Biotechnological aspects of ensuring the dairy food safety

I A Evdokimov¹, A G Khramtsov¹, S A Emelyanov¹, A D Lodygin¹,³ and D N Volodin²

¹ North Caucasus Federal University, 1, Pushkin str., Stavropol, 355017, Russia
² OOO “DMP,” office 901, 160, Dzerzhinsky str., Stavropol, 355003, Russia
³ E-mail: alldodygin@yandex.ru

Abstract. One of the urgent issues of modern food industry is the biological safety. In this regard, especially important for ensuring the safety of functional dairy food for healthy nutrition are new methods developed for the primary sanitation of raw milk, prior to technological processing, in order to reduce the bacteriological contamination and extend the shelf life of the food produced. This is of particular importance for improving the biotechnological properties of milk used in cheese making. The purpose of the work was to develop alternatives to innovative technologies for the heat treatment of raw milk by mild heating that ensures a controlled decrease in its bacterial contamination with a targeted effect on especially dangerous and heat-resistant spore-forming bacteria. Based on biotechnological principles, the theoretical foundations of the bacterial sanitation of dairy raw material prior to technological processing have been studied and developed; the parameters of mild heat treatment that provides a decrease in bacterial contamination by six orders of magnitude, with spores being converted into vegetative forms and subsequently destructed, experimentally and analytically substantiated; the suitability of cheese and other technological properties of raw materials has been increased; the shelf life has been prolonged; and innovative technologies for the production of biologically safe dairy products have been developed. There has been developed a procedure for the heat treatment of low-grade cow's milk - raw material - for further reservation or use in technological processes in order to improve its microbiological and technological properties and increase the product shelf life. Studies have been performed to inactivate spore-forming bacteria in milk, which has halved their incubation time.

1. Introduction

One of the urgent issues of modern food industry is the biological safety [1, 2]. Traditional technologies in the food industry and medicine for ensuring the biological safety are based on various chemicals used as disinfectants or antibiotics and nowadays reach a dead end due to even stronger antibiotic-resistant strains of microorganisms [3, 4], an increase in toxicity of medicines, and a decrease in the general immunity of animals and humans [5, 6]. In this situation, improving the quality and safety of food products is one of the most important and priority tasks [7, 8]. Therefore, microbiological aspects of a food production technology are of particular relevance. Insufficient knowledge of the interrelated and interdependent issues of environmental and food safety and the lack of scientific, methodological, and practical recommendations for mechanisms of sustainable environmentally balanced development of the Russian food sector must be compensated for by measures to ensure the food safety, including effective manufacturing practices, the Hazard Analysis and Critical Control Point (HAACCP) system, and international standards in the field of food safety and product quality management [9]. Bacterial
contamination of raw milk is problematic, i.e. the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) is restricted to 3.0·10^5 to 4.0·10^6 CFU/cm^3 in Russia, should not exceed 1.0·10^5 in the European Union, and the level of 1.0·10^3 CFU/cm^3 is recommended by the World Health Organization (WHO). The actual QMAFAnM of raw milk (at acceptance) reaches 2.0·10^5 – 1.0·10^6 CFU/cm^3. The smart way is to prevent contamination of milk with foreign substances on farms and industrial livestock complexes. However, under the conditions of a real biocenosis, the dairy industry is forced to solve the problem of removing contaminants from dairy raw materials after acceptance, prior to processing.

2. Materials and methods

The purpose of the study was to develop alternatives to innovative technologies for the heat treatment of dairy raw materials by mild heating that ensures a controlled reduction of its bacterial contamination with a targeted effect on especially dangerous and heat-resistant spore-forming bacteria.

Methods and technologies applied: background. At dairy enterprises, it is a custom that all raw milk supplied for processing is mechanically pre-cleaned and cooled in order to reserve or store it before processing. However, the entire bacterial pool remains and lives, especially the psychrophilic microflora. There are known attempts to solve this problem related to the quality of raw materials at the turn of the 1960s [10]. In Russia, despite the research results available, no measures were implemented. Therefore, it seems essential to search for ways of bacterial sanitation of milk before technological processing.

