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OPTIMIZATION OF THE PROCESS FOR OBTAINING ANTIOXIDANT EXTRACTS RICH IN *β*-CAROTENE FROM THE RESIDUAL BIOMASS OF *ARTHROSPIRA PLATENSIS*

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Abstract: This study aimed to optimize the process of obtaining antioxidant extracts rich in *β*-carotene from the remaining biomass of *Arthrospira platensis*. Optimal extraction procedures using ultrasound, homogenization and temperature were investigated at the laboratory level.
The results demonstrated that the highest yield of *β*-carotene in the extracts was obtained using 96 % ethylic alcohol at a temperature of +45 °C for 30 minutes and a biomass/solvent ratio of 1 : 50 g·mL^{-1} and 1 : 10 g·mL^{-1}. The activity of the antioxidant enzymes catalase and superoxide dismutase varied depending on the extraction method, and the highest activity was obtained in the experimental variants that were placed at the temperature of +35 °C and +45 °C. Considering the valuable biochemical composition and enzymatic activity, the antioxidant extracts obtained have a relevant potential for implementation in various fields such as animal husbandry, medicine and food.

Keywords: *Arthrospira platensis, antioxidant extracts, β-carotene, catalase, superoxide dismutase*

INTRODUCTION

Currently, particular attention of biotechnological research is directed towards the use of microalgae *Arthrospira platensis* which possesses high biological activity to produce extracts with a beneficial effect on human and animal health [1, 2]. A basic component of interest in cyanobacterial biomass are carotenoid pigments such as *β*-carotene and lycopene that protect vulnerable biomolecules in the cell from the damaging effects of environmental stress and reactive oxygen species [3, 4]. According to the literature, β-carotene protects humans from serious disorders associated with oxidative and inflammatory stress, including skin degeneration and aging, cardiovascular disease, certain cancers, and age-related eye diseases [5]. In addition, research has suggested that *β*-carotene extracted from *Arthrospira platensis* may have neuroprotective effects and may be useful in the prevention and treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease [6 – 8].

Extracts rich in β-carotene also play an important role in the zootechnical sector, being used to increase the productive and reproductive potential of animals. In animal studies, it has been established that β-carotene protects the plasma membrane against lipid peroxidation and loss of sperm viability [9, 10]. The susceptibility of spermatozoa to oxidative stress is a consequence of the abundance of polyunsaturated fatty acids in the sperm plasma membrane, the presence of which gives the membranes fluidity and flexibility that help sperm engage in the membrane fusion events associated with fertilization. Unfortunately, the presence of double bonds in these molecules makes them vulnerable to free radical attack and the initiation of the lipid peroxidation cascade. This results in subsequent loss of membrane integrity, creates morphological defects, impairs cellular functions, along with decreased sperm motility and induction of sperm apoptosis [11].

Also, the results of the studies provided that feeding chickens with microalgae extracts enriched with *β*-carotene contributes to a dark yellow color of the yolk [12] and significantly improves egg quality parameters such as shell thickness, albumin height, increases the ability of antioxidant and immune response of chickens [13]. Positive effects on quality have been recorded when using *β*-carotene for cattle feed and aquaculture as a supplement for shrimp and fish [14]. Consequently, the use of β-carotene to improve the quality of meat, eggs and dairy products will provide more benefits for animals as well as for human health when consumed [15 – 17].

Thus, studies on the optimization and obtaining of extracts with high *β*-carotene content for application in food, medicine and the zootechnical sector are becoming necessary, especially for stimulating the productive and reproductive potential of animals.

Based on the above, the research aims to optimize the process of obtaining antioxidant extracts rich in β-carotene from the remaining biomass of *Arthrospira platensis.*

MATERIALS AND METHODS

Objects of research

The dried biomass of *Arthrospira platensis* cyanobacteria, which remained after the production of the commercial BioR remedy, was used as research material. The biomass was provided by the company "Ficotehfarm" SRL, Chisinau, Republic of Moldova.

