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OBTAINING AND CHARACTERISTIC OF THE MAGNESIUM ORGANIC FORMS ON THE BASIS OF PRODUCTS OF BIFIDOBACTERIA PROCESSING AND THEIR METABOLITES

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Abstract. The possibility of obtaining bioavailable mixed ligand chelate complexes of Magnesium has been considered. As bioligands, it is proposed to use the metabolites and products of enzymatic hydrolysis of the peptidoglycans of the cell walls of *Bifidobacterium bifidum* AC-1670. As ligands, fragments of peptidoglycans of cell walls of bifidobacteria, which have their own immunotropic effects, were used. Destruction of bacterial cells was done by ultrasound treatment with subsequent enzymatic hydrolysis with papain. It was found that the highest content of potential ligands for chelation was obtained by ultrasound treatment at a frequency of 35 kg for 600 seconds with subsequent enzymatic hydrolisys, which lasted for 180 minutes at a ratio of the enzyme: substrate 1:100. In this case, the accumulation of amino acids in the hydrolyzate was 11.35 mg/cm^3, low molecular weight peptides - 7.54 mg/cm^3. The liquid phase of the product of the disintegration of the bacterial mass is investigated for the presence of metabolites that can participate in the formation of chelating magnesium complexes. Qualitative composition and quantitative content of organic acids are determined. It is established that in the product of disinfection of bifidobacteria the following acids are present: acetic (445.5 mg/dm^3), lactic (284.6 mg/dm^3), benzoic (1.3 mg/dm^3). It has been established that the obtained mixed ligand systems are effective chelating agents and bind magnesium in an amount of 14 mg/cm^3. The method of IR spectroscopy has proved that this system is formed with the participation of polydentant ligands. Determination of the pH stability of the complex showed that in the range of pH values 4–7, the chelate system is stable, at pH 2 only 10% of the complex is stored, at a pH of 9–60%. The thermostability of the complex was investigated by the method of differential scanning calorimetry. It was established that the complex is stable in the temperature range of 20–12 °C, and therefore can be used as a physiologically functional ingredient in the health foods, the technology of which involves high-temperature processing.

Key words: magnesium, chelate complexes, bioligands, probiotic bacteria, metabolites, muropeptides.

ОТРИМАННЯ ТА ХАРАКТЕРИСТИКА ОРГАНІЧНИХ ФОРМ МАГНІЮ НА ОСНОВІ ПРОДУКТІВ МЕТАБОЛІЗМУ ТА ПЕРЕРОБКИ БІФІДОБАКТЕРІЙ

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Анотація. Розглянуто можливість отримання біодоступних змішанолігандних хелатних комплексів магнію. У якості біолігандів пропонується використовувати продукти метаболізму та ферментативного гідролізу пептидогліканів клітинних стінок *Bifidobacterium bifidum* AC-1670. Деструкцію бактеріальних клітин здійснювали шляхом обробки ультразвуком з послідуючим ферментативним гідролізом папаїном. Встановлено, що найбільший вміст потенційних лігандів для хелатоутворення мав місце за обробки ультразвуком частотою 35 кГ протягом 600 с із послідуючим ферментолізом, який тривав 180 хв при співвідношенні фермент:субстрат 1:100. При цьому накопичення амінокислот у гідролізаті складало 11,35 мг/см^3, низькомолекулярних пептидів – 7,54 мг/см^3. Рідку фазу продукту дезінтеграції бактеріальної маси досліджено на предмет наявності метаболітів, які можуть приймати участь в утворенні хелатних комплексів магнію. Визначено якісний склад та кількісний вміст органічних кислот. Встановлено, що у складі продукту дезінтеграції біфідобактерій присутні наступні кислоти: оцтова (445,5 мг/дм^3), молочна (284,6 мг/дм^3), бензойна (1,3 мг/дм^3). Встановлено, що отримані змішанолігандні системи є ефективними хелатоутворювальними агентами та зв'язують магній у кількості 14 мг/см^3. Методом ІЧ-спектроскопії доведено, що дана система утворена за участю полідентантних лігандів. Визначення pH стабільності комплексу показало, що в інтервалі значень pH 4–7, хелатна система є стабільною, при pH 2 зберігається лише 10% комплексу, при pH 9 – 60%. Методом диференційної скануючої калориметрії досліджено термостабільність комплексу. Встановлено, що комплекс є стійким в діапазоні температур 20–122°С, а отже, може бути використаний як фізіологічно функціональний інгредієнт в рецептурі оздоровчих продуктів харчування, технологія яких передбачає високотемпературну обробку.

