

Ministry of Education and Science of Ukraine
Black Sea Universities Network

ODESA NATIONAL UNIVERSITY OF TECHNOLOGY

International Competition of
Student Scientific Works

BLACK SEA SCIENCE 2022 PROCEEDINGS



ODESA, ONUT 2022

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BLACK SEA SCIENCE 2022

Proceedings

Odesa, ONUT 2022

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INTRODUCTION

International Competition of Student Scientific Works “Black Sea Science” has been held annually since 2018 at the initiative of Odesa National University of Technology (formerly Odesa National Academy of Food Technologies) with the support of the Ministry of Education and Science of Ukraine. It has been supported by Black Sea Universities Network (the Association of 110 higher education institutions from 12 countries of the Black Sea Region) since 2019, and by Iseki-FOOD Association (European Integrating Food Science and Engineering Knowledge into the Food Chain Association) since 2020.

The goal of the competition is to expand international relations and attract students to research activities. It is held in the following fields:

- Food science and technologies
- Economics and administration
- Information technologies, automation and robotics
- Power engineering and energy efficiency
- Ecology and environmental protection

The jury includes both Ukrainian and foreign scientists. In the 4 years that the competition has been held, the jury included scientists from universities of 24 countries: Angola, Azerbaijan, Benin, Bulgaria, China, Czech Republic, France, Georgia, Germany, Greece, Israel, Italy, Kazakhstan, Latvia, Lithuania, Moldova, Pakistan, Poland, Romania, Serbia, Slovakia, Switzerland, Turkey, USA.

At the same time, every year the geography has expanded and the number of foreign jury members has increased: from 46 jury members representing 25 universities from 12 countries in 2018, to 73 jury members of the 46 universities from 19 countries in 2022.

More than a thousand student research papers have been submitted to the competition from both Ukrainian and foreign institutions from 25 countries: China, Poland, Mexico, USA, France, Greece, Germany, Canada, Costa Rica, Brazil, India, Pakistan, Israel, Macedonia, Lithuania, Latvia, Slovakia, Romania, Kyrgyzstan, Kazakhstan, Bulgaria, Moldova, Georgia, Turkey, Serbia.

The interest of foreign students in the competition grew every year. In 2018, the students representing 15 institutions from 7 countries have submitted 33 works. In 2021 the number of submitted works increased to 73, authored by the students of 40 institutions from 18 countries.

The competition is held in two stages. In the first stage, student research papers are reviewed by members of the jury who are experts in the relevant fields. In the second stage of the competition, the winners of the first stage have the opportunity to present their work to a wide audience in person or online.

All participants of the competition and their scientific supervisors are awarded appropriate certificates, and the scientific works of the winners are included in the electronic proceedings of the competition. Every year the competition receives a large number of positive responses from Ukrainian and foreign colleagues with the desire to participate in the coming years.

1. FOOD SCIENCE AND TECHNOLOGIES

FUNCTIONAL FOOD INGREDIENTS BASED ON ORGANIC COMPLEXES OF BIOMETALS WITH COMPOUNDS OF POSTBIOTIC ORIGIN**Author:** Alexander Sirotyuk**Advisor:** Antonina Kapustian

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Abstract. *Functional food ingredients based on complexes of biometals, products of postbiotic origin and dietary fiber of wheat bran were obtained in the work. Products of postbiotic origin were obtained by ultrasonic treatment and enzymatic conversion of peptidoglycans of *Lactobacillus delbrueckii* subsp. *Bulgaricus* B-3964. As a result of these transformations, a mixture of amino acids, low molecular weight peptides and mucopeptides was obtained, the concentration of which is 10.24 mg/cm³, 6.45 mg/cm³ and 2.25 mg/cm³, respectively. This hydrolyzate was used as a mixed ligand system to obtain complexes of $+Fe^{3+}$, Ca^{2+} and Mg^{2+} . The progress of complexation was monitored using nephelometry. It has been established that the studied system of bioligands binds Fe^{3+} ions in quantity $32 \text{ mol/dm}^3 \cdot 10^{-2}$, $Ca^{2+} - 28 \text{ mol/dm}^3 \cdot 10^{-2}$ and $Mg^{2+} - 24 \text{ mol/dm}^3 \cdot 10^{-2}$. The behavior of complexes at different pH and temperature values was studied. They are found to be stable in the pH range inherent in most food and digestive systems. The differential scanning calorimetry method shows that the obtained complexes are stable in the temperature range of 44–180 °C, which allows to recommend them as functional food ingredients for food products whose technology requires high-temperature processing. The feasibility of the obtained biometals complexes immobilization at dietary fibers is substantiated. It is proved that immobilization occurs only by physical sorption, which contributes to the complete release of the active components in environments that mimic the pH of the small intestine, where the absorption of biometals is been. The presence in the investigated ingredients composition of the biometals in organic form, low molecular weight mucopeptides with immunotropic activity, dietary fibers, allows to classify these preparations in the category of polyfunctional.*