The remaining *Arthrospira platensis* cyanobacteria biomass was dried at a temperature of +50 ± 5 °C after which it was subjected to grinding in a grinder (Saturn ST-CM1231) for 3 min. The procedures for obtaining the extracts with the application of temperature were carried out using 96 % ethyl alcohol and performing the extraction at the following temperatures: +20 °C with permanent stirring at to rotary agitator to 200 rpm, +35 °C and +45 °C for 30 min with manual stirring of the flasks every 5 minutes. At the end of the process, the extracts were separated from the biomass by centrifugation (MPW-310) at 3500 rpm for 10 minutes.

The following experimental variants were carried out by homogenizing (Heidolph SilentCrusher M) the biomass for 5 minutes or 10 minutes, then the *β*-carotene content was extracted using 96 % ethyl alcohol, at room temperature, by permanent stirring at 200 rpm, for 30 minutes, centrifugation at 3500 rpm for 10 minutes.

The effect of ultrasound on obtaining *β*-carotene based extracts was studied by mixing biomass with 96 % ethyl alcohol and subjecting the mixture to ultrasonic treatment using a 50 W ultrasound processor. The ultrasound was applied at 50 % amplitude for varying durations of 3 minutes or 5 minutes, all conducted at room temperature. Following ultrasonication, the samples underwent centrifugation at 3500 rpm for 10 minutes.

Methods of achieving research

The *β*-carotene content was determined spectrophotometrically (Shimadzu UV-1280) according to the method described by Delia et *al*. (2001) [18]. The method is based on extracting the pigments using 96 % ethyl alcohol and determining the absorbance of the extract at a wavelength of 450 nm.

The activity of the antioxidant enzyme catalase (CAT) was determined by the spectrophotometric method [19]. The principle of the method is based on the ability of hydrogen peroxide to interact with molybdenum salts, which in turn leads to the formation of a resistant colored complex.

Superoxide dismutase (SOD) activity was determined by the method based on the inhibition of the reduction of tetrazolium-nitro blue salt in the presence of tetramethylethylenediamine and riboflavin [20]. The protein content of the samples was determined according to the method described [21]. The principle of the method is based on the formation of a copper complex with peptide bonds and its subsequent reduction in an alkaline environment.

Statistical analysis

All experiments shown were performed in 3 replicates. The results were expressed by calculating the mean, ± standard deviation and confidence interval for a mean. All differences between calculated values were considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

In this study, the impact of ultrasound, homogenization, and temperature on the process of obtaining antioxidant extracts with high content of *β*-carotene from the residual biomass of *Arthrospira platensis* was evaluated. According to the obtained results, it was determined that the content of *β*-carotene in the extracts varies within the limits of 0.2395 ± 0.002 - 0.6875 ± 0.010 mg/100 g (Figure 1). The highest results were recorded in the experimental variant of obtaining extracts with 96 % ethyl alcohol, at a temperature of +45 °C, for 30 minutes, with periodic stirring, the content being 0.6875 mg/100g, with 187 % more compared to the control variant. The lowest *β*-carotene content was obtained in the variant in which the extraction was carried out at room temperature 0.2395 ± 0.002 mg/100 g and in the experimental variant in which the biomass was subjected to homogenization for 10 minutes 0.4591 ± 0.027 mg /100 g.

