Study of the “Pokrovskiy” cheese microstructure at various degrees of maturity before drying, after freeze drying and after vacuum drying

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Abstract. The article presents studies of the “Pokrovskiy” cheese microstructure at various stages of ripening; cheesesamples were taken at each stage of ripening and were dried by freeze-drying and vacuum drying, and then were subjected to microstructure studies. By micrographs of the cheese “Pokrovskiy” after vacuum and freeze drying, it was found that the cheese has a cellular structure from the first days of ripening. Drying the cheese allowed performing a more detailed study of the cheese structure. After drying, a cellular structure of the cheese was revealed.

1. Introduction
Cheese production technology is mainly based on biochemical and physicochemical transformations of milk constituents that turn into a product. In this case, biochemical processes are determined by bacterial enzymatic systems and enzymes of milk-clotting substances, whose nature and intensity of the action, in turn, depends on a number of conditions created during the production process. Physicochemical changes depend on the amount of products accumulating as a result of the biochemical transformation of the constituent components, as well as on the salt content, moisture, etc.

Both types of transformations are closely interrelated and interdependent. In particular, physicochemical transformations caused by biochemical changes based on the feedback principle actively affect the course of microbiological and biochemical processes [1, 2].

In the ripening of cheeses, the most important role belongs to proteins, mainly casein transformations. The change in casein begins from the moment the rennet preparation influences it, which converts casein into paracasein. Subsequently, in already molded cheese, paracasein changes under the action of lactic acid, sodium chloride and, to the greatest extent, under the action of enzymes produced by microorganisms, and partly rennet and milk enzymes [3].

During maturation, paracasein begins to decompose into simpler compounds containing nitrogen. Albumoses appear first, then peptides and amino acids. The decay of paracasein with the cleavage of amino acids to form polypeptides is possible. Apparently, in cheeses, paracasein decomposes simultaneously along these two paths, since already at the beginning of ripening; an increase in the content of both amino acids and more complex intermediate decomposition products of paracasein is noted [4].
2. **Objects and methods of the research**

In experimental studies, the cheese “Pokrovskiy” produced at an experimental cheese-making factory (Barnaul). “Pokrovskiy” cheese refers to rennet cheeses that are formed in bulk with a ripening period of one month. As objects of research, we used cheese with different ripening periods: 2, 10, 30 and 90 days. “Pokrovskiy” cheese with a ripening period of 90 days is considered as an overripe cheese.

The study of the “Pokrovskiy” cheese microstructure at different ripening periods was carried out on a scanning electron microscope JEOL JSM-6390 LA.

3. **Results of the research**

Figures 1 and 2 show micrographs and profiles of the elemental composition of the cheese “Pokrovskiy” subjected to vacuum drying.

![Micrograph examples](image1)

**Figure 1.** Micrograph of the “Pokrovskiy” cheese structure after vacuum drying. The cheese ripening period is: a - 2 days; b - 30 days; c - 90 days.
Figure 2. Profiles of the elemental composition of the “Pokrovskiy” cheese after vacuum drying. The cheese ripening period is: a - 2 days; b - 30 days; c - 90 days.
It was established that cheese has a cellular structure from the first days of ripening. Moreover, in the process of cheese ripening, the cellular structure develops and increases in size, and in overripe cheese, the cells stretch, and a net-like structure similar to a "web" is formed. Since the vacuum drying cheese has a moisture content of less than 4%. This moisture belongs to the moisture of polymolecular adsorption; it is firmly bound to the dry matter of the cheese and is located in the nutria of the protein framework, like fat globules.

The pores are a protein matrix containing moisture, fat and other elements in the pores. There was moisture in the cells before drying. When electronically scanning the surface, moisture prevents the electron beam from penetrating into the product (figure 3).

The micrograph (figure 3) shows the structure of the cheese before drying: the protein framework, the location of fat globules, pores in the thickness of the cheese. However, a comparison of the micrographs shown in figure 1 and figure 2 proves the information content of photographs of the microstructure of cheeses subjected to drying.

Drying the cheese made it possible to study the structure in more detail and in detail. Due to drying during microphotography of the surface of the “Pokrovskiy” cheese, a cellular structure of the cheese was revealed. It has been proven that cheese does not shrink during vacuum drying. Consequently, there is no fear that the capillaries are broken and the structure after drying has changed its nature.

