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DIETARY SUPPLEMENT BASED ON SELENIUM CONTAINING CULTURE OF LACTIC ACID BACTERIA

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Abstract. The article describes the role of selenium in the humankind being. The analysis based on the published data shows that the biological synthesis is a perspective way to obtain an organic form of selenium, which can be used in dietary supplements. The ability of lactic acid bacteria (*Lactobacillus acidophilus 412/307*)to accumulate inorganic forms of selenium (selenites, selenates), turning them into organic forms, with purposeful trace element enrichment of culture medium is described in the article. The main organic forms of selenium, which are being used in the process of biotransformation from its inorganic forms by microorganisms, have been reorganized. The relationship between the increasing of concentrations of sodium selenite in the culture medium and the growth of biomass of lactic acid bacteria was established. It was found the depressing effect of increasing concentrations of sodium selenite on optical density rate. It was estabilished the optimal conditions for the maximum accumulation of selenium containing culture of lactic acid bacteria. The influence of selenium concentration on the lactic acid bacteria biomass accumulation was determined also by changing the values of optical density. Due to the obtained data, the selenium containing dietary supplement «Selenolakt» was created. The main microbiological indicators that characterize the quality of the obtained product are given. The content of organic form of selenium in products reaches –195 ± 1 mkg/g.

Keywords: lactic acid bacteria, sodium selenite, inoculums, dietary supplement

ДІЄТИЧНА ДОБАВКА НА ОСНОВІ СЕЛЕНВМІСНОЇ КУЛЬТУРИ ЛАКТОБАКТЕРІЙ

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Анотація. У статті охарактеризовано здатність лактобактерій (*Lactobacillus acidophilus 412/307*) накопичувати неорганічні форми селену (селеніти, селенати), перетворюючи їх в органічні форми, при цілеспрямованому збагаченні мікроелементом середовища культивування. Перелічено основні органічні форми селену, що утворюються мікроорганізмами в процесі біотрансформації його неорганічних форм. Встановлено зв'язок між зростаючими концентраціями селеніту натрію в середовищі культивування і приростом біомаси лактобактерій. Виявлено пригнічуючий вплив зростаючих концентрацій селеніту натрію на показники оптичної щільності. Визначено оптимальні умови максимального накопичення селеновмісної культури лактобактерій. Завдяки отриманим даним створена селенвмісна дієтична добавка «Селенолакт». Наведено основні мікробіологічні показники, котрі характеризують якість отриманого продукту. Вміст органічної форми селену в продукті досягає –195±1 мкг/г.

Ключові слова: лактобактерії, селеніт натрію, інокулят, дієтична добавка

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Introduction

Nowadays, scientists are paying special attention to the study of the role of microelement selenium in maintaining a healthy population status. The development of numerous selenium preparats , selenium containing dietary supplements and artificial selenium enriched food serves as a proof of this [1].

Selenium is a 34th element in the Mendeleev's periodic table of elements, is located in the 4th period 6 main subgroup. Selenium is part of a number of enzymes, including glutathione peroxidase, yodtyronindeyodynaz, tyoreduktaz; proteins it is able to deposite in organs. Selenium acts as a catalyst for redox reactions, protects the human organism from harmful free radicals [2,3]. This microelement is delivered to human organism with foods. However, when selenium is delivered with foods the daily requirement is not always satisfied. This can cause serious violations of health (illness Keşan, Kashin-Beck, Crohn's endemic myxedema). That is why the artificial enrichment of foods by this microelement plays an important role in the prevention of selenium deficit. These products do a dual positive effect on the human body [4-8].

Formulation of the problem

Nowadays, organic forms of selenium are obtained by chemical and biological synthesis (involving bacteria, yeasts, algae). The biological synthesis of organic selenium forms is carried out by adding selenium source in the culture medium of microorganisms which can accumulate and biotransformate it [4,5]. This method compared with others requires little energy, is an ecologically safe, eliminates the possibility of the formation of harmful by-products. The obtained selenium containing preparats can be used as dietary supplements.

Literature review

The insufficient providing with micronutrients of a large part of Ukrainians determines the actuality of the problem of developing new food sources of microelements that is safe for human health. To obtain new food sources of essential microelements, a biotechnological approach is promising that uses single-cell microorganisms, yeasts, algae as a bio matrix for the incorporation of a microelement (Table 1) [9,10].

Inorganic salts addition into the cultivation medium of microelements leads to their incorporation in the microorganisms. As a result of bioconversion biomass becomes enriched with organic forms of incorporated microelements, whereby selenium is mainly included in selenium containing amino acids in composition of peptides and proteins.

Biomass of selenium containing lactic acid bacterias may be used in the production of dietary supplements and specialty products.

