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Original scientific paper

FEATURES OF MICRO- AND ULTRASTRUCTURE OF LOW-FAT BUTTER AND ITS LOW-FAT ANALOGUES

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ABSTRACT

The aim of the research was to study the features of the structure of low-fat butter and butter pastes, which, in terms of composition and properties, more fully meet the requirements of a healthy diet than high-fat types of butter. The objects of research were: butter with fat content of 72.5%; butter with fat content of 55% made with the addition of skimmed milk powder; butter of the same fat content with the addition of stabilizers based on guar and xanthan gums and emulsifiers based on mono- and diglycerides of fatty acids; butter pastes with fat content of 45% with similar additives used to increase the stability of the process of butter formation and improve the texture. The microstructure was studied using an MBI-6 microscope, and the ultramicrostructure was studied using a Phillips electron microscope. In the first case, the sample was prepared by crushing the sample, in the second one — by the method of ultrafast freeze-fracture and etching. Researches have shown that the use of the introduced ingredients improves the homogeneity of the structure of the studied products. Due to the ability of milk proteins and stabilizers to retain moisture, it is more evenly distributed and well retained in the fat matrix of the product, formed from crystalline and liquid fat in the form of a continuous phase, which is confirmed by a sufficient penetration depth of the fat-soluble dye. Plasma droplets in butter with fat content of 72.5% and 55% are more isolated than in butter pastes, as indicated by the greater penetration depth of the water-soluble dye. The average diameter of isolated moisture droplets in low-fat products was 3.3–5.4 µm, and the average diameter of the fat globules that form the basis of the crystalline framework was 5.4–7.4 µm, depending on the composition of the product. For butter with fat content of 72.5%, the values of these indicators were 2.8 and 4.0 µm. The results of the study indicate the presence of differences in the sizes of structural elements, but at the same time confirm the uniformity of the structure of low-fat products, allowing them to be attributed to dispersions «water-in-oil».

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1. Introduction

In the classical sense, butter is meant to be a product obtained from cow's milk with a mass fraction of fat of at least 80% (international standard CXS279–1971 «Codex standard for butter», as amended in 2003, 2006, 2010 and 2018). In fact, it is a fat concentrate with evenly dispersed milk plasma. The composition, ratio and distribution of phases in this product predetermines its structure and texture, physicochemical and rheological properties, flavour benefits and consumption areas [1,2,3]. In accordance with the Russian standard GOST R52253–2004 "Butter and butter paste from cow's milk. General specifications" a product of this composition is classified as a classic butter. Such butter retains its properties well during transportation under controlled conditions (not higher than 5 °C) and can be stored for a long time at low subzero temperatures, therefore it is classified as a strategic product used to provide the population in extreme conditions. However, under normal conditions, the consumer prefers butter with a lower fat content or its low-fat analogues, which, according to the international classification, are called «milk spreads», and in our country they are referred to as «low-fat butter» (with fat content from 50% to 79%), "butter pastes" (with fat content of 40 to 49%) and "cream pastes" (with fat content of 39% or less). They put less stress on the human body in terms of calorie content, saturated fatty acids and cholesterol [4,5]. Among these products, the most widespread is low-fat butter with a fraction of milk fat of 72.5%, which has a plastic texture, a pronounced flavour bouquet and good storage capacity at low temperatures above zero, therefore it is often taken as a reference standard when developing new types of low-fat products based on milk fat.

However, the true standard of comparison is butter of classic composition, produced by the traditional method — the method of cream churning. The basis of such butter, according to [2,3,6,7,8,9], is fat in various states of aggregation (in liquid and in the form of triglyceride crystals). Crystalline formations of milk fat, in contact with each other, form a spatial structure — a crystalline framework. The type of connection of structural elements in this crystal framework determines the type of structure: coagulation and crystallization. According to Rebinder P. A. et al. [9] good texture of butter of classical composition corresponds to a mixed type of structure — crystallization and coagulation one with a predominance of the latter, which characterizes the elasticity, plasticity and thixotropic recoverability of the product. The mixed type with a predominance of the coagulation structure is formed, as a rule, when it is produced by cream churning. In the case of the production of classic butter by the method of converting high-fat cream, the crystallization type of structure prevails, which determines the excessive hardness and fragility of the butter. The same method is used to produce low-fat butter and butter pastes, although in international practice, for the production of low-fat products, the homogenization (dispersion) process can be used, which is a simpler technological solution that does not require a particularly complex hardware design. With such technological methods, there are practically no restrictions on the minimum acceptable content of the fat phase in the product. However, obtained products with the use

