ANTHROPOGENIC AND NATURAL TOXICANTS OF RAW MATERIAL, FOOD PRODUCTS AND MODERN METHODS OF THEIR CONTROL

[a]INGA M. NITYAGA, [b]BORIS V. USHA, [c]NATALIA G. MASHENTSEVA, [d]POLINA V. KULACH, [e]VLADIMIR I. LUTSAY,

[a] *Moscow State University of Food Production, 125080, Moscow, Volokolamskoe avenue, 11*
[b] *Moscow State University of Food Production, 125080, Moscow, Volokolamskoe avenue, 11*
[c] *Moscow State University of Food Production, 125080, Moscow, Volokolamskoe avenue, 11*
[d] *Moscow State University of Food Production, 125080, Moscow, Volokolamskoe avenue, 11*
[e] *Moscow State University of Food Production, 125080, Moscow, Volokolamskoe avenue, 11*
Email: [a]inga99@mail.ru [b]vet-san-dekanat@yandex.ru [c]natali-mng@yandex.ru [d]Ushkalov@Mail.Ru [e]recaro21@bk.ru

Abstract. A continually expanding arsenal of anthropogenic and natural toxicants regulated in raw materials, food products, animal feed, in various veterinary surveillance facilities and food production facilities requires the development and implementation of accelerated methods and test systems that simultaneously determine several safety indicators. The risks associated with the safety of raw materials and food products can be attributed to contamination by anthropogenic or natural toxicants. The first include toxic elements, dioxins, synthetic plant growth regulators, substances used in animal husbandry: medicines (antibacterial, antiparasitic and hormonal drugs), phosphorus-and-chloroorganic pesticides, etc. To the second - toxins and metabolites of bacteria, fungi, plants. In order to prevent these risks, the control of these pollutants is carried out. Solving this problem will help the introduction of promising and highly effective methods. The aim of the work was to carry out an experimental and theoretical evaluation of some methods for accelerated control of anthropogenic and natural toxicants. As a result of the conducted studies, the analysis of international and Russian legal and regulatory documents on the criteria for the safety of raw materials and food products is given. The prospects of methods and test systems based on immunomicrochip technology, immunochromatographic indicator elements and PCR in "real time" mode with the use of robotics for accelerated control of anthropogenic and natural toxicants have been experimentally proved. As a result of the studies, the possibility of determining organophosphorus pesticides and pesticides from the class of imidazoles in plant foods and water has been demonstrated, based on the inhibition of acetylcholine-esterase activity with a colorimetric determination of the final result on a vertical photometer as well as immunomicrochip technology. It has been shown that immunochromatographic indicator elements with colloidal gold nanoparticles (IIEC) are promising for screening control of natural toxicants: microorganisms, toxins of bacteria and fungi. The high sensitivity of the PCR method in real time mode has been proven, and automation makes it promising for controlling bacteria in raw materials or products, especially for hard-to-grow or dangerous microorganisms (salmonella, campylobacteria, causative agents of anthrax, brucellosis, etc.)

Keywords: toxicants, raw materials, and food products, immunochromatographic indicator elements, polymerase chain reaction, immunomicrochip method.

1 Introduction

The decree of the President of the Russian Federation "On the approval of the doctrine of food security" predetermines the intensive development of food production (Decree No. 120 of the President of the Russian Federation of 30.01.2010 "On Approving the Doctrine of Food Security of the Russian Federation" (Electronic resource) // The President of Russia (site). URL: http://kremlin.ru/events/president/news/6752). At the same time, it is essential to use high-quality raw materials, which will preserve health for consumers, and, ultimately, will ensure the food security of our country.

The safety of raw materials is primarily determined by the absence of toxic pollutants in it. According to the generally accepted international terminology (ISO / IEC 2, paragraph 2.5), the concept "safety is a state in which there is no unacceptable risk associated with causing harm to the life or health of citizens, property of individuals or legal entities, state or municipal property, the environment, life or health of animals and plants".

The risks associated with the safety of raw materials and food products can be attributed to contamination by anthropogenic or natural toxicants. The first include toxic elements, dioxins, synthetic plant growth regulators, substances used in animal husbandry: medicines (antibacterial, antiparasitic and hormonal drugs), phosphorus-and-chloroorganic pesticides, etc. To the second - toxins and metabolites of bacteria, fungi, plants. To prevent these risks, the control of these pollutants is carried out.

