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BIOTESTING IN ASSESSING THE SAFETY OF GRAIN PRODUCTS

Abstract

The article discusses the issue of assessing the safety of food products that are traditional for the Ukrainian consumer (bakery products and wheat flakes). The relevance of the work is due to the fact that safety is an important indicator of consumer properties that all food products should have. The biological methods are widely used, including biosensor technologies and biotestingto assess the safety of food products. Biotesting is one of the research methods used to determine the degree of influence of chemicals potentially hazardous to living organisms by registering changes in biologically significant indicators (test functions) of research test objects with subsequent assessment of their condition in accordance with the selected toxicity criterion.

The work assessesthe organoleptic indicators of the test samples. As a result, it was established that the samples fully comply with the requirements of the regulatory documentation, except for sample № 7 in which dampness and an off-taste are felt; this may indicate non-compliance with the storage or sale conditions. The safety of bakery products and wheat flakes was assessed using the Colpoda Steinii ciliate test object. It was established that the test samples did not contain toxic substances, and in the sample № 7 – wheat flakes purchased by weight on the market, the death of most of the Colpoda Steinii occurred within 10 minutes. This indicates its insignificant toxicity and the inadmissibility of further sale of such a product to the population. The second method was used to test the lethality of Daphnia Magna Straus (Crustacea).The results showed that in samples № 1 – 6 the number of dead Daphnia corresponds to the "non-toxic" indicator, but in sample No. 7 the number of dead Daphnia was 25%, which indicates the toxicity of this product.

The third method was carried out according to the method based on the non-destructive biocrystallization method. It was established that samples 1 and 2 are characterized by the most symmetric biocrystallogram, which indicates their higher degree of organicity, which, in our opinion, is determined by a smaller share of external negative impact on the product and high-quality raw materials that were used in the production of these samples.

Key words: *safety, grain products, biotesting, quality assessment*

Introduction

Safety is an important indicator of consumer properties that food products should have. Unlike other consumer properties, the deterioration or loss of which leads to loss of functional purpose, an increase in the permissible level of safety indicators moves the product into a hazardous category [1]. Therefore, the quality of food products is primarily determined by safety. Currently, in the food market of Ukraine, the greatest demand is for grain products (bread and cereal flakes), which are also traditional for our population.

Literary review

Nutrition and life are two interrelated concepts. The World Health Organization and most countries in the world have identified nutrition as one of the main factors in ensuring and improving public health. The most important task to improve the structure of nutrition is to increase the production of mass consumption products with high nutritional and biological value. Cereals, namely, bakery products and cereal flakes, are the main and irreplaceable components of the Ukrainian diet; they contain a number of essential substances necessary to ensure the normal functioning of the human body.

Bakery products, along with other grain products, traditionally form the basis of nutrition in our country. Plain bread contains the nutrients that humans need. Bread contains proteins, carbohydrates, vitamins of groups B, PP, mineral compounds. And what is more important, bread has a rare property – it does not become boring, people cannot stop eat it. People eat bread every day, so it is very important that it is not only tasty, but also healthy and safe [2]. Every year more and more Ukrainians eat cereal flakes, muesli and instant cereals for breakfast. Today Ukraine is in the TOP – 10 countries-producers of cereal flakes, and every eight Ukrainian regularly consumes cereal flakes, muesli or instant cereals [3].Wheat flakes are in high demand among the

population. They are superior in nutritional value to wheat cereals. Wheat flakes are a rich source of B vitamins. One serving can contain 15% – 30% of the daily value of these substances necessary for a person. Together with them, wheat flakes contain vitamin E, choline, biotin, vitamin PP. Wheat flakes are especially rich in silicon, vanadium and manganese [4]. Nutrients of wheat flakes are highly digestible. For example, the digestibility of wheat flakes proteins is 85%, carbohydrates – 96%, fats – 94%. In this regard, wheat flakes play an important role in human nutrition [1, 4].

