be taken into account [18].

An analysis of the legislative framework for the control of mites in cheeses showed that these documents do not regulate the control of the species composition of mites during the ripening process of cheeses, but only determine their permissible number per unit of product.

The European Food Safety Authority (EFSA) regulations do not prohibit the use of live animals (including mites) in food for human consumption, but rather allow it [20]. The Codex Alimentarius [21] also does not set rules for the detection and control of mite numbers in cheese. However, legislation in various countries imposes restrictions on the import of cheeses with mites, in particular, the regulations of the United States of America [22]. Since 1940, the FDA has imposed a limit of 6 mites per square inch of cheese. Some samples of imported Mimolet cheese contained 4000 mites/square inch. In addition, the US Food and Drug Administration (FDA) provides for the use of regulatory action criteria for contaminants and extraneous materials to assess food adulteration. The criteria are divided into three categories: health hazards, sanitation indicators, and natural or unavoidable defects. Of particular importance is the category of human health hazards, which includes criteria for physical, chemical and microbiological hazards associated with contamination and the presence of extraneous contaminants. The category of consumer health hazards includes criteria for HACCP hazards (Hazard Analysis and Critical Control Points) and factors contributing to HACCP compliance. As for the sanitation indicators category, it includes criteria for visible undesirable contaminants, in particular, the penetration and infestation of commensal pests, which are associated with violations of sanitation requirements in food processing and storage facilities.

As for Brazilian legislation, Mimolet cheese, which is ripened with mites, is allowed to be imported, but its own production of a similar type of cheese is not provided for [23]. Current regulations only establish a maximum allowable number of mites in cheese, which should not exceed 25 dead mites per 225 g of cheese or 5 mite bodies on the surface of the cheese with an area of 2.5 cm^2 and a depth of up to 0.6 cm [23]. As for Canadian legislation, it is somewhat similar to Brazilian legislation and provides for the selection of three cheeses for analysis, while Brazilian legislation recommends taking only one sample [24].

The legislative framework of Ukraine, on the territory of which craft hard goat cheeses ripening with the participation of mites are produced, does not contain regulatory documents for such products, and the presence of mites on the surface of cheeses is assessed as a violation of storage conditions and damage by collar pests. Moreover, the "Methodological Guidelines on Compliance with Legislation on Food Safety and Certain Quality Indicators of Food Products at Primary Milk Production Facilities and/or Small-Scale Milk Processing Facilities" were developed and approved by the Minister of Agrarian Policy and Food of Ukraine on 1 May 2025. This guideline includes a separate paragraph 6 "Measures to control cheese mites". This paragraph states that if the cheese ageing room is infected with mites, all infected cheese, packaging and other materials must be removed from the room. After that, it is recommended to clean the room thoroughly, especially the ceiling, walls, floor, racks, shelf supports, and the shelves themselves. To eliminate mites, vacuum packaging of cheese, washing it with hydrogen peroxide, regular washing and cleaning of cheese, as well as treatment of the legs of racks with "diatomaceous earth" are recommended. All workers who have come into contact with infected cheese are allowed to perform any procedures with other uninfected cheeses only after thorough washing and changing into clean work clothes.

Regarding the use of effective means of neutralizing mites, the use of diatomaceous earth is considered the most promising not only in the food industry, but also in the production of feed. However, the search for effective acaricides is not limited to this. Testing of inert materials, in particular zeolite and kaolin for neutralizing the cheese mite *Tyrophagus putrescentiae* (Schrank) (*Astigmata*: *Acaridae*) on wheat showed their acaricidal effect. When these preparations were applied to wheat grains at doses of 100, 500 and 1000 ppm, the death of *T. putrescentiae* was detected after 3 and 7 days. This study shows the superiority of zeolite over kaolin in terms of acaricidal activity. This is evidenced by the death of 100% of adult mites found in wheat grains treated with zeolite at a concentration of 1000 ppm, already 3 days after treatment. Treatment of wheat grain with kaolin at all doses was less effective in inactivating *T. putrescentiae* [25].

Similar information on cheese mites is provided by the Codex Alimentarius, which recommends the use of various methods of eliminating mites, including chemical fumigation, biological preparations, anaerobic or refrigerated food storage conditions [26].

Thus, most Ukrainian legislative documents and international requirements treat the presence of mites in cheeses as pests rather than as a component of the biome. This is primarily due to sanitary and hygienic requirements for the production, storage, and sale of hard cheeses. Although the absence of viable or dead mites on the surface of hard cheeses does not guarantee the absence of their waste products. Therefore, another important fact must be taken into account when analyzing cheeses ripening with the participation of mites is hidden infestation by mites. Since cheese can be processed or stored in conditions unsuitable for the reproduction of mites, their number can be reduced to a minimum or not detected at all. However, their waste products can be found on the surface of cheese, which can potentially cause allergies in a certain category of consumers [15].

In addition, the identification and determination of the mite population density on the surface of hard cheeses, which are characterized by a long ripening period and do not allow the wheels to be covered with protective films or paraffin, may be important to producers of these unique products themselves. Detection of mites on the surface of the rind of hard cheeses will allow for effective regulation of their density, timely prevention of damage to the wheels and loss of marketability, as well as informing consumers about possible health risks when consuming products made with the participation of mites.

