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Использование микроводорослей: спирулины, хлореллы в производстве функциональных продуктов питания (аналитический обзор)

The use of microalgae: spirulina, nori profira, nori kelp in the production of functional foods (analytical review)

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Аннотация. *Проведенный анализ иследований отечественных и зарубежных ученных в области использования морских водрослей в производстве функциональных продуктов питания с широким спектром биологической активности: антибактериальной, противовирусной, ангтиканцерогенной и антикоагулянтной показал, что использование бурых водрослей, содержащих гетерогенные полисахариды «фукоиданы», спирулины, содержащей полиненасыщенные жирныме кислоты: γ-линоленовую, олеиновую, а также нуклеиновые кислоты и отличающуюся высоким содержанием витамина В12, β-каротина, железа, кальция и фосфора и имеющей нейтральные органолептические свойства, а также хлореллы (Chlorella vulgaris) – источника жирорастворимых витаминов, холина, пищевых волокон и минералов в качестве перспективного натурьльного сырья является актуальным и перспективным напралением функциолнального и лечебно-профилактического питания в период реабилеьации после различных заболеваний.*

Ключевые слова: морские водросли, спирулина, хлорелла, биологически активные вещества, функциональные продукты питания

Для цитирования: *Оробинская В. Н., Пушмина И. Н., Лаврова Т. Н., Писаренко О. Н., Емельянов С. А., Коновалов Д. А. Использование микроводорослей: спирулины, хлореллы в производстве функциональных продуктов питания (аналитический обзор) // Современная наука и инновации. 2023. № 4 (44). С. 118-129.* https://doi.org/10.37493/2307-910X.2023.4.13

Abstract. *The analysis of research conducted by domestic and foreign scientists in the field of the use of marine algae in the production of functional food products with a wide range of biological activity: antibacterial, antiviral, angticarcinogenic and anticoagulant showed that the use of brown algae containing heterogeneous polysaccharides "fucoidans", spirulina containing polyunsaturated fatty acids: γ-linolenic, oleic, and nucleic acids acid and characterized by a high content of vitamin B12, β-carotene, iron, calcium and phosphorus and having neutral organoleptic properties, as well as chlorella (Chlorella vulgaris) – a source of fat-soluble vitamins, choline, dietary fiber and minerals as a promising natural raw material is an urgent and promising application of functional and therapeutic and preventive nutrition during the period of rehabilitation after various diseases.*

Keywords: marine algae, spirulina, chlorella, biologically active substances, functional food products

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Introduction. Undernutrition is a public health problem, especially in developing countries and the Russian Federation.

Seaweed contains valuable bioactive molecules with a wide spectrum of action: antimicrobial, antioxidant, antiviral, antifungal, antitumor, contraceptive, anti-inflammatory, anticoagulant (Fig. 1.).

