Sanitary Control in a Research Laboratory as a Factor in Ensuring Food Safety of Innovative Dairy Products

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Abstract. Today, the production of innovative dairy products using various technological methods that improve quality is steadily growing. The formulation of such products includes various food additives, physiologically functional ingredients, plant materials, etc. Therefore, special requirements are imposed on the safety and quality of such products. The sanitary and epidemic quality of innovative dairy products is determined by the presence of pathogenic and other microorganisms in them. Development of technology for the production of innovative dairy products is usually concentrated in research laboratories. In this regard, close attention should be paid to the organization of measures aimed at creating an appropriate sanitary regime for the production of products of guaranteed quality. Such measures include careful monitoring of the content of microorganisms in the air, on laboratory equipment, utensils and utensils, work clothes and hands of laboratory staff, on the basis of which the sanitary and hygienic state of the production of innovative dairy products is assessed.

1. Introduction

Food safety determines the population’s state of health and the fate of future generations; it is a strategic task for any state. The quality and food safety are assessed by directly finding certain microorganisms, although, usually, such an analysis is performed according to indirect indicators, which provide an opportunity to judge the probable contamination of products by technically dangerous microorganisms. An indirect indicator of the pollution of environmental objects is the detection of sanitary-indicative microorganisms. The control over the safety and safety of products is carried out according to the method, in which the absence of sanitary indicative, opportunistic and pathogenic microorganisms in a certain mass or volume of the product is taken as the norm [1,2].

The quality of milk and dairy products and their hygienic preservation largely depend on the sanitary condition of technological equipment, inventory and packaging. In the production of innovative dairy products on the basis of a research laboratory, it is necessary to pay increased attention to the equipment sanitization, similar to the enterprises of the dairy industry [3]. However, it should be borne in mind that the sanitary state of the research laboratory can be applied to the norms regulated by Sanitary Rules and Regulations 2.3.4.551-96 "Production of milk and dairy products" [4].
2. Relevance, scientific significance of the issue
Dairy products are one of the main and demanded food products. They contain complete protein, essential amino acids, trace elements and vitamins. However, if the requirements for the production of these products are not met, in addition to the beneficial properties, dairy products can pose a threat to human health, cause diseases and poisoning. Therefore, when choosing innovative dairy products, the consumer must be confident in their quality and safety.

The sanitary-epidemic quality of dairy products is determined by the presence of pathogenic and other microorganisms in them. In this regard, it is necessary to strictly observe the sanitary and hygienic rules aimed at creating an appropriate sanitary regime for the production of products of guaranteed quality in the production of innovative dairy products in the research laboratory. To ensure the production of high-quality and safe products in the laboratory, it is necessary to control the content of microorganisms in the air, on laboratory equipment, dishes and utensils, work clothes and the hands of employees. Based on the results of regular sanitary and microbiological control, an assessment is made of the sanitary and hygienic state of the production of innovative dairy products.

Hygienic standards for microbiological indicators for dairy products are standardized by Sanitary Rules and Regulations 2.3.2.1078 "Hygienic requirements for the safety and nutritional value of food products", TRCU 033/2013 "On the safety of milk and dairy products", TRCU 021/2011 "On food safety". The most important controlled microorganisms include:
- sanitary indicative microorganisms - mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), bacteria of the Escherichia coli group (Coliform bacteria), enterobacteria, enterococci;
- opportunistic microorganisms, which include E. coli, Staphylococcus aureus, Bac. cereus, Clostridium perfringens, bacteria of the Proteus genus;
- pathogenic microorganisms, including bacteria of the Salmonella, Listeria, Yersinia genus;
- spoilage microorganisms: yeast and mold fungi, as well as some lactic acid microorganisms [5,6,7].

3. Statement of the problem
Based on the above, the purpose of this study is to conduct sanitary control in the research laboratory for the production of innovative dairy products at the Oryol State University named after I.S. Turgenev. In accordance with the goal, the following tasks were solved:
- to conduct a microbiological analysis of air, washes from the surface of laboratory equipment, utensils and equipment; work clothes, hands of researchers;
- to assess the content of microorganisms in the air in the morning before starting work, during work, in the evening;
- compare the research results with the criteria for assessing the air of food enterprises and the criteria for cleanliness of hands, equipment, work clothes;
- to summarize the research results and propose measures to improve the sanitary condition of the research laboratory.

