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Enhancing extraction and purification of phycocyanin from *Arthrospira sp.* with lower energy consumption

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Abstract

Phycocyanin is a light-blue water-soluble natural pigment with wide application in fields including pharmaceutical, cosmetic and food industry. *Arthrospira platensis* is considered a natural bio-factory for obtaining phycocyanin, and although suitable cultivation conditions can enhance the pigment content, the importance of extraction and purification should not be overlooked, because the chemicals used in those steps can affect the quality of phycocyanin. Further, changes in temperature, or approaches used to extract other protein types from biomass, have been reported to degrade phycocyanin, influencing its final quality, while also contributing to higher energy consumption in the extraction process. Hence, this work compares several approaches for phycocyanin extraction, and analyzed the effects of changes in operating conditions, such as exposure time, application of solvent buffer, and ultrasonication, on the extraction yield, the extracts purity, and the process energy use efficiency. Experimental results point to promising improvements in the extraction yield with high purity of phycocyanin (44.24 mg/g with purity ratio of 0.34), when suitable operating parameters were used (2 h exposure to potassium phosphate buffer with 20 min of ultrasonication).

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Keywords: *Arthrospira platensis*; Extraction; Phycocyanin; Phycobiliprotein; Purification; Solvent-buffer

1. Introduction

Microalgae are unicellular or multi-cellular microorganisms, living in various environmental conditions and habitats. Being highly adaptable, they are considered to have several potential industrial applications [1] as they can

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synthesize and accumulate a broad spectrum of different secondary metabolites and bioactive compounds [2]. In particular, currently there has been a great interest in microalgae as a source of bioactive compounds for cosmetic and biomedical applications, including for the formulation of anti-aging creams, refreshing products, anti-irritation peeler and wound healing patches [3]. Microalgae are also used in food and dietary supplements for human nutrition, as a source of protein, ω-3 fatty acids, essential amino acids, vitamins (A, B, C, E) and minerals. Bioactive compounds, especially obtained from natural sources (alternative to synthetic ones) are increasingly attracting attention of the market, industry and scientific fields. Products formulated as creams, lotions, shampoos, and colorants, containing antioxidants, pigments, unsaturated lipids, UV-screens and vitamins from natural sources, such as microalgae extracts are likely to be invested [4].

Among microalgae, the *Arthrospira* strain has been investigated for a long time. Applications include the use of *Arthrospira maxima* (*A. maxima*) for wastewater treatment [5] and as a source of lipids for biofuel [6] and the use of *Arthrospira platensis* (*A. platensis*) in the skin care market [3]. *A. platensis* is a filamentous blue–green non-toxic cyanobacterium belonging to the *Phormidiaceae* family and can be found in alkaline lakes. For several centuries, it has been consumed as food supplement, as it contains protein (55%–70%), fatty acids (3%–9%), carbohydrates (15%–30%), phycobiliproteins, minerals and vitamins, among other compounds [7]. Being approved by the Food and Drug Administration (FDA), *A. platensis* is categorized as GRAS, which stands for being "generally recognized as safe" [8]. There are various medical and pharmaceutical research studies highlighting the beneficial effects of *A. platensis* extracts on skin, as well as on hair and nail protection [3]. Besides, those studies claim that extracts from microalgae can contribute to skin wounds healing, while they can inhibit the inflammation process [8,9].

Phycocyanin, a water-soluble natural pigment–protein complex from the light-harvesting phycobiliprotein family, contributes to the pharmaceutical effects of *A. platensis*. It is a major phycobiliprotein, consisting of glutamic acid, alanine, leucine, aspartic acid, isoleucine, serine, arginine, glycine and threonine [10]. Phycobiliproteins are accessory pigments that play an important role in photosynthesis, being accumulated in cells as phycobilisomes attached to the chloroplast thylakoid membrane [9]. The tetrapyrrole chromophore phycocyanobilin attached to the apoprotein is the reason for the deep blue color of phycocyanin [11]. This compound is responsible for the free-radical scavenging activity of *A. platensis* by stimulating the production of antibodies, which is associated to regulation of genes encoding cytokines. Besides the medical and pharmaceutical applications, it has been widely used as color additive in food industry, mainly in chewing gums, dairy products and jellies. Moreover, there are cosmeceutical usages for the colorful pigments in lipsticks and eyeliners. Other application is as fluorescent reagent [12].