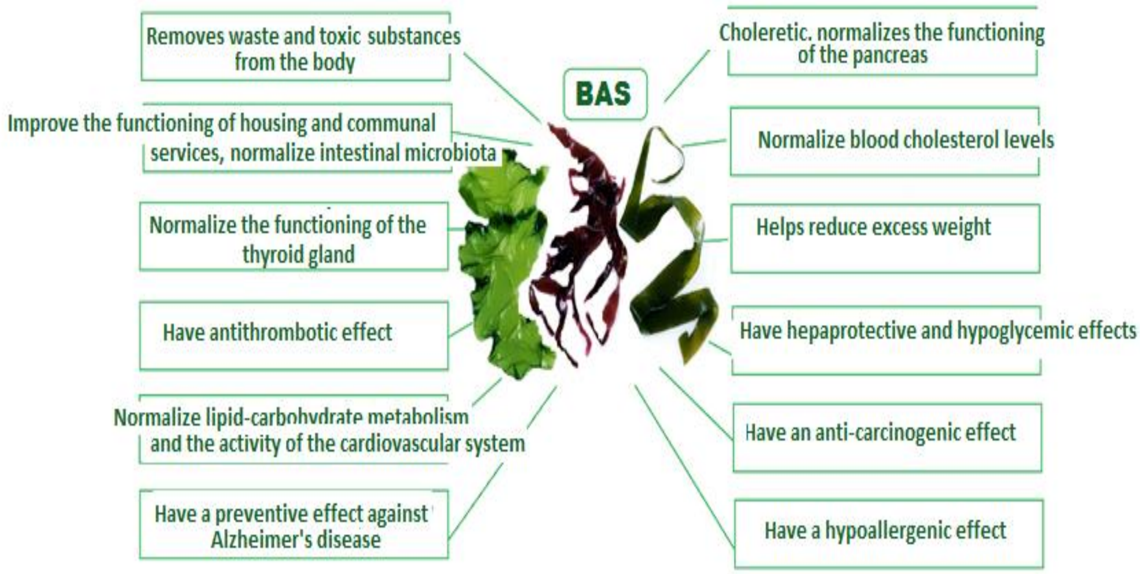

Figure 1 – Biological activity of seaweed

Materials and research methods. Algae extracts contain secondary metabolites such as fucoidan polysaccharide, sulfoquinovosyldiacylglycerols and caulerpin, with a wide range of biological activities

Brown algae are an inexhaustible source of sulfated polysaccharides with a high content of fucose of complex structure, containing FCS and SF and other monosaccharides such as galactose, xylose, mannose, glucuronic acid [1,2,3,4,5,6,7,8].

These heterogeneous polysaccharides are characterized by the term “fucoidans”.

The polysaccharide fraction obtained from algae is a mixture of biopolymers of various structures with SF as the main component. The composition may vary depending on the type and age of algae, as well as on growing conditions.

SF chains can be formed from repeating (1→3)-linked fucose residues [1,2,3,4,5,6,7,8] or from alternating (1→3)- and (1→4)- linked fucose chains [1,2,3,4,5,6,7,8], often with branches in the form of single fucose residues or multiple short oligosaccharides

Polysaccharide extract from algae usually contains other sulfated polysaccharides such as galactofucans, fucoglucuromannans and fucoglucuronans [1,2,3,4,5,6,7,8] (Fig. 2).

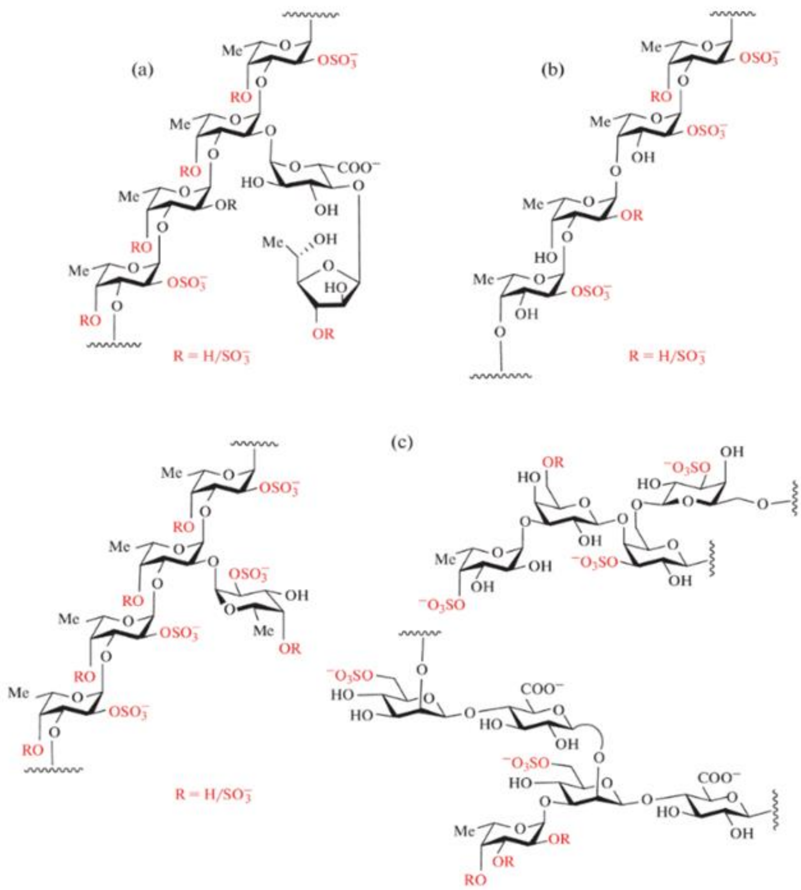

Figure 2 – Examples of sulfated polysaccharides produced by brown algae: (a) Chordaria flagelliformis [10]; (b) Fucus evanescens [11]; (c) Saccharina latissima [12]. Sulfates in fixed positions and variable substituents R are highlighted in red [Kiselevskiy, M. V., Anisimova, N. _ Y., Bilan, M. _ I. _ et al. Source: compiled by the authors based on data (Prospects for the Use of Marine Sulfated Fucose-Rich Polysaccharides in Treatment and Prevention of COVID-19 and Post-COVID-19 Syndrome // Russ J Bioorg Chem 48, 1109–1122 (2022). https://doi.org/10.1134/S1068162022060152)

Research results and their discussion. Sulfated polysaccharides exhibit various types of biological activities due to their interaction with proteins.

