Acta Aquatica Turcica
E-ISSN: 2651-5474 16(4), 506-510 (2020) DOI: https://doi.org/10.22392/actaquatr.732774

Blue colored pigment phycocyanin extraction from *Spirulina platensis*

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Research Article

Received 08 May 2020; Accepted 23 June 2020; Release date 01 December 2020.

How to Cite: Işık, O., Uslu, L., Ak Çimen, B., Gökpınar, Ş., Reddad, C., & Sayın, S. (2020). Blue colored pigment phycocyanin extraction from *Spirulina platensis. Acta Aquatica Turcica, 16*(4), 506-510 https://doi.org/10.22392/actaquatr.732774

Abstract

Phycocyanin is a blue pigment and water-soluble biliprotein from the *Spirulina platensis*. In this study, the water was used as a solvent for the extraction of phycocyanin. Wet biomass of *Spirulina*, wet biomass held for 48 hours at room temperature, frozen *Spirulina* for 48 hours, and frozen biomass for fifteen days, and dried *Spirulina* was used to determine phycocyanin concentration (PC) (mg mL^{-1}). At the same time, extract purity of phycocyanin (OD_{615}/OD_{280}) and yield (mg g^{-1}) were determined. Phycocyanin yield of 17,6497, 17,1370, and 17,0833 mg g^{-1} were obtained from frozen *Spirulina* for fifteen days, wet biomass of *Spirulina* held for 48 hours, and wet biomass of *Spirulina*, respectively. The purity ratios (OD_{615}/OD_{280}) of 4.60, 0.81, 0.85, 0.98, and 6.55 were determined for frozen for 15 days, frozen *Spirulina* for 48 hours, wet biomass of *Spirulina* (waited for 48 hours), dried *Spirulina* and wet *Spirulina*, respectively.

Keywords: Phycocyanin, extraction, solvent, C-PC yield

***Spirulina platensis*'ten mavi pigment fikosiyanin ekstraksiyonu**

Özet

Fikosiyanin, *Spirulina platensis*'ten elde edilen ve suda çözünebilen mavi pigmenttir. Bu çalışmada fikosiyanin ekstraksiyonu için çözücü olarak su kullanılmıştır. Fikosiyanin konsantrasyonunu (PC) (mg mL^{-1}) belirlemek için yaş *Spirulina* biyoması, oda sıcaklığında 48 saat beklemiş yaş biyomas, 48 saat dondurulmuş *Spirulina*, 15 gün dondurulmuş *Spirulina* ve kuru *Spirulina* kullanılmıştır. Aynı zamanda Fikosiyanin saflığı (OD_{615}/OD_{280}) ve ürün miktarı (mg g^{-1}) saptanmıştır. Onbeşgün dondurulmuş *Spirulina*,48 saat oda sıcaklığında beklemiş yaş biyomas ve yaş *Spirulina* biyomasından, sırasıyla 17,6497, 17,1370 ve 17,0833 mg g^{-1}fikosiyanin elde edilmiştir. Üründeki saflık oranları (OD_{615}/OD_{280}), 4.60, 0.81, 0.85, 0.98 ve 6.55, sırasıyla onbeşgün dondurulmuş *Spirulina*, 48 saat dondurulmuş *Spirulina*, 48 saat bekletilmiş yaş *Spirulina* biyoması, kurutulmuş *Spirulina* ve yaş biyomastan elde edilmiştir.

Anahtar kelimeler: Fikosiyanin, ekstraksiyon, çözücü, C-PC ürün

INTRODUCTION

Phycocyanin is an accessory photosynthetic blue colored pigment of *Spirulina* sp. Cyanobacteria *Spirulina platensis* is an excellent source of phycocyanin. C-phycocyanin(C-PC) is the major phycobiliprotein and may constitute up to 20% of the dry weight of *Spirulina* (Jaouen et al., 1999; Vonshak, 1997). C-PC has been widely investigated concerning its characteristics and commercial potential. The application of C-PC has been examined in a wide range of fields. Phycocyanin is a natural blue colorant, has uses as a food colorant for chewing gum, ice sherbets, soft drinks, candies, and cosmetics including lipstick and eyeliners. Phycocyanin is also used as biochemical tracers in immunoassays due to its fluorescent properties (Herrera et al., 1989; Silveira et al., 2007). Furthermore, phycocyanin has been proven to have therapeutic properties including antioxidant, anti-inflammatory, and anti-cancer activities (Romay et al., 2003; Eriksen, 2008).

