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CHANGE OF MICROBIOTAS OF MAIZE-BASED EXTRUDED PRODUCTS WITH VEGETABLE ADDITIVES DURING STORAGE

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Abstract. Recently, sugar maize has become more and more widely used for the production of new types of food. Its use in the production of cereal products for babies makes it possible to use no sugar in their composition, switch to the production of dietary and prophylactic products, which acquires high importance against the background of “rejuvenation” of diabetes and the manifestation of other nutritional diseases. To enhance the consumer properties of the finished product, to give it the appropriate taste, colour and aroma, as well as to enrich with vitamins, mineral components, flavonoids, phytoncides and glucosides, the following raw materials were selected as the main components for their preparation: maize grains, hullless barley grains in the ratio 1:1, liquorice root (2–4%), carrot roots (2–4%). The article presents the results of microbiota studies of extruded grain products with plant additives during storage. The obtained samples were stored in the laboratory in polypropylene, plastic packaging and cardboard boxes at room temperature of the air +(15–20)°C and air humidity 60–75%. The obtained results allow us to establish that with prolonged storage of commercial samples of extruded grain products with the inclusion of vegetable additives in different temperature and humidity conditions, as the shelf life increases, the number of bacteria decreases. The absolute number of spore-forming bacteria in the samples under study, which were stored in synthetic packaging, remained at the same level, and their relative content in the total bacterial flora increased. On the basis of the research, it has been established that the enrichment of products extruded from sugar maize grain with vegetable additives does not impair their microbiological and physico-chemical characteristics during long-term storage in unregulated conditions. It also allows expanding the range of this type of products.

Key words: microbiological characteristics, sugar maize, extruded products, herbal supplements, microbiological safety.

ЗМІНА МІКРОБІОТИ ЕКСТРУДОВАНИХ ПРОДУКТІВ НА ОСНОВІ ЗЕРНА ЦУКРОВОЇ КУКУРУДЗИ В ПРОЦЕСІ ЗБЕРІГАННЯ

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Анотація. Останнім часом все ширше використовують цукрову кукурудзу для виробництва нових видів харчових продуктів. Її використання при виробництві зернових продуктів для дитячого харчування дозволяє відмовитися від внесення в їхній склад цукру, перейти до виробництва дієтичних і профілактичних продуктів, що досить важливо на тлі «омолодження» цукрового діабету і прояви інших аліментарних захворювань. Об'єктами досліджень були екструдовані продукти із зерна цукрової кукурудзи. Для підвищення споживчих властивостей готового продукту, для надання йому відповідного смаку, кольору і аромату, а також для збагачення вітамінами, мінеральними компонентами, флавоноїдами, фітонцидами і глюкозидами, в якості основних компонентів для їхнього приготування обрано крупу із зерна цукрової кукурудзи, крупу із зерна голозерного ячменю в співвідношенні 1:1, корінь солодки (2–4%), коренеплоди моркви (2–4%). Представлені результати досліджень зміни мікробіоти екструдованих зернових продуктів з рослинними добавками в процесі зберігання. Отримані зразки зберігали в лабораторних умовах в поліпропіленовій, поліетиленовій упаковці та картонних коробках при кімнатній температурі повітря +(15–20)°C і вологості повітря 60–75%. Отримані результати дозволяють встановити, що при тривалому зберіганні товарних зразків екструдованих зернових продуктів із включенням рослинних добавок в різних температурно-вологісних умовах знижується число бактерій. Абсолютна кількість спороутворювальних бактерій в досліджуваних зразках, що зберігалися в синтетичній упаковці, залишалося на одному рівні, а відносний вміст їх у сумарній бактеріальній флорі підвищувався. На основі досліджень встановлено, що збагачення екструдованих продуктів із зерна цукрової кукурудзи з рослинними добавками не погіршує їх мікробіологічні та фізико-хімічні показники при тривалому зберіганні в нерегульованих умовах, а також дозволяє розширити асортимент даного виду продуктів.

Ключові слова: мікробіологічні показники, цукрова кукурудза, екструдовані продукти, рослинні добавки, мікробіологічна безпека.

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Introduction. Formulation of the problem

One of the promising technologies for obtaining high-quality products from grain raw materials is its extrusion processing, which allows obtaining food and technical products based on waste-free flexible high-efficient production [1-3].

Extrusion technology allows creating products the nutritional, biological and energy value of which can be regulated. The ability to change the composition of products by increasing the content and the bioavailability of proteins, vitamins or minerals plays an important role in the prevention of many human diseases [4,5].

