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CONTENT

POLARIZATION OF MACROPHAGES OF MICE UNDER THE INFLUENCE OF LECTIN FROM *BACILLUS SUBTILIS* IMV B-7724

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Abstract

Macrophages (Mph) are highly plastic cells that are able to change their functional activity (polarization) and perform their functions in different physiological and pathological processes (including cancer). Changes in the functional activity of Mph can occur due to the action of a number of external stimuli (cytokines, colony-stimulating factors, products of microbial synthesis, etc.).

The aim of the research was to study the effect of lectin from *B. subtilis* IMV B-7724 on the state of macrophage polarization in intact mice of the Balb/c strain.

The cytotoxic effect of lectin from *B. subtilis* IMV B-7724 on the peritoneal Mph of intact Balb/c mice was evaluated *in vitro*; indices, characterizing the functional activity of Mph with M1 and M2 phenotypes and the levels of STAT-1 and STAT-6 mRNA expression, were determined.

We have shown that the effect of bacterial lectin on peritoneal Mph is concentration-dependent: ≥0.1 **mg**/ml is cytotoxic while 0.02 and 0.05 **mg**/ml is stimulating. At low concentrations of lectin there is observed a significant increase in the ratio of NO production to the arginase activity of Mph (NO/Arg), which is characteristic of Mph with the M1 phenotype. Changes in the expression of STAT transcription factors under the influence of the lectin were similar to the changes, found under the combined action of LPS and IFN-γ on Mph. The detected changes in the functional activity of peritoneal Mph of intact mice under the influence of low concentrations of the lectin may be due to the changes in the expression of transcription factors of the JAK-STAT signaling pathway. Understanding the mechanisms of action of lectin from *B. subtilis* IMV B-7724 on Mph will open new perspectives for their modulation/polarization.

Keywords: macrophages, functional state, M1 and M2 polarization, molecular mechanisms, transcription factors.

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1. Introduction

The development of new methods of therapy, aimed at improving the effectiveness of treatment of cancer patients, is the subject of many modern studies [1]. The list of substances with antitumor activity is expanding due to agents of natural origin, which exert not only cytotoxic activity, but also are able to activate the patient's immune response. The aim of immunotherapy is to restore and maintain the proper activity of the main effectors of antitumor immunity, the formation of a full-fledged immune response to tumor antigens. It is known, that antitumor immune responses play a significant role in preventing the recurrence and metastasis of the primary tumor. Therefore, the use of medicinal agents that prevent the suppression of the immune response, caused by cancer progression or the aggressive effects of chemotherapy and radiation therapy, may be effective in the complex treatment of patients [2, 3].

In the study of the effectiveness of biotherapy Mph are thought to be the most promising effectors of natural immunity due to their ability to rapidly change the functional state depending on the stimuli they receive from the local microenvironment. There are Mph that have been activated by classical (M1) or alternative (M2) pathways. M1 subpopulations exert pro-inflammatory (antitumor) properties, while M2 subpopulations promote tumor growth (protumoral properties). It is a known fact that tumors are able to remodel the microenvironment and facilitate the switching M1 macrophages to the tumor-associated state. The study of M1↔M2 switching mechanisms and the search for possible ways to regulate the polarization process is one of the important ways for immunotherapy optimization [4, 5].

Activation of macrophages by the M1 pathway is mediated by the action of interferon-γ (IFN-γ) and/or lipopolysaccharide (LPS) on the surface receptors IFN-γR and/or Toll-like receptor 4 (TLR4), respectively, via the JAK-STAT signaling pathway. As a result, there is observed an increase in the expression of transcription factor (TF) STAT1 and activation of the transcription of STAT1-dependent genes. In addition, the binding of LPS to TLR4 results in the activation of TLR4-associated adapter proteins MyD88 and TRAK, which promotes the formation of the NF-kB-p65/p50 heterodimer and the activation of the transcription of NF-kB-p65/p50-dependent genes, including a number of factors of the acute inflammatory phase (TNFα, IL-1β, IL-6, IL-12p40, COX2) [6–9].

An alternative way of Mph activation is mediated by the action of cytokines IL-4/IL-13 or IL-10 on surface receptors IL-4Rα/IL-13Rα1 or IL-10R, respectively. Due to the activation of the corresponding signaling pathways, TFs STAT6 or STAT3/NF-kB-p50/p50 are expressed and translocated into the nucleus, which determines the direction of Mph polarization toward M2 phenotype [10].

Mph polarization is accompanied not only by altered gene transcription, but also by a rearrangement of metabolic pathways and a change in the profile of Mph-secreted factors. The marker of M1/M2 Mph polarization by their metabolic activity can be the peculiarities of L-arginine catabolism. In particular, as a result of L-arginine catabolism in M1 macrophages, the formation of NO and citrulline with the involvement of the NOS2 enzyme is observed [11, 12]. In M2 macrophages L-arginine catabolism leads to ornithine and urea production due to arginase activation. Thus, the secreted NO to arginase ratio (NO/Arg) indicates the state of M1/M2 polarization of Mph [13].

An important feature of Mph is their high plasticity, ability to change the functional properties and direction of polarization under the influence of microenvironmental signals. M2 Mph exert especially high plasticity that allows to assume a possibility of their repolarization in M1 macrophages by means of immunotherapy. Lectins as substances with immunomodulatory and antitumor properties attract special attention. These glycoproteins are able to selectively bind carbohydrates and carbohydrate components of glycoconjugates of various origins. Highly specific binding of lectins to the corresponding receptors on plasma membranes of cells mediates their cytotoxic or stimulating influence. The mechanisms of antitumor activity of lectins are associated with both direct cytotoxic effects on tumor cells and with indirect effects due to the modulation of immune responses [14–16]. Up-to-date the properties of plant lectins are described in detail, but all currently known plant lectins are quite toxic substances, causing the cytotoxic effect not only on tumor cells but also cells of healthy tissues. Lectins, produced by bacteria, have been studied to a much lesser extent. However, given their low toxicity, bacterial lectins may be considered promising as agents with antitumor and immunomodulatory properties.

The aim of the study was to investigate the effects of lectin from *B. subtilis* IMV B-7724 on the state of polarization of Mph of intact Balb/c mice.

2. Materials and methods

2. 1. Bacterial lectin

The lectin was produced by spore-forming gram-positive saprophytic bacteria of *B. subtilis* IMV B-7724 (deposited in the collection of the DK Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv) [17]. Lectin was obtained from the culture fluid of the microorganism as described in [18]. After purification and lyophilization, the substance is stored in powder form at –20 °C.

2. 2. Animals

The study has been carried out on male Balb/c mice 2–2.5 month old weighting 19–20 g, bred at the vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR).

The use and care of experimental animals have been performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [19]. The work was approved by Institutional Animal Care and Use Committee.

2. 3. Isolation and cultivation of macrophages

Mice were euthanized, and 3 ml of ice-cold PBS, supplemented with heparin (5 U/ml), was injected into the abdomen. The fluid was withdrawn and the abdomen was washed twice with the same volume of heparin-containing PBS. The resulted cells suspension was centrifuged (550 g for 10 min), and the cell pellet was resuspended in 1 ml 0.9 % NaCl solution, supplemented with 2.0 % penicillin-streptomycin. These peritoneal exudate cells were counted and cultured in 96-wells flat bottomed plates for 2 h (37 °C, 5 % CO_2, 100 % humidity). After that non-adherent cells were removed, and the adherent cells were washed two times with 0.9 % NaCl and taken for further investigation.

In all subsequent studies, Mph were cultured at 37 °C in a humidified atmosphere with 5 % CO_2 in the complete culture medium (RPMI 1640 ("SIGMA", USA), 10 % fetal calf serum (FCS), ("SIGMA", USA), and 40 μg/ml gentamicin.

2. 4. Design of experiment

Firstly, the *in vitro* sensitivity of peritoneal Mph of intact Balb/C mice to different concentrations of the lectin (2.0; 1.0; 0.5; 0.2; 0.1; 0.05; 0.02 mg/ml)) was assessed.

The assessment of the functional state of Mph was determined by the ratio of NO production levels to arginase activity, as well as by determining the expression levels of STAT-1 and STAT-6 mRNAs by qRT-PCR.

In all experiments, the corresponding indices of Mph, not exposed to stimuli, served as a control.

2. 5. Analysis of cytotoxic action of the lectin

The Mph suspension was placed into 96-well plates at a concentration of $1x10^5$ cells/well in 100 μl of the complete growth medium. Lectin was added to the appropriate wells in concentrations of 2; 1; 0.5; 0.2; 0.1; 0.05 and 0.02 mg/ml, and cells cultivation continued under standard conditions for 2 or 24 hours. Wells with the culture medium and cells, to which no lectin was added, were used as controls. All samples were analyzed in triplicates. Further evaluation of the cytotoxic effect was performed using the colorimetric MTT test as described in [20].Optical density was measured at λ=545 nm vs λ=630 nm with a MicroELISA reader (StatFax-2100, USA). Each sample was measured in triplicate.

Cytotoxic Activity Index (CTAI, %) was calculated as follows:

$$CTAI=[1-(OD_{Mph+l}-OD_{Mph})/OD_{Mph}]\times 100\ \%,$$

where OD_{Mph} – optical density of wells, in which only Mph were incubated; OD_{Mph+l} – optical density of wells, in which Mph were incubated with the lectin.

2. 6. *In vitro* reprogramming of macrophages

To polarize the Mph of intact mice toward the M1 phenotype, IFN- γ (20 ng/ml, U-Cytech, the Netherlands) in combination with LPS (100 ng/ml, Sigma, USA) was added in the complete culture medium; for polarization toward the M2 phenotype, IL-4 was used (10 ng/ml, Sigma, USA). The incubation continued for 24 hours.

2. 7. Measurement of NO production

NO production was measured by the standard Griess reaction [21]. In brief, cell suspensions ($2\cdot10^5$ cell/well) were placed in a volume of 200 μl in 96-well flat-bottom tissue culture plates and cultured for 24 hours. Each cell culture was investigated in duplicate. At the end of the incu-

bation period, supernatants were collected and NO production was assessed by the accumulation of nitrite (as stable metabolite of NO) by the Griess reaction. An aliquot of the culture supernatant (100 µl) was mixed with an equal volume of the Griess reagent (Acros Organics, Belgium) and incubated for 1 hour at room temperature in the dark. The reaction products were colorimetrically quantified at λ=550 nm. The standard curve, plotted by the results of measurements of the solutions, containing known concentration of $NaNO_2$, was used for converting the absorbance to micromolar concentrations of NO, expressed in µM NO_2 – per 10^6 cells.

2. 8. Measurement of arginase activity

Arginase (Arg) activity was determined based on urea measurement [21]. Mph were lysed by double freezing and melting. Then 50 µl of 50 mM Tris-HCl (pH 7.4) and 10 µl of 50 mM $MnCl_2$ were added to each sample. Samples were heated at 56 °C for 10 min, and upon addition of 100 µl of 0.5 M L-arginine (pH 9.7), heated for further 30 min (37 °C). The reaction was stopped with 800 µl of the acidic mixture (1:3:7, 96 % H_2SO_4:85 % H_3PO_4:H_2O). Then 40 µl of α-isonitrosopropriophenone (Sigma-Aldrich) was added to the solution, which was heated for 30 min (95 °C) and incubated for 30 min at 4 °C. The urea concentration was measured spectrophotometrically at λ=550 nm. Values of optical density were converted to the mass of urea based on the calibration curve of the standard urea solution. Arg activity was calculated as described in [22]. One unit of Arg activity means the amount of the enzyme, hydrolyzing 1 µM of arginine per 1 min. The results are expressed as units/10^6 cells.

2. 9. qRT-PCR

Total RNA was isolated from 2×10^5 Mph using NucleoZOL (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to the manufacturer's protocol. cDNA synthesis was performed using RevertAid Reverse Transcriptase, RiboLock Inhibitor, dNTP mix and Oligo(dT) primer (Thermo Scientific, USA). qRT-PCR was held on sequence detection system 7500 (Applied Biosystems, CA, USA) using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, USA) and following forward (For) and reverse (Rev) list of primers: STAT1 For 5`-TCCTTCTGGCCTTGGATTGA-3`, Rev 5`-ACCGTTCCACCCATGTGAA-3`, STAT6 For 5`-AGATGAGGCTTTCCGGAGTCA-3`, Rev 5`-CCCATATCTGAGCTGAGTTGCA-3`, TBP For 5`-CCAATGACTCCTATGACCCC-3`, Rev 5`-GTTGTCCGTGGCTCTCTTATTC-3`. The target genes` Ct values were normalized to Ct value of the internal control gene (TBP) using ddCt method.

2. 10. Statistical analysis

The statistical significance was evaluated by the nonparametric Mann-Whitney U test, and the correlation analysis was determined according to Spearman`s correlation using Prism software Version 8.0. The statistical significance between examined groups was assessed as $p<0.05$.

3. Results and discussion

First of all, the toxic *in vitro* effect of lectin from *B. subtilis* IMV B-7724 on the peritoneal Mph of intact Balb/c mice was evaluated. It is shown, that the studied lectin has a concentration-dependent cytotoxic effect (**Table 1**).

Table 1

Cytotoxic activity (CTAI, %) of lectin from *B. subtilis* IMV B-7724 against Mph of intact Balb/c mice

Concentration of the lectin, mg/ml	Cytotoxic activity (%) after incubation for	
	2 hours	24 hours
2.0	86.1±1.6	85.0±2.1
1.0	82.1±2.1	80.1±0.9
0.5	60.4±3.9	75.8±2.4
0.2	54.4±5.1	60.5±1.3
0.1	25.4±6.9	21.9±4.7
0.05	0	0
0.02	0	0

The cytotoxic effect of the lectin was observed when it is used in concentrations from 0.1 to 2.0 mg/ml. The calculation of the IC_{50} (concentration of an agent that is required for killing of 50 % target cells *in vitro*) showed that the cytotoxic effect did not depend on the duration of exposure to the lectin. The IC_{50} after 2 h of incubation of macrophages with lectin was 0,155 mg/ml; after 24 h of incubation – 0,148 mg/ml. The incubation with lectin at lower doses (0.05 and 0.02 mg/ml) had no cytotoxic effect on macrophages. Taking into account the obtained results, for further studies we have chosen the lowest concentrations of the lectin (0.05 and 0.02 mg/ml).

The effect of the lectin on the polarization of Mph was evaluated by calculating the NO/Arg ratio. When Mph were exposed to LPS in combination with IFNγ, a 1.3-fold increase in the NO/Arg ratio was observed compared to the intact control (**Fig. 1**), which is characteristic of the M1 phenotype. In contrary, the action of IL-4 resulted in a significant decrease in the NO/Arg ratio compared to the intact control (1.9 times), which indicated the predominance of arginase activity in the metabolism of L-arginine and is a feature of the M2 phenotype. The obtained results correlate with the literature on the possibility of changing the direction of the Mph polarization by the influence of LPS+IFNγ and IL-4 [10, 11].

The addition of lectin at a concentration of 0.02 mg/ml to the culture medium of macrophages led to a significant increase in NO production and a decrease in Arg activity. The NO/Arg ratio increased 1.4 times as compared with the intact control (18.6 a.u. vs 13.5 a.u.) and approached this indicator under the combined action of LPS+IFNγ (18.1 c.u.)

The incubation of Mph with lectin at a concentration of 0.05 mg/ml had no effect of the NO/Arg ratio compared to that in the intact control (14.2 vs 13.5). Instead, increasing the concentration of lectin up to 0.2 mg/ml caused downregulation in macrophages activity that reflects in 1.3-fold decrease in the NO/Arg ratio compared to the intact control (10.5 a.u. vs 13.5 a.u.) (**Fig. 1**).

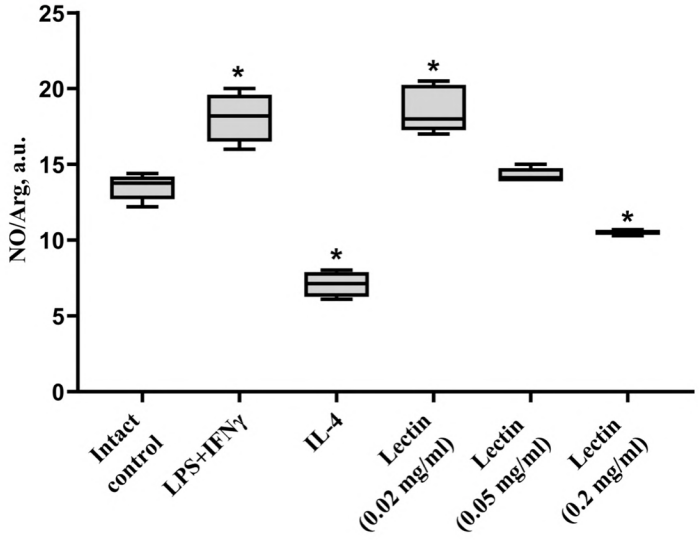


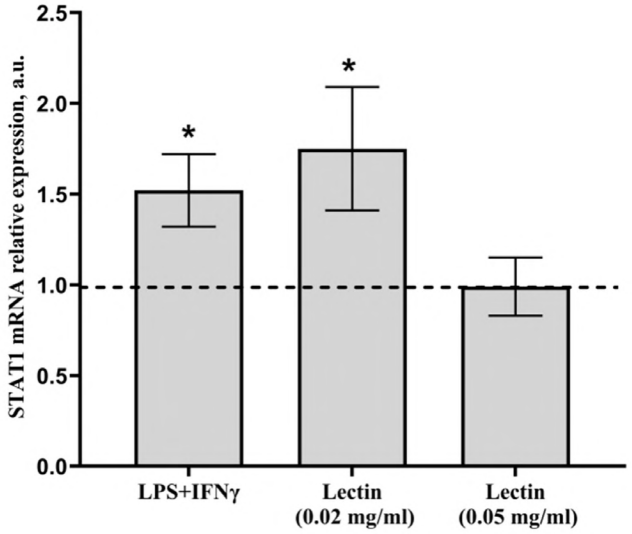
Fig. 1. Changes in the NO/Arg ratio under the action of different stimuli on the Mph of intact Balb/c mice; * – $p<0.05$ compared with the intact control

Given into account the results obtained, we have decided to focus our further research on LPS+IFN-γ, lectin(0.02 mg/ml) and lectin (0.05 mg/ml)`s effect om Mph and to study whether the mechanisms of their action are also similar.

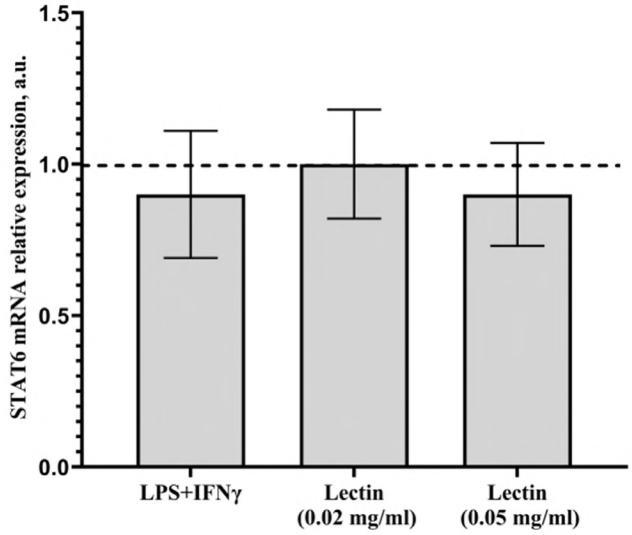
We have evaluated the mRNA expression levels of STAT1 and STAT6 in all experimental groups, as far as this TFs are known regulators of Mph polarization into M1 or M2 phenotypes, respectively.

