

# FREE FAT IN MILK AND CHEESE PRODUCTS: INFLUENCE ON QUALITY

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## ABSTRACT

The theoretical and practical aspects of the formation of free fat in milk, cheese and cheese products with vegetable fats are considered. The amount of free fat in milk depends on the integrity of the fat globules membranes, which are affected by: ineffective emulsification of fat during the synthesis of milk in the cow's udder (authentic fat) and physic-mechanical factors in the milk processing after milking (destabilized fat). Free fat, primarily subjected to oxidative processes and lipolysis, reduces the quality and ability to store milk. For cheese products, the presence of free fat, available for lipolysis and oxidation, is a prerequisite for obtaining high-quality products. Characteristics of the production of cheese products with vegetable fats, providing for the preliminary emulsification of vegetable fat in skim milk, contribute to the formation of denser membranes on the surface of fat globules, consisting mainly of casein micelles and whey proteins. This is the reason for the formation of a more closed structure of the fat phase with low availability of fat for enzymes and oxidizing agents. The low availability of fat in the structure of cheese products with vegetable fats is one of the factors that worsen their organoleptic characteristics. In order to increase the amount of available fat in the production of cheese products with vegetable fats, it is advisable to use additional enzyme preparations or cultures of microorganisms that activate proteolysis and lipolysis.

## 1. Introduction

Fat is an important component in the composition of milk and dairy products. It performs an energy function, is a source of biologically valuable polyunsaturated fatty acids, fat-soluble vitamins, provitamins and other compounds necessary for the normal functioning of the human body. The presence of fat in milk and dairy products has a positive effect on their taste and texture.

In the production of cheesemaking products, the structure of the fat phase of milk substantially changes under the influence of a complex of physical, microbiological, biochemical factors. In particular, free fat is formed, which is most susceptible to change under the influence of enzymes. This is important for the formation of the necessary organoleptic characteristics of cheese. The presence of free fat in the structure of cheese products with vegetable fats, which differ from cheeses in less pronounced cheese taste and smell, is of special significance. This is due both to a change in the fatty acid composition and to a decrease in the availability of fat for enzymatic transformations due to the formation of denser membranes on the surface of fat globules. In this regard, studies of the mechanism of formation of free fat in milk and cheese products, including with vegetable fats, are currently relevant, as they are the scientific basis for a directed influence on the quality of finished products.

## 2. Main part

Fat in milk is in the form of a natural emulsion, the structural elements of which are fat globules enclosed in a membrane — a specific biological membrane (MFGM), which is formed in the secretory cells of the mammary gland [1]. The main mission of the membranes formed on the surface of milk fat globules is to create a fine dispersion of fat to facilitate digestion by newborn offspring [2]. The membranes of the fat globules separate the fat from the aqueous medium and other structural elements and act as a stabilizer of the fat emulsion. The main components of the membrane are proteins (25–60%), phospholipids (up to 25%), cerebrosides (about 3%), cholesterol (about 2%), as well as minerals and minor organic compounds [3,4,5,6,7,8,9].

In addition to coated fat globules, milk contains free fat. There is still ambiguity in the definition of free fat [2]. So, the following can be attributed to free fat:

- fat inside damaged globules or fat that is not sufficiently enclosed in an intact membrane,
- fat that has leaked from damaged globules;
- fat extracted by centrifugation,
- solvent extracted fat.

The last two definitions assume the dependence of the amount of measured free fat on the used method.

The presence of free fat in milk is caused, firstly, by insufficiently effective emulsification of fat in the synthesis of milk in a cow udder and, secondly, by a violation of the integrity of the fat globules membranes. A. N. Petrov [10] proposed to identify free fat as «authentic» in the first case, and in the second one — as «destabilized». The amount of authentic free fat in milk depends on the genetic characteristics of the animal, the conditions of its maintenance and feeding, and the lactation stage.

Destabilized fat is formed as a result of physical and mechanical effects on fat globules during milking, filtration, mixing, transfusion, during the milk transportation. Obviously, the amount of formed free fat correlates with the degree of damage to the fat globules membranes. Damage to MFGM during stirring and agitation largely depends on the ingress of air bubbles into the milk, upon contact with which, the membrane material of the membrane is cut and subsequently spread along the interface between the milk plasma and the air. To destroy the membrane in the absence of air bubbles, large shear forces are required [1].

Stirring raw milk with a stirrer for 1 min at 600 rpm doubles the amount of free fat [11]. With mechanical shaking, an increase in the amount of free fat is accompanied by a change in the average diameter of the fat globules, depending on temperature: it increases at 5 °C (coalescence predominates), it decreases at 45 °C (dispersion predominates) [12].