Several factors can influence the phycocyanin extraction. The most important are, cellular disruption method, type of solvent, biomass-solvent ratio, and extraction time (Abalde et al., 1998;

Reis et al., 1998). Many studies on efficient extraction and purification of C-PC have been carried out. Phycocyanin extraction was evaluated in terms of phycocyanin concentration using different solvents, including distilled water, sodium phosphate, and sodium acetate buffers, NaCl, $CaCl_2$ (Abalde et al., 1998; Bermejo et al., 2003, Ilter et al., 2018).

In the present study, the water as a solvent for phycocyanin extraction from *S. platensis* was investigated. In the study we carried out, C-PC contents were determined from the wet biomass of *Spirulina*, wet biomass of *Spirulina* held for 48 hours at room temperature, frozen *Spirulina* for 48 hours, and frozen biomass for fifteen days and dried *Spirulina*. Phycocyanin concentration (PC) (mg mL^{-1}), extract purity of phycocyanin (OD_{615}/OD_{280}), and yield (mg g^{-1}) were determined in the samples.

MATERIALS and METHODS

In the study, the blue-green alga *Spirulina platensis* was cultured at the fiber-glass ponds with a size of 1x5x0.2 meter and 1 ton capacity in the greenhouse of the Algal Biotechnology Pilot Plant, Fisheries Faculty, Cukurova University, Turkey were used. There is a pedal system that provides mixing with a speed of 20 cm sn^{-1} in the ponds. The cultures were harvested by filtering. The wet biomass of *Spirulina*, the wet biomass of *Spirulina* held for 48 hours at room temperature, frozen *Spirulina* at -22 °C for 48 hours and frozen at -22 °C for fifteen days and *Spirulina* dried at 55 °C and stored at room temperature were used to extract and purify.

Phycocyanin concentration was evaluated in terms of phycocyanin concentration (Eq. (1) using distilled water. The *Spirulina* biomass samples were weighted.

Wet *Spirulina* samples of 20.09, 20.01, 20.07, 20.01 g; *Spirulina* waited 48 hours of 20.03, 20.04, 20.00, 20.04 g; frozen *Spirulina* for 48 hours of 20.04, 20.04, 20.04, 20.04 g, frozen *Spirulina* of 15.00 grams at for fifteen days and dried *Spirulina* of 2.0, 2.0, 2.03 g were weighed.100 mL distilled water was added to the samples. The samples were studied with three samples except for frozen *Spirulina* for fifteen days. The biomass was homogenized in ultra thorax at 5000 rpm for five minutes and 15000 rpm for one minute. The homogenized samples of 7 mL were placed in the centrifuge tubes and centrifuged at 5000 rpm for five minutes. After centrifugation, the optical density of the supernatant was measured at 615 and 652 nm.

Phycocyanin concentration (PC), according to Bennett and Bogorad (1973), was defined as

$$\mathrm{PC} = \frac{615\ \mathrm{nm} - 0.474(652\ \mathrm{nm})}{5.34}$$

where PC is the phycocyanin concentration (mg mL^{-1}), OD_{615} is the optical density of the sample at 615 nm, OD_{652} is the optical density of the sample at 652 nm.

The purity of phycocyanin extract was monitored spectrophotometrically by the A_{615}/A_{280} ratio (Abalde et al., 1998). This relationship is indicative of the extract purity of phycocyanin with respect most forms of contaminating proteins. Absorbance at 615 nm indicates the phycocyanin concentration, while that at 280 nm is due to the total concentration of proteins in the solution (Liu et al., 2005). The extract purity of phycocyanin (EP) was defined as

$$\mathrm{EP} = OD_{615}/OD_{280}$$

Where EP is the extract purity, OD_{615} is the optical density of the sample at 615 nm, OD_{280} is the optical density of the sample at 280 nm.

The yield of the extraction was defined as

$$\mathrm{Yield} = \frac{\mathrm{PCxV}}{\mathrm{DB}}$$

Where PC is phycocyanin concentration (mg mL^{-1}), V is the volume of solvent (mL), DB is dried biomass (g).

Data were subjected to analysis of variance (ANOVA) and means were separated using Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$.

RESULTS and DISCUSSION

In the study carried out, PC contents were determined from the wet biomass of *Spirulina*, wet biomass of *Spirulina* kept for 48 hours at room temperature, frozen *Spirulina* for 48 hours, frozen *Spirulina* for fifteen days, and dried *Spirulina*.

