THE PROCEDURE FOR OBTAINING OF PROTEOGLYCAN PREPARATION FROM THE *Arthrospira platensis* BIOMASS

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Abstract: The present work refers to the elaboration of a new procedure for obtaining of proteoglycan preparation with high content of sulfated polysaccharides from the remaining biomass of *Arthrospira platensis* from the production of remedy BioR. Proteoglycans have a great potential for application, especially in the animal husbandry, food and cosmetics industry. The process consists in the following steps: the dried at the temperature of 50±5°C remaining biomass *Arthrospira platensis* was subjected to grinding; then it was mixed with 96% ethyl alcohol in a volume of 1:10, the obtained suspension was placed in a water bath at a temperature of +45°C, for 30 minutes and subjected to centrifugation at 3500 rpm. for 15 minutes, the separation of supernatant (pigments); the sediment was mixed with 96% ethyl alcohol in a volume of 1:2, the obtained suspension was placed in a water bath at a temperature of 60°C, for 60 minutes, and subjected to centrifugation at 3500 rpm for 15 minutes, the separation of supernatant (lipids); the sediment was mixed with distilled water at a ratio of 1:3 v/v and subjected to autoclaving at a temperature of 115°C (0.5 atm.) for 30 minutes, the obtained suspension was subjected to centrifugation at 3500 rpm; EDTA was added to the final proteoglycan preparation. The proteoglycan preparation has a total content of sulfated polysaccharides 733.41±1.55 mg/L, proteins 26.50±0.06% DW, carbohydrates 20.71±0.42% DW and the total antioxidant activity 25.43±0.29% inhibition, superoxide dismutase activity 38.41±0.42 U/mg protein and catalase activity 27.20±1.2 mmol/min/mg protein.

Key words: *Arthrospira platensis*; cyanobacteria; extraction; proteoglycans; sulfated polysaccharides.

INTRODUCTION

Proteoglycans present a specific kind of polysaccharides. Proteoglycans contain protein core with covalently attached glycosaminoglycans. Fungal, yeast cyanobacterial polysaccharides are the part of many research fields. Up to present, scientific data has accumulated that polysaccharides are regulators of metabolic processes and potential immunocorrectors. Of greatest interest are the immunomodulatory, radioprotective, antitumor, antibacterial properties of algal and cyanobacterial oligo- and polysaccharides. The increased interest is explained by their low toxicity and diverse biological activity, which can be used to obtain new bioactive preparations and additives.

Of particular interest to researchers are proteoglycans, which are soluble in water and can be isolated from algae species. These polysaccharides are considered polyvalent compounds, which are characterized by the presence of a number of biological properties [3, 4, 22]. There are many priority directions and industrial applications of cyanobacteria such as food industry, animal husbandry and cosmetic industry [23, 29]. However, complex processing is required for the application of cyanobacteria in above mentioned purposes. In addition, due to the fact that environmental situation is aggravated, it is necessary to obtain natural balanced products. In this context, the reutilization of the remaining biomass from the BioR preparation production is of scientific and practical interest. It is generally known, that *Arthrospira platensis* can be used as a source of proteoglycans [33]. Proteoglycans are complex proteins consisting of proteins (the protein part represents 5-10% of the total mass) with a high degree of glycosylation (the carbohydrate part represents 90-95% of the total mass) [12, 16]. The ability of proteoglycans to stimulate cell proliferation, to possess anti-inflammatory, regulatory and antioxidant effects, has been demonstrated in a number of studies. Proteoglycans regulate a wide variety of intracellular processes and are involved in molecular interactions [5]. In the light of the above, proteoglycans are able to perform a wide range of functions: they are used for substitution therapy in medicine, for the treatment of viral diseases, including herpes; they are used, also, as an additive in animal feed for osteosynthesis, as antifungal and anti-infective, anti-inflammatory agents [2, 21, 27, 31]. One of the most current areas of sulfated polysaccharide research is the study of the anti-infective potential of these biopolymers.

Sulphated polysaccharides as well as algal extracts containing these bioactive substances have antiviral activity against various viruses (flavi-, toga-, arena-, rhabdo-, orthopoxviruses as well as the herpes virus family) [30]. In the field of cosmetics, proteoglycan-based preparations are considered innovative in terms of preventing premature aging. According to existing literature data, cartilage tissue of fish, mollusks, birds and mammals [22, 28] as well as plants [10, 16] serve as a source of proteoglycans. A combination of anti-inflammatory, immunomodulatory and antioxidant properties of proteoglycans allows them to be recommended for use in various fields. The use of spirulina for the production of proteoglycan preparations can be characterized by the low toxicity and increased biological activity of proteoglycans (including sulphated polysaccharides).

The aim of the research was to develop the procedure for obtaining of proteoglycan preparation from the remaining biomass of *Arthrospira platensis*.

MATERIALS AND METHODS

Arthrospira (Spirulina) platensis cyanobacteria biomass, left over from the production of the BioR

bioremedy, dried (at 50±5°C to constant mass), provided by the production company, specialized in biotechnological and pharmaceutical products, was used.

