C-phycocyanin extraction from *Spirulina platensis* oven-dried biomass

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Abstract

C- Phycocyanin is a natural blue pigment used in food and pharmaceutical industry. In the present study, C-phycocyanin extraction from cyanobacteria *Spirulina platensis* was investigated using various conditions. The effects of solid-liquid ratio, extraction temperature and extraction time on C-phycocyanin concentration, extract purity and yield of extraction were considered to optimize conditions for phycocyanin extraction. The optimum condition for the extraction of C-phycocyanin was the solid-liquid ratio, 1:15, 25°C and 24 h. Under this condition an extract of C-phycocyanin concentration of 6.88 mg ml^{-1} and extract purity of 3.54 were obtained.

Keywords: *Spirulina*, C-Phycocyanin, Extraction, Oven-dried

Introduction

Cultivation of S*pirulina* microalga is an effective process for obtaining several valuable biochemicals, such as polysaccharides, γ-linolenic acid, β-carotene, chlorophyll *a*, and phycobiliproteins. Phycobiliproteins, which are brightly colored pigments, function as a receiver of light for driving photosynthesis in the S*pirulina* microalga (Su et al. 2014). Phycocyanin is commonly used as a natural colorant in food and cosmetic industries because it is inherently blue. Moreover, it can be incorporated into health foods because of its physiological properties, such as antioxidant, anti-inflammatory, and hepatoprotective activities. Because of these benefits, numerous researchers have focused on developing efficient processes for mass production of phycocyanin-producing strains and extraction of phycocyanin from microalgae (Su et al. 2014).

The cyanobacteria *Spirulina platensis* is an excellent source of C-phycocyanin. The protein fraction may contain up to 20% of phycocyanin (Vonshak, 1997). Several factors can influence the phycocyanin extraction. The most important are cellular disruption method, type of solvent, biomass-solvent ratio, extraction temperature and extraction time (Silveira et al. 2007). In this study, *S. platensis* was used as a source for C-phycocyanin. The effects of the operating factors (extraction temperature and extraction time) on the aqueous solid–liquid extraction of phycocyanin from *S. platensis* were examined.

Methodology

Culture condition and sample preparation

The microalgae used in this studied was *Spirulina platensis* IFRPD 1182 that was obtained from the Institute of Food Research and Product Development, Kasetsart University, Thailand. Zarrouk medium used prepared and maintained the cultures. The inoculums were grown in 200 ml microalgae culture tube containing 50 ml of Zarrouk medium and kept for 3 days. Added 100 ml of the same medium into a culture tube for expansion inoculums and kept for 3 days or the cell concentration was 1 at OD_{560}. There were used the clear glass made microalgae culture tube, with 3.22 cm internal diameter, 0.39 cm glass thickness and 33.61 cm glass height. The microalgae culture tubes were incubated in the chamber equipment, which controlled the temperature at 30 ºC, the light intensity, was 12 klux with 18 watt daylight fluorescent, the cycle of light and dark were 16:8 h, the continuous bubble of air mixed with 1-2 % (v/v) carbon dioxide at flow rate 0.67 vvm through the PTFE membrane filter. Preparing the inoculum was inoculated amount 10 % (v/v) of working volume.

The biomass production was performed in open raceway pond containing 200 liters with an initial biomass concentration of 10 % (v/v). The cultures were mixed using paddle wheels at 15 rpm. The mass production reached it exponential phase and the biomass were harvested by filtration through 60 micron nylon and washed with tap water to completely remove residue culture medium. Then, oven-dried *S. platensis* was performed using the temperature at 70 ºC for 6-7 hours. Oven-dried *S. platensis* biomass was milled to 0.5 mm for further C-phycocyanin extraction.

C-phycocyanin extraction

C-phycocyanin was extracted from oven-dried *S. platensis* using 10 mM sodium phosphate buffer (pH 7.0) with different condition, including, solid-liquid ratio (1:15, 1:25, 1:50), extraction temperature (25, 4, -20 ºC) and extraction time (12, 24, 48 h). After the extraction, aliquot was centrifuged and the C-phycocyanin extract was collected for yield, phycocyanin concentration and extract purity analysis.

Analysis

Determination of C-phycocyanin and yield

The concentration and purity of C-phycocyanin were determined using a UV-vis spectrophotometer at the wavelengths of 280, 615 and 652 nm and calculated using the following equation:

$$\text{C-PC (mg ml}^{-1}) = \frac{A_{615} - (0.474\ A_{652})}{5.34} \quad (1)$$

The equation was established by using the simultaneous equations of Bennett and Bogorad (1973) with the extraction coefficients from Bryant et al. (1979). The purity of C-phycocyanin was calculated by the absorbance at A_{615}/A_{280} ratio.