The most studied property is the anticoagulant effect, similar to the effect of heparin [1, 2, 3, 4, 5, 6, 7, 8].

Fucoidans are non-toxic, biocompatible and relatively readily available compounds and can be used as a promising ingredient for the production of new drugs with antiviral, anti-inflammatory, antitumor activity, as well as biologically active additives with immunomodulatory and anticoagulant effects.

The biological effect of fucoidans is associated with their high degree of sulfation, although other minor features of structure and molecular weight also play a significant role in their functions.

Detailed structural analysis of fucoidans is extremely difficult due to their irregularity and heterogeneity [1, 2, 3, 4, 5, 6, 7, 8].

To date, most of the biological studies conducted on fucoidans with obtained samples, without a confirmed chemical structure, make it difficult to accurately correlate the structure and biological activity of these compounds.

Most studies are aimed at studying the biological activity of sulfated fucose-containing polysaccharides with the study of the characteristics of their anticoagulant and antithrombotic effects [1, 2, 3, 4, 5, 6, 7, 8].

However, recently, due to the spread of the SARS-CoV-2 virus, more attention has been paid to the antiviral functions of these polysaccharides [1, 2, 3, 4, 5, 6, 7, 8], which are similar to the functions of heparin [1, 2, 3, 4, 5, 6, 7, 8].

The clinical presentation of COVID-19 ranges from asymptomatic illness to potentially life-threatening pneumonia, which can ultimately lead to acute respiratory distress syndrome (ARDS) [1, 2, 3, 4, 5, 6, 7, 8].

While most COVID-19 cases can be classified as mild or moderate disease, about 15% are severe cases requiring oxygen support, and about 5% require mechanical ventilation.

The coronavirus is distinguished by the manifestation of 2 types of severe acute respiratory syndrome (SARS-CoV-2) that affects various cells, including alveolar macrophages, which activates them and can induce a cytokine storm [34 , 35].

Studies have shown that COVID-19 can significantly affect hematopoiesis and the immune system, leading to lymphopenia, thrombocytopenia, neutrophil dysfunction and anemia [1, 2, 3, 4, 5, 6, 7, 8].

Currently, drugs with different mechanisms of action are used to treat COVID-19, which, along with clinical effectiveness, may cause adverse events.

A particular challenge is the development of an appropriate treatment regimen and further rehabilitation process for patients after COVID-19 who require long-term supportive care and therapy.

Natural substances are particularly interesting because they include bioactive compounds that can be used to develop drugs and dietary supplements with a wide range of biological activities with minimal side effects [1,2,3,4,5,6,7,8].

The microalgae spirulina is of interest.

The use of microalgae dates back to the sixteenth century. For example, spirulina was harvested from Lake Texcoco and sold in markets in Tenochtitlan (today Mexico City).

C pyrulina are prokaryotic cyanobacteria capable of photosynthesis.

It has valuable potential for use as ingredients in the development of new functional foods in the prevention or treatment of disorders associated with metabolic syndrome, which is one of the leading trends in the food industry.

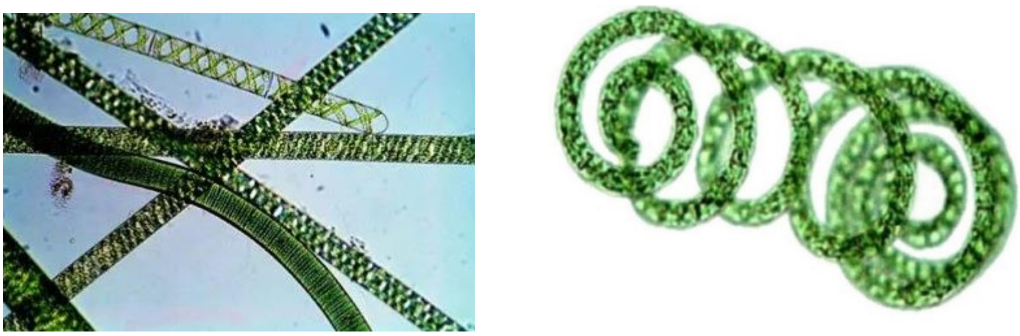

Figure 3 – Microscopy of cyanobacteria (spirulina)