2.4 Development of a method for controlling the number of mites during the ripening of craft hard goat cheeses

2.4.1 Identification of mites in hard goat cheeses

Isolation and identification of mites was carried out in the laboratory of the Department of Animal and Food Hygiene named after A. K. Skorokhodko of the National University of Life Resources and Environmental Sciences of Ukraine and in the laboratory of the Department of Zoology of Uzhhorod National University, Ukraine.

To determine the density of mite infestation of hard craft goat cheeses, three names were used: Yoghurt with a ripening period of 6 months and 18 months, Canestrato with a ripening period of 18 months, and Caciotta with a ripening period of 20 months. The cheeses were made according to the recipe described earlier by us [14, 27] under the conditions of the Eco farm "Zhuravka" in the Kyiv region (Ukraine) from the milk of one herd of Anglo-Nubian goats.

The following characteristics were used to identify and differentiate *Acarus siro:* *A. siro* has a slit on the posterior part of its body between the 2nd and 3rd pairs of legs. Male *A. siro* has tarsal and anal suckers on its body, as well as a distinct hook-like extension on the segments of the first pair of legs. Females have a claw at the end of each leg [28].

Cheeses were used to develop a method for controlling mite density during ripening and storage. During cheese production, mites were introduced to the surface of the cheese rind naturally from the ripening chamber; no special inoculation was performed.

The colonization of the surface of hard goat cheeses with mites depended on the intensity of mold growth. In the future, the ripening process of cheese wheels took place with the participation of mites, and the wheels of experimental cheese samples were not subjected to treatment.

In the example of Yoghurt cheese, superficial damage to the rind of the wheels is clearly visible, especially after washing with running water. This indicates the presence of a viable colony of *A. siro* mites (**Fig. 2.8**).

Ripening of craft hard goat cheeses with the participation of cheese mites *A. siro* provided a gradual increase in the intensity of the rind color from light yellow to dark amber. The increase in the intensity of the rind color of hard cheeses directly depended on their age.

Fig. 2.8 Surface of washed wheels of Yoghurt cheese aged 4 months, ripened with the participation of the *A. siro* mite after washing with water: 1 – superficial damage to the rind by the mite

Thus, wheels of Yoghurt cheese aged 4 months had a light amber rind with traces of superficial damage to the rind by mites on the surface (**Fig. 2.8**), and in cheeses aged 6 months the rind color noticeably changed to amber (**Fig. 2.9**). In the cross section, wheels of such cheese had a clear demarcation of the texture from the rind.

Fig. 2.9 Wheel of washed Yoghurt cheese aged 6 months, ripening with the participation of the *A. siro* mite after washing with water on the cut: 1 – texture of hard cheese on the cut with a clear demarcation of the rind

2.4.2 Methodology for determining the density of mites on the surface of cheese wheels

To count mites, the 15 × 15 grid of 1 mm × 1 mm squares, 96° pharmacopoeial ethyl alcohol, microscope slides and coverslips, a scalpel for taking scrapings from the surface of cheese rinds, and an OHAUS NV212 (OHAUS CORPORATION, USA) scale with an accuracy of 0.01 g and a microscope MBS 9 (Ukraine), which allows for observation in both artificial and natural lighting were used.

To sample the powder on the surface of the cheese wheels or perform scrapings, the cheese wheel was conditionally divided into 4 sectors, which were further divided into 3 sub-sectors and 3 samples of "brown powder" were taken from each, which was located on the upper, lower and lateral surfaces of the wheel (**Fig. 2.10**). The sampling scheme from the upper and lower surfaces of the wheel is identical. From the lateral surface of the cheese wheels, 3 samples were also taken diagonally, covering the lower, middle, and upper thirds of the wheel's height. Thus, in this case, 12 samples can be taken from each surface of the wheels of hard cylindrical cheeses for an objective assessment of the density of acarid mites.

To analyze mites, a 0.01 g sample of "brown powder" was taken, placed on a glass slide, and then a drop of 96° ethyl alcohol was applied to immobilize the mites and facilitate counting, and covered with a coverslip.

To analyze the density of mites in washed cheeses, or cheeses washed and treated with linseed oil, samples were taken from the surface of the rind by scraping with a scalpel with an area of 1 cm × 1 cm. In this case, the mass of the sample ranged from 0.01 to 0.03 g.

Some regulatory documents state that a 6 mm-deep slice of cheese rind should be selected [23], but in this study, this method was ineffective because cheese contains significant amounts of fat and protein, which interfere with microscopy. In addition, it was not detected *A. siro* mites in cheese slices, they were located on its surface, which is due to their need for oxygen for respiration.

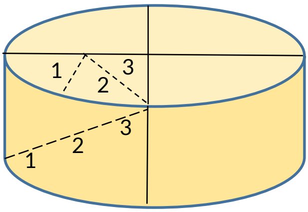

Fig. 2.10 Sampling scheme for analysis of mite density in cylindrical cheeses

The layout of the graduation grid, object and cover glass is shown in **Fig. 2.11**.

