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## Stability and Antioxidant Activity of Food-Grade Phycocyanin Isolated from *Spirulina platensis*

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Food-grade phycocyanin was obtained from *Spirulina platensis* cultured in seawater-based medium and purified by ammonium sulfate precipitation. The stability of phycocyanin under different conditions, including different pH, temperature, light, and edible stabilizing agents, was systematically investigated by spectroscopy methods. The optimum pH range for phycocyanin was found to be 5.0–6.0. Phycocyanin was kept stable at temperatures up to 45°C over short time periods (i.e., no significant changes were observed in the relative concentration of phycocyanin,  $C_R$ ). In contrast, incubation at a relatively high temperature resulted in a decrease in the  $C_R$  and half-life in a temperature-dependent manner. Constant exposure to light at  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 36 h, decreased the  $C_R$  value of phycocyanin (pH5.0) to 78.4%. Sodium chloride was an effective stabilizing agent for phycocyanin, and its efficacy increased in a concentration-dependent manner for all concentration ranges assessed in this study. Moreover, phycocyanin exhibited concentration-dependent antioxidant activities in 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) and  $\alpha, \alpha$ -Diphenyl- $\beta$ -picrylhydrazyl assays. Taken together, our results suggest that the optimal conditions for preserving the stability of food-grade phycocyanin isolated from *S. platensis* are a pH of 5.0–6.0, low temperature, darkness, and the addition of edible stabilizing agents.

**Keywords:** Antioxidant activity, Phycocyanin, *Spirulina platensis*, Stability, Temperature, Stabilizing agents.

### INTRODUCTION

*Spirulina platensis*, namely *Arthrospira platensis*, is a non-toxic cyanobacterium widely distributed in tropical and subtropical lakes characterized by a high pH and high concentrations of carbonate and bicarbonate.<sup>[1]</sup> *S. platensis* has long been used as a food source and is considered to be safe for human consumption.<sup>[2]</sup> In addition to its relatively high protein content, *S. platensis* also contains vitamins, minerals, unsaturated fatty acid, carotenoids, and other nutrients.<sup>[2]</sup> Therefore, *S. platensis* is considered a balanced natural food and is widely

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used as dietary supplement. Various processes have been developed to reduce the production costs and improve the quality of *Spirulina* for commercial purposes.<sup>[3]</sup> A large-scale *Spirulina* cultivation technique in seawater medium was established by the South China Sea Institute of Oceanology, Chinese Academy of Sciences in the early 1990s.<sup>[4]</sup> Cultivation in seawater medium not only improved the nutritional composition of *S. platensis*, but also effectively prevented such problems, as the accumulation of excess levels of heavy metals, which is a common occurrence in fresh water culturing.<sup>[5]</sup> Phycocyanin, the major phycobiliprotein of *S. platensis*, is an essential functional component. It has recently drawn much attention due to its excellent nutritional value, fluorescence specific-properties, and physiological functions.

Phycocyanin is often used as nutritional supplement, and has great potential benefits for human nutrition and health, as it contains all the essential amino acids.<sup>[6]</sup> As a water-soluble natural pigment, it is also widely used as a food coloring agent, cosmetic additives, fluorescent reagent for clinical diagnosis, and immunochemistry.<sup>[7-9]</sup> In addition, phycocyanin has significant antioxidative, anti-inflammatory, hepatoprotective, and radical scavenging properties.<sup>[10-12]</sup> Phycocyanin also prevents cisplatin-induced nephrotoxicity through the inhibition of oxidative stress.<sup>[13]</sup> Thus, it is a potential therapeutic or protective agent for the treatment of oxidative stress-induced diseases.<sup>[14,15]</sup> Moreover, phycocyanin has exhibited high therapeutic value in immunomodulation and cancer therapy.<sup>[16]</sup> However, the application of phycocyanin in different fields has been hindered by its sensitivity to treatment and storage conditions, which can easily cause denaturation, precipitation, and discoloration.