The comparative analysis of the effectiveness of various raw milk processing methods, i.e. microfiltration and sorption-desorption; bactofugation, deactivation of milk with ion-exchange resins, denitrification by biosynthesis, and cooling milk in a flow-through heat exchanger; use of solutions of hydrogen peroxide and silver ions; and wave radiation, in order to improve its physicochemical, microbiological, and biotechnological properties of milk (table 1) showed that they have not a general, but a selective positive effect on certain contaminants and also entail an increase in the cost of milk processing. The bactofugation decreases the amount of protein and, consequently, the yield of cheese. Moreover, these methods do not solve the main problem that is to improve the quality of freshly milked milk and must be provided by commodity producers.

| Method                          | Procedure                                                                 | Author, year          |
|---------------------------------|---------------------------------------------------------------------------|-----------------------|
| Production of drinking milk     | Dispersion and homogenization of milk heated at a temperature not higher than 58 °C and exposed to acoustic vibrations. | Volkov G.A. et al. (2004) [11] |
|                                 | After bactofugation, milk is cooled to a temperature not exceeding 6 °C, filled into bottles, and pasteurized in sealed bottles. | Ponomarev A. N. et al. (2004) [12] |
| Production of pasteurized milk  | Milk normalization, bactofugation, homogenization, pasteurization, cooling, additional pasteurization at 90 – 95 °C for 5 – 10 sec., recooling, and filling into bottles. | Ponomarev A. N. et al. (2005) [13] |
| Production of pasteurized milk of extended shelf life | Double vacuum processing of fresh milk by spraying it into space volumes of successively located vacuum chambers and double vacuum processing by adiabatic boiling in space volumes of successively located vacuum chambers. | Dolinsky A.A. et al. (2003) [14] |
|                                 | Separation of milk into a cream fraction and a skim milk fraction, microfiltration of skim milk and its separation into retentate and permeate, heat treatment of retentate and permeate, mixing the streams of permeate and retentate, and packaging the product. | Rogov I. A. et al. (2005) [Ошибка! Источник ссылки не найден.] |
After normalization, milk is heated to a temperature of 30 – 70 °C and subjected to two-stage purification on a milk separator and a bactofuge.

### Production of pasteurized cream
- First pasteurization at a temperature of 74 – 76 °C for 20 sec., cooling the cream, and additional pasteurization at a temperature of 92 – 94 °C for 4 min.

Mizgirev V. V. et al. (2005) [16]

### Pasteurization and homogenization of liquid food products
- Heating the product with steam and homogenization with vibration and impulse acoustic waves.

Nekipelov A. V. (2004, 2005) [17, Ошибка! Источник ссылки не найден.]

### Sterilization of liquid media
- Creation of cavitation bubbles, high pressure, and ultrasonic vibrations in the liquid by mechanical, electrical, and chemical actions (“cold” pasteurization).

Glaznev N. V. et al. (2001) [Ошибка! Источник ссылки не найден.9]

### A device for cooling and pasteurizing milk
- Removal of embryonic growth of unwanted microorganisms, using a magnetic field, specifically immobilized by magnetically controlled microcarriers.

Petrakov A. D. et al. (2003) [20]

### Preserving milk and its processing products
- It contains a reservoir for milk supplied for pasteurization, a recuperative heat exchanger, a heat exchange reservoir, insulated channels for milk and a heat carrier, and pumping units equipped with reservoirs for unpasteurized and pasteurized milk.

Darashkevich O. N. (2005) [21]

### Protecting milk from acidification
- Introduction of monovalent silver, bivalent copper, and hydrogen peroxide ions into milk.

Maryakhin F. G. et al. (2003) [22]

Adding a preservative—natural bischofite—into the original product.

Denisov V. V. et al. (2001, 2002) [23]

Effects of plants with antibacterial properties (garlic, onions, horseradish, etc.) on milk.