Ключові слова: магній, хелатні комплекси, біоліганди, пробіотичні бактерії, метаболіти, муропептиди.

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Introduction. Formulation of the problem

Magnesium is the most demanded metal in nature, it activates enzymes of oxidative phosphorylation, DNA replication and bone mineralization. In addition, with the help of magnesium cations, ribosomes are formed from RNA and proteins, and the process of protein synthesis is activated in them. In the intracellular fluid, magnesium ions form complexes with the anions ATP and ADP, which are the active form of these substrates. Magnesium ions suppress the centers of regulation of respiration and blood vessels in the brain, causing a decrease in blood pressure. They also contribute to the secretion of cholesterol from the body, increased intestinal motility and bile secretion. It is proved that a wide range of pathological conditions in humans is caused by a magnesium deficiency in the body. Among them: increased pressure, pain in the heart, irritability, depression, poor sleep, fatigue, bronchospasm and much more [1-5]. The prevention of magnesium deficiency is possible through the use of foods with high content of this biometal. But there is a category of people in whose diet there are certain restrictions, or during the recovery period after certain surgical interventions, etc. In this case, additional use of bioavailable oral forms of magnesium is necessary [3-4].

Analysis of recent research and publications

Chelate complexes of biometals have a high bioavailability. Chelates differ significantly from non-chelate compounds both in terms of chemical and physical properties, and in their influence on living organisms. For example, they have increased resistance to heating, and also in them the metal atom does not enter the oxidation reaction. It has been established that chelates are almost identical to the natural structure and can penetrate through cellular membranes, which determines their high bioavailability [3].

Chelate magnesium compounds deserve particular attention. So, in 1951, A. Popovici and co-authors suggested that the infusion of even a small amount of chelate form magnesium (10–15 mg) to patients with hypertonic disease contributed to a decrease in blood pressure (AT), which lasted from 2 up to 6 weeks. At the same time, intravenous administration of magnesium sulfate required much more magnesium to achieve the same antihypertensive effect, and in addition, the reduction in blood pressure was short-lived [6]. Guided by the results of this work, in 1961 H.G. Kelly and co-authors compared the effect of chelating and non-chelate magnesium compounds not only on blood pressure but also on renal hemodynamics [7].

The authors of the paper [8] found that when using magnesium chelate, the metal maximum content in the blood is reached for a 3 hours earlier and a better tolerability of the drug was noted. The authors suggest that this effect of the chelate compound is possible due to the use of special transport systems of enterocytes, which allow the chelates to reach the blood as easily as possible without pre-treatment in the digestive tract [9].

Despite the fact that the positive effect of the use of magnesium in chelate form has been proven for a long time, scientists devote enough attention to the search for ligands that have their own high biological values [10-15].

Most of the processes occurring in biological systems involve the interaction of metal ions with several ligands, so it is of particular interest to obtain and study the properties of mixed ligand complexes of biometals with biologically active ligands. The assimilation of the biometal will occur in that case if it is firmly connected with the chelating agent that is a participant in the metabolic processes: amino acids, polybasic acids, vitamins. The study of mixed ligand complexes of biometals has become widespread, the methods of obtaining and characteristics of some of them have been described in the literature.

New metal complexes formulated as [Mg(L)2(H2O)2]·H2O (1) and [Zn(L)2(H2O)2]·0.5H2O (2), where HL=5-hydroxyflavone (primuletin), have been synthesized and characterized by elemental and thermal analyses, molar conductance, IR, UV-Vis, ^{1}H- and ^{13}C-NMR, fluorescence and mass spectra. In solid state, complexes had shown higher fluorescence intensities comparing to the free ligand, and this behavior is appreciated as a consequence of the coordination process [11].