According to L. S. Tolstukhina [13] fresh milk supplied to dairy enterprises of the Russian Federation in the 1970s contained from 0.34 g/kg to 0.79 g/kg (from 0.03% to 0.08%) of free fat.

With further processing of milk into dairy products under the influence of various factors, the structure of the fat phase changes. The membranes on the surface of the fat globules are damaged or completely disappear, which causes an increase in the amount of free fat [6]. For example, the separation of milk increases the amount of free fat by 4 times, which is distributed between skim milk and cream in a ratio of 1:200 [11,13].

Milk homogenization causes a decrease in the size of fat globules and a change in the composition of their membranes. Compared to natural MFGM, the membranes of small fat globules formed during homogenization consist of casein micelles, individual submicelles, serum proteins, and fragments of the destroyed native membrane [7,14]. Caseins account for about 70% of the proteins in the membrane of the milk fat globule. The composition of the proteins that form the membrane on the surface of milk fat globules in homogenized milk is not affected by the homogenization pressure and fat concentration, but the heat treatment, which is applied before homogenization, has a significant effect. In milk heated to 85 °C for 20 min, the ratios of adsorbed  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin relative to adsorbed caseins were higher than in milk heated to 65 °C [14]. An increase in pressure and temperature during homogenization helps to reduce the amount of free fat in whole milk [12].

The effect of milk homogenization on the state of milk fat in Mozzarella cheese was considered in a study [15]. It has been shown that the fat in the structure of this cheese exists in two forms: in the form of small highly emulsified globules inside a dense protein matrix, and in the form of larger, free drops of fat within the serum phase. Milk homogenization has led to an increase in cheese viscosity. This is explained by the fact that small emulsified fat globules obtained as a result of homogenization are embedded in the protein structural matrix of cheese, thereby contributing to its strengthening.

During the manufacture of dairy products, milk is exposed to both low and high temperature effects. The heating and cooling of milk causes changes in the composition of MFGM due to the adsorption of surface-active components of milk plasma and the selective or non-selective desorption of components of MFGM. In turn, this can affect other properties of the fat globule, such as stability, electrokinetic or  $\zeta$ -potential [1].

Our studies [11] showed that pasteurization of milk at a temperature of 73 °C for 1 min reduces the amount of free fat by 3 times, subsequent cooling to a temperature of 9 °C with holding for 24 hours increases by 4–5 times. The decrease in the amount of free fat after pasteurization can be explained by the denaturation of whey proteins, which become more surface active and precipitate on the surface of fat globules, isolating free fat [8].

J. Norlund, M. Heikonen [16] proposed a theory of the formation of free fat during milk cooling, based on the results of electron-microscopic studies of the interaction of the inner surface of the fat globule membrane with a fraction of high-melting milk fat triacylglycerides. Its essence is as follows.

When cooled, fat triacylglycerides inside the fat globule crystallize in layers, starting from layers adjacent to the membrane. During crystallization, some fractionation of triacylglycerides occurs. The high-melting fraction, which crystallizes first, is tightly fixed on the inner surface of the membrane, as a result of which the membrane loses its elasticity. Low melting triacylglycerides are concentrated as the liquid core of the fat globule. The ratio between solid and liquid fractions depends on the degree of diffusion and crystallization of the molecules of liquid triacylglycerides. Both processes are a function of temperature. Dilatometric studies have found that crystallized fat has a smaller volume than the same mass of liquid fat. Since crystallization occurs from the surface of the fat globule inward, with decreasing temperature, the liquid triacylglycerides inside the globule experience pressure

from the crystallized fat caused by the mutual attraction between the molecules in the crystal lattice. The crystal structure is broken even with weak compression of liquid triacylglycerides. The fat ball becomes unstable and remains in this state for a long time. This theory explains why the fat of rapidly chilled milk easily becomes free as a result of mechanical stress.

I.E Veiti, T. F. Fryer [17] studied the release of free fat from whole milk when exposed to low temperatures during fast (for 1.1 s) and slow (for 1 h) cooling to a temperature of 7.5 °C. The chilled milk was then subjected to aeration for 3.5 hours in a thermostat with a temperature gradient and a fluctuation of 30 °C for 1 min. The results were as follows.

Regardless of the preliminary temperature effect on milk, an increase in the amount of free fat was noted already at a temperature of 15–20 °C, and the maximum amount at 25–35 °C. The modes of milk cooling did not affect the release of free fat; however, mixing of milk during storage after aeration was of great importance. So, the maximum amount of free fat after aeration of milk, which was stored with stirring for 2 days at temperatures of 15 and 7.5 °C (slow cooling) and 7.5 °C (fast cooling), was 78%, 66% and 80%, respectively. When milk was stored without stirring, the maximum free fat content was 56%, 59% and 63%, respectively. The authors believe that the appearance of free fat in milk and cream is associated with aeration, which takes place most intensively at temperatures of 20–40 °C. The effect of mixing milk during storage on the formation of free fat is not the same and depends on the nature of the cooling.