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of homogenization, especially with a mass fraction of fat up to 40%, are characterized by the presence of an empty, unexpressed flavour, a feeling of wateriness. To improve their flavour and aroma, milk-protein additives, stabilizers and emulsifiers [10] are added to the source raw material before heat treatment and homogenization [10], which, along with ensuring the fullness of the flavour, increase the homogeneity of the system and prevent moisture separation when the structure is destroyed. This processing provides the product with a pasty texture with good spreadability. When homogenization is used in low-fat products, an "oil-in-water" product structure is predominantly formed. The aqueous phase in direct type products is continuous, contains proteins, carbohydrates, vitamins, mineral components and therefore is a good breeding ground for the development of spoilage microorganisms. The most common cause of deterioration in this type of food is mold caused by the development of Penicillum and Cladosporium during storage. To prevent mold growth, product manufacturers have to use preservatives [10], the attitude of the consumer towards which is unambiguously negative. Considering this, many researchers propose the formation of a "water-in-oil" structure, which provides a high degree of plasma dispersion as an alternative way to prevent microbial spoilage of low-fat products. Glaeser H. in [11] indicated that to obtain an emulsion of this type, the fat content in the product must be at least 34%. However, the study of products even with a high fat content (38–40%) shows that complete closure of moisture droplets in the continuous fat phase does not occur. In products of this composition, the aqueous phase is additionally structured due to its binding to proteins. As a result, in analogues of butter with a reduced fat content, the aqueous phase is simultaneously both in emulsified form and in the form of a continuous aqueous phase, i. e. such products are mixed dispersions, which are less susceptible to microbiological deterioration in comparison with direct "oil-in-water" dispersions [10].

When fatty products are produced by the method of converting a high-fat mixture, a crystallization structure is formed, the severity of which depends on the composition of the product and the operating parameters of the butter churn. The plastic texture of a product of low fat content is formed with an increase in the intensity of mechanical treatment, achieved by a decrease in the productivity of the butter by 15–30% and the introduction of milk-protein additives, stabilizers and emulsifiers into the normalized mixture [12]. The butter plasticity, in addition to the fat phase, is influenced by the distribution of its dispersed phase — milk plasma, most of which in high-fat types of butter is in the form of tiny droplets isolated from each other, and a small part of them is connected by the finest ducts and channels that permeate the entire mass product. Part of the plasma exists in a bound state and is firmly retained on the surface of fatty aggregates formed from solid and liquid fat, and on the shells of fat globules that are not destroyed in the process of butter production [6]. With an increase in the proportion of plasma in butter from 16–25% to 30–35%, its dispersion decreases by 1.3–1.6 times [13]. This leads to a decrease in the strength characteristics of the product and an increase in its fragility [14].

With an increase in the proportion of plasma, its importance in the formation of the product structure increases. An important element of this structure in products of low fat content (40–55%), according to Gulyaev-Zaitsev S.S. and Belousov A. P. [15], is "the presence of a large number of continuous, thin liquid layers of milk plasma, permeating the entire monolith of the product and including a significant fraction of its aqueous phase. The stabilization of the thin-layer structure of the aqueous phase in the liquid fat is achieved by interfacial adsorption layers with mechanical strength, formed by milk plasma proteins and other surfactants on both surfaces of each water layer. Such a distribution of the unencapsulated aqueous phase achieves a rather strong fixation of it in the product structure, as a result of hydration of the surfaces of both interfacial layers. The combination of a large number of interfacial adsorption layers with mechanical strength and associated with both phases can be considered as the second skeletal structure of such products." This is the main distinguishing feature of low-fat butter compared to conventional butter. The better the plasma is distributed and the adsorption layers are stabilized, the more stable the structure of the low-fat butter is.