Source: compiled by the authors based on data (https://shopozz.ru/items/291744101812-organic-spirulina-500 mg- tablets-detox-immune-system-weight-loss-b-vitamins-/)

The use of algae, especially spirulina, as a functional food was proposed several decades ago due to the fact that this raw material is not only a protein-rich food source, but also has a rich amino acid composition with high biological value.

Spirulina is rich in polyunsaturated fatty acids: γ -linolenic, oleic, as well as nucleic acids and is characterized by a high content of vitamin B $_{12}$, β -carotene, iron, calcium and phosphorus, has neutral organoleptic properties, does not exhibit acute and chronic toxicity, and is safe for humans . All of the above indicators prove that spirulina is a promising source for obtaining functional products and dietary supplements.

Blue-green algae is a storehouse of not only macro- and microelements, including iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium and zinc, but it is also rich in pigments such as chlorophyll A, as well as phycobiliproteins: C-phycocyanin and allophycocyanin .

It is known that spirulina has anticarcinogenic activity and also has an antispasmodic effect. The safe recommended dosage of spirulina is 3-10 g/day for adults.

Spirulina is one of the leading trends in the food industry In 1967, the International Association of Applied Microbiology recognized spirulina as the food source of the future.

Microalgae are now being incorporated into many food formulations, resulting in a significant increase in the number of food products containing microalgae that have been released into the market, although most of them use microalgae as a colorant or as a marketing strategy.

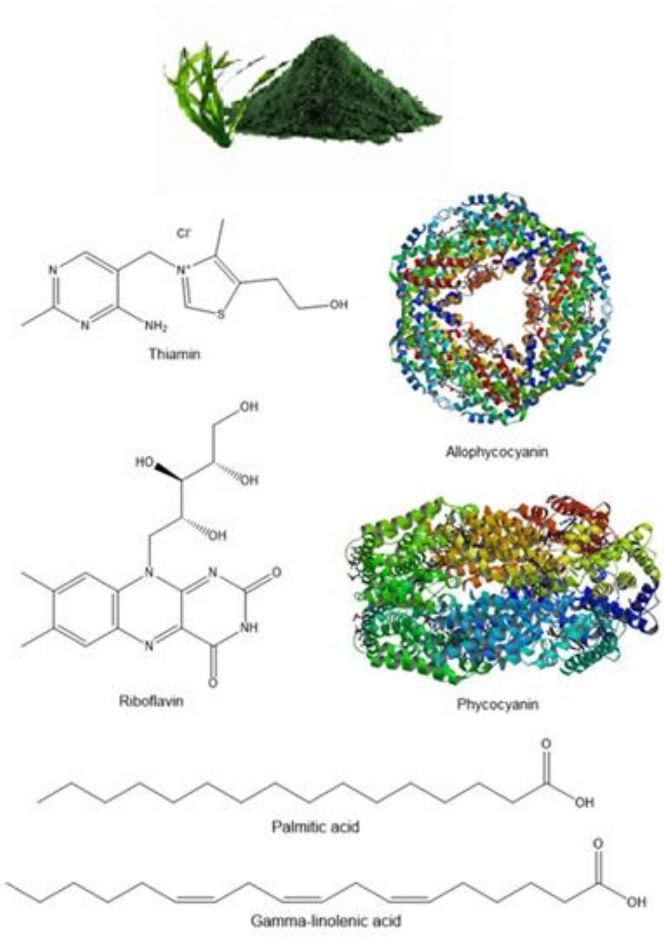

Figure 4 – Valuable molecules of biologically active substances found in *spirulina* phycobiliproteins: phycocyanin and allophycocyanin in a ratio of 10: 1 (.1-8). Along with palmitic and linolenic acids, (1-8) thiamine (vitamin B1) and riboflavin (vitamin B2)

Most spirulina biomass produced today is consumed as a dietary supplement, marketed as a “superfood,” and sold as dry powder, flakes, or capsules.

