

# DEVELOPMENT OF THE FORMULATION AND QUALITY ASSESSMENT OF IMMUNOSTIMULATING FRESH-MIXES WITH A BALANCED POTASSIUM-PROTEIN COMPOSITION

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*За допомогою методів математичного моделювання розроблено рецептури імуностимулюючих фреш-міксів. Оптимізацію рецептурного складу проводили за вмістом білку та калію. Визначено біологічну активність отриманих напоїв, вміст основних харчових нутрієнтів та проведена товарознавча оцінка якості даних продуктів. Використовуючи тест ALST визначено умови зберігання розроблених фреш-міксів*

*Ключові слова: комплексна оцінка якості, імуностимулюючий напій, біологічна активність, харчова цінність, термін зберігання*

*С помощью методов математического моделирования разработаны рецептуры иммуностимулирующих фреш-миксов. Оптимизацию рецептурного состава проводили по содержанию белка и калия. Определены биологическая активность полученных напитков, содержание основных пищевых нутриентов и проведена товароведная оценка качества данных продуктов. Используя тест ALST, определены условия хранения разработанных фреш-миксов*

*Ключевые слова: комплексная оценка качества, иммуностимулирующий напиток, пищевая ценность, срок хранения*

## 1. Introduction

To fully satisfy vital needs, food products for human consumption should contain more than 6,000 groups of different macro- and micronutrients, including more than 20 thousand different food compounds of plant and animal origin.

In recent decades, the state of health of people in many countries is characterized by the growth of morbidity and

mortality. There is a tendency for development of alimentary diseases: cardiovascular, cancer, as well as non-infectious, related to the lack of vitamins and micronutrients (iodine, iron, fluoride, selenium). There are attempts to resolve such a dissonance in nutrition by creating multi-component products, including beverages, enriched with various ingredients: food fibers, vitamins, micro- and macroelements.

The diet of modern human is characterized by a deficiency of the protein in the amount of 10–26 % of the required quantity [1]. Result of the protein deficiency in the human body is the development of protein insufficiency. This leads to the disruption of enzyme synthesis, of pancreatic and intestinal functions, to the formation of negative nitrogenous balance, atony of muscles, reduced resistance of the body to infectious diseases [2].

Marketing strategies aimed at promoting new fortified foods should be based on a high degree of consumer satisfaction [3].

Exploring the needs of consumer in terms of the quality of beverages is an important criterion for assessing their quality. However, the attitude of consumers to a product depends on many factors, both subjective (habit, prejudice etc.) and objective (economic, advertising) [4].

Studies into family of mammalian genes shed light on the molecular mechanisms underlying information coding in the olfactory system [5]. Detection of chemically different odorants is connected to the association of odorous ligands with specific receptors on the olfactory sensory neurons.

The new dessert product “Martyshka”, based on curd, exhibited high sensory and biological indicators [6]. The dessert’s formulation makes it possible to obtain a balanced composition of all macro- and micronutrients.

Given this, it is appropriate to compensate for insufficient nutrients through the creation of specialized food products. An important nutritional factor for the ergogenic orientation is to develop products with improved nutritional value and biological activity of [7].

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## 2. Literature review and problem statement

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There is the formulation and developed technology for producing a tonic beverage using traditional plant products that has an expressed adaptogenic properties [8]. The fruits of *Schisandra chinensis* were used as plant raw material. To enhance the tonic and adaptogenic effect of the beverage, technologists exploited extracts of *rhodiola rosea* root and guaraná. However, development of the formulation involved organoleptic characteristics only; it was not optimized in terms of the ratio between main micronutrients.

A formulation of the beverage for people with higher physical activity was optimized [8]. Such a drink contains muesli, sugar-free yoghurt, banana, raisins and walnuts.

The formulation of energetically balanced product consists of plant and milk raw materials, as well as succinic acid [9]. The quantity of the protein-carbohydrate product in the amount of 189 grams makes it possible to load the human body over 2 hours. The ratio of proteins and carbohydrates in the proposed mixture is consistent with the principles of nutrition and is 1:4.

It is known that the consumption of antioxidant vitamin complexes in doses exceeding the minimum level can benefit human health [10]. It explains growing popularity of multi-vitamin beverages over recent years. They are manufactured based on using both natural vitamin concentrates and synthetic vitamin substitutes as a source of vitamins. The role of such products in the human diet and their possible effects on the body have been investigated.

