Changes in the cheese "pokrovskiy" microstructure during the ripening process

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Abstract: Issues of cheese microstructure in relation to the determination of its quality characteristics at various stages of the ripening process are of considerable interest. For the correct performance of the production process, it is important to get knowledge about the changes in the capillary structure of the curd mass in the process of cheese ripening and further storage. As a result of the conducted research, it was established when, in the process of ripening, a pattern begins to appear in the Pokrovskiy cheese. It was studied how the size of fat droplets changes during its maturation. The size of the cheese microcapillaries was determined. The dynamics of changes in the elemental composition of the cheese Pokrovskiy in the process of its maturation has been investigated. The location of calcium phosphate in the curd mass at different stages of ripening is determined. The possibility of its identification using a scanning electron microscope has been proven.

1. Introduction
Cheese microstructure is formed under the influence of the processes of its production and maturation. The microstructure of cheese affects its shrinkage, diffusion of salts, lactic acid and protein breakdown products between different parts of the cheese mass, as well as crystallization of salts and other substances that make up the cheese. The main elements in the microstructure of cheese are macrostructural (macrograins, interlayers between macrograins, microcavities) and microstructural (fatty and lipoid particles, calcium salt deposits and colonies of microorganisms) elements [1-6].

The microstructure of cheeses should be used as the most important factor in assessing quality indicators, both at various stages of production, as well as in the process of its maturation and further storage.

2. Materials and methods
The current studies were carried on the analysis of the cheese brand “Pokrovskiy” produced at an experimental cheese-making factory (Barnaul). This cheese refers to rennet cheeses that are formed in bulk with a ripening period of one month. As objects of the research, we used cheese with different ripening periods: 2, 10, 30 and 90 days. "Pokrovskiy" cheese with a ripening period of 90 days is an over-ripe cheese.

The “Pokrovskiy” cheese’s package and its structure in different ripening periods are shown in figure 1 and 2 respectively.
Figure 1. The “Pokrovskiy” cheese’s package.

Figure 2. The “Pokrovskiy” cheese structure of at different ripening periods: a - 2 days; b - 10 days; c - 30 days; d - 90 days.
When cheese ripens, decay products of milk sugar protein and fat accumulate inside it. As a result, the finished product acquires a specific taste and smell, plastic consistency and pattern.

While cheese ripening, the following gases are released: ammonia, carbon dioxide and a small amount of hydrogen. Gases are partially retained in the cheese mass, and some of them are released outside. They move the cheese mass apart, resulting in free spaces. Such lacunae of the “Pokrovskiy” cheese begin to appear in the very first days and are finally formed by the age of 10-15 days. The formation of lacunae in the first days of ripening of the “Pokrovskiy” cheese is explained as follows: when sugar ferments, carbon dioxide is formed; later, as they mature, they grow large \[7\-13\].

Figure 2a shows that the cheese with a ripening period of 2 days has some micro-pores with a size of 0.5 to 2 mm. In cheese with a ripening period of 10 and 30 days, micro-pores are of practically the same size (from 2 to 5 mm). In overripe cheese (figure 2d), the micro-pores increase in size up to 7 mm. Holes of the “Pokrovskiy” cheese are round and oval.

3. Results and discussion

Figure 3 shows photographs of the “Pokrovskiy” cheese microstructure at different ripening periods, they were made on a scanning electron microscope JEOL JSM-6390 LA.

![Figure 3](image-url)

**Figure 3.** Microstructure of the “Pokrovskiy” cheese with different ripening periods (multiplicity is 100 times): a - 2 days; b - 10 days; c - 30 days; d - 90 days.

The micrographs (figure 3) clearly show the distribution of fat on the slices of cheese. The size of the fat droplets ranges from 80 to 150 microns. In overripe cheese (figure 3d), the fat droplets decrease and have a size of 30 to 100 microns. In cheeses with different ripening periods, fat droplets are evenly...
distributed throughout the entire thickness of the cheese. It is the fat droplets, their shape, the way they are located, that basically give the cheese its specific pattern.

Figure 4 shows micrographs of the “Pokrovskiy” cheesecapillary structure. With a magnification factor of 500, the micrographs show the capillary structure of the cheese mass. Moreover, the size of the micro-capillaries in the cheese decreases during the ripening process. Micro-capillaries in the “Pokrovskiy” cheese, maturing for two days, are of 5-20 microns in size; at thirty days of maturation - 1-7 mm.

![Figure 4](image)

**Figure 4.** Micrographs of the capillary structure of the “Pokrovskiy” cheese (multiplicity of magnification 500 times) with a ripening period of: a - 2 days; b - 30 days.

Micro-capillaries are rounded. The structure of micro-capillaries, the arrangement of fat droplets and the structure of the protein matrix of mature “Pokrovskiy” cheese are shown in figure 5. Most often, micro-capillaries are formed at the junction of several macrograins. The number of micro-capillaries affects the cheese pattern, which is characterized by the shape and position of the holes. It is known that these pores are formed in the gaps between grains. The most favorable conditions for the release of gases are created by the smallest micro-capillaries between the cheese grains. The presence of gases in the micro-capillaries leads to the formation of pores. At a magnification of 2000 times, the following can be detected: the size of the micro-capillaries is (1-7) microns; fat droplets are (30-100) microns in size; the protein matrix is a layer of (5-15) microns in size.

![Figure 5](image)

**Figure 5.** Micrograph of the capillary structure of a 30-day-old “Pokrovskiy” cheese, (magnification 2000 times).
At various stages of ripening of the “Pokrovskiy” cheese, profiles of trace element composition were obtained (figure 6).