Key words: *functional food ingredients, biometals, postbiotics, chelate complexes, mucopeptides, dietary fiber.*

I. INTRODUCTION

Recently, theories of nutrition, which would satisfy all aspects of physiological activity and the need for alimentary nutrients, are being transformed and improved in parallel with changes in scientific and technological progress. The optimal theory of nutrition is replaced by the theory of functional nutrition [1]. Functional nutrition allows to individualize the needs of each person, prevent the lack of essential components of food, which may occur due to certain dietary restrictions associated with diseases of various etiologies, allergic conditions, intense lifestyle that prevents regular and complete nutrition.

Today the problem of hypoelementosis is quite acute [2-3]. Lack of essential biometals, in particular, calcium, magnesium, iron, etc., can lead to irreparable consequences: metabolic disorders, iron deficiency anemia, disorders of the

cardiovascular, nervous system, immunity, etc. [2-5]. The use of functional nutrition to overcome this problem by introducing easily digestible and safe forms of biometals into the diet is very important.

II. LITERATURE ANALYSIS

There are three main approaches [2] to solving the problem of deficiency of macro- and micronutrients: the first – the consumption of products high in minerals; the second – the use of drugs and dietary supplements; third – purposeful enrichment with deficient elements of food for mass consumption. The most optimal for the prevention of food-dependent hypoelementosis is the enrichment of essential bioelements of food.

USA, England, Sweden, Holland, etc. adopted national programs for the enrichment of food with macro- and micronutrients. The experience of these countries shows that the use of inorganic compounds for these purposes does not provide the required level of their assimilation, can lead to physiological side effects, adversely affect the sensory characteristics of the product [2-4]. Therefore, it is important to develop safe and effective food ingredients based on biogenic metal compounds. It is known that organic and chelated compounds of biometals are more effective and safe compared to their inorganic forms [2-5].

Of particular interest for the prevention of trace elementosis are chelated compounds of biogenic elements with organic ligands, because in the metabolism of all minerals involved proteins, peptides, amino acids, phospholipids, carbohydrates, carboxylic acids, etc. [2,6]. Despite some advances in the development of chelated forms of biometals, the search for new, more advanced forms of functional food ingredients for the prevention of microelementosis continues, [7-11].

The use of postbiotics – products of metabolism and processing of probiotic bacteria to obtain mixed ligand chelate complexes of biometals is promising [12,13]. These systems contain a number of compounds that can be attributed to potential organic ligands for complexation with biometals, namely: organic acids, amino acids, low molecular weight peptides, muropeptides. In addition, the use of compounds of the muropeptide series for complexation with biometals will create multifunctional tools, because muropeptides have powerful immunotropic properties [14,15]. The use of postbiotics in functional nutrition is widely discussed today [16,17].

The purpose of the work is to obtain and characterize multifunctional food ingredients based on complexes of essential biometals, namely, calcium, magnesium and iron with low molecular weight degradation products of cell wall peptidoglycans and metabolites *Lactobacillus delbrueckii subsp. Bulgaricus* B-3964.

III. OBJECT, SUBJECT, AND METHODS OF RESEARCH

The next materials were used for research: biomass (BM) of lactic acid bacteria *Lactobacillus delbrueckii subsp. Bulgaricus* B-3964 with concentration $4.8 \cdot 10^9$ CFU/cm³ (SPI “Ariadna, Odesa, Ukraine); papain with a proteolytic activity of 10 U/mg (Swanson Health Products, USA); FeCl₃·6H₂O (China); CaCl₂, CaMg₂ (STAB, Netherlands); dietary fiber of wheat bran (DFWB) (Farmakom, Kharkiv, Ukraine).