Table 1 − Biotransformation of selenium by microorganisms

Microorganisms	The form of biotransformed selenium
Lb. Casei	SeCys (selenocysteine)
Lb. plantarum	SeCys (selenocysteine)
Lb. delbrueckii subsp. Bulgaricus	SeCys (selenocysteine)
Lb. rhamnosus LB3	Se(0) (zero valent selenium)
Lb. fermentum LB7	Se(0) (zero valent selenium)
Lb. bulgaricus	Se(0) (zero valent selenium)
B. animalis 01	SeMet (selenomethionine)
E. faecium LAB 1	Se(0) (zero valent selenium)
B. animalis 01	SeMet (selenomethionine)
Lb. casei	Se(0) (zero valent selenium)
Lb. acidophilus LA 5	SeCys (selenocysteine), Se(0) (zero valent selenium)
Bifidobacterium BB 12	SeCys (selenocysteine)
Streptococcus thermophilus	Se(0) (zero valent selenium)

It is proved that selenium is able to incorporate into amino acids instead sulfur with subsequent incorporation in the process of protein synthesis in microorganisms especially when sulfur supply is insufficient. Although the main selenium fractions are connected with proteins, a small portion of selenium may be associated with polysaccharides. The other part of the bio-transformed selenium is in zero-valent form Se^0 and is able to accumulate itself inside the cell, and in the space around the cell (the rose color of the microorganisms culture medium is evidence) [8].

After the biotransformation in the bacterial cell, about 32 % of selenium is in the membranes, 22 % is part of the cell wall, 52 % – is part of amino acids and soluble proteins of protoplasm (of which 72 % is contained in the group of proteins and amino acids, 1% is connected with lipids) [2].

Dietary supplements with selenium containing microorganisms are able to provide antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory effect on the host organism and inhibit the growth of pathogens. So on the one hand microorganisms due to its probiotic properties are able to improve human health and on the other hand they can be a cheap source of organic forms of selenium.

At high concentrations of selenium in the culture medium the microorganisms start protective reaction which provides adsorption of sodium selenite on polysaccharide secreted by microorganisms at first and turning it into a zero valent selenium in the future (Se^0).

Main part

The purpose of work was the selection of optimal culture conditions that would have made it possible to get the maximum yield of biomass of selenium enriched lactic acid bacteria and creation of the dietary supplements on basis of them.

In this work the museum culture *Lactobacillus acidophilus 412/307* was used. Cultivation of lactic acid bacteria was carried out on the medium with cheese whey (cheese whey, milk, corn extract, magnesium sulfate). As a source of selenium, the sodium selenite Na_2SeO_3 (Hemel) was used. Sodium selenite was dissolved in sterile distilled water and added to the culture medium at concentrations from 0.5 mg/cm^3 to 20 mg/cm^3. As a control one, it was medium without adding sodium selenite. Inoculum of daily culture brings in flasks with the cultuvation medium in quantities of 5 %.

Initially, the study determined the optimal conditions for the accumulation of biomass of selenium containing lactic acid bacterias. The colony forming units was chosen as criteria for optimal performance (CFU/sm^3). This indicator shows the overall picture of the microbial biomass yield after the cultivation process is finished (Figure 1).

According to the data the optimization functions were found as dependence between criteria and parameters. The surface is described by a polynomial degree 3, fig. 1. With Table Curve 3D we received the coefficients.

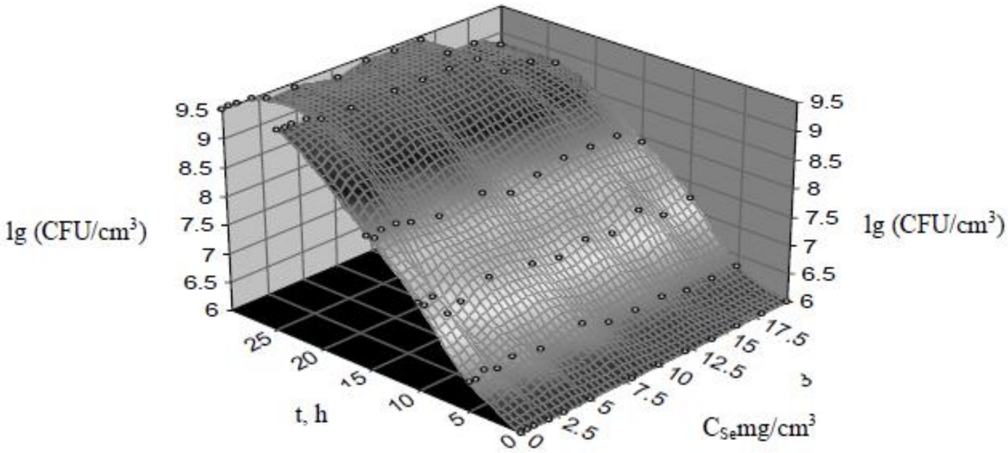

Fig. 1. Cultivation of lactic acid bacteria in the medium with cheese whey: z – lg CFU/cm³; x − C_{Se} (concentration of selenium); y – t (cultivation time).