Extraction using inorganic solvents has been discussed in several experiments as an efficient and alternative method for phycocyanin recovery. Soxhlet extraction is a popular approach to extract *Arthrospira's* contents; however, it is considered to be less favorable in two ways: high energy consumption, and employment of toxic chemicals [13]. Aqueous two-phase system (ATPS) has been evaluated as an appropriate extraction method for biomolecules (proteins, antibodies, DNA and nutraceuticals) for experimental purposes, but in industrial applications, solvent recovery is known to be an ill-posed step in ATPS [14]. Another method is the one used by Sarada et al. [15], where sodium phosphate salt is added to the crude extract (making the total weight of the system 100% w/w) and mixing thoroughly using a magnetic stirrer for achieving equilibrium. The extraction process was finished by separating the phases via gravity (centrifugation). Then, the volumes of supernatant and bottom phase were analyzed for the phycocyanin (C_p) and total protein concentrations. Availability of the solvents make this method favorable in several investigations, as [16] reported a C-phycocyanin yield of approximately 79% with 4.32 purity using this extraction technique.

For phycobiliproteins purification, several methods have been described [17]. Among all, in this experiment, we focused on the combination of techniques, namely centrifugation and precipitation with $(NH_4)_2SO_4$. This method was also intended by Kumar et al. [12], who used the precipitation with aqueous ammonium sulfate solution, obtaining a phycocyanin recovery of 80%, with 1.5 purity. The inclusion of anion exchange chromatography in combination with the application of ammonium sulfate solution, resulted in a purity increase to the analytical grade of 4.58 purity. Therefore, this work aims to study the extraction and purification methods of phycocyanin from *A. platensis*. Focusing on the compound of interest, three extraction approaches are tested and discussed, and the purification methods explained to find the proper sequence. The analysis and discussion of results aim to find an efficient and environmentally benign approach to extract and purify phycocyanin.

2. Materials and methods

2.1. Extraction and quantification of phycocyanin

The experimental study conducted in this work aims to evaluate three approaches for phycocyanin extraction from *Arthrospira sp.* and to determine which one results in the highest extraction yield. It is considered the employment of non-toxic solvents and it is evaluated the influence of operating parameters.

Materials used in this investigation consist of *Arthrospira* sp. strain obtained from commercial side, branded Neotea, 0.1 mol/L phosphate buffer solutions with a pH of 6.5 prepared using adequate volumes of 0.2 mol/L sodium phosphate dibasic (Na_2HPO_4) solution and 0.2 mol/L of monosodium phosphate (NaH_2PO_4) or 0.2 mol/L potassium phosphate dibasic (K_2HPO_4) solution and 0.2 mol/L of monopotassium phosphate (KH_2PO_4).

With the objective to investigate the effect of several parameters on the phycocyanin extraction from *Arthrospira* sp. biomass, different solvents, exposure time to solvents and ultrasonication times were studied. The experiments were carried out in two phases, each one aiming to individually enhance the total extraction yield. For samples preparation, 40 mg of lyophilized *Arthrospira* sp. sample was mixed in 20 ml of solvent, during 10 min in vortex. Each sample was then used in each phase to initiate extraction.

In the first phase, three experiments were conducted to verify the best approach among solvent-less, sodium phosphate buffer and potassium phosphate buffer. After determining the approach with the highest yield, the second phase was proceeded by employing ultrasonication to enhance the phycocyanin recovery via modifying the sonication time. Besides, another parameter being investigated is the exposure time to solvent buffer. To quantify the concentration of extracted C-phycocyanin, *Cp*, Eq. (1) is employed [18]:

$$Cp = \frac{Abs_{620} - 0.474 \times Abs_{652}}{5.34} \tag{1}$$

where ABS_{620} and ABS_{652} are respectively the samples absorbance at 620 and 652 nm wavelength. To determine the amount of phycocyanin extracted in the experiments (mg of phycocyanin per g of biomass), Eq. (2) was used:

$$EC_P = \frac{Cp \times V}{m_B} \tag{2}$$

where EC_P is the C-phycocyanin concentration (mg/mg), V is the solvent volume (mL), and m_B is the *Arthrospira* sp. dry weight mass (mg).

2.2. Effect of extracting solvent

To evaluate the effect of the solvent used for phycocyanin extraction, in the first phase of this work it was studied the effect of using as solvents: distilled water, sodium phosphate buffer solution (100 mM) at pH 6.5, and potassium phosphate buffer solution (100 mM) at pH 6.5.