The magnesium complex [Mg(hesp)2(phen)] (1), where hesp=hesperidin and phen=1,10′-phenanthroline, was synthesized and characterized by Elemental Analysis (C,H,N), atomic absorption and spectroscopic (FTIR, UV–visible, ^{1}H NMR) techniques. The complex was found to be a better radical scavenger for superoxide radical (IC50 = 68.3 μMat pH 7.8) than free hesperidin (IC50=116.68 μmol L^{-1}) and vitamin C (IC50=852 μmol L^{-1}). The strong blue fluorescence of complex 1 switches through loss of luminescence in pure water/protic organic solvents or when protected from water (in octanol for example as a model of phospholipid membranes) [12].

In [16], complexes of some d-metals ions (Co^{2+}, Cu^{2+}, Zn^{2+}) were obtained in the form of salts and in solution with ligands containing donor nitrogen and oxygen atoms to which the d-metal cations have an affinity. As an oxygen-containing ligand oxycarboxylic acid (malic acid) was used, as a nitrogen-containing ligand imidazole was used.

In [17] the formation of binary and ternary complexes of metal ions such as Cu(II), Co(II), Pb(II), Zn(II) and Cd(II) with biologically important ligand were investigated. Where nucleic acid Adenine was used as primary ligand and amino acid Histidine was used as secondary ligand.

In [18] the mixed ligand complexes of Cu(II), Ni(II) and Co(II) with uridine and amino acids L-alanine, L-phenylalanine and L-tryptophan were synthesized and characterized by the elemental analysis, conductivity data, infrared spectra, electronic spectra and magnetic susceptibility data.

In [19] mixed ligand complexes of Co(II), Ni(II) and Cu(II) with L-glutamine and succinic acid were studied. The increased stability of the ternary complexes compared to their binary complexes was believed to be due to electrostatic interactions of the side chains of the ligands, charge neutralization, chelate effect, stacking interactions and hydrogen bonding

After analyzing a number of scientific studies in the field of obtaining chelate complexes of biometals, we came to the conclusion that the literature lacks information on the possibility of using metabolites and probiotic bacteria processing products as bioligands. Taking in the account the great experience and volumes of cultivation of probiotic cultures, such an idea is very relevant. In the production of probiotic cultures a large amount of by-products is utilized. Such is the culture fluid that remains after the separation of the bacterial mass. The culture fluid contains a large number of metabolites, in particular organic acids, capable of chelate complexation with biometals. In addition, non-conditioned biomass is often disposed of, which can be sent for recycling to produce degradation products of peptidoglycans from their cell walls – compounds of the muramilpeptide series, which also contain functional groups that can form ionic and coordination bonds with metal ions. In addition, the substances of the muramilpeptide series have their own physiological effect – they are powerful immunotropic compounds [20-23].

The purpose of this work is to obtain the chelate complexes of Mg^{2+} ions with metabolites and low molecular weight degradation products of peptidoglycans of bifidobacteria cell walls.

Research tasks:

- obtaining and characteristic of bioligands – metabolic products of bifidobacteria and degradation products of their cell walls;
- obtaining chelate complexes of Magnesium with bioligands;
- studying the stability of received complexes depending on the pH medium and temperature.

Research Materials and Methods

The research was conducted on the basis of laboratory of Scientific and Production Enterprise "Ariadna" (Odesa, Ukraine), Laboratory of the Department of Food Chemistry and Expertise of the Odesa National Academy of Food Technologies (Odesa, Ukraine), the Laboratory of the State Scientific-Research Control Institute for Veterinary Medicines and Feed Additives (Lviv, Ukraine).

Materials. The strain *Bifidobacterium bifidum* AC-1670 is used in the work from the collection of Scientific and Production Enterprise "Ariadna" (Odesa).

Enzymatic degradation of biomass (BM) cell was performed by papain treatment with a proteolytic activity of 10 Un/mg. $MgCl_2$ (STAB, the Netherlands) was used as a source of Mg^{2+}.

*Obtaining of metabolic products of probiotic bacteria and degradation products of their cell walls and their characteristics.*Bacterial strain was grown in sterile conditions at 37°C on special nutrient medium developed by the "Ariadna" company. After reaching the number of bacteria up to $5–9 \cdot 10^9$ CFU and more, the culture fluid was subjected to ultrasoud treatment on laboratory device with a working frequency of 25, 35 and 40 kHz, the processing time was varied in the range 60–900 s. In samples, the content of free amino acids was controlled by the method of formolithic titration [24], soluble protein by Benedict's method [24], low molecular weight peptides (LMWP) by the Benedict method after precipitation of high molecular-weight proteins by 10% solution of trichloroacetic acid.