The total carbohydrate content was determined by a spectrophotometric method at wavelength 620 nm using the antron reagent and D-glucose as standard [11]. The protein content was measured using Folin-Ciocalteu reagent bovine serum albumin was used as a standard [18]. Catalase activity was measured by the spectrophotometric method, which is based on the ability of hydrogen peroxide to interact with molybdenum salts to form a stable color complex [17]. Superoxide dismutase activity was estimated spectrophotometrically, the method was based on the SOD-mediated inhibition of nitroblue tetrazolium (TNB) reduction to the blue formazan in presence of TEMED and riboflavine. The assay of SOD activity was carried out at pH 7.8, in 50 mM phosphate buffer containing EDTA [19]. One unit of SOD was determined as the amount of enzyme that lead to the 50% maximum inhibition of the reduction of TNB. The content of sulfated polysaccharides was estimated spectrophotometrically by the utilization of alcian blue [8]. Amino acids. Ion-exchange chromatography method was used for determination of the free amino acid content in proteoglycan preparation using amino acid analyzer AAA-339M [13]. The spectrophotometric ABTS assay was used to evaluate total antioxidant activity [24].

RESULTS

The utilization of spirulina bioactive properties has such characteristics as low toxicity and increased biological activity of proteoglycans (including sulphated polysaccharides). There is a need to develop a method of extracting proteoglycans that would have the low cost and highly efficiency while keeping these complexes intact. In the next step, the optimal extraction parameters of biologically active principles from cyanobacterial biomass were determined. After the first two stages of obtaining pigment and lipidic extracts, extracts of proteoglycan nature were obtained. The procedure for obtaining of proteoglycan extracts requires the establishment of optimal conditions for extracting of the bioactive components. Thus, 5 experimental samples and 1 control sample were selected.

1. **Sonication 1:3** (biomass to water ratio 1:3). Extraction of proteoglycans from the biomass of the cyanobacteria *Arthrospira platensis* was carried out with sonication procedure for 5 minutes. The biomass was subjected to centrifugation at 3500 rpm for 15 minutes and then the supernatant was collected.

2. **Sonication 1:4** (biomass to water ratio 1:4). Extraction of proteoglycans from the biomass of the cyanobacteria *Arthrospira platensis* was carried out with sonication procedure for 5 minutes. The biomass was subjected to centrifugation at 3500 rpm for 15 minutes and then the supernatant was collected.

3. **Hot water extraction with distilled water 1:3** (biomass to water ratio 1:3). Extraction of proteoglycans from the biomass of the cyanobacteria *Arthrospira platensis* was carried out with the procedure of heating on the water bath at temperature 100°C for 60 minutes. The biomass was subjected to centrifugation at 3500 rpm for 15 minutes and then the supernatant was collected.

4. **Hot water extraction with distilled water 1:4** (biomass to water ratio 1:4) – control sample. Extraction of proteoglycans from the biomass of the cyanobacteria *Arthrospira platensis* was carried out with the procedure of heating on the water bath at temperature 100°C for 60 minutes. The biomass was subjected to centrifugation at 3500 rpm for 15 minutes and then the supernatant was collected [32].

5. **Autoclaving 1:3** (biomass to water ratio 1:3). The biomass of the cyanobacteria *Arthrospira platensis* was autoclaved at 115°C 0.5atm for 30 minutes. The biomass was subjected to centrifugation at 3500 rpm for 15 minutes and then the supernatant was collected.

6. **Autoclaving 1:4** (biomass to water ratio 1:4). The biomass of the cyanobacteria *Arthrospira platensis* was autoclaved at 115°C 0.5atm for 30 minutes. The biomass was subjected to centrifugation at 3500 rpm for 15 minutes and then the supernatant was collected.

In this way, six proteoglycan extracts with the total dry matter of 25.0±1.7 - 37.0±1.7 mg/mL, were obtained and characterized by the content of carbohydrates, sulfated polysaccharides, proteins ans activity of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). It was important to determine the biochemical composition of the obtained proteoglycan extracts. It was found that the highest amount of protein 37.80%±0.24% DW was observed in the experimental samples - hot water extraction with distilled water (biomass to water ratio 1:3) and 39.62±0.7 DW autoclaving (biomass to water ratio 1:4). (Fig. 1). It was noted that the carbohydrate content in the experimental samples remains at the same level with the maximum carbohydrate accumulation 24.0 ± 1.4% DW in the case of autoclaving (biomass to water ratio 1:4).

The obtained proteoglycan extracts indicated a high amount of sulphated polysaccharides – from 758.0±0.86 mg/L to 799.0±1.73 mg/L for autoclaving (1:4) and (1:3) experimental samples (Fig. 2). For the samples which provide sonication of cyanobacterial biomass, lower amounts of sulfated polysaccharides were reported.

