Study of the microstructure of stained cheeses

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Abstract. A selection of methods for cheese mass coloring was performed. Cheeses stained with Sudan III according to Herxheimer's method have fat of a bright orange color, and on micrographs this fat is clearly distinguished in the form of droplets with a diameter of 30 to 100 microns. Moreover, the fatty droplets in the micrographs, regardless of the cheese type, are predominantly light in color (white). The microstructure of cheeses stained with Sudan III and Methylene Blue has a cellular structure. Their fat droplets in micrographs have a dark color (gray or black). The size of the fat droplets is the same as that of cheeses stained with Sudan III according to Herxheimer's method, from 30 to 100 microns.

1. Introduction
The purpose of the study is to select a method for coloring cheeses in order to analyse their microstructure.

The cheeses’ microstructure was stained using various techniques. Photographs of cheese microstructure were specially studied with varying degrees of magnification. When staining different cheeses with the same dye, general patterns are observed.

The fat in cheeses stained with Sudan III according to Herxheimer's method has a bright orange color. It is known that neutral fats are colored with Sudan III in this color [1, 2, 3]. This makes it possible to divide all the components of the fatty series of cheeses into two morphological groups: the group of neutral fats in the form of fatty drops and the group of lipoid and protein-lipoid macrograins, usually stained with Sudan III in a light-dark color. Lipoid and protein-lipoid micrograins make up the bulk of the fat component of cheeses, and it is they that give a yellowish tint to the general appearance of cheese preparations stained with Sudan III [4, 5, 6].

The structure of cheeses dyed with Sudan III and Methylene Blue is clearer and more pronounced in comparison with the structure stained with Sudan III according to Herxheimer's method. When dry cheese is stained with Sudan III according to Herxheimer's method, fat is melted. Therefore, this technique cannot be used to color fat in dry cheeses. Fat in dry cheeses should be stained with Sudan III and Methylene Blue. In stained cheeses, the size of calcium phosphate before drying varies from 5
to 10 microns, in cheeses after drying from 30 to 100 microns. The microstructure of stained cheeses was investigated. The cheeses stained with Sudan III and Methylene Blue have a clear pattern on micrographs. The sizes of capillaries (from 5 to 30 microns) and protein layers (up to 50 microns) of stained cheeses were determined. Before drying there were found particles of calcium phosphate with dimensions of 5-15 microns, after drying 10-20 microns. The spectra and maps of elements’ distribution in stained cheeses are investigated. The structure of dry cheese samples after vacuum treatment is more vivid in comparison with the structure of freeze-dried cheese samples, which is determined by the peculiarities of the drying processes. Profiles of the elemental composition of the “Pokrovskiy” cheese samples before drying, after vacuum and freeze drying treatment were obtained. The dry cheese showed, that calcium phosphate was in the form of characteristic formations, similar in size and shape to formations in the cheese samples before drying treatment [7, 8, 9, 10, 11].

2. Experimental section
One of the most important components of the cheese mass is milk fat [12, 13, 14]. Two methods were used to stain the fat of the cheeses:
- Sudan according to Herxheimer’s method (alkaline) is characterized by high coloring power. It strongly and brightly colors fats within (3-5) minutes [1, 15, 16].

| Components                                | Quantity, ml |
|-------------------------------------------|--------------|
| Ethanol or its 96° solution                | 70           |
| 10 % sodium hydroxide solution (NaOH)     | 20           |
| Distilled water                           | 10           |
| Sudan III                                | until saturation while boiling (up to 2g) |

- Sudan III and Methylene Blue. The presence of fat is determined by setting a sample with Sudan III. The coloring procedure was the following: 70 ml of 95% ethyl alcohol were heated up to 60°C (bain-marie) and 0.2 g of crushed Sudan III paint and Methylene Blue were dissolve in it. Then 10 ml (20-25)% ammonia solution and 20 ml of distilled water were added. This solution can be stored in a tightly closed bottle in the refrigerator for 6 months.

To check the sample for the fat presence, it is necessary to apply the required amount of the reagent, wetting the surface with it. After 10 seconds everything is washed off with water. The appearance of blue spots and streaks indicates the presence of fat.

3. Result section
Before drying, cheeses stained with Sudan III according to Herxheimer’s method (Fig. 1a; Fig. 2a) have a lot of free fat on the surface due to the fact that Sudan III according to Herxheimer’s method melts fat from the protein structure.
Dry cheese colored with Sudan III according to Herxheimer's method does not intensively melt fat. The cheeses stained with Sudan III and Methylene Blue have a clear pattern in micrographs (Fig. 1 b, d; Fig. 2 b, d). Capillaries of cheeses "Yaroslavskiy" and "Pokrovskiy" have sizes from 5 to 30 microns. The thickness of the protein layers is up to 50 microns. There were found particles of calcium phosphate with a diameter of (10-20) microns.
The study of the spectrum and maps of the elements’ distribution in the cheese “Yaroslavskiy”, colored with Sudan III and Methylene Blue before and after drying, revealed the content of: nitrogen, oxygen, phosphorus, sulfur, chlorine, calcium. The distribution maps of elements clearly show the content of phosphorus and calcium.