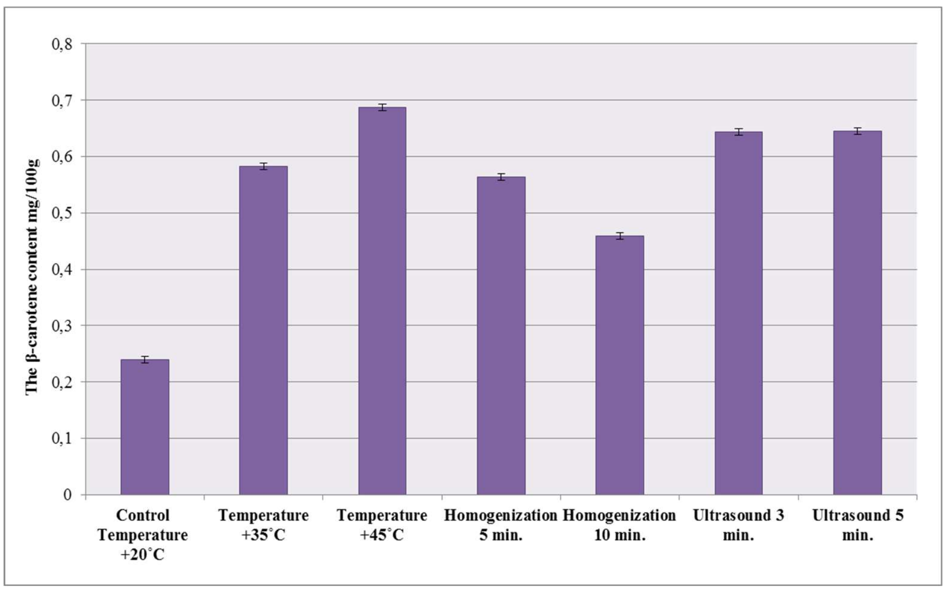

Figure 1. *The content of β-carotene in the ethanolic extracts obtained from the remaining biomass of Arthrospira platensis*

Similar findings regarding *β*-carotene extraction methods from *Arthrospira platensis* were obtained by other researchers, who determined that temperature significantly influences the extraction process. The results presented by Palma et *al*. (2013) suggest that temperature influences solubility, solute diffusivity and liquid viscosity and should be selected according to the applied organic solvent [21]. Additionally, the study presented by Soumen et *al*., 2013 demonstrates that the low temperature of +10-20 °C decreases the extraction yield of *β*-carotene content, as the temperature increased to +30 °C, the extraction capacity becomes relatively higher [22]. The use of ultrasound in the extraction of β-carotene from *Arthrospira platensis* also presents a promising way to improve the efficiency and effectiveness of the extraction process. The remarkable ability of ultrasound to destroy cell walls and membranes plays a key role in its impact on the extraction of *β*-carotene [23] and other bioactive compounds.

One of the most important parameters for the optimization of the extraction process is the biomass-to-solvent ratio. Therefore, the extraction yield was measured at different biomass-to-solvent ratios, ranging from 1 : 10 to 1 : 200 g·mL^{-1}, at a temperature of +20 °C. The results obtained are presented in Figure 2.
It was determined that the biomass/solvent ratio of 1 : 50 g·mL^{-1} and 1 : 10 g·mL^{-1} gave maximum extraction yield, the content being 1.1685 ± 0.01 mg/100 g and 1.0247 ± 0.025 mg/100 g. The increase in extraction yield with changes in the biomass-to-solvent ratio can be attributed to the initial conditions, where a high solvent volume at a 1 : 200 g·mL^{-1} ratio resulted in a low concentration of extracted *β*-carotene.
As the biomass concentration increases the concentration gradient between the solid biomass and the liquid ethanol becomes more favorable, enhancing the mass transfer of *β*-carotene from the solid phase to the liquid phase, thereby resulting in increased extraction yields.

