



## **Ensuring Raw Dairy Biological Safety by Tyndallization**

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Milk; Whey; Microbial contamination; Heat treatment; Thermized milk; Technological properties; Biosafety.

### **Abstract**

The article provides a theoretical substantiation of the technological platform for ensuring the biological safety of raw milk by the method of controlled thermal cleaning before technological processing. Investigated alternative options for bacterial purification of raw milk by heating, with exposure to a soft regime, improved its microbiological, physicochemical, and technological properties. The technology of low-temperature heat treatment of whey has been substantiated, making it possible to achieve microbiological purification, stabilization, and preservation of the quality of raw materials, with the possibility of its preservation further use in obtaining functional food products and feed means of a new generation.

**Disciplinary:** Biotechnology, Agriculture, Dairy Science & Technology.

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## **1 Introduction**

The result of the general deterioration of the ecological and epidemic situation in the world is the accumulation of foreign substances of chemical and micro-biological origin in dairy raw materials, which inevitably pass into dairy products [11, 3]. An urgent problem is the bacterial contamination of raw milk and by-products of its processing, for example, milk serum [18, 10]. A logistically sound and reasonable way is to prevent raw milk contamination by foreign substances on farms and industrial complexes. However, in the current epizootic situation, the dairy industry is forced to solve eliminating "contaminants" from raw milk and food products obtained from it [12]. The most effective way to combat bacterial contamination of raw milk is controlled heat treatment, but not all modes ensure the preservation of milk's physicochemical properties [16]. Complex universal technology is needed that allows the use of raw milk processed specially, in the

future, for any technological processes of dairy production [4]. Cognitively (comprehension), it seems logical to use the implementation of the problem posed known in the dairy industry and scaled in practice methods of heat treatment for the sanitation of dairy raw materials, implemented in automated plate pasteurization cooling installations of the fifth generation. In the future, the need to search for convergent (combination) solutions for a directed and controlled impact on the undesirable microflora of dairy raw materials by modern methods-laser treatment, pressure, permissible radiation, and other achievements of fundamental sciences seems to be quite justified.

The purpose of the study: development of a Technological Platform for ensuring the biological safety of raw milk by the method of controlled thermal cleaning, which allows the necessary reduction of the bacterial contamination of milk raw materials.

The field of innovation is agricultural enterprises and specialized dairy factories.

Practical significance: the article proposes, using specialized or existing equipment, by the method of fractional low-temperature processing (thermization) with intermediate exposure, it is guaranteed to reduce the microbiological contamination of milk, including spore forms of microorganisms, while fully preserving the technological properties of the original raw materials, including the system of dispersed phases of raw milk and native protein structure.

The research's scientific novelty lies in the adaptation of the tyndallization method used for the disinfection of nutrient media in microbiology to the dairy industry's needs. Classically, this process is carried out within 36 hours and consists of triple boiling of raw materials (with the destruction of vegetative forms of bacteria) with intermediate exposure at 20 °C for 12 hours (to provoke spores with their further destruction during boiling). Our production schedule has been reduced to 1.5 hours with the same effect of the destruction of bacteria. It is adapted for dairy production and existing standard equipment, bringing raw milk in terms of microbiological and technological properties to the level of requirements of the European Union.

## 2 Materials and Methods

In the process of realizing the goal of the study, we used generally accepted GOSTs, standard, standardized, and modified methods of physicochemical and microbiological analyses of dairy raw materials (Table 1), and methods of mathematical modeling and processing of experimental data.

## 3 Results and Discussions

Analyzing the available information on the quality of the so-called "raw" or "harvested" milk, we consider it reasonable to propose options for a possible solution to reducing microbiological contamination before processing.

Practically some operations-cleaning from mechanical impurities and inactivation of microflora are implemented by filtration or centrifugation and heat treatment-pasteurization and sterilization [13]. These processes are very effective and well equipped with equipment. Still, it has not a general but a selective positive effect on certain pollution and entails an increase in milk processing cost. Besides, they do not solve the main goal: to improve the quality of freshly milked

raw milk and be provided by commodity producers. For our country, this problem is excruciating due to the practically low quality of raw milk. This problem has become noticeably aggravated in the collected milk from small commodity producers. The severity of the situation in this area indicates that, on the whole, the problem requires a state approach, an immediate and systemic solution.