Studies carried out at VNIIMS on the effect of the composition of low-fat types of butter and butter pastes in the fat content range of 45–55% on the process of butter formation and the formation of the main structural, mechanical and rheological characteristics are described in [16]. These studies have shown a positive effect of the introduced skim milk, stabilizers and emulsifiers on the process of butter formation and the formation of a good texture of low-fat products. Of interest is to study the effect of these additives on the micro- and ultrastructure of foods that reflect the internal structure of low-fat foods. This article is devoted to this issue.

2. Materials and methods

The objects of research were experimental samples of butter with a mass fraction of fat of 72.5% (control), experimental samples of butter with a mass fraction of fat of 55%, as well as butter pastes with a mass fraction of fat of 45%, made by converting the corresponding normalized mixtures in a cylindrical butter churn. To ensure the stability of the structure formation process and good moisture retention in low-fat products, the Palsgaard 0291 emulsifier based on mono- and diglycerides with an iodine number of 60 g I_2/100 g and the complex stabilizer Palsgaard 5232, based on guar and xanthan gums, in the following quantities: for a product with fat content of 55% — 0.5% and 0.2%; for a product with fat content of 45% — 0.5% and 0.25%, were added to the normalized mixtures. The manufacturer of these additives is the Danish company of the same name.

Low-fat butter and butter pastes with the addition of skimmed milk powder were also used as objects of research. For butter, its amount was calculated in such a way as to obtain the mass fraction of milk-solids-non-fat (MSNF) of 7.5% in the product, and for butter pastes — 15.0%. Processing parameters of mixtures: pasteurization temperature of normalized mixtures, composed on the basis of high-fat cream, buttermilk and added additives, was 85 °C with holding for 15 minutes; temperature of the normalized mixture at the inlet to the laboratory cylindrical butter churn — 75 °C; product outlet temperature — 15–17 °C; coolant temperature (ice water) — 2 °C; intensity of mechanical action on the processed product — 35.8–40.3 W/kg.

The continuity of the fat and water phases of the studied products was assessed by the method of diffusion of fat- and water-soluble dyes described in [15]. To do this, a glass tube with a diameter of 10 mm and a height of 5 cm was carefully pressed into the product monolith to a depth of 3 cm; the tube with the product was removed, and placed vertically in a stand rod. A fat-soluble dye Sudan III and a water-soluble dye of methylene blue in an amount of 3 cm^3 were applied to the surface of the product. The sample was left at room temperature for 20 days and the depth of penetration of the dyes was measured.

Microstructure studies were performed using an MBI-6 (Russia) and an analogue-digital camera «Olympus». The specimens were prepared by crushing: a small amount of the product was placed on the slide with a metal needle; a cover slip was carefully placed on top, which was pressed against the slide with a 100 g weight; left for 1–2 minutes at room temperature. Microscopic examination was performed in transmitted light in a bright field with direct illumination of a transparent light filter. During the work, a 40^x objective with a $K7^x$ eyepiece was used. The prepared specimens were examined under a microscope and the most typical fields were photographed. Electron microscopic studies were carried out using a transmission electron microscope EM-410 «Phillips» (Netherlands). Specimens for research were prepared by the method of ultrafast freeze-fracture and etching with carbon replication and contrasting with platinum according to the methods described in [17] modified by Smykov I. T. [18]. For this, a sample of butter and butter pastes was frozen with liquid nitrogen at minus 190 °C at a rate of up to 300 °/s. The frozen sample was placed in a vacuum chamber, where a vacuum of 10^{-6} mm Hg was created. Then the sample was split, and the plane of its split was sputtered with platinum 4–6 nm thick at an angle of 30 °. Then a carbon layer with thickness of 20–30 nm was deposited orthogonally onto the split plane. After deposition, the sample with the replica was removed from the vacuum chamber; the replica was taken onto the liquid surface and washed with a hypochlorite solution. The resulting replica was analyzed using an electron microscope at an accelerating voltage of 80 thousand watts and a magnification of 6600 times.