Phycocyanin, an accessory pigment to chlorophyll, is a pigment-protein complex of the phycobiliprotein family that occurs in many cyanobacteria and some red algae.<sup>[17]</sup> It is formed by  $\alpha$  and  $\beta$  subunits and has linear tetrapyrrole phytychromobilin covalently attached to its polypeptides via a thioether linkage to a conserved Cys residue.<sup>[18]</sup> Phycocyanin exists as a complex interacting mixture of trimer, hexamer, and decamer aggregates which has been reported to be a function of pH and ionic strength of the medium, protein concentration, and algal origin.<sup>[1,19-21]</sup> Furthermore, the stage of aggregation affects the molecular weight as well as the position and intensity of the maximum absorbance peak of phycocyanin.<sup>[22]</sup> The stability of phycocyanin depends on its origin, pH, temperature, light, and some exogenous substances.<sup>[22-27]</sup> Minor differences in the amino acid composition of phycocyanin affect the stability and structural properties of these proteins. Phycocyanin derived from the thermophiles is generally more stable than that derived from the mesophiles.<sup>[23,24]</sup> Stabilizing agents are usually added to phycocyanin solutions to ensure the long-term stability.<sup>[23,25]</sup> Besides optimizing the physical and chemical parameters mentioned above, phycocyanin microencapsulation has been developed to improve the storage stability of phycocyanin.<sup>[28]</sup>

The phycocyanin used in bioactivity studies usually of the high purity, and few reports have investigated food-grade phycocyanin. To enhance the storage stability and maintain the quality of phycocyanin, the anti-microbial agent sodium azide ( $\text{NaN}_3$ ) and the protein reducing agent dithiothreitol (DTT) are commonly used as preservatives in C-phycocyanin (CPC) for analytical purposes.<sup>[22]</sup> Although these compounds have strong preservative effects, they are also toxic and, therefore, cannot be used in preparations intended for human consumption. Therefore, it is necessary to develop an effective, green, and non-toxic method for the stabilization of food-grade phycocyanin. In the present study, to determine the optimal conditions for maintaining phycocyanin stability, we studied the effects of those factors, including pH, temperature, light, and the use of edible stabilizing agents, on the stability of food-grade phycocyanin derived from *S. platensis* that cultured in seawater-based medium. The antioxidant activity was also determined. This study provides not only suggestions for maintaining the stability and biological activity of phycocyanin during the storage process, but also useful information for the development and application of *S. platensis* cultured in seawater-based medium.

## MATERIALS AND METHODS

### Materials and Culture Conditions

The *S. platensis* used in this experiment was a seawater domesticated strain and cultured in seawater-based medium in open raceway ponds in Sanya, as previously described.<sup>[4]</sup> A semi-continuous culture mode was adopted, and the *S. platensis* cells were harvested by filtration daily.

### Extraction and Purification of Phycocyanin

The *S. platensis* was suspended in phosphate buffer (50 mM, pH 6.8) and subjected to several freezing (−20°C)/thawing (4°C) cycles in darkness. The mixture was then centrifuged at  $8000 \times g$  (5804R, Eppendorf, Hamburg, Germany) for 30 min at 4°C to collect the phycocyanin-containing supernatant. The supernatant was subsequently fractionated by precipitation with two saturations of ammonium sulfate at 30 and 65%. The precipitate from the 65% saturation of ammonium sulfate was collected by centrifugation at  $8000 \times g$  for 20 min at 4°C. The precipitate was resuspended in phosphate buffer (50 mM, pH 6.8) and dialyzed against double distilled water at 4°C for 48 h in darkness by intermittently changing the distilled water at regular intervals. The phycocyanin containing solution was then centrifuged at  $10,000 \times g$  for 20 min at 4°C to remove the denatured protein. A food-grade phycocyanin powder was obtained after lyophilization.

### Assessment of Phycocyanin Stability

For the stability assessment, the food-grade phycocyanin powder was solubilized in phosphate-citrate buffer to a final concentration of 0.4 mg/mL.

### pH Stability

Phycocyanin solutions were prepared in phosphate-citrate buffer at pH values of 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0. The ultraviolet-visible (UV-Vis) absorption spectra of phycocyanin solutions were recorded on a UV-Vis spectrophotometer (UV-2550, Shimadzu, Tokyo, Japan) between 250 and 700 nm with a 1-cm-path-length quartz cuvette. The purity ratio of phycocyanin ( $A_{620}/A_{280}$ ) was calculated using the UV-Vis absorption spectra data. Based on the UV-Vis absorption spectra, phycocyanin solutions at pH 5.0, 5.5, 6.0, 6.5, and 7.0 were chosen to further investigate the effects of pH at two temperatures (55 and 65°C) for the indicated durations.