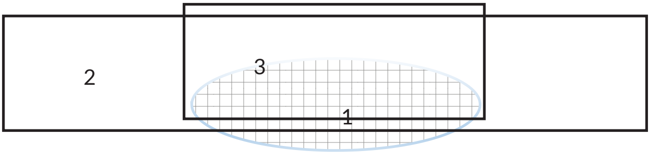

Fig. 2.11 Scheme of microscopy of cheese samples for counting the number of mites: 1 – calibration grid; 2 – slide; 3 – cover glass

For mite counting, the use of a microscope slide is not essential; however, in its absence, the mesh must be cleaned of any remaining cheese crumbs and mites, and degreased, after each count.

The slide with the cheese sample was placed on a calibration grid (**Fig. 2.12**) and microscopy was performed at a magnification of ×8.

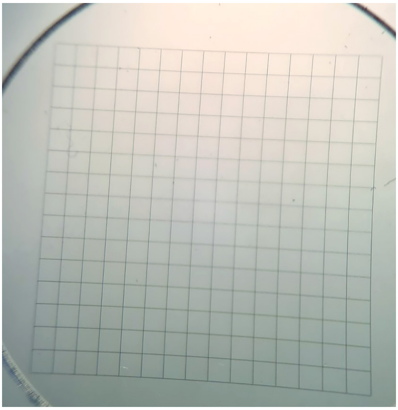

Fig. 2.12 View of the calibration grid for counting mites in the field of view of the microscope (magnification ×8)

The method of counting immobilized mites is as follows: in the first row, all mites were counted from left to right, as well as all those located in the center, on the upper and lower lines of the horizontal rows of the grid. In the second and all subsequent rows, all mites located in the center and on the lower line of the row were counted (**Fig. 2.13**). The counting direction for odd lines was from left to right, for even lines – from right to left.

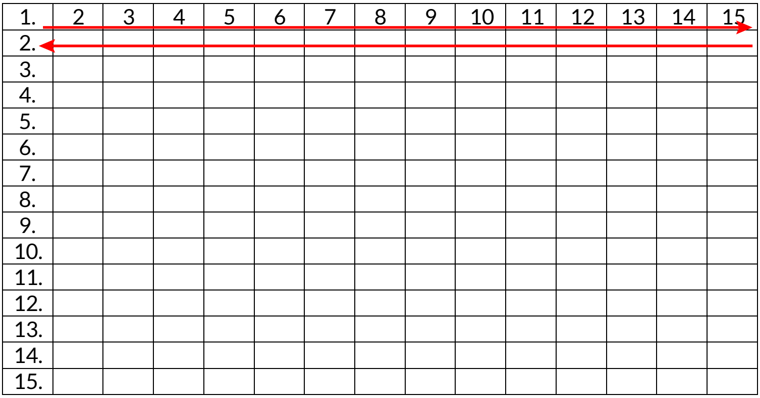

Fig. 2.13 Scheme of counting mites in horizontal rows of the calibration grid

The microscope lens clearly shows the lines of the graduation grid at different magnifications, on which immobilized mites at different developmental stages are placed. They are located in the center and on the lines of the grid cells. Around the mites, a powdery mass is visible, taken from the surface of the cheese wheels (**Fig. 2.14**).

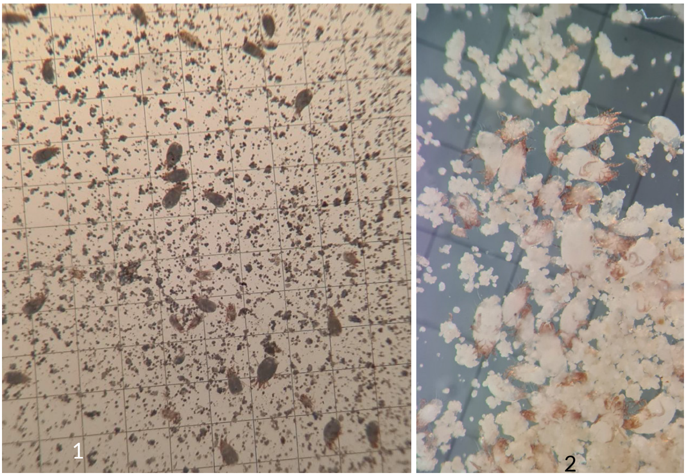

Fig. 2.14 Fragment of a calibration grid with immobilized mites *A. siro* under a microscope: 1 – sample 0.01 g; 2 – sample 0.02 g

For ease of counting, it is possible to select a mass of "brown powder" from cheese wheels that will allow an objective assessment of the density of mites using a calibration grid. In this study, a mass of 0.01 g was the most acceptable, while a weight of 0.02 g or more caused the accumulation of mites and cheese grains in the field of view of the calibration grid, which made it difficult to count them.

Counting the density of *A. siro* mites in the field of view of a microscope using a graduated grid showed that in 0.01 g of "brown powder" taken from the upper surface of the wheel of Yoghurt cheese, there were from 34 to 59 individuals of different stages of development. On the lower surface of the cheese wheel, their number was somewhat lower, ranging from 16 to 31 mites. The lowest density of mites found in the "brown powder" from the lateral surface of the cheese wheel was from 6 to 21 individuals (**Table 2.1**).