The yield of the C-phycocyanin extraction was calculated by following equation (2) from Silveira et al. 2007.

$$\text{Yield (mg g}^{-1}\text{)} = \frac{\text{(C-PC)V}}{\text{DB}} \quad (2)$$

Yield is the extraction yield of phycocyanin in mg of C-phycocyanin/dry biomass (g), C-PC is C-phycocyanin concentration (mg ml^{-1}), V is the solvent volume (ml) and DB is the dried biomass (g).

Statistical analysis

The experiments were performed in triplicate and results were expressed as mean ± SD. Analytical data were tested by factorial design and followed by suitable post hoc Duncan's multiple range test (DMRT) was applied to calculate the statistical significance between various groups using SPSS statistical program. A value of $P < 0.05$ was considered to be statistically significant.

Results and Discussions

C-Phycocyanin and extract purity

The C-Phycocyanin extraction was reported by various methods. Several factors can influence the C-PC extraction from *S. platensis*. In this study, C-Phycocyanin was extracted from *S. platensis* oven-dreid biomass by using phosphate buffer with different parameter, including, solid-liquid ratio, extraction temperature and extraction time. The C-phycocyanin content (C-PC) and extract purity (EP) were shown in Figure 1. The C-PC varied from 1.03 to 6.88 mg ml^{-1}, while the extract purity varied from 2.38-3.83. The maximum C-PC was found at solid-liquid ratio of 1:15, extraction temperature at 25ºC and extraction time 24 h, while the extract purity of C-PC was highest value at solid-liquid ratio of 1:15, extraction temperature at 4ºC and extraction time 48 h. All of the various solid-liquid conditions (1:15, 1:25 and 1:50) found that the C-PC was increased when the increasing of extraction temperature at the same extraction time. The variable solid-liquid ratio strongly influenced the C-PC, and the maximum value was obtained using the largest solid-liquid ratio, which obtained the same direction the report C-PC extraction from dried biomass (Silveira et al. 2007). These results are superior to the report by Abalde et al. (1998) obtaining C-PC from *Synechococcus* sp. of 27 µg ml^{-1}, Minkova et al. (2003) extracting C-PC from fresh biomass of *S. fusiformis* of 1.28 mg ml^{-1}, and Silveira et al. (2007) optimizing C-PC extraction from *S. platensis* LEB 52 of 3.68 mg ml^{-1}.

The purity of C-PC preparations is evaluated base on the ratio between absorbencies from phycocyanobilin at 615 nm, A_{615} and aromatic amino acids in all proteins in the preparation at 280 nm. C-PC preparations with A_{615}/A_{280} greater than 0.7 was considered food grade, while A_{615}/A_{280} of 3.9 was considered reactive grade and A_{615}/A_{280} greater than 4.0 analytical grade (Eriksen. 2008). The cost of phycocyanin product varies widely and is dependent on the purity ratio (Chaiklahan et al. 2011). The highest EP from C-PC preparation was obtained using the condition of solid-liquid ratio at 1:15, extraction temperature at 4ºC and extraction time 48 h. These results were the ranges of 2.38 to 3.83, which were considered in various applications.