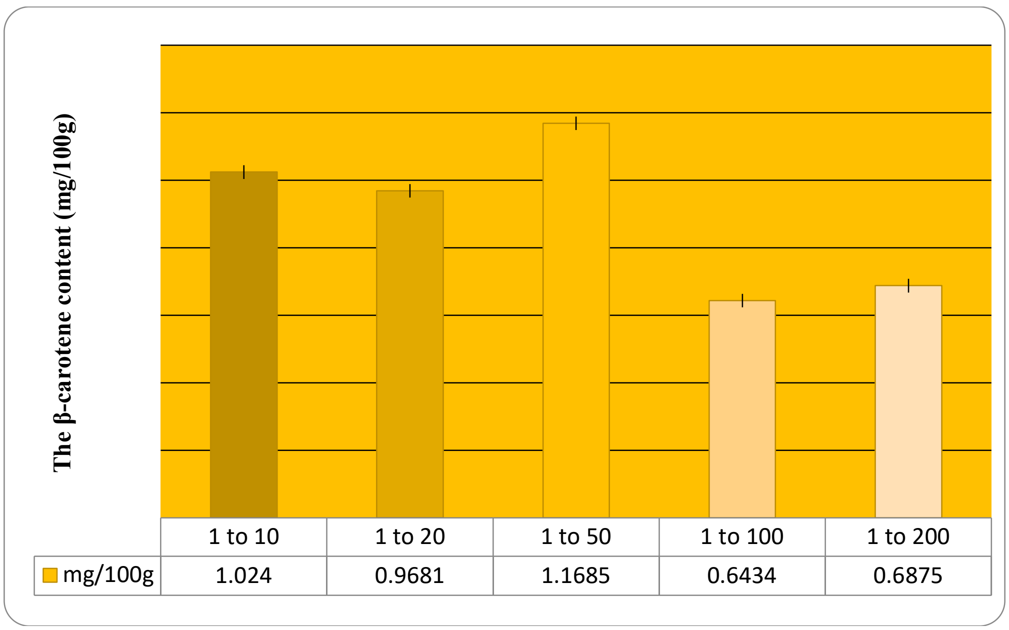

Figure 2. *The content of β-carotene in the ethanolic extracts obtained from the remaining biomass of the Arthrospira platensis cyanobacteria depending on the biomass-solvent ratio*

In the next step, the activity of the antioxidant enzymes CAT and SOD, which are indispensable in many biochemical processes including intracellular signaling, defense and cell function was evaluated [24, 25]. As a result, it was established that in the experimental variants, CAT activity varies within the limits of 165.6 ± 8.9-763.85 ± 8.13 mmol·min^{-1}·mg^{-1} protein and SOD activity is within the limits of 89.89 ± 4.41-660.04 ± 1.49 U·mg^{-1} protein. The highest activity of CAT and SOD was recorded in the extraction variants at the temperature of +35 °C and +45 °C (Figure 3). Conversely, the variant in which homogenization was performed for 10 minutes showed an inhibitory effect on enzyme activity.
Comparing the results of this study with other sources, we can see that the levels of CAT and SOD activity vary depending on the extraction methods [26]. In the study presented

by Ramos-Romero et *al*., (2017) it was determined that the activity of SOD and CAT enzymes in *Spirulina platensis* extract was affected by the extraction temperature and time. Higher enzyme activity was recorded at temperatures of +40 -60 °C which is consistent with the findings of this study [27].

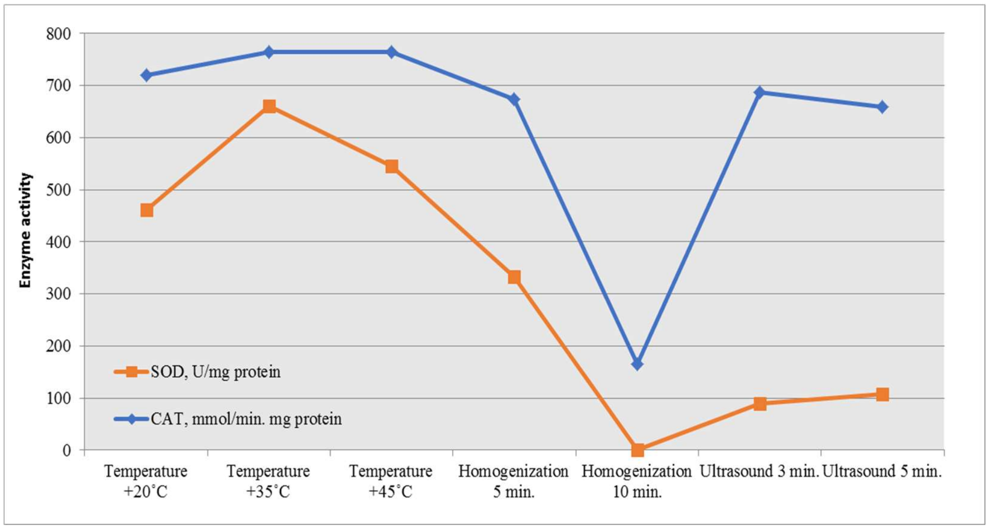

Figure 3. *The activity of the antioxidant enzymes CAT and SOD in the ethanolic extracts obtained from the remaining biomass of Arthrospira platensis cyanobacteria*

Furthermore, the inhibitory effect of homogenization on enzyme activity was also observed in the study reported by Kim et *al*., (2014) who established that excessive homogenization led to a decrease in SOD activity in *Spirulina platensis* biomass [28].
Based on the aforementioned information, the results obtained present substantial advantages from both an environmental standpoint, as they can contribute to reducing expenses related to industrial waste management and from the perspective of biological properties and economic potential.