3. Results and discussion

The results of microstructure studies (Figure 1, left row) illustrate that with a decrease in the mass fraction of fat in butter and butter pastes, an increase in the size of plasma droplets was observed with a simultaneous increase in the unevenness of their distribution. However, due to the high moisture-binding capacity of the introduced components (milk-protein additives, stabilizers), milk plasma was well retained in low-fat products. No release of free moisture droplets was observed on their cut. Considering the nature of the distribution of milk plasma, it can be concluded that from normalized mixtures with skimmed milk powder, as well as from mixtures with stabilizers and emulsifiers, it is possible to obtain low-fat butter and its analogues with a structure similar to that of butter with fat content of 72.5%, i. e. “water-in-oil” type. However, the pictures show that both the prototypes with an increased content of MSNF (Figure 1, a and c) and the samples with stabilizers and emulsifiers (Figure 1, b and d) differed from the control samples (Figure 1, e) by an increased amount and less dispersion of plasma drops. If the average diameter of plasma droplets in butter with a mass fraction of fat of 72.5% under the conditions of the experiment was 2.8 µm, then in experimental samples of butter pastes with fat content of 45% it was 4.9 µm (for a product with an increased content of MSNF) and 5.4 µm (for product with structure stabilizers). The average diameter of plasma droplets in butter with a fat/MSNF ratio of 55.0%/7.5% was 3.0 µm, and butter of similar fat content with structure stabilizers — 3.3 µm (Table 1).

The nature of the distribution in the studied products of the main structural elements of the crystalline framework — fat globules — can be traced both in the photographs with a magnification of 6600 times (Figure 1, right row), and according to Table 1.

Comparison of the ultramicrostructure of experimental samples of butter pastes of 45% of fat and butter of 55% of fat with the addition of skimmed milk powder, stabilizers and emulsifiers with control samples of 72.5% of fat made it possible to reveal both the similarity of their structure and some differences. Thus, in the control butter (Figure 1, e), against the background of a continuous fatty phase, which is both in a crystalline and in a gel-like state, individual fat globules are seen, which make up the main structural framework of the product; there are individual fat globules that are not destroyed in the process of thermomechanical action on the original cream in the butter, as well as fragments of the shells of fat globules and milk plasma in the form of small droplets and interlayers, which is consistent with the results of studies of the microstructure of butter products carried out by other researchers [15,19,20].

The presented images demonstrate that in the test samples of products (both with an increased content of MSNF, and with stabilizers and emulsifiers), fat globules are larger and less evenly dispersed in the volume of the product, which causes a weakening of the structural bonds between them. In these products the weakening of the structural bonds between the fatty components and the increased role of the non-fatty component, which is milk plasma with an increased content of MSNF, can be traced by the change in their structural, mechanical and rheological parameters relative to the control sample of butter, described in [16]. It reflects in a decrease in the hardness and recoverability of the structure, complex shear modulus, elastic modulus and dynamic viscosity of butter and butter pastes made with the addition of MSNF. The weakening of structural bonds between fatty components in products with a high content of MSNF is comparable to that observed in samples of butter and butter pastes of similar fat content, developed with structure stabilizers.

Of great importance in the formation of the structure of low-fat butter and butter pastes with the addition of MSNF is the non-fat phase, which forms less rigid structural bonds than those formed mainly by fat components in butter of 72.5% of fat. The increased content of MSNF in the product contributes to an increase in heat resistance and emulsified fat content, noted in [16]. It should be noted that if the differences in the structural, mechanical and rheological parameters of butter pastes made with MSNF and structure stabilizers are insignificant, then they are present in butter samples: butter with an increased content of MSNF is characterized by higher values of rheological parameters, including hardness.