It is known [11] that in order to enrich coffee drinks, technologists use pectin, which not only improves consumer

properties, but also contributes to the creation of a beverage capable of removing toxic substances from the human body.

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The biologically active substances of plant raw materials were examined, the bioavailability determined and plant species identified. These include citrus fruits, spicy-aromatic raw materials, herbs. A unique source of biologically active additives (BAA) are the bee-keeping products (honey and propolis), which contain flavanones and can be used to adjust the composition of food products [12].

There is a known fermented beverage based on whey [13]. Technologists used a water extract of *Melissa officinalis* as a plant component, which was introduced prior to the stage of leaven introduction in a concentration of 2.7 %. The resulting product has high organoleptic characteristics. However, biologically active components in the extract of *Melissa officinalis* were not sufficiently studied, which makes it impossible to accurately determine whether the product actually possesses antioxidant properties.

In order to create functional compositions based on whey, technologists used plant raw materials, taking into account its availability and adaptability. The fillers included carrot and pumpkin puree (pretreated with fermentation by the fermenting preparation “Fruktocim M”), and concentrated apple juice. Cinnamon, cloves and vanilla were applied as spicy-aromatic raw materials [14].

Extracts from cranberry and cowberry are also used as sources of biologically active components [15]. To obtain the extracts, water is exploited at a ratio of water: raw materials =4:1. With the aim of intensifying the process of extracting, the raw materials are treated with the enzymes “Fruktocim Color”, “Fruktocim P6L”, and “Biocelluloza”. The obtained extracts contain 1.5 and 1.6 % of solids for cranberry and cowberry, respectively.

The antioxidant activity of berries, fruits and citrus fruits was investigated [16]. The data acquired showed that orange, blueberry, and apple concentrates have the highest anti-radical properties.

The contribution of beverages to a total energy consumption in summer and winter was also examined [17]. The data were obtained using a questionnaire on water balance (WBQ) in Athens, Greece ( $n=984$ ): 473 people aged from 18 to 42 in summer, and 511 people aged from 20 to 38. The resulting data revealed that beverages reduce energy consumption by  $\frac{1}{4}$ . The largest share of energy drinks belongs to coffee, dairy and alcoholic beverages.

It was proven that fruits and vegetables contain flavonoids that protect against heart diseases [18]. Phenolic substances were extracted using acid hydrolysis followed by colorimetric measurements. Antioxidant activity of the extracts was determined by inhibiting the oxidation of low-density lipoproteins.

Scientific studies show that whole grains reduce risk of chronic diseases, including cancer and heart diseases [19]. Whole grains provide a wide range of nutrients and phytochemicals that can work synergistically to improve human health. A study was conducted to compare the antioxidant activity of whole grains, breakfast cereals with fruits and vegetables. The antioxidant activity was determined by dispersing finely-ground samples in a 50-% aqueous methanol solution of the stable free radical 2,2-diphenyl-1-picrylhy-

drazyl. The data obtained revealed that breakfast cereals exceed fruits by 3.67–5 times in terms of antioxidant activity. However, the antioxidant activity of red plum was comparable to that of the breakfast cereals.

Epidemiological studies have demonstrated interrelation between fruit and vegetable consumption, morbidity and mortality from degenerative diseases [20]. Fruits, vegetables, drinks and vegetable oils were examined for the content of antioxidants of different origin. The highest antioxidant activity was demonstrated by asparagus, blackberries, red currants and raspberries, as well as the juice of citrus fruits and olive oil. However, the authors did not investigate the antioxidant activity of blended juices, which are very important in the human diet.

It is recommended that the content of carbohydrates in sports drinks for rehydration should be low (not larger than  $80 \text{ g}\cdot\text{l}^{-1}$ ) when the loss of sweat is maximal. Sodium is added to the formulations of beverages in order to maximize the absorption of fluid and carbohydrate. A series of electrolyte values to replace sweat caused by loss were studied, depending on the procedure of washing out from the body [21].

Due to a combination of physical and chemical characteristics, soft drinks, fruit juices and canned fruits represent a well-defined and unique ecosystem [22]. Compositional characteristics of these products were explored, as well as their effect on the microflora of raw materials and resulting products. Fruits as the main raw materials for fruit juices, soft drinks and canned goods typically have pH between 2.0 and 4.5. This is due to the high level of contained organic acids, for example, 0.2 % in pear, 8.5 % in lime. The highest pH values are found in tomato (4.3 on average). In addition to citric acid (which may comprise 95 % of the total acid content in citrus fruits), they contain malic, tartaric and quinic acids.

