

# STUDY OF FATTY ACID COMPOSITION OF MILK FOR CHEESE PRODUCTION

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## KEY WORDS:

*milk, cheese, milk suitability for cheese, fat phase of milk, fatty acid composition*

## ABSTRACT

The article shows that the studied samples of raw milk in terms of composition (mass fraction of fat, protein, lactose and milk solids-not-fat) met the criteria of suitability for cheese, and its physico-chemical properties (titratable acidity, density, freezing point) were within acceptable limits. Moreover, rennet sample corresponded to the first and second classes. It was proved that the number of spores of lactating fermenting microorganisms met the requirements for milk for the production of any kind of cheese. It was established that the fatty acid composition of raw cow's milk used to produce cheeses at various enterprises varied significantly. The data indicate the variability of the content of all groups of fatty acids — low molecular weight, saturated, monounsaturated and polyunsaturated. As a result of gas chromatography studies using the chromatographic complex «Chromos GX-1000» with a flame ionization detector and a CP 88 quartz capillary column — Sil 88 for FAME100 m×0.25 mm×0.2 μm, it was found that the largest absolute fluctuations were for saturated (±7.03% of the average value) and monounsaturated fatty acids (±3.77% of the average value). Absolute fluctuations in the group of low molecular weight fatty acids amounted to ±2.62%, and in the group of polyunsaturated — ±1.02% of the average value. The calculation of the relative deviation showed that the most varied groups were the ones of low molecular weight fatty acids (±28.40 rel.%) and polyunsaturated fatty acids (±25.11 rel.%). At the same time, a relatively high content of certain fatty acids: myristoleic, palmitic, palmitoleic and low levels of stearic and oleic fatty acids, was revealed in individual milk samples.

## 1. Introduction

The quality of any dairy product directly depends on the composition and properties of the raw milk used, as well as on the features of technological methods and processing modes used in its manufacture.

In the cheese production, it is extremely important to use «milk suitable for cheese». This concept means a set of properties associated with the usefulness of the milk composition, its ability to coagulate under the action of rennet and to be a favorable environment for the development of starter microorganisms used in the cheese manufacture [1]. Considering this, during acceptance tests of milk at cheesemaking enterprises, its physical and chemical properties are evaluated: density, titratable acidity, and freezing point. The composition indicators are determined: the content of milk dry fat-free substances, the mass fraction of fat, and protein. The rennet test is determined and evaluated according to the indicators of bacterial contamination, the absence of inhibitory substances, including antibiotics.

Moreover, for cheesemaking it is important to know not only the total protein content in raw milk, but also the amount of true protein, as well as the ratio of whey proteins and caseins. These milk quality criteria are not mandatory for milk acceptance, but directly affect the yield of cheese and the quality of the finished product. Therefore, with a low yield of cheese from processed milk, it is advisable to determine the content of protein and non-protein nitrogen, calculating the content of the true protein, including casein, as the main structural component of cheeses [2].

Scientific studies give great attention to the protein component of cow's milk. In addition, the content of various fractions of casein and whey proteins is studied depending on the breeds of animals and their feeding diets [3,4]. The presence of genes that contribute to a simultaneous increase in milk yield and its protein content is established [5,6]. For improving these particular indicators, corresponding diets for feeding lactating animals are being developed.

Moreover, there is insufficient scientific data on how the conditions of keeping and feeding animals affect the fat phase of milk and its fatty acid composition.

Currently, when milk is accepted at domestic enterprises, studies of the fat phase are extremely rare. In the European practice of cheesemaking, it is quite common to determine free fatty acids in raw milk intended for making cheese, which can cause not only a decrease in organoleptic characteristics and cheese spoilage, but also serve as inhibitors of the lactic acid process in its manufacture [7].

When identifying cheeses in circulation, the fatty acid composition is a very important indicator of this group of products in terms of their naturalness. It is determined along with the organoleptic characteristics of cheese and indicators of its physico-chemical composition. Many fatty acids and their derivatives are involved in the formation of a characteristic cheese flavor and odor [8].

In this regard, the study of the fatty acid composition of milk suitable for cheese is relevant. The data obtained will be the starting point for studying changes in the fatty acid composition in the process of cheesemaking and substantiating the identification indicators of cheeses made using various technologies related to their fat phase.

Based on the relevance of the issue, the aim of this work was to study the fatty acid composition of milk used at different enterprises of the industry for the manufacture of cheeses of different species groups at different times of the year.

## 2. Materials and methods

Raw cow's milk obtained in different regions and in different seasons of the year served as research objects. 25 samples of raw cow's milk were examined.

To assess its suitability for cheese, standardized methods were used to determine the composition (mass fraction of fat, protein, lactose and milk solids-not-fat) and physico-chemical properties of raw milk (titratable acidity, density, freezing point).