Meat products are one of the most critical elements of the population's nutrition. The issues of improving methods for assessing the safety of raw materials and products of animal origin, as well as the harmonization of safety criteria with international requirements, are very relevant in the light of technical regulation and integration processes within the framework of the Eurasian Union and the WTO.

Currently, the safety criteria are specified in the Technical Regulations of the Russian Federation and the Customs Union (TR TS 021/2011 "On the safety of food products" (Electronic resource) // Rosstandart (Offic. site). URL: http://www.gost.ru/wps/portal/pages/directions?WCM_GLOBAL_CONTEXT=/gost/gostru/directions/technicalregulation/technicalregulationses/teh%20reg%20tc%20o%20bez%20pizh%20prod). However, in them, as well as in the Sanitary Rules and Norms (SanPiN 2.3.2.1078-01), initially not all requirements were fully harmonized with such international documents as the European Union Directives and the Codex Alimentarius. It should be noted that the introduction of the significant additions and changes to date has not been fully completed.

The most significant group of pollutants are the remains of agricultural pesticides. This group includes pesticides (bactericides, fungicides, insecticides, herbicides), fertilizers, plant growth regulators, plant protection products. Pesticides are substances of various chemical nature used in agriculture to protect cultivated plants from weeds, pests, and diseases, among them: organochlorine, organophosphate, carbamate, organomercuric, synthetic pyrethroids and copper-bearing pesticides. Although many of them are currently banned in most countries, they are produced and used for their intended purpose in agriculture. It is impossible to abandon the use of pesticides entirely. Therefore, it is vital to control their production and use, since they are capable of bioaccumulation in plant foods, they can later enter the animal organism and then accumulate in the form of residual quantities in raw materials and animal products. Studies conducted in several countries have shown the presence of residual amounts of pesticides in animals and food (Zheltov VA 2015;Melnikov N.N. 1994; Yaremchuk V.P. Monitoring the content of pesticides in meat raw materials and 2016).

The international criteria for toxicological safety are established in the directives of the European Union (EU), Codex Alimentarius. According to these documents, the content of antibiotics (levomycetin (chloramphenicol), tetracycline group, grazing, bacitracin) is not allowed in meat, meat and meat products, poultry meat and offal); pesticides (hexachlorocyclohexane (alpha, beta, gamma isomers), DDT and its metabolites).

Sanitary Rules and Norms "Food raw materials and food products. Hygienic requirements for the safety and nutritional value of food products "prescribe that animal products are controlled by residual amounts of animal growth stimulants (including hormonal drugs), medicines (including antibiotics) used in animal husbandry for fattening, treatment, and prevention of diseases livestock and poultry. According to Appendix No. 12 SanPin 2.3.2.1078-01 and the documents of the Joint FAO / WHO Expert Committee on Food Additives and Contaminants, the maximum levels of residues of veterinary (zootechnical) preparations in food products of animal origin are given, where, in particular, the residual amounts of antimicrobial agents, spectinomycin, neomycin, gentamicin, ceftiofur, sulfadimidine, flumequine, lincomycin, thiamphenicol, danofloxacin, spiramycin, sarafloxacin. Remaining quantities of anthelminthic agents - closantel, ivermectin, flubendazole, thiabendazole, triclabendazole, levamisole, febantel, fenbendazole and oxfendazole, moxidectin, doramectin, abamectin, eprinomectin.

In meat, meat products and by-products of slaughter cattle and poultry, fodder antibiotics - grisin, bacitracin, and curative antibiotics, which are most often used in veterinary medicine - antibiotics of the tetracycline group, levomycetin, are approved for use in agriculture. In milk and dairy products, penicillin, streptomycin, tetracycline antibiotics, and levomycetin are controlled.