After ultrasoud treatment the enzymatic hydrolysis was conducted. The constant parameters of hydrolysis were: temperature – 37°C and pH=5. The ratio of the enzyme to the substrate (dry matter content of BM) was varied in the range from 1:50 to 1:150 and the duration of the incubation of the reaction mixture was varied in the range 10–300 min. In the obtained hydrolysates, the content of free amino acids, soluble protein, low molecular weight peptides (LMWP) were investigated. Enzymatic hydrolysis was stopped by heating at the temperature 100°C during 15 min, the mixture was cooled, centrifuged for 10 min at 8000 min^{-1}, decanted, further the supernatant containing BB metabolites and low molecular weight soluble biological active substances are used for chelate complex formation.

Qualitative and quantitative content of organic acids was determined by the method of capillary electrophoresis (device Capel 105/105M). Up to 0.5 g of the 50 cm^3 preparation of distilled water heated to 70°C was added. The mixture was stirred on a laboratory shaker for 10 min. After that, 1 cm^3 of filtrate was taken out, centrifuged and the determination of quantitative and qualitative content of organic acids was carried out. Detection was performed at wavelengths of 190 nm [25].

Obtaining of chelate complexes of Magnesium with bioligands. For obtaining chelate complexes of Magnesium, as a source of bioligands, a supernatant obtained after ultrasoud treatment and enzymatic hydrolysis is used. The complexing ability of Magnesium ions was determined by a nephelometric method in the presence of Na_2CO_3 on a spectrophotometer SF-2000 at a wavelength of 450 nm [26].

To the aliquot of the mixtures containing the bioligands, various volumes of 0.5n $MgCl_2$ were added,

stirred and left for 15 minutes to complete chelation. Thereafter, an equimolar amount of Na_2CO_3 was added to the solutions. Ions of Mg^{2+}, which did not participate in complex formation, in interaction with sodium carbonate form insoluble particles of $MgCO_3$ of white color, which provoked turbidity of the system.

Investigation of the stability of the received complexes depending on the pH of the medium. The stability of chelate complexes of Magnesium, depending on the pH of the medium, was determined by changing the intensity of absorption of light at a wavelength of 270 nm using a spectrophotometer SF-2000. The required pH of the solutions was created by solutions of NaOH and H_2SO_4 "ap". The constancy of the ionic strength (I≈0.1) was maintained by a solution of Na_2SO_4 "ap". The activity of hydrogen ions was measured on an ionometer I-160 using a working electrode ES-10601/7 and a reference electrode ESR-10101. The instrument was calibrated using standard buffer solutions prepared from fixanal. The measurements were carried out at room temperature 20±2°C. Distilled water was used as the reference solution.

Investigation of the stability of obtained complexes depending on temperature. The research was conducted using the method of differential scanning calorimetry (DSC) in dynamic mode. Thermograms of DSC were obtained in the temperature range of 25–250°C at a constant heating rate of 5°C/min on a Derivatorgaph Q1500-D. In order to determine under what conditions the complete destruction of the samples will occur, the heating was extended to a maximum temperature of 450°C. A weight of 500 mg was placed in a ceramic tigel. The accuracy of the temperature determination was ±1°C, the thermal effect – ±3%.

IR spectra of samples were recorded in the range of wavelengths from 4000 to 400 cm^{-1} in a spectrometer with a Fourier transformer FTIR IR Affinity-1, Shimadzu (Japan).

Results of the research and their discussion

Obtaining of metabolic products of bifidobacteria and degradation products of their cell walls and their characteristics.

It is known that bifidobacteria (BB) produce a number of organic acids that play a decisive role in maintaining colonization resistance and antagonistic activity against pathogenic microflora. In the aspect of this work, the functional groups of organic acids synthesized by BB are potential donors for the formation of ionic and coordination bonds in chelate structures of Magnesium. That is why it was expedient to determine the qualitative and quantitative content of organic acids in the BB culture fluid. The content of metabolites was investigated in the culture fluid after cultivation. Definition of these indicators was carried out by capillary electrophoresis. The study showed that there is a number of organic acids in the composition of the BB culture fluid. The following organic acids were identified and their quantitative content was determined: acetic (445.5 mg/dm^3), lactic (284.6 mg/dm^3), benzoic (1.3 mg/dm^3).