The rich chemical composition and average values are given in Table 1 [1, 2, 3, 4,5,6,7,8]. Spirulina *is* certified as a Generally Recognized as Safe (GRAS) - GRN number .127 - by the US Food and Drug Administration (FDA) and, due to its long history of use, it can also be commercialized in the European Union (EU) without the need to comply with Novel Foods Regulation (EU) 2015/2283 (EU, 2015).

Table 1 – Chemical composition of spirulina powder [1, 2, 3, 4, 5, 6, 7, 8].

Name of biologically active substance	**Content per 100 g**	**Name of biologically active substance**	**Content per 100 g**
Macronutrients		Vitamin B2, mg	3.7
Calorie content, kcal	290	Vitamin B3, mg	12.8
Water, g	4.7	Vitamin B6, mg	0.4
Total amount of lipids, g	7.7	Vitamin E, mg	5.0
Total protein, g	57.5	Amino acids	
Carbohydrates, g	23.9	Tryptophan, g	0.93
Ash, g	6.2	Threonine, g	2.97
Micro-macroelements		Isoleucine, g	3.21
Calcium, mg	Vitamin C, mg	Leucine, g	4.95
Iron, mg	28.5	Lysine, g	3.02
Magnesium, mg	195.0	Methionine, g	1.15
Phosphorus, mg	118.0	Cysteine, g	0.66
Potassium, g	1.4	Phenylalanine, g	2.77
Sodium, g	1.0	Tyrosine, g	2.58
Zinc, mg	2.0	Valin, g	3.51
Copper, mg	6.1	Arginine, g	4.15
Manganese, mg	1.9	Histidine, g	1.08
Selenium, mcg	7.2	Alanin, g	4.51
Vitamins		Aspartic acid, g	5.79
Vitamin A, IU	570	Glutamic acid, g	8.39
Vitamin K, mcg	25.5	Glycine, g	3.09
Vitamin B_1, mg	2.4	Proline, g	2.38
		Serin, Mr.	2.99

The chemical composition of spirulina can vary depending on environmental and cultivation conditions (temperature, light, salinity, etc.).

For example, insufficient content of nitrogen compounds contributes to the accumulation of high lipid content and affects the amount of polyunsaturated fatty acids such as γ-linoleic and linolenic acids and varies with content per 100g in the range from 13.1 to 34.9% of the total fatty acids.

These biologically active compounds help prevent cardiovascular diseases, have a positive effect on the central nervous system, prevent the harmful effects of cholesterol, and accelerate metabolic processes.

The advantage of using spirulina in the production of functional foods rich in proteins is the content of essential amino acids, according to the definition of the "ideal protein"

Phycobiliproteins: C-phycocyanin and allophycocyanin and phycoerythrin (Fig. 2) are high molecular weight compounds used in the nutraceutical, cosmetic, pharmaceutical and food industries.

Spirulina contains carotenoids, a class of pigments naturally synthesized by microalgae, in the form of provitamin A, which is biotransformed in the body into vitamin A. Natural carotenoids are a mixture of trans and cis isomers, unlike synthetic compounds, which are completely trans isomers.

Vitamin A functions: growth promotion, regulation of visual function, differentiation of epithelial tissues and embryonic development (Tang, 2010).

Carotenoids make up 30% of daily vitamin A intake (Tang, 2010). Carotenoids present in *spirulina:* astaxanthin, zeaxanthin and β-carotene.

Lubich et al. (2018) found the contents of zeaxanthin, β-carotene and astaxanthin in *A. platensis* to be 1.46, 1.74 and 0.43 mg/g on a dry weight basis. Canthaxanthin and lutein detected, but in lower concentrations

A valuable biologically active substance is chlorophyll, which is present in a concentration of approximately 6.0–20.0 mg/g dry weight.

This green pigment can be used not only as a dye, but also as an ingredient with antimutagenic, chemoprotective, antioxidant, anti-inflammatory and antimicrobial properties.

The limited use of spirulina in the food industry is due to its intense green color and distinctive flavor, but with the transition in the last decade of many large foreign companies to the "green" path and the commercialization of green food and beverages. These innovative products are becoming popular.

An example is the Spanish company **Fitoplancton Marino SL (Cádiz, Spain)** commercializing freeze-dried *Tetraselmis chuii biomass* under the name *Plancton Marino Veta la Palma* to enhance the marine flavor of the products.

