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Tushuri Guda Cheese and EU Food Safety Regulations

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ABSTRACT. The risk of Georgian Tushuri Guda cheese contamination with mycotoxins is investigated in the paper. Mycotoxins are produced by different genera of filamentous fungi and cause serious health hazards such as carcinogenicity and mutagenicity. Toxigenic fungi produce mycotoxins which contaminate the lactating sheep's feedstuff. During metabolism, the mycotoxins undergo biotransformation and are secreted in milk. Studies show that there is a seasonal trend in the levels of mycotoxins in ewe milk. In the cold months sheep feeds provide favorable conditions for fungal growth. Good agricultural and storage practices are therefore fundamental for controlling the toxigenic species and mycotoxins. Although aflatoxins (especially aflatoxin M1) are the mycotoxins of greater incidence in milk and cheese. It was found that other mycotoxins, such as fumonisin, ochratoxin A, trichothecenes, zearalenone, T-2 toxin, and deoxynivalenol, can also occur in these product. Our investigation show that there are all favorable conditions in Georgia for safe production of Tushuri Guda Cheese to be exported to Europe. *© 2016 Bull. Georg. Natl. Acad. Sci.*

Key words: specific technology, hygienic standards, GHP, EU Regulations, mycotoxins, enzymes

Introducion

Archaeologists discovered that a variety of cheese similar to Tushuri Guda was produced in Georgian part of the Caucasus in the 4th millennium BC. There is a documented evidence that cheese was produced as early as the 4th century in this location of Europe, the mountainous region in northern Caucasus that extends through the present-day regions of North Georgia. Each region of old Georgia developed its own variety of cheese: sheep milk cheese was produced in dry interior areas, and in the pastures of big Caucasus mountains in the North, and in more recent history in some of the Black see regions. Goat's milk cheese was produced throughout all Georgia [1].

A rich creamy Tushuri Guda cheese was made entirely from sheep's milk. The ewe milk is slightly sweeter than the cow milk and very rich, since it contains more than twice the amount of butterfat than the cow's milk. The array of herbs growing in the areas often grazed by the sheep in subalpine pastures of Caucasus gives pleasant flavor to milk. It also provides mild overtones of lanolin resulting in a distinctive and very specific aroma to the cheese. Sweet milk combined with natural nutty flavors developed from the maturing sheep results in a rich tasting cheese that can range in flavor from mild to very sharp. Common variety of cheese is well known in South Caucasus region as can be served as a dessert, as an appetizer or snack, and as a complement to salads.

In Georgia sheep were raised for milk for thousands of years and were milked long ago than the cows. The world's commercial dairy sheep industry is concentrated in Europe and in the Mediterranean and Black Sea countries.

Most of the sheep milk produced in the world is made into cheese. Sheep milk is also made into yogurt and ice cream. Some of the most famous cheeses made from sheep milk are Feta (Greece, Italy, and France), Ricotta and Pecorino Romano (Italy) and Roquefort (France). Since 24.01.2012 Tushetian Guda cheese of Georgia is registered as a brand by "Saqpatenti" for International trade. Sheep milk is highly nutritious, richer in vitamins A, B, and E, calcium, phosphorus, potassium, and magnesium than cow's milk. It contains a higher proportion of short- and medium-chain fatty acids, recognized to be beneficial to health. For example, short-chain fatty acids have little effect on cholesterol levels in people. They make milk easier to digest. According to German researchers, sheep milk has more conjugated linoleic acid (CLA) than the milk from pigs, horses, goats, cattle, and humans. CLA is cancer-fighting and fat-reducing. The fat globules in sheep milk are smaller than the fat globules in cow's milk, helping sheep milk to be more easily digested [2].

Sheep's cheeses have always been a part of Georgia's cheese-making history with small producers in mountain regions keeping up the tradition even when the country was going through a period of modernization and industrialization. Throughout the 20th century, the advances and discoveries achieved in the fields of bacteriology, chemistry and technology had the effect on modernization of the cheese producing sector to a certain degree. However, because of their very nature, the most traditional cheese like Tushuri Guda are still made in a highly artisan way to this day.

Materials and Methods

While lactating the ewes of any breed can be milked, as any other species of livestock, there are specialized dairy sheep breeds. There are more than a dozen dairy sheep breeds worldwide, but only two breeds area revealed in Georgia: Tushetian and Imeretian. Specialized dairy breeds produce from 240 to 550 kg milk per lactation, whereas the milk production from conventional sheep breeds is only 120 to 300 kg milk per 220 to 240-day lactation. Tushetian and Imeretian endemic breeds of Georgian sheep are selected for making famous Tushuri Guda cheese.