In contrast, the activity of antioxidant enzymes CAT and SOD in these samples was higher and constituted 20.0±0.5 - 53.3±0.8 mmol/min/mg protein and 54.7±2.8 - 66.3±2.3 U/mg protein, respectively (Fig. 3). According to literature data, SOD is active at 25°C and remains stable up to 45°C [6]. This may explain the decrease in enzyme activity at elevated temperatures in other experimental samples, as well as in the control variant.

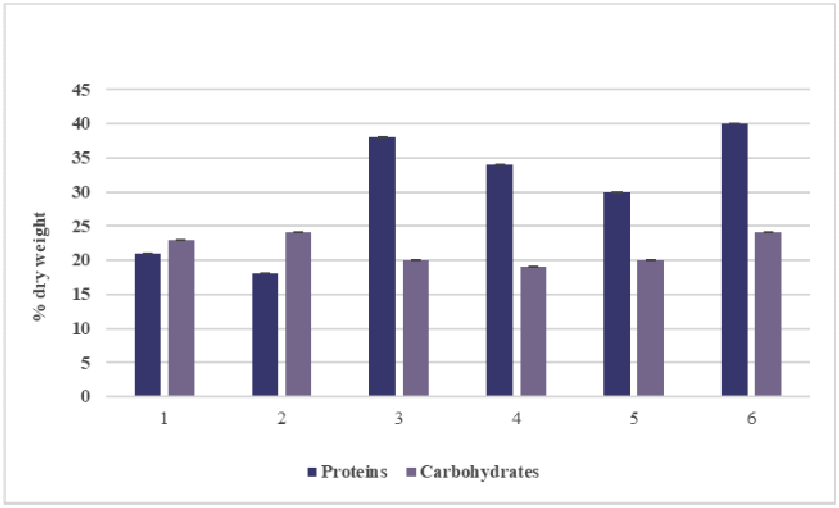

Figure 1. Content of proteins and carbohydrates in proteoglycanic extracts from the biomass of *A. platensis*, where: 1 - Sonication 1:3; 2 - Sonication 1:4; 3 - Hot water extraction 1:3; 4- Hot water extraction 1:4; 5 - Autoclaving 1:3; 6 - Autoclaving 1:4.

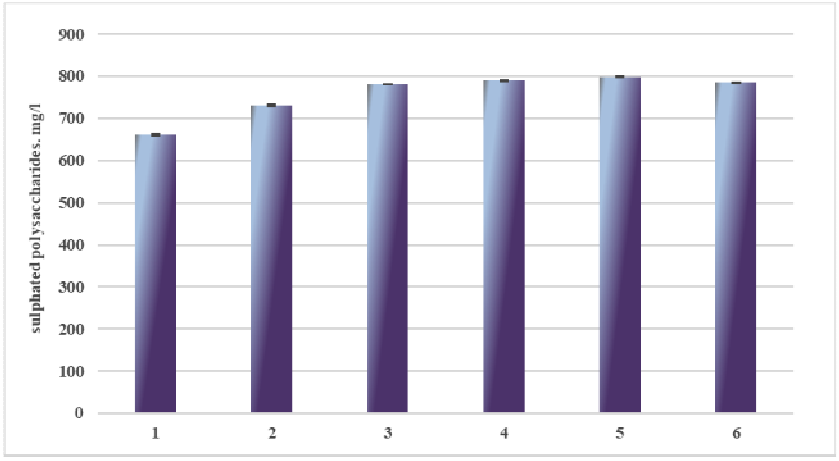

Figure 2. Content of sulfated polysaccharides in proteoglycanic extracts from the biomass of *A. platensis*, where: 1 - Sonication 1:3; 2 - Sonication 1:4; 3 - Hot water extraction 1:3; 4 - Hot water extraction 1:4; 5 - Autoclaving 1:3; 6 - Autoclaving 1:4.

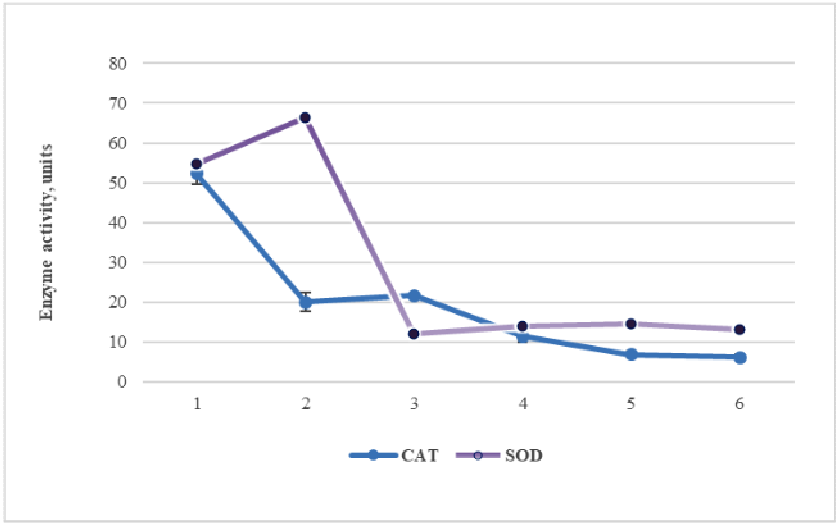

Figure 3. The activity of SOD and CAT enzymes in proteoglycanic extracts from the biomass of cyanobacteria *A. platensis*, where: 1 - Sonication 1:3; 2 - Sonication 1:4; 3 - Hot water extraction 1:3; 4- Hot water extraction 1:4; 5 - Autoclaving 1:3; 6 - Autoclaving 1:4.