Table 2.1 *A. siro* mite density in the "brown powder" on the surface of craft hard goat cheese Yoghurt

Sample No.	Pieces/225 grid squares (in 0.01 g of "brown powder")		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	59	25	17
2	34	22	13
3	46	16	21
4	38	17	15
5	55	29	7
6	35	23	12
7	39	31	10
8	46	18	19
9	44	17	14
10	40	29	6
11	36	19	9
12	50	24	11
$x \pm SD$	43.5 ± 8.0^{a}	22.5 ± 5.2^{b}	12.8 ± 4.6^{c}

Note: different superscript letters indicate a significant difference between the values in a row of the table using the Tukey's test ($p \leq 0.05$)

Accordingly, the density of *A. siro* mites on the underside of the cheese wheels was 48.3% lower, and on the lateral surface it was 3.4 times lower compared with the upper surface. This pattern is due to the shape and placement of the wheels in the ripening chambers. From the sides and lower surfaces of the cheese wheels, part of the mites fall together with the powder onto the drainage mat, whereas from the upper surface this does not happen.

Further ripening of craft hard goat cheeses in a ripening chamber, as well as preparation of finished wheels for sale, involves washing with running water followed by drying on drainage mats.

As the study results demonstrated, on the surface of the freshly washed wheels of Yoghurt cheese, the density of *A. siro* mites sharply decreased compared with the corresponding values recorded prior to washing (**Tables 2.1, 2.2**). However, it should be noted that washing the wheels of Yoghurt cheese under laboratory conditions did not allow achieving the standard mite density on their surface. Density of *A. siro* mites in 1 cm^2 scrapings from the upper and lower surfaces of the wheel of Yoghurt cheese did not differ significantly, while on the lateral surface, it was lower by 1.88–2.00 times, respectively. No eggs of *A. siro* mites were detected in scrapings from the surfaces of the cheese wheels.

To monitor the rate of mite colonization on cheese surfaces, Caciotta cheese wheels were used; after washing, the wheels were dried on a draining mat and left in the maturation chamber. Studies have shown a slightly lower density of *A. siro* mites on the surface of aged Caciotta cheese.

Table 2.2 *A. siro* mite density in scraping from the surface of craft hard goat cheese Yoghurt immediately after washing with running water

Sample No.	Pieces/225 grid squares		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	6	8	4
2	15	12	3
3	7	10	6
4	11	11	7
5	10	13	4
6	14	11	5
7	12	14	6
8	8	12	7
9	6	7	4
10	9	7	8
11	11	7	9
12	12	12	11
x ± SD	9.8 ± 3.1^a	10.4 ± 2.3^a	5.2 ± 1.6^b

Note: a scraping from 1 cm^2 of cheese was taken for analysis, different superscript letters indicate a significant difference between the indicators in the table row using the Tukey's test ($p \leq 0.05$)

Washing of Caciotta cheese wheels followed by drying on drainage mats and storage in a refrigerated state for 3 months in a craft factory environment showed low densities of *A. siro* mites in crust scrapings (**Table 2.3**).

This cheese was characterized by the absence of a significant difference in the density of mites on the upper, lower, and lateral surfaces of the wheels.

It should be noted that the analysis of the number of *A. siro* mites in scrapings from different parts of the cheese wheel indicates a slightly lower density than in Yoghurt cheese. Probably, *A. siro* mites inhabit hard goat cheeses in different ways, which depends on their recipe and manufacturing technology.

Thus, on the upper surface of the wheels of Caciotta cheese, the number of mites per 1 cm^2 of scrapings ranged from 0 to 5 individuals, on the lower surface – from 0 to 4, and on the side – from 0 to 5 in 225 squares of the calibration grid.

No statistically significant difference was observed between the mean densities of *A. siro* mites on different areas of the cheese wheels. Moreover, when considering the regulatory limits for mite density in cheeses, this value did not exceed the permissible level established by Brazilian legislation, which specifies a maximum of five mites on a 2.5 cm^2 surface of cheese to a depth of 0.6 cm [23].

Table 2.3 *A. siro* mite density in scrapings from the surface of craft hard goat cheese Caciotta 3 months after washing with running water

Sample No.	Mite density, pieces/225 grid squares		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	3	1	4
2	2	3	0
3	5	4	4
4	0	1	2
5	2	3	3
6	0	2	2
7	1	3	0
8	3	0	5
9	4	4	0
10	1	2	1
11	4	0	2
12	0	0	0
x ± SD	2.1 ± 1.7^a	1.9 ± 1.5^a	1.9 ± 1.8^a

Note: a scraping from 1 cm^2 of cheese was taken for analysis, different superscript letters indicate a significant difference between the indicators in the table row using the Tukey's test ($p \leq 0.05$)

Analysis of scrapings of Caciotta cheese after washing with running water and storing for 3 months in a refrigerated state showed the presence of eggs of the *A. siro* mite in scrapings from the upper, lower, and lateral surfaces of the cheese wheels (**Fig. 2.15**).