Extremums of function are located at the points where the partial derivatives are equal to 0:

$$\begin{cases} \frac{dz}{dx} = 0 \\ \frac{dz}{dy} = 0 \end{cases} \quad (1)$$

$$\frac{d}{dx}Z = \frac{d}{dx}\left(a + b\cdot x + c\cdot y + d\cdot x^2 + e\cdot y^2 + f\cdot x\cdot y + g\cdot x^3 + h\cdot y^3 + i\cdot x\cdot y^2 + j\cdot x^2\cdot y\right) \quad (2)$$

$$\frac{d}{dy}Z = \frac{d}{dy}\left(a + b\cdot x + c\cdot y + d\cdot x^2 + e\cdot y^2 + f\cdot x\cdot y + g\cdot x^3 + h\cdot y^3 + i\cdot x\cdot y^2 + j\cdot x^2\cdot y\right) \quad (3)$$

$$3\cdot g\cdot x^2 + 2\cdot j\cdot x\cdot y + 2\cdot d\cdot x + i\cdot y^2 + f\cdot y + b = 0 \quad (4)$$

$$j\cdot x^2 + 2\cdot i\cdot x\cdot y + f\cdot x + 3\cdot h\cdot y^2 + 2\cdot e\cdot y + c = 0 \quad (5)$$

Solution:

$$y = \begin{pmatrix} \dfrac{f - 2\cdot e + \sqrt{f^2 - 4\cdot e\cdot f + 4\cdot e^2 + 12\cdot b\cdot h - 4\cdot b\cdot i - 12\cdot c\cdot h + 4\cdot c\cdot i}}{6\cdot h - 2\cdot i} \\ -\dfrac{2\cdot e - f + \sqrt{f^2 - 4\cdot e\cdot f + 4\cdot e^2 + 12\cdot b\cdot h - 4\cdot b\cdot i - 12\cdot c\cdot h + 4\cdot c\cdot i}}{6\cdot h - 2\cdot i} \end{pmatrix} \quad (6)$$

$$x = \begin{pmatrix} -\dfrac{d + \sqrt{d^2 + 2\cdot d\cdot j\cdot y + j^2\cdot y^2 - 3\cdot g\cdot i\cdot y^2 - 3\cdot f\cdot g\cdot y - 3\cdot b\cdot g} + j\cdot y}{3\cdot g} \\ -\dfrac{d - \sqrt{d^2 + 2\cdot d\cdot j\cdot y + j^2\cdot y^2 - 3\cdot g\cdot i\cdot y^2 - 3\cdot f\cdot g\cdot y - 3\cdot b\cdot g} + j\cdot y}{3\cdot g} \end{pmatrix} \quad (7)$$

It was defined extremes where y – cultivation time and x – sodium selenite concentration. Thus, in the cultivation of lactic acid bacterias in cheese whey environment we identified that optimum time of cultivation is 25.5 hours and quantitative of sodium selenite − 1.9 mg/cm³.

The optimum cultivation conditions were the basis for a dietary supplement based on selenium containing lactic acid bacteria.

Effect of selenium concentration on the accumulation of biomass by lactic acid bacteria was determined also by changing the values of optical density (fig. 2). The optical density was determined using a KFK-2-UHL 4.2 photocolorimeter at a wavelength of 590 nm.

Correlation between the optical density rate (OD) and the concentration of sodium selenite is noted. Thus, when the concentration of sodium selenite was 0.5 – 3 cm³ the OD parametrs were equal to the control unit and were 2,18; when the concentration of sodium selenite was 5 – 20 cm³ a gradual decline in the OD was noted that in the sample of 20 cm³ reached 2.09 units.

The quantitative content of lactobacilli accumulated selenium was determined by the fluorimetric method, using 2,3-diaminonaphthalene (Table 2).

It was established that with increasing of concentrations of sodium selenite in the culture medium the amount of selenium in lactic acid bacterias was increased too. Thus, the maximum exposure observed in a sample containing the sodium selenite was 20 mg/sm^3.