Since the aim of this research was to develop the procedure for consecutive use of the remaining biomass of *A. platensis*, the proteoglycanic preparation was obtained as a result of the whole process. After extraction of the pigment and lipid preparations, the proteoglycan preparation was obtained. The scheme presented below (Fig. 4) consists of the following steps: the dried at the temperature of 50±5°C remaining

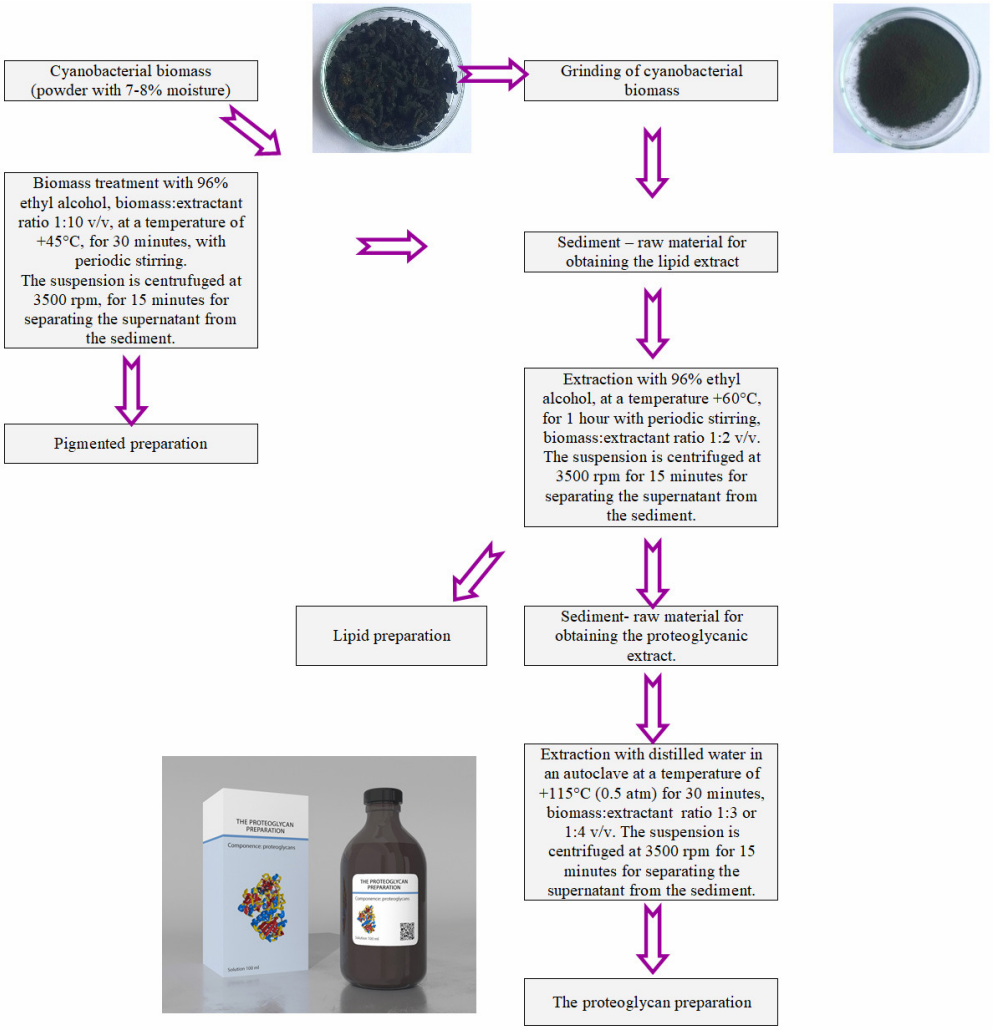

Figure 4. The procedure for obtaining of proteoglycan preparation from the remaining biomass of *A. platensis*

biomass of *Arthrospira platensis* was subjected to grinding; then it was mixed with 96% ethyl alcohol in a volume of 1:10, the obtained suspension was placed in a water bath at a temperature of +45°C, for 30 minutes and subjected to centrifugation at 3500 rpm. for 15 minutes, the separation of supernatant (pigment preparation); the sediment was mixed with 96% ethyl alcohol in a volume of 1:2, the obtained suspension was placed in a water bath at a temperature of 60°C, for 60 minutes, and subjected to centrifugation at 3500 rpm for 15 minutes, the separation of supernatant (lipid preparation); the sediment was mixed with distilled water at a ratio of 1:3 v/v and subjected to autoclaving at a temperature of 115°C (0.5 atm.) for 30 minutes, the obtained suspension was subjected to centrifugation at 3500 rpm; EDTA was added to the final proteoglycan preparation. The scheme presenting the obtaining of proteoglycan preparation is presented below (Fig. 4).