In connection with the harmonization of safety indicators with international standards and the need to monitor all potential pollutants, a National Laboratory Monitoring Plan for the Remains of Prohibited and Harmful Substances in Live Animals, Animal Products and Feedstuffs has been developed and is being implemented in our country. In particular, Group A (A) - substances that have an anabolic effect and forbidden substances: stilbenes, their derivatives (derivatives), salts and ethers; hydrostatics; steroids; lactones of resorcic acid, including zeranol; beta - agonists; substances included in Annex V of Council Regulation (EEC) No. 2377/90 of 26 June 1990; chloramphenicol; nitrofurans (including furazolidone); nitroimidazoles. Group B (B) - veterinary medicinal products and environmental contaminants: antibacterial substances, including sulfonamides and quinolones. Other veterinary medicinal products: anthelmintics coccidiostatics; carbamates and pyrethroids; sedatives; non-steroidal anti-inflammatory drugs; other pharmacologically active substances; other substances and environmental contaminants: organochlorine compounds, including PCBs; organophosphorus compounds; chemical elements, mycotoxins, dyes, other materials, including non-proprietary medicines that could be used for veterinary purposes (histamine, radionuclides, agrochemicals, microbiological indicators, etc.).

Also in the meat of animals, and the products of its processing, antibacterial and antiparasitic agents can accumulate. Antimicrobial and anthelmintic preparations are widely used in veterinary medicine, and residual amounts of these medicines represent a risk to human health, since they can cause allergic reactions, can lead to the emergence of resistant to these drugs, microorganisms, nematodes and trematodes, and have an adverse effect on the blood and hereditary systems of man and animals. Thus, the control of these residues plays a crucial role in ensuring food safety (Melnikov N.N. 1994).

To natural toxicants of bacterial origin are themselves pathogenic bacteria and the toxins they produce. They contaminate food and cause acute food intoxication.

To determine anthropogenic and natural toxicants, various approaches are used based on physicochemical, microbiological, molecular-biological and immunochemical methods.

The work aimed to carry out an experimental and theoretical evaluation of some methods for accelerated control of anthropogenic and natural toxicants.

2 Methodology

As objects in the validation of test systems based on the inhibition of enzymatic activity "in vitro" and immunomicrochip analysis for screening control of pesticides, samples of plant feed and drinking water from open sources artificially contaminated with organophosphorus pesticides were selected. Preliminary samples were checked by HPLC (High-Performance Liquid Chromatography) for the absence of detectable toxicants. For artificial contamination of samples, standardized diazinon samples were used. The "Abraxis OP / C" test system was used to determine the organophosphorus pesticides. The essence of the method of determination was the inhibition of acetylcholinesterase by organophosphorus compounds and a change in the degree of coloration, which was fixed by the reaction of the enzyme with 5,5'-dithio-bis (2) nitrobenzoic acid. The reaction was carried out in the wells of a plate for enzyme immunoassay. The final result was recorded by changing the degree of staining with a colorimetric determination using a vertical photometer at 405 nm.

Immunomicrochip analysis of the detection of pesticides from the imidazoles class was carried out with the help of the Anthelmintics Array test system using the Evidence investigator semi-automatic chemiluminometer "Randox" (UK).

For experimental confirmation of the use of indicator immunochromatographic elements with colloidal gold nanoparticles (IIHE) for monitoring toxicants in meat raw materials, immunochromatographic indicator elements (IIHE) manufactured by FSUE "State Research Institute of Biological Instrumentation" of FMBA Russia were used.

The subjects of the study to improve the methods of controlling pathogens in raw materials and food products using real-time PCR were – chicken`s meat and meat semi-finished products (pelmeni), as well as artificially contaminated S.typhimurium samples of these products. Peptone water was used to grow salmonella. Cell destruction and DNA isolation were carried out using a Qiagen EZ1 Advanced XL robotic station using magnetic particle technology, PCR was carried out on a RotorGene® 6000 (Corbett Research) amplifier using the AmpliSens® Salmonella spp. The test system (TsNIIE Rospotrebnadzor). By reference methods of identification we used: classical bacteriological analysis and the technique with the miniVidas device.

3 Research results

At present, active physicochemical reference methods exist for the determination of pesticides. However, when carrying out production daily, these methods are very laborious, expensive and require the availability of sophisticated equipment. For screening control of livestock facilities, especially for small businesses and farms, accelerated techniques can be used based on inhibition of enzymatic activity "in vitro" and immunomicrochip analysis. Earlier, it was shown the possibility of applying this approach for the detection of toxicants in honey and critical control points of biotechnological productions (Babunova V.S. 2011, Yarov OA, Svetlichkin VV, Mikhaleva LP, 2010).