STAT1 mRNA expression levels in Mph after their co-coutivation with LPS+IFN-γ and lectin in concentration 0.02 mg/ml were significantly higher compared to the intact control – 1.52 a.u. and 1.75 a.u. vs 1 a.u., respectively (**Fig. 2, *a***). Instead, the influence of lectin in con-

centration 0.05 mg/ml did not cause the changes in STAT1 mRNA expression level. As expected, we did not observe any difference in the STAT6 mRNA expression level after the application of LPS+IFN-γ and lectin in both concentrations because this TF is involved in M2 polarization not at M1 (**Fig. 2, *b***).



a



b

Fig. 2. mRNA expression levels after co-cultivation of Mph with various stimuli: *a* – STAT1; *b* – STAT6; – – – – – the intact control; **p*<0.05 compared with the intact control

Similar to the NO/Arg index, the analysis of the ratio of the expression level of STAT1 mRNA to the expression level of STAT6 mRNA (STAT1/STAT6 ratio) allows to assess the state of M1/M2 polarization. The combined effect of LPS+IFN-γ as well as lectin in concentration 0.02 mg/ml but not in concentration 0.05 mg/ml led to a significantly increase of the STAT1/STAT6 ratio compared to the intact control and were similar to those, observed in NO/Arg ratio (**Fig. 3**).

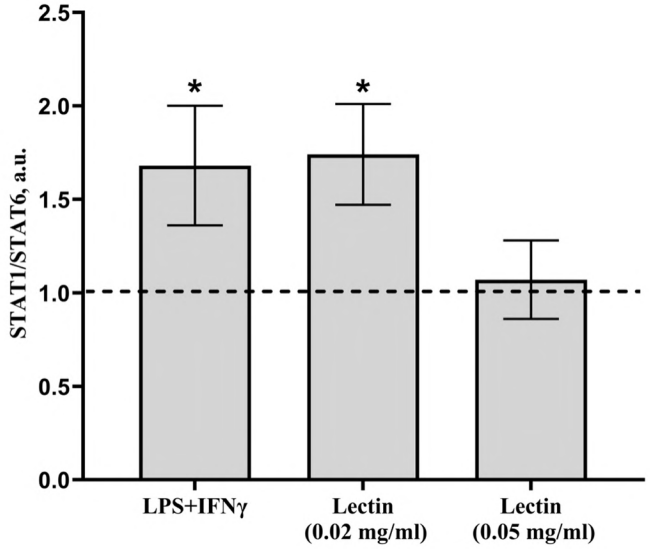


Fig. 3. STAT1/STAT6 ratio in Mph under the action of different stimuli. – – – – the intact control; *p<0.05 compared with the intact control

Our results are consistent with the literature on the study of molecular mechanisms of Mph activation. In particular, it is known, that the action of IFN-γ and/or LPS in Mph cause up-regulation of the NOS2 enzyme expression (via STAT1-dependent pathway), which metabolizes L-arginine to NO and citrulline and is characteristic for the M1 phenotype. Under the action of IL-4, the expression of the enzyme arginase, which hydrolyzes L-arginine to ornithine and urea, increases, which is a characteristic functional feature of the formation of a subpopulation of cells with M2 phenotype [10, 11]. Therefore, when Mph are polarized in the M1 direction, there is a shift in the NO/Arg ratio toward an increase in NO production, probably due to the increased NOS2 expression and decreased arginase activity. The detected changes in the metabolic activity of peritoneal Mph of intact mice under the influence of low concentrations of bacterial lectin may be caused by the changes in the expression of TFs of the signaling pathway JAK-STAT. This evidence opens the prospects for studying the mechanism of action of the lectin as a means of mediating the increased antitumor immune response.

4. Conclusions

1. The effect of lectin from *B. subtilis* IMV B-7724 on the peritoneal Mph of intact the Balb/c mice *in vitro* is concentration-dependent: ≥0.1 mg/ml – cytotoxic; 0.02 and 0.05 mg/ml – stimulating (in terms of functional activity).

2. Under the action of low concentrations of the lectin there is observed a significant increase in the NO/Arg ratio, which is characteristic of Mph with the M1 phenotype.

3. Changes in the STAT1 mRNA expression under the influence of the lectin in concentration 0.02 mg/ml on Mph are similar to the changes under combined action of LPS and IFN-γ.

Conflicts of interest

The authors declare no conflicts of interest.

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INFLUENCE OF PHILAZONIT BIOPREPARATION ON THE MYCOBIOME OF SOYBEAN PLANTS RHIZOSPHERE

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Abstract

The article presents the results of analysis of biodiversity and spatial-functional structure of the microbial complex in the soil rhizosphere, and development of ways to regulate the number of phytopathogenic micromycetes in the rhizosphere of soybean plants in organic production. Varieties of plants of soy were grown using the biopreparation «Philazonit", which was developed in the company "Philazonit of Ukraine". The study determined the effect of phylazonite biopreparation on the mycobiome of the rhizosphere of soybean plants. The interaction of plants of soy varieties Kent and Suzir'ja with phytopathogenic micromycetes in conditions of the organic production in the Central Forest-Steppe of Ukraine (Research farm "Skvyrske" of Institute of Agroecology and Nature Management of the National Academy of Agrarian Sciences of Ukraine (IANM of the NAAS) was investigated. The number of phytopathogenic micromycetes in the rhizosphere of plants of different varieties of soy is determined depending on the variety and technology of its cultivation. It was established that the biopreparation Philazonit inhibits the formation of the number of phytopathogenic micromycetes in the rhizosphere of plants of the soy variety Suzir'ja and Kent during the growing season. It is proved that the representatives of genera: Alternaria, Fusarium, Penicillium, Aspergillus are dominated in the rhizosphere of plants of soy of both varieties. In the rhizospheres of plants of the soy Suzir'ja variety the representatives of genus Penicillium (32.8 %) most often occurred. The smallest number of micromycetes were members of the genus Aspergillus (9.5 %). In the rhizosphere of plants of the Kent variety dominated by representatives of the genera Alternaria and Fusarium (35.6 % and 34.1 % respectively). Representatives of the genus Penicillium were 15 % and Aspergillus – 15.3 % It is proved noted that during the studies there was a

correlation between the development of micromycetes and the hydrothermal coefficient – in the flowering phase (r=0.8) and in the ripening phase (r =0.9) except for the germination phase.

Keywords: soybeans, phytopathogenic micromycetes, rhizosphere, hydrothermal coefficient, ecology, agrocenosis.

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1. Introduction

Fertile land and favorable climate determined the rapid spread of production of soy in Ukraine. At the same time, there is a growing of the mass accumulation of phytopathogenic microorganisms in the agrocenoses of soy, among which genera: Fusarium, Alternaria, Penicillium, Aspergillus are dominated [1–3]. They lead to the development of harm-causing diseases of soybean plants during the growing season and can intensively inhabit the seed material. This causes a deterioration in the quality of the crop by reducing the indicators of protein and fat contents [4]. These micromycetes are toxin-forming. They can damage of the various organs of plants, animals, fungi and are toxic to humans.

A wide range of phytotoxic action have deuteromycetes toxins, which belong to the genera: Penicillium, Aspergillus and Fusarium. In the functioning of the system (soil – microbial community – plants), toxin-forming micromycetes are characterized by greater competitiveness compared to other microorganisms [5–8].

Therefore, more and more attention is paid to the organic production of soy in the world, which is aimed at regulating the number of phytopathogenic microorganisms in agrocenoses. Biological preparations are one of the most powerful factors in regulating of the biotic relationships in agrocenoses, which allows to solve the issue of ensuring a balanced nutrition of plants and resistance to phytopathogenic micromycetes. Retrospective analysis of literature showed that the problems of the effectiveness of the influence of biological preparations on the functioning of agroecosystems were studied by various scientists [9–15]. Their research revealed the main problems such as: optimization of the nutritional regime under the influence of biopreparations, increasing of the crop yields, reproducing of the soil fertility, regulating their microbiological activity. High efficiency of the various biopreparations using was found, among which Philazonit biopreparation has proven itself.

Philazonit is a complex biopreparation based on beneficial soil bacteria.

Philazonit contains several groups of bacteria: nitrogen-fixing bacteria, phosphate-mobilizing bacteria, celluloso-destroying bacteria. The composition of Philazonit also includes natural B vitamins, which reduce the sensitivity of plants to diseases, as well as hormones that accelerate seed germination and plant growth, in particular gibberellins and auxins. Antipathogenic bacteria prevent and protect plants from fungal diseases, especially *Fusarium oxyporum* and promote the production of immunity in plants. Soy plants respond well to the introduction of biopreparations that contribute to: the productivity of soy; humic acids enhance the properties of plant immunoregulation, biosynthesis of protective substances; contribute to the growth of auxins and cytoquinins; regulate of the physiological processes in cells [16, 17].

Consequently, soil microbial cenoses have complex genetic and spatial organization and high biodiversity, the structure of which depends on farming systems, agrotechnical measures and physiological biochemical properties of cultivated plants.Therefore, the study of the effect of the biological product Filazonit on biodiversity and the spatial-functional structure of the microbial complex in the soil rhizosphere is important for regulating the number of phytopathogenic micromycetes in the rhizosphere of plants of soy varieties in conditions of organic production.

So, the goal of the investigation was to develop of the ways to regulate the number of phytopathogenic micromycetes in the rhizosphere of plants of soy varieties in conditions of organic production.

2. Materials and Methods

2. 1. Characteristics of place, soil of researches

Experimental research was carried out during 2018-2020 in the Central Forest-Steppe of Ukraine (Research farm “Skvyrske” of organic production of the IANM of the NAAS) and in

the Department of Agrobioresources and Environmentally Safe Technologies of the IANM of the NAAS. The best soils for soy are black humus earth, dark gray and chestnut. The most suitable for this crop are soils with a neutral reaction (pH 6.5–7.0). The soil of the research field of the "Skvyrske" research station of organic production is represented by low-humus black humus earth, and in the mechanical composition – large dusty-medium loamy. The humus content in the upper layer of soil (0–20 cm) is 3.6 %, lightly hydrolyzed nitrogen – 6.6 mg, easily digestible phosphorus – 14.0 mg and metabolic potassium – 15.2 mg per 100 g of soil. The reaction of the soil solution is slightly acidic (pH=6.0).

2. 2. Hydrothermal coefficient (HTC)

Hydrothermal coefficient (HTC) was calculated according to the methodology [18].

$$HTC=\Sigma R/0,1\cdot\Sigma t_{act>10},$$

where:

HTC<0.4 – very strong drought,
HTC from 0.4 to 0.5 – severe drought,
HTC from 0.6 to 0.7 – average drought,
HTC from 0.8 to 0.9 – weak drought,
HTC from 1.0 to 1.5 – sufficiently moisture,
HTC>1.5 – excessively moisture.

2. 3. The object of the study

Samples of plants of different varieties of soy were selected during the growing season in the phase: shoots, flowering, maturation. The studies were carried out on plants of soybean varieties: Suzir'ja, selection of the National Scientific Center of the Institute of Agriculture of the National University of Ukraine and Kent variety, selection company "SAATBAULINZ" in Austria. The technologies with using of the biopreparations of various actions is the alternative at the organic production, because the use of mineral fertilizers and plant chemical protection products is prohibited. Therefore, the varieties of plants of soy were grown using the biopreparation "Philazonit", which was developed in the company "Philazonit of Ukraine".

We used a biological product Filazonit before sowing soybeans (tillage). Consumption rate – 1.5 l/ha.

To compare the results, we used the control option (soybean varieties without processing of the biological product).

The sampling of the biological samples and counting the number of colony-forming units (CFU) were carried out according to methods, generally recognized in mycology [19].

2. 4. Analysis of soil mycobiome

Analysis of soil mycobiome was carried out by soil dilution method [20]. The rhizosphere soil was taken from the roots of plants. Samples were transferred in laboratory conditions to sterile flasks of 90 ml of sterile distilled water and for 5 minutes were shaken on a horizontal orbital shaker (Wise Shake SHO, SHR 2D) to a homogeneous suspension. 1 ml of suspension was transferred from the flask to a sterile test tube of 9 ml of sterile water. From the test tube of the 3rd dilution, 1 ml of the suspension was seeded into sterile Petri cups. After that, 10 ml of Chapek medium was poured, mixed and incubated at a temperature of 25 ºC for 3 days, after which colonies were counted on the automatic counter SCAN 4000 (Intercience, France) and transferred them into test tubes for further identification.

2. 5. Identification of isolates

Identification of isolates was carried out on 15-day cultures using a trinocular microscope (DN-200 M) using the Mycobankdatabase base [21].

2. 6. The count of colonies

The average number of colonies of fungi per 1 g of dry soil was determined by the formula [22]:

$$A = \frac{b \cdot c \cdot K}{g},$$

where:

A – the number of infectious units of micromycetes in 1 g of dry soil;
b – the average number of colonies in a cup;
c – dilution of the solution, from which inoculation is made;
K – humidity amendment;
g – soil weight (10 g).

2. 7. Statistical analysis

Statistical analysis of the obtained results was carried out using dispersion and correlation analysis (p=0,05) [23]. For the process of the results, standard mathematical methods of data analysis and charting were used using the Microsoft Office Program Package, Statgraphics Plus for Windows, Excel 2000.

3. Results

The air temperature exceeded the norm of indicators during the field research and ranged from 14 ºC to 29 ºC (**Fig. 1**).

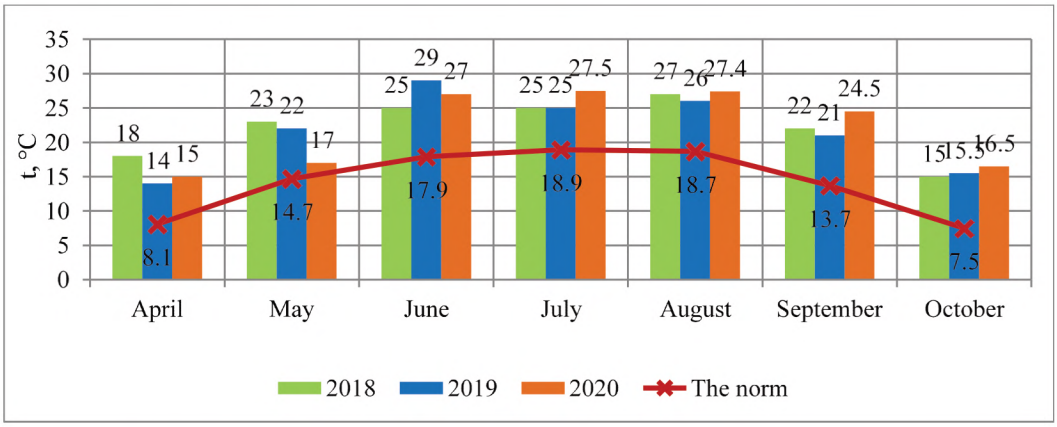


Fig. 1. Air temperature during field research (Research farm "Skvyrske" of IANM of the NA)

It is known that the optimum temperature for germination of soy seeds is 15–20 °C; for flowering – 19–25 °C; for the formation of beans and seeds – 17–23 °C; for maturation – 13–20 °C. The average daily temperature at which the reproductive organs of soy are formed – 18–23 °C [24].

The weather conditions for growing soy during the study were within the normal range, despite the fact that the air temperature indicators exceeded the norm. The amount of precipitation during field research was quite contrasting and ranged from 8 mm (lower than the norm by 38 mm) to 229 mm (above the norm by 128.9 mm) (**Fig. 2**).

The varieties of soy plants Kent and Suzir'ja are characterized by medium resistance to drought. Given this, 2018 year was the least favorable for growing soy.

Climatic conditions perform both direct and indirect effects on the vital activity of microorganisms. At the same time, temperature and moisture are important components of environmental conditions that regulate the course of soil and biological processes. According to the results of retrospective analysis of literature, hydrothermal coefficient (HTC) determines the vital activity of soil organisms and the activity of soil biochemical processes.

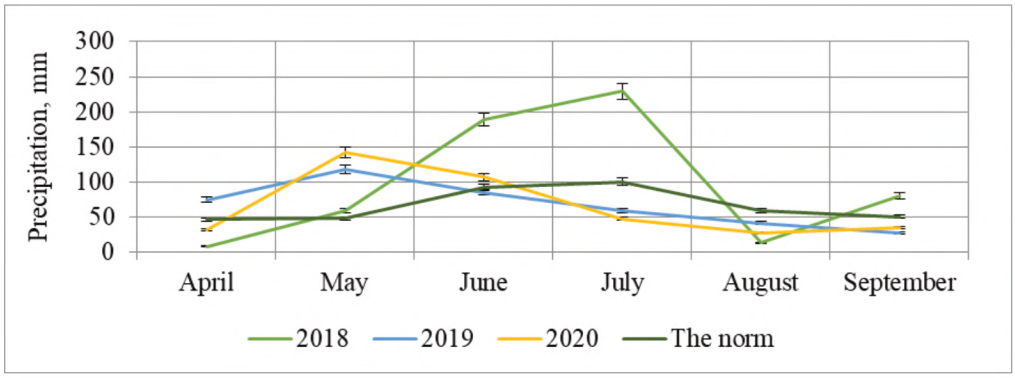


Fig. 2. The amount of precipitation during the field research (Research farm "Skvyrske" of IANM of the NA)

According to the calculated values of the HTC, it was established that the growing season of 2018 was wet enough (HTC 1.35), in 2019 – slightly arid (HTC 0.9) and in 2020 – wet enough (HTC 1) (**Fig. 3**).

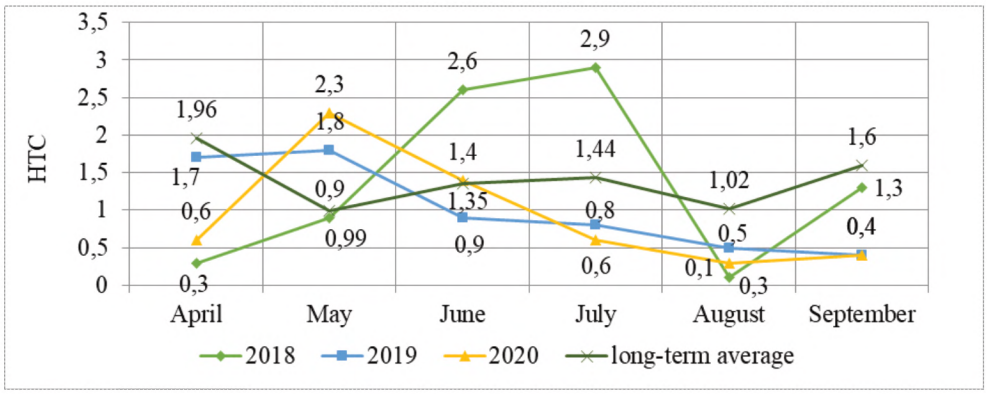


Fig. 3. Dynamics of indicators of hydrothermal coefficient (HTC) of the vegetation period in the years of research (Research farm "Skvyrske" of IANM of the NA)

According to the results of studies, it was found that there was a restrained formation of the number of micromycetes in the phase of shoots and maturation in 2018 during the action of the biopreparation Philazonit on plants of soy varieties Kent and Suzir'ja (**Fig. 4)**

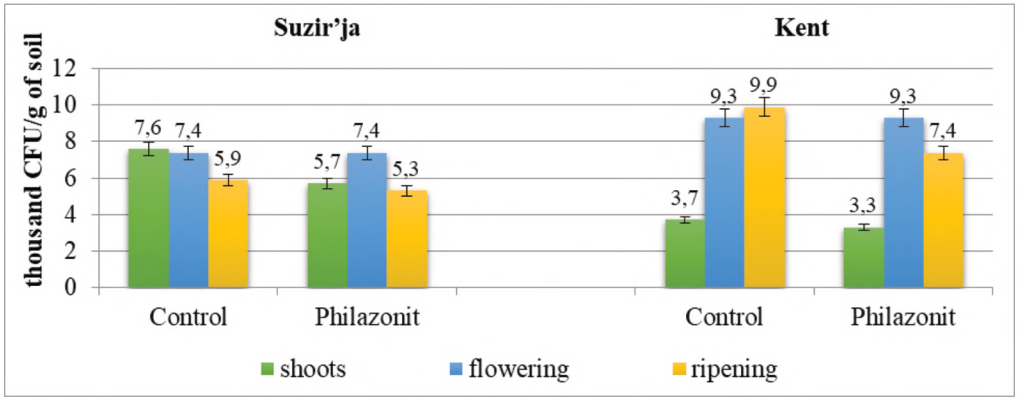


Fig. 4. The number (CFU/g of soil) of micromycetes in the rhizosphere soil of soybean plants of the varieties Suzir'ja and Kent under the action of the biological product Philazonit (2018)

On plants of soy variety Suzir'ja in the phase of shoots, the number of micromycetes was less by 1.9 thousand CFU/g of soil and in the ripening phase – by 0.6 thousand CFU/g of soil compared to control. At the same time, on plants of soy variety Kent in the phase of shoots the number of micromycetes was less by 0.4 thousand CFU/g of soil, and in the ripening phase – by 2.5 thousand CFU/g of soil compared to control.

