Determination of the number of somatic cells in milk by biotesting method

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Abstract. Food products must meet the physiological needs of man in the necessary substances and energy, meet the requirements for organoleptic, physical and chemical parameters, as well as meet the requirements established by regulatory documents for the permissible content of chemical, radioactive, biological substances and its compounds, microorganisms that pose a danger to the health of present and future generations. Specific safety indicators are set and controlled for certain groups of food raw materials and products. Milk and dairy products have several specific safety indicators, which include the number of somatic cells. To determine this index in the raw milk, biotesting method is used for determining the total toxicity on behavioral responses of infusoria Paramecium caudatum.

1. Introduction
Food safety – the state of reasonable assurance that food products under normal conditions of use are not harmful and do not pose a risk to the health of present and future generations. Safe for the health of the consumer is considered to be products that do not contain toxic substances.

Sanitary and hygienic standards use the following definitions:
Food quality – a set of characteristics of food products that can meet human needs in food under normal conditions of use.
Food safety – the state of reasonable assurance that food products under normal conditions of use are not harmful and do not pose a risk to the health of present and future generations.
Certificate of quality and safety of food products – a document in which the manufacturer certifies compliance with the quality and safety of each batch of food products, the requirements of normative, technical documents.

Thus, food products must meet the physiological needs of man in the necessary substances and energy, meet the requirements for food products in terms of organoleptic and physical and chemical parameters and meet the requirements established by normative documents for the permissible content of chemical, radioactive, biological substances and its compounds, microorganisms that pose a danger to the health of present and future generations.

The main criteria of food safety include the following:
1. Ecology of food as a criterion determining the economic and social welfare of society.

The modern attitude to the ecology of food has emerged relatively recently. So in 1992, an international conference was held in Rome, which discussed the issues of nutrition and food safety for humans.
In industrialized countries, the most pressing problem is the problem of quality of food safety. And over-nutrition among rich people in these countries is the cause of very serious diseases. The environmental situation and these factors, in turn, determine the need to solve the problem of therapeutic and preventive nutrition.

At the same time, in underdeveloped countries, in the context of food shortages, the most important issue is to ensure the minimum required amount of basic food.

Along with this, with the development of food technology, biotechnology, microbiology and food chemistry, a huge number of food additives appeared, the level of environmental pollution increases, and other factors, which necessitated the strengthening of control over food safety. The European Union has adopted Directive 93/43/cee “on the adoption of measures to ensure the safety of food products, taking into account the genetic safety for future generations”.

In 2001, Moscow hosted an international conference for the first time, which also addressed issues that determine the main aspects of food safety: environmental, social, environmental protection, ensuring appropriate control over the quality and safety of food and others.

2. Regulatory and legislative - framework of food safety in Russia. Security in the Russian Federation (RF) of food products is also governed by the applicable laws: RF law “On protection of consumer rights” № 2300-1, the Federal law “About state regulation in area gene-engineering activity” № 3348; law of the RF “On the sanitary-epidemiological welfare of population” № 52-FL; the Federal law “About quality and safety of food products” № 29-FL.

State regulation, supervision and control over the quality of food products is carried out by establishing standards of sanitary rules, norms, hygienic standards, mandatory for food producers. The problem of quality, nutritional value and food safety includes not only the development of normative documentation, but also control methods. Currently, the Russian Federation has developed guidelines for conducting of chemical and microbiological methods of analysis of food products, in accordance with the rules of ISO.

For the same purpose, the state sanitary and epidemiological service of the Russian Federation conducts social and hygienic monitoring.

Social monitoring includes the analysis and generalization of data on food consumption, epidemiological surveys of nutrition and nutritional status of different population groups, about the demographic situation and health status of the population, etc.

Hygienic monitoring determines the degree of pollution of the environment, raw materials and food products. The following types of potential hazards of toxic substances can be identified: microbiological and viral origin, lack or excess of nutrients, foreign substances and natural components of food products; genetically modified microorganisms; technological and food additives, biologically active additives.

3. Certification and environmental certification of food products. The main purpose of certification of products, works, services is to control the safety of products for human life and health. The regulatory framework of certification includes the laws of the Russian Federation, interstate and international standards, sanitary norms and rules, safety standards, technical specifications and instructions, guidelines and other documents that establish mandatory requirements for products in accordance with the legislation of the Russian Federation.

The legislative base of certification in the Russian Federation is formed by the whole package of laws of the Russian Federation defining a basis and system of certification. The main international and regional organizations in the field of standardization and product quality management are: ISO, IEC, BIPM, OIML, EPAC, CASCO, CEN, EOQ, UNECE, UN, etc.