The biochemical composition of the obtained proteoglycan preparation was further studied (Table 1). According to the data obtained, the preparation was characterized by the increased protein (26.50±0.06% DW) and carbohydrate (20.71±0.42 DW) content, which corresponds to the method for its obtaining. The sulphated polysaccharide content was 733.4±1.55 mg/L.

Table 1. The biochemical composition of proteoglycan preparation.

Protein content, % DW	26.50±0.06
Carbohydrate content, % DW	20.71±0.42
Sulphated polysaccharide content, mg/L	733.4±1.55

Then, the antioxidant activity, as well as the activity of antioxidant enzymes presented great interest. The results from the Table 2 demonstrated the antioxidant activity of the obtained proteoglycan preparation. Studies on total antioxidant activity showed moderate activity (25.43±0.29% inhibition). Activity of SOD and CAT constituted 38.41±0.42 U/mg protein and 27.20±1.2 mmol/min, mg protein, respectively.

Table 2. Antioxidant activity of proteoglycan preparation.

Antioxidant activity, % inhibition	25.43±0.29
SOD, U/mg protein	38.41 ± 0.42
CAT, mmol/min, mg protein	27.20 ± 1.2

Interest to the amino acid research is conditioned by the possibility of solving a number of fundamental problems in the field of protein structure and their application in practice. Proteoglycan preparations with

a high amino acid content holds the prospect of the application in different economic fields. The study of amino acid content in proteoglycan preparation is demonstrated in Figure 5 and 6.

The sum of free amino acids was 793.3 mg/100 mL of which the sum of essential amino acids was 362.8 mg/100 mL (Fig. 5). The content of proteinogenic amino acids was 793.3 mg/100 mL, immunoactive - 415.5 mg/100 mL (Fig. 6).

DISCUSSION

The high rate of consumption of different types pharmaceutical preparations from natural sources worldwide has increased the demand for the utilization of residues of their production. Thus, the increased interest to obtaining of a high source of proteoglycans enriched in sulfated polysaccharides has appeared. Pharmaceutical residues management is an important aspect of pharmaceutical industries for the futher extraction of bioactive substances such as proteins, pigments, polysaccharides, lipids, antioxidants. According to previous studies, proteoglycans are widely used in cosmetic industry for increasing collagen density and promoting proliferation of dermal blast [25]. *Arthrospira platensis* biomass residues are obtained after the production of BioR bioremedy. The biomass contains protein, carbohydrate, sulfated polysaccharides, lipid and some components with antioxidant properties. Previously studies have been reported that spirulina residues from phycocyanin extraction can be used as a protein source for