### Temperature Stability

Phycocyanin solutions at pH 5.0, 6.0, and 7.0 were incubated in a hot water bath for 30 min at 11 temperatures between 25 and 75°C in 5°C intervals. To further investigate the effects of short-term incubation, the phycocyanin solution was incubated at four temperatures (60, 65, 70, and 75°C) for 1, 2, and 4 min.

### Light Stability

Phycocyanin solutions at pH 5.0, 6.0, and 7.0 were exposed to lamps emitting light of 50 and 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  total intensity at room temperature (25°C) for 2, 4, 8, 12, 24, and 36 h.

### Stability in the Presence of Stabilizing Agents

Sucrose, glucose, or sodium chloride were added to the phycocyanin solutions at pH 5.0, 6.0, 7.0, and 8.0 at a concentration of 20% (w/v). The phycocyanin solutions containing stabilizing agents were incubated in a hot water bath at 65°C for 30 min. To further investigate the effect of the stabilizing agents, the phycocyanin solution at pH 6.0 with the indicated concentrations of sucrose, glucose, or sodium chloride within their maximum permissible levels was incubated at 65°C for 30 min.

### Analysis

The absorbance of each sample was measured at 620 and 652 nm. The C-phycocyanin (CPC) and allophycocyanin (APC) concentration of the solution were calculated using the following equations:<sup>[29,30]</sup>

$$\text{CPC (mg/mL)} = \frac{A_{620} - 0.474(A_{652})}{5.34}$$

$$\text{APC (mg/mL)} = \frac{A_{652} - 0.208(A_{620})}{5.09}$$

The phycocyanin concentration in the solution was the sum of the CPC and APC concentrations. The relative concentration of phycocyanin ( $C_R$ ) used to quantify the stability was expressed as the percentage of the remaining phycocyanin concentration of the treated samples over that of the initial concentration, as previously described.<sup>[25]</sup>

In the temperature stability assay, the degradation rate constant ( $\kappa$ ) used to estimate the thermal degradation of phycocyanin solution and half-life value ( $T_{1/2}$ ) which is the time required for phycocyanin concentration to fall to half its initial concentration was calculated as described by Chaiklahan.<sup>[25]</sup>

### Determination of Antioxidant Activity of Phycocyanin

#### *2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical-scavenging assay*

The ABTS scavenging activity was measured using spectrophotometric method with minor modifications.<sup>[31,32]</sup> Briefly, the ABTS stock solution was prepared by mixing ABTS (7 mM) and potassium persulfate (2.45 mM) in equal quantities. After being stored in the dark for 18 h, the stock solution was diluted with phosphate buffer saline (PBS) (0.02 M, pH 7.4) to a working solution yielding an absorbance of  $0.70 \pm 0.05$  at 734 nm. To measure the antioxidant capacity, 20  $\mu\text{L}$  aliquots of the phycocyanin solutions of different concentrations were mixed with 180  $\mu\text{L}$  of the ABTS working solution. The absorbance of the mixture was then measured at 734 nm after 6 min of incubation in darkness at room temperature. The ABTS radical-scavenging activity of the samples was expressed as  $S\% = ([A_{\text{control}} - A_{\text{test}} - A_{\text{sample}}] / A_{\text{control}}) \times 100$ , where  $A_{\text{control}}$  is the absorbance of the blank control (ABTS working solution with PBS),  $A_{\text{test}}$  is the absorbance of ABTS working with sample solution, and  $A_{\text{sample}}$  is the absorbance of sample solution with PBS. In this experiment, Trolox was used to generate a standard curve. The Trolox equivalent antioxidant capacity (TEAC) of sample was calculated from the Trolox standard curve and expressed as millimoles of Trolox equivalents (TEs) per gram of sample (mmol TE/g). The  $\text{IC}_{50}$ , defined as the concentration of sample necessary to cause 50% inhibition, was calculated using the relationship between the percentage scavenging activity and the sample concentration.