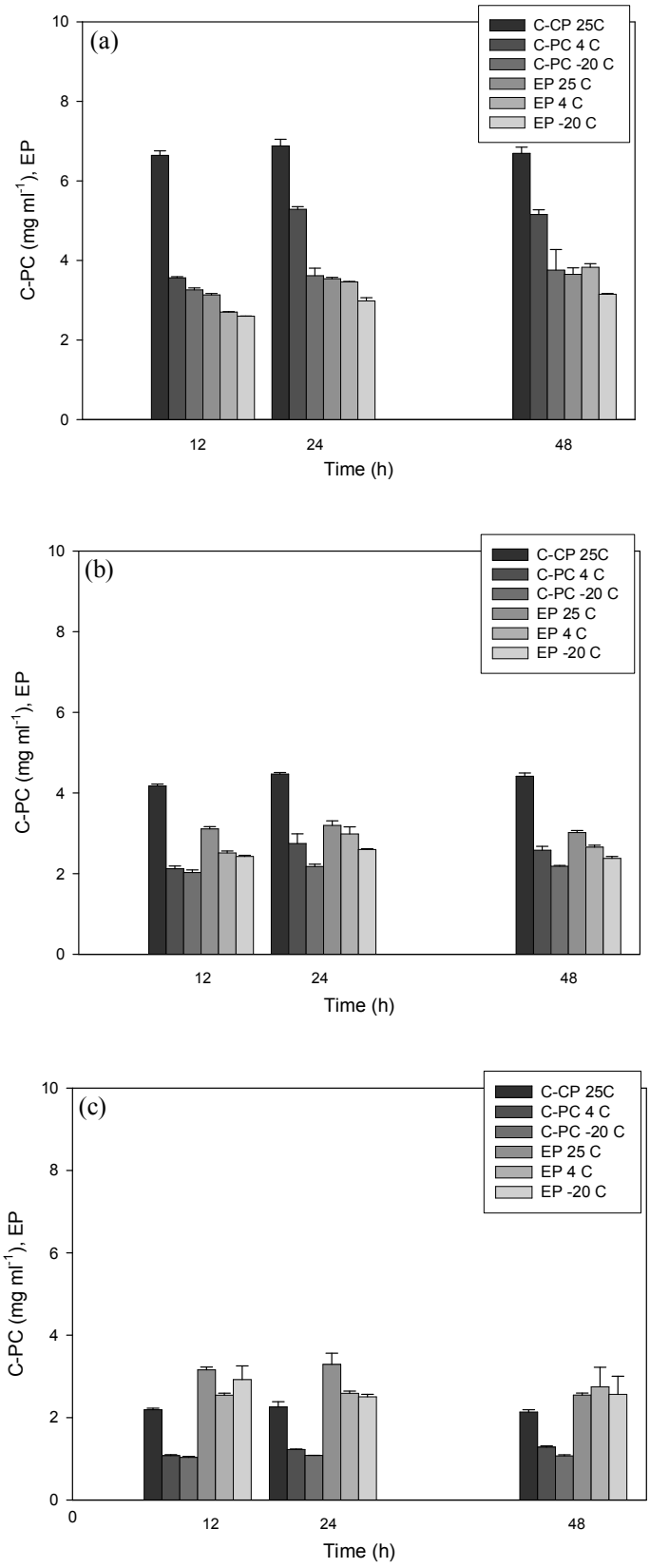

Figure 1 C-phycocyanin concentration and extract purity with different ratio solid-liquid as a function of time. (a) solid-liquid ratio at 1:15 (b) solid-liquid ratio at 1:25 (c) solid-liquid ratio at 1:50

Yield of C-Phycocyanin extraction

The great commercial interest on C-PC is mainly due to the high protein yield and the relatively easy extraction procedures (Martelli et al. 2014). Figure 2 showed the yield of C-Phycocyanin extraction from *S. platensis* oven dried biomass at different condition. From the result found the yield of C-PC extraction was increased at almost condition, when increasing extraction time. Compared to the results from difference extraction temperature, the yield from highest extraction temperature (25°C) showed a better extraction yield. This may be attributed to the increased diffusion efficient of solutes at a higher temperature (Su et al. 2014).

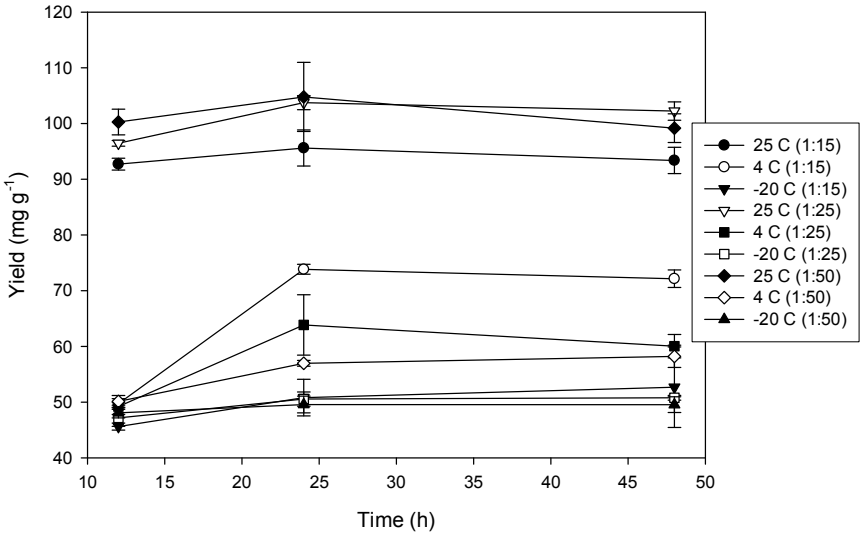

Figure 2 Yield of C-phycocyanin extracted with different condition.

From the result, the factorial analysis of variance detected an interaction among solid-liquid ratio, extraction temperature and extraction time ($P<0.05$). There was significant interaction between the three factors. Hence, interaction among factors was used for the Duncan test (Table 2). C-PC value was obtained highest at solid-liquid 0.06 g ml^{-1}, extraction temperature at 25°C and extraction time 24 h, which was no significant difference with 48 h. Considering EP value from C-PC extraction was obtained highest at solid-liquid 0.06 g ml^{-1}, extraction temperature at 4°C and extraction time 48 h, which was no significant difference with temperature extraction 25°C and extraction time 48 h. The extraction yield value was obtained highest at solid-liquid ratio 0.02 g ml^{-1}, extraction temperature at 25°C and extraction time 24 h.