In the modern world, the requirements for food safety have noticeably increased, for which purpose biological methods are widely used, including biosensor technologies and biotesting [5]. Biotesting is one of the research methods used to determine the degree of influence of chemicals potentially hazardous to living organisms by registering changes in biologically significant indicators (test functions) of research test objects with subsequent assessment of their condition in accordance with the selected toxicity criterion [5].

At the present stage, the range of test organisms has expanded and includes various hydrobionts (green water plants), macrophytes, protozoa (ciliates, flagellates), coelenterates (hydras), worms (planaria, leeches), molluscs (lamellibranchs, gastropods), crustacea (daphnia, gamma), fish, etc. Nowadays, toxicological methods of analysis, namely biotesting, are widely used in ecoanalytical laboratories, together with methods of analytical chemistry. Biotesting of food raw materials, food products and feed, as well as various environmental objects (water, air, soil, polymer and building materials, etc.) takes, along with other research methods (physicochemical, biochemical, microbiological), one of the most important places, since it allows one to reveal the influence of the objects under study on a living organism and to determine the possible adverse consequences of their use [6].

Biological analysis makes it possible to reveal the effect of food and non-food components in their interconnection and interdependence and to obtain an integral expression of this effect in the form of a reaction of a living organism. Since the use of higher animals for these purposes is often difficult or even impossible for a number of reasons (methodological, economic, ethical), all over the world there is a tendency towards the maximum possible replacement by alternative living models (plants, tissue cultures, invertebrates, microorganisms, etc.), among which the one-celled ones are of undoubted interest – ciliates, which are similar to higher animals in the spectrum of basic metabolic parameters [7, 8].

Nowadays, the biotesting method is widely used for rapid assessment of water quality. In a number of scientific publications, leading foreign experts substantiate the important role of biotesting for an objective assessment of water quality. In particular, Takashi Kusui (Japan) emphasizes that the existing and growing number of created chemicals require ecotoxicological characterization in order to assess their risk to human health [9]. Therefore, Japan develops legislation that provides for biotesting of all substances that have already entered and are entering the country's market: "premarketing and rostmarketing risk assessments". There are also a number of countries where biotesting is leading in water quality testing. Thus, the governments of Chile, Mexico and Argentina have already introduced biotesting into national standards when assessing the quality of water and feed [10].

Daphnia magna Straus has become a classic object as an analytical indicator in biotesting. *D. magna* as a test object is included in most national and international standards for the study of water quality. The Daphnia genus includes about 50 species, among which the most common are *Daphnia magna, D. longispina, D. carinata.* These organisms belong to the filtration group and live mainly in the water column. They play an important role in the processes of self-purification of water bodies from substances suspended in water. Freshwater crustaceans *Daphnia magna Straus* are currently considered the most sensitive and versatile test objects; therefore, they are used for biotesting of waste and natural waters, bottom sediments, soils and industrial waste. *Daphnia* is used to determine both acute and chronic toxicity of controlled objects [11].

In the practice of toxicological laboratories for biotesting of feed, the ciliate *Colpoda Steinii* is also widely used. These are widespread species found in soils and freshwater bodies. *Colpoda Steinii* culture is intended for toxicological control of food products, feed, feed mixtures, feed additives that do not contain drugs, disinfectants, preservatives, salts, heavy metals, and patmaterial from animals and poultry to confirm the fact of their poisoning with toxic substances.

In recent years, the advantages of the biotesting method have attracted the attention of not only ecologists, specialists in the preparation of fresh water for soft and alcoholic beverages, but also scientists studying the potential danger of food products due to the presence of toxic substances.

Biotesting of food is understood as the analysis of the toxicity of aqueous extracts of the product using living test objects. Test objects (test organisms) are experimental biological objects (organisms) that are used to determine toxicity. As a rule, the test object is a sensitive biological element capable of responding to external influences. It can be enzymatic systems, isolated organelles, cells, tissues, individual organs of multicellular organisms, unicellular and multicellular organisms of one biological species or several species. The revealed toxic effect is registered and assessed in the experiment. Test objects allow you to replace complex chemical analyzes and to quickly establish the fact of product toxicity [6]. The method for determining toxicity with test objects is quite fast, does not require the use of experimental animals or expensive equipment, and has the potential to accelerate the control of the safety of raw materials and food products [13]. Ciliates, hydras, planaria, leeches, molluscs, crustaceans, representatives of different groups of plants and algae, insects, etc. are used as test objects in the assessment of food products [14-15]

Formulation of the problem

The object of the research is bread and cereal flakes of various brands.