### 3. The aim and objectives of the study

The aim of present work is to develop a formulation for fresh-mixes with increased content of potassium.

To accomplish the aim, the following tasks have been set:

- to develop formulations for fresh-mixes with the optimal content of potassium, protein, and antioxidants of plant origin;
- to determine the antioxidant activity of juices that are included in the composition of fresh-mixes and ready drinks;
- to determine the content of main macro- and micronutrients, as well as biologically active substances;
- to perform a QFD-quality assessment of the developed fresh-mixes.

### 4. Materials and methods for developing fresh mixes

We used the following equipment for conducting the study:

- to prepare fresh-mixes – electric juice extractor KENWOOD JE850 (Japan), blender (PHILIPS HR-1633/80,

China), refrigeration chamber (ShH-0.4 MC, Mari El Republic, Russia), a washing machine, a tabletop electric scale (Rotex RSK 10-P, China);

- to determine antioxidant activity – the photometric potential colorimeter KFK-2MP (Russia).

In order to determine quality of fresh mixes, we applied a QFD-technique (Quality Function Deployment), which is an original Japanese procedure for the systematic and structured implementation of consumer preferences in technical specifications of product quality. The use of QFD at a food making enterprise makes it possible to:

- to formalize a procedure for determining the main characteristics of a product, which is developed in accordance with the wishes of the user;
- to take justified decisions on managing the quality of processes aimed at creating a new product;
- to minimize adjustment of product parameters following its introduction to the market;
- to ensure high value and, at the same time, product quality by minimizing non-production costs.

The optimization of formulation of beverages for the basic indicators of chemical composition that meet the needs of a particular group of consumers was carried out using mathematical modeling. We took into consideration requirements to the physiological norms of nutrition for different groups of adult population [23].

## 5. Results of the study into quality indicators of immunostimulating drinks

In order to develop drinks, we employed a Delphi method, which implies obtaining optimal information with a high degree of reliability in the process of developing fresh-mixes (Fig. 1).

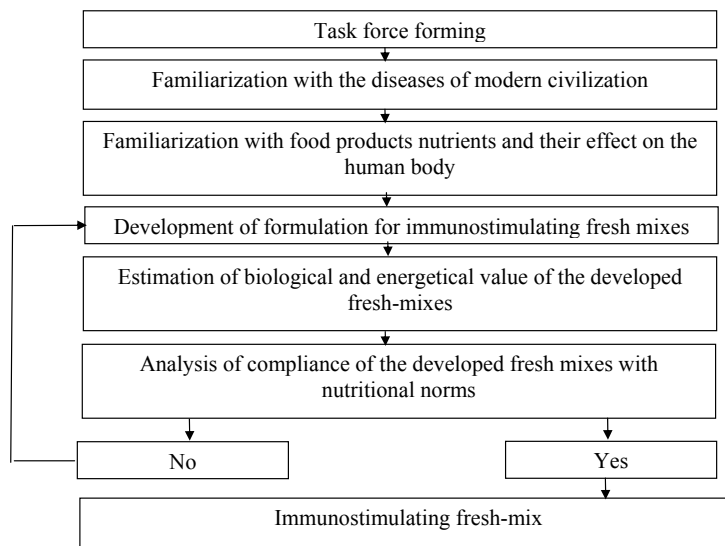


Fig. 1. Algorithm for development of new immunostimulating fresh-mixes

Taking into consideration the multiple components of formulation composition, predetermined by the need to achieve the recommended ratio between essential nutrients, as well as to control the content of indispensable aminoacids, the procedure for selecting the components is a complicated one.

One of the main stages that preceded the development of formulations involved prioritizing the requirements for a resulting beverage. Summarizing data on the strength of relations between technical characteristics of immunostimulating beverages and consumer requirements, given importance of the latter, we prioritized the optimization of new drinks.

First of all, when developing new immunostimulating beverages with respect to consumer requirements, considerable attention should be paid to the use of the original raw materials.

The main criteria for selecting the raw materials for fresh-mixes were a high nutritional value and the possibility of combining components in order to obtain a product with enhanced biological value and improved antioxidant effect, with high organoleptic indicators.

