ASSESSMENT OF GENOTOXIC AND MUTAGENIC EFFECTS OF FOOD PRODUCTS WITH BIOASSAY METHODS

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Keywords: food safety, meat products, genotoxicity, mutagens, express bioassay, allium test

Abstract
The current state of studies on application and safety of food additives in various technologies for food production is examined. Considerable attention should be given to studies dedicated to analysis of food safety criteria due to a possibility of appearance of adverse consequences for human health and the trend towards increasing life quality. Special emphasis is placed on such parameters as genotoxicity and mutagenicity. It is shown that the most rapid and convenient tool for complex monitoring of product toxicity can be the bioassay procedure. Based on the review of the literature on bioassays for edible meat and meat products, canned foods, carbonated soft drinks, beer, milk and milk containing products as well as seasonings, the authors show that above mentioned products had the cyto- and genotoxic potential when tested on animal and human cell cultures, microorganisms and plants. With that, it was found that a list of relevant publications is quite small despite a significant growth in scientific research dedicated to food toxicity assessment using bioassays. A review on the conducted research on assessment of genotoxic and mutagenic effects of foods by bioassay methods will make it possible to extend the understanding of the processes and mechanisms of this toxicity and form more rational concept of consumption.

Introduction
Increasing globalization and acceleration of the global food market development impose new requirements for finished products [1]. According to this trend, a spectrum of food product types and their components is expanding, new production technologies and food additives, which extend shelf life and improve consumer characteristics of foods, are appearing. At the same time, according to the “Strategy for improving the quality of food products in the Russian Federation until 2030” [11], adequate nutrition of the population remain to be the key requirement for increasing life expectancy and quality. Therefore, the problem of controlling the risk for food safety has been actively discussed in the scientific community [2].

For example, several studies were published that assessed the correlation between red and processed meat consumption and the development of colorectal cancer [3,4]. Substances in meat with the proved carcinogenic potential (polycyclic and heterocyclic aromatic hydrocarbons) and metabolites increasing the proliferative activity of the intestinal epithelial cells and triggering the process of lipid peroxidation can initiate processes of the malignant tumor development.

The main part
As for now, the methodological aspects of the solution to the problem of food safety assessment are still debatable. One of the realized approaches is aimed to studying certain product components and assessing their individual toxicity or combined effects of every component on each other. This approach is also directed to studying products of component breakdown in the body [5–7]. For example, it was shown that food colorants widely used in production of soft drinks and confectionary products can facilitate the development of allergic reactions, as well as pathological changes in the gastrointestinal tract [8,9]. Also, nano-food additives that are more and more frequently used in the food industry are not classified as fully safe for consumption. Furthermore, when testing zinc nanoparticles and ascorbic acid in vivo and in vitro, it was found that cytotoxicity in the mixture was significantly higher than upon their individual incorporation [10]. The American scientists showed in their review on the toxicity of the biologically active compounds that 47 known chemical compounds (alkaloids, hormones) in 55 different plant species from 46 families demonstrated harmful side effects [11]. The negative side effects such as hepatic toxicity, the development of pathologies of the cardiovascular, central nervous and digestive systems in the animal and human bodies were revealed after consumption of products with the indicated components.

On the other hand, studies dedicated to assessment of the toxicity effects of complex food matrices using the bioassay methods on different objects such as human and animal cell cultures [12], plants [13] and microorganisms [14] have appeared recently. This approach can provide the complex analysis in food biosafety assessment, first of all, with account for interaction of all indicated components. The undeniable advantage of these methods is also a possibility to obtain the indicated experimental data without using labo-
ratory animals, which keeping and handling require adherence to modern bioethical standards and significant material expenditures. In addition, a significant period of time is required to obtain results of animal experiments.

Today, analyses of the genotoxic and mutagenic potential of food matrices have become topical in assessment of risks for safe food consumption [15]. Moreover, the recommendations of the European Food Safety Authority (EFSA) indicate the necessity of cytotoxic and genotoxicological studies in assessment of the finished products when considering the risk of safety of food product consumption for human health [16].

Therefore, the aim of the review was to analyze approaches to assessment of genotoxic and mutagenic effects of foods using bioassays.

