Safety and quality assessment of cheeses with mold

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Abstract. In many Russian regions, increased cheese production has allowed to satisfy the population needs. Cheeses made with the participation of mold fungi are very popular. In violation of technological regimes, sanitary-hygienic conditions of production, storage, various types of cheese defects arise. The aim of this work is to assess the quality and safety of cheeses with mold according to physicochemical and microbiological indicators. Six samples of mold cheese from various manufacturers were examined according to standard methods. The mass fraction of moisture and active acidity in the investigated samples of cheese with mold were within the specified values in the regulatory documentation. Mass fraction of fat in terms of dry substance in two samples below the stated. In terms of quality and safety, among the six samples of cheese with mold, been sold in the Irkutsk distribution network, were detected Escherichia coli Bacteria and Listeria groups, therefore all do not correspond the requirements. We believe that it is necessary to include in-state standard 32263-2013 requirements for organoleptic and physicochemical indicators for cheeses with the development of molds. The pH limits for such cheeses are set from 4.0 to 7.0, since this indicator, in general, is involved in the formation of the quality and safety of this product.

1. Introduction
A variety of cheeses on the market can satisfy the population needs. Cheeses made with the participation of mold fungi are very popular. They have special organoleptic properties, their spicy, full-bodied taste does not allow, even the most sophisticated gourmet, to confuse them with cheeses of other groups. Soft cheeses with mold contain a variety of vitamins and minerals. Moreover, for the maturation of such cheeses that do not require long periods, the manufacturing process is highly mechanized. The category of «blue» noble cheeses includes cheeses with differences in the preparation technology, type and color of the mold, and composition. In general, cheeses with mold are represented by soft cheeses, where molds grow on the surface of the cheese (Russian Camembert, White Dessert, Camembert, Brie, and others), and blue cheeses developing throughout the entire mass of cheese (Roquefort, blue, Blue-veined, Styleton and others) [1, 2, 3].

Noble mold is distinguished, which gives the cheese a unique taste and distinctive features and an alien, degrading product quality and causing harm to the body. Therefore, it is very important in the production of cheeses with mold to create the conditions for the necessary growth and suppress the development of harmful microflora and ensure the safety of this product [4].
The import substitution policy, introduced since 2014, gave impetus to the development, including cheese production, in many regions of Russia. Entrepreneurs began to actively adopt the best world practices in cheese making, supplementing, created their original flavoring properties. Of course, copying the taste and quality of cheeses, which are the hallmark of European countries, is quite difficult, because these indicators are influenced by the climatic conditions, the food supply, in particular the composition of herbs, and the breed of cows and several others. An important role is played by the milk quality. In Russia, they already make good cheeses of their own brand, but yet the problem of quality and safety exists [5, 6].

In violation of technological regimes, sanitary-hygienic conditions of production, storage, various defects of taste and smell, consistency, appearance, color of cheese arise. Inspections conducted by supervisory authorities regularly identify food products, labeling violations, and falsification on the shelves of distribution networks of inadequate quality. This also applies to cheeses, including those with mold. Therefore, it is important to conduct a veterinary and sanitary examination of cheeses with mold to identify a poor-quality product.

It is worth noting that the Mercury electronic veterinary certification system allows to reduce the share of counterfeit products on the Russian cheese market (young cheeses from November 1, 2019, for other cheeses - from July 1, 2019), because the production path from raw materials to a graded product is completely traced [6].

Thus, it is very relevant to determine the quality and safety of cheeses with mold sold in retail chains.

The aim of this work is to assess the quality and safety of cheeses with mold according to physicochemical and microbiological indicators.