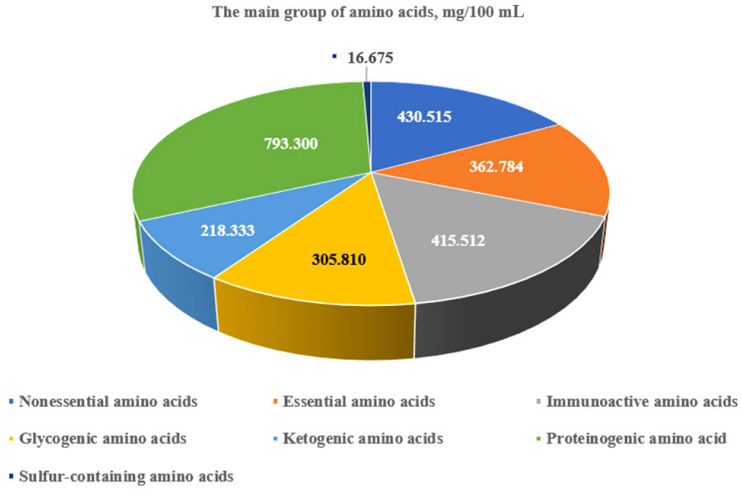

Figure 5. The main groups of amino acids in the proteoglycan preparation

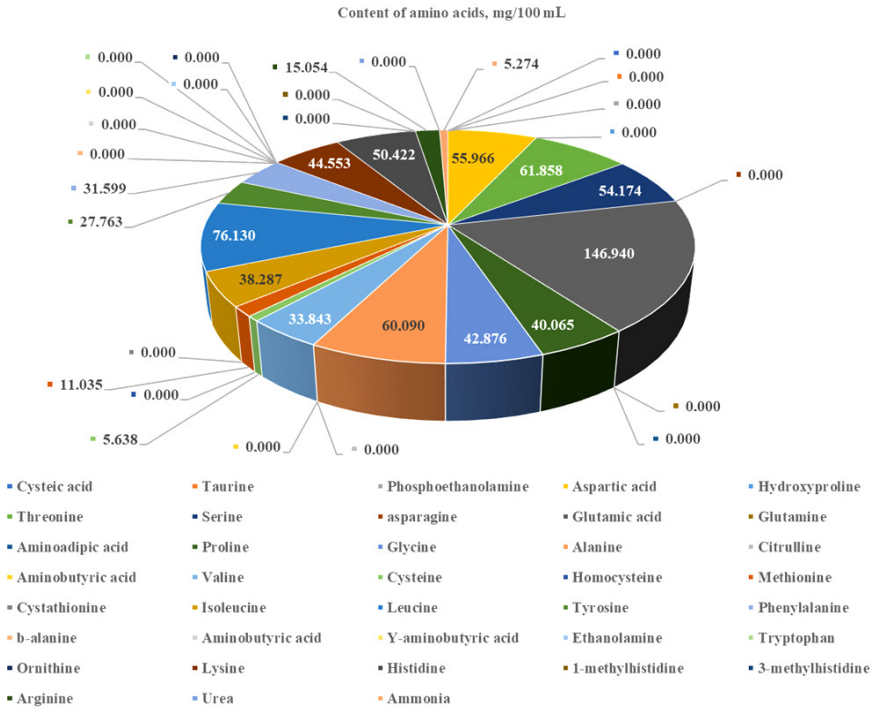

Figure 6. Content of amino acids in the proteoglycan preparation

Cordyceps militaris culture to replace the use of commercial Eri silk worms [15]. Spirulina has already been reported as a sustainable protein source because of high protein content- up to 70% protein per dry mass, has high protein yield than other sources such as plants, soybean, wheat. In this study, we evaluated the possibility of the utilization of remaining biomass of *Arthrospira platensis* for the obtaining of proteoglycan preparation.

The purification or extraction of byproducts from cyanobacteria by using ethanol is a common protocol in bioengineering and biochemical processes for industrial use [7]. In our research the use of the *A. platensis* biomass, environmentally safely production technologies have a potential for the use of proteoglycan preparations in research and practice. The proposed process for obtaining of proteoglycan extracts based on cyanobacteria remaining biomass is suitable for use in veterinary medicine and animal husbandry as an active component of dietary supplements. Obviously, the low toxicity and high biological activity of proteoglycans allow them to be widely used in various fields.

Previous studies have established that brown algal sulfated polysaccharides can prevent the development of oxidative stress and protect cells from free radical damage in various ways [27]. It is known from the literature that sulphated polysaccharides are able to neutralize superoxide radicals [32]. According to literature data, sulfated polysaccharides and extracts of *U. latuca* provide enzymatic (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic (reduction of glutathione and total thiols) antioxidant protection [14]. Sulphated polysaccharides obtained from seaweed have the function of protecting cells from free radical damage [9].

However, the majority of the studies reported the production of proteoglycans from animal (from aquatic animal tissues, especially fish head) sources and only a few have documented the production of proteoglycans from the remaining biomass of cyanobacteria and microalgae. Recent previous studies have been reported that raw material for proteoglycan extraction is animal tissue, especially salmon nasal cartilage [20], shrimp waste extract [1]. Also, previous studies have demonstrated the results that refers to the isolation of proteoglycans from bovine nasal cartilage. This procedure included the utilization of guanidinium chloride in the presence of protease inhibitors [26]. Extraction of proteoglycans from animal tissues as raw materials is limited. Furthermore, the extraction procedure often is complicated, needs additional purification from the components of animal tissues and includes the utilization of solvents unsuitable for utilization in food industry and are nonsuitable for medicine. So, the utilization of the proteoglycans are limited and the price is high. The method proposed in this research was based on the application of the autoclaving procedure at the temperature + 115°C (0.5 atm.) during 30 minutes that allows the utilization of residual biomass from the production of bioremedy BioR thus making the process environmentally and economically valuable.

The results of this research revealed that the remanent biomass of the cyanobacteria *Arthrospira platensis* left over from the bioremedy preparation process is a complete source of proteins and sulphated polysaccharides and a promising raw material for the production of proteoglycan preparations. A complex sequential technology that prevent environmental pollution by bioremediation for processing *Arthrospira platensis* cyanobacteria remaining biomass to obtain proteoglycan extracts has been developed. Moreover, rational parameters for the extraction of proteoglycans from the remaining biomass of the cyanobacterium *Arthrospira platensis* were established: autoclaving at 0.5 atm, 115°C, for 30 min. The proteoglycan preparation has a total content of sulfated polysaccharides 733.41±1.55 mg/L, proteins 26.50±0.06% DW, carbohydrates 20.71±0.42% DW and the total antioxidant activity 25.43±0.29% inhibition, superoxide dismutase activity 38.41±0.42 U/mg protein and catalase activity 27.20±1.2 mmol/min/mg protein.

The elaborated proteoglycan preparation has high potential for application in animal husbandry as dietary supplement for the regulation of productivity of livestock. Proteoglycans obtained from cyanobacteria are distinguished by a wide range of pharmacological effects and low toxicity. Also, the use of obtained proteoglycan preparation presents vast prospects for other fields such as the food, cosmetic and pharmaceutical industries and contributes to reducing the risks of environmental pollution.