Research subject – safety indicators.

The purpose of the work is to study the safety of

bread and cereal flakes, which are in the greatest demand among consumers. To achieve it, the following objectives were set:

– to conduct an organoleptic assessment of the quality of test objects;

– to carry out studies to determine the toxicity of bread and cereals.

Materials and methods

Experimental studies were carried out in the laboratories of the Department of Ecology and Environmental Technologies of the Odessa National Academy of Food Technology as part of the continuation of research on biotesting of grain products [16]. In order to determine the fact of the safety of wheat flakes and bread, which are sold in retail chains in Odessa, studies were carried out using the biotesting method. For research, we selected the following samples of bread of "Cherkas khlib LTD" LLC and Bakery № 1 in Odessa: honey yeast-free bread (sample 1); organic yeast-free wheat bread (sample 2) and not packaged long loaf «Smachnyi» (sample 3). Biotesting was carried out on wheat flakes of TM "Kozub Product", TM "Hercules", TM "Nordic" (samples 4–6) and wheat flakes, which were purchased on the city market.

Determination of toxicity using a test object of ciliate *Colpoda Steinii.* The studies were carried out in three replicates in accordance with GOST R 52337-2005 [17]. In a nutrient medium, live dry ciliates *Colpoda Steinii* have the ability to exist after a 16-hour incubation at 28°C. Upon contact with toxic substances, they lose their mobility. The time after which the ciliates stop moving indicates the degree of toxicity of the test sample.

Determination of toxicity by the death of crustaceans *Daphnia Magna Straus.* The studies were carried out on a synchronized *Daphnia* culture. Synchronized is a culture of the same age obtained from one female by acyclic parthenogenesis in the third generation. Such a culture is genetically homogeneous. The technique is based on establishing a discrepancy between the number of dead Daphnia in the analyzed sample (experiment) and that cultivated in water. Biotesting is the criterion for acute lethal toxicity [18–20].

Determination of naturality by biocrystallization. Biocrystallization is one of the experimental methods that was chosen to determine the organic nature of grain products. The crystals of various configurations formed from a test sample are the result of the biocrystallization process. The symmetry of the pattern and size of the crystals indicates the natural origin of this type of product, which did not experience a significant negative impact. The asymmetry and deformation of the crystal pattern indicate an aggressive effect on the food product (for example, chemical processing, genetic modification of raw materials, addition of toxic improvers, etc.) or plant diseases used as raw materials for the manufacture of these products [5, 20, 21].

Results of the study and their discussion

In accordance with the objectives set, first, an organoleptic quality assessment was carried out. The bread was assessed for compliance with DSTU 4587: 2006 "Bakery products. General technical conditions", which is now valid for bakery products. The results of the organoleptic assessment of the quality of three samples of bread are shown in table 1.

Table 1 – Organoleptic assessment of bread quality

Indicator	Sample № 1	Sample № 2	Sample № 3	Requirements of DSTU 4587:2006
Form	Rectangular, corresponds to the baking form, without falling out on the sides	Roundish, corresponds to the type of product, with slight falling out on the sides	Oval, corresponds to the type of product, with slight falling out on the sides	Products baked in the hearth: correspond to the type of product. Products baked in the baking form: corresponds to the baking form, without falling out on the sides
Surface	Corresponds to the type of product, clean, with signs of cut	Corresponds to the type of product, has traces of flour	Corresponds to the type of product, clean	Corresponds to the type of product, clean. Slight wrinkling is allowed for packaged products; for cut products – signs of cut
Color	Dark brown	Light brown	Light yellow	From light yellow to brown, with no burnt
Crumb state	Soft, elastic, not wet to the touch, without under mix-ingtraces, but with gaps between the crumb and crust	Dryish, easily crumbles, not wet to the touch, without under mix-ingtraces, without gaps	Not elastic enough, wet to the touch, with under mix-ingtraces and gaps	Baked, elastic, not wet to the touch, without under mix-ingtraces
Taste	Good taste, typical of this type of products, without foreign taste	Good taste, typical of this type of products, slightly sour, without foreign taste	Weak taste, without foreign taste	Typical of this type of products, without foreign taste
Smell	Pronounced flavor, typical of this type of products	Good, typical of this type of products	Weak smell, not typical of this type of products	Typical of this type of products, without foreign smell