2. Conditions, materials and methods
We have studied six samples of cheeses with mold purchased from the retail network of the Irkutsk city:

- Soft cheese «Brie ATON» with white mold. Manufacturer LLC "Renard", Krasnodar region, Leningrad district, f. Korgi;
- Cheese «Brie Alti». Manufacturer LLC «MILKPROM», Moscow;
- Cheese «Camembert Alti». Manufacturer LLC «MILKPROM», Moscow;
- Cheese soft «Camembert ATON» with white mold. Manufacturer LLC «Renard», Krasnodar region, Leningrad district, f. Korgi;
- Cheese with blue mold «DORBLU CLASSIC», 50% (by weight). Manufacturer «Kaserei Champignon Hofmeister GmbH & Co.KG», Germany, Lauben;
- Blue cheese “Blue cheese”, 53% (by weight). Manufacturer «Kalleh Dairy production» Iran, Amol.

Because cheeses with mold belong to the soft cheeses category examination and quality assessment provided according to GOST 32263-2013 Soft cheeses. Specifications, in compliance with the TR CU 033/2013 «Safety of milk and dairy products» requirements.

Physicochemical parameters were determined by standard methods: mass fraction of moisture and dry substance according to GOST 3626-73 Milk and milk products. Methods for determination of moisture and dry substance, a mass fraction of fat was determined by the acid method according to GOST 5867-90 Milk and dairy products. Method of determination of fat; active acidity was measured on a potentiometric analyzer according to GOST 32892-2014 Milk and dairy products. Method of pH determination.

The presence of microorganisms was determined by safety indicators: Escherichia coli Bacteria (colimorphs) according to GOST 32901-2014 Milk and milk products. Methods of microbiological analysis; Salmonella spp. - GOST ISO 6785-2015 Milk and milk products. Detection of Salmonella spp.; Staphylococcus aureus - GOST 30347-2016 Milk and milk products. Methods for determination
of Staphylococcus aureus; Listeria monocytogenes - GOST 32031-2012 Food products. Methods for detection of Listeria monocytogenes.

3. Results and discussion
We determined the acidity, amount of fat, the mass fraction of moisture, and dry substance (table 1).

Table 1. Physicochemical results of researches.

| Samples | pH | Mass fraction of fat in terms of dry substance, % | Mass fraction of moisture and dry substance, % |
|---------|----|-----------------------------------------------|---------------------------------------------|
| №1     | 6.70±0.02 | 52.4±0.21                                      | 46.5                                        |
| №2     | 6.25±0.06 | 61.0±0.30                                      | 39.0                                        |
| №3     | 5.45±0.05 | 49.0±0.20                                      | 49.4                                        |
| №4     | 7.14±0.07 | 47.1±1.10                                      | 35.5                                        |
| №5     | 5.70±0.04 | 47.1±0.85                                      | 40.2                                        |
| №6     | 6.91±0.06 | 51.5±0.20                                      | 39.6                                        |

The active acidity of all cheese samples is within the specified values in the regulatory documentation and varies from 5.45 for sample №3 to 7.14 for sample №4.

The active acidity of cheeses may vary depending on the stages of the process. So, in the enzymatic phase, the pH should be kept within fairly narrow boundaries, the violation of which affects the cheese quality. To prevent the propagation of unwanted microflora, which poses a threat to the cheese quality, accelerate the decrease in pH to 4.5-4.6. Lactic acid bacteria produce lactic and acetic acid. These organic acids penetrate into the bacterial cell, causing irreversible cytoplasm phenomena, and, as a result, an inhibitory effect is achieved. But at the same time, mold fungi increase the pH of cheeses [2, 7].

The indicator of active acidity depends on the nature and intensity of lactic acid fermentation, the conditions for the ripening of cheese, and the quality of the finished product. An excess acidic reaction characteristic of the production of soft cheeses with mold. But at the same time, too high active acidity of the cheese has a bad effect on the consistency and taste of the cheese - crumbling and sour taste appear. At low acidity (5.5 and higher), the consistency of the cheese mass becomes blurry with the outflow of the liquid mass, such a defect occurs in overripe cheeses. Regulation of the active acidity of the finished cheese is one of the means of enhancing the fermentation of protein substances of cheese. At certain stages of the process, active acidity is monitored. It is regulated by adding different doses of bacterial starter cultures, the duration of individual technological operations, temperature, and other parameters. It is noted that high-quality soft cheese occurs at a pH of 5.2-5.3 on the third or fifth day of self-pressing [7].