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Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Abun, Widjastuti, T., Haetami, K., (2015): The characteristic of proteoglycans as naturalize product of shrimp waste extract with ion sulfate on broiler. Scientific Papers. Series D. Animal Science LVIII: 71-76.

[2] Araújo, I.W.F., Vanderlei, E.S.O., Rodrigues, J.A.G., Coura, C.O., Quinderé, A.L.G., Fontes, B.P., Queiroz, I.N.L, Jorge, R.J.B, Bezerra, M.M., Silva, A.A.R., Chaves, H.V., Monteiro, H.A.S., Paula, R.C.M., Benevides, N.M.B., (2011): Effects of a sulfated polysaccharide isolated from the red seaweed *Solieria filiformis* on models of nociception and inflammation. Carbohydrate Polymers, 86(3): 1207-1215. https://doi.org/10.1016/j.carbpol.2011.06.016.

[3] Arivuselvan, N., Radhiga, M., Anantharaman, P., (2011): *In vitro* antioxidant and anticoagulant activities of sulfated polysaccharides from brown seaweed (*Turbinaria ornata*) (Turner) J. Agardh. Asian Journal of Pharmaceutical and Biological Research, 1: 232-239.

[4] Besednova, N.N., Zvyagintseva, T.N., Andriukov, B.G., Zaporozhets, T.S., Kuznetsova, T.A., Kryzhanovsky, S.P., Guseva, L.G., Shchelkanov, M.Y.U., (2021): Seaweed-derived sulfated polysaccharides as potential agents for prevention and treatment of influenza and COVID-19. Antibiotics and Chemotherapy, 66(7-8): 50-66. https://doi.org/10.37489/0235-2990-2021-66-7-8-50-66.

[5] Bhaumik, M., Dhanarajan, G., Chopra, J., Kumar, R., Hazra, C., Sen, R., (2020): Production, partial purification and characterization of a proteoglycan bioemulsifier from an oleaginous yeast. Bioprocess and Biosystems Engineering, 43: 1747-1759. https://doi.org/10.1007/s00449-020-02361-1.

[6] Boon, H.T., Thean, C.L., Hooi, L.F., Raha, A.R., (2014): Molecular characterization of a recombinant manganese superoxide dismutase from *Lactococcus lactis* M4. BioMed Research International, 4: 469-498. https://doi.org/10.1155/2014/469298.

[7] Brezeștean, I., Bocăneală, M., Gherman, A.M.R., Porav, S.A., Kacsó, I., Rakosy-Tican, E., Dina, N.E., (2021): Spectroscopic investigation of exopolysaccharides purified from *Arthrospira platensis* cultures as potential bioresources. Journal of Molecular Structure, 1246: 131228. https://doi.org/10.1016/j.molstruc.2021.131228

[8] Bulimaga, V., Olan, O., (2011): Procedure for the quantitative determination of acidic and sulphated algal exopolysaccharides in culture fluid. Patent for invention 230 MD. [in Romanian]

[9] Coura, C.O., Araújo, I.W.F, Vanderlei, E.S.O., Rodrigues, J.A.G., Quinderé, A.L.G., Fontes, B.P., Queiroz, I.N.L., Menezes, D.B., Bezerra, M.M., Silva, A.A.R, Chaves, H.V., Jorge, R.J.B., Evangelista, J.S.A.M., Benevides, N.M.B., (2012): Antinociceptive and anti-inflammatory activities of sulphated polysaccharides from the red seaweed *Gracilaria cornea*. Basic & Clinical Pharmacology & Toxicology, 110(4): 33541. https://doi.org/10.1111/j.1742-7843.2011.00811

[10] Dantas-Santos, N., Gomes, D.L., Costa, L.S., Cordeiro, S.L., Costa, M.S.S.P., Trindade, E.S., Franco, C.R.C., Scortecci, K.C., Leite, E.L., Rocha H.A.O., (2012): Freshwater plants synthesize sulfated polysaccharides: heterogalactans from Water Hyacinth (*Eicchornia crassipes*). International Journal of Molecular Sciences, 13(1): 961-976. https://doi.org/10.3390/ijms13010961.

[11] Dey, P., Harborn, J., (1993): Methods in plant biochemistry. Carbohydrats. Academic Press, Cambridge, 2: 529.

[12] Gandhi, N.S, Mancera, R.L., (2008): The structure of glycos-aminoglycans and their interactions with proteins. Chemical Biology & Drug Design, 72(6): 455-482. https://doi.org/10.1111/j.1747-0285.2008.00741.

[13] Garaeva, S.N., Redkozubova, G.V., Postolati, G.V. (2009): Aminoacids of live organisms. Academy of Sciences of Moldova. Institute of physiology and sanocreatology, pp. 552.

[14] Hassan, S., El-Twab, S.A., Hetta, M., Mahmoud, B., (2011): Improvement of lipid profile and antioxidant of hypercholesterolemic albino rats by polysaccharides extracted from the green alga *Ulva lactuca Linnaeus*. Saudi Journal of Biological Sciences, 18(4): 333-340. https://doi.org/10.1016/j.sjbs.2011.01.005.