The extrusion process lasts a short time, during which there is no intensive destruction of vitamins, at the same time inactivation of anti-nutritional enzymes occurs [6]. At the same time, extrusion processing of grain provides high sanitary and hygienic indicators of the product: bacteria of the *Escherichia coli* group, mould fungi and salmonella are almost completely destroyed, which increases the shelf life of the product [7,8].

The use of extrusion technology has led to a significant expansion of the range of food products from grain [9]. The behaviour of the microbiota, changes in the physical properties and chemical composition of such products are quite well studied [10]. However, the use of new grain raw materials requires additional study of technological modes, the behaviour of the microbiota and the correction of the storage modes of new grain products.

Recently, the use of sugar maize for the production of new types of food products has been increasing [11]. Its use in the production of cereal products for baby food makes it possible to use no sugar in their composition, to switch to the production of dietary and prophylactic products, which acquires high importance against the background of "rejuvenation" of diabetes and other nutritional diseases [12].

Sugar maize differs in its physical properties and chemical composition from other varieties of maize, which requires additional study of the behaviour of the microbiota during storage of food products based on it.

Analysis of recent research and publications

One of the important tasks of the production of extruded products is the safety of their nutritional and biological value, as well as their delivery to the consumer without deterioration.

Currently, quite intensively, work is underway to expand the range of new types of grain products of functional purpose, at the expense of flour mixtures, bakery, pasta and confectionery products, food concentrates, enriched with additives from medicinal plants, vegetables, fruits, etc. [13-17].

Consumer safety is the most important indicator of food quality. Unlike other properties, the reduction or loss of which downgrades the functional or social value of food, exceeding the permissible levels of safety indicators makes food dangerous. According to the Law of

Ukraine "On the fundamental principles and requirements for food safety and quality" [18], a product that does not adversely affect the human body is safe.

Obtaining new types of extruded cereal products is closely related to the problem of the duration of their storage. The storage process is almost always accompanied by a deterioration in the quality of these products, their nutritional value, the development of microflora and other undesirable processes. In addition, the storage time is determined by the individual composition of the products, the intensity of biochemical processes in them, as well as the nature of the additives introduced, the effect of which may be different [19]. That is why, when choosing packaging for enriched extruded cereals, it is necessary to eliminate contact with oxygen, moisture and light. In this regard, it is necessary to study the effect of packaging materials on the stability of the quality of new types of enriched extruded grain products during storage and to determine the recommended term for their storage.

According to the results of the analysis of the market of packaging materials that are used by various enterprises to package snacks, namely extruded products, and after studying specialized literature on the subject, it was found that manufacturers use different types of packaging: cardboard packs with an internal package of heat-sealing polymeric materials, cardboard boxes of extruded or corrugated cardboard, bags of cellophane, vacuum packaging and metallized bags with bioaxially-oriented polypropylene films [20-21].

For the packaging of extruded breakfast cereals manufacturers impose the following requirements: it must have high barrier properties, namely, be impenetrable for oxygen, moisture, ultraviolet rays, and foreign odours; be not heavy; prolong the storage time of the product; be an advertising medium for brand attributes [20].

An economical packaging is corrugated cardboard boxes packed in vacuum film. This package protects the product from the effects of the environment and mechanical damage. However, the product after unpacking has a limited shelf life, it quickly absorbs moisture, as a result of which its consumer properties change [21].

Cellophane bags are resistant to fats and temperature, are environmentally safe, but very sensitive to moisture [21,22]. That is why extruded products packed in cellophane should be stored in rooms with low relative humidity of air, which is not always possible when selling these products in a commercial network.

The films viscothene, cellothene, melathene, lamithene protect extruded products from exposure to oxygen, but are not resistant to ultraviolet rays and do not prevent changes in the organoleptic properties of products [22].

Packs of folding boxboard (FBB) with an internal package of heat-sealing polymeric materials successfully protect extruded products from mechanical damage and light, and the internal package from contamination, and, besides, make the product resistant to grease and moisture.

Vacuum packaging for extruded products is used very rarely, since these products can destructively affect the integrity of the packaging material, reduce its barrier properties and even break the tightness of the packaging [20].

Nowadays, for packaging breakfast cereals, domestic manufacturers widely use metallized bags with bioaxially-oriented polypropylene film (GM-200, GM-201, GM-210, GM-212 with a thickness of 20–35 microns). The advantages of this film is that it does not change the taste properties of the product, does not cause the mutual migration of the components of the material and the product, does not let moisture through, protects the products from sunlight. It should be noted that this film does not contain harmful additives and impurities and does not create problems when disposing of packaging waste [20-22].