Worldwide, most sheep are milked seasonally by hand. This is because many dairy sheep are raised in remote areas where no cow could survive. In Georgia dairy ewes are managed in mountain pastures. On some farms, ewes are not milked until their lambs are weaned at the age of 30 to 40 days. Another system allows ewes to suckle their lambs for 8 to 12 hours per day, after that they are separated for a night and the ewes are milked the following morning. After the lambs are weaned at 28 - 30 days, the ewes are milked twice per day. Optimal milk yield is obtained when the lambs are removed from their dams within 24 hours of birth and raised on artificial or cow milk replacer, as is common in most European countries.

Recently, Georgia joined the EU and has already started introducing the designation of origin classification to premium foods, which controls the quality and traditional characteristics of those products. In 2011 the Georgian Association of Cheese makers updated the original catalogue of cheeses, it recorded 42 different types according to provenance and production method, with a view to preserving the particular characteristics and quality of Georgian cheeses. Currently, 16 varieties of cheese dominate, from which 4 are Guda type.

Cheese production is fully based on primitive technology and equipment developed in high mountains pastures of the Tusheti region of North-East Caucasus region of Georgia. The manufacture of specially pressed cheese at the plant involves the following main processing stages in field conditions: sheep milking, milk treatment, starter culture addition, curd making, cooling / brining in natural condition, transportation from mountains by horses, finishing and storage in basements. The more precise scheme of technology is as follows [3].

After milking, the milk is processed in a number of steps including field pasteurization for eliminating all non-sporogenous pathogenic and coliformic bacteria (the cheese milk is heated at 71°C to 72°C for 15 seconds). The milk is then treated by filtration to remove spores and other unwanted micro particulates before being fat standardized to achieve a specific ratio between fat and total solids content. Protein standardization of the cheese milk is then carried out using filtration by special cotton cloth to achieve a uniform protein content. Some cheese are produced at the herder's buildings, some in open fields. Milk homogenization is recommended to give a paler look to the final cheese and to make the fat more susceptible to fat cleavage by means of lipase enzymes, which contributes to their characteristic flavor.

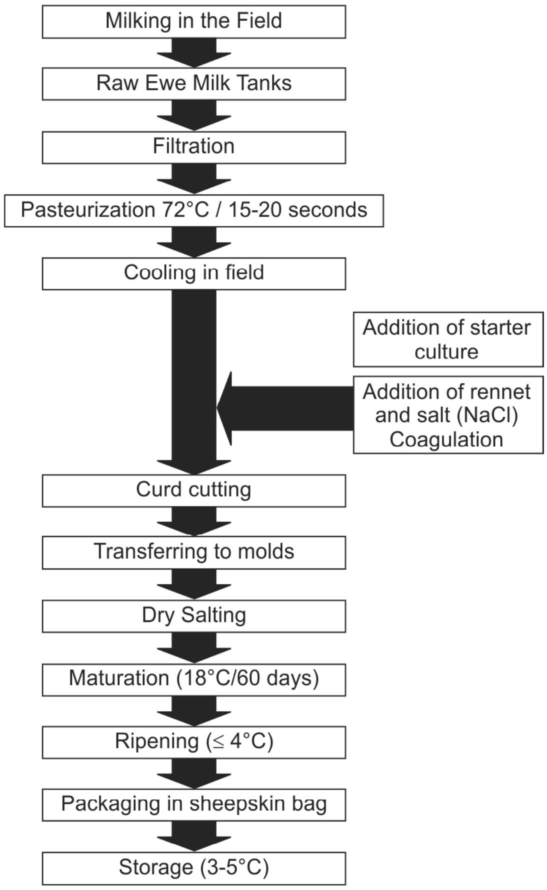

Fig. 1. Diagram of Georgian ewe cheese -Tushetian Guda production

Results and Discussion

Contamination of the external surface of the teat with fecal and other environmental organisms is scarcely avoidable, but is minimized in compliance with the highest standards of hygiene at milking. However, if initial contamination levels are low and subsequent milk storage conditions (hygiene and temperature) are more or less correct (is not easy in field conditions), then further bacterial growth will be minimized.

Mycobacterium Bevis organism has a broad host range and is the principal agent of tuberculosis in wild and domestic animals. This organism can also infect humans causing zoonotic tuberculosis. The transmission of tuberculosis to humans in Georgia follows from consumption of unpasteurized milk [4].