Genotoxicity is a property of a substance (agent) manifested in its ability to damage a DNA molecule. This can happen as a result of the direct impact or indirectly, for example, by acting on enzymes taking part in DNA replication. It is necessary to pay close attention to these indicators mainly due to the fact that mutations in cells can be linked with an increased risk of cancer development [17]. In case of chronic exposure, compounds with mutagenic properties can lead not only to appearance of chromosomal aberrations, but also to an increased rate of their accumulation [18]. Based on the above, it can be concluded that the determination of the genotoxic and mutagenic potential of food products can be of utmost importance when assessing risks of safe food consumption [15].

Meat and products of its processing are important sources of protein in the human diet. Taking into consideration this fact, assessment of mutagenicity and genotoxicity of meat and cooked meat dishes is an important component in monitoring food safety of the daily diet. The research was carried out to assess studies that determined mutagenicity of different types of prepared meat using the Ames test (Table 1). Augustsson et al. demonstrated the pronounced mutagenic effect of extracts of six different fried meat dishes on Salmonella typhimurium TA98. Apparently, the revealed mutagenic effect can be linked with the development of heterocyclic amines during frying [19]. In another study, effects of frying temperature (100, 150, 200, 250 and 300 °C) and duration (2, 4, 6, 8, 10, 12 min at 250 °C) of cooking patties from ground lamb on mutagenicity was investigated also using the Ames test [20]. The maximum number of revertant colonies per 10 g sample was found in case of frying meat for 10 min (nine times higher than in uncooked meat) and in case of frying at maximum temperature 300 °C (eight times higher than in uncooked meat). It is also necessary to emphasize the presence of the dose-dependent mutagenic effect when considering an influence of both factors.

Gocke et al. analyzed mutagenicity of the extract from fried pork sausages on several cell cultures and microorganisms [21]. They showed that the Ames test was more sensitive to mutagens formed during meat frying compared to other used tests. For example, only slight mutagenic activity was noticed in analysis of the sister chromatid exchange in the cell line V79. Also, the mutagenic action of the extracts was not recorded in analysis of gene mutations (thioguanine resistance) in V79 cells and in analysis of sex-linked recessive mutations in Drosophila.

Vikse and Joner studied the mutagenic activity in 16 extracts of different meat types compared to the control beef sample using Salmonella typhimurium TA98 [22]. It was found that all extracts had less pronounced mutagenic effect than the control sample. Mutagenicity of extracts varied from 36% in seal meat to 81% in goat meat compared to the control sample. Additionally, the content of water, protein, fat and carbohydrate was measured in the meat samples. The significant correlation coefficients between these indicators and mutagenicity were not revealed.

Furthermore, data on toxicity of commonly consumed canned fish and vegetables were obtained using the yeast cell culture (S. cerevisiae) and the standard Ames test [14]. The results of these investigations showed that four selected ready-to-eat products (canned fish, canned spinach, canned tomato and canned fruit cocktail) showed the cytotoxic and genotoxic effects on these test objects. Canned tuna fish had the highest cytotoxic and genotoxic potential; namely, the number of spontaneous mutations in the experimental samples was two times higher than the control values. Moreover, a significant dose-dependent mutagenic effect was observed for all concentrations of this product (2, 5, 10, 50, 100 ml of the sample in a well). The authors linked the pronounced mutagenic effect largely with contamination of tuna meat with a high amount of heavy metals (mercury, lead, cadmium). Among canned plant products, canned spinach turned to be most toxic. The researchers suggested that these products contain food additives (mainly, coloring agents) with the pronounced toxic effect.

Biotesting of beer and the reference aqueous solution (7% of ethanol and sugar added according to the quantity indicated on product labels) was carried out on tumor and normal animal and human cells [12]. It is worth noting that in the experimental conditions survival of both tumor and normal cells decreased upon exposure to these low-alcohol beverages. After 72 hours of treatment, the number of normal cells reduced up to 67% and the number of tumor cells up to 3–4% compared to the control, while the exposure to the equivalent doses of the reference solution did not show the cytotoxic effect. Based on these results, the authors concluded that ethanol is not the determining component affecting manifestation of toxicity.

The other test object being used in studies on genotoxicity of foods is onion (Allium cepa L.). The onion apical meristem is convenient for cytological investigations as its chromosomes have the large size and are well seen under a microscope [23]. Furthermore, advantages of this method also include its low cost. Therefore, the field of Allium-test application for different foods has been extending recently (Table 1).