The depth of penetration of a fat-soluble dye (Sudan) into the structure of control samples of butter under the experimental conditions was 12 mm, in experimental samples of low-fat butter and butter pastes it was 1.1–1.2 times lower. Methylene blue penetrated into the butter of control and experimental samples in trace amounts. The water-soluble dye penetrated into butter pastes with fat content of 45% to a depth of 1 mm, which characterizes the lower closure of plasma drops in products of this composition compared to butter and indicates a greater development of thin milk plasma capillaries in it, which is consistent with the data of other authors [16]. At the same time, the

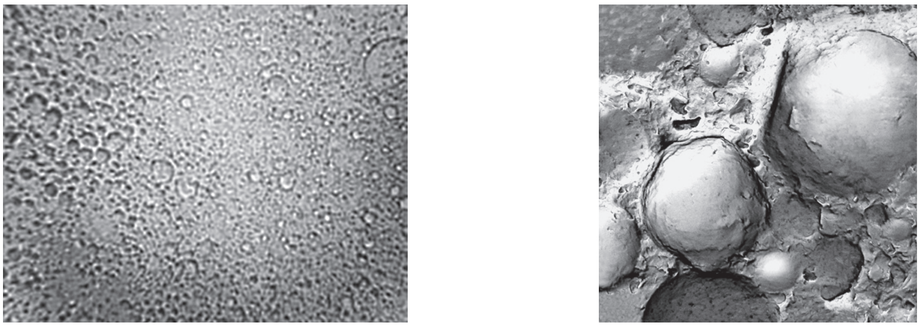
Table 1

Parameters of distributions of plasma droplets and fat globules by size

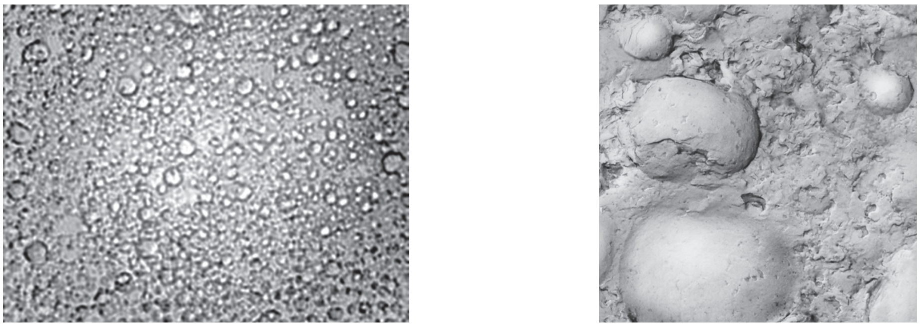
Name indicator	Indicator value for a product sample, µm ± standard deviation				
	Butter with m. f. f.* of 72.5%	Butter with m. f. f.* of 55.0% MSNF of 7.5%	Butter with m. f. f.* of 55.0% with stabilizers and emulsifiers	Butter paste with m. f. f.* of 45% MSNF of 15%	Butter paste with m. f. f.* of 45% with stabilizers and emulsifiers
Average diameter of milk plasma droplets, µm	2.8 ± 0.9	3.0 ± 1.1	3.3 ± 1.3	4.9 ± 1.8	5.4 ± 2.3
Average diameter of fat globules, µm	5.0 ± 1.9	5.4 ± 2.1	5.6 ± 2.2	7.4 ± 3.1	6.0 ± 2.4

* mass fraction of fat

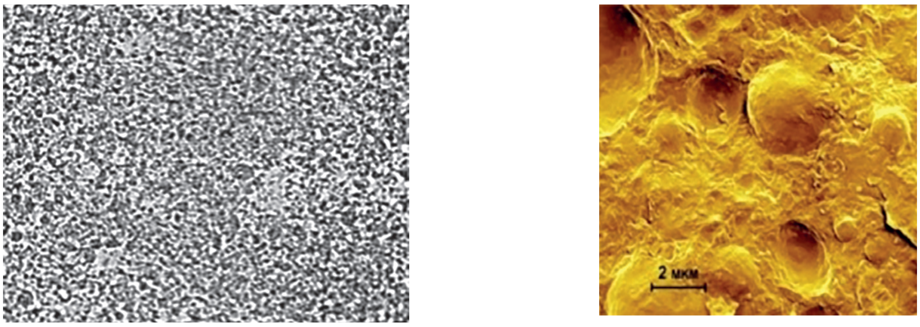
Left row: 280 x magnification Right row: 6,600 x magnification