Obtaining the destruction products of peptidoglycans Lactobacillus delbrueckii subsp. Bulgaricus B-3964. Isolation of cells from the culture fluid was performed by centrifugation for 15 min at 8000 min⁻¹. The cell pellet was washed with distilled water, resuspended and sonicated using PSB-1335-05 ultrasonic baths with an operating frequency of 40 kHz and a treatment duration of 300 s. Enzymatic destruction of BM cell walls was performed by treatment with papain at a temperature of 37°C and pH=7.4. The ratio of enzyme: substrate (dry matter content BM) was 1: 200, the incubation time of the reaction mixture was 300 minutes. The enzymatic hydrolysis was stopped by emergency heating to 100 ° C., the mixture was cooled, and the liquid phase was separated from the solid by centrifugation for 10 min at 8000 min⁻¹. In the liquid phase, the content of free amino acids was controlled by formol titration [18]. The content of low molecular weight peptides (LMWP) was determined by the Benedict method [18] after precipitation of high molecular weight proteins with 10% solution of trichloroacetic acid (TCA), the content of mucopeptides was determined after purification of hydrolysate on Anthrone method [18].

Obtaining organic complexes of biometals. The complexes were obtained by combining solutions of the hydrolysate of *Lactobacillus delbrueckii subsp. Bulgaricus B-3964* and biometals with vigorous stirring for 180 s, the temperature of complexation was 40°C. The complexing ability of metal ions in relation to bioligands was determined by nephelometric method in the presence of Na₂CO₃ on a spectrophotometer SF-2000 at a wavelength of 450 nm [19,20].

Study of the complexes pH stability. The behavior of the complexes was studied in the range of pH values of 1–10 units. The required pH value was achieved using standard solutions of NaOH and H₂SO₄. The concentration of free Fe³⁺ was determined by the thiocyanate method [21], the concentration of magnesium and calcium complex was determined spectrophotometrically at wavelengths $\lambda = 340$ nm [20]. The stability of chelate complexes was calculated by formula (1):

$$C = \frac{a-b}{a} 100, \% \quad (1)$$

where a – initial concentration of the complex, mg/cm³;

b – the concentration of the complex after incubation at certain pH values, mg/cm³.

Investigation of heat resistance of the obtained complexes. The studies were performed using the method of differential scanning calorimetry (DSC) in dynamic mode. DSC thermograms were obtained in the temperature range 25–250°C at a constant heating rate of 5°C/min on a calorimeter Derivatograph Q1500-D. In order to determine the conditions under which the complete decomposition of the samples will take place, the heating was continued to a maximum temperature of 450°C. A portion weighing 500 mg was placed in a ceramic crucible. The accuracy of determining the temperature was $\pm 1^\circ\text{C}$, the thermal effect – $\pm 3\%$.

Functional food ingredients (FFI) obtaining. FFI was obtained by immobilization of organic complexes of biometals on DFWB, which were previously removed from water and salt-soluble protein residues by three treatments with physiological NaCl solution at a hydromodule (HM) 1:20 (the mixture was stirred for 10 min at room temperature). DFWB was washed with distilled water and dried in a convective dryer

at 70°C to a humidity of 10–12%. Immobilization was performed by combining a solution of an organic complex of biometal with DF WB (HM 1: 5). The mixture was kept for 120 min and dried in a convective dryer at a temperature of 50°C until a humidity of 10–12% was reached.

Determination of the degree of complexes desorption from the matrix DF WB. The degree of desorption of organic complexes of biometals from the matrix was determined by indicating the amount of protein substances transferred to the external environment of the extractant from the total content of these substances in the complex (%) by reaction with ninhydrin [18]. To determine the degree of biometals complexes desorption from DF WB as extractants where used distilled water (HM 1:5, process duration 300 min) and buffer systems corresponding to the pH of the of the gastrointestinal tract medium, namely the stomach (pH 1.2) and the small intestine (pH 7.4). FFI was incubated for 120 min in a solution with a pH of 1.2 at a GM of 1: 5, after which the liquid phase was separated, buffer with a pH of 7.4 was added and the mixture was incubated for 180 min with stirring. The concentration of protein in the liquid phase of the mixture was determined every 30 minutes

The absolute error of the measurements was determined using the Student's test, the confidence interval $P = 0.95$, the number of repetitions in the definitions 3–4, the number of parallel samples of the experimental samples – 3.