In the experiment in which water was used as solvent, the wet sample was frozen for 24 h and thawed, for two consecutive times. In the other two experiments in which the buffer solvents are employed, there is no need for freeze/thaw. Subsequently the samples were taken to the vortex (VELP SCIENTIFICA Zx3) for 10 min. The suspension was placed in an orbital shaker (Certomat® R) at 100 rpm for 2 h, followed by sample centrifuging (Ecco 2S 8501 centrifuge) at 4000 rpm for 20 min.

The supernatant was collected and a spectrophotometric reading (Jasco V-660 UV–VIS) at 620 and 652 nm was performed to assess the phycocyanin concentration and extraction yield.

2.3. Effect of contact time and ultrasonication of microalgae with extraction solvent

The second phase of the process comprises the evaluation of the effect of exposure time to the solvent-buffer, as well as of using ultrasonication. In this regard, each sample was contacted with potassium phosphate buffer for 2, 4 and 24 h, in the orbital shaker. After the required time for shaking, the suspension was subsequently placed into the ultrasonication bath, configured at 50 kHz for 20, 30 and 60 min. Since the vibrations of ultrasonication waves are converted into heat, it causes an increase in the temperature of the medium. Thus, to avoid further consequences (e.g. phycocyanin degradation), the solution temperature was controlled at 23 °C, using laboratory thermocouples and adding cold water to the bath. After extraction, the samples were centrifuged (as described before) and the supernatant collected to further purify the extracts. The concentration of the extracted phycocyanin was evaluated using spectrophotometric measurements (Jasco V-660 UV–VIS) at 620 and 652 nm.

2.4. Extraction and quantification of phycocyanin

To make a preliminary study finding the best concentration of $(NH_4)_2SO_4$ for precipitation, an experiment was conducted, using the extract obtained with the best configuration in extraction procedure. The test consisted of preparing different salt concentrations (20, 30, 40, 50, 60 and 70%). After resting for 24 h at 4 °C in the dark, the solutions were centrifuged at 2500 rpm for 30 min. Subsequently, the supernatants were collected, and the precipitates were resuspended in 20 ml of potassium phosphate buffer solution 100 mM. Absorbances at 280 and 620 nm of supernatants and precipitates were measured.

In this study, to determine the purity ratio of phycocyanin (R_p) it was divided the absorbance at 620 nm (maximum absorbance of phycocyanin pigment) by the absorbance at 280 nm (maximum absorption peak of proteins in solution) as shown in Eq. (3) [19].

$$R_p = \frac{Abs_{620}}{Abs_{280}} \tag{3}$$

where Abs_{620} and Abs_{280} are the sample absorbance at 620 and 280 nm wavelength, respectively. Phycocyanin preparations with purity ratio greater than 0.7 are considered as food-grade. Ratios up to 3.9 are considered reagent grade and above 4.0 are analytical grade [20].

3. Results and discussion

The photo of Fig. 1 shows the blue color phycocyanin extract after centrifuging. Table 1 reports the average extracted phycocyanin (from three samples per each solvent), as well as the purity ratio calculated from Eq. (3).

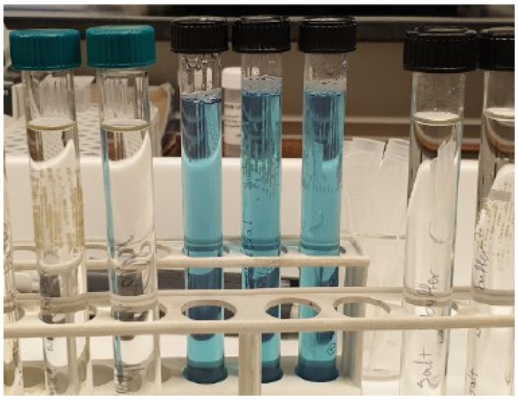

Fig. 1. Extracted phycocyanin samples (blue tubes in the middle) using water as solvent via freeze-thaw approach. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Concerning the results reported in Table 1, the best approach, leading to the highest yield is by using the potassium phosphate buffer (pH 6.5), not only in what concerns the extraction yield but also in what concerns the purity of the extracted phycocyanin.

Table 1. Extraction yield and purity ratio of phycocyanin after phase I.