As can be seen from the data presented, sample 1 and sample 2 fully comply with all the requirements of regulatory documents in terms of organoleptic characteristics, such as form, surface, crumb state. Not packaged long loaf «Smachnyi» does not meet the requirements of organoleptic quality indicators for the state of the crumb; also, the sample has a weakly expressed taste and smell.

The organoleptic assessment of the quality of wheat flakes of the test samples was carried out in accordance with GOST 4634: 2006 "Concentrates for dry food breakfasts. Cereal flakes. General technical conditions". The results obtained are shown in Table 2. In the course of the organoleptic assessment, it was established that samples № 4–6 in all respects meet the requirements of regulatory documents. Sample № 7 does not correspond to the "taste" and "smell" indicators, dampness and foreign taste are felt. In our opinion, this may indicate non-compliance with the requirements of the storage conditions or product sale.

The next stage of the study was to determine the integral toxicity of grain products using biosensors – *Colpoda Steinii* culture. These cultures are highly sensitive and versatile. The toxicity rating scale is shown in Table 3.

The toxicity level of the bread was assessed in accordance with the scale for assessing the toxicity of products. In the experiment, an aqueous and acetone sample with an extract of test samples was used to determine the effect of toxic substances of a polar and non-polar nature. The results of assessing the finished product are shown in Table 4.

According to the results of biotesting using *Colpoda Steinii* biosensors, it was determined that the samples are non-toxic, because most of the *Colpoda Steinii* remain mobile after 3 hours. Good results were found in sample 1, in samples 2 and 3 there is some death of the *Colpoda Steinii*, but the vast majority remain mobile after three hours, which meets the requirements of a non-toxic sample (Table 3).In order to determine the presence and degree of toxicity of substances of a polar nature, an aqueous sample was made from the squeeze residues of the test samples of bakery products, and to determine non-polar ones – an acetone one. The results of the analysis of aqueous and acetone samples of bread of test samples indicate their non-toxicity.

When determining the safety of cereal flakes using *Colpoda Steinii*, it was determined that Samples № 4 – 6 do not contain toxic substances, because most of the

Table2 – Organoleptic assessment of the quality of wheat flakes

Indicator	Sample № 4	Sample № 5	Sample № 6	Sample №7	Requirements of DSTU 4634:2006
Color	Light brown	Light brown	Light brown	Brown	From light brown to dark brown in different shades
Flavor	Typical of wheat grits, without foreign smell, quite pronounced	Typical of wheat grits, without foreign smell, quite pronounced	Typical of wheat grits, without foreign smell, pronounced	Typical of wheat grits, smell of dampness, weak smell	Typical of this type of products, without foreign taste and smell
Taste	Typical of wheat grits without foreign taste	Typical of wheat grits, without foreign taste	Typical of wheat grits without foreign taste	Typical of wheat grits, foreign taste is slightly felt	Typical of oatmeal without bitterness and off-flavors

Table 3 – Scale for assessing the toxicity of finished products [6, 13]

Toxicity	***Indicators***
Very toxic	The death of most of the *Colpoda Steinii* occurs within 3 minutes
Toxic	The death of most of the *Colpoda Steinii* occurs within 10 minutes
Slightly toxic	The death of most of the *Colpoda Steinii* occurs within 1 hour
Non-toxic	Most *Colpoda Steinii* remain mobile after 3 hours.