As a result of development that employed the Microsoft Excel software, by using the add-on Solver, we made up formulations for the fresh-mixes “Potassium-mix” and “Potassium-mix plus” (Fig. 2).

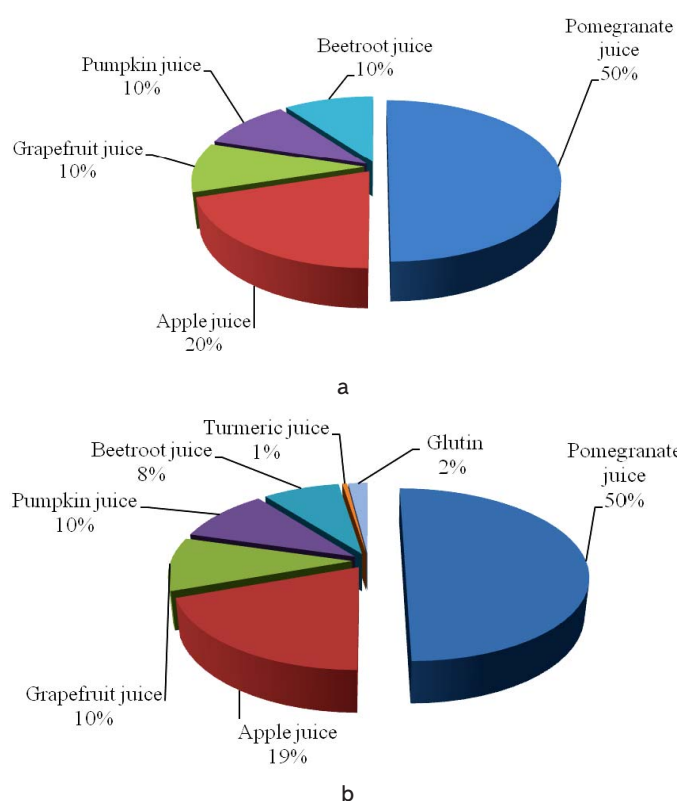


Fig. 2. Fresh-mixes formulations: a – “Potassium-mix”; b – “Potassium-mix plus”

Most often, the main criterion for the final product is both organoleptic and physical-chemical characteristics. These criteria, however, do not explain the synergistic and antagonistic effects, observed in practice, of systemic influence that biologically active components of the mixture exert on a living organism.

It is therefore interesting to study the developed product in terms of biological activity.

The magnitude of a given indicator takes into consideration two major factors:

- intermolecular interaction between ingredients that are included in the composition of plant raw materials;
- cooperative contribution of biologically active components to the electron transport intensity, which models energy homeostasis of the organism.

Experimental data on the biological activity of samples indicate that the biologically active substances of juices are capable of oxidizing  $NAD \cdot H_2$  to  $NAD$  in a different way. All samples are biologically active because the speed of electron transfer in the system  $NAD \cdot H_2 - K_3[Fe(CN)_6]$  increases in their presence by 50–350 times, indicating the existence of the antioxidant effect of fresh mixes.

The biological activity of the examined samples of fresh-mixes “Potassium-mix” and “Potassium-mix plus” is significantly different (Fig. 3).

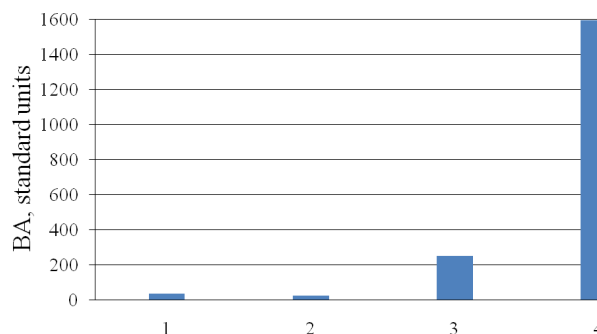


Fig. 3. Study into biological activity (1 – turmeric, 2 – glutin, 3 – “Potassium-mix”, 4 – “Potassium-mix plus”)

The fresh-mix “Potassium-mix” demonstrates a biological activity of 250 standard units; “Potassium-mix plus” – 1,596 standard units. This suggests that the introduction to the formulation of a fresh mix of spicy-aromatic raw material, turmeric (35 standard units), and of raw material of protein nature, glutin (25 standard units), increases the indicator of biological activity by 6.5 times.