Food safety for consumers and their storage stability among many other indicators primarily depend on the size and species composition (group composition) of its microbiota [23].

When storing grain products of standard moisture content, the main type of deterioration is rancidity due to fat hydrolysis and further oxidation of the formed fatty acids to aldehydes and ketones, which give the product an unpleasant odour and taste, and as a result, deterioration in consumer properties of the product [22].

The composition and development of the microbiota of flour, cereals and extruded products are significantly influenced by various technological methods (hydrothermal processing, technology and modes of extrusion, etc.). Microbial contamination of products is on average tens of thousands of bacteria per gram [24]. In products that do not use heat treatment in their production, *Erwinia herbicola* bacteria prevail, their number reaching 70–90% of the total number of bacteria, and there are only 5–15% of spore-formers and cocci. After heat treatment, spore-forming bacilli and micrococci prevail. The content of fungal spores ranges from fractions of a percent to 1–5% of the total number of microorganisms [25]. On maize products (groats), the number of fungi is much larger and reaches 30 %. The fungal flora of the groats is mainly represented by *Penicillium* species [26]. With long-term storage under conditions where the product humidity and temperature exclude the possibility of microbiota development, there is a gradual decrease in the total number of bacteria as a result of the die-off of non-sporulating forms [27].

The purpose of this study was to assess the changes in the qualitative and quantitative composition of the microbiota of products made from sugar maize grain with vegetable additives after extrusion and during storage in uncontrolled conditions.

Objectives of the study:

1. carrying out microbiological studies of samples before storage, and during the next six months, using modern and classical techniques;
2. studying packaging extruded cereal products;
3. selection of the optimum packaging for storing samples of extruded cereal products.

Research materials and methods

Objects and research methods. The objects of research were extruded products from sugar maize. The main components for their preparation were cereals from sugar maize grain, cereals from hull-less barley grain in the ratio 1:1, liquorice root 2–4%, carrots 2–4% [11].

Packaging materials for new types of grain products were selected taking into account their protective properties, cost and the possibility of using them for these types of products.

After analysing various types of packaging materials and examining their properties, we selected and investigated how cellophane, cardboard boxes and metallized bags from bioaxially oriented propylene film (BOPP) GM-200 effected on the changes in consumer properties of new types of enriched extruded products during storage.

The obtained samples were stored in the laboratory in polypropylene, plastic packaging and cardboard boxes at room air temperature +(15–20)°C and air humidity 60–75%. We chose these types of packaging because they are most often used for the storage of extruded grain products [19-22].

Microbiological studies of the samples were carried out before storage, after 1 month of storage and then every three months, using modern methods of determination (BakTrak 4300 microbiological analyser, which is based on recording changes in the electrical resistance of the nutrient medium resulting from the activity of microorganisms), as well as classical techniques. The samples were taken in a sterile container under aseptic conditions that excluded contamination of the product with microbes from the environment.

The study of the selected samples was carried out according to the following main microbiological parameters: the total number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in 1 g, mould fungi in 1 g, the presence of coliform bacteria (BHCC) in 0.1 g, of pathogenic staphylococcus in 1 g, of *B. cereus* in 1 g, of sulphite-reducing clostridia in 0.1 g, and of bacteria of the genus *Salmonella* in 25 g of the product.

The quantitative and qualitative composition of microbiota and the presence of microorganisms were determined by inoculation on special nutrient media with subsequent cultivation and characterization according to DSTU 2903:2005 “Concentration of food crops. Dry breakfasts”; GOST 10444.9-88 Food products. Method for the determination of *Clostridium perfringens*; GOST 10444.12-88 Food products. Method for the determination of yeasts and moulds; GOST 10444.15-94 Food products. Methods for determining the amount of mesophilic aerobic and facultative-anaerobic microorganisms.

The total number of bacteria was determined by inoculating tenfold dilutions of washoffs under meat-peptone agar (MPA), mould fungi and yeast – into wort agar (WA) with subsequent cultivation at a temperature +30±1°C for 24–48 hours and +28±1°C for 5–7 days, respectively. Spore forms of bacteria were determined in the washoffs of the samples, which were inoculated on

the complex nutrient medium MPA and WA in the ratio 1:1; for the determination of clostridia, cultures were inoculated in liver broth and a Kitt-Tarozzi nutritional medium; for the determination of staphylococcus – on milk-salt agar; for the detection of salmonella - on bismuth sulphite agar. The presence of *E. coli* in the Kessler medium was detected by the turbidity of the medium and the occurrence of gas bubbles in the floats, which are formed during the fermentation of sugar, with further re-inoculation on the Endo differential diagnostic medium for accurate identification. The cultivation was carried out at a temperature of $37 \pm 1^\circ\text{C}$ for 24 hours.