Table 4 – Assessment of complex indicators of the safety of finished products by biotesting using biosensors (Colpoda Steinii)

Sample name	Aqueous sample	Acetone sample	Toxicity
	Colpoda Steinii		
Sample № 1	All the *Colpoda Steinii* remain mobile after 3 hours	All the *Colpoda Steinii* remain mobile after 3 hours	Non-toxic
Sample № 2	Most of the *Colpoda Steinii* remain mobile after 3 hours (95%)	Most of the *Colpoda Steinii* remain mobile after 3 hours (95%)	Non-toxic
Sample № 3	Most of the *Colpoda Steinii* remain mobile after 3 hours (85%)	Most of the *Colpoda Steinii* remain mobile after 3 hours (85%)	Non-toxic

Colpoda Steinii survived after three hours, and in sample № 7, most of the *Colpoda Steinii* died within 10 minutes. This indicates its toxicity and the inadmissibility of further sale of such a product to the people. The results obtained coincide with the results that were obtained during the organoleptic analysis of wheat flakes.

The next stage of the study of the toxicity of grain products was carried out according to the method of biotesting by the lethality of crustaceans *Daphnia Magna Straus*. At the end of the biotesting, the number of live *Daphnia* was visually counted (Fig. 1). According to GOST 32536-2013 [19], *Daphnia* is considered alive, if it freely moves in the vessel 15 seconds after it is lightly shaken. The rest of the *Daphnia* is considered dead. The degree of toxicity of the test product when testing an aqueous solution of the test samples was determined by the proportion of surviving crustaceans in accordance with Table 5.

Based on the studies conducted, it was established: in sample 1 – honey yeast-free bread, the number of dead *Daphnia* was 3%, in sample 2 – organic yeast-free wheat bread, the value of the number was – 5%; in sample 3 – long loaf «Smachnyi», the number of dead *Daphnia* was 7 % (the indicator is on the border of "non-toxic" and "slightly toxic" indicator); in sample 4 – wheat flakes, the number of dead *Daphnia* was 4 %, in samples № 5 and № 6 –5 %, and in sample № 7 the number of dead *Daphnia* was 25%, which indicates a slight toxicity of this product.

One of the experimental methods for determining the organicity of grain products is biocrystallization, which belongs to non-destructive methods of analysis. As a result of the process of biocrystallization of the

Table 5 – Scale of toxicity of the test samples when testing an aqueous solution [22]

Degree of toxicity of the test product	Survival of crustaceans *Daphnia Magna Straus*,%
Non-toxic	93-100
Slightly toxic	62-92
Toxic	0-61

sample extract of the test samples, crystals of various configurations are formed. The symmetry of the pattern and size of the crystals indicates the natural origin of this type of product, which did not experience a significant negative impact. For the most complete extraction and adequate decoding of the information capacity of a biomaterial, it is necessary to use the achievements of chemistry (nature of crystallogenesis, features of crystalline and amorphous structures), physics (assessment of the energy component and physical bases of dehydration), mathematics and computer science (modeling of crystal formation in order to create equations for predicting the state of the product) and biomedical direction [5].

As a result of the experiment, the patterns of the formed crystals of a mixture of a 10% $CuCl_2$ solution and an extract of test samples were obtained. Biocrystalograms were created using a microscope in 10x and 40x magnification. The assessment of indicators of "organicity" based on the method of biocrystallization was carried out visually. Biocrystallograms of samples for bakery products are shown in Fig. 2 – 4.

Biocrystalogram is characterized by the presence of a significant number of branches of needles of different lengths. Crystals mainly form at the droplet