Phycocyanin contents of 0.3119 mg mL^{-1} obtained from wet biomass of *Spirulina* waited for 48 hours was found higher than 0.3108, 0.3043, 0.2997 and 0.24096 mg mL^{-1} obtained from wet *Spirulina*, frozen *Spirulina* for 48 hours, dried *Spirulina* than biomass frozen for 15 days, respectively (Table 1).

Phycocyanin yield of 17,6497, 17,1370, 17,0833, 16,7208 mg/g obtained from frozen *Spirulina* for fifteen days, wet biomass of *Spirulina* waited for 48 hours, wet biomass and frozen *Spirulina* for 48 hours respectively were higher than 14,8935 mg/g from dried samples (Table 1).

The purity of phycocyanin extracts of 4.60 and 2.55 were found high in the samples of *Spirulina* frozen for 15 days and wet *Spirulina* of, respectively.

Table 1. Main parameters values of phycocyanin concentration (PC), extract purity of phycocyanin (EP), and yield. Values with the different letter in each column showed a statistically significant difference between and within groups ($p \leq 0.05$)

Parameters	PC (mg/mL)	EP(OD615/OD280)	Yield (mg/g)
Frozen For 15 Days	$0.240967 \pm 0.004315^{b}$	$4.606667 \pm 1.158462^{a}$	17.6497^{a}
Frozen *Spirulina* for 48 hours	$0.304325 \pm 0.014516^{a}$	$0.814275 \pm 0.080285^{c}$	16.7208^{b}
Wet biomass of *Spirulina* (held for 48 hours)	0.3119 ± 0.010455^{a}	0.85225 ± 0.050554^{c}	17.1370^{a}
Dried *Spirulina*	0.29975 ± 0.004282^{a}	0.98205 ± 0.02608^{c}	14.8935^{c}
Wet *Spirulina*	0.3108 ± 0.007143^{a}	2.5567 ± 0.092880^{b}	17.0833^{a}
Prob > f	0.0001	0.0131	0.0001
LSD % 0.05	0.014	3.671	0.116

Many studies on efficient extraction and purification of C-phycocyanin have been carried out. The phycocyanin content during the extraction with different solvents was investigated. The study was carried out by Silveira et al. (2007) about the optimization of phycocyanin extraction from *Spirulina platensis* and used different solvents, including, distilled water, 10 mM sodium phosphate buffer (pH 7.0), 10 mM sodium acetate buffer (pH 5.0), NaCl 0.15 M and $CaCl_2$ 10 g L^{-1} (Abalde et al., 1998; Bermejo et al., 2003). Silveira et al.(2007) were recorded that the higher phycocyanin contents of 3.73, 4.20 3.32, and 3.48 mg mL^{-1} with different solvents of water, phosphate buffer pH 7.0, NaCl 0.15 M, and $CaCl_2$ 10 g L^{-1} than 1.84 mg mL^{-1} of acetate buffer. In conclusion, the work described a suitable method for the extraction of phycocyanin from the *S. platensis*. Water was chosen as the extractant because it produced a high phycocyanin concentration. Besides, it is a low cost extractant (Silveira et al., 2007). The reason for the low PC values (mg mL^{-1}) in our study can be explain strains of the species and the mechanical disruption of cells, which caused increased release of phycocyanin.

At the study carried out that the slurry of *Spirulina* dried at different temperatures (T) and relative humidity (RH). The PC values of air-dried fresh *Spirulina* and freeze-thawed and air-dried *Spirulina* were compared and no significant differences were found, so it can be confirmed that the preservation of *Spirulina* in a freezer did not cause serious changes in its properties. The phycocyanin contents for three protocols of T30/RH50, T50/RH20/T80/RH20 were found similar (Nakagawa et al., 2016). In this study, the PC values were found similar except the *Spirulina* frozen for fifteen days.

In the other study carried out with *Spirulina*, different phycocyanin extraction methods were compared. Spectral properties of phycocyanin were observed that extracted with water, hydrochloric acid, and homogenization in the blender. The quality of phycocyanin required would influence the selection of suitable extraction method. It was reported that fresh biomass was suitable for phycocyanin extraction (Sarada et al, 1999). In our study, we also observed that the best PC values with the wet samples of 0.31 mg/mL.

The relationship is indicative of the extract purity of phycocyanin with respect to most forms of contaminating proteins. The results obtained from the studies demonstrated that the purity of the extract is significantly influenced by temperature. In the study carried out phycocyanin concentration and extract purity were shown that as a function of time. The results obtained in that work demonstrated that the purity of the extract was significantly influenced by temperature. High

temperature resulted in reduced purity because it facilitated the extraction of other proteins (Silveira et al., 2007).