CONCLUSIONS

The optimization of the methods for obtaining antioxidant extracts rich in *β*-carotene resulted in the highest yield in the experimental variant of extraction with 96 % ethyl alcohol, at a temperature of +45 °C, for 30 minutes and a biomass/solvent ratio of and 1 : 10 $g \cdot mL^{-1}$. The obtained extract is also characterized by high activity of the antioxidant enzymes CAT and SOD. It was established that the values of CAT enzyme activity are 763.85 ± 8.13 $mmol \cdot min^{-1} \cdot mg^{-1}$ protein and SOD 545.95 ± 1.16 $U \cdot mg^{-1}$ protein. Considering the valuable biochemical composition and the established enzymatic activity, the biologically active extract based on *β*-carotene obtained from the remaining biomass of *Arthrospira platensis* cyanobacteria has a relevant potential for application in various fields. Therefore, it represents a valuable resource for future research and development.

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REFERENCES

1. Hoseini, S.M., Khosravi-Darani, K., Mozafari, M.R.: Nutritional and medical applications of *Spirulina* microalgae, *Mini-Reviews in Medicinal Chemistry*, **2013**, **13**, 1231-1237;
2. Wu, Q., Liu, L., Klímová, B., Wan, D., Kuca, K.: The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: an overview, *Archives of Toxicology*, **2016**, **90**, 1817-1840;
3. Beșliu, A.: Carotenoid biosynthesis potential and morphological characters of *Rhodotorula gracilis* yeast under the action of Fe_3O_4 nanoparticles, Studia Universitatis Moldaviae, **2018**, **1** (111), 66-70;
4. Kim, J.Y., Lee, S.Y., Kang, S.H.: Lycopene suppresses oxidative stress-induced mitochondrial dysfunction and apoptosis by upregulating the Nrf2 pathway in human neuroblastoma cells, *Biomolecules & Therapeutics*, **2019**, **27** (4), 343-353;
5. Rao, A.V., Rao, L.G.: Carotenoids and human health, *Pharmacological Research*, **2007**, **55**, 207-216;
6. Wang, X., Liu, R., Zhang, W., Zhang, X.: The neuroprotective effects of carotenoids on Alzheimer's disease development and progression: A systematic review, *Molecules*, **2016**, **21** (10), 1312;
7. Zhang, Y., Xun, P., Wang, R., Mao, L., He, K.: Can dietary beta-carotene intake decrease the risk of Alzheimer's disease?, A meta-analysis of observational studies, *Brain and Behavior*, **2017**, **7** (10), e00812;
8. Uysal, O., Bucak, M.N.: Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen, *Acta Veterinaria Brno*, **2007**, **76** (3), 383-390;
9. Rao, B., Soufir, J.C., Martin, M., David, G.: Lipid peroxidation in human spermatozoa as related to midpiece abnormalities and motility, *Gamete Research*, **1989**, **24** (2),127-134;
10. Ben-Amotz, A., Levy, Y.: Bioavailability of a natural isomer mixture compared with synthetic all-trans β-carotene in human serum, *The American Journal of Clinical Nutrition*, **1996**, **63** (5), 729-34;
11. Bucak, M.N., Sariözkan, S., Tuncer, P.B.: The effect of antioxidants on post-thawed Angora goat (*Capra hircus ancryrensis*) sperm parameters, lipid peroxidation and antioxidant activities, *Small Ruminant Research*, **2010**, **89**, 1-24;
12. Wang, Y.F., Han, Y., Hu, W., Yang, Y., Zhang, X., Zhao, X., Wang, M., Zhou, Z., Cheng, Z.: Effect of β-carotene on productive performance, egg quality, antioxidant capacity, and immune response of laying hens, *Poultry Science*, **2019**, **98** (2), 719-727;
13. Ben-Amotz, A.: Industrial production of microalgal cell-mass and secondary products major industrial species *Dunaliella* in: *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, (Editor: Richmond, A.), Blackwell Publishing Ltd, **2004**, 273-280;
14. Saleh, A.A., Metwally, H.M.: Effects of β-carotene on growth performance, antioxidant capacity and meat quality in broiler chickens, *Journal of Animal Physiology and Animal Nutrition*, **2014**, **98** (5), 945-955;
15. Barkia, I., Saari, N., Manning, S.R.: Microalgae for high-value products towards human health and nutrition, *Mar Drugs*, **2019**, **17** (5), 304;
16. Saadaoui, I., Rasheed, R., Abdulrahman, N., Bounnit, T., Cherif, M., Al, Jabri H., Mraiche, F.: Algae-derived bioactive compounds with anti-lung cancer potential, *Marine Drugs*, **2020**, **18** (4), 197;
17. Rodriguez-Amaya, D.B.: *A guide to carotenoid analysis in food*, International Life Sciences Institute, One Thomas Circle, N.W., Washington, D.C, **2001**, 64;