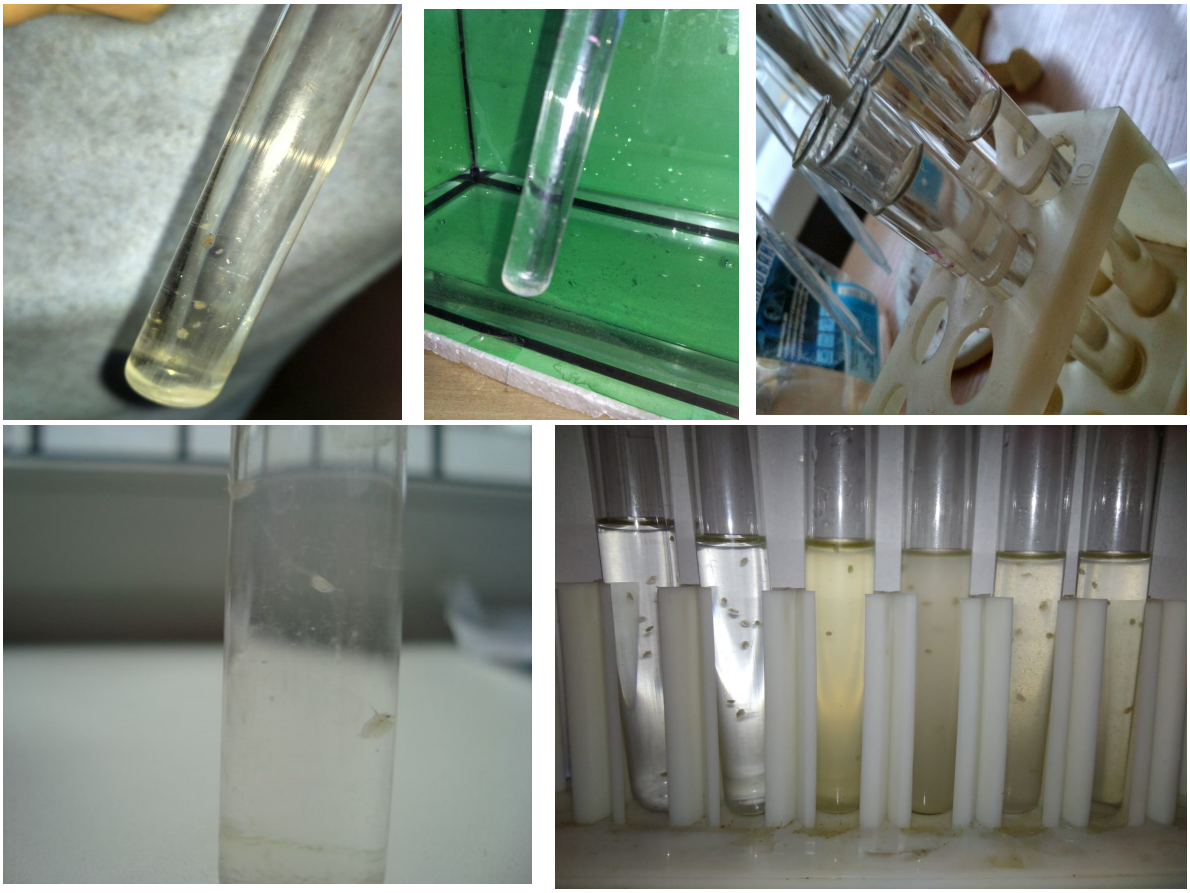

Fig. 1. Daphnia magna Straus culture in the medium of the test samples.

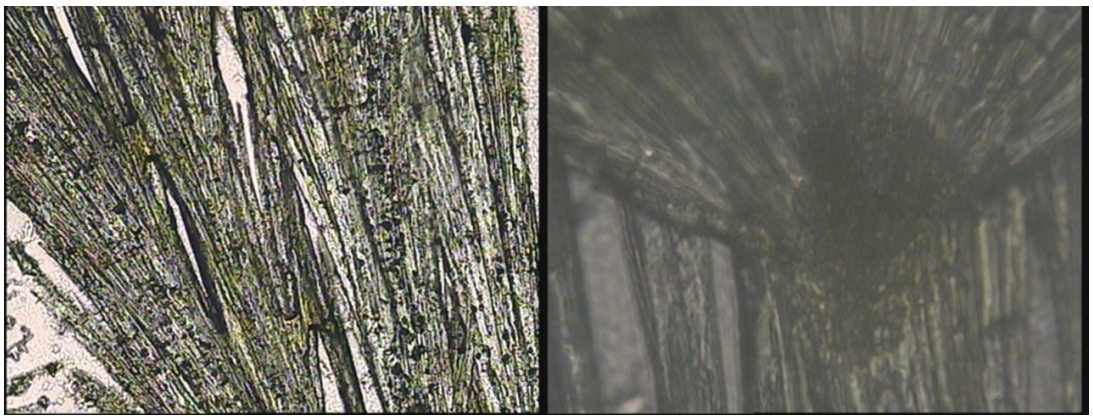

Fig. 2. Biocrystallogram of Sample № 1.