One of the research tasks was the validation of test systems based on inhibition of enzymatic activity "in vitro" and immunomicrochip analysis for screening control of pesticides in plant foods and drinking water from open sources.

During the research, adapted techniques were developed. The method for determining organophosphorus pesticides in feeds included: sample homogenization, FOS extraction with double methanol volume, homogenate precipitation by centrifugation at 3500g, dilution and filtration of supernatant through a 0.02 micron filter cartridge and with Anotop 25 Plus fiberglass prefilter, sequential incubation in wells of the plate of the test aliquots of the filtrate with reagents of the test system. Determination of the absorbance of the wells at 405 nm after addition of the Stop Solution. Interpretation of the results was carried out to determine the decrease in the optical density values in comparison with the control. As a blank control sheet, non-contaminated samples of these test subjects were taken, as well as methanol, which was used for the extraction of FOS and dilution of contaminants. In the experiments, an internal negative control was also applied in a 50% solution of methanol.

For the control of water quality, the following modified procedure was developed. Centrifugation at 500g was used for preliminary purification from coarse particles. The redemption from possible low-molecular impurities of protein nature was carried out in the following way: zinc sulfate and potassium ferrocyanide were added to the samples, which are known to form zinc ferrocyanide, which binds proteins well, and are also extracted with FOC by 50 percent methanol. To this end, the water was transferred to a centrifuge tube, successively solutions of Carrez 1, Carrez 2, and methanol was added to a final concentration of 50%, vortexed vigorously after each reagent and centrifuged for 10 minutes at 5000 g and 4-12 ° C 39 - 54 ° F). Aliquots of the sample solutions, after appropriate sample preparation, were added to the wells of the plate and similar

reactions were carried out with the reagents of the Abraxis OP / C test system. Negative control and samples that did not contain FOS in a detectable quantity gave a dark yellow color. The samples containing FOS had a weaker color than the negative control. A 20% color inhibition (decrease in optical density) indicated the presence of FOS in the amount at the detection limit or above. The established detection limits of the OPC made it possible to detect nanogram quantities of the substances to be determined. The sample preparation procedure for analysis with the Anthelmintics Array test system for the detection of imidazoles in feeds included: homogenization, successive addition to acetonitrile homogenate, sodium chloride and anhydrous magnesium sulfate, centrifugation at 2840 rpm for 12 minutes, transferring 5 ml of supernatant to a glass vial, drying at 50 ° C, resuspension in diluted wash buffer. The sample preparation procedure for water was practically the same, except the homogenization procedure.

The essence of the method of immunochromatography consists in the immune reaction "antigen-antibody" occurring between the analyzed liquid sample and antibodies immobilized in the test and control zones, conjugated with colloidal gold. The resulting immune complex under the action of capillary forces moves along the nitrocellulose membrane of the test strip, reaches the analytical zone where it is immobilized by binding to antibodies of the analytical zone. In this zone, a brightly colored "sandwich" of the conjugate of colloidal gold is formed, associated with the desired antigens by specific antibodies immobilized on the membrane. The presence of two colored bands on the layer of immunochromatographic indicator elements indicates the presence of the desired antigen in the sample. The final result is recorded visually or using an OTDR. Reflectometry allows reducing such factors of subjectivity when evaluating a conclusion by a person, such as a workplace illumination, operator fatigue or individual features of color perception and immunohromatogram line saturation (Yarkov S.P. 2005).

The experimental confirmation of the use of IIHE domestic and foreign production for controlling Salmonella in meat raw materials and food products has been carried out. To manage the meat raw materials for the maintenance of bacteria, a modified procedure has been developed that makes it possible to improve the efficiency of sample preparation and reduce the effect of stained meat elements on the interpretation of the analysis results (Figure 1).

The polymerase chain reaction (PCR) method is compassionate and specific (Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. Kinetic PCR:1993). When using real-time PCR, the amount of time spent in analysis and quantification is significantly reduced. Means for detecting the result in real-time PCR are based on changes in fluorescence, which is proportional to the increase in the amount of PCR product during the reaction. Fluorescence is measured during each PCR cycle, and a graph is constructed based on the measurement data to monitor the progress of the response.

