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**DEVELOPMENT OF BIOMETRIC METHODS FOR DETERMINING
XENOBIOTICS IN MILK**

ABSTRACT

on the dissertation work of a doctoral student A.O. Utegenova
for the degree of Doctor of Philosophy (PhD)
in the specialty 6D073500 – Food safety

The relevance of the research. One of the main conditions for ensuring the national security of the Republic of Kazakhstan, formation of a strong nation, successful long-term development and economic growth is food safety, which is enshrined in the Law of the Republic of Kazakhstan "National Security of the Republic of Kazakhstan" dated January 6, 2012.

According to the State Program for the development of the agro-industrial complex of the Republic of Kazakhstan over the period from 2017 to 2021, approved by the Decree of the Government of the Republic of Kazakhstan No. 423 dated 12.07.2018, today the current system does not sufficiently allow monitoring the reliability of procedures for confirming compliance with the requirements of technical regulations, including united sanitary-epidemiological and hygienic requirements for products which are subjected to sanitary and epidemiological supervision (adopted by the decision of the Customs Union Commission No. 132 dated 06.08.2019).

The program emphasizes that it is necessary to equip raw materials laboratories with modern express methods of research on the safety of raw materials and food products. This area of work also corresponds to the implementation of one of the main indicators of the National Project for the development of the agro-industrial complex of the Republic of Kazakhstan over the period from 2021 to 2025, namely, increasing the amount of food products subjected to monitoring by safety indicators.

It is known that various chemicals - insecticides, which have high toxic properties, are used in agriculture to increase yields. If the rules for the use of pesticides in agriculture are violated, there is a threat of these substances entering food in an amount exceeding the maximum permissible concentration.

In most cases, organophosphate pesticides (OPP) are used in agriculture because of their low cost and relative ease of use. It should be noted that organophosphate compounds (OPC) have a toxic effect and the ability to inhibit the action of enzymes of the cholinesterase group. Inhibition of the activity of enzymes of the cholinesterase group leads to serious disorders of the nervous system of the human body. The most common organophosphate compounds include parathion, diazinon, chlorophos, carbophos, disulfoton and malation.

Based on the above, it should be noted that the development of accelerated methods for determining the sufficient amount of organophosphate compounds in animal products, including milk, is relevant and promising. At the same time, research on the development of accelerated methods for determining the content of toxic substances will allow solving the main tasks of ensuring food safety specified in the State Program for the development of the agro-industrial complex of the Republic of Kazakhstan over the period from 2017 to 2021 and the National Project for the development of the agro-industrial complex of the Republic of Kazakhstan over the period from 2021 to 2025.

The purpose of the dissertation is to develop an express method for determining the residual amounts of organophosphate pesticides, carbophos in milk, to ensure the food safety of dairy products.

To achieve this **goal**, the following tasks were formulated and consistently solved:

- conducting research and selection of an enzyme for the development of test systems based on the results of determining their specific activity in the dairy products;

- conducting research on the selection of a method and material for the immobilization of the enzyme;

- development of a method for obtaining a biosensor test system;

- development of technological parameters of milk purification from xenobiotic;

- development of technology of a dairy product using the method of purification of milk from xenobiotic based on the determination of critical control points;

- development and approval of regulatory and technical documentation (organization standard and technological instructions) for a new dairy product.

The objects of the research: milk, a dairy product, organophosphate pesticide (carbophos), hydrolytic enzymes (acetylcholinesterase, butyrylcholinesterase), biosensor test system.

The methods of the research. Theoretical and experimental studies were applied in this work. Experimental studies were conducted on the basis of generally accepted, modified and standard research methods of physical-chemical, organoleptic, rheological, hygienic safety indicators of research objects, as well as the specific activity of enzymes.

Mathematical processing of the results of experimental studies was carried out by the method of mathematical statistics with the calculation of the coefficient of determination.

Experimental studies were carried out on the basis of laboratories of the scientific center "Food Biotechnology" of the Semey State University named after Shakarim, and the testing laboratory of a branch of the stock company "National Center for Expertise and Certification" of Semey.

Scientific novelty of the research.

For the first time, an accelerated biometric method for determining the residual amounts of organophosphate pesticide – carbophos in milk based on a test system with acetylcholinesterase inhibition has been developed:

- an enzyme for immobilization in test systems was studied and selected;
- biosensory test systems (on a glass surface and paper-based) for the determination of organophosphate pesticides (carbophos) in milk have been developed;
- technological parameters of milk purification from carbophos with the use of zeolite as a filter material have been developed;
- the technology and control scheme of the technological process of the production of cottage cheese produced from milk with a high content of carbophos based on the determination of critical control points has been developed.

The novelty of the main technical solutions is confirmed by a patent for the utility model of the Republic of Kazakhstan No 4295 "Biosensory test systems based on an immobilized enzyme for the determination of carbophos in milk", dated 13.09.2019, bulletin No. 37.

The main provisions submitted for thesis defence:

- development of a biosensor test system based on an immobilized enzyme for the qualitative determination of carbophos in milk;
- development of technological parameters of milk purification from carbophos;
- development of cottage cheese technology using milk purified from carbophos and research of nutritional value, safety indicators and quality of the finished product;
- cottage cheese quality management based on HACCP principles based on a risk analysis system and critical control points.