When developing the fresh-mix “Potassium-mix plus”, we established a synergistic effect due to the interaction between biologically active substances from all ingredients of beverages for the antioxidant effect indicator (amino acids–antioxidants: methionine, tyrosine, cysteine, taurine, etc., turmeric – vitamin C).

The overall estimation of the drink “Potassium-mix” for organoleptic indicators is 34.01; that of “Potassium-mix plus” is 34.37 points. Profiles of sensory indicators for the fresh-mixes are shown in Fig. 4.

Research into vitamin composition of the obtained fresh-mixes revealed a high content of vitamins-antioxidants (Table 1). Owing to the use of fresh mixes, the resulting beverages contain food fibers in the amount of 0.29 and 0.37 g/100 ml, respectively, for “Potassium-mix” and “Potassium-mix plus”.

The use of spicy-aromatic raw materials increases the content of monosaccharides, as well as mineral substances. The introduction of glutin to the composition of the drink “Potassium-mix plus” increases the content of protein fraction by 4.15 times compared to that of “Potassium-mix”.

The content of fats and carbohydrates in the drinks “Potassium-mix” and “Potassium-mix plus” is 0.18 and 0.23 g/100 ml; 12.54 and 12.30 g/100 ml; respectively. The content of the antioxidant vitamin C in the beverages satisfies, on average, 10 % of the daily need when consuming 100 ml.

The nutritional value of the fresh-mixes “Potassium-mix” and “Potassium-mix plus” in this case was 58.8 and 64.65 kcal, respectively.



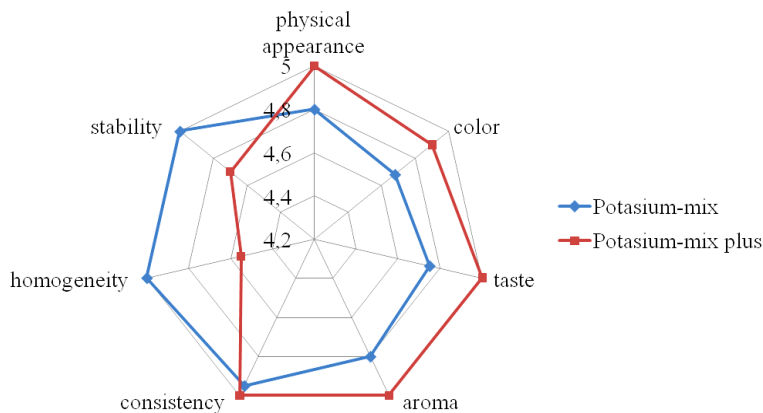


Fig. 4. Sensory indicators of fresh-mixes

Table 1  
Chemical composition of the developed fresh-mixes

Indicator	Potasi-um-mix	Potasi-um-mix plus	Adequate consumption level, g, mg/day	
Water, g	84.71	82.77	1,500...2,000	
Proteins, g	0.45	1.866	58...117	
Fats, g	0.18	0.23	60...154	
Carbohydrates, g, incl.:	12.54	12.30	257...586	
Monosaccharides, g	9.9	12.30	207...486	
Cellulose, g	0.29	0.37	10...15	
Mineral substances, mg per 100 g	Na	15.53	14.63	4,000...6,000
	K	171.83	179.51	2,500...5,000
	Ca	15.77	16.19	800
	Mg	8.47	9.03	400
	P	11.64	12.54	1,200
	Fe	1.22	1.39	10...18
Vitamins, mg per 100 g	A	1.45	1.41	800...1,000
	β- carotene	0.08	0.087	5...10
	B <sub>1</sub>	0.03	0.03	1.1...2.1
	B <sub>2</sub>	0.018	0.019	1.3...2.4
	PP	0.26	0.28	14...28
C	8.42	8.44	70...100	
Energy value, kcal	58.8	64.65	1,800...4,200	

Microbiological indicators of fresh-mixes are given in Table 2.

The microbiological indicators obtained have shown that the developed fresh-mixes are in conformity with the requirements of acting legislation of Ukraine.

The next stage of our work implied obtaining weight coefficients for each intra- and inter-group indicators of beverages.