**Table 1: Standard and adjusted methods of experimental physicochemical and microbiological research.**

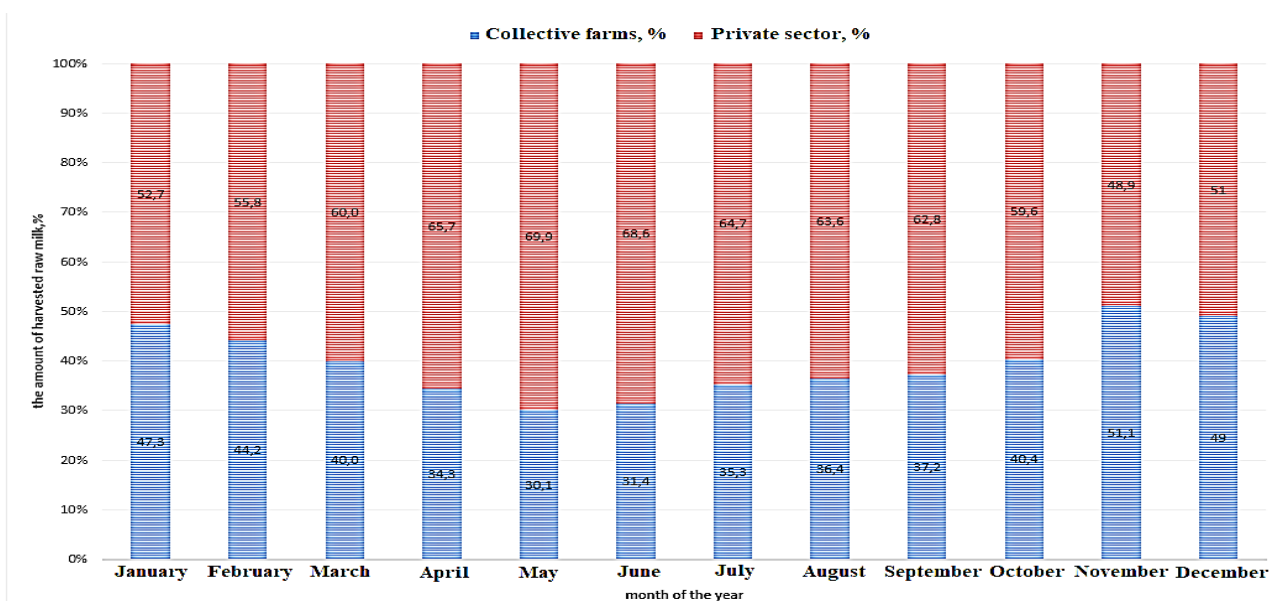
Indicators	The name of the method, features of the analysis.	Normative document
Titrateable acidity	Protolithometry. Titration 0.1 N. NaOH solution.	GOST 3624-92; GOST 2874-93
Active acidity (concentration of hydrogen ions, pH)	Potentiometric method using pH meters; pH-121, pH-150, pH-340, and glass electrode EVL-15-11 paired with silver chloride EVL-1M4 by GOST 26781-85; in the measurement range from 4 to 9 pH units with the basic permissible error within $\pm 0.05$ units.	GOST 26781-85 and according to the instructions for the device
Sampling and preparation of samples for microbiological analysis	According to standard techniques.	GOST 26668-85; GOST 26669-85
Total bacterial count (TBC)-determination of the total microbial count	Sowing on agar with hydrolyzed milk. Koch's method: determination in 1 cm <sup>3</sup> of the total content of mesophilic, mesotrophic aerobes and facultative anaerobes that can grow on agar at $t = (37 \pm 0.5) \text{ }^{\circ}\text{C}$ for (24 $\pm$ 2) h.	GOST 9225-84
Microbiological contamination	Generally accepted methods of microbiological examination of milk and dairy products.	GOST 9225-84
The number of mesophilic aerobic and facultative anaerobic microorganisms (KMAFAnM)	According to standard techniques.	GOST 26670-85; GOST 10444.15-94;
The presence of bacteria of the group of Escherichia coli (BGKP)	According to standard techniques.	GOST 9225-84 and GOST R 50474-93; GOST 30518-97
Lactic acid microorganisms	According to standard techniques.	GOST 10444.11-89
The presence of pathogenic microorganisms, including salmonella	According to standard methods: for Salmonella according to GOST 30519-97 and GOST 10444.2-94; for Listeria monocytogenes according to MUK 4.2.1122-02; for Staphylococcus aureus according to GOST 30347-97	GOST 30519-97 and GOST R 50480-93;
Determination of the presence of proteus	According to standard techniques.	GOST 28560-90
Yeast and Molds	According to standard techniques.	GOST 10444.12-88; GOST R 50474-93