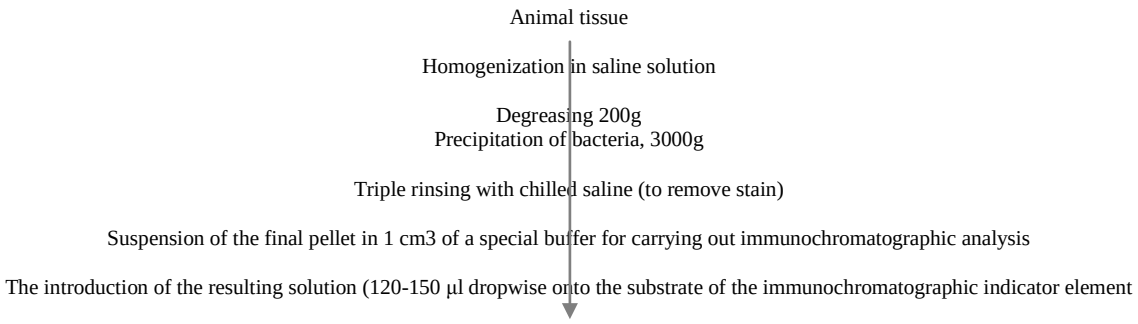

Figure 1: Scheme of a modified method for determining bacteria in animal tissues using immunochromatographic indicator elements with colloidal gold nanoparticles

Studies have been carried out to improve the control of salmonella in poultry meat using real-time PCR and robotics. During the studies, the daily culture of S. Typhimurium bacteria before growth in peptone water and seeding of neutral samples was titrated at a factor of 10. A total of 4 dilutions were prepared, where each subsequent dilution contained 10 times less microbial cells than the previous one. So, if in the first dilution there were 1000 microbial cells, then in the last one cells. For the PCR study, the raw material (a mixture of plant cells) and 4 samples of chicken meat artificially seeded with S. Typhimurium (25 grams homogenized with 225 grams of peptone water and the addition of S. Typhimurium cells, respectively, 1.10, 100 and 1000, respectively) were taken and thermostated at T = 37 ° C for 2.4.6.8 and 18 hours. Next, the mixture of plant cells was stored at minus 20 ° C until the time of the study. So did the material taken after 2, 4, 6 and 8 hours from seeded samples. Interpretation of PCR results was based on the presence (or absence) of the intersection of the fluorescence curve with the threshold line established at the appropriate level, which determined the presence (or absence) of the value of the threshold cycle (Ct) for this sample of DNA equal to 38.

4 Discussion

As a result of the studies, validation of the test systems was carried out and modified and adapted screening techniques were developed to determine organophosphorus pesticides and pesticides from the imidazoles class in plant feeds and water based on the inhibition of acetylcholinesterase activity with colorimetric determination of the final result on a vertical photometer as well as immunomicrochip technology. Techniques are highly sensitive and relatively low cost. Also, the analysis time is 1-2 hours, including sample preparation, which makes them promising for the control of pesticides in plant foods and drinking water from open sources.

Analysis with the help of IIEC does not require sophisticated equipment, which allows it to be used in laboratories with low technical equipment. However, direct examination of the extract of food homogenates utilizing the immunochromatography method is possible only with an extremely high level of contamination of products or raw materials with Salmonella, which is a rare case. The duration of such an analysis scheme, including sample preparation, does not exceed 1h. Sample preparation involves homogenizing the product, extracting the cells with assay buffer, filtering or centrifugation under mild conditions, and then applying the filtrate or supernatant to the immunochromatographic test. To increase the sensitivity, pre-

enrichment (for bacteria) or concentration (for toxins) can be used.

The experimental estimation of multiplex PCR in real time mode and robotics for control of salmonella in meat raw materials and products was carried out. Enrichment of the isolated bacteria from the objects in the primary accumulation environment for 4 hours made it possible to identify S. Typhimurium by real-time PCR reliably

Robotics allowed in an automatic mode to carry out deproteinization, separation of fractions of lipid-protein debris and DNA. This approach allowed for 4 hours to display Salmonella in artificially contaminated sites. The results were correlated with bacteriological analysis data.