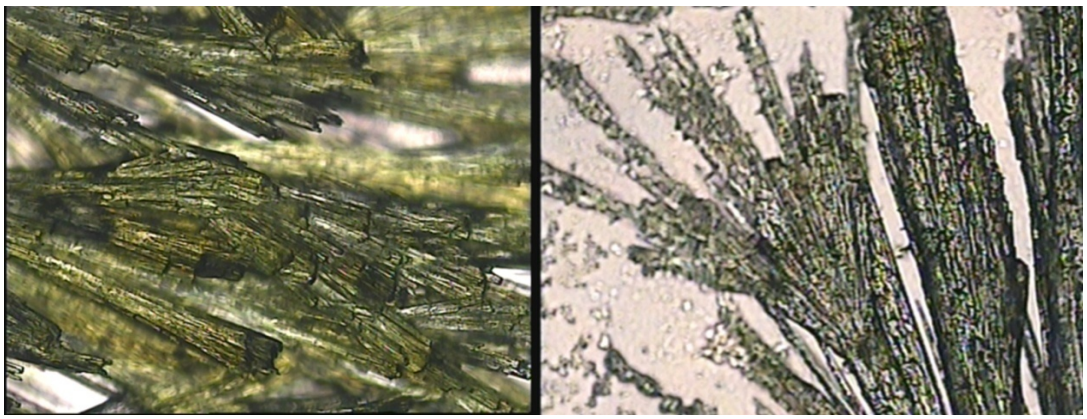

Fig. 3. Biocrystallogram of Sample № 2.

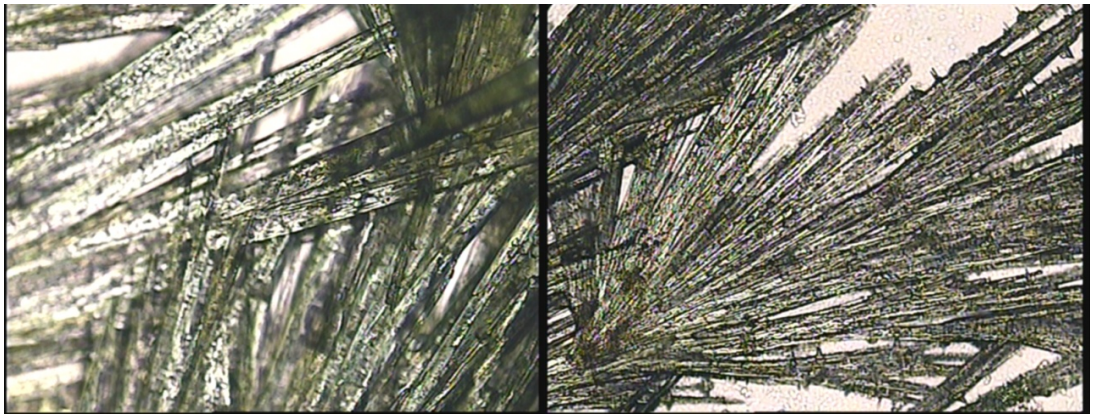

Fig. 4. Biocrystallogram of Sample № 3.

periphery and grow from one edge to the other. The tips of all branches are sharp, the structure of the branches is dense and clearly visible without aids. As can be seen from the above experimental data, samples 1 and 2 are characterized by the most symmetric biocrystalogram, which indicates their higher degree of organicity, which, in our opinion, is determined by a smaller share of negative impact on the product and high-quality raw materials that were used in the production of these samples.