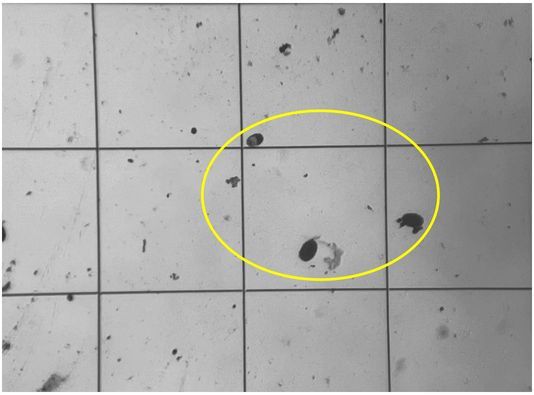

Fig. 2.15 Fragment of a calibration grid under a microscope with mite eggs in scrapings of Caciotta cheese after washing with running water and storing for 3 months in a refrigerated state

Under the microscope, eggs of *A. siro* mites have a clear, oval shape, allowing them to be differentiated from the cheese mass grains and other components in the field of view of the grading grid. The grains, which contain the remains of cheese and mite waste products, are characterized by irregular shapes, different sizes, and colors. This is clearly visible in the microscope's field of view and allows them to be separated from mite eggs. At the same time, this method does not allow to differentiate live mites from dead ones, since under the influence of 96° ethyl alcohol they have an immobilized appearance and do not differ from each other.

Despite the fact that no regulatory documents worldwide contain restrictions on the density of mite eggs in cheeses, this study conducted such an analysis because it is a necessary condition for predicting the risks of mite reproduction and spread in cheeses.

The results of the study showed that the density of mite eggs did not differ significantly in samples taken from the upper, lower and lateral surfaces of Caciotta cheese wheels. It ranged from 5 to 16 on the upper surface, from 4 to 17 on the lower surface and from 7 to 17 on the lateral surface in 225 squares of the calibration grid (**Table 2.4**).

Table 2.4 Mite egg density in scrapings from the surface of craft hard goat cheese Caciotta 3 months after washing with running water

Sample No.	Pieces/225 grid squares		
	Upper surface of the wheel	Underside of the wheel	Lateral surface of the wheel
1	7	4	14
2	12	11	10
3	11	10	15
4	10	16	12
5	9	12	17
6	5	6	9
7	14	17	10
8	10	10	15
9	16	8	8
10	12	9	11
11	7	11	7
12	9	5	13
x ± SD	10.2 ± 3.1^{a}	9.9 ± 3.4^{a}	11.7 ± 3.1^{a}

Note: a scraping from 1 cm^2 of cheese was taken for analysis, different superscript letters indicate a significant difference between the indicators in the table row using the Tukey's test ($p \leq 0.05$)

Thus, it can be concluded that washing the cheese wheels with running water not only fails to completely remove mites from the surface but also leaves viable individuals capable of reproduction.

Calculation of the mite population density on the surface of hard cheese indicates a fairly significant error in the results, which ranges from 26.5% to 34.3%. However, the developed method may satisfy the purpose of most studies, since it is quite difficult to achieve high accuracy in calculating the mite density. This is primarily due to the movement of mites on the surface of cheese wheels, and their density can vary both spatially and temporally.

Among methods for isolating and recording mite density in feed and food products, the method developed by M. Solomon [29] is still used. This method involves determining the density of mites in bulk samples of feed or food products, followed by counting them in sectors of a Petri dish. The results are expressed in units of volume, which for hard cheeses cannot be an objective indicator of mite infestation, since their highest density is on the surface, i.e. in the scraping or "brown powder" on the surface of the wheels. This does not reflect the density of infestation of the entire wheel of hard cheese and does not take into account the uniformity of the distribution of mites, which is due to the peculiarities of its texture, in particular the high content of moisture, protein and fat, which interfere with the microscopy process.

To analyze the density of mites on the surface of hard cheeses, it is more appropriate to use two methods:

– counting the number of mites in the "brown powder" that is on the surface of the wheels. In this case, the calculation can be made per unit mass;

– counting the density of mites on the surface of washed or washed and oiled cheeses. In this case, it is advisable to scrape from the surface of the rind and calculate per unit area.

The developed method is simpler, more convenient, and takes less time. In addition, this method is non-destructive, which allows samples to be taken from the surface of cheese wheels and then placed in chambers for further ripening or sale to consumers.

The reagents, equipment and materials used in this method do not require preparation, are quite cheap and do not require a special recipe or permit for purchase. This also indicates the possibility of its use in any cheese factory. The use of a calibration grid manufactured industrially eliminates the error in the calculations due to the unified size of the cells and their number, which allows for an increase in the accuracy of the calculation. At the same time, the calibration grid is fully within the microscope's field of view, reducing errors in counting mites within cells or rows. Furthermore, the developed method allows for the enumeration of not only mites but also their eggs, thereby enabling the prospective prediction of their distribution on food products. This method also does not depend on the correctness and accuracy of the researcher in manually making a graduated card for a Petri dish with division into sectors, which involves the method developed by M. Solomon [29].

2.4.3 Methods for reducing mite density during ripening and storage of craft hard goat cheeses

Among the methods proposed to reduce the density of mites on the surface of cheeses during their ripening process, the use of chemicals is proposed, in particular, fumigation with methyl bromide (currently banned by the Montreal Protocol), as well as the use of registered organophosphorus insecticides. At the same time, it is also prohibited for neutralizing the *A. farris* mite that damages Cabrales cheese. This is due to the insecticide's toxicity, primarily to consumers.

There is an ongoing discussion in the literature about the effectiveness of using acaricidal agents based on insect growth regulators, inert dust, plant-based preparations, and individual representatives of the pyrethroid insecticide group. However, such agents must meet the requirements of the "One Health" concept, i.e., take into account the impact on human, animal, and environmental health [30]. This can be achieved by meeting the following criteria:

– high acaricidal effectiveness;
– harmlessness to human health;
– no effect on the organoleptic characteristics of cheese;
– low cost, ease of use, dosage and storage;
– harmlessness to the environment and non-target organisms.