BB belong to gram-positive microorganisms, the share of peptidoglycan in which reaches 70% of their total mass, which makes them extremely resistant to the effects of degrading factors. Therefore, a series of experiments with the use of ultrasound, which is the most effective physical method of primary degradation of microorganisms with subsequent enzymatic hydrolysis by papain, has been performed for the violation of the anatomical integrity of the cell membrane of the BB [27]. It is established that rational treatment of a suspension of the BB by ultrasound at a frequency is 35 kHz for 600 seconds. The product obtained by the ultrasound treatment was subjected to enzymatic hydrolysis by papain. The results of hydrolysis have shown that treatment with papain provides more efficient accumulation of target low molecular weight degradation products in the reaction mixture compared to samples that were not subjected to enzymatic treatment. Thus, in sample obtained only by ultrasound treatment, the amount of amino acids in the reaction medium reaches 1.8 mg/cm^3, low molecular weight peptides (LMWPs) – 0.03 mg/cm^3, whereas in the sample obtained with the combination of ultrasound and enzymatic hydrolysis, the amount of amino acids reaches 11.35 mg/cm^3, LMWPs – 7.54 mg/cm^3. At the same time, enzymatic hydrolysis of the biomass, without preliminary ultrasound treatment, leads to the accumulation of low molecular weight peptides in the reaction medium in the amount 3.23 mg/cm^3.

So, proceeding from the above, it has been found out that organic acids, amino acids, LMWPs are contained in the composition of the culture liquid of BB, and amino acids and LMWPs are contained also in the hydrolysate of the bacterial cell wall peptidoglycans that can serve as biologically active ligands for the formation of chelate mixed ligand complexes of Magnesium.

Obtaining of Magnesium chelate complexes with bioligands. The preparation of Magnesium chelate structures was carried out according to the scheme given in the section "Methods of investigation". In fig. 1 the results of nephelometric titration are shown, where the arrow indicates the point of equivalence of the maximum binding of Mg^{2+} ions with a mixed ligand system. The use of the classical method for Magnesium determining by means of complexometric titration was not possible, since the chelating agent of this reaction (EDTA) competes for the binding of Magnesium with bioligands of the investigated system. The use of this method in determining the amount of Magnesium that participates in complex formation would not be correct.

Analyzing the data of Figure 2 it can be stated that the highest ability to bind magnesium ions is 14 mg/cm^3. Presumably, in the formation of a chelate complex are involved organic acids, which are donors of anions for ionotropic binding of Magnesium ions and amino groups of free amino acids and peptide bonds of LMWPs, which form coordination bonds with metal. Due to the amphoteric

properties of free amino acids and peptide, the stability of Magnesium chelate complexes can improve at different values of pH media.

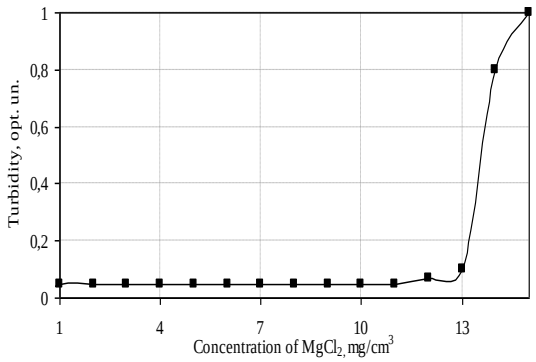


Fig.1. Maximum binding of Mg^{2+} ions by mixed ligand systems (λ=450 nm)

Complex formation was also proved by changing of the spectrum of the mixed ligand system in the ultraviolet region depending on the content of Magnesium ions in it (Fig. 2).