Conclusion

As a result of the studies, the possibility of determining organophosphorus pesticides and pesticides from the class of imidazoles in plant foods and water has been demonstrated, based on the inhibition of acetylcholine-esterase activity with a colorimetric determination of the final result on a vertical photometer as well as immunomicrochip technology.

Indicator immunochromatographic elements with colloidal gold nanoparticles (IIEC) are promising for screening control of natural toxicants: microorganisms, toxins of bacteria and fungi.

The high sensitivity of the real-time PCR technique and automation makes it promising for bacterial control in raw materials or products, especially for those that are difficult to grow or are dangerous (salmonella, campylobacteria, causative agents of anthrax, brucellosis, etc.)

The carried-out researchers have shown perspective of the considered innovative directions for introduction in practice of the control of the toxicological and microbiological safety of raw materials, food products and objects of food manufactures.

Source of Support: Research was carried out within of the state contract 15.7831.017 / BC “Search for living systems and substances of natural origin with the analysis of their biological activity for the creation of functional food and feed products”.

Literature:

1. Babunova V.S. Application of immunomicrochip technology to control the safety and quality of food products. Babunova, D.A. Onischenko, OA Yarov, A.V. Artemov, M.V. Kondratieva, S.V. Lutsyk // Russian Journal "Problems of Veterinary Sanitation, Hygiene and Ecology", 2011, No. 1 (5), pp. 12 - 16.
2. Decree No. 120 of the President of the Russian Federation of 30.01.2010 "On Approving the Doctrine of Food Security of the Russian Federation" (Electronic resource) // The President of Russia (site). URL: http://kremlin.ru/events/president/news/6752
3. Higuchi, R., Fockler, C., Dollinger, G., and Watson, R. Kinetic PCR: Real-time monitoring of DNA amplification reactions. Biotechnology, 1993, no. 11, pp. 1026-1030.
4. Melnikov N.N. The current situation with the use of pesticides / N.N. Melnikov // Chemical Industry, 1994, N 2, Pp.14-18.
5. Nityaga I.M. Prospects of application of methods and means of nanotechnology for ensuring food production safety // Storage and processing of agricultural raw materials, 2016, № 7, pp.22-23.
6. Svetlichkin V.V. Test systems and technical means for accelerated control of the safety and quality of veterinary surveillance facilities / V.V. Svetlichkin, A.B. Kononenko, S.P. Yarkov, A.A. Strelkov, M.V. Kondratieva, A.I. Panyushkin // Problems of Veterinary Sanitation, Hygiene and Ecology. M .: VNIIVSGE, 2010, No. 1, pp. 26 - 30.
7. TR TS 021/2011 "On the safety of food products" (Electronic resource) // Rosstandart (Offic. site). URL: http://www.gost.ru/wps/portal/pages/directions?WCM_GLOBAL_CONTEXT=/gost/gostru/directions/technicalregulation/technicalregulationses/teh%20reg%20tc%20o%20bez%20pizh%20prod
8. Zheltov VA Priority technogenic pollutants of the environment / V.A. Zheltov V.A., V.I. Dorozhkin // Problems of Veterinary Sanitation of Hygiene and Ecology, 2015, No. 2, P. 101.
9. Yaremchuk V.P. Monitoring the content of pesticides in meat raw materials and veterinary and sanitary measures to reduce them / Yaremchuk VP, Alieva I.Kh., Udavliev DI, Rodin VI, Nityaga I.M., Glazkova I.V. , Vanner N.E. // Meat technologies, 2016, №1, P. 58.
10. Yarkov S.P. Indication of dangerous pathogens based on immunochromatography with video-digital recording / S.P. Yarkov, S.I. Tretyakov, L.A. Basharova, V.N. Zlobin // Theses of the II International Conference "Molecular Medicine and Biosafety", Moscow, 2005, p. 295.
11. Yarov OA, Svetlichkin VV, Mikhaleva LP, Immunomicrochip technology as an express method for monitoring the level of residual amounts of antimicrobial drugs in honey. // Proceedings of the collection of the VIII International Scientific and Practical Conference "Living Systems and Biological Security of the Population", Moscow: MGUPB, 2010.- pp. 221-222.

Primary Paper Section: A

Secondary Paper Section: AC, FQ