The recommended means of preventing the spread of mites on food products, in particular cheeses, primarily include natural components. To neutralize mites that damage food products, such as cured ham, coating with vegetable oils or hot lard is used. It is also considered quite effective to use essential oils that contain biologically active compounds – monoterpenes. Monoterpenes of essential oils of plants showed high acaricidal activity and did not have a significant effect on the sensory properties of food products. They are also considered safe for consumer health.

Another strategy to control mite populations in cheeses is to regulate the relative humidity in the ripening chamber. Results showed that *A. farris* population density decreased at relative humidity levels below 70%. However, this was ineffective at controlling mite populations on Cabrales cheese [15]. The researchers concluded that the cheese's high water content provided the mites with suitable conditions for reproduction, and that the humidity in the ripening chamber was not significant.

Comprehensive studies on the effectiveness of coating with fatty acid, monoterpene (eucalyptol) at low temperatures to control the population density of *A. farris* in cheeses indicate that their population density on cheeses ripened at temperatures of 2, 4 and 6°C was significantly reduced. Thus, at a temperature of 2°C, 10 mites/cm^2 were detected, at a temperature of 4°C – 11.4 mites/cm^2 and at 6°C – 14.7 mites/cm^2 against 174.33 mites/cm^2 in the control. For all temperature regimes, these values were also significantly lower than the initial values (10 versus 21.3 mites/cm^2 at 2°C, 11.4 versus 22.4 mites/cm^2 at 4°C, and 14.7 versus 26.5 mites/cm^2 at 6°C) [16].

Analysis of Ras cheese samples showed that it was infested simultaneously with three mite species: *A. siro* and *A. farris* (family *Acaridae*), as well as *Carpoglyphus lactis* (family *Carpoglyphidae*). In this study, the authors showed that the dominant mite species infesting Ras cheese was *A. siro*, with an abundance of up to 82% of the other mite species. Another study also found that cheeses were infested with *A. siro* [31].

Also, the main means for neutralizing mites in cheese were proposed to use essential oils of cloves, citrus crops, thyme and rosemary. Their effectiveness was proven in the treatment of Ras cheese from *A. siro* mites. The acaricidal effect depended on the type and concentration of the essential oil used to treat the cheese. Among the essential oils, clove oil was the most effective. LD50 (oral dose for mites, providing 50% mortality of mites) for a 0.1% concentration of clove oil provided 95% mortality of mites. A higher concentration of clove oil guaranteed 100% death of mites on cheese. Slightly lower efficiency in terms of LD50 for mites was found in thyme and rosemary oils. They were effective at a concentration of 1.0%, which provided mortality of mites at the level of 75% and 55%, respectively. With increasing concentrations of both oils, the mortality of mites increased. It is believed that the essential oils of the above-mentioned plants can be an effective means of protecting Ras cheese from mite infestation during ripening, storage and sale. The authors also emphasize that the essential oils of plants can exhibit a bactericidal effect, which can have an effect on microorganisms necessary for the life of mites. At the same time, such treatments can affect certain properties of the cheese and the health of consumers. Treatment of cheese with essential oils does not exclude a change in its sensory characteristics, which can in some way affect consumer preferences. This study confirmed that essential oils of plant origin change the organoleptic properties of Ras cheese: the taste and smell of this cheese improved when treated with citrus or thyme essential oils [28].

To control mite density in hard cheeses, our study used two strategies (**Fig. 2.16**):

– reducing the density of mites in cheeses subject to further ripening by washing with running water;

– decontamination of mites in cheeses to be sold by washing with running water and treating with linseed oil.

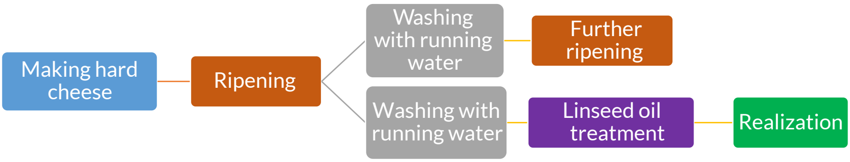

Fig. 2.16 Scheme of processing of craft goat hard cheeses to control the density of *A. siro* mites

The use of linseed oil for the processing of craft hard goat cheeses, Yoghurt and Canestrato, showed its high acaricidal efficiency. This is confirmed by the analysis of scrapings from different surfaces of their wheels (**Table 2.5**).

No mites or their eggs were detected on the surface of Yoghurt cheese 2 months after washing and treatment with linseed oil, as well as Canestrato cheese 3 months after washing and treatment with linseed oil.