IV. RESULTS

For the formation of biometals chelated complexes used bioligands of probiotic origin, namely, the destruction products of peptidoglycan *Lactobacillus delbrueckii subsp. Bulgaricus B-3964*. Destruction of peptidoglycan of BM cell walls was performed according to the procedure described above. As a result, a mixture of amino acids, LMWP and muropeptides was obtained, the concentration of which was 10.24 mg/cm³, 6.45 mg/cm³ and 2.25 mg/cm³, respectively. The complexing capacity of mixed ligand organic systems with respect to metal ions was investigated by nephelometry, namely by detecting insoluble forms of carbonates / metal hydroxides formed by the interaction of free metal ions with sodium carbonate after saturation of the ligand system with biometals. Free metal ions provoke turbidity after interaction with sodium carbonate. Determining the complexing capacity of the ligand system in relation to a particular biometal will allow to obtain a complex in which the presence of metal in an undesirable inorganic form is impossible. The results of the study are shown in Fig. 1.

As can be seen from Fig. 1, the turbidity of mixed ligand systems in the presence of biometal ions and Na₂CO₃ is minimally stable (0.05 opt. units) until a certain metal concentration is reached in the mixture. For the studied systems, this value has a significant difference. Thus, a rapid increase in the turbidity of the system containing Mg²⁺ ions occurs at their concentration in a mixture of 24 mol/dm³·10⁻², Ca²⁺ – 28 mol/dm³·10⁻², Fe³⁺ – 32 mol/dm³·10⁻². This behavior of the studied systems indicates that before reaching these concentrations, the metal is in a bound state in mixed ligand complexes, which prevents its interaction with sodium carbonate presented in the system and the appearance of insoluble particles of iron hydroxide or magnesium / calcium carbonate, which cause turbidity of the system.

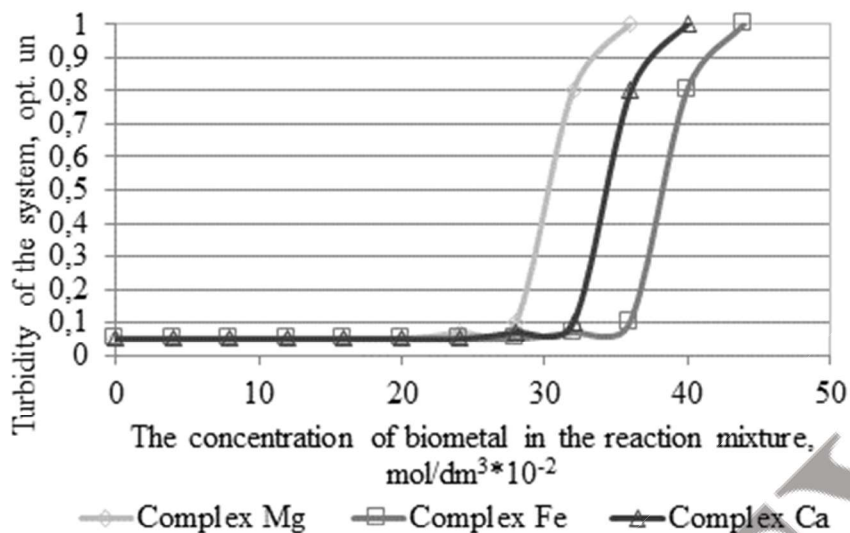


Fig. 1. Dependence of turbidity of mixed ligand system of probiotic origin on the content of biometal ions in the presence of Na_2CO_3

Since chelated complexes of biometals with the processing products of BM *Lactobacillus delbrueckii subsp. Bulgaricus* B-3964 is planned to be used as a dietary supplement and functional food ingredients, it is advisable to study their behavior at different pH values and temperatures. Figure 2 shows the dependence of the complexes stability on different pH values.

Based on the data of Fig. 2, the greatest stability at different pH values has a complex formed with the participation of Fe^{3+} . Complexing agent Fe^{3+} has d^2sp^3 hybridization of atomic orbitals and can cause the formation of an octahedral shape of the complex with bioligands, which explains the significant stability of the complexes in mediums with different ion activity. The stability of the obtained complex in the pH range 8–10 allows us to predict the possibility of the presence of ferric ions in the dissolved state in the small intestine, where it is absorbed by enterocytes.