Scientific and practical significance of the research. The results of scientific research are of practical importance, since a test system has been developed for an accelerated method for determining the content of the residual amount of organophosphate pesticide (carbophos) in milk, the possibility of using a hydrolytic enzyme (acetylcholinesterase) for its immobilization into a test system has been proved, technological parameters for cleaning milk from carbophos using zeolite as a filter material have been developed, cottage cheese using a filtration process to purify milk from carbophos, a control scheme for the technological process of the production of cottage cheese made from milk with a high content of carbophos has been developed based on the determination of critical control points.

An organization standard and technological instruction for the production of cottage cheese using a filtration process for cleaning milk from an organophosphorus pesticide has been developed and approved.

An industrial approbation of the technology of cottage cheese was carried out using the filtration process to purify milk from carbophos in the dairy shop of the peasant farm “Nur”.

Connection of the work with scientific research projects. The work was carried out within the framework of a scientific project funded by the Ministry of

Education and Science of the Republic of Kazakhstan in the priority area "Sustainable development of the agro-industrial complex and safety of agricultural products", the priority "Technical support for the modernization of the agro-industrial complex" on the topic "Development of a biosensor for the determination of highly cumulative xenobiotics in milk and dairy products based on regional food safety monitoring" (2021-2023).

The author's personal contribution lies in the theoretical substantiation of the research objectives, in the choice of methods and in the conduct of experimental studies on the development of biosensor test systems for the qualitative determination of organophosphorus pesticide (carbophos) in milk, in the development of cottage cheese technology using the method of cleaning milk from xenobiotics and in the development of regulatory technical documentation.

Approbation of the results of the dissertation. The main results of the dissertation work were reported at the VII International Scientific and Technical Conference "Kazakhstan - Cold 2018" (Almaty, 2018); at the International Scientific and Practical Conference dedicated to the memory of Vasily Matveyevich Gorbatov, "Innovative and technological development of the food industry - trends, strategies, challenges" (Moscow, 2018).

Publications. 11 papers were published on the topic of the dissertation, including 3 articles in journals included in the Scopus database (1 article with a percentile of 9; 1 article with a percentile of 51 and 1 article with a percentile of 56); 4 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan; 3 articles in the materials of the International Scientific and Practical Conference, patent for the utility model of the Republic of Kazakhstan No 4295 "Biosensory test systems based on an immobilized enzyme for the determination of carbophos in milk", dated 13.09.2019, bulletin No. 37.

The structure and volume of the dissertation. The dissertation work consists of an introduction, four chapters, a conclusion, a list of references and appendices. The work is presented on 119 pages, it includes 17 tables, 37 figures and 6 appendices, the list of references includes 162 sources.

Evaluation of the completeness of solutions to the tasks. The obtained data allows to consider that all the tasks set in the dissertation work have been completed and the goal of the dissertation has been achieved:

1. In order to develop a biosensor test system, the hydrolytic enzyme acetylcholinesterase was selected from two hydrolytic enzymes with the optimal amount of this enzyme being 0.2 mg, with a buffer solution pH of 8.4; at a thermostating temperature of 37 °C and an inhibition time of 30 minutes, since the specific activity of acetylcholinesterase in milk was 11 mmol/ml, and the specific activity of butyrylcholinesterase was 7.9 mmol/ml.

2. For immobilization of the acetylcholinesterase enzyme, the method of inclusion in the gel was chosen with the additional use of one of the chemical

methods - cross-linking. As a carrier for the immobilization of the acetylcholinesterase enzyme, 2% sodium alginate with a bifunctional crosslinking agent, calcium chloride, was chosen. It has been established that within 30 minutes at a dynamic viscosity of 620 MPa*s, the final state of the reaction mixture is observed, in which the gel based on sodium alginate acquires a solid state.

3. Methods have been developed for obtaining biosensor test systems on a glass surface and paper base for the determination of organophosphorus pesticide (carbophos) in milk. The shelf life of the biosensor test system was established (30 days at a temperature of 5-6°C; on the basis of a glass rod - 30 days, and on a paper basis - 20 days). Instructions for the use of biosensor test systems on a glass surface and paper base for the determination of organophosphorus pesticide (carbophos) in milk were developed.

4. Technological parameters of milk purification from carbophos in the process of milk filtration using zeolite as a filter material were developed. The content of carbophos in the initial raw material is reduced to the maximum permissible concentration when the content in the filters is 200 g of zeolite (80% filling), with a pump volumetric capacity of up to 6 l/min (200 rev/s⁻¹) and a temperature of 20-25 °C.

5. The technology for the production of cottage cheese using a filtration process with a filter material - zeolite from milk containing carbophos in quantities above 0.05 mg/kg was developed. In accordance with the Standard of the Republic of Kazakhstan 1179-2003 "Quality system. Food quality management based on the principles of HACCP", the block diagram of the technological process for the production of cottage cheese was developed and critical control points were determined, a control scheme for the technological process of the production of cottage cheese was drawn up to determine the frequency of control.

6. The regulatory and technical documentation for the production of cottage cheese using the filtration process with a filter material - zeolite was developed and approved. The industrial approbation of the technology for the production of cottage cheese was carried out using the filtration process with a filter material - zeolite from milk on the basis of the dairy shop of the peasant farm "Nur".