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Research Article

Impact of Spectral Character on Quality Assurance and Stability of Spirulina Phycocyanin -

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ABSTRACT

Phycocyanin extracted from S*pirulina platensis* is purified by ion-exchange chromatography. The purity of chromatographed phycocyanin is measured by their UV vis spectral absorbance which exhibited (A620/280 = 4.53). The purified pc was preserved at 4˚C with intermittent exposure to 25˚C for 3 months. At the end of the storage the purity of phycocyanin (A620/280 = 1.3). The phycocyanin with unstable spectral ratio may affect the quality and reliability of results concerning time. Whereas under preservation phycocyanin is lowly loses its purity. The pure form of phycocyanin has been used in the bioactive assay and electrochemical studies. This report illustrates the screening of spectral ratio of phycocyanin primarily at each time of application.

Keywords: Phycocyanin; Spectral quality; Storage; Stability

INTRODUCTION

Cyanobacterochrome phycocyanin a photosensitive water-soluble photosynthetic accessory pigment widely reported in *Spirulina platensis*. It is an oxygenic photosynthetic cyanobacterium present in fresh and marine water environments [1]. Phycocyanin receives more importance in the food and beverage industry as a natural blue colorant [2]. PC can be used as a color additive in numerous foods and coatings for dietary supplements (U.S. Food and Drug Administration). C-PC can be used as a fluorescent reagent, fluorescent probe, and fluorescent tracer, in the field of medicine [3]. C-PC is also acting as a nontoxic photosensitizer that can be involved in adjuvant therapy in photodynamic therapy (PDT) of tumors [4]. Phycocyanin is an oligomeric protein and it also consists of two subunits (α and β) that can be trimer (αβ) or hexamer (αβ). Phycocyanin is a trimer and it contains three chain tetrapyrrole that it is covalent binds to cysteine thioether [5]. Phycocyanin is composed of phycobilins (tetrapyrrole chromophores) linked with cysteine residues. Phycocyanin can be easily extracted using buffers due to its water-soluble nature. The purification of PC is facilitated by proper control of pH and ionic strength of buffers to keep the complex in a stable form [6]. The C-PC purification methods are the combination of fractional precipitation, column chromatography followed by gel filtration. Several methods have been adopted for phycocyanin extraction and purification. Therefore it is essential to develop a single step chromatography to yield PC with high purity [7]. The purity of pc is monitored by measuring the absorbance ratio at A620/A280 nm. Optimizing light conditions at the time of cultivation also increases the purity and yield [8].

C-PC in an aqueous solution is sensitive to heat and light. It often undergoes free-radical oxidation and unfolding of protein subunit at high temperature (47˚C) [9]. The stability of phycocyanin is one of the limiting factors for wide application. Stabilizing agents like sucrose, glucose, sodium chloride, ammonium sulfate, citric acid were widely used to extend the stability of phycocyanin. The protein conformation of phycocyanin changes in response to external factors pH, temperature and light [10]. This study aims to determine the phycocyanin storage stability by analyzing their spectral property.

MATERIALS AND METHODS

C-phycocyanin extraction and purification

The wet biomass of *Spirulina platensis* (1g) was suspended in 5ml of 0.1 mM phosphate buffer (pH 7). The cell suspension was frozen at -20˚C for 12 h with subsequently the suspension thawed at 25˚C for every 12 h for a period of 48 h. Then it was centrifuged at 10,000 rpm (4˚C) for 15 min to extract phycocyanin (C-PC). The supernatant with PC was subjected to further purification. The crude PC extract was fractionated with 30% - 50% Ammonium Sulfate (NH_4SO_3) (w/v). The precipitate containing mainly pc and mixed with 2-3 ml of 0.01M Na- Phosphate buffer (pH 7) was dialyzed against the same buffer (0.001M) at 4˚C for overnight. The dialyzed PC (1 mL) was loaded on top of the CM-Sephadex (A 25X) column then it was washed with 10 mL of the 0.1 M NaCl buffer (pH 7). The blue-colored fractions (5mL) were collected and analyzed under a spectrophotometer at 620 and 280 nm.

Analysis of spectral quality of phycocyanin

The chromatographed phycocyanin fractions were stored in the refrigerator at 4˚C for 3 months. For every month the PC was exposed to 25˚C for 1 h. The absorption spectrum of C-phycocyanin has determined under UV- Visible spectrophotometer (200 nm - 900 nm). The purity was evaluated based on absorbance ratio A620/A280 [11]. Declining in the spectral property of PC was analyzed for every month.

