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BIOASSAY IN SAFETY ASSESSMENT OF NEW GRAIN PRODUCTS

Maryna Mardar[1*], Galina Krusir[2], Rafaela Znachek[1], Larisa Agunova[3]

[1]*Department of Marketing, Business and Trade, Odessa National Academy of Food Technologies, Odessa, Ukraine*
[2] *Department of ecology and environmental protection technologies, Odessa National Academy of Food Technologies, Odessa, Ukraine*
[3] *Department of Technology of meat, fish and seafood, Odessa National Academy of Food Technologies, Odessa, Ukraine*
Corresponding author: marinamardar2003@gmail.com

Abstract

The article is devoted to the issue of food safety as an important indicator of consumer properties and a decisive criterion of their quality. It describes that various alternative toxicological methods of research using the biological test-objects, the so-called bioassay methods are used along with the traditional toxicological control methods for safety assessment of food products. Safety of new grain crisp bread on the basis of spelt using bioassay methods were evaluated in experimental studies. The first method is based on determining the toxicity of crisp bread using test-object of *Colpoda steinii infusoria*, the second – on testing for a mortality rate of Daphnia Magna Straus crustaceans, the third – by method of determining the toxicity of objects using bioassay methods on the test-object Drosophila melanogaster fruit flies. On the basis of the performed bioassay the safety of new grain crisp bread was determined based on spelt. Ecotoxicological studies allowed assessing the safety of products being developed, as well as the prospect of introduction of new grain crisp bread into the market as a safe food product will be described in future.

Key words: *grain crisp bread, product safety, toxicity, ashberry, brier*

INTRODUCTION

Over the last decade the number of toxicants that affect the safety of raw materials and foodstuffs has increased, moreover, methods of their determination are quite complicated. In 2016 the Act On the Basic Principles and Requirements for Food Safety and Quality was adopted in Ukraine. One of the main priority lines is to prevent losses, preserve quality and guarantee food safety at all stages of production and storage [3]. Pursuant to Act a safe foodstuff is a foodstuff which has no harmful effects on human health and is fit for use.

Contaminants may occur in our food from various sources. They typically pose a health concern, resulting in strict regulations of their levels by national governments and internationally by the Codex Alimentarius Commission. Therefore, analysis of relevant chemical contaminants is an essential part of food safety testing programmes to ensure consumer safety and compliance with regulatory limits. Modern analytical techniques can determine known chemical contaminants in complex food matrices at very low concentration levels. Moreover, they can also help discover and identify new or unexpected chemical contaminants [4].

Many strategies to detect biological and chemical contaminants in foodstuffs have been developed to solve food safety problems [5]. Bioassay is one of the methods of study which is used to determine the degree of negative impact of chemicals being potentially dangerous to living organisms through recording of changes of biologically significant parameters (test functions) of the experimental test-objects with a subsequent assessment of their condition in accordance with the chosen toxicity criterion [6].

Foodstuff bioassay means toxicity studies of aqueous extracts from the product using live test-objects. Test-objects (test organisms) are experimental biological objects (organisms) that are used in determining toxicity. The discovered toxic effect is registered and evaluated in the experiment. Test-objects allow replacing complex chemical analyses and quickly identify the toxicity of the product [6, 7]. The method of determining the toxicity with the test-objects is fast enough; it does not require the use of experimental animals or expensive equipment and has prospects for accelerating safety control of raw materials and foodstuffs [4]. Infusoria, hydras, planarians, leeches, molluscs, crustaceans, representatives of different groups of plants and algae, insects etc. are used as test objects [13]. The choice of the test object for each case is determined by standards, sensitivity level to toxic substances and environment you want to study [6]. In the study we used test objects from different systematic groups: *Colpoda steinii* infusoria, *Daphnia Magna Straus* crustaceans and *Drosophila melanogaster* fruit-flies. The specific properties of these life forms allow obtaining detailed information on the likely negative impact or food stuff safety.

MATERIAL AND METHODS

Bioassay in evaluation of grain crisp bread safety

The objective of the study was to determine the safety of consumption of new grain crisp bread with ashberry and brier using bioassay methods on aquatic organisms and insects.

New types of grain crisp bread made with spelt including enriching additives. Spelt is specie of soft wheat, which is in contrast to the traditional wheat has a high content of proteins, nutrient rich fibres, mineral substances and vitamins [15]. Dry powders of ashberry and brier were used as nutrient additives. Their inclusion into the composition of the crisp bread contributes to giving curative and preventive effect, improving the organoleptic properties to the finished products.