It was also found that in the flowering phase both on plants of soy varieties of Suzir'ja and Kent, the indicators of the number of CFU micromycetes were at the level of control and amounted to 7.4 thousand CFU/g of soil and 9.3 thousand CFU/g of soil, respectively. This indicates the low efficiency of the Philazonit biopreparation, which may be due to exceeding temperature standards (by +11.1 ºC) and a large amount of precipitation, exceeding the norm by more than 2 times in the period from June to July (flowering phase), which caused the formation and development of phytopathogenic micromycetes and significantly reduced the effectiveness of Philazonit biopreparation on plants of soy varieties Kent and Suzir'ja in the flowering phase.

According to the results of the research, it was established that the formation of mycobiome in the rhizosphere of soy plants was suppressed during ontogenesis during the action of the Philazonit biopreparation on plants of soy varieties Suzir'ja and Kent in 2019 (**Fig. 5**).

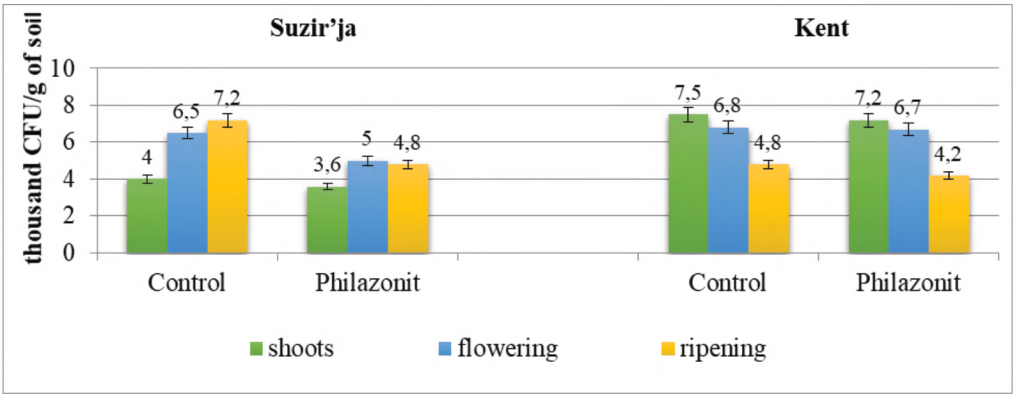


Fig. 5. The number of micromycetes (CFU/g of soil) in the rhizosphere of soybean plants Suzirya and Kent under the action of the biological product Philazonit (2019)

The smallest number of phytopathogenic micromycetes was observed in the phase of shoots on plants of soy varieties of Suzir'ja (3.6 thousand CFU/g of soil), and in the ripening phase on plants of soy of Kent variety (4.2 thousand CFU/g of soil). Similar results were obtained in 2020 (**Fig. 6**).

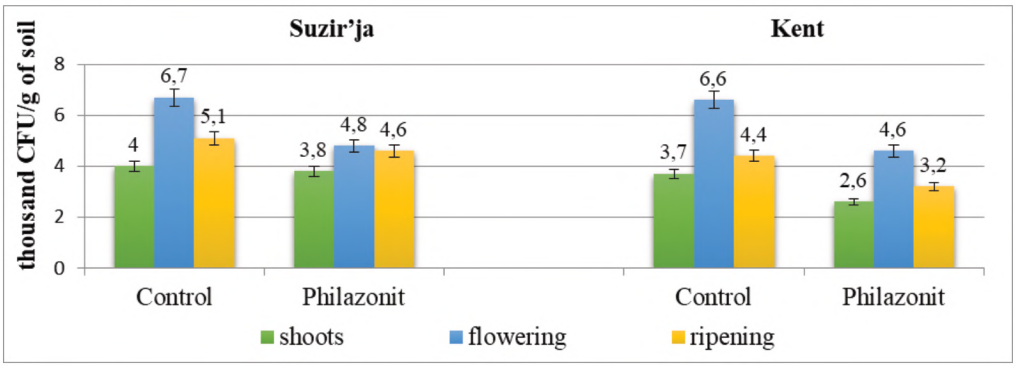


Fig. 6. The number (CFU/g of soil) of micromycetes in the rhizosphere soil of soybean plants of the varieties Suzir'ja and Kent under the action of the biological product Philazonit (2020)

It should be noted that the most effect of biopreparation was found in the phase of shoots on both varieties, where the number of micromycetes in the mycobiome of rhizosphere plants of the soy Suzir'ja was 3.8 thousand CFU/g of soil, and on plants of soy of Kent variety – 2.6 thousand CFU/g of soil. The efficiency of the Philazonit biopreparation was high despite the fact that 2020 was characterized as wet enough (HTC 1) and the air temperature exceeded the norm. According to statistical analysis, presented in Table 1, the biopreparation Philazonit showed efficacy during the study in all phases of ontogenesis of plants and reduced the number of micromycetes in the mycobiome of the rhizosphere of the plants studied (**Table 1**).

Table 1

Dynamics of the number of micromycetes in the rhizosphere of soybean plants during the growing season

Varieties	Biopreparation	The average number of CFU in the rhizosphere of soybean plants, thousand									The average		
		2018			2019			2020					
		shoots	flowering	Ripening	shoots	Flowering	ripening	Shoots	flowering	ripening	shoots	flowering	ripening
Suzir'ja	Control	65	62	49	35	54	60	35	56	44	45	57,3	51
	Philazonit	47	62	36	26	44	41	25	40	32	32,6	48,7	36,3
insignificant difference p = 0.05	over the years										19,5	9,8	21,4
	for technologies										7,8	8,9	11,7
Kent	Control	37	78	83	62	57	40	31	55	37	43,3	63,3	53,3
	Philazonit	28	78	62	52	45	26	22	42	22	34,0	55,0	36,7
insignificant difference p = 0.05	over the years										11,5	16,0	17,1
	for technologies										8,4	10,7	12,7

Note: The difference is significant at the level of p = 0.05

It should be noted that during the studies there was a correlation between the development of micromycetes and the hydrothermal coefficient – in the flowering phase (r=0.8) and in the ripening phase (r =0.9) except for the germination phase. A significant number of phytopathogenic micromycetes were isolated and identified from the rhizosphere of plants of soy varieties (**Fig. 7**).

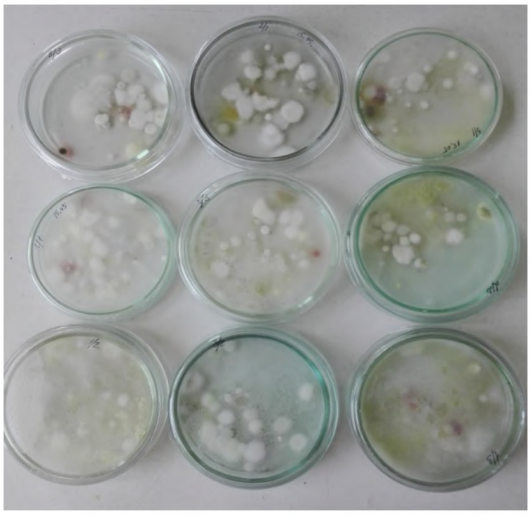
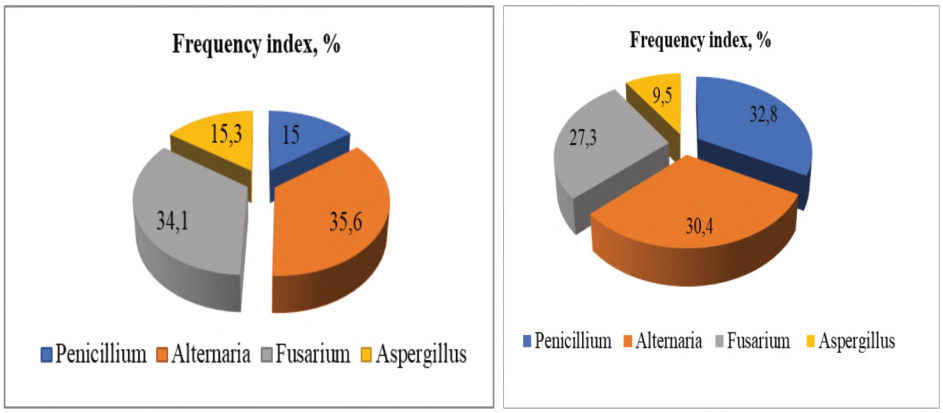


Fig. 7. The spectrum of micromycetes in the rhizosphere soil of soybeans plants of the Kent variety (5 days after seeding)

The number of solates isolated from the rhizosphere of soybean varieties

Analysis showed that the species, belonging to four genera of micromycetes: Alternaria, Fusarium, Penicillium, Aspergillus in the rhizosphere of plants of both varieties of soy are dominated. In the rhizospheres of plants of the soy Suzir'ja variety the representatives of genus Penicillium (32.8 %) most often occurred. The smallest number of micromycetes were members of the genus Aspergillus (9.5 %). In the rhizosphere of plants of the Kent variety dominated by representatives of the genera Alternaria and Fusarium (35.6 % and 34.1 % respectively). Representatives of the genus Penicillium were 15 % and Aspergillus – 15.3 % (Fig. 8 *a*, *b*).



a ***b***

Fig. 8. Frequency of isolates isolated from the rhizosphere of soybean plants of varieties: *a* – Suzir'ja; *b* – Kent in the laboratory (2018–2020)

Biotic environmental factors significantly affect the number and dynamics of the population of phytopathogenic micromycetes in agrocenoses. One of the important factors is the selective pressure of cultivated plant varieties, which can affect the quantitative and qualitative indicators of the phytopathogenic background in agrocenoses.

Thus, it was established that in the rhizosphere of plants of soy varieties Suzir'ja and Kent the species of phytopathogenic micromycetes, belonging to the genera Alternaria, Fusarium, Penicillium, Aspergillus are dominated, which are factors of biological contamination of agrophytocenoses and decrease in product biosafety.

4. Conclusions

The possibility of regulating the number of phytopathogenic micromycetes in the mycobiome of soy plants by using biopreparation Philazonit has been experimentally proved, which will increase biosafety in soy agrocenoses and improve of the crop quality. According to statistical analysis, the biological product "Filazonit" has shown efficiency in research at all stages of plant ontogenesis.

It is proved that the representatives of genera: Alternaria, Fusarium, Penicillium, Aspergillus are dominated in the rhizosphere of plants of soy of both varieties. In the rhizospheres of plants of the soy Suzir'ja variety the representatives of genus Penicillium (32.8 %) most often occurred. In the rhizosphere of plants of the Kent variety dominated by representatives of the genera Alternaria and Fusarium (35.6 % and 34.1 % respectively). Biotic environmental factors significantly affect the number and dynamics of the population of phytopathogenic micromycetes in agrocenoses. One of the important factors is the selective pressure of cultivated plant varieties, which can affect the quantitative and qualitative indicators of the phytopathogenic background in agrocenoses.

Therefore, the control of microscopic fungal contamination of the main contaminants of biotic origin is relevant in the issue of feed safety. Systematic mycological studies of feed and feed materials for the presence of mold saprophytes will not only determine the taxonomic affiliation and identify toxin-forming species, but also contribute to the creation of a system of measures to prevent human poisoning and feed toxicosis in farm animals.

Conflicts of interest

The authors declare no conflicts of interest.

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Number 3

IMPROVEMENT OF THE TECHNOLOGICAL TREATMENT SCHEME OF IRON-CONTAINING WASTEWATER FROM ETCHING OPERATIONS

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Abstract

The aim of the research is to improve the technological scheme of treatment of iron-containing wastewater from etching operations by creating combined systems, including reagent wastewater treatment, their mutual neutralization, regeneration of etching solutions, deep post-treatment using a magnetic device. The main volume of wastewater is treated in centralized systems with partial return of water to the production process. Spent solutions from etching operations are subject to regeneration with return to the production process and partial dosing into the main wastewater stream from flushing operations. The consumption of commercial HCl is reduced by 50 %. The use of hydrogen peroxide can increase the effect of extracting iron from etching solutions by 30% (total purification effect of 70 %). Given that deep purification from iron-containing impurities is provided using a magnetic device, the possibilities of practical implementation of reverse osmosis to obtain «pure» water in centralized systems, which can be used for preparation of process solutions and in a mixture with technical water – for flushing operations, increase. In experimental and industrial conditions the expenses of reagents, their concentrations, dosing time are established.

Keywords: combined schemes, technological schemes, wastewater, etching operations, iron - containing impurities, magnetic device, regeneration of etching solutions.

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1. Introduction

Etching of steel surfaces involves treatment with acid solutions (HCl, H_2SO_4) at elevated temperatures, resulting in the formation of liquid iron-containing waste – wastewater (WW): spent etching solutions (SES), which are characterized as highly concentrated solutions, and washing water (WasW), which belong to the category of concentrated solutions [1].

The main problem of the etching area is the processing of SES, for this purpose local cycles of their disposal, utilization, regeneration are created [2]. The most progressive is the creation of combined systems, in which the bulk of wastewater is treated in centralized systems with partial

return of water to the production process. SES are subject to utilization or regeneration with partial return of chemical reagents to the production process and are partially dosed into the main WW stream from flushing operations. At such wastewater treatment there is a task of decrease in the general concentration of iron less than 1 mg / l. That is why in accordance with the requirements of environmental legislation on nature management there is a need for deep treatment of such wastewater.

WW treatment with an alkaline reagent, including lime milk, which allows to provide the final total concentration of iron in WW up to 3 mg /l is widespread in practice. To ensure a concentration of iron in purified water less than 1 mg/l requires additional (deep) water purification.

Since a significant part of iron-containing impurities has magnetically sensitive properties, it is possible to use the magnetic method in order to purify such WW [3]. For deep purification of water systems from iron-containing impurities, the introduction of magnetic devices is promising, in which the main element is a granular filter load, which is magnetized by an external device that generates a magnetic field. This direction has received intensive development, the thorough theoretical and experimental base of process of deposition is developed, a number of effective designs of magnetic filtration devices is created [4].

2. Analysis of literature data and formulation of the problem of magnetic purification of water systems

Innovative technologies and equipment for microarc treatment in rotating magnetic fields [5] for various technological processes: drinking water treatment, treatment of various types of wastewater, sludge elimination, oxidation of phenols, alcohols in WW, neutralization and utilization of water after washing tankers and tanks with oil products, etc are known. The versatility of the proposed technologies is determined by the complexity of the combined system, which provides the following effects: mechanical (shocks of working fluids, intensive dispersion of components and particles and their mixing); destructive, weakening intramolecular and interatomic bonds; hydrodynamic, expressed in significant shear stresses of the fluid of developed turbulence, pressure ripples and flow velocity; hydroacoustic due to small-scale pressure pulsations, intense cavitation of shock waves and secondary nonlinear acoustic effects; microarc and electromagnetic field of eddy current; thermal exposure; hydrolysis reactions. At that the magnetic devices are included in the main system of treatment plants, which also include an ozonation unit, a hydrocyclone, an electrolytic reactor, a settling tank with thin-layer blocks and the like. The application of this technological system is expected to improve the environment, expand opportunities for pollution disposal, for the production of organic fertilizers, building materials, etc. [5].

We believe that more promising is the use of magnetic and magneto-electric wastewater treatment for the removal (separation) of iron-containing impurities as an element of auxiliary systems that provide deep after-treatment of wastewater.

The main element of the magnetic device is a granular load. The load is magnetized by an external electromagnetic field generator.

Effective capture zones are formed between the granules due to high values of the generated magnetic field H, which is much higher than the magnetic field, created by the external magnetizing system, and mostly a high degree of its heterogeneity gradH. The product of these two parameters H·gradH, the so-called force factor, is essential just near the contact points of the granules. The disadvantages of the known devices are the limited possibilities of creating localized zones of high-gradient electric fields in the working zone of deposition of electrically charged impurities, which leads to low precipitating force on impurity inclusions [4].

The results of research on the technology of magnetic-electric wastewater treatment, conducted earlier, allowed to develop a device that creates a high-gradient magnetic field in the working zone of deposition in combination with a branched filtration surface. This increases the force on electrically charged impurities, especially with a low specific electric charge

and, as a consequence, provides an increase in the overall efficiency and degree of wastewater treatment [6].

In this technological solution we propose to implement deep purification of wastewater from the etching area from iron-containing impurities using a magnetic device, followed by desalination by reverse osmosis.

3. Research aim and tasks

The aim of the research is to improve the technological scheme of treatment of iron-containing wastewater from etching operations by creating combined systems, including a WW treatment reagent, their mutual neutralization, regeneration of etching solutions, deep after-treatment using a magnetic device.

To achieve this goal it was necessary to solve the following tasks:

– to study the technology of wastewater treatment using a magnetic device, in which the main element is a granular filter load, which is magnetized by an external device that generates a magnetic field;

– improvement of the technological scheme of wastewater treatment from surface preparation operations from iron-containing impurities in combined systems, which includes a magnetic device as an auxiliary element.

4. Research materials and methods

The research was carried out on the spent technological solutions and washing waters of etching of steels of the enterprise "Hardware Plant" LTD under laboratory and research-industrial conditions.

Acid-base and redox properties of WasW and conditions of their processing for purification, utilization, regeneration were studied by potentiometric titration and chemical precipitation in a batch reactor with intensive stirring of the reactants.

Quantitative analyzes for the content of iron ions were performed in the laboratory by photometric determination with sulfosalicylic acid. Potentiometric titration was performed on a potentiometer EV 74 (in the laboratory) and under industrial research conditions using a portable pH meter «pH 602». The magnetic purification of PV was studied in an experimental setup for magnetic deposition.

5. Results of research on wastewater treatment technology using a magnetic device

Improvement of the technological scheme of treatment of iron-containing wastewater from etching operations is achieved by providing deep purification with the use of a magnetic device. The proposed technological solution is the result of the conducted research that meets the conditions for the creation of new equipment (technology).

Studies have been conducted to study the effect of the length (height) of the nozzle layer L, the magnetic field strength H, the filtration rate , using model suspensions of magnetite on an experimental setup including granular filter load, which is magnetized by an external device.

The parameters under consideration can be divided as follows: state parameters particle size of impurities δ, magnetic susceptibility of impurity particles χ, dynamic viscosity of wastewater η; technological indicators – external magnetic field strength H, filtration rate , , degree of purification ψ; design parameters – packing density of the nozzle granules γ, nozzle granule diameter d, length (height) of the filter nozzle layer.

As a result of the conducted researches it has been established, that the greatest degree of purification of water environments ψ is observed in the following ranges of the basic technological parameters of magnetic deposition: to 5060 kA/m, , to 100150 m/h, to 0,60,8 m.

The disadvantage of the known magnetic treatment devices, containing granular loading, is the low force on the impurities that are separated (removed) from the wastewater. Researchers (Yatskov M. V., Mysina O. I.) patented the design of the magnetic device, which allows to separate magnetic and non-magnetic impurities of wastewater due to the fact that a high-gradient electric field in combination with a branched magnetic surface is generated in the working area

of the device. This increases the force on the impurities of wastewater to be separated (especially with a low specific electric charge), and as a result increases the degree of wastewater purification ψ [6].

At the same time, the magnetic device provides for the separation of wastewater impurities into ferromagnetic and non-ferromagnetic fractions by acting on the medium of the magnetized force, generated by the solenoid.