Furthermore, data on analysis of ultra-pasteurized milk and milk containing products in the conditions of the
Allium-test were published [26,27]. It was found that all analyzed samples had the significant anti-proliferative effect. It was also established that the mitotic index in the cells of the onion bulb reduced by 2.5 to 7 times compared to the control value depending on a producer upon the daily exposition of bulbs to the samples of ultra-pasteurized milk. When treating onion bulbs with samples of dry milk diluted with water, the frequency of cells in mitosis also reduced to several tenths of a percent. Moreover, an increased number of micronuclei compared to the control was observed in the studies. According to the authors’ opinion, these disorders can indicate the presence of the potential toxicity of this product, which can provoke tumor development, as a positive correlation between the increased frequency of micronuclei and cancer development was demonstrated [32]. The authors also explained such negative changes by the presence in the products of fruit processing and milk-containing products produced in Brazil, as well as by the presence of food additives (anti-caking and alkylating agents, colorants, preserving agents and sweeteners). It is necessary to note, that some of these additives are forbidden for using in production of juice and dairy products in Russia according to the requirements of the Technical Regulations of the Customs Union2-3.

Analysis of the toxic effect of domestic apple juice using the Allium-test, showed that juice diluted in a ratio of 1:5 reduced the growth of root biomass by two times and the mitotic index by 18 times, while the MDA level in the root tissue increased by 11% compared to the control [31]. At the same time, fructose and the model juice solution prepared from the main components of its dry solids were less toxic both regarding an effect on cell mitosis and in the process of root development. Thus, the leading role of the minor juice components in inhibition of cell proliferation and the following growth and development of roots was demonstrated.

Mitotoxicity, genotoxicity and mutagenicity were recorded in analysis of the other frequently used soft drink, Coca-Cola, in the experimental conditions using the onion apical meristem [28–30]. The mitotic index decreased in a dose-dependent manner with the extension of treatment duration and the level of aberrant cells increased when roots were treated with soft drinks. For example, in the experiment with a short-term impact, the 8-hour exposure of roots were treated with soft drinks. For example, in the experiment with a short-term impact, the 8-hour exposure to the drink reduced the mitotic index from 8.5% to 1.3%. The mitotic index reduced almost by half (7.6% vs. 4.4% in the control) in the root cells exposed to the drink over two days. Among the whole spectrum of the revealed chromosomal aberrations in cells upon treatment with drinks, an increased frequency of stickiness of chromosomes in mitosis was recorded. The authors suggested that an increase in the frequency of stickiness of chromosomes during cell division took place due to depolymerization of the DNA molecule and partial dissolution of nucleoproteins in the metaphase. This process can be irreversible and lead to cell

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2 TR TU023/2011 Technical Regulations of the Customs Union “Technical regulations for juice products from fruits and vegetables” (as amended on December 15, 2015, Decision of the Council of the Eurasian economic Commission of December 09, 2011, № 882 .Moscow, 2015. (In Russian)

3 TR TU033/2013 Technical Regulations of the Customs Union “On the safety of milk and dairy products” (as amended on July 10, 2020, Decision of the Council of the Eurasian economic Commission of October 09, 2013, № 67. Moscow, 2020. (In Russian)
death. With that, significant differences were not found when comparing the response of the bio-tester on drinks of two different trademarks. It is worth noting that in the above mentioned examples, the roots were exposed to the undiluted drink or drink diluted with water in a ratio of 1:2 for 2–48 hours. We assume that testing over such a short period of time can give little information as the average duration of the cell cycle of the onion apical meristem is 18 hours. It is also known that the limits of Allium-test application in terms of the hydrogen indicator begin from 3 units [33], while cola pH is within a range of 2.5–3.5 units [34]. Therefore, bio-testing of the undiluted drink can be associated with inaccurate experimental data as well as with problems in interpretation of indicators.

Conclusion

At present, the problem of food safety assessment, including their toxicological load, is topical. When analyzing studies devoted to this question, it was found that a list of studies on food toxicity assessment by bioassay methods is still quite small despite the growing interest to such investigations. Onion (Allium cepa) should be mentioned among frequently used test objects. Several studies showed cytotoxic- and genotoxicity of products in the concentrations being analyzed with this biotester. Extension and addition of studies on food toxicity using a wide spectrum of test objects can be useful for understanding mechanisms of this toxicity and formation of the risk-oriented concept of consumption.

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Completely prepared the manuscript and is responsible for plagiarism.

The author declare no conflict of interest.