Table 1 Summary of yield, C-PC and EP with different condition.

Solid-liquid ratio ($g\ ml^{-1}$)	Temperature (°C)	Time (h)	C-PC ($mg\ ml^{-1}$)	EP	Yield ($mg\ g^{-1}$)
0.06	25	12	6.64 ± 0.11^{i}	3.13 ± 0.03^{gh}	92.72 ± 1.06^{h}
0.06	25	24	6.88 ± 0.17^{j}	3.54 ± 0.03^{jk}	95.61 ± 3.24^{hi}
0.06	25	48	6.69 ± 0.16^{ij}	3.65 ± 0.16^{kl}	93.37 ± 2.36^{h}
0.06	4	12	3.56 ± 0.03^{e}	2.70 ± 0.01^{bcde}	49.71 ± 0.25^{abc}
0.06	4	24	5.29 ± 0.07^{h}	3.46 ± 0.01^{ijk}	73.84 ± 0.89^{g}
0.06	4	48	5.16 ± 0.12^{h}	3.83 ± 0.09^{l}	72.15 ± 1.57^{g}
0.06	-20	12	3.26 ± 0.05^{d}	2.60 ± 0.01^{abc}	45.62 ± 0.63^{a}
0.06	-20	24	3.62 ± 0.19^{e}	2.98 ± 0.08^{efgh}	50.81 ± 3.29^{bc}
0.06	-20	48	3.76 ± 0.52^{e}	3.15 ± 0.02^{gh}	52.68 ± 7.22^{cd}
0.04	25	12	4.18 ± 0.04^{f}	3.11 ± 0.06^{gh}	96.45 ± 0.51^{hi}
0.04	25	24	4.47 ± 0.04^{g}	3.20 ± 0.11^{ghi}	103.75 ± 1.26^{jk}
0.04	25	48	4.42 ± 0.08^{g}	3.02 ± 0.05^{fgh}	102.25 ± 1.65^{jk}
0.04	4	12	2.12 ± 0.07^{b}	2.51 ± 0.05^{abc}	49.21 ± 1.41^{abc}
0.04	4	24	2.75 ± 0.24^{c}	2.99 ± 0.17^{efgh}	63.86 ± 5.42^{f}
0.04	4	48	2.58 ± 0.10^{c}	2.66 ± 0.05^{abcd}	60.04 ± 2.11^{ef}
0.04	-20	12	2.03 ± 0.07^{b}	2.42 ± 0.03^{ab}	47.18 ± 1.52^{ab}
0.04	-20	24	2.18 ± 0.06^{b}	2.60 ± 0.02^{abc}	50.55 ± 1.28^{abc}
0.04	-20	48	2.18 ± 0.02^{b}	2.38 ± 0.05^{a}	50.79 ± 0.43^{bc}
0.02	25	12	2.19 ± 0.04^{b}	3.16 ± 0.07^{gh}	100.27 ± 2.30^{ijk}
0.02	25	24	2.26 ± 0.13^{b}	3.29 ± 0.27^{hij}	104.78 ± 6.19^{k}
0.02	25	48	2.14 ± 0.06^{b}	2.55 ± 0.05^{abc}	99.17 ± 2.57^{ij}
0.02	4	12	1.08 ± 0.02^{a}	2.55 ± 0.05^{abc}	50.13 ± 1.05^{abc}
0.02	4	24	1.23 ± 0.01^{a}	2.59 ± 0.06^{abc}	56.97 ± 0.52^{de}
0.02	4	48	1.28 ± 0.03^{a}	2.75 ± 0.47^{cdef}	58.22 ± 1.99^{e}
0.02	-20	12	1.03 ± 0.02^{a}	2.92 ± 0.33^{defg}	48.07 ± 0.90^{abc}
0.02	-20	24	1.08 ± 0.00^{a}	2.50 ± 0.06^{abc}	49.57 ± 1.50^{abc}
0.02	-20	48	1.07 ± 0.03^{a}	2.56 ± 0.44^{abc}	49.55 ± 1.42^{abc}

Data in the same column with different letters are significant ($P<0.05$)

Conclusion

The present work investigates a suitable condition for the extraction of C-phycocyanin from the cyanobacteria *Spirulina platensis*. The biomass-solvent ratio of extraction affected to C-phycocyanin concentration and extract purity. C-phycocyanin concentration and extract purity gave the value inversely with yield of extraction.

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