An example of a column heading	EC_P (mg/g)	R_p
Water	22.70 ± 0.79	0.31 ± 0.00
Sodium Buffer	32.58 ± 1.00	0.27 ± 0.00
Potassium Buffer	32.82 ± 0.12	0.31 ± 0.02

Evaluation of results obtained in the first phase of extraction reveals that the selection of an adequate solvent buffer can enhance the extraction rate. In this phase, potassium phosphate buffer solution was evaluated to surpass water (via freeze-thaw) and sodium phosphate buffer.

Concerning the effect of contact time, results for each set of configurations are shown in Table 2.

Table 2. Extraction yield and purity ratio of phycocyanin after phase II.

Solvent contact time (h) in orbital shaker		2		4		24	
		EC_P (mg/g)	R_p	EC_P (mg/g)	R_p	EC_P (mg/g)	R_p
Ultrasonication time (min)	0	32.82 ± 0.12	0.31 ± 0.02	42.35 ± 0.40	0.33 ± .0.00	40.56 ± 1.58	0.24 ± 0.02
	20	44.24 ± 2.35	0.34 ± 0.01	42.84 ± 0.62	0.31 ± 0.02	42.38 ± 0.16	0.23 ± 0.01
	30	44.06 ± 5.71	0.33 ± 0.00	41.07 ± 7.71	0.26 ± 0.01	35.29 ± 2.52	0.19 ± 0.00
	60	45.94 ± 2.10	0.27 ± 0.02	37.78 ± 2.38	0.21 ± 0.00	27.34 ± 1.90	0.15 ± 0.01

In the second phase, two conclusions can be taken, as only 2 h has been demonstrated to be sufficient to extract phycocyanin from biomass using the solvent buffer. The reported concentrations in Table 2 show that longer exposure time to solvent can degrade the extracted phycocyanin.

Comparing the obtained extraction results with those from the experiment by Moraes et al. [18] it shows that it is possible to obtain higher extraction yields from wet biomass, compared to 43.75 mg/g reported in their work. Nevertheless, this value is only about 59% the one reported by Tavanandi et al. [21] of 74.51 mg/g who also reported a purity of 0.56, and 40% the one reported by Ferreira da Silva et al. [22], of 115 mg/g.

Evaluation of ultrasonication accompanied by solvent-buffer shows an interesting trend. Although placing the suspension in ultrasonic bath up to an hour can enhance the extraction yield up to 45.94 mg/g, the reported purity rate indicates a significant decrease from 0.34 to 0.27. Therefore, the second phase of the experiment can be concluded by choosing a 2 h contact time of biomass to solvent buffer in orbital shaker, and 20 min to place the suspension in the ultrasonic bath. It should be noted that it is even possible that lower than 20 min ultrasonication time could be enough to provide high extraction yield with high purity ratio, and this lower ultrasonication time contributes to a lower energy consumption, as reported in a previous study by Tavanandi et al. [21].

The graph of Fig. 2 shows the results from the preliminary test, which was intended to find an appropriate saturation level of $(NH_4)_2SO_4$ salt. In other words, it reveals that the effect of saturation levels of the employed salt during the precipitation step.

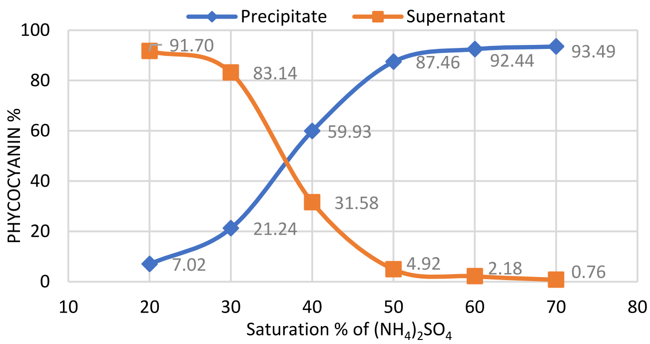

Fig. 2. Effect of the saturation level of $(NH_4)_2SO_4$ on the phycocyanin purification.

By increasing the saturation level of the salt, the phycocyanin contents found in precipitate increased, contrarily to the content measured in the supernatant. Based on the obtained results, employing solutions with $(NH_4)_2SO_4$ concentration more than 60% does not result in a significant increase of phycocyanin content in precipitate. Therefore, an efficient $(NH_4)_2SO_4$ concentration can be defined based on the applied desired rate. In this work, we used $(NH_4)_2SO_4$ 60% solution to obtain the reported R_p in Tables 1 and 2.