The ferrous wastewater predicted purification degree ψ depending on the intensity of the external magnetic field at a filtration speed of 100 m/h and L=0.8 m is 0.60.7. Assume that the particle size of the impurities is in the range from 2 to 4 μm.

As a result of the research in the article a technological solution was proposed, which consists in the fact that the magnetic device is used as an auxiliary element in technological systems of wastewater treatment, namely: wastewater after reagent treatment with coagulant, flocculant, alkaline reagent and after expanded styrofoam filter is fed to a magnetic filter with a granular load. The results of studies of wastewater treatment from the etching area (chloride solution) are given in Table 1.

Table 1

Parameters of wastewater treatment from the etching area (chloride solution)

Volume of 40 % NaOH, l/m^3	pH	Iron concentration after reagent treatment, g/l	Iron concentration after an magnetic device with =0.7, g/l
0	0.7	2,5	–
50	1	–	–
100	1.8	$0.32 \cdot 10^{-3}$	$0.096 \cdot 10^{-3}$
200	2	$0.24 \cdot 10^{-3}$	$0.072 \cdot 10^{-3}$
250	2.6	$0.12 \cdot 10^{-3}$	$0.036 \cdot 10^{-3}$
450	12	$0.12 \cdot 10^{-3}$	$0.036 \cdot 10^{-3}$

Thus, the use of an electromagnetic device (filter) will provide deep purification of wastewater from iron ions, which is very important for further desalination by reverse osmosis [7] and allows to solve the problem of returning washing water to production processes.

6. Improvement of the technological scheme of iron-containing wastewater treatment from etching operations

The basic concept of improving the technological scheme of wastewater treatment from surface preparation operations was formulated in accordance with the environmental requirements of chemical technologies the main principle of which is to reduce the consumption of chemical reagents at the inlet and outlet of the process, including water, which is provided by its deep purification with the return to production and disposal of waste [8].

The surface preparation area includes the following operations: degreasing, two-stage washing (hot and cold), post-degreasing, etching, cold washing after etching.

In order to reduce the consumption of chemical reagents and ensure effective subsequent disposal of toxic waste, we recommend the inclusion of additional process equipment for local cycles of purification of process solutions (Fig. 1).

Spent process solutions from etching operations are fed to local regeneration cycles with a volume of 50 % of the total. The rest of the spent process solution enters the next local cycle (which operates in batch mode). In the local cycle, they are mutually neutralized with spent process solutions from degreasing operations, followed by dosing into the total wastewater stream, which contains flushing wastewater from degreasing and etching operations. The total flow of wastewater together with technological solutions from the operations of etching and degreasing after their mutual neutralization in the local cycle enters the centralized treatment system, which operates semi-continuously..

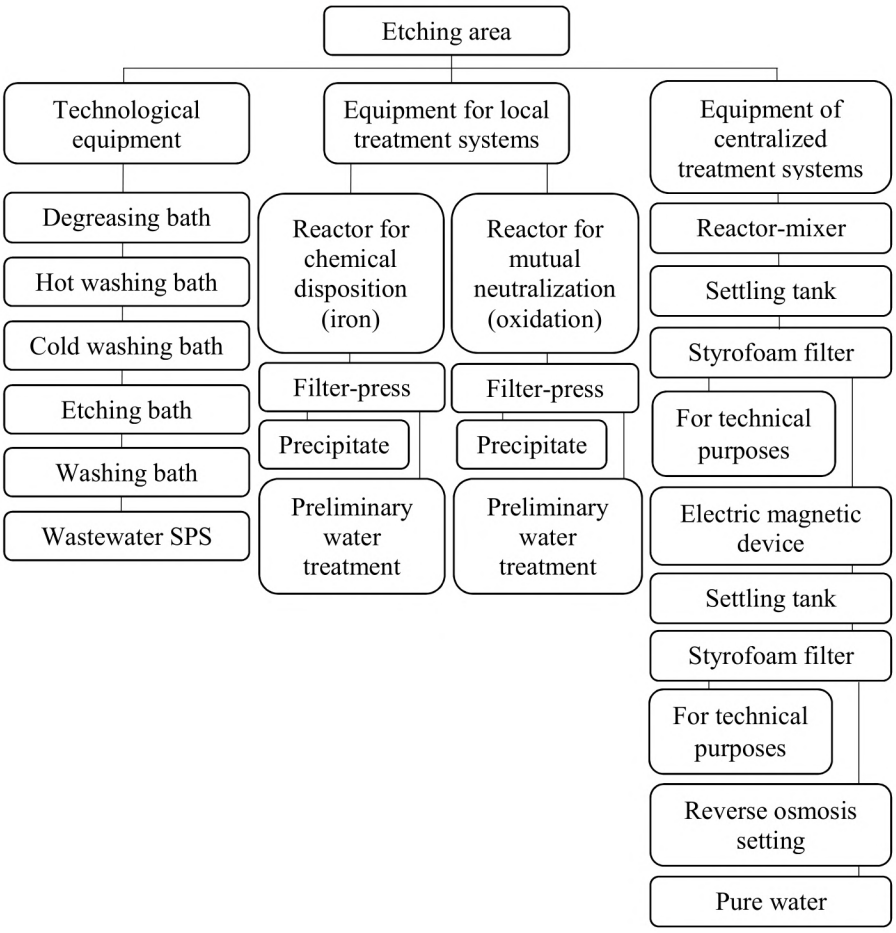

Fig. 1. Block diagram for improving the treatment of iron-containing wastewater from etching operations using a magnetic device

In order to ensure deep wastewater treatment, it is recommended to use additional equipment, namely a magnetic device that provides electromagnetic separation of wastewater impurities (Fig. 1).

Regeneration of solutions from etching operations is carried out by treatment with an alkaline reagent and hydrogen peroxide in an acidic oxide environment in order to precipitate iron ions in the form of hydroxy compounds, followed by separation on a vacuum filter with a chemically resistant fabric. Hydrogen peroxide promotes the formation of hydroxycomplexes of iron (III), which are able to form insoluble compounds in an acidic environment. As an alkaline reagent, it is recommended to use 1020 % NaOH solution. It should be noted, that the use of lime milk adversely affects the surface of the metal to be etched (see Fig. 2).

As can be seen from the presented data in Fig. 2, application of hydrogen peroxide allows to increase the effect of extracting iron from etching solutions by 30 % (total purification effect 70 %) under the same conditions at a consumption of an alkaline reagent as 1.5 mol NaOH per 1 mol Fe^{2+}. The clarified solution to restore the ability to digest as is enhanced with concentrated HCl in a ratio of 1:1. As a result of the proposed technological solutions, a reduction in the consumption of commercial HCl by 50 % is provided. The costs of reagents, their concentrations, dosing time, which ensures the extraction of the precipitate of hydroxy compounds of iron of stable composition for its further utilization (processing) are established in experimental and industrial conditions.

Thus, at an acid consumption of 1000 kg per day, there is a need to discharge the spent solution with a volume of 500 liters, followed by replenishment with new a commercial reagent. Thus, the cost savings of the commercial reagent per year is up to 500,000 conventional units.

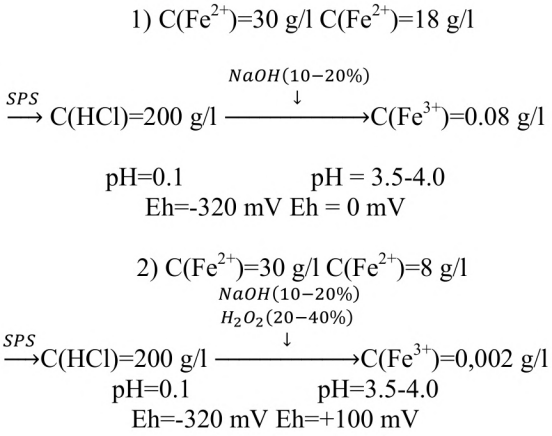

Fig. 2. Scheme of regeneration of spent process solutions (SPS) from etching operations

Approximately one third of the clarified solution (0.7 m of clarified filtrate is formed from 1 m^3 of spent etching solution, of which 0.5 m^3 is returned to the technological process, and 0.2 m^3 for mutual neutralization) is sent to the local cycle of mutual neutralization with the technological solution from degreasing operations. The presence of residual concentrations of iron ions and hydrogen peroxide in an alkaline environment provides the conditions for coagulation of surfactants and oils, entering the process solution during degreasing [9]. When using separate streams of spent technological solutions from etching and degreasing operations as chemical reagents, the saving of an alkaline reagent is 80 %, the use of an acidic commercial reagent is excluded. Thus, 1,2 kg/m^3 of a sodium hydroxide commercial reagent is consumed per 1 m^3 of SPS after their mixing, and without mutual mixing (neutralization) this consumption is 6 kg/m^3. Thus, the savings are about 17,000 conventional units (at the minimum price). The treatment effect increases from 80 to 99 % [9].

For subsequent treatment, wastewater, generated in local cycles, enters the construction of centralized treatment systems.

The main purpose of wastewater treatment (80 % of flushing operations and 20 % of local treatment cycles) is to remove iron ions in the form of insoluble hydroxy compounds, which is achieved by dosing an alkaline reagent (NaOH solution with a concentration of 1 %). At that in order to accelerate the formation phase (insoluble hydroxy compounds), flocculant dosing is recommended. The practical implementation of this process at one of the enterprises of Ukraine allowed to make recommendations for improving the treathemt technology, namely:

– dosing of an alkaline reagent and flocculant can be carried out in one apparatus, such as reactor-mixer - settling tank;

– the subsequent separation of the formed suspension is carried out on the expanded styrofoam filter, which provides additional purification from iron ions in the thickness of the filter load and effective separation of suspended solids;

– for deep purification from iron ions (see Table 2) we recommend using a magnetic device [5]. The use of this device increases the overall threatment efficiency by additional formation of the solid phase of salts, which determine the hardness in the volume of wastewater as it passes through a system of magnets;

– after separation of the additionally formed salt suspension (settling filtration), it is recommended to apply part of the flow to the reverse osmosis unit.

Thus, deep purification from iron-containing impurities with the use of a magnetic device is provided, the possibilities of practical implementation of reverse osmosis to obtain pure water, which can be used for preparation of technological solutions and in a mixture with technical water for washing operations, increase.

Table 2

Indicators of treatment of iron-containing wastewater from etching operations

Name of inicator	Measuring un.	Before treatment	After treatment	MPC Technical water, category II [١٧]
pH	un.	5÷6	7÷8	6÷9
Ferrum, Fe^{2+} ,	Mg/l	600	0,096	0.3
Chlorides, Cl^-	Mg/l	627	98	100.0
Sulphates, SO_4^{2-}	Mg/l	220	69.1	150.0
Magnesium, Mg^{2+}	Mg/l	26.75	1.8	3.0
Hardness	Mg-equ/l	10	0.8	2.0

7. Conclusions

To improve the technological scheme of wastewater treatment from etching operations from iron-containing impurities in combined systems, it is recommended to use a magnetic device. The main element of the magnetic device is a granular load. The load is magnetized by an external electromagnetic field generator. The use of a magnetic device will provide deep purification of wastewater from iron ions, which is very important for further desalination by reverse osmosis. This solves the problem of returning washing water to production processes, which is the most difficult for chloride etching solutions.

In order to reduce the consumption of chemical reagents and ensure effective subsequent disposal of toxic waste, we recommend the following:

to include additional technological equipment for local cycles of treatment of process solutions and wastewater from flushing operations. This will allow the regeneration of solutions from etching operations (total regeneration volume up to 50 %) and mutual neutralization of solutions from etching operations and degreasing (total volume up to 50 %).

Regeneration of solutions from etching operations is carried out by treatment with an alkaline reagent and hydrogen peroxide in an acid-oxide medium in order to precipitate iron ions in the form of hydroxy compounds, followed by separation on a vacuum filter with a chemically resistant fabric.

As a result of the proposed technical solutions, a reduction in the consumption of commercial HCl by 50 % is provided. In experimental and industrial conditions, the costs of reagents, their concentrations, dosing time, which ensures the extraction of the precipitate of hydroxo compounds of iron of stable composition for its subsequent utilization (processing), are established.

The use of hydrogen peroxide can increase the effect of extracting iron from etching solutions by 30 % (total purification effect 70 %) under the same conditions at a consumption of an alkaline reagent as 1,5 mol NaOH per 1 mol Fe^{2+}.

We recommend using a magnetic device for deep cleansing from iron ions. The use of this device increases the overall treatment efficiency, including from iron-containing impurities, which expands the implementation of reverse osmosis to obtain «pure» water.

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PROSPECTS OF USE OF VEGETABLE RAW MATERIALS IN THE TECHNOLOGY OF SOUR-MILK DESSERT

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Abstract

Prospects for the use of vegetable raw materials in the technology of sour-milk desserts have been substantiated. According to the results of organoleptic and physicochemical parameters, a rational ratio of recipe components in the product has been established.

The results of the organoleptic evaluation show that the introduction of sublimated fruits in an amount of less than 5 % does not give a pronounced taste and smell. And the introduction of dry whey protein concentrate in an amount of more than 6 % contributes to a heterogeneous consistency with the presence of lumps.

It has been investigated, that with increasing the amount of sublimated fruits in the sour-milk dessert, the active acidity slowly decreased in the range of 4.8…5.4 pH units. Due to which the process of fermentation of the mixture is slow. The moisture holding index increased with the addition of sublimated fruits by an average of 4 %. The presence of a dry whey protein concentrate gives the products a delicate plastic consistency due to the high dispersion of whey protein micelles and the peculiarities of gelation.

Therefore, it is recommended to add sublimated fruits to the sour-milk dessert not more than 7 %, dry whey protein concentrate not more than 6 %.

The nutritional value of the sour-milk dessert in accordance with the most important components according to the daily requirement has been studied. The consumption of sour-milk dessert will ensure the content of easily digestible biologically complete milk protein within 5–8 g/100 g. The combination of the sour-milk base with raw fruits will provide potassium content by 166–267 g/100 g, magnesium content by 16–24 g/100 g, calcium by 135–180 g/100 g, sodium by 53–70 g/100 g.

Keywords: dessert, apple, banana, water activity, dry whey protein concentrate, moisture holding capacity.

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1. Introduction

Herbal ingredients are used to expand the range of food and enrich the nutritional value of the product. Fruit and berry raw materials are the main source of biologically active

substances, such as vitamins, phenolic compounds, minerals, etc. Such substances have immunomodulatory, radioprotective, antioxidant properties. In addition, the plant raw material has technological properties, gives the color of the product, exhibits stabilizing properties and others [1, 2].

The most available raw material is apple fruit, which has a high content of low molecular weight phenolic compounds. Their content (by chlorogenic acid) averages 1720 mg/100 g. They are natural antioxidants and immunomodulators. In addition, apples contain a significant amount of biologically active substances, such as ascorbic acid (75 mg/100 g), β-carotene (0.11 mg/100 g), pectin, tannins and others [3, 4].

It has been found, that the addition of 1 % apple powder before yogurt fermentation promotes the aggregation of casein micelles. As a result, it causes the onset of gelation at higher pH (5.9). Also, there is an increase in the stability and cohesion of the gel during storage. As evidenced by the strengthening of the structure of intact casein gels. This effect can be explained by the gelation ability of pectins and other soluble fiber, secreted from apples into milk [5].

Another most popular fruit in the world is the banana. The energy value of 100 g of banana pulp is very high – from 80 to 240 kcal. Raw banana pulp contains 30 % of dry matter, 27 % of carbohydrates, including 15–25 % of sugars, 7–20 % of starch, 0.5 % of fiber and pectin, 0.3–0.6 % of essential oil. The pulp contains up to 1.3 % of proteins, which contain the essential amino acid tryptophan. Isovaleric, isoamyl esters and isoamyl acetate give a peculiar aroma to fruits. The vitamin complex consists of vitamin C – 37–53 mg/100 g, β-carotene – up to 30 mg/kg, vitamins B1 – 0.04–0.07 mg/100 g, B2 – 0.02 and B3 – 0, 2–0.3 mg/100 g, as well as vitamins B6, PP, E. The mineral composition of bananas is rich and varied. It is represented by mg/100 g of calcium (8–33), phosphorus (21–38), iron (0.4–1.4), sodium (1–5), magnesium (42), copper (0.16) , zinc (0.2), potassium (370–401) [6, 7].

Biologically active substances of vegetable raw materials, their functionality determine the urgency of creating new products.

A promising area is the development of resource-saving food technologies, namely the development of innovative products using raw materials of plant and animal origin.

2. Research aim and tasks

The aim of the study is to develop a sour-milk dessert. This will make it possible to improve typical products using raw materials of plant and animal origin.

This goal was solved by solving the following tasks:

– substantiate and establish a rational ratio of components in the composition of sour-milk dessert;

– calculate the nutritional value of fermented milk product.

3. Materials and Methods

Model samples were prepared as follows: dry whey protein concentrate and sublimated fruits were added in skimmed pasteurized milk at a temperature of 40–45 °C,. The gelatin was pre-soaked in cold water for at least 30 minutes, then the solution was heated to a temperature of 55–65 °C with stirring until completely dissolved.

The resulting milk mixture was heated to 80 °C with stirring, a solution of gelatin was added and heated to 90 °C for 50–60 s for pasteurization. The mixture was then cooled to a temperature of 55–60 °C, mixed thoroughly, cooled to a fermentation temperature of 38–42 °C, and a direct application yeast was added. It consists of microorganisms of bifidobacteria and lactobacilli that include *Streptococcus thermophilus*, *Lactobacillus delbrueckii ssp. Bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Bifidobacterium infantis.*

The mixture was stirred for 15–20 min and fermented for 8 h until a strong clot formed. Subsequently, the samples were cooled for 8–16 h to a temperature of 4±2 °C.

The organoleptic evaluation of the sour-milk dessert was performed by the method of describing open tastings, using a 10-point scale.

The nutritional value of the sour-milk dessert was determined by calculating the percentage of compliance of the integrated score of each of the most important components according to the daily requirement [8].

The energy value was calculated by the formula:

$$E=4\times P+9\times F+4\times C,$$

where P, F, C – mass fraction of proteins, fats and carbohydrates in the product, respectively (g/100 g); 4, 9, 4 – thermal energy, released during the burning of 1 g of proteins, fats, carbohydrates, respectively (kcal).

The determination of active acidity (pH) was carried out according to DSTU 8550: 2015. The determination of active acidity was carried out at a product temperature of 20±2 °C. In a clean, dry beaker, about 40 cm^3 of the fermented milk product is taken, electrodes are immersed in it and after 10…15 s the readings are taken on the scale of the device.

Moisture holding capacity (MHC) was determined by the gravimetric method of Grau-Hamm in the modification of A. A. Alekseev, which is based on determining the amount of moisture, released from the product by light pressing. To do this, a batch weighing 0.3 g, weighed to the nearest 0.001 g was placed on a soft waterproof plate with a diameter of 40 mm, covered with a slowly absorbing ashless filter with a diameter of 40 mm, then covered with a glass plate with a diameter of 100 mm and placed on it a weight of 500 g. After 7 minutes, the plate was removed and the plate with the batch was weighed. The MHC was determined by the formula:

$$MHC=(100\cdot(a-b))/a,$$

where MHC – moisture holding capacity, %; a – the amount of moisture in the batch, g; b – the amount of moisture, released from the batch of cheese, g.

$$a=0.3\ M_h/100,$$

where 0,3 – cheese batch, g; M_h – moisture mass fraction, % [9].

The study of water activity (Aw) (relative humidity, %) was performed on a water activity analyzer «HygroLab 2» (Rotronic, Switzerland) at a temperature of 20 °C in the measuring range 0…1 Aw (0…100 % rh).

HygroLab 2 (Rotronic, Switzerland) is a desktop laboratory humidity and temperature analyzer with a display and control keys, to which 1–4 water activity probes are connected simultaneously. The analyzed sample is taken in a container and placed in the measuring chamber. A water activity probe is installed on top. The measurement cycle lasts 3–5 minutes, after which the display shows the values of water activity and temperature for each probe.

The obtained measurement results and graphical presentation of experimental data were performed using standard statistical processing programs Microsoft Excel 2010. The accuracy of the obtained results was ensured by the three-fivefold repetition of experiments.