During the ripening of cheeses, an increase in pH occurs, which accelerates the formation of special organoleptic indicators due to the development of the main microorganisms on the surface of the cheeses. However, too high a pH level of cheese in adulthood contributes to the contamination of its dangerous extraneous microflora.

Mass fraction of fat is an indicator of the nutritional value of the product. By the number of the mass fraction of fat in terms of dry substance, cheese samples №4 and №5 do not correspond to the information specified by the manufacturer. So, in samples №4 and №5, the amount of mass fraction of fat is less than declared by 2.9%.

The high or low-fat content of cheese affects the taste, smell, texture, appearance, maturation, and can cause various defects. First of all, the fat content of cheeses affects their energy value, since excessive or insufficient fat content increases or, conversely, lowers the calorie content of the product.
Soft cheeses have a fairly high moisture content. GOST 32263-2013 indicates the mass fraction of moisture for the Russian Camembert cheese, which is not more than 55%. In soft cheeses, according to the classification of A.V. Gudkova [2] the mass fraction of moisture varies between 46-82%. Moreover, in fungus cheeses, which are part of soft cheeses, the mass fraction of moisture in the cheese mass without fat is not lower than 67%. Perhaps such a wide range of moisture in this classification is associated with a variety of soft cheeses having certain subtleties of the technological process. In TR CU 033/2013, the mass fraction of moisture is 55-80%.

According to our research, the mass fraction of moisture in all cheese samples is within acceptable limits.

Soft cheeses with high moisture content ripen faster than other cheeses, and taste, smell, and texture, in turn, depend on this indicator. In addition, the moisture content of the cheese mass is an important condition for the breakdown of proteins. The amount of moisture depends on the indicator of the cheese active acidity [7, 8].

Analysis of the literature and normative documentation shows that in GOST 32263-2013 «Soft cheeses. Specifications» provides requirements for organoleptic and physicochemical parameters only for the following soft cheeses: Russian Camembert, Amateur, Moale, Ostankino, Klinkovy, Adygea, Adygea Smoked. There are no indicators for cheeses with mold (fungus). Meanwhile, in the technological process of making these cheeses there are certain subtleties that affect the formation of special organoleptic indicators, which in turn depend on biochemical processes, physical properties, and cheese chemical composition.

According to GOST 32892-2014 «Milk and dairy products. Method of pH determination» the active acidity indicator extends to a wide range of dairy products - cottage cheese and curd products, soft cheeses and cheese products, processed cheeses and processed cheese products, dairy products, except sour cream, hard, semi-solid, brine cheeses and cheese products and varies also in a wide range from 3 to 8.

In our opinion, it is necessary to include in GOST 32263-2013 the requirements for organoleptic and physicochemical indicators for cheeses with the development of molds; reduce pH limits, and set 4.0 to 7.0.

According to safety indicators, the number of microorganisms, including pathogenic. In the production and ripening of some soft cheeses, in addition to lactic acid bacteria, cultural molds, the most popular Penicillium Camemberti and Penicillium Roqueforti take part.

Mucor often grows on the surface of Camembert type cheeses, the development of which causes product defects, and it has a narrow pH range for development (5.0), in contrast to *P. Camemberti*, which grows in a wider range [2].

In cheeses ripened under aerobic conditions, harmful aerobic microbes are suppressed by low pH at the beginning of ripening, a high salt concentration, and cultured aerobic microflora, which multiplies during ripening, the growth of which gives the cheese specific organoleptic characteristics. Cultural microflora includes - cheese mucus, consisting of *Brevibacterium linens*, yeast and micrococcus, and mold fungi *P. Camemberti* and *P. Roqueforti*. Cheeses ripening with the participation of aerobic microflora are characterized by high proteolysis and lipolysis, which determines their sharp specific taste and aroma.