From the theory proposed by Finnish researchers [16], it follows that free fat released through damaged membranes of globules consists mainly of low-melting fractions of triacylglycerides and contains more low-molecular and unsaturated fatty acids than total fat. Therefore, milk containing destabilized fat is more sensitive to the development of defects of a chemical origin. Liquid fusible fractions of fat globules emerging on the surface of the fat globule through damaged membranes become accessible to hydrolytic enzymes and to substances that cause its oxidation. Therefore, milk containing a large amount of free fat spoils faster [18,19]. During long-term storage, such milk acquires an oleous taste, which occurs due to the accumulation of oxidation products of linoleic and arachidonic fatty acids [16].

This is consistent with the results of research by Russian scientists [13], which showed that the destabilized fat of milk and cream was always characterized by greater oxidation than all fat extracted from the product, and the degree of oxidation depended on the season of the year (Table 1).

Table 1  
Fat oxidation in milk obtained in different seasons [12]

Season	Fat oxidation, oxidation degrees	
	total	destabilized
Summer	19.00	41.35
Autumn	20.60	27.02
Winter	7.80	10.44
Spring	5.04	18.79

To an even greater extent, the effect of free fat on the quality and storage capacity is manifested in whole milk powder. V. D. Kharitonov, L. V. Petrova and S. V. Petrova [22] found that the quality of whole milk powder is affected only by the fraction of free fat that is on the surface of the particles, since the oxidation of free surface fat is 5–8 times greater than the oxidation of all fat of whole milk powder. A 50% increase in free surface fat content contributes to a 20% increase in the oxidation of all product fat.

When storing whole milk powder with plenty of free fat, a rancid taste appears. Therefore, manufacturers are trying to reduce its content in the finished product. It is believed that when

the total fat content in milk powder is less than 26%, its free fat content is low (less than 10% of the total fat content), with an increase in the mass fraction of fat above 26%, the free fat content sharply increases [23].

It should be noted that the desire to reduce the free fat content in milk powder is not always justified. For example, in technological processes for the production of confectionery products using milk powder, a high free fat content (up to 80%) reduces the viscosity of the intermediate product, which reduces the energy costs of production and contributes to a uniform texture of the finished product [24,25].

Numerous research works have been devoted to the study of free fat in whole milk powder obtained by various methods and to its effect on the quality and storage capacity [20,26,27,28,29,30,31,32,33,34,35,36,37,38,39]. The most systematic studies of the influence of various factors on the state of free fat in milk powder are presented in a series of articles and thesis paper by T. J. Buma [30,31,32,33,34,35,36,37,38,39]. The division of the general concept of «free fat» into separate categories depending on the condition and location in the structure of the product belongs to him. Based on the fact that the term «free fat» actually means recoverable fat (since most methods for determining its amount involve extracting fat from a dairy product) T. J. Buma proposed a physical model, dividing the recoverable fat into four forms:

- ❑ free fat on the surface of the particles;
- ❑ free fat in the surface layer of the product;
- ❑ free fat located in the capillaries of the product, which can be extracted with solvents through capillary pores or cracks;
- ❑ buried fat, consisting of fat globules located deep in the product that can be removed by solvents through openings left by dissolved fat globules in the outer layer or close to the wide capillaries in the product.

Obviously, there is also fat that is not recoverable by solvents in whole milk powder. This is the fat inside the fat globules with an intact dense membrane. In addition, drops of milk fat without membranes can be enclosed within the protein matrix of the product so that they remain inaccessible to solvents.

Proposed by T. J. Buma classification of the state of the fat phase in the structure of whole milk powder can be used in relation to cheese products, which, like milk powder, are a concentrate of dry substances and have a dense structure formed by the removal of large amounts of water.

In the protein matrix of cheese, fat is in the form of: coated fat globules; coated conglomerates of fat globules; small homogenized fat globules with a diameter of less than 4 microns, mainly coated with casein; accumulations of fat globules, as well as free fat [40]. Globules of natural milk fat, globules of fat with a membrane of caseins and whey proteins and free fat have different physicochemical composition of surface membranes. Because of this, milk fat can interact differently with the protein matrix and therefore have different effects on the organoleptic and physicochemical properties of cheese [7].

As in other dairy products, free fat in cheese is most susceptible to lipolysis and oxidation. However, if this causes spoilage in milk, then in cheese the products of lipolysis and oxidation are involved in the formation of taste and aroma [9,21,41,42,43]. However, in dry cheese, obtained in the form of a powder, a high content of free fat, which increases even more during storage, can limit its shelf life [44].