Ilter et al. (2018) was carried out a study in which different solvents of distilled water, Na-Phosphate pH:7,4 suspension, and 1.5% $CaCl_2$ (w/v) water solution were used for dry, frozen, and wet *Spirulina*. In that study, the highest phycocyanin content of frozen biomass was obtained using 1.5% $CaCl_2$ (w/v) as extraction medium with 55.33±3.23 mg/g PC. With distilled water as a solvent, 24.67±2.26 mg/g PC was obtained for frozen *Spirulina* biomass. In our study, a higher PC content of 17.64 mg/g was found with frozen *Spirulina* biomass for fifteen days with distilled water ($p \leq 0.05$).

The extract purity was favored when using low temperatures. High temperature resulted in reduced purity because it facilitated the extraction of other proteins. Since the phycocyanin concentration was only slightly affected by temperature, the increase of temperature did not improve phycocyanin extraction (Silveira et al., 2007). In that study, the room temperature was 24 ºC and extract purity (OD_{615}/OD_{280}) values were determined 4.60, 0.81, 0.85, 0.98, and 6.55 for frozen for 15 days, frozen *Spirulina* for 48 hours, wet biomass of *Spirulina* (waited for 48 hours), dried *Spirulina* and wet *Spirulina*, respectively.

C-phycocyanin extraction process for large-scale use were studied and the optimum conditions for extracting C-PC from dried, frozen biomass, milled to a small diameter, were an extraction time of 1 h, a biomass-to-solvent ratio 0.16:1, and without agitation, obtaining a C-PC concentration of 13.20 mg/mL, purity of 0.603 and extraction yield of 82,48 mg/g (Moraes et al., 2010).

Oğuz et al. (2011) were carried out the experiment in the fiber-glass ponds in the greenhouse during the months of April, July, and September to determine the effect of seasonal temperature and light intensity on the blue pigment C-phycocyanin and protein content of *Spirulina platensis*. While C-phycocyanin content was found higher in Autumn (332.7 ±1 μgmL^{-1}), lower and similar in Spring and Summer (327.5 ±2 and 323.4±1 μgmL^{-1} respectively. In the study, $NaNO_3$ was used as a solvent and read at 620 nm CP values was calculated with the equation of $CP=OD_{620nm}$ x 137= μgmL^{-1} (Boussiba and Richmond, 1979). The CP values were similar to the CP contents of this study, however, the water as a solvent for extraction is not harmful to health and environment, at the same time costless.

In the other study, the effect of ultrasonication process time on the extraction of phycocyanin and chlorophyll-a prior to the application of solvent extraction with methanol and aqueous sodium nitrate solution (1.5% $NaNO_3$), and the antioxidant activity (FRAP, Ferric Reducing Ability of Plasma) of extracts were determined. Ultrasonication for 1, 3, 5, 10, 15, 20, 30, 45, and 60 minutes were applied prior to methanol and $NaNO_3$ solvent extraction for 120 minutes at room temperature. Chlorophyll *a* concentration with 6.75 mg/g dry *Spirulina* for control sample increased to 7.70 mg/g dry *Spirulina* by 30 minute sonication process, and it remained constant at further sonication times. Moreover, phycocyanin concentration with 34.52 mg/g dry *Spirulina* for the control sample increased to 51.83 mg/g dry *Spirulina* up to 45 minute sonication process and remained constant at further sonication. Antioxidant activity of chlorophyll *a* and phycocyanin were 15.74 mg/g and 11.98 mg/g, respectively for 60 minutes sonic application followed by solvent extraction. In conclusion, prior to solvent extraction, 30 minute sonication process is recommended for chlorophyll *a* while 45 minute process for phycocyanin (Aksay and Arslan, 2018). In that study, phycocyanin extraction was performed using 1.5% sodium nitrate aqueous solutions and the phycocyanin concentration was higher than in our results because of the different solvents probably. However, as stated in this study, prior to solvent extraction sonication process will increase the yield of phycocyanin, so the sonification seems necessary in this study.

CONCLUSION

In the study, water as a solvent was used for extraction of phycocyanin. It can be said that in that study phycocyanin amount is not high as the other studies used different solvents or methods for disruption to the cells. In the trials used water as a solvent, disruption of the cells, agitation, different temperatures can be added for more effective extraction. Phycocyanin, the blue color, organic coloring, not harmful to human health, can be used in the food industry widely. However the cost of the product should be as low as possible, so the water can be preferable.

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