18. Komina, A.V., Korostileva, K.A., Gyrylova, S.N., Belonogov, R.N., Ruksha, T.G.: Interaction Between Single Nucleotide Polymorphism in Catalase Gene and Catalase Activity Under the Conditions of Oxidative Stress, *Physiology Research*, **2012**, **61**, 655-658;
19. Nekrasova, G.F., Kiseleva, I.S.: Guide to laboratory and practical exercises, *UMKD Ecological plant physiology*, **2008**, 157;
20. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent, *Journal of Biological Chemistry*, **1951**, **193**, 265-275;
21. Palma, M., Barbero, G., Pineiro, Z., Liazid, A., Barroso, C., Rostagno, M., Prado, J., Meireles, M.: Extraction of natural products: principles and fundamental aspects. Rostagno MA (ed) Natural product extraction principles and applications, *Royal Society of Chemistry*, **2013**, 56-58;
22. Soumen, D., Virendra, K.: Ultrasound assisted extraction of β-carotene from *Spirulina platensis*, *Ultrasonics Sonochemistry*, **2013**, **20** (1), 271-276;
23. Ashwini, J., Purohit, P., Gogate, R.: Ultrasound-Assisted Extraction of *β*-Carotene from Waste Carrot Residue: Effect of Operating Parameters and Type of Ultrasonic Irradiation, *Separation Science and Technology*, **2015, 50** (10), 1507-1517;
24. Lassegue, B., Griendling, K.K.: NADPH oxidases: functions and pathologies in the vasculature. Arteriosclerosis, *Thrombosis, and Vascular Biology*, **2010**, **30**, 653-661;
25. Ighodaro, O.M., Akinloye, O.A.: First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid, *Alexandria Journal of Medicine*, **2018**, **54** (4), 287-29;
26. Alzahrani, A.M., Alamri, M.S., El-Bassossy, H.M., El-Ansary, A.K.: In vitro and in vivo antioxidant activities of *Spirulina platensis* extract, *Journal of Dietary Supplements*, **2019**, **16** (2), 139-151;
27. Ramos-Romero, S., Otero, A., Garcia-Salinas, C., Jimenez-Escrig, A., Sánchez-Muniz, F.J., Benedi, J.: Production of protein hydrolysates from *Spirulina platensis* by enzymatic hydrolysis: Effects on antioxidant activities and functional properties, *Journal of Food Science and Technology*, **2017**, **54** (10), 3118-3128;
28. Kim, S.K., Karadeniz, F., Ahn, B.N., Kim, Y.J.: In vitro antioxidant properties of solvent extracts from cultured marine microalgae and evaluation of their synergistic effects with antioxidant vitamins and synthetic antioxidants, *Food chemistry*, **2014**, **142**, 62-69.