The following samples were the test objects:

- sample 1 – grain crisp bread without additives (control);
- sample 2 – grain crisp bread with ashberry;
- sample 3 – grain crisp bread with brier.

When conducting ecotoxicological control of grain crisp bread bioassay methods with test organisms from different taxonomic groups were used. The first stage of the study was to determine the toxicity of grain crisp bread by bioassay method on the death of Daphnia Magna Straus crustaceans. These test-organisms are widely used in determining the toxicity of soils, waste water, feed and food products [12]. Studies were conducted on a synchronized Daphnia culture. A synchronized culture is one age culture obtained from one female by using acyclic parthenogenesis in the third generation. Such a culture is genetically homogeneous [8]. The technique is based on identifying the differences between the number of dead Daphniae in the test sample (experience) and that which is cultivated in water. Bioassay is the criterion of the acute lethal toxicity.

10 cm^3 of dechlorinated drinking water was poured into each test tube where Daphniae are cultured, 1 cm^3 of water extract of the test sample and 10 Daphniae into each tube. The bioassay was performed in diffused light at a water temperature of (20±2) °C for 96 hours.

According to GOST 32536 – 2013 [9] live Daphniae are the one which move freely in the water column or emerge from the bottom of the vessel after its slight shaking. The rest of Daphniae are considered dead.

RESULTS AND DISCUSSION

Daphniae were not fed during bioassay of samples, at the end of the experience the number of live test-objects was counted at the end of the experiment (Fig. 1).

The toxicity level of the experimental product during testing of aqueous solution of test samples was determined by % of maxillopods that survived in accordance with table 1.

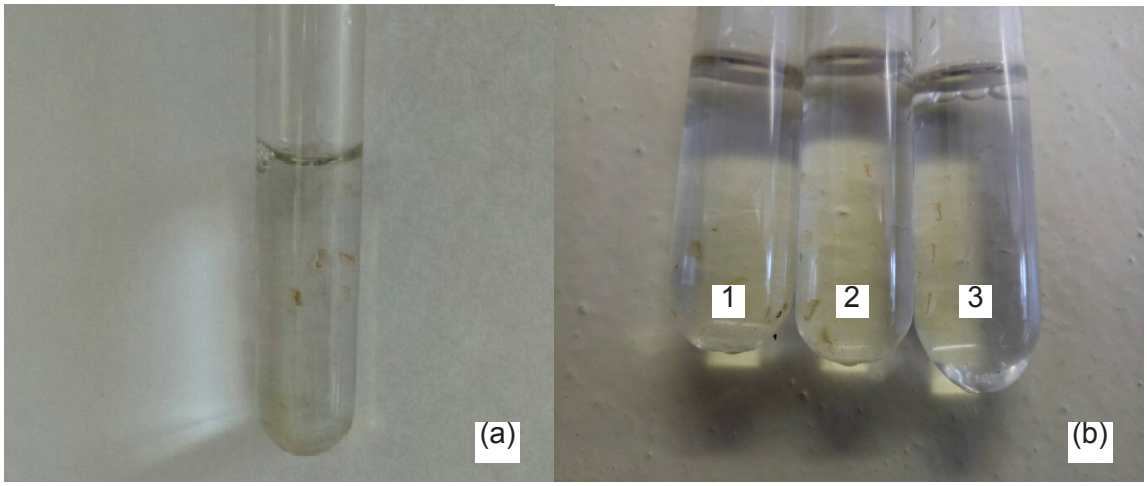

Figure 1. (a) Daphnia magna Straus culture in water; (b) Daphnia magna Straus culture in medium of the tested samples: 1 – grain crisp bread without additives; 2 – grain crisp bread with ashberry; 3 – grain crisp bread with brier

Table 1. The scale of toxicity level of grain crisp bread when testing an aqueous solution

Toxicity level of the experimental product	Survival rate of Daphnia magna Straus maxillopods, %
Non-toxic	93–100
With low toxicity	62–92
Toxic	0–61

The results of study were as follows: in sample № 1 – grain crisp bread without additives (control) the number of dead Daphniae was 1 %, sample № 2 and № 3 – grain bread with ashberry and brier – 3 % on each, that the evidence of new product safety.

The second method is based on determining the toxicity of grain crisp bread using the test-object *Colpoda steinii* infusoria (Fig. 2).