In accordance with DSTU 2903:2005 “Food concentrates. Dry breakfast cereals. General specifications” dry breakfast cereals (extruded cereal products) should look different in size and shape, be of different shades of white and yellow colour, taste and smell typical of this type of product (a pronounced smell and taste of the additives used), be of a crispy, porous, not coarse structure.

As for the physico-chemical parameters, the mass fraction of moisture in breakfast cereals (extruded grain products) should not exceed 7%, the mass fraction of fat – not more than 11.5%, the mass fraction of metal-magnetic impurities – not more than $3 \cdot 10^{-4}\%$.

Results of the research and their discussion

Liquorice root is used in food industry as a sweetener of natural origin [28]. It contains vitamins, minerals, natural amino acids, polysaccharides, essential oils, resins and tannins, as well as other biologically active compounds [29]. Using powdered liquorice root gives the finished product a sweet taste, and enriches it with vitamins (A, E, B1, B2, B6, B9, PP, beta carotene), macronutrients (phosphorus, calcium, magnesium, sodium, potassium) and microelements (iron, iodine, manganese, fluorine, etc.) [29].

The use of cereals from hull-less barley grain increases consumer properties and enriches the product with microelements [30]. Barley grain contains a complex of vitamin E, magnesium, omega-3 fats, as well as a large amount of β -glucans, which helps cleanse the body of cholesterol, slags and toxins, and has fortifying and immunostimulating effects on the body [31].

Introduction of blanched shredded carrots into the product contributes to the improvement of consumer properties of the finished product, giving it a special taste, aroma and colour, enriches it with vitamins and minerals [32]. Carotene, which is found in large quantities in carrots, helps to normalize the metabolism, affects the physical and mental state of a person, normalizes the functions of the organs of vision, has antioxidant properties, cleanses the body of toxins and slags [33,34].

We have studied the qualitative and quantitative composition of the microbiota of a grain product with herbal additives, obtained by extrusion [11] during its storage in order to establish its safe storage periods. Be-

sides, the above-listed products have been analysed for the presence of mycotoxins (aflatoxins B1, zearalenone, deoxynivalenol) using the Veratox test system. The immunoenzyme method convenient in use and interpretation, based on attaching an enzyme label to antibodies, makes it possible to take into account the result of an antigen-antibody reaction by detecting enzyme activity or by a change in its level [35]. The test is a direct immunoenzyme method (ELISA – enzyme linked immunosorbent assay) that provides the accuracy of determination, with the mycotoxin content of a few $\mu\text{g}/\text{kg}$ (ppb) [35].

Evaluation of microbiological indicators of the studied samples depending on storage conditions are given in Table 1.

In all studied samples, the content of aflatoxins, zearalenone, DON (deoxynivalenol) did not exceed the permissible limits.

It should be noted that in all samples and under different storage conditions (temperature $+(15-20)^\circ\text{C}$ and relative air humidity 65–75%), *E. coli*, *staphylococcus*, *Salmonella*, *Proteus*, sulphite-reducing clostridia were not detected. The presence of micromycetes was within the normal range. This indicates the compliance of the production of extruded grain products with sanitary and hygienic standards [36].

The obtained results allow us to establish that with long-term storage of commercial samples of extruded grain products with the inclusion of herbal supplements in different temperature and humidity conditions, as the shelf life increases, the number of bacteria decreases, mainly due to the death of the epiphyte grain – *Herwinia herbicola*. These data are consistent with those available in the professional literature on the storage of various types of natural cereals [36,37].

The absolute number of spore-forming bacteria in the samples under study, which were stored in synthetic packaging, remained at the same level, and their relative content in the total bacterial flora increased. In samples that were stored in polyethylene and polypropylene packaging for six months, the total number of bacteria and micromycetes decreased, and the yeast did not develop.

In the process of storing samples of extruded grain products in cardboard boxes, already after one month, yeast was found in all the samples under study. Its content increased due to the possibility of access of moisture. Yeast is known to cause fermentation, as a result of which substances such as alcohol, aldehydes, ketones, organic acids and other compounds accumulate in the products, which leads to a decrease in the nutritional and biological value of the product [38]. It should be noted that by the end of the third month of storage of the samples in cardboard boxes, the total number of bacteria increased, too.