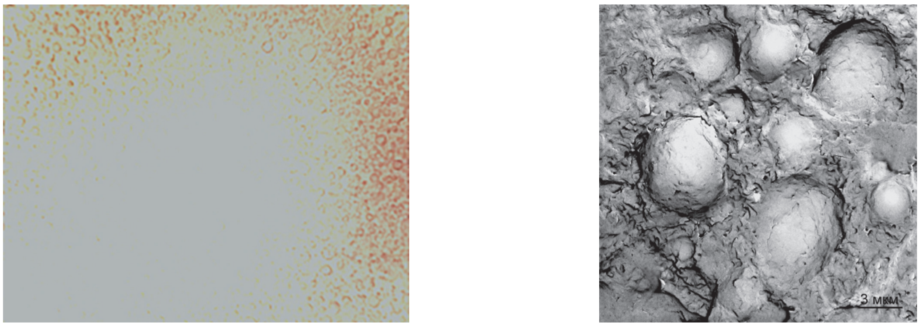
a) butter paste with a mass fraction of fat of 45%, MSNF of 15%;



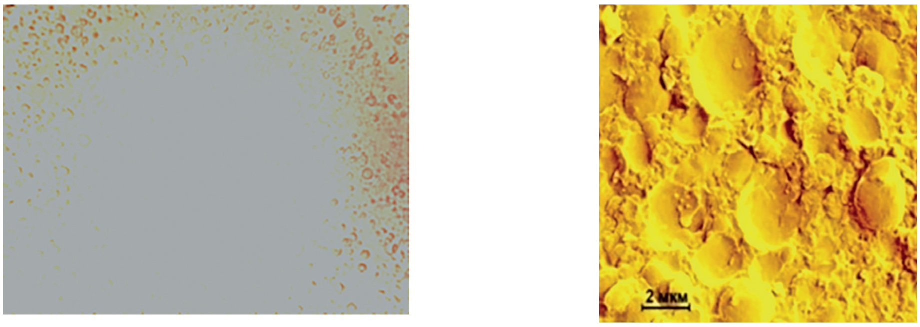
b) butter paste (mass fraction of fat of 45.0% with stabilizers and emulsifiers);



c) butter with a mass fraction of fat of 55%, MSNF of 7.5%;



d) butter with a mass fraction of fat of 55% with stabilizers and emulsifiers;



e) butter with a mass fraction of fat of 72.5% (control).

Figure 1. Microstructure (left row, 280^x magnification) and ultramicrostructure (right row, 6600^x magnification) of products of low fat content

prevailing penetration of the fat-soluble dye into the product over the water-soluble one confirms the nature of the structure, close to the structure of butter with fat content of 72.5%. Low-fat butter and butter paste are "water-in-oil" products, which allows predicting good storage properties of these products due to the predominant moisture content in the encapsulated form. At the same time, moisture in these products is partially associated with proteins and stabilizers, therefore, its activity will be significantly reduced in comparison with the moisture found in products without adding water-binding ingredients.

4. Conclusion

Based on the results of the studies, it was found that the use of a milk-protein supplement in the form of skimmed milk powder, as well as food additives in the form of stabilizers and emulsifiers in the production of low-fat butter and butter pastes with a mass fraction of fat of 55% and 45%, respectively, makes it possible to obtain a product with the type and nature of the structure close to butter of 72.5% of fat, made without using these additives. Due to the higher plasma content, its more significant effect on the structure of the product appears, which consists in the appearance of additional structural elements (channels and ducts) between its drops, dispersed in the fat phase of the product, which affects the structure of the finished product and the depth of penetration of the water-soluble dye into the product. The structural features of low-fat butter and butter pastes are also the less fine distribution of milk plasma droplets and the presence of larger fat globules involved in the formation of the fat matrix of these products.

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