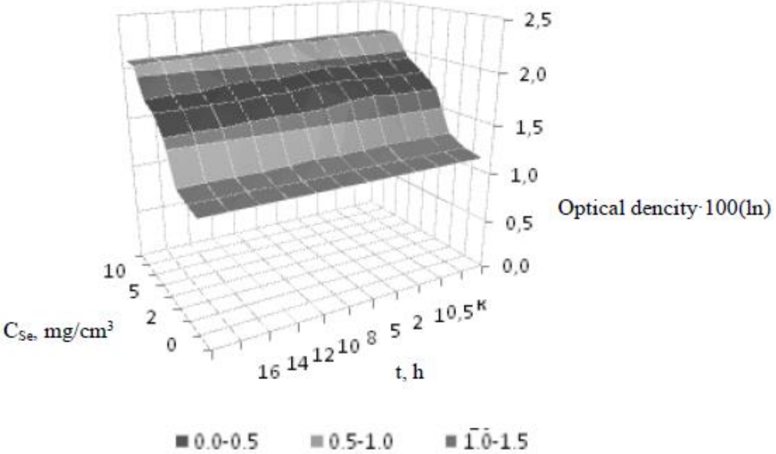

Fig. 2. Changing of optical density in the process of accumulation of selenium containing lactic acid bacteria biomass.

Table 2 − Dynamics of accumulation of selenium by bacteria cultures (n=3, P≥0,95)

The concentration of sodium selenite before cultivation, mg/sm^3	The intensity of luminescence	The content of selenium mkg/g of dry biomass
control	3	37,5
0,5	10	105,0
1	13	195,0
3	18	450,0
5	25	975,0
10	48	2116,0
14	70	3252,0
20	98	4698,0

The data was used in the development of biotechnology obtaining dietary supplement based on selenium containing lactic acid bacteria culture – «Selenolakt». For this the sterile aqueous solution of 0.1 % sodium selenite was injected in the culture medium with lactic acid bacteria in an amount 1 mg/cm^3. Lactic acid bacteria inoculum culture was injected in 5 %. Cultivation was carried out at 37 °C for 24 hours. Selenium containing biomass of microorganisms was separated from the culture medium by centrifugation at 10,000 rpm for 10 minutes. The received biomass separated from the non-accumulated sodium selenite, which was contained in the culture medium by washing in sterile water, followed by centrifugation at 10,000 rpm for 10 minutes. The next step was the introduction of a protective environment, which incorporates contained milk, sucrose and zhelatoza followed by freeze-drying. Microbiological parameters of obtained product are shown in Table 3.

Table 3 − Microbiological characterization of product

Indicator	Characteristics
The quantitative content of bifidobacteria	2,0×10^9 CFU /cm^3
Mold fungi CFU/cm^3	missing
Pathogenic microorganisms, including salmonella	missing
Coliform bacteria 0.1 g	missing

Quantitative selenium content in the resulting product is 195±1 mkg. The resulting dietary supplement characterized beige powdery structure, the specific taste and smell.

Conclusions

The biotransformation is an effective way to obtain the organic form of selenium that can be used in selenium containing dietary supplement. The lactic acid bacteria (*Lactobacillus acidophilus 412/307*) can transform selenium from the sodium selenite Na_2SeO_3 (Hemel) into organic form. The increasing of sodium selenite concentrations in the culture medium leads to the growth of the amount of selenium in lactic acid bacterias. On the same time, it was found the depressing effect of increasing concentrations of sodium selenite on optical density rate and hence on the bacteria biomass. Consequently, there are the optimal parameters of selenium containing lactic acid bacteria biomass accumulation in their cultivation on the environment from cheese whey. The best time of cultivation of lactic acid bacteria is 25.5 hours, and the optimal content of sodium selenite in the culture medium – 1.9 mg/cm^3. Based on the data the dietary supplement was developed. The quantity of lactic acid bacteria in the resulting product is 2.0×10^9 cfu/cm^3, and quantitative content of selenium is 195±1 mg. The microbiological analysis of the obtained product shows the pathogenic microorganisms, mold fungi, and coliform bacteria were not observed in it.

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ДИЕТИЧЕСКАЯ ДОБАВКА ИЗ СЕЛЕНСОДЕРЖАЩЕЙ КУЛЬТУРЫ ЛАКТОБАКТЕРИЙ

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Аннотация. В статье описана способность лактобактерий (*Lactobacillus acidophilus 412/307*) накапливать неорганические формы селена (селениты, селенаты), превращая их в органические формы, при целенаправленном обогащении микроэлементом среды культивирования микроорганизмов. Перечислены основные органические формы селена, образуемые микроорганизмами в процессе биотрансформации его неорганических форм. Установлена связь между растущими концентрациями селенита натрия в среде культивирования и приростом биомассы лактобактерий. Выявлено угнетающее влияние растущих концентраций селенита натрия на показатели оптической плотности. Определены оптимальные условия максимального накопления селенсодержащей культуры лактобактерий. Благодаря полученным данным создана селенсодержащая диетическая добавка «Селенолакт». Приведены основные микробиологические показатели, которые характеризуют качество полученного продукта. Содержание органической формы селена в продукте достигает - 195 ± 1 мкг/г.

Ключевые слова: лактобактерии, селенит натрия, инокулят, диетическая добавка

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