The rennet test, the presence / absence of antibiotics and inhibitory substances were determined. The number of somatic cells, the number of mesophilic aerobic and facultative anaerobic microorganisms, and the number of spores of lactic-fermenting microorganisms determined the evaluation of the microbiological suitability of milk for the cheese manufacture. Its organoleptic characteristics were evaluated as well.

To determine the fatty acid composition of raw milk, a fat phase was isolated from it. With this purpose, the analyzed samples were placed in two centrifuge tubes of 50 cm<sup>3</sup>, centrifuged at 10,000 min<sup>-1</sup> for 15 min, and the upper fat fraction was taken after centrifugation. The resulting fat fraction was placed in a glass with a capacity of 250 cm<sup>3</sup>, 150 cm<sup>3</sup> of hexane were added (Vekton JSC, Russia), carefully mixed with a blender at maximum speed for 1 min, and the hexane layer with the dissolved fat was separated.

The resulting solutions of milk fat fractions were transferred to round-bottom flasks with a capacity of 250 cm<sup>3</sup> for subsequent removal of the solution, which was carried out at a temperature of 70 °C using a rotary evaporator.

To obtain methyl esters of fatty acids, 0.1 g of fat was transferred to a 10 ml centrifuge tube, 2 cm<sup>3</sup> of hexane and 100 µl of a 2 M solution of sodium methylate in methanol (Sigma-Aldrich, USA) were added; the tube was closed with a stopper, vigorously mixed for 2 min, it was settled for 5 min to separate the transparent upper phase containing methyl esters, which was then transferred to the vial and used to determine the fatty acid composition.

Fatty acid composition tests were performed on a Chromos GX-1000 chromatographic complex with a flame ionization detector (Chromos LLC, Russia) and a CP 88 silica capillary column Sil 88 for FAME100m×0.25mm×0.2µm (Agilent Technologies, USA). During the research, the following chromatographic modes were established: the volume of the introduced

sample 1 mm<sup>3</sup>; injector temperature 220 °C; temperature program of the thermostat: 1) 100 °C — 4 min, then a temperature increase of 5 °C for 20 min; 2) 170 °C — 20 min, a temperature increase of 5 °C for 9 min; 3) 215 °C — 30 min (analysis duration — 77 min); carrier gas — nitrogen (pressure in front of the column — 2.7 kgf/cm<sup>2</sup>). A standard mixture of Supelko 37 Component FAME Mix fatty acid methyl esters (Supelko, USA) was used as an identification mixture. Processing of the obtained data was carried out by the method of internal normalization using the «Chromos» program.

### 3. Results and discussion

Quality indicators of raw cow's milk used to make cheese are shown in Table 1.

According to the data of the table, all the milk used for the cheeses manufacture met the criteria of suitability for cheese in terms of organoleptic, physico-chemical and microbiological indicators. Rennet, as a specific indicator of suitability for cheese, corresponded to the first and second classes. The number of spores of lactate-fermenting clostridia met the requirements for milk for the production of any kind of cheese.

The results of evaluating the fatty acid composition of the studied milk are shown in Table 2. According to the data obtained, the fatty acid composition of raw cow's milk used to produce cheeses at various enterprises varied significantly. The data indicate the variability of the content of all groups of fatty acids — low molecular weight, saturated, monounsaturated and polyunsaturated.

The largest absolute fluctuations were found for saturated ( $\pm 7.03\%$  of the average) and monounsaturated fatty acids ( $\pm 3.77\%$  of the average) groups. Absolute fluctuations in the group of low molecular weight fatty acids amounted to  $\pm 2.62\%$ , and in the group of polyunsaturated —  $\pm 1.02\%$  of the average value. The calculation of the relative deviation showed that the

Table 1

Indicators of the investigated raw milk for the cheese manufacture

| Nº (item number) | Name of indicator  | Indicator value  | Value of the admissible indicator  |
|------------------|--|--|--|
| 1.               | Organoleptic indicators:   |  |  |
|                  | — flavor and odor  | Clean, free from foreign odors and flavors, not peculiar to the fresh natural milk | Clean, free from foreign odors and flavors, not peculiar to the fresh natural milk. Mild weedy flavor and odor are allowed   |
|                  | — color  | From white to light cream  | From white to light cream  |
|                  | — consistence  | Homogeneous liquid without sediment and flakes                                     | Homogeneous liquid without sediment and flakes. Freezing is not allowed.   |
| 2.               | Mass fraction, %:  |  |  |
|                  | — solid-not-fat  | 8.3–9.2  | Not lower than 8.2   |
|                  | — fat  | 3.2–4.6  | Minimum 3.2  |
|                  | — protein  | 3.0–3.4  | Not lower than 3.0   |
| 3.               | Density, kg/m <sup>3</sup>   | 1027–1028  | Not lower than 1027  |
| 4.               | Titrate acidity, oT  | 16.0–19.0  | From 16 to 18  |
| 5.               | Freezing point, °C   | 0.520–0.528  | Not lower than 0.520   |
| 6.               | Rennet, class  | I–II   | Not lower than I–II  |
| 7.               | Number of mesophilic aerobic and facultative anaerobic microorganisms, CFU/cm <sup>3</sup> | $3.3 \cdot 10^4$ – $5.0 \cdot 10^5$  | Maximum $5 \cdot 10^5$   |
| 8.               | Number of spores of lactic-fermenting microorganisms, spore/dm <sup>3</sup>                | 11–600   | For cheeses with a low temperature of the second heating — not more than 13000 spores/dm <sup>3</sup> ; For cheeses with a high temperature of the second heating — no more than 2500 spores/dm <sup>3</sup> . |
| 9.               | Number of somatic cells, cells / cm <sup>3</sup>   | $5 \cdot 10^4$ – $4 \cdot 10^5$  | Maximum $5 \cdot 10^5$   |
| 10.              | Inhibitory substances  | Not found  | Not allowed  |
| 11.              | Antibiotics  | Not found  | Not allowed  |