As can be seen from Fig. 2, the absorption spectrum of a solution of magnesium chloride has no peaks in the ultraviolet region, in contrast to the spectrum of the studied mixed-ligand system, which has a clear peak in the region of 260–280 nm. Namely in this area of the spectrum organic acids, amino acids and proteins are absorbed. When adding magnesium chloride in the amount of 4–19 mg/cm^3 to the bioligand system, the intensity of spectral absorption gradually decreases with increasing magnesium ion concentration (Complex I is 4 mg/cm^3 $MgCl_2$, Complex II is 9 mg/cm^3 $MgCl_2$, Complex III is 14 mg/cm^3 $MgCl_2$, Complex IV is 19 mg/cm^3 $MgCl_2$). Such changes in the spectrum indicate that the number of free functional groups responsible for oscillations in the spectrum is reduced due to their participation in the formation of magnesium chelate complexes. Moreover, with the addition of magnesium chloride in the amount of 4–14 mg/cm^3 to the system, the absorption intensity decreases linearly with a step of ≈12%, with further increase of magnesium chloride in the system (19 mg/cm^3), the absorption intensity decreases by only ≈2%, this confirms that the saturation of bioligands with Magnesium take place at a concentration of magnesium chloride equal to 14 mg/cm^3.

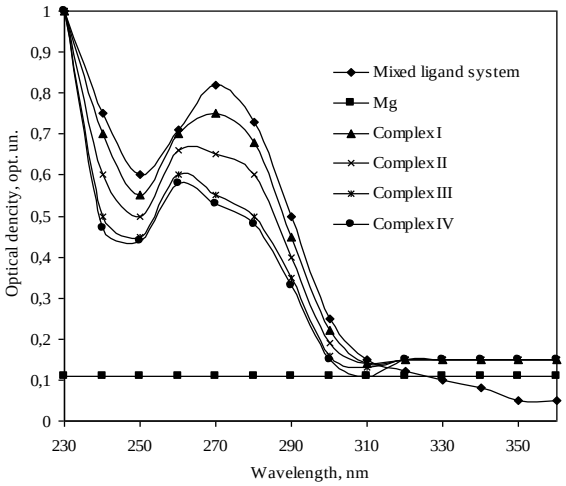


Fig. 2. Absorption spectrum of a mixed ligand systems with different Magnesium content

Chelating magnesium complexes were also investigated using IR spectroscopy (Fig. 3).

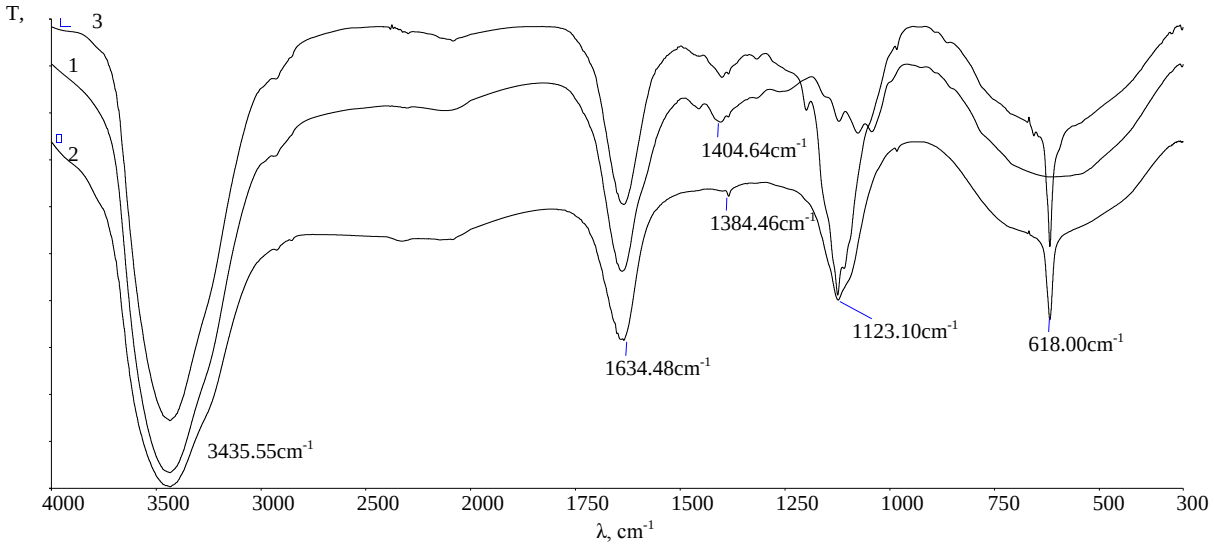


Рис. 3. IR-spectra : 1 – Mg Cl_2; 2 – mixed ligand system (metabolites and hydrolysis products of peptidoglycans of cell walls of BB; 3 - a complex of magnesium and a mixed ligand system