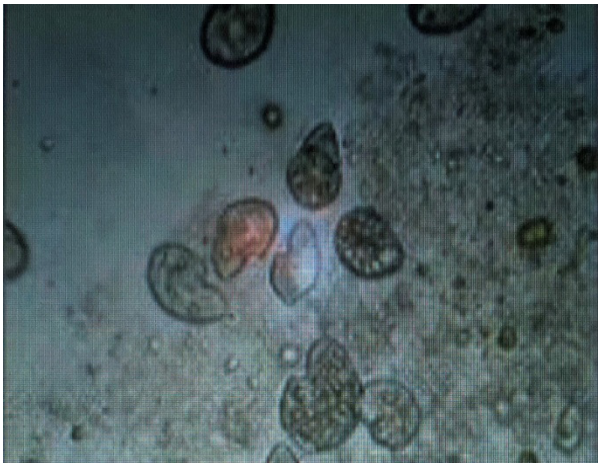

Figure 2. *Colpoda steinii culture*

The method is based on extraction of different fractions from the experimental products – polar and non-polar toxic substances respectively with water and with hexane and the subsequent impact of these extracts (hexane was previously evaporated) on Colpoda steinii culture. Dry culture *Colpoda stenii* represents o colpoda cysts and *Bac. Subtilis* spores attached to the wall of the vial and visible on increase of 80 – 150 min. Developed standardized rating scale of toxicity is given in table 2 [10]. The specimen used in study is manufactured in a

certified laboratory (Vidrodzhennya m LLC, Odessa), in accordance with TU U 46.15.243-97 requirements and [14]. Colpoda culture is harmless to humans and animals; it was stored dry up to 6 months at a temperature of 10 – 25 °C. Vials with Colpoda culture and nutrient medium were opened for studies not earlier than 12 – 24 hours before use. 2 cm^3 of nutrient medium were poured into each vial with Colpoda culture. One made certain of relevance of Colpoda culture immediately before the use. This culture was studied by pendent drop method under the microscope. Colpodae in maximum number of 6 cells per high power field should actively move. Weighing of 20 g was added to a flask with a capacity of 250 cm^3 and poured 100 cm^3 of distilled water. The flasks with the content have been shaken for 20 min, and then the mixture was filtered. 2 cm^3 of extract were added to the tube containing the active culture of Colpoda and stirred. 2 cm^3 of nutrient medium were brought to the control sample with the active culture of Colpoda. In 10 min and then in 3 hours one drop of the mixture was selected from the experimental and control test tubes and reviewed them under the microscope using pendent drop method. Available live and dead infusoriae were considered in the test samples. The criterion of toxicity is the time from beginning of exposure of the experimental extract till death of the majority (90 %) of Colpodae. The fact of death of Colpodae was stated on the basis of a complete termination of their movement and the presence of decay. In the control sample all Colpodae should remain movable in a control sample [10].

The product under study is considered toxic, if death of Coolpodae occurs within 10 min after introduction of extract in a live culture of Colpodae. The product under study is slight toxic, if death of Colpodae occurs within the interval up to 3 hours of studies. The product under study is non-toxic, if all Colpodae remain movable in 3 hours of studies.

Table 2. Toxicity assessment scale of finished product [10]

Toxicity	Criteria
Very toxic	Death of the most Colpodae occurs within 3 minutes
Toxic	Death of the most Colpodae occurs within 10 minutes
Slight toxic	Death of the most Colpodae occurs in 3 hours
Non-toxic	Most Colpodae remain movable within 3 hours

Fig. 3 shows the obtained results of determination of ecological and toxicological parameters of grain crisp bread safety by bioassay method using Colpoda Steinii.

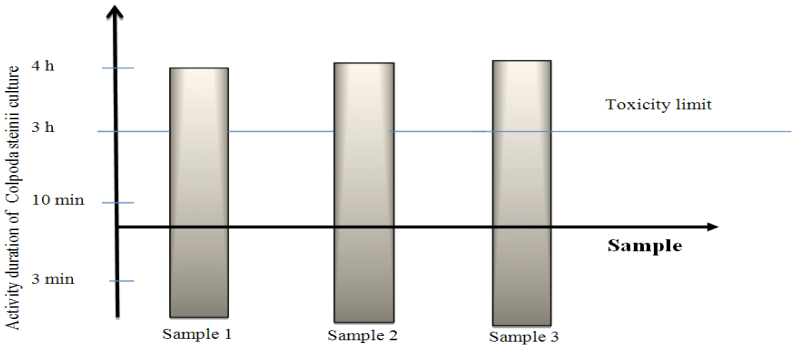

Figure 3. Grain crisp bread toxicity by bioassay method using Colpoda Steinii

On the basis of the performed studies it is established that the sample № 1 – grain crisp bread without additives (control) and samples № 2 and № 3, namely grain crisp bread with ashberry and brier do not contain toxic substances, because most Colpodae remained alive in all the experimental samples within three hours. The results correlate with the studies performed by bioassay method using *Daphnia magna Straus*.