4. Results

The creation of functional dairy products with the introduction of secondary resources is relevant. Therefore, at the first stage of work model samples were prepared in order to determine the rational ratio of components in the composition of the sour-milk dessert.

The research results were systematized and shown in **Table 1**.

Model samples with different content of recipe components are shown in **Fig. 1**.

Table 1

Characteristics of organoleptic parameters of model samples with different content of component composition

Sample, No.	Mass fraction, %			Taste and smell	Color and consistence
	Dry whey protein concentrate	Sublimed fruits			
		apple	banana		
1	2	1	0.5	Pure, milky, moderately sweet, with an inexpressible taste and aroma of sublimated fruit	The consistency is homogeneous, tender, not glossy enough. The color is milky, uniform throughout the mass
2	4	3	1	Pure, milky, moderately sweet, with a pronounced taste and aroma of sublimated fruit	The consistency is homogeneous, tender, the surface is glossy, jelly-like. Milk color with a cream tint, uniform throughout the mass
3	6	5	2	Pure, milky, moderately sweet, with a pronounced taste and aroma of sublimated fruit	The consistency is homogeneous, tender, the surface is glossy, jelly-like. Milk color with a cream tint, uniform throughout the mass
4	8	7	3	Pure, sweet, with a pronounced taste and aroma of sublimated fruit	The consistency is heterogeneous, the presence of lumps, the surface is matte, jelly-like. The color is cream, uniform throughout the mass

a *b* *c*

Fig. 1. Model samples with different content of recipe components:
a – sample No. 1; *b* – samples No. 2, 3; *c* – sample No. 4

According to the results of the organoleptic evaluation of model samples, it is advisable to choose a sample No. 2 and No. 3. These results show that the introduction of sublimated fruit in the amount of 1.5 % does not give a pronounced taste and smell. And the introduction of a dry concentrate of whey protein in the amount of 8 % contributes to the heterogeneous consistency with the presence of lumps.

Due to the balanced ratio of basic food components: proteins, fats, carbohydrates, minerals, vitamins, fermented milk products have dietary properties. Therefore, determining the nutritional value of the sour-milk dessert is important and relevant.

The calculation of the chemical composition and nutritional value according to the content of essential nutrients [10] are shown in **Table 2**.

The consumption of the sour-milk dessert allows to ensure the content of easily digestible biologically complete milk protein in the range of 5–8 g/100 g. Sour-milk dessert contains such important mineral elements as calcium, phosphorus, magnesium, potassium, iron. Therefore, the combination of the sour-milk base with fruit raw materials allows to provide the content of potassium at 166–267 g/100 g, magnesium at 16–24 g/100 g, calcium at 135–180 g/100 g, sodium at 53–70 g/100 g.

This combination will also enrich the product with vitamins A – up to 0.03 mg/100 g, C – up to 2.5 mg/100 g, group B up to 1.6 mg/100 g.

Table 2

Chemical composition and nutritional value of the sour-milk dessert with different content of the component composition

Food substances	The content of nutrients in 100 g of the sour-milk dessert			
	Sample, No.			
	1	2	3	4
proteins	5.0	6.0	7.0	8.8
fats	–	–	–	–
carbohydrates	4.0	4.0	10.0	13.0
moisture	86.0	81.0	75.0	69.0
Mineral substances, mg				
sodium	53.0	56.0	65.0	70.0
potassium	166.0	185.0	235.0	267.0
calcium	135.0	150.0	165.0	180.0
magnesium	16.0	18.0	22.0	24.0
iron	0.6	0.6	0.7	0.8
Vitamins, **mg**				
C (ascorbic acid)	1.5	1.5	2.0	2.5
B^1(thiamine)	0.3	0.3	0.3	0.3
B^2 (riboflavin)	1.6	1.6	1.6	1.6
A (retinol)	0.03	0.03	0.03	0.03
Essential amino acids, **mg**				
valine	100.0	94.0	87.0	80.0
leucine	192.0	180.5	168.0	154.0
isoleucine	84.0	79.0	73.0	67.0
tryptophan	168.0	158.0	146.0	134.0
threonine	94.0	88.0	82.0	75.0
lysine	165.0	154.7	144.0	132.0
methionine	52.0	48.9	45.0	42.0
phenylalanine	181.0	170.0	157.0	144.0
Energetic value, kcal/100 g	36.0	40.0	68.0	87.2

The calculation of the energy value indicates that the caloric content of the sour-milk dessert is in the range of 36–87.2 kcal/100 g.

Thus, the consumption of the sour-milk dessert will halve the caloric content of foods.

The next step was to investigate the effect of recipe components of the sour-milk dessert on its physicochemical parameters.

There is studied the active acidity of model samples by different content of components (**Fig. 2**), the ratio of which is shown in **Table 1**.

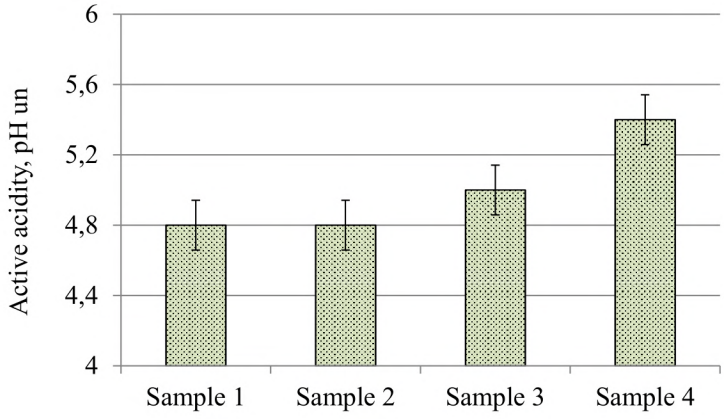

Fig. 2. Active acidity of model samples

It has been found, that the studied samples were characterized by values of active acidity in the range of 4.8…5.4 pH units. As the increased amount of sublimated fruits was added to the sour-milk dessert, the active acidity index slowly decreased and thus the fermentation process of the mixture was slow. Therefore, we can recommend the amount of sublimated fruit no more than 7 % of the total amount of milk mixture.

The main characteristic of fermented milk products is MHC and water activity index, so the effect of recipe components at different ratios on these parameters was determined in further studies (**Fig. 3, 4**).

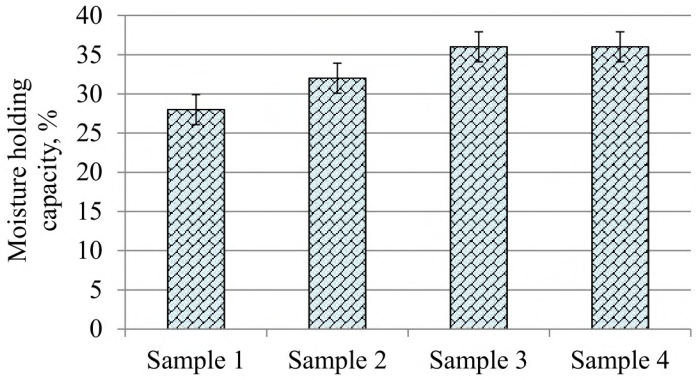

Fig. 3. Moisture holding capacity of model samples

In **Fig. 3**, we see that the rate of MHC increased with the addition of sublimated fruits by an average of 4 %. This is because the apple and banana contain soluble fiber, which allows you to retain free moisture. The presence of dry whey protein concentrate gives the products a delicate plastic consistency due to the high dispersion of whey protein micelles and gelation, and have the high biological value, so they can serve as an additional enriching component.

A similar relationship was observed for water activity.

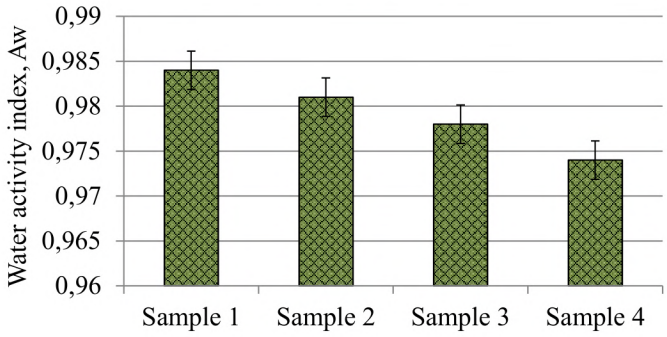

Fig. 4. Water activity index of model samples

If the increased amount of sublimated fruit was added to the sour-milk dessert, the water activity index decreased. Decreasing the activity of water increases the binding energy in the material and, as a rule, reduces the ability of microorganisms to use moisture for metabolism, reduces the rate of most chemical reactions, responsible for spoilage of fermented milk products.

5. Conclusions

According to the results of organoleptic and physicochemical studies, the rational ratio of components in the composition of the sour-milk dessert has been substantiated and established. It is recommended to add no more than 7 % of sublimated fruits, no more than 6 % of dry whey protein concentrate to the sour-milk dessert.

The nutritional value of the sour-milk dessert in accordance with the most important components according to the daily requirement has been studied. The consumption of the sour-milk dessert will halve the caloric content of foods.

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RESEARCH OF QUALITY AND SAFETY INDICATORS OF ORGANIC RAW MATERIALS FOR DEVELOPMENT OF NEW COOKIE RECIPES

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Abstract

Studies of quality and safety of organic flour and oils for the development of cookies are considered. The objects of the study are samples of organic spelt, corn and coconut flour; and samples of organic oils (camelina and hemp). The purpose of the study is to substantiate the feasibility of using organic raw materials for the development of new cookie recipes. To determine the quality and safety of organic flour, the nutritional value, physicochemical parameters and safety indicators (content of salts of heavy metals) have been analyzed. To determine the quality of organic oils, the fatty acid composition has been analyzed. Standard methods have been used for the study. The moisture content of the flour has been determined by the drying method. Ash content – by the method of organic matter charring. Atomic absorption and flameless atomic absorption methods have been used to determine the safety indicators of the flour. The fatty acid composition of oils has been determined by gas chromatography. It has been proved, that organic raw materials meet the safety requirements of the Regulation of maximum levels of certain contaminants in food, approved by the Order of the Ministry of Health of Ukraine № 368 of 13.05.2013 and can be used as a promising enrichment for the nutritional value of cookies.

Keywords: organic cookies, organic raw materials, coconut flour, spelt flour, camelina oil, hemp oil.

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1. Introduction

Excessive use of synthetic chemicals that pollute the environment, as well as mechanical disturbances of the soil and irrigation have led to negative consequences. These include the generation of resistant insects, fungi, weeds, the accumulation of chemicals in crops and soil, water and

air pollution. All this contributes to some extent to the greenhouse effect and global warming [1]. Just that is why in many countries, such as the United States, Germany, Italy, Greece, France, Poland and a number of others, programs and legislative initiatives have been adopted to stimulate organic production. The EU has developed and is implementing an action plan for the introduction and expansion of organic production, most EU member states have their own national programs for the development of this area of agricultural activity [2]. The main forms of support for organic agriculture in the EU are grants for rural development programs, legal protection of producers, national action plans. Stimulating the development of organic agriculture in the world has led to an increase in organic production [3]. Thus, according to official data, in 2018 there were 71.5 million hectares of organic land in the world. The largest number of them is recorded in Oceania (36 million hectares), the European Union (15 million hectares) and Latin America (8 million hectares) [4]. In addition, consumers themselves note that they are increasingly choosing organic products. The above data indicate the relevance of the study of organic raw materials as a promising component of finished food products. In particular, the issue of developing flour products based on organic raw materials is poorly studied.

Flour products are quite popular among the population and are an integral part of the diet. The works of authors [5, 6] are devoted to the improvement of recipes and the search for new types of cookies. There are data on organic cookies, which include:

– spelt flour;
– rice molasses;
– butter;
– banana powder;
– sodium bicarbonate baking powder;
– vitamin B1.

Recipes for cookies based on organic raw materials are also known:

– whole-wheat flour;
– unrefined cane sugar;
– non-hydrogenated vegetable oils;
– rice flour;
– eggs;
– skimmed milk powder [7].

However, the assortment of organic raw materials is expanding every year. This encourages the search for new recipes using organic raw materials. Based on this, the purpose of the study is to substantiate the feasibility of using organic raw materials for the production of new types of cookies.

2. Materials and Methods

The objects of the study are samples of organic spelt, corn and coconut flour (**Fig**. 1) produced by the company «Organic Original» TM «Ecocord» (Ukraine); and samples of organic oils (camelina and hemp). Detailed characteristics of the samples are described in source [8].

a *b* *c*

Fig. 1. Visual image of the objects of study: *a* - organic spelt flour; *b* – organic corn flour; *c* – organic coconut flour

The determination of the moisture and gluten content in the flour was performed according to standard methods. Studies of the baking properties of the flour were performed using a direct method, ie quality assessment by trial baking, which [9] provides a steamless method of preparing dough from flour, water, baker pressed yeast and salt.

The nutritional value was determined from the information on the package.

The moisture content of the flour was determined by drying 10 g of flour, ground in a mill, in an electric semi-automatic oven at 130 ° C for 40 minutes

The ash content was determined by the method of organic matter charring with low heating in a muffle furnace.

To study the content of toxic elements in new products, there were used conventional methods: copper, zinc, lead and cadmium were determined by the atomic absorption method, arsenic – by the colorimetric method, mercury - by the flameless atomic absorption method.

The fatty acid composition of oils was determined by gas chromatography on a gas chromatograph HP 6890 (USA) **Fig. 2.**

Fig. 2. Gas chromatograph HP 6890

3. Results

Coconut flour, corn flour and organic spelt flour were chosen to develop the recipes for the new cookies. There are data on the prospects of using coconut flour as a functional additive. Clinical studies have proven the usefulness of coconut flour to lower blood cholesterol [10]. Increased attention to semi-wild varieties of wheat, namely spelt (Triticum spelta) is due to a number of reasons, among which we can highlight its suitability for low-cost agriculture, as well as some food and technological properties.

The nutritional and energy value according to the information on the label is given in **Table 1**.

Table 1

The nutritional and energy value of the flour

Sample	Content, g/100 g			
	fats	proteins	carbohydrates	Energetic value (kJ)
Spelt organic flour	2.7	11.3	72.5	1429
Corn organic flour	1.5	7.2	70.9	1381
Coconut organic flour	26.6	14.7	17.0	1772

As can be seen from **Table 1**, coconut flour has a high fat content and low carbohydrate content. At the same time, coconut and spelt flour have a high protein content – 14.7 and 11.3 g/100 g, respectively. The introduction of these flour samples in the cookie recipe can significantly enrich its amino acid composition.

Table 2 shows the results of the study of flour quality indicators.

Table 2

The results of the study of flour quality indicators

Indicator, %	Spelt organic flour	Corn organic flour	Coconut organic flour
Moisture content	10.5	11.7	12.8
Ash content	1.3	1.2	0.75
Gluten content	32.0	29.0	0.00

These physico-chemical parameters are within normal limits. The highest moisture content is recorded in coconut flour, the lowest - in spelt flour. In terms of ash content, corn and spelt flour are almost identical. Coconut flour has the lowest gluten content.

As the object of research is organic raw materials, an important step is the study of safety indicators. In particular – the content of toxic elements, because just contaminated soils and agrochemicals can be a source of their accumulation in raw materials. **Table 3** shows the content of toxic elements in the flour.

Table 3

The content of toxic elements in the flour

Toxic element name	Permissible level, mg/kg, no more	Spelt organic flour	Corn organic flour	Coconut organic flour
Lead	0.5	0.24	0.31	0.30
Cadmium	0.1	0.04	0.05	0.06
Arsenic	0.3	0.1	0.1	0.26
Mercury	0.02	<0,001	<0,001	<0,001
Copper	10.00	9.1	9.3	9.4

As can be seen from table 3, the content of safety indicators of the flour meets the normative ones. In particular, the lead content in all samples is almost twice less than the norm. The mercury content is almost invisible in all flour samples. The content of arsenic is the lowest in corn and spelt flour – only 0.1 mg/kg, while the permissible level is 0.3 mg/kg.

Since an important component of cookies is the fat base, the fatty acid composition of oils was studied. The results are shown in **Table 4.**

Table 4

The fatty acid composition of organic oils

Fatty acid name	Camelina oil	Hemp oil
Palmitic$_{(C16:0)}$	5.78	9.9
Stearic$_{(C18:0)}$	2.45	6.15
Palmitoleic$_{(C16:1)}$	0.16	0.23
Oleic$_{(C18:1)}$	15.7	16.7
Linoleic$_{(C18:2)}$	49.2	61.2
Linolenic$_{(C18:3)}$	11.59	15.4

The results in **Table 4** indicate a balanced fatty acid composition of vegetable oils. Their introduction into the recipe of products can affect the biological value of the lipid basis of cookies, enriching it with essential fatty acids. However, the comparison of the obtained results with [10] does not give grounds to believe that organic oils are superior to traditional ones in fatty acid composition.

4. Conclusions

The proposed samples of organic corn, coconut and spelt flour are of high nutritional value. In particular, coconut and spelt flour are characterized by the high protein content – 14.7 and 11.3 g/100 g, respectively. That is why the samples should be considered promising in terms of enrichment of the protein composition of finished products. The moisture, ash and gluten content have been determined in the products. Coconut flour does not contain gluten. It can be used in recipes of gluten-free products. According to the content of toxic elements, all flour samples meet the standards. Samples of organic oils – camelina and hemp – have been also studied. Vegetable oils are a promising source of essential acids, which is why the fatty acid composition has been studied. It has been found, that the studied oils contain a significant amount of poly- and monounsaturated fatty acids, in particular palmitic, linoleic, linolenic. The use of oils in product recipes can have a positive effect on the fatty acid composition.

Prospects for further research are to develop new recipes for organic cookies and to study its nutritional value.

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RESEARCH OF THE CONTENT OF PHENOLIC COMPOUNDS, FLAVONOIDS AND IODINE IN CHOCOLATE USING NON-TRADITIONAL RAW MATERIALS

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Abstract

A study to determine the total content of phenolic compounds, flavonoids and mass fraction of iodine in chocolate using non-traditional raw materials has been conducted. The object of the study was chocolate products, containing stevioside, erythrol, germinated soy flour, enriched with iodine.

Carrying out this set of studies is important because it will determine the content of phenolic compounds, flavonoids and iodine in the developed chocolate. Based on the obtained patterns, it is possible to determine the percentage of satisfaction of the recommended daily requirement and to set the recommended amount of consumption of the developed product per day.

As a result of research, it has been found, that the total content of phenolic compounds in the control type of chocolate per 198 mg of GA/100 g of the initial raw material is less compared to the sample of chocolate, where non-traditional raw materials were used. The results of the study correlate with the results of determining the total content of flavonoids. The developed types of chocolate exceed control samples by 229 mg of C/100 g of the initial raw material. That satisfies 88.6% of the recommended daily amount. It has been found, that the developed type of chocolate is a carrier of iodine in the amount of 63 μg/100 g. In the control sample of chocolate there were found only traces of mass fraction of iodine. It has been determined, that the use of germinated soy flour, enriched with iodine in the amount of 10 %, due to the reduction of cocoa mass satisfies 42 % of the recommended daily amount of iodine for a woman aged 30… 40 years of average labor intensity.

The obtained results are useful and important because they will allow to develop recommendations for the consumption of chocolate using non-traditional raw materials. What will affect the solution of an important social problem - maintaining high efficiency, confrontation with environmental factors under the pandemic due to the influx of test substances.

Keywords: chocolate, stevioside, erythrol, germinated soy flour enriched with iodine.

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1. Introduction

According to the forecasts of the world's leading experts in the field of nutrition and medicine, the issue of developing technologies for special dietary products is quite acute and is relevant [1]. Modern research focuses on the production of specialized foods enriched with essential microelements [2, 3].

Chocolate is a popular confectionery product among children and adults [4]. Research work [5] proves the positive properties of chocolate as a product for specialized nutrition due to the content of phenolic compounds and flavonoids. Deterioration of the human body is associated with the deterioration of its own antioxidant defense system. Deficiency of phenolic compounds, as substances that exhibit antioxidant properties, negatively affects the health of modern people, living in the metropolis, and people who need a special diet.