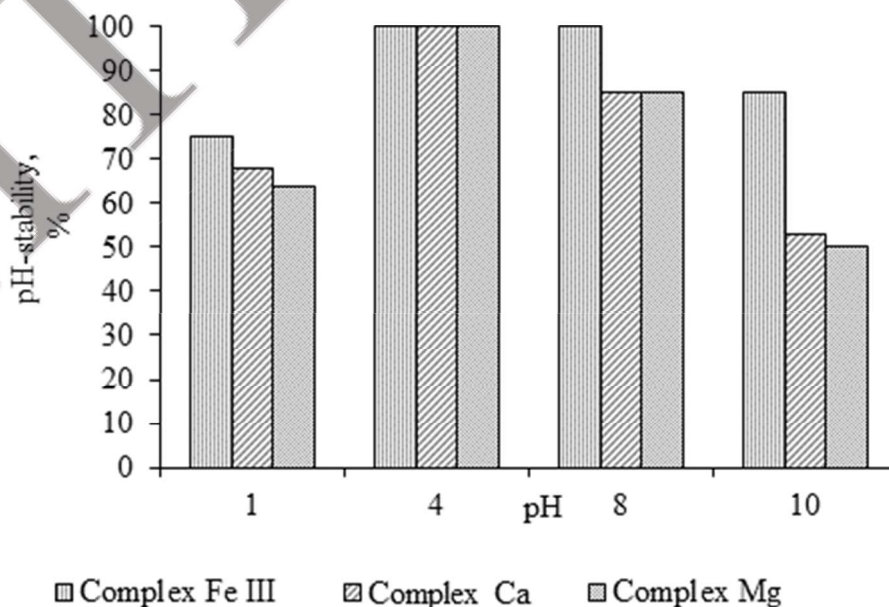


Fig. 2. Complexes pH-stability

The Ca and Mg complexes are also quite stable over a wide pH range. Thus, at the most aggressive values of pH 1 and pH 10, the stability of the Mg complex is 64 and 50%, Ca – 68 and 53%, respectively. The rather high stability of the obtained complexes can be explained by the fact that mixed ligand polydentant systems cause a stable "chelate effect", because the complexation of metals with polydentant ligands is more advantageous from a thermodynamic point of view than monodentant ligands. The stability of chelates at elevated pH values is also due to competition between metal ions and the proton of the solution for the anion of the chelate ligand. Thus, the obtained chelated structures are stable in the range of pH values of the environment, inherent in most food systems and digestive systems of the body, which determines the prospects of their use as components of dietary supplements and functional food ingredients.

In order to predict the behavior of the obtained chelate complexes in the composition of food systems that can be subjected to heat treatment, their analysis was performed by the DSC method (Table 1).

When comparing the DSC analysis data, it can be stated that the initial weight loss of the complexes begins at a temperature of 44–52 °C, samples of the mechanical mixture – at 55–60 °C. The first weight loss is not accompanied by thermal effects, which indicates that at these temperatures there is no destruction of the chelated bonds of the complexes, which can provoke a change in the enthalpy of the process and the appearance of thermal effects. In the temperature range 118–180 °C, an endothermic reaction is observed during heat treatment of complexes, and no thermal effects are observed during treatment of the mechanical mixture. The presence of endothermic thermal effects on the thermograms of the complexes may indicate the presence in their structure of chelated bonds, the destruction of which changes the enthalpy of the process.

Table 1 – Thermal effects and weight loss in thermogravimetric study of samples

Samples	Temperature range, °C	Maximum, °C	Thermal effects	Weight loss, %
Complex Ca	49–122	–	–	3.00
	122–178	140–145	endothermic	18.50
Mechanical mixture of components of the complex Ca	59–122	–	–	7.00
	122–178	–	–	16.00
Complex Mg	44–118	–	–	4.00
	118–173	139–143	endothermic	17.80
Mechanical mixture of components of the complex Mg	55–120	–	–	6.25
	120–173	–	–	16.40
Complex Fe (III)	52–125	–	–	3.80
	125–180	140–145	endothermic	19.60
Mechanical mixture of components of the complex Fe (III)	60–125	–	–	7.30
	125–180	–	–	17.50

The results of the research allow us to state that the obtained metal complexes are promising components of dietary supplements and functional food ingredients intended for the prevention of hypoelementosis, as they contain metal in safe organic (chelated) form, are stable in a wide range of pH and temperature.