The third method is based on bioassay of grain crisp bread samples by determining the availability or absence of acute toxic action on the test-objects. Chronic toxicity was determined in toxicological analysis of grain crisp bread quality. Biological tests on *Drosophila melanogaster Meig* flies were used during bioassay [11] shown in Fig. 4.

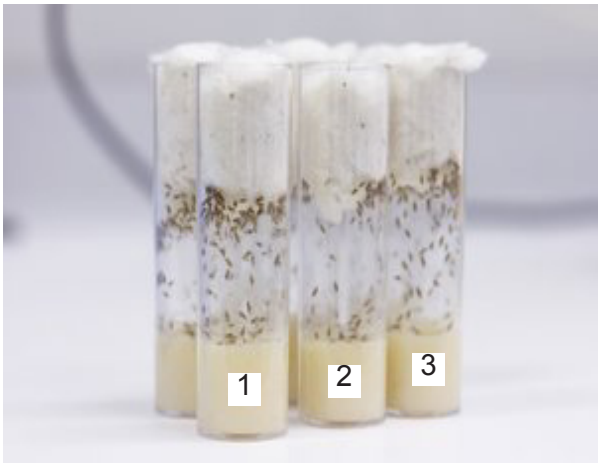

Figure 4. *Drosophila melanogaster Meig* in medium of the experimental samples; 1 – grain crisp bread without additives; 2 – grain crisp bread with ashberry; 3 – grain crisp bread with brier

The toxicity criterion in bioassay method on fruit flies is a probable deviation from frequency control of occurrence of dominant lethal mutations

Determination of toxicity of objects using bioassay method on Drosophila melanogaster fruit flies has as compared to other test organisms (bacteria, plants, tissue culture) a number of advantages due to the fact that it is possible to find all types of mutations in Drosophila. It has a small number of chromosomes, short life cycle and great fertility; metabolic activation of substances proceed the organism which is the same as in humans. Data obtained using this test organism can be extrapolated to highly organized animals including mammals and used as forecast of risk to human health [11].

Grain crisp bread was tested with the aim of establishing the use of bioassay method on *D. melanogaster* to determine the genotoxicity. The presence or absence of genotoxic and mutagenic actions to *D. melanogaster* during bioassay was determined in samples. According to the results of testing of samples of grain crisp bread genotoxic and mutagenic actions were not found in either of the samples that the evidence of the safety of the product.

CONCLUDING REMARKS

Based on ecotoxicological control of grain crisp bread with the test-organisms from different taxonomic groups, namely *Colpoda steinii* infusoria, *Daphnia Magna Straus* crustaceans, *Drosophila melanogaster* fruit flies it was established that the consumption of new cereal products is safe.

Biological analysis of all test objects showed that the samples under study of the crisp bread do not have a negative impact on living form and can be recommended for consumption by a human. The conducted studies are the evidence of expediency of the further work and industrial production of new types of grain crisp bread

based on spelt with ashberry and brier that will allow expanding the assortment and fills the market with safe food stuffs.

The lack of significant material costs, high sensitivity of methods and the obtained results with a high degree of reproduction demonstrate the feasibility to recommend the conduction of bioassay for all new types of food stuffs, particularly on introduction of non-traditional raw materials and additives.

REFERENCES

Biotesting as a method of assessing the quality of drinking water. (2006). Bulletin of the National Academy of Sciences of Ukraine. 10. 55-57. (in Ukrainian).

Bochkov, N., & Chebotarev, A. (1989). Human heredity and mutagens of the environment. Moscow, Russia, Medicine. (in Russian).

Braginsky, L. (2000). Methodological aspects of toxicological biotesting at Daphnia magna Str. and other branching crustaceans (critical review). Hydrobiological Journal. 36(5), 50-70. (in Russian).

Eremeeva, A. S., Donchenko, M. I., Buchelnikov, V. S., Peregudina, E. V., & Azarova, S. V. (2015). Review of bioindication and biotesting methods for environmental assessment. Young Scientist. 11, 537-540. (in Russian).