4. Theoretical part
Experimental studies of the assessment of the content of microorganisms in the air on laboratory equipment, dishes and utensils, work clothes and hands of employees were carried out in accordance with the tasks in the laboratory of milk and dairy products at the Department of Food Technology and Organization of Restaurant Business at Oryol State University. The studies were carried out in accordance with the experiment design matrix (table 1).

The total number of microorganisms in the air (in 1 m3 of air) was estimated using the sedimentation method (Koch's method) [8]. Counting the number of colonies of microorganisms in Petri dishes was carried out after 4 days. To determine the microbial count, the Petri dish was placed upside down on a dark sheet of paper. First, colonies in 100 fields of view were counted in the dishes, then the arithmetic mean value for 1 field was determined.
Table 1. Experiment design matrix.

| Sample № | Object of sampling | Condition / location of sampling | Investigation |
|----------|--------------------|----------------------------------|---------------|
| 1-6      | Air in the laboratory | In the morning (before work) During the work In the evening (after work) Hands washed with water without soap (before starting work) | Total bacterial contamination (TCB), Coliform bacterias |
| 7-10     | Hands of researchers | Hands washed with water with soap (before starting work) Hands washed with soap and water and disinfected with 10% bleach solution (before starting work) | Total bacterial contamination (TCB), Coliform bacterias, starch iodine test |
| 11-13    | Hands of researchers | Hands after going to the lavatory/after break | Total bacterial contamination (TCB), Coliform bacterias |
| 14-15    | Hands of researchers | 1st researcher 2nd researcher | Total bacterial contamination (TCB), Coliform bacterias |
| 16-19    | Work clothes        | 1st researcher 2nd researcher | Total bacterial contamination (TCB), Coliform bacterias |
| 20-24    | Equipment           | Cheese dairy "Bergmann" Butter churn "Salute" | Total bacterial contamination (TCB), Coliform bacterias |

The number of colonies was determined by the formula:

\[
K = P \cdot p, \quad (1)
\]

where \( P \) is the area of the Petri dish;

\( p \) is the area of the field of view.

Counting the number of cells per 100 cm² (equivalent to 10 l, or 0.01 m³ of air) was carried out according to the formula:

\[
S = \pi r^2, \quad (2)
\]

where \( r \) is the radius of the Petri dish [9].

If the colonies are evenly distributed, they can be counted over half or quarter of the plate area. If there are many colonies (more than 600), it is better to use Wolfgügel's camera to count the colonies [10]. With a uniform distribution of colonies, they were counted in 10 squares located in different parts of the dish. The calculation of the number of microbial bodies in 1 ml or 1 g of the test material was carried out according to the formula:

\[
C = N \cdot V \cdot S, \quad (3)
\]

where \( N \) is the number of counted colonies;

\( V \) is the volume of the solution taken for sowing;

\( S \) is the area of the plate on which the colony count was carried out (3.14 * 52 = 78.5 cm²).

The identification of microorganisms was carried out by the method of describing their cultural characteristics [11]. Staining of microorganisms’ cells was carried out according to Gram, microscopic examination of microorganisms Edge was also carried out, the structure of the colony was determined using a magnifying glass or using a microscope at low magnification. For this, the cup was placed on the table with its lid upward [12]. Colony consistency was determined by touching its surface with a loop. Describing the growth of microorganisms by stroke, the following features were noted: scanty, moderate or abundant, solid with a smooth or wavy edge, feathery, bead-like, resembling chains of isolated colonies, diffuse, treelike or rhizoid [8,10].
Washes from surfaces (laboratory equipment, glassware, inventory) were performed in accordance with the sampling schedule (Table 1). The total surface area was 100 cm². To limit the surface, a pre-sterilized metal stencil with an area of 25 cm² was used.

Bacterial contamination of hands and clothes is determined by examining the microflora of washes. At the same time, the bacterial contamination and the presence of E. coli bacteria were examined. The workers' hands were washed as follows: first, palms and fingers were wiped with a tampon (both, and the tampon was carried out at least five times), and the interdigital areas and nails were also wiped. Additionally, after treating the hands with a disinfectant solution, a starch iodine test was made for the effectiveness of the treatment. Washes from sanitary clothes were taken in the following way: they selected and wiped with a swab 4 areas with an area of 25 cm² on the right and left sleeves, as well as from the front and top of the work clothes [13].