After analyzing long-term statistical data on the amount of milk procured from collective farms and the private sector by enterprises of the North Caucasian Federal District (Figure 1), we can conclude that on average, 62.7% of the population comes from the population, and in some months-up to 70% of raw materials, which can potentially contain pathogens. That is why raw milk in food enterprises without preliminary processing can lead to biological accidents.

As one of the first steps on the path of bacterial purification of milk raw materials, it is possible to offer "mild" heat treatment by heating, which does not significantly affect the composition and especially the biological properties of milk as a system, and at the same time provides a noticeable decrease in the total bacterial contamination [7].

Reimerdes [19] recommended keeping the milk for 20-30 minutes to compensate for the loss of casein. At 60-65°C. Heat treatment of milk at 65°C for 10 s and its subsequent storage at 7 °C allows preserving milk's initial technological properties for up to three days [19]. Cogill et al. [6]

found that thermization significantly reduces the total number of bacteria and the number of psychrotrophic bacteria, increases the shelf life of milk up to four days at 4 °C without subsequent growth of bacteria [6]. Thermization does not cause denaturation of whey proteins, does not change the composition of the casein complex, and stabilizes the pH during milk storage. Abroad, during long-term storage of milk on farms, thermization is used at 65°C with an exposure of 15-25 s and subsequent cooling to a temperature not exceeding 10°C. Before processing, milk is repeatedly subjected to short-term pasteurization. Thermization to a large extent (by 85%) inhibits the development of psychrotrophic bacteria and allows milk to be stored at a temperature of 5°C for up to 3 days. Milk lipases are partially destroyed. The main microflora of thermized milk after storage at 6-11°C for 18-42 hours are psychrotrophic bacteria and BGKP. The leaven added in an amount of 0.2% inhibits their development.



**Figure 3:** The ratio of the raw milk amount procured from collective farms and the private sector, %.

In recent years, double heat treatment of milk has been used at cheese factories: first, milk is thermized, stored for one to three days (if necessary, backing up) at low temperatures, then pasteurized under the usual modes of cheese making. Such processing does not negatively affect the physicochemical and technological properties. The milk's biological value improves its sanitary and hygienic parameters and allows you to keep the milk's cheese suitability up to three days. NR Karlikanova [5] concluded that the pasteurization process at generally accepted temperature conditions (including 63 °C with exposure for 30 minutes and 72 °C with exposure for 15 s) usually allows destroying microorganisms the genus *Listeria* in milk if their number does not exceed  $1 \times 10^5$  CFU/cm<sup>3</sup>. Moreover, the use of modes of double heat treatment of milk significantly increases thermal exposure efficiency even with significant doses of contaminants. E. Zakrnewski notes that thermization promotes the transition of certain types of *Bacillus cereus* spores into a vegetative form, which then dies during pasteurization [20].

In principle, the thermization process is known, is used in foreign practice, was the subject of research concerning cheese making in the 80s of the last century at SKF VNIIMS [2]. Still, the results obtained, unfortunately, are not in demand by the industry.

Currently, in the situation of a pandemic of new coronavirus infection, and the implementation of the quality management methodology according to the HACCP system [9], there are two alternative options in terms of importance:

1. Thermization of milk received at the place of production (farm, collective farm, collection, and collection point), followed by immediate, deep cooling, designed to maintain the quality of raw materials for the period of collection and delivery to a specialized processing enterprise;

2. Thermization of all incoming (procured) milk-naturally not thermized-directly at dairy factories, regardless of the form of ownership, production volumes, and an assortment of products, with subsequent processing according to the accepted technological regulations.

A first option is a state approach of a departmental scale, capable of resolving the issue on its merits. Its economic component is the quality of products (raw materials) and regulating milk delivery frequency to specialized enterprises. The second option is a pure branch level within the dairy industry of the agro-industrial complex's food industry. It stabilizes the quality and allows you to specifically reserve milk before processing without developing the main microflora, especially psychrophiles.