In the review of D. W. Everett, M. A. E. Auty [45] evaluated the role of fat in the formation of organoleptic characteristics of cheese. To date, discussions on the mechanism of the effect of fat on the taste and aroma of cheese are conducted. Several reasons are considered: the presence of fat-soluble flavoring substances, the participation of components of the fat globules membranes in the formation of taste, the formation of free fatty acids as a result

of lipolysis, and the presence of free fat bordering the aqueous phase, which is necessary to maintain high activity of lipolytic enzymes and the formation of flavor compounds. In all probability, all these mechanisms are involved in the formation of the taste and aroma of cheese, and the positive role of free fat is undeniable. Moreover, it practically does not affect the consistency [46].

The presence of free fat is a prerequisite for lipolysis of fat, resulting in the formation of substances involved in the formation of the taste and aroma of cheese during its maturation. This is especially important for cheese products with vegetable fat, which differ from cheeses in less pronounced cheese taste and smell. The production of cheese products with vegetable fats is widespread in the Russian Federation along with the production of cheese [47,48].

The fundamental difference between the technologies of cheese products and cheese is that it includes an additional block of operations for the preparation of an emulsion of vegetable fat. The resulting fat emulsion is then introduced into a mixture of whole and skim milk, from which cheese products are made with partial replacement of milk fat, or mixed with skim milk, if a full replacement of milk fat is provided.

As a rule, emulsification of vegetable fats is carried out in skim milk, sometimes using skim milk powder, specially introduced into the mixture in small quantities [49,50,51]. Emulsification of vegetable fats can be carried out in whey and buttermilk. However, whey proteins have a lower emulsifying ability compared to skim milk and buttermilk proteins [52,52,54]. Buttermilk is the best medium for the manufacture of emulsions [54,55,56]. Its emulsifying effect is due to the cooperative effect on the formation of membranes of the fat globule of whey proteins, caseins and envelope proteins of milk fat globules. However, protein isolates of the membranes of the milk fat globules are poor emulsifiers. Apparently, this is the result of heat treatment, as a result of which  $\beta$ -lactoglobulin interacts with the proteins of the fat globules membranes, which negatively affects the manifestation of their emulsifying properties [57].

Despite the best emulsifying properties of buttermilk, its use for emulsification of vegetable fats is undesirable, since the subsequent use of such an emulsion in the production of cheese products results in a flabby gel, which is unacceptable in cheesemaking.

If by-products of the production of dairy products (skim milk, whey, buttermilk) containing milk proteins are not used for emulsification of vegetable fats, then, as it was established by Pechenik N. V. and Tereshchuk L. V. [58], for direct emulsions (butter / water) the best emulsifying ability is possessed by a mixture of phospholipids with monoglycerides in a ratio of 1:1. However, this practice is not applied in industry. Basically the emulsification of milk fat replacers is carried out in skim milk.

In this case, milk proteins act as emulsifiers of fat, forming a dense membrane on the surface of fat globules, which prevents the contact of fat with enzymes. This is one of the reasons that cheese products with vegetable fats have a less pronounced taste and smell [45,59,60,61,62]. To achieve good organoleptic characteristics, they must ripen for a longer time compared to cheeses [59]. This gives time for the production by starter microorganisms of a sufficient amount of lipases, phospholipases, proteinases and glycosidic hydrolases, which can not only ensure the accumulation of lipolysis products of fat, but also damage the dense membranes of fat globules, providing access of enzymes to open fat [1].

A long ripening process is economically disadvantageous. This process can be accelerated if, in addition to the milk-clotting enzyme preparation, additional enzymes are used that enhance the hydrolytic processes that occur during the maturation of cheese products with vegetable fats [63,64,65,66,67], or by using, in addition to the main starter culture, additional microorganisms whose enzymes enhance proteolysis and / or lipolysis [68,69,70,71,72,73].



### 3. Conclusion

An analysis of the above research works shows that free fat in milk and dairy products plays an important but ambiguous role in shaping the quality of milk and dairy products. Its presence in the structure of the product affects organoleptic characteristics and storage capacity. For whole milk products, an increase in the amount of free fat in the fat phase is undesirable, since it is most susceptible to oxidation and enzymatic transformations that cause spoilage. In cheese products, on the contrary, the presence of a sufficient amount of free fat available for lipolysis and oxidation is a prerequisite for obtaining high-quality products. The high availability of fat for cheese products with

vegetable fats, which, in contrast to cheeses, have a more closed structure of the fat phase, due to the peculiarities of emulsification of vegetable fat before introduction into the structure of the product is of particular relevance. In order to increase the amount of available fat in cheese products, it is advisable to use additional enzyme preparations or cultures of microorganisms that activate proteolysis and lipolysis.

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