5. Results of experimental studies

Conducting sanitary control in the research laboratory of the OSU was held in December 2019. At the first stage, the number of microorganisms in the air was counted using the senimentation method [14]. After 4 days, the number of colonies of microorganisms in Petri dishes was counted. The results of the study of the air microflora (determination of the total bacterial contamination) are shown in Figure 1.

The next stage of the study was to determine the total bacterial contamination of the researchers' hands. The determination of the total number of microorganisms was carried out by inoculation of washes into Petri dishes on agar-meat infusion medium, determination of microorganisms of the Escherichia coli group - by inoculation on Endo medium [13]. The results are shown in Figure 2.
Determination of the total bacterial contamination of the work clothes of researchers was carried out by taking swabs from the clothes of the researchers at the end of the working day.

The results of determination of the total number of microorganisms are presented in Table 2. Also, a microscopic examination of the grown colonies was carried out using the Gram stain technique. Based on the identification of cultural signs, the belonging of microorganisms to the main types of sanitary indicative microorganisms was determined. The degree of cleanliness was established in accordance with the criteria for the sanitary assessment of air, equipment and personal hygiene of employees in the production of dairy products [10,15].

| Object of sampling | Condition / location of sampling | Number of microorganisms | Level of purity |
|--------------------|----------------------------------|--------------------------|----------------|
| Air in the laboratory | In the morning (before work) | 2000 | clean |
| | During the work | 3900 | clean |
| | In the evening (after work) | 8100 | dirty |
| | Hands washed with water without soap (before starting work) | 8900 | satisfactorily |
| Hands of researchers | Hands washed with water with soap (before starting work) | 4304 | well |
| | Hands washed with soap and water and disinfected | 708 | excellent |
| Hands of researchers | Hands after going to the lavatory/after break | 11304 | bad |
| Work clothes | 1st researcher | 2512 | well |
| | 2nd researcher | 3611 | well |
| Equipment | Cheese dairy "Bergmann" | 4553 | well |
| | Butter churn "Salute" | 3297 | |

The results of this study were compared with the criteria for assessing indoor air. The level of air pollution with microorganisms in the test room exceeds in the evening, after a working day. Since the study was carried out in winter, the recommended indoor air purity indicators are up to 4500 (the total number of bacteria in 1 m³) is clean air. Over 7000 is heavily polluted air [12].

Klebsiella was found in samples 7 and 8 by morphological characteristics. This is a conditionally pathogenic microorganism that is part of the normal microflora of the intestines, skin and...
mucous membranes, but in large quantities causes Klebsiella infection. The main mechanism for the penetration of bacteria is elementary non-observance of hygiene, namely, dirty hands [15].

Determination of sanitary - indicative microorganisms revealed the presence of E. Coli in samples 14 and 15. Pore-free bacteria Proteus vulgaris was found on unwashed hands, poorly treated hands without soap, on the clothes of the researchers. Micrococcus was found on the skin of the hands (samples 7 and 8). Most often, micrococci do not cause any disease. As representatives of normal microflora, they have a share of pathogenicity, which must be taken into account when working with dairy products. It is imperative to carry out disinfection before starting production with approved preparations. Conditionally pathogenic microorganisms were found on sanitary clothes (samples 18.19) Bac. cereus. The opportunistic bacteria Bacillus megaterium were found in small quantities at the cheese factory (samples 23, 24).

6. Conclusions
Having studied the microflora of the air in a research laboratory during production, saprophytic microorganisms were identified in small quantities - Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Micrococcus. They do not cause disease in humans and are permanent inhabitants of the air [12]. But their number is standardized at production shops and should not exceed the requirement for contamination of the air. The smallest number of bacteria was detected in sample 1, this sample was taken in the morning. The air was more polluted during work and in the evening, which is explained by the intensity of research during the working day.

We identified microorganisms that are significant in infectious diseases that live on contaminated surfaces: E. coli, Clostridium perfringens. Enterococcus, Bacillus subtilis. We compared the results of sanitary and microbiological studies done on equipment, work clothes, and hands. It was found that Bacillus seereus is more common on equipment, Bacillus sereus, Bacillus megaterium on work clothes, and Bacillus subtilis, Micrococcus, Klebsiella oxytoca, E. Coli, Proteus Vulgaris, Enterococcus on hands after visiting the toilet. One of the main factors in the quality of obtaining safe dairy products is the thorough processing, washing and disinfection of laboratory equipment, as well as the employees' compliance with the rules of personal hygiene regulated in the production of dairy products [1,15, 16].

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