Food safety. Fact sheet (October 2017). Retrieved from http://www.who.int/mediacentre/factsheets/fs399/en/ (in Russian).

GOST 13496.7-97 Feed grain, grain by-products, compound feeds. Methods for determination of toxicity (with Change No. 1). (in Russian).

GOST 32536-2013 Test methods for chemical products that present a danger to the environment. Determination of acute toxicity for daphnia. (in Russian).

Kohajdová, Z., & Karovičová, J. (2008). Nutritional value and baking applications of spelt wheat. Acta Scientiarium Polonorum, Technologia Alimentaria. 7 (3), 5-14.

Law of Ukraine "On basic principles and requirements for the safety and quality of food products" Retrieved from http://zakon3.rada.gov.ua/laws/show/771/97-%D0%B2%D1% 80 (in Ukrainian).

Lyashenko, O. (2012). Bioindication and biotesting in environmental protection. St. Petersburg, Russia, SPb GTURP. (in Russian).

Man, Lv, Liu, Y., Geng, J., Kou, X., Xin, Z., & Yang, D. (2018). Engineering nanomaterials-based biosensors for food safety detection. Biosensors and Bioelectronics, 106, 122-128. Retrieved from https://www.sciencedirect.com-/science/article/pii/S0956566318300605.

Omarov, R., Agarkov, A., Rastovarov, E., & Shlykov, S. (2017) Modern methods for food safety. Engineering for rural development, Jelgava, Latvia Proceedings: 960-963.

Shaw, J. R., Pfrender, M. E., Eads, B. D., Klaper, R., Callaghan, A., Sibly, R. M., Colson, I., Jansen, B., Gilbert, D., & Colbourne, J. K. (2008). Daphnia as an emerging model for toxicological genomics. Advances in Experimental Biology. 2, 165-219, 327-328.

Vinokhodov, D. (2007). Scientific fundamentals of bit-testing using infusorians. The dissertation of the Doctor of Biological Sciences. St. Petersburg, Russia, St. Petersburg Institute of Technology. (in Russian).

Yevlash, V., Samoilenko, S., Otroshko, N., & Buryak, I. (2016). Express-methods of studying the safety and quality of food products. Kharkiv, Ukraine, KhDUHT. (in Ukrainian).

БИОТЕСТОВИ ЗА АНАЛИЗА НА БЕЗБЕДНОСТ НА НОВИ ЗРНЕСТИ ПРОИЗВОДИ

Марјана Мардар[1*], Галина Крусир[2], Рафаела Значек[1], Лариса Агунова[3]

[1]Оддел за маркетинг, бизнис и трговија, Национална академија за прехранбени технологии во Одеса, Одеса, Украина

[2]Оддел за екологија и технологии за заштита на животната средина, Национална академија за прехранбени технологии во Одеса, Одеса, Украина

[3]Оддел за технолгоја на месо, риба и морска храна, Национална академија за прехранбени технологии во Одеса, Одеса, Украина

**Контакт автор: marinamardar2003@gmail.com*

Резиме

Трудот е посветен на прашањето за безбедноста на храната како важен показател за потрошувачкит есвојства и одлучувачки критериум за нивниот квалитет. Во трудот се опишува дека се користат различни алтернативни токсиколошки методи за истражување со користење на биолошки тестови, таканаречени биолошки методи, заедно со традиционалните токсиколошки методи за контрола за проценка на безбедноста на прехранбените производи. Безбедноста на новиот двојнопечен леб базиран на житната култура спелта со методи за биоанализа беа евалуирани во експериментални студии. Првиот метод се базира на утврдување на токсичноста на свеж леб, користејќи биолошки тест од *Colpoda steinii infusoria*, вториот – за тестирање на стапката на смртност на рак од *Daphnia magna* Straus, третата – со метод на одредување на токсичноста на предметот на проучување, кои користат методи за биоанализа на тест-објектот *Drosophila melanogaster*. Врз основа на извршениот биотест, безбедноста на новиот двојнопечен леб беше определена врз основа на спелата. Екотоксиколошките студии овозможија проценка на безбедноста на производите што се развиваат, како и можноста за воведување на нов двојнопечен леб на пазарот како производ за безбедна храна, кои ќе бидат опишани во иднина.

Клучни зборови: *двојнопечен леб, безбездност на производ, токсичност, природни конзерванси*