Gorlov I. F. et al. (2003) [24]

Kulikov E. A. (2004) [25]

The most effective way to combat bacterial contamination of raw milk is purposeful heat treatment; however, not all modes ensure the preservation of the physicochemical properties of milk, especially in cheese making (table 1). Current analogue productions of milk with extended shelf life due to double heat treatment of raw materials are energy-intensive, require additional equipment, greatly change the initial properties of raw milk, and are quite expensive. The innovative technology developed eliminates these disadvantages.

### 3. Results and discussion
The study results of the quality of raw milk purchased by the processing industry proved its indicators changing seasonally (figures 1-2).
Figure 1. Monthly analysis of raw milk supplies to dairy enterprises from private and collective producers.

The comparative analysis of seasonal changes in whole milk purchased by dairy factories in the Stavropol and Krasnodar Territories revealed the fact that 75% of raw material came to the plant from private farmers and only 25% from collective farms (figure 1). Considering that the probability of contamination with pathogenic microflora in milk from individuals considerably increases, the problem of its preliminary preparation is very acute.

The monthly analysis of the quality (grades) of raw milk (figure 2) found that the actual QMAFAnM of raw milk at acceptance reached $2.0 \times 10^7 – 1.0 \times 10^9$ CFU/cm$^3$. Taking into account the current epidemic situation in the Russian Federation, including diseases transmitted by the alimentary route, the problem of the quality of purchased milk is acute and needs further serious study.

Figure 2. Monthly analysis of the quality (grades) of raw milk.
In this regard, new methods of primary processing of raw milk, prior to technological processing, in order to reduce its bacteriological contamination and extend the shelf life of finished products, especially to improve the biotechnological properties of milk used in cheese making, are particularly important for ensuring the safety of dairy products.

To compare and develop optimal technological parameters of raw milk processing, we chose several modes.

*Milk processing control mode* that included double pasteurization at a temperature of not higher than 72 – 74 °C and intermediate cooling for up to 6 hours is usually used in cheese making to prepare raw material for further technological processing, in order to improve its microbiological parameters that directly affect the quality of the finished product (figure 3).

The control mode data showed (figure 3) that the initial QMAFAnM of n·10^7 CFU/cm^3 in raw milk can be reduced only to n·10^4 CFU/cm^3. A series of microbiological experiments made it possible to develop two most effective modes of processing raw milk. The first one is optimal for cheese making, and the second one is proper for low-grade milk. The first production mode (figure 4) included thermization of raw milk at 65 °C for 20 sec; holding in a container with thermal insulation without cooling for 3 hours and pasteurization at 72 °C for 10 sec. There was noted a decrease in QMAFAnM from 2.5·10^7 to 4.2·10^3 CFU/cm^3, somatic cells from 5.7·10^5 to <9.0·10^4 in 1 cm^3, and butyric acid bacteria from 2.5 to 0.6 cells per 1 cm^3, with the acidity and the number of heat-resistant microorganisms not changing. Escherichia coli bacteria were not detected after the first heat treatment.

![Figure 3](image-url)  
**Figure 3.** Microbiological points and acidity of milk in the control mode: double pasteurization with intermediate cooling.
Based on the results of the experiments, we came to the conclusion that the primary temperature treatment of raw milk at 65 °C did not allow milk to be hold without cooling for more than 3 hours, since the temperature in the container dropped to 56 °C by this time, and this was the threshold when the thermophilic streptococcal microflora sharply grew. In this regard, we decided to raise the temperature of the primary milk processing to 72 °C.

The second production mode of the temperature treatment of raw milk (figure 5) included the primary temperature treatment of milk at 72 °C for 10 sec., holding for 4 hours in a container with thermal insulation without refrigeration, and secondary pasteurization at 72 °C for 10 sec. The acidity and heat resistance did not increase, and the QMAFAnM decreased from 3.1·10⁸ to 1.0·10³ in 2 hours and was 3.0·10² CFU/cm³ after the second heat treatment. Somatic cells decreased from 5.8·10⁵ to <9.0·10⁴ per cm³, so did butyric acid bacteria from 25.0 to 2.5 cells per cm³.