Brucella spp. are pathogens, which are highly infectious and capable to cause disease in both animals and humans. The pathogenic strain *Brucella abortusis* is more associated with cows, whereas *Brucella melitensis* is more commonly found in sheep and goats. Transmission of bacteria to humans can be (amongst other routes) via milk and milk products. Regulation 853/2004 (EU, 2004) (Annex III, Section IX) states that raw milk must come from animals that do not show symptoms of infectious diseases communicable to humans through milk. In particular, tuberculosis and brucellosis, this regulation states that raw milk must come from sheep belonging to a herd, which within the meaning of Directive 64/432 (EU, 1964) is free or officially free of tuberculosis and brucellosis, and if not, the milk may only be used with the authorization of the competent authority. In addition to compliance with directivities on milk quality, perhaps, the most effective means of ensuring the safety of milk from a public health perspective can be implementation of ongoing training for grassland dairy farmers and their employees in the areas of sheep management, milk handling and storage procedures, fundamentals of toxin and disease transmission, and pathogen effects on human health. In addition, field pasteurization of milk represents possibly the most significant and successful contribution to milk safety [5].

Toxic residues/contaminants of these compounds in the animal's body may shed into milk and thus pose a threat to human health. Chemical residues are remnants of purposeful additions to the food chain, whereas contaminants represent any biological or chemical agent and Color.

The most common chemical residues found in milk are antibiotics, administered for treatment of mastitis. Regulation 853/2004 (EU, 2004) states that raw milk must come from animals to which no unauthorized substances have been administered, and in respect of which, where authorized products or substances have been administered, the withdrawal periods for those products have been observed. The most effective means of controlling the toxic residues/ contaminants is by legislation, voluntary codes of practice, monitoring and surveillance of animal feeds, and prudent use of all animal inputs.

One specific Regulation 853/2004 (EU, 2004) lays down specific hygiene rules for food of animal origin, with Annex III containing specific requirements for raw ewe milk and cheese. Specifically, with regard to plate count standards, milk-processing operators must ensure that raw milk meets strong criteria. By the point of safety, carried out lab analyses show microbiological data below in Table 1.

The ewe milk itself must also satisfy specified hygienic standards in terms of bacterial numbers present, for example the plate count at 30°C for raw ewe milk is ≤100 × 103 cells mL - 1. Milk is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources: from within the udder, the exterior of the udder (sheep environment) and the surface of milk handling and storage equipment. The health and hygiene of the sheep, the environment, in which the sheep is housed and milked, the procedures used in cleaning and sanitizing the milking and storage equipment, and the temperature and length of time of storage are all key factors in influencing the level of microbial contamination of raw milk.

External microbial contamination of the mammary gland and the udder can originate from two main sources, namely the environment of the sheep and milk contact surfaces. Potential for microbial contamination of milk during the pasture production process is present in the general environment. Microbes may be transferred to milk through the medium of grass, hay, silage, bedding material and soil, and, if not removed prior to milking, are washed into the milk during milking.

The influence of dirty sheep on total bacterial count in milk depends on the extent of soiling of the teat surface and the teat cleaning procedures used immediately before milking. Contamination of milk by unclean teats can potentially contaminate the milk with heat-resistant bacterial spores, which are problematic for the cheese manufacturing.

A further source of microorganisms in milk and frequently the principal cause of consistently high bacterial counts is the build-up of contaminated deposits within the milking crockery plate. Milk residue left on plate contact surfaces supports the growth of a variety of bacteria (Micrococcus, Streptococcus and Bacillus spp.) Except in very cold and dry weather, bacteria can multiply on these surfaces during the interval between milking. This risk can only be corrected by an appropriate plate washing routine. This is particularly relevant for thermoduric bacteria, which may be removed with hot water. Insufficient cleaning may result in persistent growth of thermoduric bacteria on surfaces.