The problem of thermization in both variants is simple at first glance. It is necessary to consider the most complex biotechnological process with the small (farm) hardware design and factory production. Also, it is necessary to develop safe regimes that prevent the development of thermophilic microflora and a sharp increase in acidity.

During the production of dairy products, based on the separation and concentration of fat and milk proteins, significant amounts of milk whey are formed, which is a valuable raw material. The exclusion of its processing significantly reduces the economic efficiency and environmental component of production. With large volumes of whey production, the question arises about its storage and transportation before processing, since during storage, the composition and properties of milk whey change [1]. Lactic acid bacteria's action facilitates this in the production process and contamination by extraneous microflora [15]. Lactose undergoes enzymatic hydrolysis, the pH of the medium, and the turbidity of the serum change. Also, hydrolysis of proteins and fat occurs, the taste of whey changes and unwanted and harmful substances can accumulate. It is theoretically believed that milk serum, when stored without processing, loses 25% of its energy value within 12 hours [17]. That is why the process of primary processing and preservation of milk whey is relevant. Milk whey accumulated in the process of cheese and curd production, on the principles of waste-free technology, with the use of bacterial sanitation of milk raw materials, can be purified, stored, and used to obtain innovative feed bifidogenic and anti-salmonella products.

Based on the analysis of the available information, we propose a concept of alternative options for the heat treatment of milk whey in a mild regime, which ensures a controlled decrease



in its bacterial contamination and long-term preservation of physicochemical properties composition.

The study of the effect of heat treatment on the physicochemical parameters of whey shows that when it is heated from 50°C, agglomeration of protein globules occurs due to their denaturation the turbidity of the serum decreases markedly [8]. Having lost their stability at 75-80°C, denatured proteins form flakes, which slowly settle. This process takes place most intensively when the acidity of whey is 30-35°T (pH 4.4-4.6), which coincides with the isoelectric point of the lactoalbumin fraction whey proteins. It has been established that the denaturation threshold of whey proteins is at the level of 50-65°C, visible coagulation is observed at 75-80°C, and the optimum thermal effect corresponds to 90-95°C. When the whey is heated to 65-70°C, a protein is intensively released on the apparatus's surfaces, which forms a hard-to-remove burn-in layer, which impairs the heat exchange process. When thickening whey under vacuum, temperatures of 55-65°C are used. Thus, during heat treatment of whey to reduce its bacterial seed, the temperature limit is 62±2°C [10]. This will avoid the denaturation of whey proteins and their adhesion to heat transfer surfaces.

The production experiments' results to ensure the biological safety of dairy raw materials are reflected in Tables 2 and 3.

**Table 2:** Concentration of microbes (CFU / g) in milk under different heat treatment modes.

Heat treatment modes						The concentration of microbial cells, CFU/cm <sup>3</sup> in milk (QMAFAnM *)	Percentage of surviving bacteria,%
First treatment		Storage after 1st treatment		Second treatment			
t °C	Time, s	t °C	Time, H	t °C	Time, s		
Raw milk without treatment						1.0·10 <sup>9</sup>	100
62	20	-	-	-	-	8·10 <sup>5</sup>	0.080
65	1800	-	-	-	-	2·10 <sup>5</sup>	0.020
75	15	-	-	-	-	5·10 <sup>4</sup>	0.005
85	10	-	-	-	-	2·10 <sup>4</sup>	0.002
Raw milk without treatment						2.5·10 <sup>7</sup>	100
62	20	6±2 (with cooling)	0	-	-	3.5·10 <sup>5</sup>	1.40
-	-	6±2	1	-	-	2.8·10 <sup>5</sup>	1.12
-	-	6±2	2	-	-	2.1·10 <sup>5</sup>	0.84
-	-	6±2	3	-	-	2.0·10 <sup>5</sup>	0.80
-	-	-	-	75	15	3.3·10 <sup>4</sup>	0.13
62	20	62 (without cooling)	0	-	-	3.5·10 <sup>5</sup>	1.40
-	-	60	1	-	-	1.2·10 <sup>5</sup>	0.48
-	-	58	2	-	-	5.6·10 <sup>4</sup>	0.22
-	-	56	3	-	-	4.0·10 <sup>4</sup>	0.16
-	-	-	-	75	15	2.2·10 <sup>3</sup>	0.01
75 (control)	15	-	0	-	-	4.2·10 <sup>3</sup>	0.02

\* Abbreviations: QMAFAnM-the quantity of mesophilic aerobic and facultative anaerobic microorganisms.