Table 2.5 Density of *A. siro* mites in scrapings from the top, bottom, and side surfaces of craft hard goat cheeses after washing with running water and treating with linseed oil

Sample No.	Yoghurt cheese (2 months after treatment)		Canestrato cheese (3 months after treatment)	
	Presence of mites	Presence of mold fungi	Presence of mites	Presence of mold fungi
1	0	+	0	+
2	0	–	0	–
3	0	–	0	+
4	0	+	0	–
5	0	–	0	+
6	0	+	0	+
7	0	–	0	–
8	0	+	0	–
9	0	–	0	+
10	0	–	0	–
11	0	+	0	–
12	0	+	0	+
x ± SD	0 ± 0^a	White mold	0 ± 0^a	Blue mold

Note: a scraping of 1 cm^2 of cheese was taken for analysis; "+" – presence of mold growth, "–" – absence of mold growth

Considering that linseed oil is a valuable food product and does not affect the organoleptic characteristics of hard cheeses, it can be considered a natural acaricidal agent for storing craft cheeses intended for sale to consumers.

At the same time, the presence of white and blue mold was noted on their surface, which indirectly confirms the absence of mycoid mites *A. siro* on the wheels of cheese (**Fig. 2.17**).

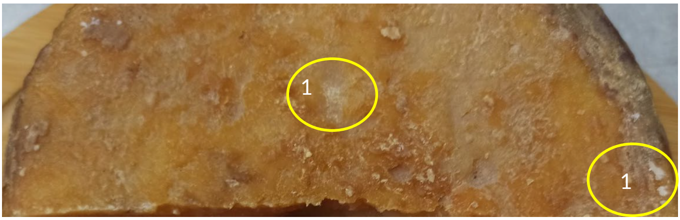

Fig. 2.17 Yoghurt cheese washed with running water and treated with linseed oil: 1 – traces of white mold

The growth of mold fungi on the surface of craft hard goat cheeses can recur due to their contamination with spores during the ripening process. The absence of mycoid mites or a reduction in their population density on the surface of cheeses to a critically low level can promote the germination of spores on the surface of cheese wheels. This phenomenon is also observed in craft hard goat cheeses used for this study. Many researchers believe that the growth of fungi on the surface of hard cheeses can have certain benefits, since it provides the creation of more complex aroma and taste compositions of cheeses, thereby forming original sensory properties.

At the same time, an important criterion for assessing the safety of hard cheeses on which mold fungi grow is the production of mycotoxins. Their formation depends on the ripening period of the wheels and requires strict control of the temperature and humidity regime in the ripening or storage chambers. Therefore, characteristics such as the level of relative humidity and the air temperature of the ripening chambers are key for the growth of fungi. No less important are the indicators of the physicochemical composition of the cheeses themselves, in particular the pH value, the content of table salt and the activity of water. These parameters can also be used to regulate the intensity of the growth of mold fungi on the surface of cheeses [32].

If fungal mycelium appears on the surface of the cheese wheels, they can be mechanically removed. This technique was used to clean the craft hard goat cheeses in this study. A similar practice is used for Brazilian craft cheese, which is ripened in the cheese factory premises to remove white filamentous fungi.

It is believed that the presence of fungal growth on the surface of cheeses does not always indicate the production of mycotoxins by them. For example, PR-toxin, as one of the strongest, synthesized by certain strains of the fungus *P. roqueforti*, does not accumulate in cheeses during ripening in quantities dangerous to consumers. This is due to the lack of optimal conditions for the synthesis of this mycotoxin, since such conditions are not provided in cheeses [33].

Among the most common fungi isolated from craft cheeses, the main ones are representatives of the genera *Aspergillus* and *Penicillium*. For the synthesis of mycotoxins, a fairly high water activity is required, which should be 0.96–0.99, as well as a low pH level of about 5.0. These conditions are able to ensure the activation of genes that determine toxinogenesis. In addition, the intensity of mycotoxin formation depends on the ambient temperature, which should be 25–30°C [34]. These parameters differ significantly from the parameters provided in the ripening chamber of hard cheeses. In this study, hard craft goat cheeses were used, which ripen at a temperature of 8–11°C, which is significantly below the optimal regime.

It should be noted that the ripening period of hard cheeses also affects their mycobiome. In this study, the Caciotta cheese used was characterized by the presence of mold fungi only at the age of 1 month, and at the age of 12 and 24 months, they were not detected, while in Canestrato cheese, the growth of mold fungi was recorded at the age of 10 days, 3 and 12 months [14]. As for the Alpine and Yoghurt cheeses, which were also used in this study, they had their own characteristics of mold fungus growth. In Alpine cheese, the peak of mold fungi occurred at the 6th month of ripening, but they were also detected at the age of 12 months. In Yoghurt cheese, mold fungi were isolated only at 6 months of age, and during the ripening periods of 7 days and 18 months, their colonies were not detected [27]. Thus, it can be assumed that the growth of mold fungi on hard craft goat cheeses, even those made from the same raw milk, differs significantly. This may depend on the recipe and the manufacturing technology.

Fresh cheeses are thought to contain more aflatoxins than mature or aged cheeses, due to the presence of proteins capable of binding them. The ripening process of cheeses is also associated with certain chemical processes that cause changes in the mycotoxin molecules, which may reduce the risk to the health of consumers [35]. Although cheese is a nutrient medium for many species of filamentous fungi, they do not always produce toxins or produce them in quantities that do not pose a health risk. Among the mycotoxins found in cheeses, the most common are penicillic acid, mycophenolic acid (MPA), cyclopiazonic acid (CPA), roquefortine C, sterigmatocystin (STC), aflatoxin, PR-toxin and citrinin, which are not able to be stored for a long time. The low content of mycotoxins on the one hand and the small portion of consumption of old-ripened cheeses also on the other hand reduce the risk of mycotoxin poisoning of the body [36]. Thus, it can be concluded that the appearance of mold on the rind of cheese wheels does not always indicate their danger to humans.