Table 1 – Group composition of microbiota of the samples under study, CFU/g

Package type	Storage time, months.	Bacteria			Mould fungi				Yeast
		Total	In particular		In particular				
			<i>Erwinia herbicola</i>	<i>Subtilis li-cheniformis</i>	Total	<i>Aspergillus</i>	<i>Penicillium</i>	Other fungi	
Extruded maize groats and hull-less barley cereals (1:1 in the control)									
Polyethylene	0	380	330	30	40	10	–	30	–
	1	340	290	30	30	10	–	20	–
	3	270	200	40	40	20	20	10	–
	6	190	130	40	50	21	21	8	–
Polypropylene	0	380	330	30	40	10	–	30	–
	1	230	200	20	30	10	–	20	–
	3	190	160	20	30	10	10	10	–
	6	150	120	20	40	20	12	8	–
Cardboard	0	380	330	30	40	10	–	30	–
	1	350	290	40	50	10	20	20	30
	3	290	240	40	50	22	10	18	50
	6	240	160	60	60	30	20	10	70
Extruded maize and hull-less barley cereals with the addition of a liquorice root									
Polyethylene	0	60	30	20	40	–	–	40	–
	1	50	20	20	30	10	–	20	–
	3	50	10	30	40	10	20	10	–
	6	60	10	40	50	22	21	7	–
Polypropylene	0	60	30	20	40	10	–	30	–
	1	50	20	20	20	–	–	20	–
	3	50	10	30	30	10	10	10	–
	6	40	10	20	29	10	10	9	–
Cardboard	0	60	30	20	40	10	–	30	–
	1	80	30	40	60	20	20	20	20
	3	90	40	40	50	22	12	16	30
	6	90	30	50	60	30	20	10	40
Extruded maize and hull-less barley cereals with the addition of carrots and a liquorice root									
Polyethylene	0	300	210	70	50	–	–	50	–
	1	200	150	40	40	10	10	20	–
	3	200	110	40	40	10	10	20	–
	6	100	40	30	60	40	10	10	–
Polypropylene	0	300	210	70	50	–	10	40	–
	1	200	150	40	40	10	10	20	–
	3	200	80	60	40	10	10	20	–
	6	100	30	50	30	20	–	10	–
Cardboard	0	300	210	70	50	–	10	40	–
	1	200	120	60	40	10	10	20	30
	3	200	110	80	50	20	10	20	30
	6	200	100	75	80	60	10	10	50

Since polypropylene packaging was the best in terms of microbiological indicators, therefore, in the studied samples, and physico-chemical quality characteristics after 6 months of storage in this package (Table 2). the extruded grain products also determined organoleptic

Table 2 – The main organoleptic and physico-chemical characteristics of the quality of extruded cereal products after six months of storage in polypropylene packaging

No	Parameter	Characteristics
1	Appearance	The form is correct, the surface is rough, without deformation and tears, the products have the appropriate dimensions, attractive appearance
2	Colour	Uniform, homogeneous, yellow
3	Taste	Pleasant, pronounced sweet
4	Smell	Pleasant, pronounced
5	Structure	Crispy, uniform, porous, tender
6	Moisture content, %	4.0
7	Mass fraction of carbohydrates, %:	
	– starch	28.9
	– mono and disaccharides	24.0
8	Mass fraction of proteins, %	12.7
9	Mass fraction of fat, %	5.1
10	Mass fraction of ash, %	0.7
11	Mass fraction of metal-magnetic impurities (particles not more than 0.5 mm in the greatest dimension), %, not more	not revealed
12	Energy value, kcal	118

As can be seen from table 2, the samples of extruded cereal products, which were stored in polypropylene packaging, after 6 months of storage, are characterized by the correct shape, uniform colour, have a pleasant, pronounced sweetish taste and smell and a delicate, crisp structure. The energy value of the investigated samples of the extruded products is 118 kcal, which is 3.9 times less than the that of extruded products (maize sticks) represented on the Ukrainian market (the energy value of which averages 467 kcal).

Conclusion

When storing extruded grain products in polypropylene packaging, their microbiological characteristics

remained almost unchanged, which is not the case when storing samples in plastic packaging and cardboard boxes. That is why, in the samples that were stored in polypropylene, organoleptic and physico-chemical quality parameters were also determined after six months of storage (Table 2), as a result of which it has been established that these parameters remained almost unchanged. In the course of the research, it was established that the enrichment of extruded cereal products with vegetable additives during storage in polypropylene packaging allows expanding the range of extruded products based on cereal grain. Further studies on the storage of the samples are being carried out.

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