Two production modes of processing raw milk (Table 2) include:

1. Heating milk up to 65±2°C for 20 s; cooling to 6±2°C and storage for 1.5 hours; pasteurization at 72±2°C for 10 seconds;

2. Heating milk up to  $65\pm 2^{\circ}\text{C}$  for 20 s; holding without cooling at a temperature of  $60\text{-}58^{\circ}\text{C}$  for 1.5 hours; pasteurization at  $72\pm 2^{\circ}\text{C}$  for 10 seconds.

The results of production experiments on the development of optimal modes of heat treatment of raw milk (Table 2) showed that, depending on the temperature and exposure time, it is possible to reduce the number of bacteria by 2-4 orders of magnitude. On the one hand, the higher the temperature and pasteurization time, the more bacteria are killed during processing. The opposite effect of this process is the loss of raw milk's technological properties due to a violation of the structure of proteins and the Maillard reaction (melanoid processing). Therefore, the optimal mode of cleaning raw milk from bacterial contamination using heat treatment is thermization at  $65^{\circ}\text{C}$  and double heat treatment withholding without cooling for no more than one hour or reservation at a low temperature of  $6\pm 2^{\circ}\text{C}$ .

The study of the effect of heat treatment on the physicochemical parameters of whey (Table 3) shows that after low-temperature heat treatment at  $65^{\circ}\text{C}$ , whey can be stored without changing its properties for 48 hours at a temperature of  $6\pm 2^{\circ}\text{C}$ . Simultaneously, the initial bacterial contamination (QMAFAnM) of whey is reduced by two orders of magnitude after thermization and by another two orders of magnitude due to holding without refrigeration.

**Table 3:** Microbiological and physicochemical changes in the mixture (1/1) of curd and cheese whey during its heat treatment.

HEAT TREATMENT MODES				Acidity, $^{\circ}\text{T}$	pH	The concentration of microbial cells in $1\text{ cm}^3$ of milk	
First treatment		Storage after 1st treatment				QMAFAnM CFU / $\text{cm}^3$	Percentage of surviving bacteria, %
Temperature, $^{\circ}\text{C}$	Time, s	Temperature, $^{\circ}\text{C}$	Time, h				
Control (crude whey)				14.0	6.28	2.3.107	100
65	20	62	0	12.5	6.28	5.6.105	2.40
-	-	60	1	12.5	6.28	8.8.104	0.38
-	-	58	2	12.5	6.28	5.3.103	0.023
-	-	56	3	12.5	6.28	9.6.103	0.041

The production model of whey processing is reflected (Table 3) includes: heating at  $62\pm 2^{\circ}\text{C}$  with possible exposure (for 1.5 hours) or cooling to a temperature of  $6\pm 2^{\circ}\text{C}$  for redundancy (up to 48 hours).

Thus, as a result of the experiments carried out, a comprehensive universal technology is proposed that ensures the preservation of milk's physicochemical properties and allows the use of raw milk processed specially, in the future, for any technological processes of dairy production incl. For cheese making. The whey obtained as a normal by-product of milk processing into protein-fat products must also be sanitized before technological processing.

## 4 Conclusion

As a result of the analysis of the available information, we proposed to carry out preliminary heat treatment of raw milk immediately after acceptance before reservation (maturation) for the production of all dairy products in the technological cycle of a dairy enterprise with subsequent aging, which will allow obtaining a cleaner microbiological term of raw materials, to stabilize and

ensure the biosafety (quality) of the produced dairy products. A similar procedure should logically take place when processing the normal by-product of the main production-whey.

In general, according to preliminary expert assessment, the possible economic potential from the implementation of only bacteriological sanitation of raw milk by heat treatment by heating in the technological cycle of a dairy enterprise when processing 30% of the raw material (10 million tons per year) is about 30 billion rubles a year.

## 5 Availability of Data and Material

Data can be made available by contacting the corresponding authors.

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