At the IR spectrum of magnesium in inorganic form (1) and in organic form (3) there is an absorption band at 618.00 cm^{-1}, and in absorption spectrum 3, the absorption intensity is somewhat lower. The paper [28] also confirms that in the manganesium chloride spectrum absorption bands are present at wavelengths of ≈ 600 and 1600 cm^{-1}. Obviously, the intensity of absorption decreases in connection with the participation of magnesium ions in the formation of a chelate complex. This assumption is confirmed by the fact that in the work of K. Nakamoto [29] it is indicated that fluctuations of complex compounds of magnesium with the participation of polydentant ligands appear for wavelengths of 300-600 cm^{-1}. The presence of intensive absorption bands at 1634 cm^{-1} and 3435 cm^{-1} in all three spectra can be due to deformation and valence fluctuations of crystalline water [30], in addition, these bands are also characteristic for fluctuations of amino groups that are part of amino acids and carboxylate- anions (3400–3500 cm^{-1}) [31]. In spectrum 3 there is a decrease in the intensity of the vibration of these groups, which may indicate their participation in the formation of ionic and coordination relations with magnesium and the formation of, respectively, a chelating complex.Since chelate complexe of Magnesium is planned to use as dietary supplement and biologically active food ingredient, it is expedient to study their behavior at different pH values of the medium and temperatures.

Investigation of the stability of the obtained complexes depending on the pH of the medium

The research methodology of the magnesium chelate complex pH stability is described in detail in the section "Research Methods". The intensity of absorption of the complex was determined in the range of pH values 2–10 at a wavelength of 270 nm (Fig. 4), in which the maximum absorption of chelate mixed ligand systems (Fig. 2) was defined.

Fig. 4 indicates that in the range of pH values of 4–7, the intensity of absorption of optical density by the complex is 0.53 opt. un., this corresponds to the value of the maximum absorption peak of optical density by a complex with a 14 mg/cm^3 concentration of Magnesium in it (Fig. 1). This means that in this range of pH values of the medium, the complex retains its chelate structure and its concentration in the mixture is maximal – 100%. If the pH value is deviated to a more acidic side, the stability of the complex decreases sharply. Thus, at pH 3, the concentration of the complex in the mixture is 24%, at pH 2–12%. With the deviation of the pH to the alkaline side, the stability of the complex is also somewhat lost, at pH 8 ≈ up to 18% and at pH 9 ≈ up to 35%. Consequently, the obtained magnesium chelate structures are stable in the range of pH values of the medium inherent for most food systems, which determines the promising use of them as biologically active food ingredients.

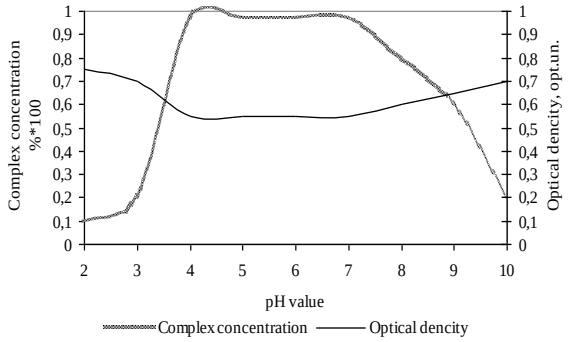
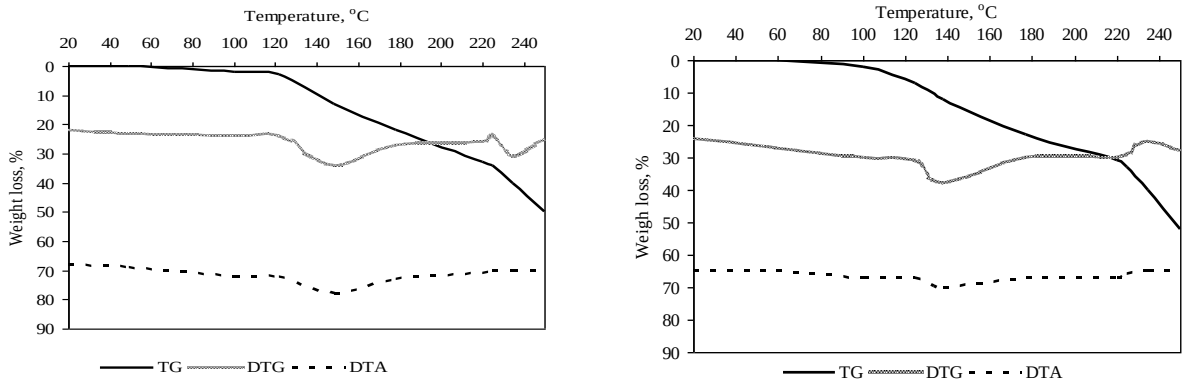