### $\alpha,\alpha$ -Diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical-scavenging assay

The DPPH scavenging activity was measured as previously described with minor modifications.<sup>[33]</sup> First, 20  $\mu$ L aliquots of phycocyanin solution at different concentrations were mixed with 180  $\mu$ L of DPPH in methanol solution (0.06 mM). An equal amount of PBS and DPPH served as the control. After incubation for 30 min in darkness at room temperature, the absorbance was recorded at 517 nm. The calculations of the DPPH radical-scavenging activity and IC<sub>50</sub> were conducted analogously to the ABTS scavenging calculations previously described.

### Statistical Analysis

The data obtained were expressed as the mean  $\pm$  standard deviation, and all experiments were completed in triplicate. Differences of  $p < 0.05$  and  $p < 0.01$  were considered statistically significant. All statistical analyses were performed using OriginPro 8.6 software.

## RESULTS AND DISCUSSION

### Effect of pH on Phycocyanin Stability

Phycocyanin is generally graded according to its purity ratio ( $A_{620}/A_{280}$ ), which is indicative of the purity of the preparation with respect to contaminating proteins.<sup>[34]</sup> A purity of 0.7 is considered to be food grade, 3.9 is considered to be reactive grade and 4.0 or greater is considered to be analytical grade.<sup>[35]</sup> Various methods have been developed for the separation and purification of phycocyanin, such as chromatography, gradient centrifugation, ammonium sulfate precipitation, and membrane process.<sup>[36–38]</sup> Food-grade phycocyanin with a purity ratio ( $A_{620}/A_{280}$ ) of approximately 1.5 was obtained in this study due to the more effective removal of contaminating proteins in the ammonium sulfate precipitation step. UV-Vis spectroscopy is a popular analytical method used for the characterization of phycocyanin. The decrease in the relative intensity of the peaks at 620 and 652 nm for the same protein concentration indicates decrease in the phycocyanin content of the aqueous solution. The pH of the dissolvent is one of the most important factors influencing the stability of phycocyanin. Therefore, in the present study, UV-Vis spectroscopy was used to determine the stability of phycocyanin dissolved in phosphate-citrate buffer at different pH values to determine the optimal pH range for the stability of phycocyanin isolated from *S. platensis* cultured in seawater-based medium. All the phycocyanin solution samples exhibited a characteristic absorption peak at 620 nm and strong absorbance at approximately 280 nm, as shown in Fig. 1A. At the same concentration of phycocyanin, the relative intensity of the peaks at 620 and 652 nm was lowest at pH 3.0, followed by pH 4.0. In acidic solutions ( $\text{pH} \leq 4.5$ ), the phycocyanin structure is unfolded, resulting in protein precipitation.<sup>[35]</sup> This phenomenon explains why the phycocyanin solutions at pH 3.0 and 4.0 exhibited lower intensities at 620 and 652 nm for the same protein concentration. There were no significant differences among the spectra of phycocyanin at pH 5.0, 6.0, and 7.0. As shown in Fig. 1B, the purity ratio ( $A_{620}/A_{280}$ ) calculated using the UV-Vis absorption spectra data exhibited a similar trend.

The  $C_R$  values of the phycocyanin solution at five different pH levels (5.0, 5.5, 6.0, 6.5, and 7.0) incubated at 55 and 65°C were shown in Fig. 1C and 1D. The phycocyanin at pH 7.0 showed the lowest stability, as evidenced by its lowest  $C_R$  value. After 30 min of incubation at 55 and 65°C, the  $C_R$  values of phycocyanin at pH 7.0 decreased to 81.4 and 50.2%, respectively, which were significantly different from the values for the other treated groups ( $p < 0.05$ ). At 65°C, phycocyanin was found to be more stable at pH 5.0, but it was more stable at pH 5.5 and 6.0 at 55°C. This result consistent with the findings of Antelo et al., who reported that phycocyanin