Research by Niccolai (2019) and others, when used as an ingredient in the production of toasted bread at a concentration of 2 to 6%, enriched the product with high protein content and polyphenols.

The introduction of spirulina at a concentration of 1.5% into the product enriches it with a high content of iron and selenium.

The inclusion of *spirulina* in a concentration of 5-20% allowed Fradinho to develop a gluten-free pasta.

Useyin Bozkurt (2019) in his work emphasized the high potential *of spirulina* for the use of microalgae as an ingredient in the production *of ayran*, which increases the protein content and promotes the growth of probiotic cultures even in small concentrations (from 1%).

The second promising source is chlorella

Chlorella is a green unicellular algae, a source of high-quality protein and bioactive molecules with potential use against disorders of lipid-carbohydrate metabolism (obesity and two types of diabetes).

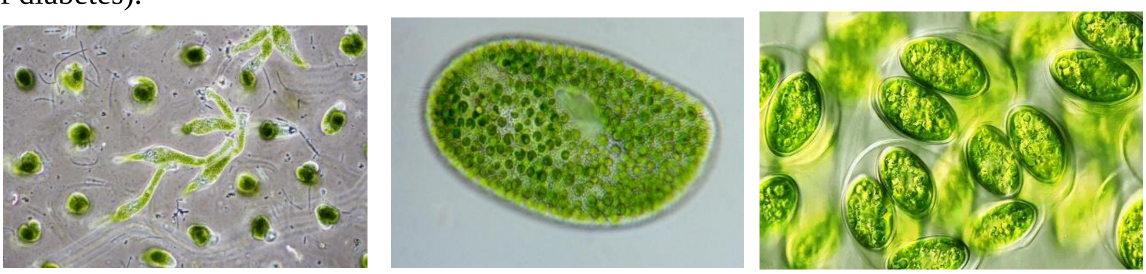

Figure 5 – Microscopy of chlorella

Source: compiled by the authors based on data (https://pro-dachnikov.com/uploads/posts/2021-11/1638013581_55-pro-dachnikov-com-p-khlorella-foto-61.)/

Chlorella has long been a popular functional food in Asian countries, including Korea, Japan and Taiwan, due to its digestibility and bioavailability (Cherng & Shih, 2005a).

Chlorella vulgaris is a source of fat-soluble vitamins, choline, dietary fiber and minerals.

Chlorella is rich in proteins, the protein contains essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine, dietary soluble and insoluble fiber, carotenoids, chlorophyll.

Studies by Sherafati N, Bideshki MV, Behzadi M, Mobarak S, Asadi M, Sadeghi O. have proven that dietary supplements based on chlorella normalize lipid-carbohydrate metabolism [9].

In experimental studies in vivo Kang H, Lee CH, Kim JR have proven the positive effect of chlorella extract as a dietary supplement on atopic dermatitis, a chronic inflammatory disease.

A dietary supplement from Chlorella vulgaris modulates allergy-causing factors when ingested [10].

Experimental studies were carried out in experiments in vivo on an experimental model of atopic dermatitis in Wister rats for six weeks, a decrease in dermatitis rates was noted throughout the entire period of the experiment.

Histological analysis showed positive dynamics - a decrease in DFE-induced eosinophil infiltration in the epidermis of skin cells and a decrease in the expression level of IL-4 and IFN-γ mRNA, which proves the possibility of using Chlorella vulgaris CV extract as a nutraceutical ingredient for the prevention of atopic dermatitis [11].

Previous studies in a model of type 1 diabetes have proven that chlorella does not affect insulin secretion but improves insulin resistance, which does not explain the hypoglycemic effect of chlorella in type 2 diabetes, and there was a need to study the effect of chlorella in a model of type 2 diabetes. In experimental studies, Hyejin Jeong et al. proved the hypoglycemic effect of chlorella in in vivo experiments on experimental Wistar rats. By using 3 diets containing different concentrations of powdered chlorella composite: (0%, 3%, 5%) with the same amount of calories, corn starch, sucrose, dextrinized corn starch (manufacturer Dyets, Inc., USA) were used as carbohydrate sources), soybean oil (manufactured by CJ Co., Korea) was used as a source of lipids. Optimal glucose reduction occurred with diets containing 3 and 5% chlorella. Chlorella may have a plasma glucose-lowering effect, but this has no effect on insulin secretion. Which requires further research [12].