Experimental studies [6] show that phenolic compounds and flavonoids are safe and necessary for people with diabetes mellitus type II and endocrine disorders of various genesis.

The question of studying the content of flavonoids and phenolic compounds in chocolate is little studied and relevant, therefore, the purpose of the experiment was to study the content of phenolic compounds, flavonoids and iodine in chocolate for a special dietary direction.

A recipe for chocolate with natural sugar substitutes: stevioside and erythrol in combination with germinated soy flour, enriched with iodine, has been developed at the Department of International E-Commerce and Hotel and Restaurant Business of Kharkiv National University named after V. N. Karazin. Chocolate, made with the above ingredients, was the subject of research.

2. Analysis of literary data and statement of the problem

There are publications [7] about harmful effects of chocolate on human health. Chocolate consumption is linked to diseases, such as diabetes and obesity. With this in mind, many nutritionists advise people with high blood sugar, or to lose weight, to exclude chocolate from their diet by replacing white sugar with natural or synthetic sugar substitutes.

There is a well-known method of chocolate production [8], using rebaudioside A as a sweetener. The difference between the proposed method of chocolate production from well-known ones is the increased content of whole milk powder (by 2 %) and the use of soy phosphatide concentrate. The disadvantage of the proposed technologies is that non-traditional food ingredients are considered in terms of only improving the structure-forming characteristics of chocolate products. Scientists have paid little attention to increasing consumer value, as required by specialized products and principles of nutritiology.

There is a well-known method of dietary milk chocolate production [9], using, in addition to the classic food ingredients, mare's milk, roasted grated walnut kernels, dried fruits. The invention allows to obtain chocolate, which is a source of essential amino acids. The presence of dried fruit allows you to get chocolate with high levels of phenolic compounds and flavonoids. However, the disadvantage of the proposed method of chocolate production is the partial content of white sugar and the lack of essential micronutrients, especially iodine. Almost 20 % of Europe's population is in deficit of it. The antioxidant potential of new products has not been studied. One of the methods to overcome the above difficulties may be the use of non-traditional recipe ingredients, namely: germinated soy flour, enriched with iodine, erythrol and stevioside.

Given that chocolate is a popular confectionery product among children and adults and can take a worthy place in the diet of modern human, it is necessary to conduct research, aimed at studying the content of mass fraction of iodine, phenolic compounds and flavonoids. Consumption of chocolate with the use of non-traditional raw materials will solve an important social problem: to maintain high efficiency in the pandemic, to resist diseases and environmental factors.

Purpose and objectives of the study. Study of the content of phenolic compounds, flavonoids, iodine in chocolate using non-traditional raw materials. To achieve this goal, the following tasks were set:

– to investigate the total content of phenolic compounds;
– to investigate the total content of flavonoids;
– to investigate the content of mass fraction of iodine.

3. Materials and methods of the study

The recipe of the developed chocolate for specialized nutrition is shown in Table 1.

Table 1

The recipe of the developed chocolate for specialized nutrition

Recipe ingredients	Mass, kg (%)
Grated cocoa	700
Flour of germinated soy, enriched with iodine	10
Cocoa butter	275
Stevioside	2.5
Erythrol	25
"Vanilla" flavor	10
Output, kg (%)	1000

A well-known recipe was used as a control one, the difference of which is the use of 710 kg (%) of grated cocoa and the exclusion of germinated soy flour, enriched with iodine, from the recipe.

Characteristics of the used non-traditional recipe ingredients of raw materials. Erythrol is a polyhydric sugar alcohol, made from corn. The sweetness coefficient is 0.7, the energy value is 0.02 kcal/gr. According to the FAO/WHO Committee, it is safe for human health. It is registered in the food industry as a food supplement, under the code E 968, [10]. Stevioside is a sugar substitute of natural origin. It is produced from the leaves of perennial herb «Stevia». Stevioside is registered in the food industry as a food supplement E 960, as a sweetener. Sweetness coefficient – 250. Energy value – 1 kcal/g [11]. Sprouted soy flour, enriched with iodine [12].

3. 1. Study of the total content of flavonoids

The flavonoid content was determined spectrophotometrically on a Lambda 35 UV/VIS spectrophotometer "(USA). The absorption spectrum was taken at a wavelength of 510 nm in a cuvette with a liquid layer thickness of 10 mm. Distilled water was placed in the reference cuvette. The calculation of flavonoids in mg of catechin (C)/100 g of product was performed on a calibration curve.

3. 2. Study of the total content of phenolic substances

The studies were performed by the colorimetric method to determine the total content of phenolic substances using Folin's reagent. 0.25 ml of a 50 % aqueous solution of Folin's reagent, 0.50 ml of saturated sodium carbonate solution and 4.00 ml of distilled water were added to sterile 0.25 ml tubes with 0.1 mg/cm^3 chocolate extract. 0.25 ml of distilled water was added to the control sample instead of chocolate extract. The mixture was kept for 25 min at 25 °C with constant stirring to complete the reaction. The samples were centrifuged for 10 minutes at a speed of 2,000 rpm The content of phenolic substances in a clear solution of chocolate extract was determined by the spectrophotometric method using a spectrophotometer "Lambda 35 UV/VIS" (USA). The absorption spectrum was taken at a wavelength of 725 nm in a cuvette with a liquid layer thickness of 10 mm.

3. 3. Study of the iodine mass fraction

The mass fraction of iodine was determined using a voltammetric analyzer «AVA-2» (Russia), which is equipped with an indicator electrode, auxiliary electrode, type comparison electrode. A portion of the sample was treated with a solution of potassium hydroxide, burned on an electric stove, then using a system for microwave ashing «PHOENIX» (Daewoo, China). The resulting ash was mixed in water, neutralized to pH 4… 6, centrifuged. The resulting mass was added to an electrochemical cup with a background solution and measured. According to the results, the mass fraction of iodine was calculated.

4. Research results and their discussion

The results of the study of the total content of phenolic compounds, flavonoids and the mass fraction of iodine in chocolate are shown in Table. 2.

Analyzing the experimental studies, it can be stated, that the total content of phenolic compounds in the control type of chocolate per 198 mg of GA/100 g of the product is less compared to the sample of chocolate, where non-traditional raw materials were used. It is known [14], that phenolic compounds in the body are not synthesized, but come with plant foods and are included in

the composition – adrenaline, thyroxine, serotonin. Scientists believe [15] that the total content of phenols is a determining indicator of the level of antioxidant activity of food systems. According to literature data [16], germinated soy flour has a high content of phenols, so it is possible to assume that due to its content the content of phenols in the test sample of chocolate increases.

Table 2

The total content of phenolic compounds, flavonoids and the mass fraction of iodine in chocolate

Studied parameter	Recommended daily amount	Control	Experiment
Phenolic compounds, mg of gallic acid/ 100 g of the initial raw material	≤1000	998±0.4	1196±0.4
Flavonoids, mg of catethin/100 g of the initial raw material	≤500	214±0.4	443±0.3
Mass fraction of iodine, mcg/100 g	150	traces	63±0.2

Note* *The recommended daily amount for a woman aged 30... 40 years of medium labor intensity, according to the source [13].*

The obtained results correlate with the results of determining the total content of flavonoids. The developed types of chocolate exceed control samples by 229 mg of C/100 g of the initial raw material.

That satisfies 88.6 % of the recommended daily amount. It is known, that flavonoids depending on the degree of oxidation of the propane fragment are divided into catechins, anthocyanins, chalcones, flavanones, flavones, flavonols. Which have a wide range of biological activity on the human body due to the fact that they participate in redox processes. Performing the antioxidant function, they show P-vitamin activity, choleretic, antispasmodic, diuretic, hypoglycemic, sedative, estrogenic action. Scientists who have studied berries and germinated grains have found that they contain a significant amount of catechins, anthocyanins and flavnones.

The developed type of chocolate is a carrier of 63 μg/100 g of iodine. It satisfies 42 % of the recommended daily amount, as required by the principles of nutritiology. Chocolate is enriched with iodine due to the fact that sprouted soy flour is a carrier of iodine. Analyzing the research results, the recommended amount of consumption of the developed chocolate, which is 100 g per day, was determined.

When consuming this amount, the human body will receive 20… 60 % of the daily requirement for the studied substances.

The prospect of further research is to study the antiradical activity of the developed chocolate with a content of 10 % germinated soy flour, enriched with iodine; because radicals are highly reactive substances that lead to cell mutations or death.

5. Conclusions

1. The total content of phenolic compounds has been studied. It has been found, that the control sample of chocolate is a carrier of 998 mg of GA/100 g, and the test sample exceeds this amount by 198 mg of GA/100 g of the initial raw material. This is 1196 mg of HA/100 g of the product.

It has been substantiated, that the use of germinated soy flour, enriched with iodine, in the amount of 10 % due to the reduction of cocoa mass increases the total content of phenolic compounds, which is an indicator of increased levels of antioxidant activity.

2. The total content of flavonoids has been studied. It has been found, that the control sample of chocolate is a carrier of 214 μg of C/100 g of the initial raw material, and the test sample exceeds this amount by 229 mg of C/100 g, and is 443 mg of HA/100 g of the initial raw material.

The use of germinated soy flour, enriched with iodine, in the amount of 10 % provides a supply of flavonoids to the human body by 45.8 % more than the control.

3. The content of mass fraction of iodine has been investigated. It has been found, that the developed type of chocolate is a carrier of iodine in the amount of 63 μg/100 g. Only traces of mass fraction of iodine were found in the control sample of chocolate. It has been determined, that the use of germinated soy flour, enriched with iodine, in the amount of 10 % due to the reduction of cocoa mass satisfies 42 % of the recommended daily amount for a woman aged 30… 40 years of average labor intensity.

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DEVELOPMENT OF A METHOD FOR PRODUCING NEW GENERATION OF PROTEIN SNACKS USING THE PROCESSES OF CRYO AND MECHANICAL DISTRUCTION

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Abstract

The aim of research is to develop a method for the production of a new generation of protein snacks using a protein vegetable and milk base and vegetable fortifiers from spicy and carotene-containing vegetables using cryo and mechanical destruction processes. The method is based on the use of a deep processing method as an innovation, which consists in a complex effect on vegetable and protein raw materials of cryo and mechanical destruction processes in modern quick-freezing and low-temperature crushing equipment. The proposed method allows to get new protein snacks with a high content of protein, BAR and phytocomponents in an easily digestible form.

A new generation of protein snacks has been developed to strengthen the immune system using cryogenic protein base and vegetable raw materials. Nano-additives from dried peas and specially processed soft brine cheese are used as a protein base. Pea nanoadditives obtained using non-enzymatic catalysis – mechanolysis, mechanical destruction contain 21.5 ... 23.0 % of complete protein, which includes 49 % of amino acids in a bound state and 51 % in a free state. During the special processing of soft brine cheese with the help of mechanical destruction, 50 ... 55 % of the bound amino acids of the protein are transformed into free α-amino acids in an easily digestible form.

The proposed method makes it possible to more fully reveal the biological potential of vegetable and protein raw materials, to transform protein, BAS and phytocomponents in a bound form into easily digestible nanocomponents into a free easily digestible form. In addition, the processed protein base and raw materials acquire new properties structure formation, gelation, coloring ability.

The obtained protein snacks are natural, differ from the traditional ones by their high content of complete protein and the absence of synthetic impurities (preservatives, thickeners, colorants, etc.). In addition, 100 g of new products can satisfy the daily requirement for biologically active substances (β-carotene, L-ascorbic acid, phenolic compounds).

Keywords: vegetable processing, protein snacks, vegetable fortifiers, health products, pea nano-additives.

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1. Introduction

The relevance of the studies presented in the work is associated with the need to solve the problem of reducing the immunity of the population [1–3]. It can be increased by including in the daily diet of combined dairy and vegetable products containing a significant amount of biologically active substances (BAS) and phytocomponents of plant raw materials [4–5], and also have a significant content of complete protein [6, 7]. Powdered milk and milk whey have traditionally been used as a dairy base in the manufacture of such products [8]. Recently, legume flour has been used as a milk base and a

source of protein [9, 10]. Powders, pastes, frozen additives, concentrates from various types of fruit and vegetable raw materials are used as enrichment of recipe components [11]. Among the combined dairy and vegetable products that are popular among the population, one can distinguish protein snacks – spreads, the protein base of which is made up of additives from legumes, as well as processed fermented milk cheeses [12]. The main disadvantages of such combined products are the low content of complete protein, biologically active substances [13], high fat content and the presence of harmful food additives in the composition (structure-formers, transgenic fats, flavors, dyes, etc.) [14, 15], which correspond in organoleptic attractiveness, and short shelf life of the product [11, 16, 17]. The low content of biologically active substances is associated with the use in the manufacture of enriching prescription components in the form of powders, pastes, frozen additives from fruit and vegetable raw materials of traditional technologies [18]. Losses of BAR when receiving additives from fruit and vegetable raw materials using traditional technologies range from 20 to 80 % [19].

A promising raw material for enriching herbal supplements is spicy, carotene-containing vegetables (sweet peppers, carrots, pumpkin, tomatoes, parsley root, garlic) and natural spices, the therapeutic and prophylactic properties of which are well known [13, 14].

One of the promising directions of processing raw fruits and vegetables is "shock" freezing with subsequent storage at a temperature of 18 °C [20, 21]. The analysis of literature data on the study of the effect of freezing temperature regimes on the quality of the product in terms of the content of biologically active substances, organoleptic, thermophysical indicators. It has been established that the disadvantage of the most common method of "shock" freezing by a flow of cold air is the loss of vitamins and other biologically active substances, as well as the loss of cell sap during thawing [22]. It has been shown that the loss of BAS and cell sap during thawing of the product depends on the freezing rate [23, 24]. The higher the speed, the less the violation of the cellular structure of fruit and vegetable raw materials and the less the loss of BAS [25] and cell juice [26] during defrosting [27, 28]. In leading countries, to obtain high-quality frozen products, "shock" freezing is used using cryogenic liquids (nitrogen, carbon dioxide). The analysis of the data showed that there is little information in the scientific literature about the effect of cryogenic "shock" freezing and fine grinding [29] of fruit and vegetable raw materials when receiving additives for biochemical, physicochemical, microbiological processes, cryo and mechanical destruction processes [30]. The exception is the results of fundamental and applied research obtained by the authors of this work for some types of fruit and vegetable raw materials [13].

The aim of research is to develop a method for obtaining a new generation of protein snacks to strengthen immunity using a specially processed protein base and vegetable fortifiers with a high content of amino acids, BAS and phytocomponents in an easily digestible nanoscale form. The new method is based on the use as an innovation of a complex effect on the protein and raw materials of cryoprocessing and the processes of cryo and mechanical destruction. They occur in modern quick-freezing and low-temperature crushing equipment (at a temperature of 35 °C), which makes it possible to obtain a new generation of protein snacks to increase immunity.

2. Materials and Methods

The study was carried out at the Kharkiv State University of Food Technology and Trade (KSUFTT, Ukraine) on the basis of the research laboratory "Innovative cryo and nanotechnologies of herbal supplements and health products" of the Department of Food Technologies of Products from Fruit, Vegetables and Milk and Innovations in Health Nutrition.

As research materials, when developing a new method for the production of protein snacks, the following were chosen: nano-additives from peas and specially processed soft brine cheese in an easily digestible form as a protein base; sweet pepper, parsley root, garlic, carrots, pumpkin, tomatoes and vegetable cryo-frozen fortifiers obtained from them; a new generation of natural protein snacks to strengthen the immune system "Bogatyr", "Carotone", "Svitliachok" (Fig. 1).

Cryogenic freezing was carried out using a cryogenic freezer using liquid and gaseous nitrogen as a refrigerant [9]. For fine grinding, let's use homogenizer-grinders made in France "Robot Couper" and an innovative food processor "ThermoMix" (France) [13]. Steam-thermal processing in the manufacture of protein snacks was carried out using modern Italian-made equipment a UNOX vapor convection oven of the XVC series [9].

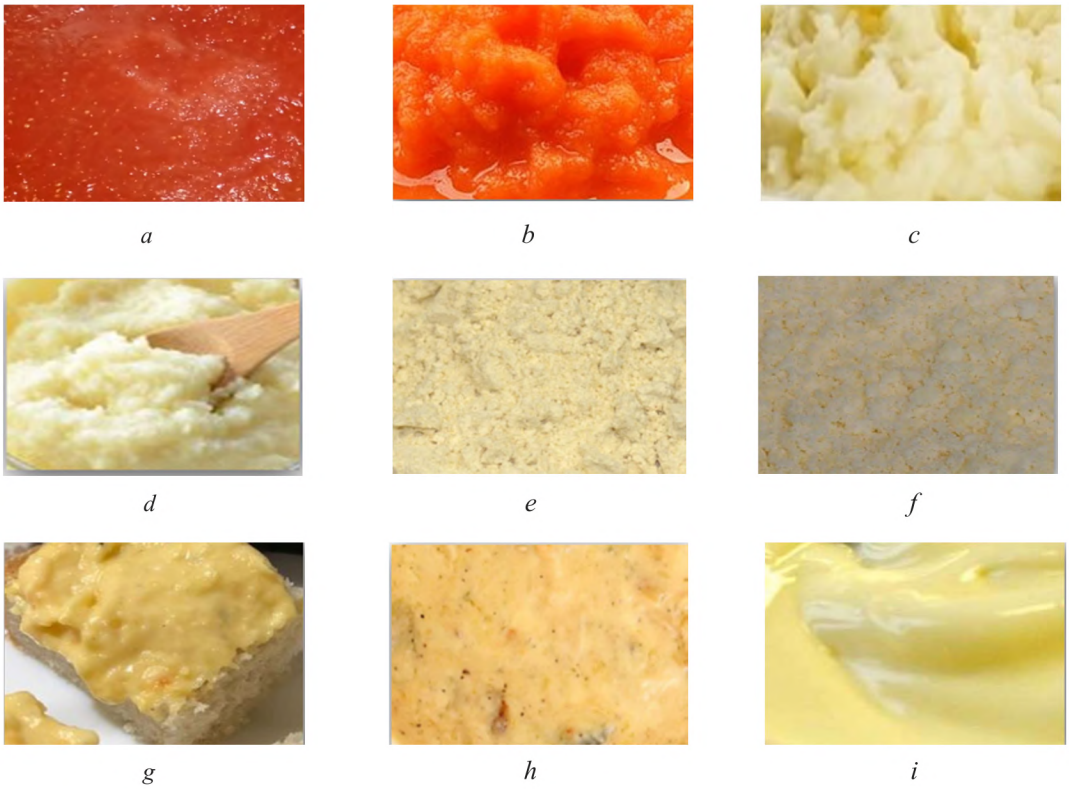

Fig. 1. Materials of research in the development of a method for obtaining a new generation of protein snacks using a protein base and vegetable fortifiers: a, b, c, d – cryo-enrichers from sweet pepper (a), carotene-containing vegetables "Multicarotin" (b), parsley root (c) , garlic (d); e – Nanoadditives from peas, there f – soft brine cheese after special processing, g, h, i – protein snacks "Bogatyr" (g), "Carotone" (c), "Sviliachok" (and)

A new generation of natural protein snacks has been developed to strengthen the immune system, it is recommended for implementation in large and small food industry enterprises, in restaurant business establishments.

3. Experiment procedures

In vegetable raw materials and additives from spicy and carotene-containing vegetables and natural spices, the quality was determined by the content of biologically active substances, in particular:

– L-ascorbic acid, by the method of visual and potentiometric titration with a solution of 2,6-dichlorophenolindophenate Na;

– β-carotene, by the colorimetric method of Moore after extraction of carotene from the product with an organic solvent and purification of carotene from accompanying colored substances using column chromatography;

– low molecular weight phenolic compounds (for rutin and chlorogenic acids separately), by the colorimetric method of Folin-Denis in terms of rutin and separately for chlorogenic acid;

– polyphenolic compounds, by a titrometric method based on the properties of polyphenolic compounds to oxidize in the presence of the indicator indigo carmine, the calculation of tannins was carried out in terms of tannin.