[15] Iamtham, S., Kaewkam, A., Chanprame, S., Pan-Utai, W., (2022): Effect of *Spirulina* biomass residue on yield and cordycepin and adenosine production of *Cordyceps militaris* culture. Bioresource Technology Reports, 17: 100893. https://doi.org/10.1016/j.biteb.2021.100893.

[16] Kanyamas, C., Chidchanok, S., Supawita, B., Kanlayanee, C., Jariya, C., Thunyaluk, M., (2018): Investigation of sulfated glycosaminoglycans and their agarose gel electrophoresis patterns from plant extracts. Journal of Associated Medical Sciences, 51(1): 11-18.

[17] Komina, A.V., Korostileva, K.A., Gyrylova, S.N., Belonogov, R.N., Ruksha, T.G., (2012): Interaction between single nucleotide polymorphism in catalase gene and catalase activity under the conditions of oxidative stress. Physiology Resources, 6: 655-658. https://doi.org/10.33549/physiolres.932333.

[18] Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., (1951): Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193: 265-275.

[19] Nekrasova, G.F., Kisileva, I.S., (2008): Educational and methodical complex of the discipline "Ecological physiology of plants". Guidelines for laboratory and practical classes. Ural State University, Yekaterinburg, pp. 157.

[20] Okamoto, Y., Higashi, K., Toida, T., (2021): A novel preparation method for a proteoglycan in a matrix with collagen from salmon (*Oncorhynchus keta*) nasal cartilage and its affinity to L-selectin. Japanese Journal of Food Chemistry and Safety, 28(1): 9-15.

[21] Pomin, V.H., (2015): Sulfated glycans in inflammation. The European Journal of Medicinal Chemistry, 92: 353-369. https://doi.org/10.1016/j.ejmech.2015.01.002.

[22] Prydz, K., Dalen, K.T., (2000): Synthesis and sorting of proteoglycans. Journal of Cell Science, 113: 193-205. https://doi.org/10.1242/jcs.113.2.193.

[23] Ragusa, I., Nardone, G.N., Zanatta, S., Bertin, W., Amadio, E., (2021): Spirulina for Skin Care: A Bright Blue Future. Cosmetics, 8(7): 19. https://doi.org/10.3390/cosmetics8010007.

[24] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, 26(9-10): 1231-1237. https://doi.org/10.1007/978-3-319-03880-3_8.

[25] Ruiz Martinez, M.A., Peralta Galisteo, S., Castan, H., Morales Hernandez, M.E., (2020): Role of proteoglycans on skin ageing: a review. International Journal of Cosmetic Science, 42(6): 529-535. https://doi:10.1111/ics.12660.

[26] Sama, B.A., (2011): The extraction of tropocollagen and proteoglycans from bovine byes and investigation of their recombination using a new method to quantify fibrillogenesis. Thesis for the degree Master of Science, Department of Mechanical and Industrial Engineering, Northeastern University, Boston, Massachusetts.

[27] Si, M., Wenjian, Y., Gangliang, H., (2021): Antioxidant activities and mechanisms of polysaccharides. Chemical Biology & Drug Design, 97(3): 628-632. https://doi.org/10.1111/cbdd.13798.

[28] Smith, R.A., Meade, K., Pickford, C.E., Holley, R.J., Merry, C.L.R., (2011): Glycosaminoglycans as regulators of stem cell differentiation. Biochemical Society Transactions, 39(1): 383-387. https://doi.org/10.1016/j.colsurfb.2016.11.022.

[29] Vanthoor-Koopmans, M., Cordoba-Matson, M.V., Arredondo-Vega, B.O., Lozano-Ramírez, C., Garcia-Trejo, J.F., Rodriguez-Palacio, M.C., (2014): Microalgae and cyanobacteria production for food and food supplements. Biosystems Engineering: Biofactories for Food Production in XXI Century. pp. 253-275. https://doi.org/10.1007/978-3-319-03880-3_8.

[30] Vo, T.S., Kim, S.K., (2010): Potential anti-HIV agents from marine resources: an overview. Marine Drugs, 8: 2871-2892. https://doi.org/10.3390/md8122871.
[31] Volpi, N., (2006): Therapeutic applications of glycosaminoglycans. Current Medicinal Chemistry, 13(15): 1799–810. https://doi.org/10.2174/092986706777452470.
[32] Zhang, W., Wang, J., Jin, W., Zhang, Q., (2013): The antioxidant activities and neuroprotective effect of polysaccharides from the starfish *Asterias rollestoni*. Carbohydrate Polymers, 95(1): 9-15.
[33] Zosim, L., Bulimaga, V., Rudic, V., Gulea, A., Tapcov, V., (2019): Procedeu inovativ de sporire a conținutului de polizaharide acide la cianobacteria *Spirulina platensis*. Intellectus, 1-2: 139-142.

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