**Figure 4.** Microbiological points and acidity of milk of the first production (cheese-making) mode: double low-temperature treatment (thermization) without intermediate cooling.
Figure 5. Microbiological points and acidity of the second production mode milk (for low-grade milk): double pasteurization without intermediate cooling.

The QMAFAnM limit was $n \cdot 10^7$ CFU/cm$^3$ ($n$ is any value in the range from 1 to 9), with the QMAFAnM concentration achieving up the permissible SanPiN standards of not more than $5.0 \cdot 10^5$ CFU/cm$^3$ in Mode I; and $n \cdot 10^8$ CFU/cm$^3$, with QMAFAnM decreasing to $n \cdot 10^2$ CFU/cm$^3$, which did not exceed the EU standard ($1.0 \cdot 10^3$ CFU/cm$^3$), for Mode II.

The acidity limit of raw milk in these procedures was considered 19 °T for Mode I and 20 °T for the Mode II.

The combined use of both modes improved the quality of raw milk for cheese making, destroyed of the E. coli bacteria, and considerably reduced the concentration of butyric acid bacteria and spore forms of microorganisms.

The conducted studies of the storage capacity of raw milk processed according to our modes showed (figure 6) that the storage time of thermally processed raw milk subjected to the heat treatment according to production Mode II (72 °C $\rightarrow$ (4 h) $\rightarrow$ 72 °C) was 2.5 days, and according to production Mode I (65 °C $\rightarrow$ (3 h) $\rightarrow$ 72 °C) 1.5 days.
Figure 6. Changes in titratable acidity of heat-treated raw milk during storage (at 4 °C).

The possibility to inactivate spore-forming bacteria in milk by prolonged heat treatment was studied (figure 7). The research was performed at the Stavropol Research Anti-Plague Institute. For the experiments, the Bacillus cereus strain 16 was used.

The following heat treatment modes were selected:

- No. 1: +72 °C (10 sec) → +65 °C (6 h); No. 2: +65 °C (10 sec) → +58 °C (6 h);
- No. 3: +72 °C (10 sec) → +5 °C (6 h); No. 4: +65 °C (10 sec) → +5 °C (6 h);
- No. 5: +72 °C (10 sec) → +51 °C (6 h); No. 6: +72 °C (10 sec) → +37 °C (6 h).

The research study of inactivated spore-forming bacteria in milk showed that:
Bacillus cereus spores were not provoked to germination into vegetative forms of a microbe (0%) during the first five treatment modes.

Bacillus cereus vegetative forms were formed for the 5th-6th hours of incubation at a temperature of 37 °C, with vegetative forms being formed by the end of the sixth hour of incubation (35±5) %.

Our technology allowed halving the time accepted in the literature (10 – 12 hours) for the Bacillus cereus spore forms converted into vegetative ones. Therefore, the preliminary heat treatment is a promising way to provoke spore germination for their further destruction.

There was verified the fact that spores do not germinate into vegetative forms while holding milk at a temperature of 72 – 65 °C for an hour and a half, which prevents the accumulation of metabolic products in the raw material and preserves its original biochemical properties.

4. Conclusions
The production modes of primary processing established by the large-scale investigative study made it possible to remove the E. coli bacteria from raw milk and considerably reduce the concentration of butyric acid bacteria. The exposure time did not allow thermophilic microflora to develop, so the temperature for spore microflora, which prevented the accumulation of metabolic products. At the quantum level, molecular biological processes of spore opening are triggered and followed by their further destruction. The selected modes accelerated the ripening process and increased the cheese yield due to better use of whey proteins, the absence of lipolysis by unwanted microflora, and greater moisture retention of the cheese mass. Preliminary heat treatment of milk can be considered a promising method of provoking spore germination for their further destruction due to their incubation time reduced by half. The QMAFAnM of raw milk was reduced to the EU and WTO limits. The economic effect was achieved by reducing the energy consumption during the technological process and increasing the grade of raw materials purchased.

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