The processing of the results of experimental studies was carried out using methods of mathematical processing using computer programs MathCad and Microsoft Excel.

4. Results

A new method has been developed for obtaining a new generation of protein snacks to strengthen immunity using a protein base and cryo-frozen vegetable fortifiers from spicy and car-

otene-containing vegetables. The new method is based on the use as an innovation of the method of deep processing of raw materials, which consists in a complex effect on vegetable and protein raw materials of cryo and mechanical destruction processes in modern quick-freezing and low-temperature crushing equipment (at a temperature of –35 °C).

The proposed method allows to preserve, additionally remove and transform into a free easily digestible form of protein, BAS and phytocomponents. So, when receiving cryo-frozen vegetable fortifiers from spicy and carotene-containing vegetables, the mass fraction of BAR and phytocomponents increases 5.0 ... 5.7 times compared to the original fresh raw material. When a protein plant base is obtained from peas in the form of a nanopowder, the amino acids of the protein are transformed into a free, easily digestible form, the percentage of which in the powder is 51 %. When a protein milk base is obtained from soft brine cheese, 50 ... 55 % of the amino acids bound in the protein molecule are transformed into α-amino acids, which are in a free easily digestible form. In addition, it allows the protein base and vegetable enrichment to be provided with new functions of structure formation, gelling, coloring ability, and the like. This makes it possible to obtain new natural protein health-improving snacks with a high content of biologically active substances, phytocomponents, protein and without the use of artificial food additives. The proposed method makes it possible to more fully reveal and use the biological potential of these types of protein and vegetable raw materials, significantly increase the efficiency of the resulting product when consumed by the human body.

When receiving cryo-frozen vegetable fortifiers, it was found that the use of cryogenic "shock" freezing at a rate of 2 °C/min to a product temperature of 32 ... 35 °C leads to inactivation of oxidative enzymes. The mechanism of the process is associated with the denaturation and destruction of the protein part of the enzymes, as well as with the inactivation of the active centers of the enzymes.

In addition, the effect of cryo and mechanical destruction processes on the content of biologically active substances and phytocomponents in the production of cryo-frozen vegetable fortifiers from spicy and carotene-containing vegetables was studied in terms of the mass fraction of L-ascorbic acid, β-picture, phenolic compounds, flavonol glycosides, polyphenols (Table 1).

Table 1

Influence of cryo and mechanical destruction processes on the content of biologically active substances and phytocomponents of spicy and carotene-containing vegetables

Product	Mass fraction of BAS, mg per 100 g				
	L-ascorbic acid	β-carotene	phenolic compounds (by chlorogenic acid)	flavonol glycosides (by rutin)	polyphenolic tannins (by tannin)
Parsley root					
fresh	28,4…30,1	0,2…0,3	305,0…321,4	50,6…56,7	380,2…385,4
cryoprocessed	58,6…62,5	0,3…0,4	506,2…529,2	82,7…90,3	556,0…570,2
A mixture of carotene-containing vegetables (carrots, pumpkin, tomatoes) «Multicarotine»					
mix of fresh KBO*	18,2…20,4	8,2…10,3	170,1…182,3	48,2…51,4	240,3…250,2
cryoprocessed	40,1…45,6	30,5…32,4	295,3…304,2	108,2…125,4	390,1…425,3
Sweet pepper					
fresh	310,0…335,0	5,0…5,4	132,0…150,1	28,5…32,4	118,3…126,2
cryoprocessed	720,2…800,4	12,8…15,5	204,2…210,6	41,0…46,3	190,2…195,4

**CCW – carotene-containing vegetables*

It has been established that the use of cryogenic "shock" freezing of spicy and carotene vegetables to a temperature of 32 ... 35 °C and further fine grinding leads to additional removal of previously unknown forms of BAS from raw materials into a free form, the mass fraction of which is 5.0 ... 5 7 times more compared to fresh raw materials.

The mechanism of increasing the content of biologically active substances and phytocomponents is associated with the use of cryo and mechanical destruction processes, mechanocracking and transformation of biologically active substances in an easily digestible form.

The obtained vegetable fortifiers were used in the production of protein snacks not only as BAR fortifiers, but also as natural structurants, gelling agents, dyes and flavorings.

Nano-additives from dried peas and specially processed soft brine cheese were used as a protein base in the manufacture of a new generation of protein snacks to strengthen the immune system.

Additives from peas in the form of powder and puree obtained with the use of nanotechnology based on the use of non-enzymatic catalysis – mechanolysis, mechanical destruction, which makes it possible to obtain additives in an easily digestible nano-sized form. It was shown that the obtained nanoadditives from peas contain 21.5 ... 23.0 % of complete protein, of which 51 % of amino acids are in a free state and 49 % are in a bound state.

When using cheese, special processing was carried out using mechanical destruction. It is shown that in soft brine, raw protein for special processing is in a bound form. The use of mechanical destruction leads to the transformation of 50 ... 55 % of the amino acids bound in the protein molecule into α-amino acids, which are in a free easily digestible form.

A new generation of natural protein snacks has been developed that help to strengthen the immune system using vegetable fortifiers from spicy and carotene vegetables in combination with a protein base (from legumes and soft brine cheese) as recipe components. Natural spice additives in the form of powders and extracts were used as natural preservatives. New types of protein snacks differ from the traditional ones in their high content of biologically active substances, complete protein (more than 18 %) and the absence of synthetic impurities (preservatives, thickeners, dyes, etc.). In addition, 100 g of new products can satisfy the daily requirement for biologically active substances (β-carotene, L-ascorbic acid, phenolic compounds). New types of protein snacks are unique in chemical composition, natural products, superior in quality to analogues and are recommended for immunoprophylaxis of all segments of the population. In terms of BAS content, the new snacks meet the recommended FAO/WHO criteria for foods for a healthy diet. The obtained protein base and vegetable fortifiers are recommended for use in the manufacture of a wide range of products for a healthy diet.

Health-improving protein snacks have been tested in production conditions at the enterprises of Kharkiv, in particular in LLC" Lisova Kazka ", LLC" KHPK ", UE "Baby food combine", received a positive assessment and are waiting for their consumer and investor.

5. Conclusions

A method for obtaining a new generation of protein snacks for strengthening immunity using a protein vegetable and dairy base and vegetable cryo-frozen fortifiers from spicy and carotene vegetables has been proposed and developed. The new method is based on the use of the method of deep processing of raw materials as an innovation, which consists in the complex effect of cryo and mechanical destruction processes on vegetable and protein raw materials.

The proposed method allows to preserve, additionally remove and transform into a free easily digestible form of protein, BAR and phytocomponents. So, when receiving cryo-frozen vegetable fortifiers from spicy and carotene vegetables, the mass fraction of BAR and phytocomponents increases 5.0 ... 5.7 times compared to the original fresh raw material. When a protein plant base is obtained from peas in the form of a nanopowder, the amino acids of the protein are transformed into a free, easily digestible form, the percentage of which in the powder is 51 %. When a protein milk base is obtained from soft brine cheese, 50 ... 55 % of the amino acids bound in the protein molecule are transformed into α-amino acids, which are in a free easily digestible form. In addition, it allows the protein base and vegetable enrichment to be provided with new functions of structure formation, gelling, coloring ability, and the like. This makes it possible to obtain new natural protein health-improving snacks with a high content of biologically active substances, phytocomponents, protein and without the use of artificial food additives. The proposed method makes it possible to more fully reveal and use the biological potential of these types of protein and vegetable raw materials, significantly increase the efficiency of the resulting product when consumed by the human body.

When receiving cryo-frozen vegetable fortifiers, it was found that the use of cryogenic "shock" freezing using high speeds (including 2 °C/min) up to a temperature inside the product 32...35 °C leads

to complete inactivation of oxidative enzymes. The mechanism of the process is associated with denaturation and destruction of the protein part of enzymes and inactivation of active centers of enzymes.

It has been established that the use of cryogenic "shock" freezing of spicy and carotene vegetables to a temperature of 32 ... 35 °C and further fine grinding of cryo-frozen vegetables leads to additional withdrawal from raw materials of previously hidden unknown forms of BAS into a free form, the mass fraction of which is 5,0 ... 5.7 times more compared to fresh raw materials. The mechanism of the processes is associated with the use of cryoprocessing processes, cryomechanical destruction, mechanocracking and transformation in an easily digestible form.

A new generation of natural protein snacks has been developed to strengthen the immune system using vegetable fortifiers from spicy and carotene vegetables in combination with a protein base (from legumes, soft brine cheese). The obtained protein snacks differ from the traditional ones in their high content of biologically active substances, complete protein (more than 18 %), the absence of synthetic impurities (preservatives, thickeners, dyes, etc.) and an extended shelf life. Consumption of 100 g of new products can satisfy the daily requirement for biologically active substances (β-carotene, L-ascorbic acid, phenolic compounds). New types of protein snacks are unique in chemical composition, natural products, superior in quality to world analogues and are recommended for immunoprophylaxis of all segments of the population.

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RESEARCH OF CHEMICAL COMPOSITION OF VEGETABLE RAW MATERIALS FOR USE IN INNOVATIVE TECHNOLOGIES OF BEVERAGES FOR CHILD NUTRITION

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Abstract

The relevance of the study is due to the establishment of scientific data on the inferiority of child nutrition and the possibility of improving the diet through innovative technologies of beverages based on vegetable raw materials.

The data on the substantiation of the choice of vegetable raw materials for the production of child smoothies are given.

Their expediency, nutritional and energy value are characterized. Data on the chemical composition of the created beverages are given, which confirm the effectiveness of used raw materials.

Functional foods should meet 1050 % of daily needs. When creating functional compositions, a key place is occupied by a set of predicted functional and technological characteristics and properties, compatibility and specificity of interaction with other components of recipe mixtures and finished products. Just this complex determines the feasibility of using functional compositions for culinary products.

Functional products are created on the principle of food combinatorics by reasonable quantitative selection of basic raw materials, ingredients, food supplements, the combination of which provides the formation of the desired organoleptic and physicochemical properties, as well as a given level of nutritional, biological and energy value.

The aim of the study: to establish the chemical composition of vegetable raw materials and the development of innovative technologies for child food beverages based on functional compositions, recipe mixtures and finished products.

Keywords: vegetable raw materials, sweet beverages, child nutrition, smoothie, preschool children, protein-vegetable mixture

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1. Introduction

Experience in the development of technologies that ensure the rational use of raw materials has been accumulated in the scientific and practical activities of the production of beverages for child nutrition [1].

Drinks and desserts are gaining popularity among children. Smoothies are a cold dessert drink in the form of berries or fruits, crushed and mixed in a blender, (usually one type) with the addition of pieces of ice, juice or milk.

In recent decades, a fundamentally new direction of the drink has appeared drinksbreakfast (breakfast drinks or smoothies). Smoothies are a dense viscous product, which is both a drink and a quick breakfast with the addition of dairy and fruit parts, dietary fiber and vitamins, ie the

chemical composition of the product is very close to the requirements of nutrition for children. The market of «healthy» drinks in Ukraine and the world as a whole is constantly growing, its segment of special purpose drinks is not fully formed. Along with sports and tonic drinks, the functional beverage segment is still being formed in the world.

High-quality, natural raw materials are used to make smoothies. In Switzerland, for example, popular smoothies contain 20 % of skimmed sour milk and 52 % of fruit juice (TM CoopBettyBossi); in the Netherlands, smoothies based on low-fat yogurt, low sugar and 60 % of natural lime or peach juice (TM SisiFrutmania) are common, the UK prefers products with soy extract and high content of fruit pieces (TM TomSoya), with natural carrot juice (TM), Ireland produces drinks with more than 50 % of fruit pieces in combination with low-fat yogurt (TM Tropicana Smoothies).

Wide involvement of local and non-traditional raw materials will increase the quality and biological value of child food. This gives grounds to claim that drinks for child nutrition have potentially favorable prospects for development due to the introduction of innovative technologies based on functional compositions, despite the negative impact of the external environment. The relevance of the study is due to the establishment of scientific data on the inferiority of child nutrition and the possibility of improving the diet.

The main problem of creating drinks for child food with a given set of quality properties is a complex process of ensuring the most complete balance and structure of products. The right choice of a raw material base plays an important role in solving this problem.

2. Materials and methods

The object of research is the smoothie technology based on model functional compositions (MFC).

The subject of research: almonds (GOST 16831-71), walnut (GOST 16832-71), sesame (TC U 01.1-24435116-002-2010), protein-vegetable mixture «Milkosoy-1», smoothies: «Carotelka», «Nut «,»Fig milk «,»Beta-charge «.

Preparation of test samples based on model functional compositions (MFC) was carried out in accordance with the traditional technology, control samples according to the classical recipe [2].

3. Research results

The lack of scientific research to meet the demand for functional foods for preschool children determines the relevance of the search for new types of raw materials with a high biological value.

Important issues of food development for preschool children are revealed in scientific works of Peresichny M.I. (2016), Peresichna S. M. (2014), Pyatnytska N. O. (2012), Nyankovsky S. L. (2018), Korzun V. N. (2020), Podrushnyak A. E. (2020), Kaprelyants L. V. (2021).

Berries and fruits are rich in various acids (caffeic, citric, tartaric and malic) and tannins [3]. They are a valuable raw material for the content of flavonoids, which is important for the child organism. Flavonoids are one of the most diverse and common groups of phenolic compounds.

Today, more than 8,000 flavonoids are known. They are widespread in the plant world and are characterized by an exceptional diversity of species [4].

Flavonoids can also be called natural plant dyes. However, they are contained only in plants, but in no case in the animal organism. Most flavonoids are contained in bilberries, blueberries, sea buckthorn, apricot [5].

Consider the influence of vegetable raw materials on the basic functions of the preschool children's organism.

Pumpkin contains large amounts of vitamin K, which is present in almost none fruit and vegetable. Vitamin K deficiency leads to nosebleeds and bleeding gums [6].

Pumpkin juice also has the ability to remove radionuclides from the organism. This ability is achieved due to the presence of water-soluble dietary fiber, which exponentially enhances the work of the intestine, its motor function. Pumpkin juice is generally suitable for cleansing the organism. It cleanses blood vessels from excess cholesterol, normalizes the water-salt balance [7].

When designing model functional compositions of smothies, it is taken into account, that calcium reduces magnesium absorption, vitamin C restores oxidized vitamin E, and zinc reduces

calcium absorption, B vitamins are incompatible with vitamin PP, calcium and zinc reduce iron absorption, calcium and iron reduce zinc absorption in the intestine, zinc reduces the absorption of copper, copper increases the benefits of iron many times [8, 9]. The recommended ratio of calcium: phosphorus: magnesium for preschool children is 1:1:6.7.

Lack of nuts and seeds in the diet of preschool children has been recognized as one of the systematic errors in nutrition, according to a study, published in the journal The Lancet in 2019. Why should nuts be included in the daily diet of children of different ages?

Nuts and seeds are useful, first of all, by: high protein content (1020 % depending on the type of nuts or seeds), full protein composition, high content of unsaturated fatty acids, including long-chain polyunsaturated omega-3 and omega-6 fatty acids. Nuts contain tocopherols, B vitamins, fiber (3.510 % depending on the type of nuts or seeds), trace elements (iron, selenium, zinc, calcium, magnesium, potassium)

We'll conduct a comparative analysis of the chemical composition of cow milk and milk from nuts and seeds (Table 1).

Table 1

Analysis of the chemical composition of nuts, 100g

Element name		Cow milk	Almond	Difference, %	Walnut	Difference, %	Sesame	Difference, %
Proteins, g		2.8	18.6	664.2	16.2	578.5	19.4	692.8
Fats, g		3.2	57.7	1803.1	60.8	1900	48.7	1521.8
Saturated fatty acids, g		2	5	250	5.5	275	6.6	330
Polyunsaturated fatty acids		1.2	7.2	600	30.4	2533.3	44	3666.6
Monounsaturated fatty acids, g		18	31	172.2	18.2	101.1	48	266.6
Carbohydrates, g		4.7	13	276.5	11.1	236.1	26.04	554
Vitamins, mg	A. mcg	0.02	3	15000	8	40000		
	B1	0.04	0.3	625	0.4	975	1.3	3125
	E		24.6		2.6		2.3	
	B2	0.2	0.7	433.3	0.1	80	0.3	166,6
	B6	0.05	0.3	600	0.8	1600	0.4	720
	B5	0.4	0.04	10	0.8	200		
	B9. mcg	5	40	800	77	1540	97	1940
	Beta-carotene	1	0.02	2	0.05	5		
	C. mcg	1.3	1.5	115.3	5.8	446.1		
	PP	0.1	4	4000	4.8	4800	4	4000
	B4		52.1					
Mineral substancesr	Calcium	120	273	227.5	89	74.1	1474	1228.3
	Magnesium	14	234	1671.4	120	857.1	540	3857.1
	Sodium	50	10	20	7	14	39	78
	Potassium	146	748	512.3	474	324.6	497	340,4
	Phosphorus	90	473	525.5	332	368.8	720	800
	Chlorine	110	39	35.4	25	22.7		
	Manganese	0.006	1.92	32000	1.9	31666	2.46	41000
	Iodine, mcg	9	2	22.2	3.1	34.4		
	Iron	0.06	4.2	7000	2	3333.3	61	101667
	Sulfur	29	175	603.4	100	344.8		
	Selenium		2.5		4.9			
	Zinc	0.4	2.1	530	2.5	630	7.2	1790
	Copper, mcg	12	142	1183.3	527	4391.6	4.08	34
	Fluorine, mcg	20	91	455	685	3425		
	Silicon		50		60		199	
Energetic value		58	649	1118.9	630	1086.2	605	1043.1

The comparative analysis of the raw materials for the smoothie showed that protein in almonds and walnuts is 5-6 times more than in cow milk. Fat in almonds, walnuts is 1519 times more than in the control sample, in sesame – 15 times. Carbohydrates in almonds and walnuts are 2 times more, in sesame 5 times. Vitamin A in almonds is 15 times more, in walnuts 40 times. Vitamin B9 in nuts is 19 times more, and vitamin B1 in sesame 31 times. Vitamin C in almonds and walnuts is 24 times more. Calcium in almonds is 2 times more, in walnuts by 89 %, in sesame 12 times. Magnesium in almonds is 16 times more, in walnuts 8 times, in sesame 38. Phosphorus in almonds and walnuts is 35 times more, in sesame 8 times.

Thus, nut milk is not biologically inferior to cow milk and significantly exceeds it by the content of minerals and vitamins. Comparing the chemical composition of almonds and walnuts, it was found, that almonds contain more vitamins and trace elements than walnuts, but as far as almonds are more valuable raw material, walnuts are better to use for smoothies due to economic benefits.

Given the above, we consider it appropriate to use sesame seeds, walnuts and berries to develop innovative smoothie technologies that increase the resistance and adaptability of the child organism, due to the content of functional ingredients of natural origin.

Studies of the chemical composition of the dietary supplement «MILKOSOY-1» for the preparation of child milkshakes have shown that this product is non-toxic, low-energy, non-addictive, successfully used as a milk substitute for human intolerance to dairy products. Milkosoy 1 has been clinically tested and recommended for use as a substitute for dairy products [10].

Today there is a wide range of soy protein supplements, which differ in composition, properties, purpose and price, which creates a problem of choice in a variety of proposals. When choosing soy protein supplements for use in beverages for functional purposes, along with functional and technological properties, such as water and fat-binding capacity, emulsifying properties, etc., one focused on their high nutritional value and safety, affordability, ease of use, while giving preference to domestic products. The above requirements are met by soybean products ‘’Super’’, ‘’Milkosoy’’, ‘’Tonus’’ under the ECO trademark.

The Ministry of Health of Ukraine classified ECO products from awakened grain as special foods that have therapeutic and prophylactic properties and can be used in the following areas: for feeding children in organized educational and health children’s groups; for nutrition of patients in treatment and treatment-and-prophylactic institutions; at medical and preventive nutrition of persons, working in harmful conditions, including at NPPs, as well as living in areas, contaminated with radionuclides; in the therapeutic and prophylactic nutrition of persons with pathologies of the digestive organs and thyroid gland; to improve the quality of nutrition of the elderly, pregnant women and nursing mothers.