Table 2

Fatty acid composition of milk used in different regions for cheese production

| Name of fatty acid   | Indicators for the fatty acid composition of the investigated milk (n** = 25) |               |  |
|----------------------|---|---------------|--|
|                      | Range   | Average value | ± Standard deviation at P = 0.95 (for n determination results) |
| C4:0                 | 2.58–3.78   | 3.05          | 0.25   |
| C6:0                 | 1.73–2.71   | 2.08          | 0.21   |
| C8:0                 | 1.06–1.66   | 1.25          | 0.13   |
| C10:0                | 2.45–3.69   | 2.83          | 0.32   |
| C10:1                | 0.21–0.37   | 0.25          | 0.04   |
| C12:0                | 2.78–4.14   | 3.30          | 0.37   |
| C14:0                | 9.84–12.71  | 11.06         | 0.89   |
| C14:1*               | 1.27–2.28   | 1.64          | 0.21   |
| C16:0                | 24.80–35.93   | 29.13         | 3.01   |
| C16:1*               | 1.85–2.84   | 2.36          | 0.29   |
| C18:0                | 7.69–12.86  | 10.07         | 1.39   |
| C18:1*               | 18.78–27.89   | 24.26         | 2.87   |
| C18:2*               | 2.60–4.55   | 3.50          | 0.55   |
| C18:3*               | 0.22–0.77   | 0.54          | 0.16   |
| C20:0                | 0.15–0.31   | 0.18          | 0.03   |
| C22:0                | 0.05–0.15   | 0.08          | 0.02   |
| Others               | 3.56–5.74   | 4.43          | 0.48   |
| Low molecular weight | 7.88–11.84  | 9.22          | 0.23   |
| Saturated            | 59.01–70.06   | 63.03         | 3.46   |
| Monounsaturated      | 22.41–32.03   | 28.26         | 2.96   |
| Polyunsaturated      | 2.92–5.06   | 4.04          | 0.63   |

Notes: \* the calculation was made by the sum of the isomers; \*\* n is the number of determination results.

most varied groups were the ones of low molecular weight fatty acids ( $\pm 28.40$  rel.%) and polyunsaturated fatty acids ( $\pm 25.11$  rel.%). Fluctuations in the fatty acid composition of the studied milk is consistent with data from other scientists, whose publications indicate the dependence of this indicator on many factors: season of the year and climatic conditions [9,10,11,12,13], breed of animals [14,15,16] and their feeding diets [17,18,19,20,21].

An increase in the content of polyunsaturated fatty acids was characteristic of summer milk. This is consistent with similar ideas of foreign and domestic researchers, who attribute the increase in this indicator to the consuming of green food by lactating animals in the summer period [10,12]. Studies have also found that individual milk samples were characterized by an increased content of the following fatty acids: capric, myristoleic, palmitic and palmitoleic in relation to the values of these indicators specified for milk fat in national regulatory documents. In some samples, a reduced content of stearic and oleic acids was found in relation to these documents. Such deviations may be associated with special diets for feeding lactating animals, including the use of highly concentrated feeds, sunflower and

other meal, glycerin, palm fat and other energy supplements described in the literature [17,22,23,24].

All identified features of the fatty acid composition of milk can affect the nutritional value of the cheeses made from it and affect the formation of organoleptic characteristics of cheese.

#### 4. Conclusion

Significant fluctuations were found in the fatty acid composition of cow's milk used to make cheese. The greatest relative fluctuations were registered at the levels of polyunsaturated fatty acids and low molecular weight fatty acids.

As there is no possibility to determine the fatty acid profile in each batch of milk at the acceptance control, it is advisable to adjust the standard values of the fatty acid profile for cheese, including variations associated with a change in the fatty acid profile of milk depending on the conditions of keeping and feeding animals. It requires the expansion of research in the collection of more extensive statistical material on the fatty acid profile of milk suitable for cheese and cheeses made from it of different species groups.

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