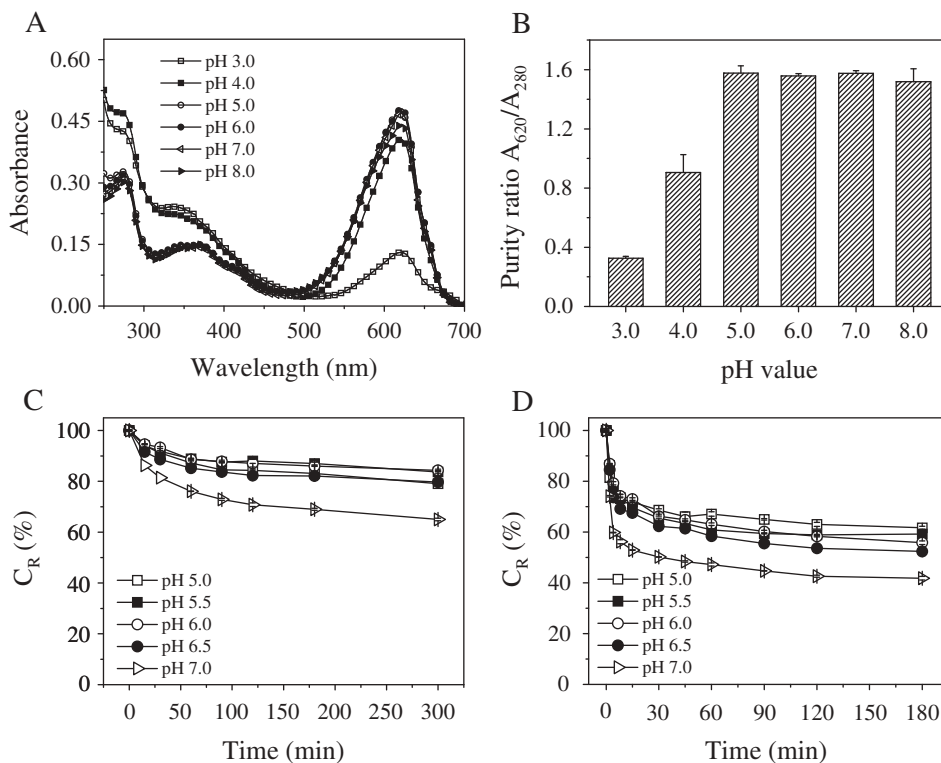


FIGURE 1 Effect of pH on the spectra of phycocyanin in phosphate-citrate buffer at A: room temperature and B: the corresponding purity ratios ( $A_{620}/A_{280}$ ).  $C_R$  values of phycocyanin in phosphate-citrate buffer at C: different pH values after incubation in a water bath at 55°C and D: 65°C for different durations in darkness.

was more stable at pH 6.0 between 50 and 55°C, but at pH 5.0 between 57 and 65°C.<sup>[26]</sup> Taken together, these results indicate that the optimal pH range for maintaining the stability of food-grade phycocyanin is between 5.0 and 6.0, in agreement with previous work.<sup>[22]</sup>

### Effect of Temperature on Phycocyanin Stability

Temperature is also a decisive factor in determining the stability of phycocyanin. Phycocyanin in aqueous buffers at pH 5.0, 6.0, and 7.0 was thermally stressed from 25 to 75°C for 30 min. Phycocyanin was found to be stable at temperatures of up to 45°C, with no significant changes in the  $C_R$  values observed. The  $C_R$  values of phycocyanin decreased in a temperature-dependent manner. When the temperature was increased from 45 to 75°C, the  $C_R$  values of phycocyanin at pH 5.0, 6.0, and 7.0 decreased from 99.0, 99.1, and 94.8% to 61.0, 55.8, and 48.4%, respectively, as shown in Fig. 2. The phycocyanin solution at pH 7.0 showed the lowest stability of all the tested temperatures. This was particularly evident in the temperature range between 55 to 75°C. Between 45 and 60°C, the phycocyanin solution at pH 6.0 was more stable than that at pH 5.0, but above 60°C the phycocyanin solution at pH 5.0 was more stable. These results were similar to those reported by Antelo et al., who reported that the relationship between the degradation temperature and the pH is inversely proportional with respect to the degradation of the phycocyanin protein.<sup>[26]</sup> The degradation rate constant ( $\kappa$ ) at the indicated temperature increased in a