![Profiles of microelement composition of the “Pokrovskiy” cheese with different ripening period: a - 2 days; b - 10 days; c - 30 days; d - 90 days.](image)

**Figure 6.** Profiles of microelement composition of the “Pokrovskiy” cheese with different ripening period: a - 2 days; b - 10 days; c - 30 days; d - 90 days.

Dynamics of changes in the elemental composition of the cheese "Pokrovskiy" in the process of maturation is given in table 1.

**Table 1.** Dynamics of the elemental composition of the “Pokrovskiy” cheese during maturation, %.

| Element | Ripening period, days | 2     | 10    | 30    | 90    |
|---------|-----------------------|-------|-------|-------|-------|
| O₂      | 40.81                 | 43.09 | 50.58 | 62.50 |
| Na      | 1.59                  | 1.47  | 1.66  | -     |
| P       | 17.15                 | 19.51 | 14.40 | 7.54  |
| Cl      | 11.98                 | 8.62  | 11.92 | -     |
| Ca      | 28.47                 | 28.32 | 21.44 | 25.42 |
| Total   | 100.0                 | 100.0 | 100.0 | 100.0 |

The study of the elemental composition dynamics of the “Pokrovskiy” cheese in the process of ripening made it possible to establish some regularities. In the process of maturation, the content of
oxygen and sodium increases; decrease in phosphorus. The amount of chlorine and calcium practically does not change during maturation.

The increase in oxygen during maturation steadily increases from 2 to 10 days from 40.81 to 43.0%; from 10 to 30 days from 43.09 to 50.58%; from 30 to 90 days from 50.58 to 62.50%. Changes in the elemental composition of cheeses are associated with the characteristics of the ripening process. As a result of complex biochemical processes, products are formed that determine the organoleptic characteristics and pattern of the cheese.

Figure 7 shows the distribution maps of individual elements in the curd mass of the “Pokrovskiy” cheese of 2 days ripening and the distribution profile of these elements. In two-day ripening cheese, elements such as O2, Na, P, Cl, Ca are dispersed fairly evenly throughout the structure. On the distribution maps of P and Ca (figure 7 d, f), two “white spots” were found. Moreover, if to superimpose the two figures (7d and 7f), these spots coincide symmetrically with each other.
Figure 7. Distribution map of elements in the cheese mass of the “Pokrovskiy” cheese: a-profile of the trace element composition; b-oxygen; in-sodium; g-phosphorus; d-chlorine; e-calcium.

Compounds of P and Ca are calcium phosphate that is cleaved from paracasein during maturation. The deposition of calcium salts in the “Pokrovskiy” cheese was found at the early stages of maturation (2 days). These deposits of calcium salts are oval in size (8-10) microns. It is known that their size increases during maturation. The bulk of the salts are located in interlayers between macrograins, less - in macrograins. Lactic acid cleaves calcium phosphate and organic calcium from casein in the form of calcium lactate, resulting in an increase in the amount of soluble calcium in the cheese towards the end of maturation. With an excessively large accumulation of lactic acid, casein loses a significant part of calcium and binds moisture poorly, while acquiring a brittle crumbly consistency and poor pattern. If little lactic acid is formed, then the elimination of calcium from casein is delayed, as a result of which the cheese has a rubbery consistency. Therefore, in the process of ripening, the cheese must reach the acidity that is optimal for each type [14-20].

Figure 8 shows a linear spectrum of the elements’ distribution in the “Pokrovskiy” cheese with a ripening period of 90 days. Table presents the trace element composition of the “Pokrovskiy” cheese with a ripening period of 90 days at a distance of 1.83 mm.

Figure 8. Linear spectrum of the elements’ distribution in the “Pokrovskiy” cheese with a ripening period of 90 days: 1 - phosphorus; 2 - calcium; 3 – oxygen.
The linear spectrum of the distribution shows three characteristic peaks for P and Ca, that is, for calcium phosphate. It should be noted that the line characterizing P repeats the rises and falls of the Ca line. This fact confirms the presence of calcium phosphate in mature cheese and the possibility of its identification using a scanning electron microscope.

The line characterizing the oxygen content in mature “Pokrovskiy” cheese is evenly distributed. Consequently, oxygen as a component is distributed one-dimensionally throughout the entire thickness of the cheese.

As the cheese ripens, calcium salt deposits move inside the grain. At first, they are distributed evenly throughout the entire mass of curd grains, but after pressing, the bulk of the deposits are often distributed over the interlayers between the macrograins, and inside the macrograins they are smaller and smaller. The movement of calcium salts in the early stages of cheese production is associated with the diffusion of moisture from the center to the surface of the cheese. When moisture escapes to the outside, the interlayers between the macrograins allow moisture to pass and retain calcium salts, which are concentrated in the interlayers.

The increase in calcium salts during maturation is associated with the redistribution of salt granules and as a result of further destruction of the paracaseinate calcium phosphate complex and proteolysis of proteins under the action of bacterial enzymes.

4. Conclusions

Thus, based on the research carried out on the “Pokrovskiy” cheese, it was established:

- the pattern of the “Pokrovskiy” cheese begins to appear in the first days and is finally formed by 10-15 days of age;
- in the process of maturation, the size of fat droplets ranges from 80 to 150 microns. In overripe cheese (90 days) fat droplets decrease and have a size of 30 to 100 microns;
- in two-day ripening cheese, micro-capillaries are (5-20) microns in size; thirty days of maturation - (1-7) microns;
- the protein matrix is a layer of (5-15) microns in size;
- investigated the dynamics of changes in the elemental composition of the cheese "Pokrovskiy" in the process of maturation;
- the location of calcium phosphate in the curd mass at different stages of ripening is determined. The possibility of its identification using a scanning electron microscope has been proven.

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