The ineffective fight against molds that grow on cheese rinds ultimately led producers to diversify their cheese production. This led to increased investment in the production of cheeses that have a surface-ripened rind with molds, which contributed to the creation of much more complex aromatic compositions and flavors compared to traditional craft cheeses. Such cheeses have reached a new stage of development in the food market due to their increased value.

The problem of contamination of hard cheeses with fungal spores has not yet been solved [37]. However, this situation has served to achieve success in the production of cheeses with a swollen crust, which occurs only with the participation of autochthonous mycoflora on the surface of the cheese crust. In this case, harmful fungi have been used to produce unique cheeses with unique sensory characteristics. Although, to assess the risk, it is necessary to conduct a study not only of the mycobiota of hard cheeses, but also to clarify their relationship with the mites that spread to such cheeses. It is known that certain preferences regarding the species composition of fungi also characterize acarid mites. For example, *A. siro* mites did not consume the mycelium of black-colored fungi, while they preferred the mycelium of white- and blue-colored fungi on the rind of craft hard goat cheeses. In this study, significant attention was not paid to the species composition of mold fungi growing on the rind of hard goat cheeses during ripening, although in production conditions, there is a practical method that allows for mechanical cleaning of the mycelium of black-colored fungi. In this case, the cleaned areas of the rind of hard cheeses are colonized by other species of mold fungi.

As for the danger of mycotoxins, which are capable of producing mold fungi in cheeses, the vast majority of studies have not confirmed the presence of mycotoxins, and the assumption that they are dangerous to the health of the consumer, which is based only on the detection of mold fungi on their surface, is questionable [38].

Thus, undesirable and sometimes dangerous representatives of mycobiota, as well as acarobiota on hard cheeses [39, 40] have shown their important role in the creation of new dairy products – hard cheeses with original sensory properties. Therefore, it is worth noting the need to review the hazard criteria for craft hard goat cheeses, which are made in compliance with good manufacturing practices. In this regard, it is also important to distinguish the following concepts in regulatory documents:

– contamination of hard cheeses with mites as collar pests;

– inoculation of mites as a component of the microbiome of hard cheeses.

The results obtained in this study can be the basis for developing effective methods for controlling the number of mites during the ripening process of hard cheeses and assessing their participation in the creation of original, authentic products that meet the criteria of taste pleasure.

2.5 Conclusion

It was established that for the correct ripening of craft hard cheeses from goat milk with the participation of acarid mites, it is necessary to control their population density. An effective method for counting the number of mites for unwashed and washed cheese wheels has been developed. The essence of the method for determining the number of mites for unwashed cheeses is to establish the optimal weight of powder from the surface of the wheels, and for washed cheese wheels – to scrape an area of 1 cm^2 from the surface of their rind, then immobilize the mites using 96° ethyl alcohol and count them on a graduated grid in the field of view of a microscope. This method also provides for the possibility of counting mite eggs, which allows predicting the intensity of their reproduction and spread.

Craft hard goat cheeses, Caciotta, Canestrato, Alpine and Yoghurt are colonized by *A. siro* mites starting from 3–4 months in ripening chambers. The density of *A. siro* mites in the "brown powder" on the surface of cheese wheels reaches 12.8–43.5 in 0.01 g. The uncontrolled process of reproduction and spread of mites on the surface of hard goat cheeses during ripening causes their surface damage, which is characterized by deformation of the rind and the appearance of surface holes. Subsequently, mites penetrate into the core of the cheese wheels with subsequent damage and unusability.

To control the density of acarid mites on the surface of the wheels of craft hard cheeses intended for further ripening, they are washed with running water. This ensures a decrease in the density of mites on the surface of the cheese rind 3 months after washing to the level of 1.9–2.1/cm^2, and their eggs to 9.9–11.7/cm^2 of rind.

For wheels of hard cheeses intended for sale, it is possible to treat with cold-pressed linseed oil, which ensures complete neutralization of *A. siro* mites and their eggs. During storage of wheels of hard cheeses treated with linseed oil, regrowth of mold fungi on the surface of the rind was observed.

For unwashed and untreated hard goat cheeses that ripen with the involvement of acarid mites, the density of mites on the surface of the cheese wheels is not regulated. For hard goat cheeses that ripen with the involvement of acarid mites and are subsequently washed with running water, a density of mites not exceeding 2.5/cm^2 and an egg density not exceeding 15 per cm^2 may be regarded as acceptable.

For hard cheeses washed with running water and treated with oils intended for sale, the presence of mites on the surface of the wheels is not permitted.

The use of *A. siro* mites for ripening craft hard goat cheeses may be one of the criteria for their authenticity and involves, in the future, instrumental research of their sensory characteristics and assessment of consumer demand.

Conflict of interest

The authors declare that there is no conflict of interest regarding this article or the published research results, including the financial aspects of conducting the study, obtaining and using its results, as well as any non-financial personal relationships.

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The manuscript has no associated data.

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Authors' contributions

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Olena Semenko: Validation, Language editing, Interpretation of results, Writing – review and editing.
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