Products for which the certificate is issued, marked with a mark of conformity adopted in the system, which is placed on the tare, packaging and accompanying technical documentation. The basis for the sale of products on the market is only a certificate of conformity.

In case of statement of new types of production at the stage of coordination of normative documentation (ND) the enterprise must receive the hygienic certificate performing function of ND.
This certificate is issued by the bodies of Gossane pidnadzor (Russian State Committee for Sanitary and Epidemiological Oversight).

It is not allowed to sell food products, raw materials and materials that do not comply with normative documents (Russian State Standard SanPin 2.3.2.1078-01 “Hygienic requirements for the quality and safety of food raw materials and food products. Sanitary rules and regulations”). Such products are withdrawn from circulation by the State Supervision and control bodies and are subject to disposal or destruction.

For goods and food products, the production of which minimally pollutes the environment, as well as guarantees the consumer the safety of the life, an environmental certificate is issued. Such a certificate is also a determining factor in the competitiveness of these products. Unfortunately, in Russia, eco-certification is at the beginning of its development, although in Europe it is quite widely developed. For example, in France, agricultural products are marked with a conformity mark. Introduced eco-signs on its species, called “Red marks”. Products with the NF mark guarantee consumer safety in all respects. In Germany, common eco-label “Green dot”, which is placed on the package and indicates the possibility of its processing. Sometimes this sign indicates that the product is made from recycled materials. In the USA, Great Britain and the Nordic countries the “recycling” sign is used.

The issues of ecological marking and labeling are dealt with by the international certification organization-Subcommittee of RCS (regulations of cryptographic security) ISO/TC 207 “Labeling (marking) in the field of environment”.

Food safety is the main property of the product, which the manufacturer is obliged to provide to the consumer. Unlike other consumer properties, the deterioration or loss of which leads to the loss of functional or social purpose, exceeding the permissible level of safety indicators translates products into the category of dangerous. For goods and products, the use of which after a certain period is dangerous, set expiration dates. The normative documentation (technical regulations) provides for mandatory requirements that ensure and guarantee safety.

Safety indicators characterize the features of food products that ensure consumer safety for customer under all conditions and methods of consumption, as well as transportation, storage and disposal. By its nature, the safety indicators of food products and raw materials are divided into: chemical, microbiological and biological. According to the importance of control, product safety indicators are divided into: general (mandatory) and specific.

General safety indicators include the content of the following xenobiotics: radionuclides, heavy metals, mycotoxins, pesticides, for which the criteria for safe consumption according to the norms of maximum permissible concentrations are established.

Specific safety indicators are set and controlled for certain groups of food raw materials and products. For example, the content of nitrates, nitrates and nitrosamines is controlled in fruits and vegetables, especially those grown in greenhouses.

Control of the presence of hormonal drugs is carried out for raw materials of animal origin, as its use is possible in the cultivation and fattening of animals and poultry.

Fusel oils, aldehydes, methyl alcohol are specific safety indicators for alcoholic beverages.

For fish and fish products specific safety indicators include histamine [5]. Histamine (a biologically active substance belonging to the class of biogenic amines) can accumulate in fish products as a result of decarboxylation of the amino acid of histidine with the participation of enzymes of the microflora developing in violation of storage conditions.

Milk and dairy products are perishable goods and also have several specific safety indicators, including antibiotics [1] and somatic cells [3, 5]. Moreover, the number of somatic cells is controlled only in milk-raw materials.

According to G. Berndt and others [2] the number of somatic cells isolated from a healthy udder is in the range of 10000-100000 in 1 ml, depending on the state of health and the stage of lactation, its number may vary. By its nature, somatic cells are tissue cells of the body and a priori cannot show toxicity. However, it is the increase in the number of somatic cells in milk that is associated with the
presence of inflammatory processes in the cow's body. G. M. Sviridenko and E. G. Semova [4] note that with an increase in the content of somatic cells, the frequency of detection of pathogenic staphylococci and streptococci in milk and dairy products increases. Therefore, an increase in the level of somatic cells in milk indicates the presence of the disease, as the number of leukocytes in the blood increases, which is a response to the presence of the pathogen.

To establish the most efficient regulations of the release of products for human food and feed products for animals the particular importance is the implementation in practice of express-methods its biological assessment, most adequately reflecting the chemical composition, nutritional value, safety and specific properties. Therefore, it is important to identify the presence or absence of the toxin during express examination. For this purpose the method of biotesting for determination of the general toxicity (T) has proved well enough.