RESULTS AND DISCUSSION

Extraction and purification of C-phycocyanin

Phycocyanin is the water-soluble accessory photosynthetic pigment embedded in the phycobilisome. To extract phycocyanin the cell-wall ruptured by physical (high pressure, gas bubbles and chemical methods (enzyme, acid and alkali). Among the various methods for phycocyanin extraction, an efficient method is freezing and thawing [12]. The crude phycocyanin extract was purified for various applications such as food colorant (0.7), and pharmaceutical drug (4.0). The purity of extract is systematically monitored by UV-vis spectroscopy [13]. Phycocyanin in the crude extract was concentrated by precipitation to exclude other water-soluble components [14]. For further purification of CPC, The crude extract was precipitated with 30% and 50% of Ammonium sulfate [15]. The precipitate recovered from 50% saturation was dialyzed and chromatographed. At each step of purification (Table 1), there was an increased concentration of CPC. The final purification of CPC was achieved by ion-exchange chromatography on cation column CM Sephadex A 25x. The column was eluted with 0.1M NaCl buffer which is more specific to PC. Phycocyanin eluted from the column with purity ratio (A620/280 >3.0) collected and lyophilized for further use (Table 1).

Spectral analysis of purified phycocyanin on storage

Lyophilization is commonly adopted by industries to commercialize phycocyanin to conserve the functional properties [16]. The phycocyanin was freeze-dried and stored in the refrigerator to reduce degradation. However, lyophilization causes degradation of some active molecules which affects the antioxidant property. Storage conditions may affect the chemical composition of the material [17]. In this study, the purity of phycocyanin was highest in the freeze-dried powder form at the time of storage, (Figure 1b) (A620/280 =

4.53). A decline in the purity of phycocyanin on storage was evident from 2nd month (Figure 1a) (A620/280 = 1.3) to 3rd month (Figure 1c) (A620/280 = 0.58). This illustrates the actual ability of phycocyanin to resist storage conditions. The degradation in phycocyanin proteins was analyzed by UV-vis spectroscopic property. Decreasing absorbance after the storage is evident for the conformational changes in phycocyanin [18]. In a normal state phycocyanin chromophore lies in the linearly stretched form whereas during degradation it changes to cyclic form [19]. These conformational changes in the protein were monitored by measuring the absorbance at 620 nm [20]. The stability of phycocyanin is improved by the addition of preservatives like sucrose, ammonium sulfate, citric acid, sodium chloride and glucose [21]. High purity phycocyanin is preferred for bioactivity studies [22].

To maintain the purified form some chemical stabilizers (sodium azide, dithiothreitol) were used. However, these preservatives are toxic and harmful to human consumption [23]. The changes in the spectroscopic property of freeze-dried phycocyanin are due to the changes in the conformational state of bilin protein [24]. Similar to this study the previous report illustrated the importance of the optical spectroscopic feature of PC to monitor the urea-induced loss of trimeric to the monomeric form. This unfolding of protein is observed through CD spectra in the visible range. This Uv- vis spectral analysis is very simple and not time-consuming when compared to FTIR and CD spectra (Figure 1).

Table 1: Data of *phycocyanin* extraction and purification from *Spirulina platensis*.

Methods	Absorbance ratios (A620/A280)
Cell extract	0.9
Precipitation with ammonium sulfate	1.26
Dialysed phycocyanin	2.39
CM - Sephadex G25x	4.53

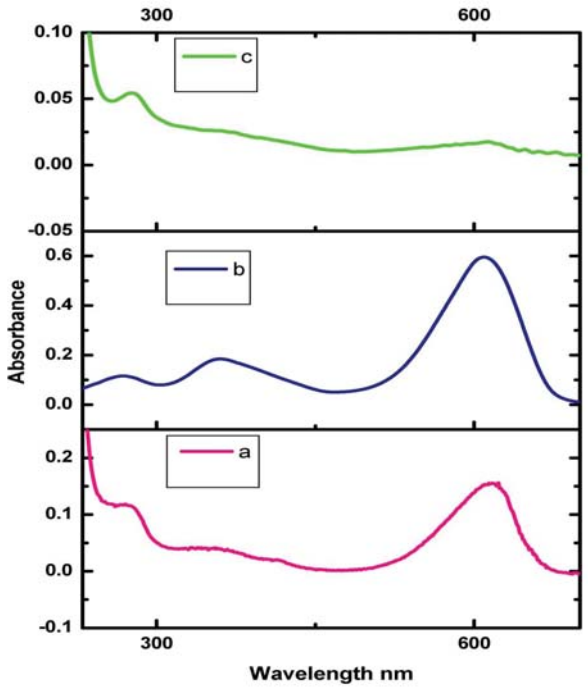

Figure 1: Absorption spectra of purified phycocyanin (lyophilized) obtained by ion exchange chromatography that were recorded from 200- 800nm for 1st month (b) 2nd month (a) 3rd month (c). All spectra were measured at room temperature.

CONCLUSION

Pure phycocyanin in a native form is highly recommended for chemical analysis, bioactive assays and electrochemical studies. The protein backbone and chromophore in the phycocyanin with good stability and purity is measured by its specific absorbance (A620/280 nm). Even though the purified phycocyanin in the freeze-dried form under storage, the bioactivity slowly reduces concerning deviation in their absorbance ratio. The nature and stability of phycocyanin before each application should perform this spectral screening as a primary step. Changes in the spectral value an indicator of protein unfolding and denaturation can be screened primarily by UV-vis spectral property. This spectral quality analysis indicates the nature of phycocyanin with time which has an impact on the quality and reliability of the result.

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