The size of the pores in 2-day ripening cheese is (5-20) microns, the thickness of the layers is (5-10) microns; in 30-day ripening cheese - (20-60) microns, (2-5) microns; 90-day ripening cheese - (30-100) microns, (1-3) microns, respectively. Moreover, the overripe “Pokrovskiy” cheese (90 days) has practically no pores as such, they merge and unite into a single mass. Thus, it was found that in the process of cheese ripening, the cellular structure increases in size, and the thickness of the protein layers between them decreases.

The profiles of the elemental composition of the “Pokrovskiy” cheese are no less informative. Table 1 shows the elemental composition of the “Pokrovskiy” cheese at various stages of maturation subjected to vacuum drying.

| Element | Ripening period, days |
|---------|-----------------------|
|         | 2                     | 30                  | 90                  |
| C       | 86.40                 | 83.21               | 73.55               |
| N       | 7.62                  | 8.15                | 7.85                |
| O₂      | 2.42                  | 3.55                | 5.32                |
| Na      | 0.20                  | 0.48                | 1.70                |
In the process of cheese ripening, the mass fraction of all elements increases as well, they are sodium, phosphorus, sulfur, chlorine, potassium, calcium. There is a stable increase in oxygen in the cheese during ripening from 2 to 30 days from 2.42 to 3.55%; from 30 to 90 days from 3.55 to 5.32%. At the same time, there is a decrease in carbon in cheese from 2 to 30 days from 86.40 to 83.21%; from 30 to 90 days from 83.21 to 73.55%. The amount of nitrogen during the ripening of the “Pokrovskiy” cheese practically does not change.

These changes in the elemental composition of the “Pokrovskiy” cheese can be explained as follows. When cheese ripens, paracasein begins to break down into simpler compounds containing nitrogen. Since table 1 shows the values of the elemental composition of the “Pokrovskiy” cheese of vacuum drying, and the process of electronic scanning of the microstructure proceeds under a deep vacuum, therefore, oxygen is not atmospheric, but a structural element of the cheese. The increase in oxygen during cheese ripening is associated with proteolysis. In the process of cheese ripening, the paracasein complex gradually decomposes into water-soluble peptides of various molecular weights and free amino acids [5].

Decay of proteins occurs with the participation of water. During decay of proteins, the OH group attaches to a free amino acid, that is, water from a free state passes into a bound one. Consequently, the increase in oxygen in the cheese during ripening is associated with the transition of moisture into more energy-intensive forms of bond. In the process of cheese ripening, along with the formation of amino acids, their deamination occurs, as a result of which acids and ammonia are formed. Deamination can occur not only of amino acids, but also of proteins and peptones of cheese. Along with this process, decarboxylation is also observed in cheeses, with the formation of carbon dioxide and new decomposition products - ammonia [6, 7, 8].

The decarboxylation of amino acids and fatty acids produces carbon dioxide (carbon dioxide). Part of it is absorbed by the cheese mass, and part of it accumulates in the voids of the cheese mass, gradually expanding them, turning them into eyes (at macro level). At micro level, carbon dioxide expands the cellular structure of the cheese (figure 1).

Minerals in the cheese mass change as a result of the formation of acids (lactic, acetic, propionic, etc.). Lactic acid cleaves calcium phosphate and organic calcium from casein in the form of calcium lactate, as a result of which the amount of calcium in the cheese increases during maturation. With a ripening period of 2 days, the amount of calcium is 1.14%; 30 days - 1.35%; 90 days - 2.6%.

When cheese ripens, gases are released: ammonia, carbon dioxide and a little hydrogen. A decrease in the mass fraction of carbon is associated with the release of carbon dioxide during maturation. The mass fraction of nitrogen in cheese during ripening has an almost constant value.

Ammonia is formed during the deamination of amino acids. Its part enters into compounds with acids, part of it accumulates in a free state and evaporates. Hydrogen is released in the process of lactic acid fermentation of lactic acid salts (lactates) by aromatic lactic acid, propionic acid, butyric acid bacteria, bacteria of the E. coli group. It dissolves poorly in the cheese mass, diffuses easily through loose areas, so it does not accumulate in the cheese.