The method is based on the observation (registration) of differences in the values of one or more quantitatively measured, factor sensitive reactions (test reactions) in laboratory grown low-organized organisms, such as infusoria, sensitive to this factor compared to the control. Infusoria in its biological parameters is very close to higher animals and humans, which makes it possible to extrapolate the data obtained in biotesting with infusoria to humans. At the same time, with the availability of special equipment and inexpensive reagents, the use of this method makes it possible to obtain results within a few hours.

2. Purpose, objects and methods of research

Determination of total toxicity (T) of raw milk was carried out on the device “Biotester-2” (figure) on the behavioral response of Paramecium caudatum infusoria. “Biotester-2” is a specialized pulse photometer with a set of photometric cuvettes.

The method of determining the toxicity of aqueous extracts is based on the ability of test objects to react to the presence in the aquatic environment of substances that are dangerous to its life, and to move in the direction of changes in the concentration of these substances (chemotactic reaction), avoiding its harmful effects. Chemotactic reaction is realized under the condition of presence of a time-stable gradient of chemical substances concentrations. Such a gradient is created by layering in a vertical cuvette (tube) of infusoria in the thickener of the tested liquid.

In this case, a stable border of section is formed in the measuring cuvette, which is preserved throughout the biotesting time. This border does not prevent the free movement of infusoria in its preferred direction, while preventing mixing of liquids from the lower and upper zones. After creating two zones in the cuvette, the infusoria are redistributed by zones within 30 minutes.

An important feature of the behavioral response of infusoria is the mass movement of organisms into the upper layers of the liquid. If the test sample did not contain toxic substances in the cuvette will be observed concentration of infusoria cells in the upper zone. The presence of toxic substances in the
test sample leads to a different nature of redistribution of infusoria in the cuvette, namely: the higher the toxicity of the sample, the smaller proportion of infusoria moves to the upper zone (test sample).

The criterion of toxic effect is a significant difference in the number of cells of infusoria observed in the upper zone of the cuvette in a sample that does not contain toxic substances (control), compared with this indicator observed in the test sample (experience).

Quantitative evaluation of the test reaction parameter characterizing the toxic effect is performed by calculating the ratio of the number of infusoria observed in the control and test samples, and is expressed as a dimensionless value – toxicity index (T). Evaluation of research results is carried out according to the established for this method toxicity indices: index of permissible toxicity-0.00-0.40; index of moderate toxicity-0.41-0.70; index of high toxicity-0.71 and above.

3. Results and discussion.

In the subclinical form of mastitis, the composition of milk differs slightly from normal precast milk, except for the number of somatic cells (table 1).

Table 1. Composition and physical and chemical properties of normal and abnormal milk.

| Type of milk          | Fat, % | dry skim milk residue, % | protein, % | density, °A | Number of somatic cells, thousand/cm³ |
|-----------------------|--------|--------------------------|------------|-------------|---------------------------------------|
| Normal                | 3.40   | 8.20                     | 3.02       | 28.5        | 368                                   |
| abnormal (subclinical form of mastitis) | 3.05   | 7.78                     | 2.87       | 26.4        | 775.0                                 |

In milk obtained from cows with subclinical form of udder mastitis, the number of somatic cells is more than 2.4 times higher than in normal milk, so the presence of subclinical mastitis in the herd significantly reduces the quality of milk.

As noted, for raw milk, the number of somatic cells is one of the specific safety indicators. Milk samples with different levels of somatic cells were analyzed. The results of the studies are presented in table 2.

Table 2. Toxicity of milk with different levels of somatic cells.

| Number of somatic cells, thousand/cm³ | Toxicity               | Index of toxicity |
|---------------------------------------|------------------------|-------------------|
| > 1500                                | High                   | 0.82-0.96         |
| 1000-1500                             | From medium to high    | 0.65-0.75         |
| 700-1000                              | Medium                 | 0.45-0.65         |
| 500-700                               | Permissible            | 0.03-0.38         |
| < 500                                 | Permissible            | 0.00-0.35         |

A high toxicity index was observed in milk samples with the number of somatic cells more than 1500 thousand / cm³, as well as in the same samples after centrifugal purification, despite the fact that the number of somatic cells decreased to the level (700-1000 thousand/cm³). This confirms the assumption that somatic cells are not toxic, but the toxicity is due to an increased content of pathogenic microorganisms [6].

4. Conclusion

Using the example of raw milk, it is shown that the method of biotesting can be used to determine the number of somatic cells by the general toxicity determined by the behavioral reaction of Paramecium caudatum infusoria. The application of this express analysis allows timely identification of samples of any food raw materials with a high level of toxicity, unsuitable for further use and processing.

References

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