By using the computer modeling method in Excel Anistratova O.V., Onikenko V.G. and others developed recipes for enriched bio-yogurts with the addition of chlorella, grapefruit, ginger, by searching for solutions to nonlinear problems using the enriched decreasing gradient method [12]

Products are being developed using spirulina and chlorella in the production of bakery products and introducing them into the human diet [13, 14].

Krolevets A.A., Glotova S.G. used a nanostructured alcoholic extract of chlorella to produce ice cream for prophylactic purposes for iron and iodine deficiency conditions [15].

Chlorella is widely used in the production of therapeutic and prophylactic confectionery products. Chlorella vulgaris due. high iodine content, more than 10 types of vitamins, micro- and macroelements (Ca, K, Fe, Na, Mg, Zn, Cu, P, Se, etc.) are used to prevent iodine deficiency diseases, anemia, reduce blood cholesterol levels and etc., also contain.

Scientific developments Nikonovich S.N., Tarasenko N.A., Novozhenova A.D. in the field of using powdered chlorella composite in the production of confectionery products, are a promising direction in the production of functional food products [16, 17, 19, 20]

Table 2 – Food value chlorella [16,17, 19,20]

Indicators of chlorella powder	**Contents per 100 g of product**	**Amino acids,%**
Proteins, g	56	lysine 10.2
Fats, g	6.7	methionine 1.4
Carbohydrates, g	11.2	tryptophan 2.26
Alimentary fiber	2.5	arginine 15.8,

Calorie content, kcal	326	histidine 3.3,
		leucine 6.1
		isoleucine 3.5
		phenylanine 2.8
		threonine 2.9
		valine 5.5

As Irgalieva K.S. notes in her research. "...Chlorella represents an attractive alternative to currently well-known bacterial, yeast and mammalian cell-based expression systems for the production of recombinant proteins (e.g. enzymes, vaccines, monoclonal antibodies and growth factors). Unlike bacteria, Chlorella is a eukaryotic organism and can perform the post-transcriptional and post-translational modifications necessary to produce functional eukaryotic proteins; therefore, Chlorella has long been used as a medicinal food and has been approved as safe for humans..." [18].

Although chlorella is one of the few microalgae that have been commercialized for health food, animal feed and fine chemicals, the global market for chlorella is still small, largely due to the high costs associated with existing production systems and processes. Another R&D priority should be given to the development of a stable and reliable gene transformation system in Chlorella, so that this organism becomes a true cellular factory for the production of high-quality recombinant proteins [18,19,20].

Conclusion. Having analyzed preclinical and clinical studies, domestic and foreign scientists have shown that spirulina has antioxidant, immunomodulatory and anti-inflammatory activity.

These studies were conducted on cell lines, tissue homogenates and animal models

Spirulina has an activating effect on the cellular system of antioxidants and enzymes, promoting the removal of free radicals, reducing the harmful effects of oxidative stress.

Research in vivo showed that biologically active substances (BAS) spirulina, used as a dietary supplement for fish, protect against oxidative stress by inhibiting lipid peroxidation and preventing DNA damage.

Further research is needed to determine the threshold concentration of biologically active substances, exceeding which reduces the antioxidant activity of spirulina.

Since spirulina has been proven to have an effective antioxidant effect against mycotoxins: trichothecenes and fumonisins, which increase oxidative stress. Thus, spirulina may be a potential treatment for mycotoxin intoxication in animals and humans.

Spirulina prevents skeletal muscle damage under conditions of exercise-induced oxidative stress.

Animal studies of the dietary supplement Spirulina have shown that it reduces the effects of toxicity caused by heavy metals, especially lead, making spirulina a potential treatment for heavy metal poisoning.

The main active compound, phycocyanin, has immunomodulatory and anti-inflammatory properties, stimulates the production of antibodies and activates genes encoding cytokines.

Phycocyanin and β -carotene are among the most important active ingredients of spirulina, which have antioxidant, immunomodulatory and anti-inflammatory properties.

The ERK 1/2, JNK, p38 and IkB signaling pathways but more research into the underlying mechanisms is needed.

Research is needed on the interaction between the biologically active compounds of spirulina and the biologically active substances of food products to which it is added.

Chlorella is a microalgae recommended for healthy diets and as an additive in animal feed.

Developments are underway to stabilize the gene transformation system in Chlorella for the production of highly purified recombinant proteins.

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