The high pH on the surface of Camembert type fungus cheeses in adulthood makes it dangerous to contaminate it with extraneous microflora. It was revealed that on the surface of such cheese enterococci contains $4.6 \times 10^5$, coliforms - $3.5 \times 10^8$ CFU / g [2].

We carried out the inoculation of the studied cheese samples on nutrient media to detect bacterium of intestinal bacillus (coliforms), bacteria *Salmonella*, *S. aureus*, *L. Monocytogenes*.

Sowing results were evaluated visually by the nature of the colonies formed (table 2).

| Table 2. Microbiological results of researches. | Samples |
|-----------------------------------------------|---------|
| Indicators According to TR CU 033/2013 | №1 | №2 | №3 | №4 | №5 | №6 |

In samples, №№ 1, 3, 4, 6 coliforms (bacterium of intestinal bacillus) were identified - red and dark red colonies with a metallic sheen - lactose-positive enterobacteria (figure 1). In sample № 2, translucent pale pink colonies formed, which indicates that microorganisms belong to lacto-negative enterobacteria, including pathogenic enterobacteria (figure 2).

The development of undesirable microflora in mold cheeses is associated with changes in physicochemical parameters at various stages of the cheese manufacturing process. This is acidity, humidity, temperature, and others. It was revealed that due to the strong antagonistic activity of molds, the growth of such bacteria, in particular bacterium of intestinal bacillus, is suppressed.

In all the cheese samples studied, typical salmonella colonies on brilliant green/phenol red agar were not found.

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**Figure 1.** Colonies of lactose-positive enterobacteria (sample №3).

**Figure 2.** Colonies of lacto-negative enterobacteria (sample №2).

Unfortunately, it was not possible to differentiate staphylococci, the results of seeding were inaccurate.

**Figure 3.** Colonies of Listeria (sample № 4). *Listeria monocytogenes* is the most dangerous foodborne pathogen.
Positive results on listeria were obtained for samples №№ 1, 4, 5. On a selective dense ALOA medium, bacteria of the genus *Listeria*, grown in the form of blue-green colonies, without a halo of bacteria were found (figure 3). The bacteria *L. monocytogenes* and *L. ivanovii* differentiate failed.

It was found that *P. Camemberti* stimulated the reproduction of this bacterium with increasing pH. Also, listeria, which came to the surface of soft cheeses during ripening, multiplies in the same way as when it enters milk. At pH 4.5-4.6, the propagation of undesirable bacteria ceases, but in case of an increase in the acidity level in cheeses, *Listeria monocytogenes*, *Hafnia alvei* can multiply. The growth of *L. monocytogenes* can be inactivated by adding to the composition of the starter cultures of low-forming strains of lactococci [2, 7, 8, 9, 10].

Therefore, it is important to strictly observe sanitary rules and the milk pasteurization regime in the production of these cheeses.

4. Conclusions
Active acidity and mass fraction of moisture of the investigated cheeses with mold is within the specified values in the regulatory documentation. In terms of the fat mass fraction and dry substance, samples №4, Camembert ATON soft cheese with white mold (Russia, Krasnodar region), and №5 Dorblu Classic blue cheese (Germany) do not correspond to the information specified by the manufacturer. In these cheeses, the amount of fat mass fraction is less than declared by 2.9%.

No *Salmonella* genus bacteria have been detected in the cheeses. But in samples №№ 1, 3, 4, 6 were found bacteria - lactose- positive enterobacteria, in cheese №2 - lactose-negative enterobacteria, including pathogenic enterobacteria. Bacteria of the genus *Listeria* were found in samples №№ 1, 4, 5.

Thus, in terms of quality and safety, all six samples of cheeses with mold sold in the Irkutsk distribution network do not occur the regulatory documents requirements.

We believe that it is necessary to include in GOST 32263-2013 the requirements for organoleptic and physicochemical indicators for cheeses with the development of molds; the pH limits for such cheeses are reduced and set to 4.0 to 7.0. The indicator of active acidity is involved in the formation of indicators of the quality and safety of this product.

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