Compared to other gases, carbon dioxide is emitted in much larger quantities (the carbon dioxide content is 60-90% of all gases). It is formed during the fermentation of milk sugar and lactic acid salts (lactates) by aromatic lactic acid, propionic acid, butyric acid bacteria, bacteria of the E. coli group, as well as during the decarboxylation of amino acids.

Figure 4 shows photomicrographs of the “Pokrovskiy” cheese after freeze drying. These micrographs were made under the action of secondary electron beams at a magnification of 100 times. For
comparison, photographs of the microstructure of the “Pokrovskiy” cheese of vacuum drying (figure 1) were taken under a beam of reflected electrons.

![Micrographs of the microstructure of the “Pokrovskiy” cheese of vacuum drying](image1.png)

**Figure 4.** Micrograph of the freeze-dried “Pokrovskiy” cheese structure. The ripening period is: a - 2 days; b - 30 days; c - 90 days.

As well as vacuum-dried cheese with different ripening periods, freeze-dried cheese has a cellular structure. In the process of maturation, the cells increase in size. The size of the cells in cheese with a ripening period: 2 days - (5-30) microns; 30 days - (20-80) microns; 90 days - (30-150) microns.

The micrographs show that part of the surface of the freeze-dried cheese is covered with a thin layer of fat (1-3 microns). The release of fat on the surface of the sublimated cheese is caused by the low residual pressure during sublimation (10-30 Pa).

Thus, according to micrographs of freeze-dried cheese with different ripening periods, an increase in protein cells in the ripening process was established, which fully confirms the studies of vacuum-dried cheese with different ripening periods. Figure 5 shows the microstructure of the freeze-dried “Pokrovskiy” cheese with a ripening period of 90 days.
Figure 5. Microstructure of the freeze-dried “Pokrovskiy” cheese with a ripening period of 90 days.

The protein framework is penetrated over the entire surface by capillaries ranging in size from 10 to 60 μm. Protein particles between capillaries are (50-250) microns in size. This size is comparable to the size of individual paracasein particles. Apparently, during coagulation of casein with rennet, casein particles only grow larger, but do not completely lose their individuality even in mature cheese [7].

4. Conclusion
Based on the studies carried out, the following conclusions can be drawn:

- micrographs of the “Pokrovskiy” cheese structure were obtained after vacuum and freeze drying. The microelement composition of the vacuum-dried "Pokrovsky" cheese has been investigated. By micrographs of the “Pokrovskiy” cheese after vacuum and freeze drying, it was found that the cheese has a cellular structure from the first days of ripening. Moreover, in the process of cheese ripening, the cellular structure develops and increases in size, and in overripe cheese, the cells stretch, and a net structure is formed;
- the mass fraction of moisture in dry cheeses does not exceed 4.0%, this moisture belongs to the moisture of polymolecular adsorption, it is firmly bound to dry matter and is located inside the protein framework;
- drying the cheese made it possible to study the structure of the cheese in more detail. After drying, a cellular structure of the cheese was revealed. The size of the cells of the 2-day ripening cheese is (5-20) microns, the thickness of the protein layers is (5-10) microns; 30-day ripening cheese - (20-60) microns, (2-5) microns; 90-day ripening cheese - (30-100) microns, (1-3) microns. Moreover, the overripe “Pokrovskiy” cheese (90 days) has practically no such cells; they merge and unite into a single mass. As the cheese ripens, the cellular structure increases in size, the thickness of the protein layers between them decreases. When cheese ripens, the mass fraction of oxygen, sodium, phosphorus, sulfur, chlorine, potassium, calcium increases; reduction of carbon content; the nitrogen content practically does not change;
- increase in oxygen during cheese ripening is associated with proteolysis. Protein breakdown occurs with the participation of water. During protein breakdown, the OH group attaches to a free amino acid, that is, water from a free state passes into a bound one. The increase in oxygen in the cheese during ripening is caused by the transition of moisture into more energy-intensive forms of bond;
- minerals in the cheese mass change as a result of the formation of acids (lactic, acetic, propionic, etc.). Lactic acid cleaves calcium phosphate and organic calcium from casein in the form of calcium lactate, as a result of which the amount of calcium in the cheese increases during
maturation. With a ripening period of 2 days, the amount of calcium is 1.14%; 30 days - 1.35%; 90 days - 2.6%.

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