Fig. 4. pH-stability of chelate complexes of magnesium (λ=270 nm)

Investigation of stability of the obtained complexes depending on temperature. In order to predict the behavior of the magnesium chelate complexes in the composition of food systems that can be subjected to temperature processing, they were analyzed by the DSC method (Figs. 5a, b).



a) b)

Fig. 5. Termograms DSC: a) chelate complex of Magnesium; b) a mechanical mixture of complex components

TG is the thermogravimetric curve that characterizes the mass loss of the sample, depending on the temperature; DTG is the curve of differential thermogravimetry based on the registration of the rate of change in mass with continuous heating, which is a more accurate interpretation of the TG curve; DTA is the curve of differential thermal analysis, which serves to fix the presence of certain thermal effects

In fig. 5a the curves of TG, DTG and DTA are shown, which were obtained as a result of research of Magnesium chelate complex by DSC method, and in Fig. 5b the curves obtained as a result of studies of the mechanical mixture (MM) of the components of the complex are depicted. When comparing the data of the DSC analysis, we can state that the initial mass loss of the complex begins at the temperature of 49°C, and MS at 59°C. From these figures it can be seen that the first mass loss is not accompanied by thermal effects, which indicates that at these temperatures there is no destruction of the chelate bonds of the complex, which can provoke a change in the enthalpy of the process and the appearance of peaks on the DTA curves. Consequently, the first loss of mass is associated with the removal of free moisture in the sample. When the temperature reaches 122–125°C, the mass loss is 3% for the complex and 7% for the MM. In the temperature range of 122–178°C, an endothermic reaction is observed at the heat treatment of the complex, while thermal effects at the treatment of MM is not observed. The mass loss of the complex in this range of temperatures is 18%, MM – 16%. The presence of an endothermic peak on the DTA curve of the complex may indicate the presence of chelate bonds in its structure, with the destruction of which there are changes in the enthalpy of the process.

Consequently, the results of the analysis of the DSC have shown that the obtained complex is stable in the temperature range of 25–122°C, and therefore can be used in the composition of health foods, the technology of which involves high-temperature processing.

Conclusions

1. It has been established that the mixed ligand system obtained by processing the bacterial mass of *Bifidobacterium bifidum* AC-1670 contains organic acids (acetic (445.5 mg/dm^3), lactic (284.6 mg/dm^3), benzoic (1.3 mg/dm^3), amino acids (11.35 mg/cm^3), low molecular weight peptides (7.54 mg/cm^3) that have their own immunotropic activity.

2. By the methods of nephelometry and spectrophotometry in the ultraviolet region, it has been found that obtained mixed ligand systems are effective chelating agents and bind magnesium in amounts of 14 mg/cm^3.

3. By the method of IR spectroscopy has proved that this system is formed with the participation of polydentant ligands.

4. Identification of the pH stability of the complex showed that in the range of pH values 4–7 chelate system is stable, at pH 2, only 10% of the complex is stored, at pH 9–60%.

5. According to the results of the DSC analysis, it has been proven that the obtained complex is formed with the participation of chelate bonds of bioligands with magnesium, at the destruction of which an endothermic effect is observed. The complex is stable in the range of temperatures of 20–122°C, and, consequently, can be used in the formulation of healthy foods, the technology of which involves high-temperature processing.

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