To compare the purification process, Kumar et al. [12] carried out the precipitation with 65% $(NH_4)_2SO_4$ solution, which resulted in phycocyanin with a purity ratio of 1.5. Although their results represent higher yield, it is important to note that their experiment was conducted for several steps of extraction to obtain more than 80% content.

Further research should be performed to evaluate the potential effect of the combination of serial extraction + ultrasonication steps, not only on the extraction yield, but also on the purity ratio.

3.1. Energy consumption perspective

Considering the amount of energy required in the extraction and purification process, the proposed method pursues the efficient use of energy both for shaking and for ultrasonication. The optimization of these parameters has been intended to increase the yield and productivity of the targeted product. Having set the parameters for shaking time for 2 h, and the ultrasonication to 20 min, phycocyanin can be obtained with 50% or 0.92% less (comparing to the studies used 4 or 24 h, respectively for shaking), and 33% or 66% less (instead of 30 or 60 min, respectively for ultrasonication).

4. Conclusion

A. platensis, as one of the cyanobacterial species, has been investigated for commercial production of phycocyanin. Although colorful pigments of *A. platensis* are rich in phycocyanin, proper extraction approaches must be considered for three main purposes: first to obtain the highest extraction (based on purity ratio), second, to avoid pigment degradation during the procedure, and third, to reduce the energy consumption in the extraction process. In this research study, several extraction parameters were analyzed in two phases at which, for the first phase the highest yield was selected using different solvents: distilled water, sodium phosphate buffer and potassium phosphate buffer. In the second phase it was evaluated the exposure time to solvent (2, 4 and 24 h) required to perform the extraction, using the best solvent buffer, as well as the use of ultrasonication time (0, 20, 30 and 60 min) as extraction enhancer. Finally, an analysis was conducted to determine the appropriate concentration of $(NH_4)_2SO_4$ required for the extracted phycocyanin purification. Experimental results indicate that the best operating conditions include: to use potassium buffer (pH 6.5), suspending lyophilized microalgae biomass for 2 h, followed by 20 min of ultrasonication (50 kHz), which resulted in 44.24 mg phycocyanin per gram of biomass with a 0.34 purity. Following this procedure, using $(NH_4)_2SO_4$ with 60% of saturation percentages can lead to more than 92% purification of the obtained phycocyanin, while reducing the energy consumption in the phycocyanin extraction and recovery process.

CRediT authorship contribution statement

Malihe Gorgich: Investigation, Formal analysis, Validation, Writing - original draft. **Marieta L.C. Passos:** Writing - review & editing. **Teresa M. Mata:** Data curation, Writing - review & editing. **António A. Martins:** Data curation, Writing - review & editing. **M. Lúcia M.F.S. Saraiva:** Writing - review & editing. **Nídia S. Caetano:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. Renew Sustain Energy Rev 2010;14:217–32. http://dx.doi.org/10.1016/j.rser.2009.07.020.

[2] Morais Junior WG, Gorgich M, Corrêa PS, Martins AA, Mata TM, Caetano NS. Microalgae for biotechnological applications: Cultivation, harvesting and biomass processing. Aquaculture 2020;528:735562. http://dx.doi.org/10.1016/j.aquaculture.2020.735562.

[3] Gunes S, Tamburaci S, Dalay MC, Deliloglu Gurhan I. In vitro evaluation of *Spirulina platensis* extract incorporated skin cream with its wound healing and antioxidant activities. Pharm. Biol. 2017;55:1824–32. http://dx.doi.org/10.1080/13880209.2017.1331249.

[4] Kim SH, Shin C, Min SK, Jung S-M, Shin HS. In vitro evaluation of the effects of electrospun PCL nanofiber mats containing the microalgae *Spirulina* (*Arthrospira*) extract on primary astrocytes. Colloids Surf B 2012;90:113–8. http://dx.doi.org/10.1016/j.colsurfb.2011.10.004.

[5] Cañizares Villanueva RO, Domínguez AR, Cruz MS, Ríos-Leal E. Chemical composition of cyanobacteria grown in diluted, aerated swine wastewater. Bioresour Technol 1995;51:111–6. http://dx.doi.org/10.1016/0960-8524(94)00099-M.

[6] Gorgich M, Mata TM, Martins AA, Branco-Vieira M, Caetano NS. Comparison of different lipid extraction procedures applied to three microalgal species. Energy Rep 2020;6:477–82. http://dx.doi.org/10.1016/j.egyr.2019.09.011.