The quality of milk for cheese manufacture including the quality of big stainless steel metal plate is essential in order to minimize bacterial contamination. Milk storage conditions in low temperature and storage of milk is conducive to the growth of psychrotrophic bacteria. These bacteria typically come from the sheep's environment, such as dirt and manure. The extent to which the bacterial count increases in milk during storage depends on both the

Table 1. Microbiological Critical Points in Ewe Milk

#	Enzymes	N	C	M	M
1	*Listeria monocytogenes*	3	0	Absence in 25 g	
2	*Salmonella spp.*	3	0	Absence in 1 g	
3	*Staphylococcus aureus* (cfu/g)	3	2	$=1x10^2$	$=1x10^3$
4	*Esherichia coli* (cfu/g)	3	2	$=1x10^2$	$=1x10^3$
5	*Coliforms* (cfu/g)	3	2	$=1x10^4$	$=1x10^5$

n is the number of sample units comprising the sample;
m is the threshold value for the number of bacteria;
M is the maximum value for the number of bacteria;
c is the number of sample units where the bacteria count may be between 'm' and 'M'.

temperature and duration of storage as well as the number and types of bacteria present in the milk. The total bacterial count of milk at the end of a cooling storage period on-farm is also influenced by the initial count of that milk. When milk is stored at low temperature, one and two doublings of bacterial growth occur after 2 days of storage, respectively.

Efficient cooling of milk immediately after production in conjunction with good milking hygiene makes it possible to maintain good quality milk for up to 2 days on the farm, provided that the milk container is well insulated. While milk produced under ideal conditions may have an initial psychrotrophic bacterial population of 10% of the total bacteria, psychrotrophic bacteria become the dominant microflora after 2 days at ~10°C.

Heat treatment of milk (thermisation, pasteurization) at the dairy may destroy the psychrotrophic bacteria, but not necessarily the products of their metabolism (FFA) or their enzymes that can adversely affect rennet coagulation properties of the milk, cheese yield and quality. Psychrotrophic bacteria commonly produce extracellular enzymes capable of hydrolyzing proteins and fats of milk and milk products. Thus, they can increase the likelihood of off-flavors and odors and cause changes in body, texture and color.

Storage of milk at 10°C for periods of 2 or more days resulted in a significant reduction in cheese yield, with a considerable loss in revenue to the cheesemaker. The latter also found that cheese manufactured from stored milk gave a significant reduction in quality, with the results being more pronounced after 3 days of storage.

Conclusion

Considering all the EU Regulations the technology of cheese production is being developed in Georgia in order to come as close as possible to the standards of the EU requirements.

სოფლის მეურნეობა

თუშური გუდის ყველის ტექნოლოგია და ეკ სურსათის უვნებლობის რეგულაციები

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შესწავლილია თუშური გუდის ყველის მიკოტოქსინებით დაბინძურების რისკები მისი წარმოების ტექნოლოგიის ეტაპებზე ევროკავშირის რეგულაციების მოთხოვნების შესაბამისად, ზოგიერთი მიკოტოქსინის ცხვრის რძეში მოხვედრის წყაროები და მისი მინიმიზაციის შესახებ ძირითადი რძის პროდუქტში - თუშური გუდის ყველში, რომელიც წარმოადგენს ცხვრის რძის მთავარ პროდუქტს და აქვს დიდი პერსპექტივა ევროკავშირის ქვეყნებში ექსპორტისათვის. მიკოტოქსინებს წარმოქმნიან მიცელიარული სოკოები, რომლებიც ქმნიან ჯანმრთელობისათვის სერიოზულ საფრთხეებს კანცეროგენული და მუტაგენური დაავადებების სახით. ტოქსიკური სოკოები წარმოქმნიან მიკოტოქსინებს, რომლებიც რძეში წარმოიქმნებიან ცხვრების საკვებიდან ლაქტაციის პროცესში და აბინძურებენ რძეს და შესაბამისად მისგან წარმოებულ ყველს. კვლევებმა აჩვენა, რომ მეტაბოლიზმის პროცესში ცხვრის რძეში წარმოქმნილ მიკოტოქსინებს ახასიათებს სეზონურობა - ზამთრის პერიოდში ისინი მატულობენ, ვინაიდან ცხვრები იკვებებიან ზამთრისათვის დამზადებული საკვებით, რომელშიც მიცელიარული სოკოების რაოდენობა გაზრდილია. კარგი სასოფლო-სამეურნეო პრაქტიკა საკვების შენახვის დროს არის ძირითადი ფაქტორი ტოქსიკური სოკოებისა და მიკოტოქსინების წინააღმდეგ საბრძოლველად. როგორც კვლევებით გამოვლინდა, აფლატოქსინი (განსაკუთრებით აფლატოქსინი M 1) წარმოადგენს მთავარ პრობლემას რძესა და ყველში, აგრეთვე სხვა მიკოტოქსინებიც, როგორიცაა ფუმონისინი, ოქრატოქსინი A, ტრიქოთესენესი, ზეარალენონი, ტოქსინი T-2 და დეოქსინივალენოლი შესაძლებელია აღმოჩნდეს ამ პროდუქტში.

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