The composition of the protein components of «MILKOSOY-1» includes all essential amino acids, it has a balanced mineral composition, presented in **Table. 2.**

The studies on the content of macro- and microelements of the dietary supplement «MILKOSOY-1» are presented below (**Fig. 1).** The results of the study of the chemical composition are shown in **Table 3.**

After performing the experiment to determine the content of macro and microelements in the dietary supplement «MILKOSOY-1», it was found, that the content of minerals is almost the same as in milk.

The studies on the dependence of the dietary supplement «MILKOSOY-1» on the specified proportions and temperatures were also performed. The research results are given below in **Table. 4.**

After conducting the experiments to determine the dependence of the dietary supplement «MILKOSOY-1» on the specified proportions and temperatures, it was found, that at a ratio of 1:2 and at a temperature of 55 °C, this product dissolves best in boiled water, the consistency is homogeneous and almost without lumps and the presence of sediment.

Table 2

Nutritional and energy value of "MILKOSOY-1" (g/100 g)

Parameters, (g, mg)	Amount
I	II
Dry substances	95.0
Proteins, g	25.3
Including essential amino acids:	10.3
valine	1.46
isoleucine	1.43
leucine	2.26
lysine	1.85
methionine	0.41
threonine	1.36
tryptophan	0.38
phenylalanine	1.17
Fats, g	10.2
Including polyunsaturated fatty acids	5.7
Carbohydrates, g, including dietary fiber	51.2
	3.6
Mineral substances:	5.4
calcium, mg	770
phosphorus, mg	950
iron, mg	8.2
zinc, mg	4.8
magnesium, mg	190
iodine, mg	0.041
Energetic value, kcal	398

Table 3

Concentration of macro- and micronutrients in the sample (mcg/g)

Macro- and microelements	Concentration	Average statistical error
K	6542.9270 +_	198.74000
Ca	2537.2370 +_	86.09200
Cr	1.3497 +_	0.52273
Mn	2.7534 +_	0.72171
Fe	34.2822 +_	2.34470
Ni	1.9957 +_	0.45128
Zn	16.1357 +_	1.45820
Br	10.5392 +_	0.83494
Rb	5.3791 +_	0.50677
Sr	13.1857 +_	0.80528
S	3345.9660 +_	567.38000
Cu	2.2664 +_	0.54612
Zr	0.6223 +_	0.16502

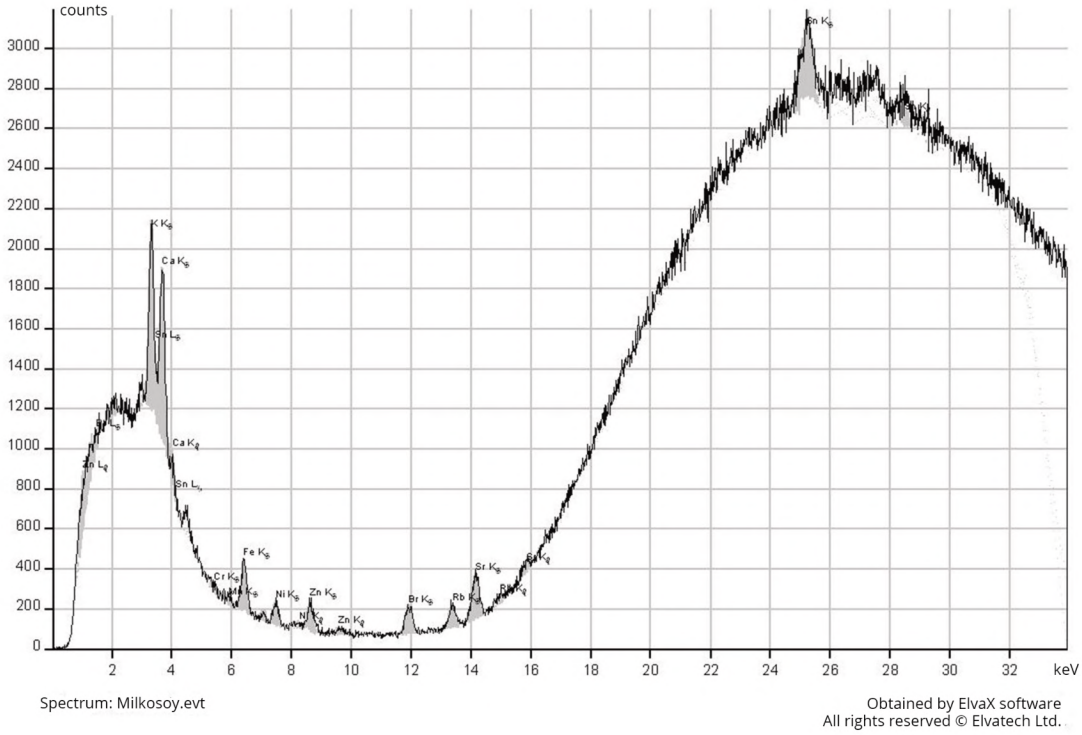

Fig. 1. Content of macro- and microelements of the dietary supplement «MILKOSOY-1»

Table 4

Determination of the dependence of the state of the dietary supplement "MILKOSOY-1" on the specified proportions and temperatures

	Proportions (dietary supplement : boiled water)		
temperature	1:1	1:2	1:3
40 °C	Heterogeneous, thick consistency, a large number of lumps after thorough mixing. The presence of sediment.	Heterogeneous, a bit thick consistency, a significant number of lumps. The presence of sediment.	Heterogeneous consistency, a large number of lumps after thorough mixing. The presence of sediment.
45 °C	Heterogeneous, thick consistency, a large number of lumps after thorough mixing. The presence of sediment.	Heterogeneous, a bit thick consistency, a significant number of lumps. The presence of sediment.	Heterogeneous consistency, a large number of lumps after thorough mixing. The presence of sediment.
50 °C	Heterogeneous, thick consistency, a large number of lumps after thorough mixing. The presence of sediment.	More homogeneous consistency, much less lumps. A small amount of sediment.	Heterogeneous consistency, a large number of lumps after thorough mixing. The presence of sediment.
55 °C	**Heterogeneous, thick consistency, a large number of lumps after thorough mixing. The presence of sediment.**	**Homogeneous consistency, all most no lumps. A small amount of sediment.**	**Heterogeneous consistency, a large number of lumps after thorough mixing. The presence of sediment.**
60 °C	Heterogeneous, thick consistency, a large number of lumps after thorough mixing. The presence of sediment.	More homogeneous consistency, less lumps. A small amount of sediment.	Heterogeneous consistency, a large number of lumps after thorough mixing. The presence of sediment.

Thus, given the high technological properties and positive effects of soy products on all types of metabolism protein, fat, carbohydrates, on the mechanisms of regulation of life support

of the mucous membranes of the gastrointestinal tract, ECO soy products can be considered an important component in functional food technology.

New technologies of preparation of smoothies for children of preschool age (46 years) on the basis of the dietary supplement "MILKOSOY" are developed. Below are technological schemes, technologies and functional compositions of beverages.

The technology of the «Beta-charge» smoothie includes bringing water to a boil, cooling it to a temperature of 55 °C, adding the dietary supplement «MILKOSOY», thorough mixing to a uniform consistency. Pumpkin is washed, cut, cleaned of seeds and pulp, cut into pieces, baked, ground in a blender to a puree.

Sea buckthorn is washed, crushed, seeds and skins are removed. The puree is brought to a boil and cooled. Berry and vegetable puree, protein-vegetable mixture is whipped to a homogeneous consistency with honey (**Fig. 2**).

The technology of the «Fig milk» smoothie includes bringing water to a boil, cooling it to a temperature of 55 °C, adding the dietary supplement «MILKOSOY - 1», thorough mixing to a homogeneous consistency. Figs are thoroughly washed, rubbed with sugar, poured with boiling water and infused for 4 hours, filtered. The finished mixtures are mixed to a homogeneous consistency (**Fig. 3**).

The "Carotelka" smoothie technology includes the preparation of a protein-vegetable mixture and carrot puree with cardamom and honey. The finished mixture is whipped to a homogeneous consistency (**Fig.4).**

To prepare the «Nut» smoothie, dried apricots are thoroughly washed and chopped. Walnut kernels are cleaned and passed through a meat grinder. Pour dried apricots with nuts with boiling water and leave to infuse for 1015 minutes, then strain. The finished mixture is whipped to a homogeneous consistency (**Fig.5**).

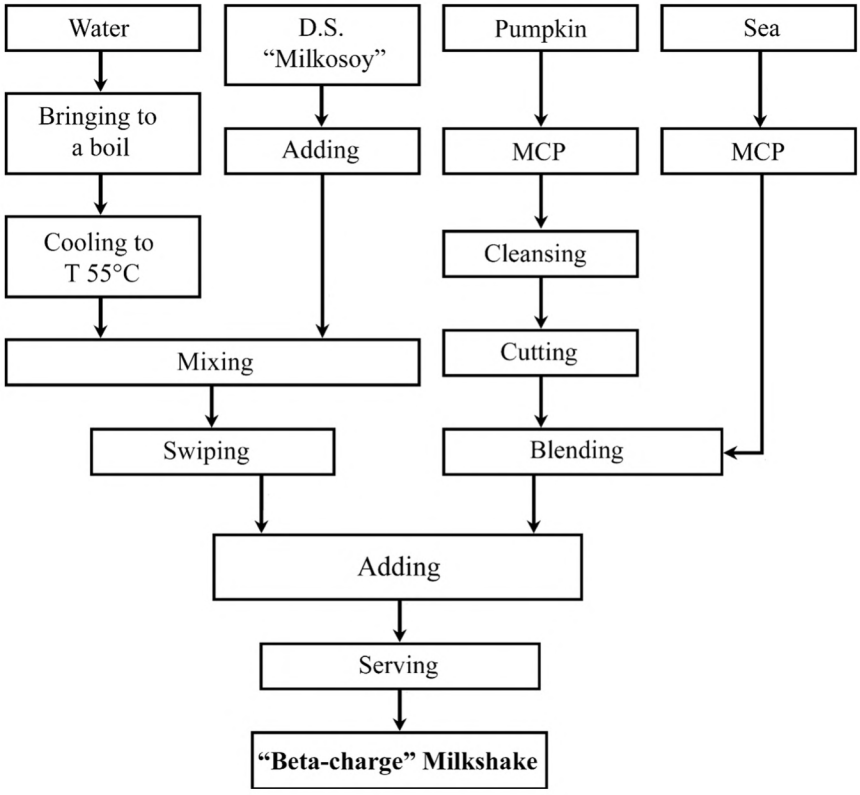

Fig. 2. Technological scheme of "Beta-charge" milkshake

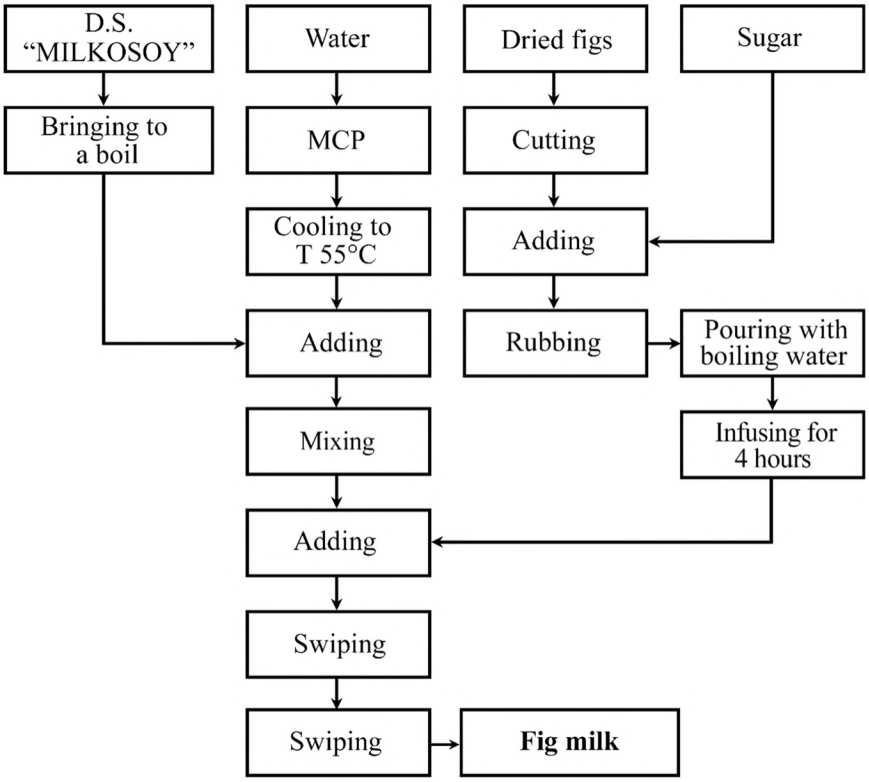

Fig. 3 Technological scheme of "FigMilk" milkshake

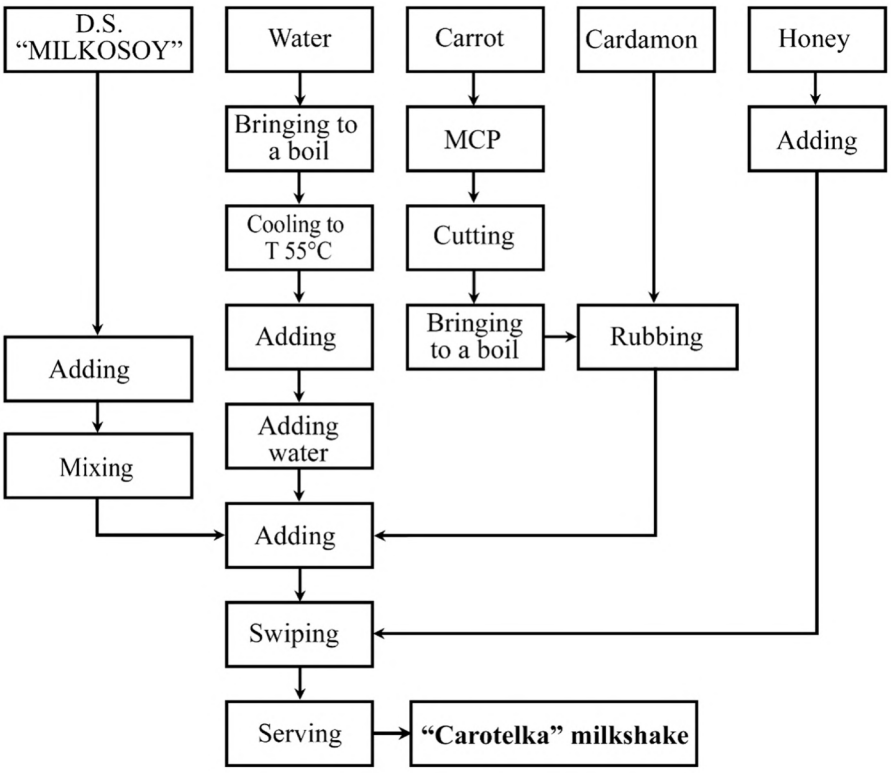

Fig. 4. Technological scheme of "Carotelka" milkshake

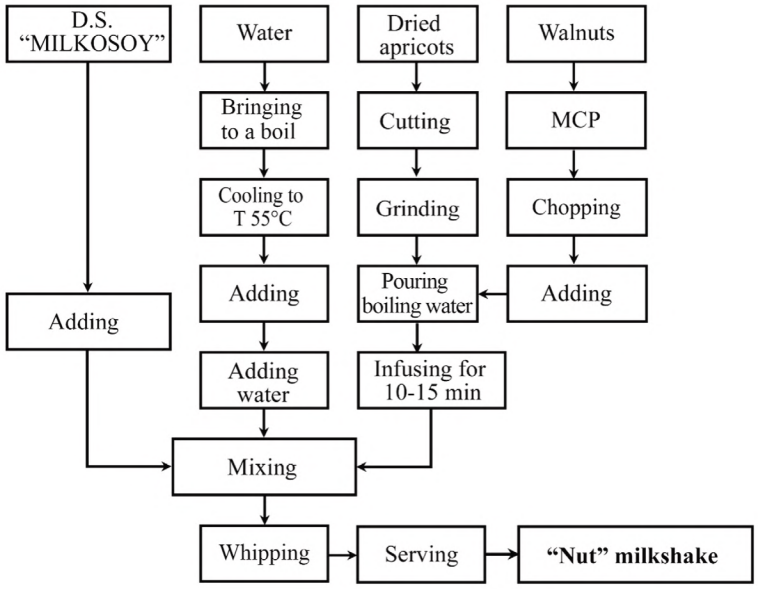

Fig. 5. Technological scheme of "Nut"milkshake

The developed technologies of the smoothies reflect the interaction between vitamins and trace elements (**Table 5).**

Table 5

Design of beverage technologies for child nutrition

Names	Synergism of nutrients	Daily need in nutrients	1050 %	Composition of ingredients for 200 g of the output (1portion)	Content of microelements
1	2	3	4	5	6
(control)	–	A-500 mcg Potassium – 150 mg C-55 mg Ca -1000 mg	50…250 15…75 5.5…27.5 100…500	Sea buckthorn 85 g water 100 g lemon 5 g cinnamon 5 g mint 5 г	A – 0.18 mg Potassium – 6.9 mg C – 0.19 mg Ca – 12.15 mg
"Beta-charge"	$Ca_{+}Mg$ +C+F= assimilation of microelements for the development of teeth and bones	Ca -1000 mg Mg-15 mg C-55 mg F-1 mg	100…500 1,5…7,5 5.5…27.5 0.1…0.5	"Milkosoy-1"100 ml or nut milk pumpkin 50 g sea buckthorn 50 g flower honey 5 g	Ca – 1474 mg Mg – 21mg Potassium – 6.9 C- 30mg F-0.15 mcg
"Fig milk"	C+Zn+A= assimilation of microelements for the development of vision	E-10 mg Potassium – 150 mg B^٠ - ٩ mcg C-55 mg Zn-6 mg	1…5 15…75 8…40 5,5…27.5 0.6…3	"Milkosoy – 1" 100 ml Dried figs – 35 g Flower honey 5 g	E- 22.14mg Potassium – 121.8 mg B9 – 7 mg C –35 mg Zn – 4.7 mcg
"Carotel-ka"	B+٤B+٩I+A= assimilation of microelements for the development of digestive organs, metabolism	B4-100 mg B9-80 mcg I-0.09 mg A-500 mcg	10…50 8…40 0,009… 0,045 50…250	"Milkosoy – 1" 100 ml Carrot – 80 g Cardamon – 20 g	B4 – 5.8mg B9 –7.6mcg A – 6.6mcg
"Nut"	B+٦B+٩I = assimilation of microelements for the development of digestive organs, metabolism	B6 -100 mg B9-80 mcg I-0.09 mg	–	"Milkosoy -1" 100 ml Walnuts – 80 g Dried apricots – 20 g	B6 – 5.8mg B9 – 7.6mcg I- 3.1mg

Thus, the chemical composition of the vegetable raw materials was studied and innovative technologies of the child nutrition smoothies were developed.

4. Conxlusions

The studies of the chemical composition of the protein-vegetable mixture «MILKOSOY-1» have found that this product is non-toxic, low-energy, non-addictive, successfully used as a substitute for milk in case of human intolerance to dairy products. The protein components of «MILKOSOY-1» include all essential amino acids, it has the balanced mineral composition.

The ratio of the protein-vegetable mixture to water (1:2) and the temperature of the best dissolution in boiled water 55 ºC, which provides a uniform consistency, have been established.

New technologies of preparation of drinks for child nutrition on the basis of the dietary supplement «MILKOSOY-1» have been developed. Technological schemes of beverage production are given.

According to the results of the research, the improved technology of beverage production based on functional compositions has been proposed.

The rational number of constituent compositions has been proved, as well as their influence on functional-technological properties, technological parameters and modes of advanced production.

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