Since the conditions for obtaining complexes involve the use of dilute solutions of their components, in the development of technologies for dietary supplements and food ingredients based on them, there are problems of concentration and drying of such systems. Upon receipt of soluble chelate complexes according to the proposed scheme, the moisture content can be up to 95%. These systems are difficult to dry and are quite hydrophilic after drying, which complicates their storage and possible dosing when added to food systems. In addition, due to the fact that the effective daily intake of the complexes is quite low, there may be a problem of even distribution of such a functional food ingredient throughout the product. In order to get rid of such shortcomings, it is proposed to immobilize the obtained complexes on classical matrices – dietary fiber of wheat bran. Modes of immobilization are shown above, and Figure 3 shows the regularity of desorption of the Fe (III) complex from the DFWB matrix during extraction with water and solutions corresponding to the pH of the gastrointestinal tract (regularities of desorption of Ca and Mg complexes correspond to those for the Fe (III) complex).

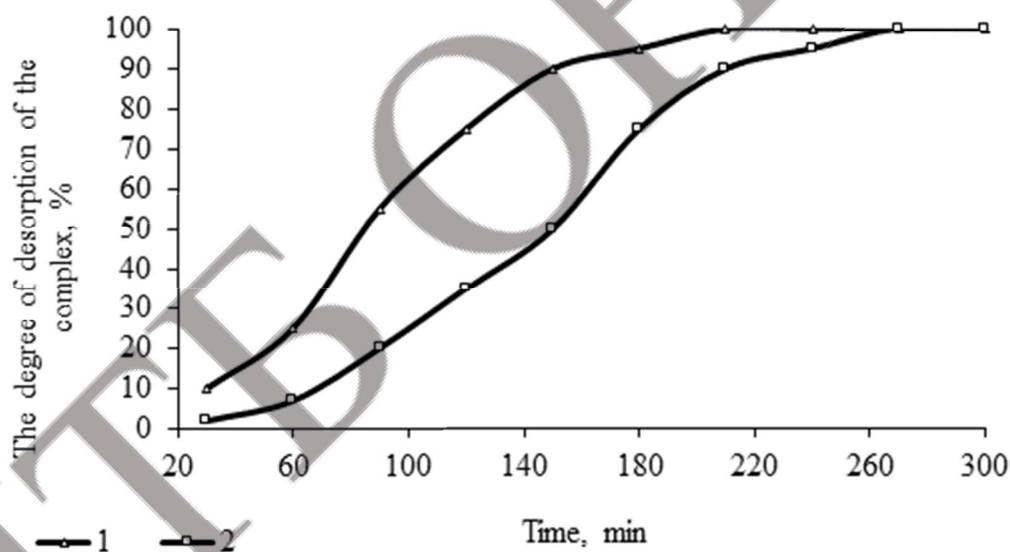


Fig. 3. Desorption of Fe (III) complex from DFWB matrix, depending on time and type of extractant

The nature of the release of the complex from the DFWB matrix during extraction with distilled water and solutions corresponding to the pH of the gastrointestinal tract is slightly different. The desorption of the complex is more intensive during the incubation of FFI with water, its complete release takes place after 210 min of extraction. Desorption of organic form of Fe (III) during extraction with solutions corresponding to the pH of the gastrointestinal tract, in the first 120 min of incubation is quite slow (only 35%, in contrast to the experiment with water – 75%). Complete release of the complex occurs after 270 minutes of extraction. The slow desorption of the complex at low pH values (the first 120 min of the experiment) can be explained

by the low water-binding capacity of DF WB under these conditions, which is a certain obstacle to the free extraction of the complex. Complete release of the complex from the matrix suggests that immobilization occurs only through physical sorption. The time of complete desorption of the biometal complex from the DF WB matrix in both experiments corresponds to the idea of the duration of the process of transporting food lumps from the upper gastrointestinal tract to the site of absorption of bimetals by enterocytes of the small intestine.

V. CONCLUSIONS

1. The results of research indicate the effectiveness of the use of polydentant mixed ligand systems of postbiotic origin for complexation with biometals.
2. According to research, the resulting complexes are stable in the pH medium inherent in most food systems and digestive systems, which determines the prospects of their use as components of dietary supplements and functional food ingredients.
3. The DSC method proved that the obtained complexes are stable in the temperature range 44–180 ° C, which allows to recommend them as FFI for food products, the technology of which provides for high-temperature processing.
4. The expediency of immobilization of the obtained biometals complexes on DF WB is substantiated. It is proved that immobilization occurs only by physical sorption, which promotes the complete release of active ingredients in media that mimic the pH of the small intestine, where the absorption of biometals takes place
5. The presence in the composition of the studied FFI biometals in organic form, low molecular weight muopeptides with high immunotropic activity, DF WB, allows to refer these tools to the category of multifunctional.

The prospect of further research is to study the physiological activity of the obtained FFI in animal experiments.

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