The generalized quality indicators, based on results obtained in the course of research and their subsequent processing, are, for the fresh-mixes “Potasium-mix” and “Potasium-mix plus”, 0.98 and 0.94 units, respectively. Comprehensive quality assessment showed that the developed drinks are characterized by enhanced nutritional and biological value.

In order to plan the optimal time for the preparation of raw materials, as well as preparing the drink to be introduced for sale at restaurant establishments, we used a method for building a Gantt chart. The network diagram constructed has showed that the preparation of the fresh mix “Potasium-mix” takes 15 minutes; “Potasium-mix plus” – 20 minutes, taking into consideration all the preparatory stages.

The preparation and storage of beverages were conducted under similar conditions, changing storage parameters according to the established ALST test plan, under conditions typical for modern restaurant business.

Table 2

Microbiological indicators of fresh-mixes

Microbiological biomass	Indicators	
	«Potasi-um-mix»	«Potasi-um-mix plus»
Bacteria of coliform group, 333 cm <sup>3</sup>	Not detected	Not detected
Number of mesophilic anaerobic and facultative-anaerobic microorganisms, CFU/cm <sup>3</sup> , not exceeding	Not detected	Not detected
Pathogens, including Salmonella genus, per 25 cm <sup>3</sup>	Not detected	Not detected
Number of mold fungi and yeast (total), CFU/cm <sup>3</sup>	Not detected	Not detected
Number of lactic acid bacteria, CFU/cm <sup>3</sup>	Not detected	Not detected

It was found that the overall organoleptic quality indicator for the developed drinks during storage over 24 hours at different temperatures did not undergo any changes. Over the following 12 hours, all indicators decreased, except for the color. Quality indicators remained high enough for 72 hours. The data obtained allow us to argue the beverage can be stored for 5 days at a temperature of (5±1) °C in an air-tight container.

## 6. Discussion of results of the research into quality indicators of fresh mixes

An analysis of sensory indicators testifies to the high quality characteristics of the developed fresh mixes. A study into stability indicators of the drink “Potasium-mix plus” allows us to state that the improved consistency and homogeneity are due to the presence of gluten. Gluten acts as a hydrocolloid, and consumers determine quality of the beverage based on these very indicators.

An analysis of nutritional composition of the developed beverage revealed that using the chosen fresh-mixes at optimal ratio makes it possible to obtain food products high in potassium, which allows recommending them to people with cardiovascular diseases. The antioxidants contained, as well as their high biological activity, makes it possible to position these beverages as the immunostimulating ones.

For their microbiological indicators, the developed fresh-mixes comply with the requirements of acting sanitary legislation of Ukraine. Storage modeling allows us to argue that the beverages can be sold both at restaurant establishments and in retail shops.

A comprehensive quality assessment of the developed fresh-mixes showed that they are characterized by high nutritional and biological value.

When formulating the beverages, we considered the optimization criterion – the maximum concentration of potassium; the content of sodium, however, was not accounted for in terms of salt balance. When developing the drinks of hypertensive category, one must take into account the ratio between potassium and sodium in the formulation or for human diet. Beverage formulations must take into consideration the age category and consumption norms for basic macro- and micronutrients.

In order to implement the technology in practice, we plan to run a SWOT-analysis of the resulting product. Further work will include determining an integrated quality indica-

tor for the developed beverages and estimating the indicator of competitiveness.

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## 7. Conclusions

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1. The optimization of the component composition of the fresh-mixes “Potassium-mix” and “Potassium-mix plus” was carried out using the tab Solver in the software MS Excel (WINDOWS-2010 operating systems). The introduction of spicy-aromatic and protein raw materials to the formulation contributes to the enrichment of beverages with antioxidants and aminoacids, which, in turn, significantly improves biological activity of the beverages.

2. It is shown that the introduction to the formulation of the drink, based on fresh juices, of spicy-aromatic raw materials (turmeric) and gluten increases the antioxidant activity by 6.5 times. We established the synergistic effect exerted by the interaction among biologically active substances of all components of the drinks in terms of the antioxidant action.

3. The data obtained in the study into nutritional composition of fresh-mixes allow us to state that beverages are a source of easily digestible macro- and micronutrients and could serve as the immunostimulating fast food products.

4. Studying storage conditions in terms of changing the microbiological and organoleptic indicators demonstrated that the rational storing period is 5 days at a temperature of ( $5\pm 1$ ) °C in an air-tight container.

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