Conclusion

1. According to organoleptic indicators, it was determined that samples 1–2 and 4–6 fully comply with the requirements of the normative documentation, samples № 3 and № 7 do not correspond in taste, smell, dampness and foreign taste are felt, this may indicate non-compliance with the requirements of storage or sale conditions.

2. On the basis of studies to determine the toxicity of cereal flakes using the test object of the ciliate *Colpoda Steinii*, it was determined that the samples of trademarks studied do not contain toxic substances, and in sample № 7 – wheat flakes purchased on the market by weight, the death of most of the *Colpoda Steinii* occurred within 10 minutes. This indicates its insignificant toxicity and the inadmissibility of further sale of such a product to the population. In the study of the bread, it was determined that the bread samples are non-toxic, because most of the *Colpoda Steinii* remain mobile after 3 hours. Good results were found in sample 1, in samples 2 and 3 there is some death of the *Colpoda Steinii,* but the vast majority remain mobile after three hours, which meets the requirements of a non-toxic sample.

3. The results of a study to determine the toxicity of bread and flakes by the death of crustaceans *Daphnia Magna Straus* showed that in samples 1 – 6 the number

of dead *Daphnia* corresponds to the "non-toxic" indicator, but in sample 7 the number of dead *Daphnia* was 25%, which indicates the toxicity of this product.

4. On the basis of biocrystallization, it was determined that samples 1 and 2 are characterized by the most symmetric biocrystalogram, which indicates their higher degree of organicity, which, in our opinion, is determined by a smaller portion of negative impact on the product and high-quality raw materials that were used in the production of these samples.

The results of the organoleptic assessment of bakery products and cereal flakes correlate with the results carried out using biotesting, namely, it was determined that samples that are completely safe have the best sensory indicators.

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БІОТЕСТУВАННЯ В ОЦІНЮВАННІ БЕЗПЕЧНОСТІ ЗЕРНОВИХ ПРОДУКТІВ

Анотація

Стаття присвячена питанню оцінювання безпечності харчових продуктів, які є традиційними для українського споживача (хлібобулочні вироби та пшеничні пластівці). Актуальність роботи обумовлена тим, що безпечність – це важливий показник споживних властивостей, яким повинні володіти всі продукти харчування. Для оцінки безпечності харчових продуктів широко застосовують біологічні методи, що включають біосенсорні технології та біотестування. Біотестування – це один із прийомів дослідження, який використовується для визначення ступеня впливу хімічних речовин, потенційно небезпечних для живих організмів, шляхом реєстрації змін біологічно значимих показників (тест-функцій) дослідних тест-об'єктів с наступною оцінкою їх стану у відповідності з обраним критерієм токсичності. У роботі оцінено органолептичні показники дослідних зразків. У результаті визначено, що зразки повністю відповідають вимогам нормативної документації, за

винятком зразка № 7 у якого відчувається вогкість та сторонній присмак, це може свідчити про недотримання умов зберігання або реалізації. Оцінено безпечність хлібобулочних виробів та пшеничні пластівців за допомогою тест – об'єкта інфузорії Colpoda Steinii. Встановлено, що зразки дослідних зразків не містять токсичних речовин, а у зразку №7 – вагових пшеничних пластівцях, придбаних на ринку, загибель більшості колпод наступила впродовж 10 хвилин. Це свідчить про його незначну токсичність та про неприпустимість подальшої реалізації такого продукту населенню. На основі другого метода проводили тестування за летальністю ракоподібних Daphnia Magna Straus. Результати показали, що у зразках 1 – 6 кількість загиблих дафній відповідає показнику «нетоксичний», але у зразка №7 кількість загиблих дафній склала 25%, що свідчить про токсичність даного продукту. Третій метод здійснювали за методикою, заснованою на недеструктивному методі біокристалізації. Встановлено, що найбільш симетричною біокристалограмою характеризуються зразки 1 та 2, що свідчить про їх вищу ступінь органічності, яка на наш погляд визначається меншою часткою зовнішнього негативного впливу на продукт та якісною сировиною, яка використовувалась при виробництві даних зразків.

Ключові слова:*безпечність, зернові продукти, біотестування, оцінка якості.*

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