Before drying, the cheese "Yaroslavskiy" contained calcium phosphate in the form of irregular clusters along the cheese structure with dimensions (5-15) microns. After drying, calcium phosphate is evenly distributed over the entire surface of the cheese. The particle size of calcium phosphate is (10-20) microns.

Linear spectra of elemental composition were made on one sample of the cheese "Yaroslavskiy" in different sections. On the linear spectra of elemental composition, the curves of the phosphorus and calcium content have the largest peaks. Moreover, the discontinuous nature of the phosphorus and calcium lines exactly coincides with each other.

Linear spectra of elemental composition fully confirm the uneven distribution of calcium phosphate in cheeses before drying.

Of all the other elements, the oxygen content curve stands out. Oxygen is contained in almost all elements and structural formations of the cheese mass. Nitrogen, sulfur, chlorine are evenly distributed in the cheese.

Figures 3, 4 and 5 show micrographs (magnification factor is of 500 times) and profiles of the elemental composition of the “Pokrovskiy” cheese before drying, after vacuum and freeze drying treatment, respectively.

Figure 3. Micrograph (magnification factor is of 500 times) of the “Pokrovskiy” cheese structure and elemental composition profile before drying treatment.
With a multiplicity of magnification of 500 times, as well as with 100 times magnification, it is seen that the structure of the cheese before drying treatment is closed and contains a large amount of moisture (Fig. 3). There are fat globules on the cheese surface.

In vacuum-dried cheese, the protein matrix and the capillaries structure of the cheese mass are clearly visible. The structure of freeze-dried cheese is more acicular compared to vacuum-dried cheese. In the dry "Pokrovs'kii" cheese after vacuum and freeze drying treatment, fat is distributed in a thin film over the surface, and is also inside the protein structure. During the drying process, fat globules that are less than 2 microns in size are dispersed.

The presented micrographs of the “Pokrovs'kii” cheese structure confirm that fat is not melted out during vacuum and freeze drying treatment. This, in turn, testifies to the correct choice of operating parameters and high quality indicators of dry cheeses. When the operating parameters of the drying process deviate from the rational ones (an increase in the drying temperature or heat load), destabilization and melting of fat occurs. Free fat is susceptible to oxidation, which leads to premature deterioration of the product.

According to micrographs of dry cheeses, the thickness of the protein layers was determined to be from 5 to 15 microns. In large capillaries, the thickness of interlayers is greater than in small ones.
Comparison of the “Pokrovskiy” cheese micrographs before drying, after freeze-drying and vacuum drying treatment, prove that the cheese does not shrink during the process of vacuum and freeze-drying treatment. Since during freeze-drying, moisture is removed from the frozen product, and during vacuum drying treatment, the rate of moisture removal is intense and uniform.

Thus, based on the study of the microstructure of stained cheeses, the following conclusions can be drawn:
- cheeses stained with Sudan III and Methylene Blue have a clear pattern on micrographs;
- the sizes of capillaries (from 5 to 30 microns) and protein layers (up to 50 microns) of stained cheeses have been established;
- particles of calcium phosphate were found, before drying their dimensions were 5-15 microns, after drying 10-20 microns;
- the spectra and distribution maps of elements in stained cheeses have been investigated;
- according to the “Pokrovskiy” cheese micrographs before drying, after freeze-drying and vacuum drying treatment, it is certain that vacuum and freezedrying do not cause cheese shrinkage. The structure of dry cheese after vacuum drying is more developed in comparison with the structure of freezedried cheese, which is explained by the peculiarities of the drying processes;
- the capillary size of dry cheese samples after vacuum drying treatment is set from 40 to 100 microns; after freeze drying- from 5 to 60 microns;
- based on micrographs of dry cheeses, the protein thickness layer was determined to be from 5 to 15 microns. The thickness of the protein layers between large capillaries is greater (10-15 microns) than between small ones (5-10 microns);
- profiles of the elemental composition of the “Pokrovskiy” cheese were analysed before drying, after vacuum and freeze drying treatment. According to the profiles obtained, it is determined that the mass fraction of carbon, phosphorus, sulfur and calcium after freeze-drying and vacuum drying treatment practically does not change. The content of sodium, chlorine and potassium in dry cheese of vacuum and freeze drying treatment increases compared to cheese before drying treatment;
- in dried samples of cheese, Calcium phosphate was detected as particular formations, similar in size and shape to formations in the cheese before drying treatment.

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