[7] Wu H-L, Wang G-H, Xiang W-Z, Li T, He H. Stability and antioxidant activity of food-grade phycocyanin isolated from *Spirulina platensis*. Int J Food Prop 2016;19:2349–62. http://dx.doi.org/10.1080/10942912.2015.1038564.

[8] Bari E, Arciola C, Vigani B, Crivelli B, Moro P, Marrubini G, Sorrenti M, Catenacci L, Bruni G, Chlapanidas T, Lucarelli E, Perteghella S, Torre M. In vitro effectiveness of microspheres based on silk sericin and chlorella vulgaris or *Arthrospira platensis* for wound healing applications. Materials (Basel) 2017;10:983. http://dx.doi.org/10.3390/ma10090983.

[9] Arad S (Malis), Yaron A. Natural pigments from red microalgae for use in foods and cosmetics. Trends Food Sci Technol 1992;3:92–7. http://dx.doi.org/10.1016/0924-2244(92)90145-M.

[10] Gang Y, Zheng L, Fei L, Chen L, Fu-xin D, Nai-ju Y. Isolation and Characterization of C - phycocyanin from *Spirulina platensis*. Chem Res Chin Univ 1998;1:35–8.

[11] Hirata T, Tanaka M, Ooike M, Tsunomura T, Sakaguchi M. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis*. J Appl Phycol 2000;435–9. http://dx.doi.org/10.1023/A:1008175217194.

[12] Kumar D, Dhar DW, Pabbi S, Kumar N, Walia S. Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). Indian. J Plant Physiol 2014;19:184–8. http://dx.doi.org/10.1007/s40502-014-0094-7.

[13] Chemat F, Vian MA, Cravotto G. Green extraction of natural products: Concept and principles. Int J Mol Sci 2012;13:8615–27. http://dx.doi.org/10.3390/ijms13078615.

[14] Zhang H, Wang Y, Zhou Y, Chen J, Wei X, Xu P. Aqueous biphasic systems formed by deep eutectic solvent and new-type salts for the high-performance extraction of pigments. Talanta 2018;181:210–6. http://dx.doi.org/10.1016/j.talanta.2018.01.014.

[15] Sarada R, Pillai MG, Ravishankar GA. Phycocyanin from *Spirulina sp*: Influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. Process Biochem 1999;34:795–801. http://dx.doi.org/10.1016/S0032-9592(98)00153-8.

[16] Chethana S, Nayak CA, Madhusudhan MC, Raghavarao KSMS. Single step aqueous two-phase extraction for downstream processing of C-phycocyanin from *Spirulina platensis*. J Food Sci Technol 2015;52:2415–21. http://dx.doi.org/10.1007/s13197-014-1287-9.

[17] Silva LA, Kuhn KR, Moraes CC, Burkert CAV, Kalil SJ. Experimental design as a tool for optimization of C-phycocyanin purification by precipitation from *Spirulina platensis*. J Braz Chem Soc 2009;20:5–12. http://dx.doi.org/10.1590/S0103-50532009000100003.

[18] Moraes CC, Sala L, Cerveira GP, Kalil SJ. C-Phycocyanin extraction from *Spirulina platensis* wet biomass. Braz J Chem Eng 2011;28:45–9. http://dx.doi.org/10.1590/S0104-66322011000100006.

[19] Liu L-N, Chen X-L, Zhang X-Y, Zhang Y-Z, Zhou B-C. One-step chromatography method for efficient separation and purification of R-phycoerythrin from *Polysiphonia urceolata*. J Biotechnol 2005;116:91–100. http://dx.doi.org/10.1016/j.jbiotec.2004.09.017.

[20] Rito-Palomares M, Nuñez L, Amador D. Pratical application of aqueous two-phase systems for the development of a prototype process for c-phycocyanin recovery from *Spirulina* maxima. J Chem Technol Biotechnol 2001;76:1273–80.

[21] Tavanandi HA, Mittal R, Chandrasekhar J, Raghavarao KSMS. Simple and efficient method for extraction of C-Phycocyanin from dry biomass of *Arthospira platensis*. Algal Res 2018;31:239–51. http://dx.doi.org/10.1016/j.algal.2018.02.008.

[22] Ferreira da Silva A, Brazinha C, Costa L, Caetano NS. Techno-economic assessment of a Synechocystis based biorefinery through process optimization. Energy Rep 2020;6:509–14. http://dx.doi.org/10.1016/j.egyr.2019.09.016.