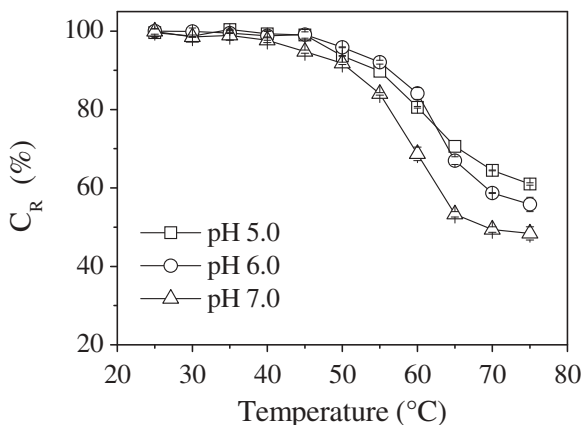


FIGURE 2  $C_R$  values of phycocyanin in phosphate-citrate buffer at pH 5.0, 6.0, and 7.0 after incubation in a water bath at various temperatures for 30 min in darkness.

temperature-dependent manner, as shown in Table 1. At 50°C, the half-life values of phycocyanin at pH 5.0, 6.0, and 7.0 were 321.3, 495.0, and 243.6 min, respectively, but the corresponding values decreased to 42.1, 35.7, and 28.6 min at 75°C, as shown in Table 1. The phycocyanin solution at pH 7.0 showed the most degradation at all the temperatures studied, as supported by the decrease in  $C_R$  and half-life values.

Phycocyanin is very sensitive to heat and undergoes rapid changes when exposed to high temperatures.<sup>[27]</sup> The  $C_R$  values of the phycocyanin solution at pH 5.0, 6.0, and 7.0 incubated between 60 and 75°C for short periods are shown in Table 2. The  $C_R$  values decreased in a time- and temperature-dependent manner. The  $C_R$  values of phycocyanin at pH 5.0, 6.0, and 7.0 were in the range of 96.3–98.9% after incubation at 60°C for 1 min. At 75°C, the  $C_R$  values of the phycocyanin solution at pH 7.0 decreased to 81.9, 57.9, and 51.4% for 1, 2, and 4 min of treatment, respectively. Between 60 and 65°C, the phycocyanin solution at pH 6.0 was most stable, but at 70 and 75°C it was most stable at pH 5.0, as evidenced by the increased  $C_R$  values. This result is consistent with those for the 30 min treatment previously described.

### Effect of Light Exposure on Phycocyanin Stability

The light sensitivity of the phycocyanin solution was studied using light exposure at room temperature (25°C). After exposure to lamps emitting light at 50 and 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  total intensity, the photochemical effect was measured by spectrophotometry. The  $C_R$  values of the phycocyanin solution at pH 5.0, 6.0, and 7.0 decreased in a time-dependent manner, as shown in Fig. 3. At the same light intensity, the phycocyanin solution at pH 6.0 showed a slightly lower degradation than that at pH 5.0 and 7.0. A slightly higher level of degradation was observed after exposure to 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light than after exposure to 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light. Although light influenced the stability of phycocyanin, the  $C_R$  values of phycocyanin at pH 5.0, 6.0, and 7.0 were still approximately 80% after continuous exposure to 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light for 36 h.

### Effect of Stabilizing Agents on Phycocyanin Stability

The degradation of the protein fraction was reported to have a significant impact on the color maintenance of phycocyanin.<sup>[39]</sup> To preserve the color and avoid phycocyanin denaturation due to



TABLE 1  
 Degradation rate ( $\kappa$ ) and half-life ( $T_{1/2}$ ) of phycocyanin in phosphate-citrate buffer at pH 5.0, 6.0, and 7.0

Temperature (°C)	pH 5.0		pH 6.0		pH 7.0	
	$\kappa$ (min)	$T_{1/2}$ (min)	$\kappa$ (min)	$T_{1/2}$ (min)	$\kappa$ (min)	$T_{1/2}$ (min)
50 ± 1	0.0022 ± 0.0000	321.3 ± 5.5	0.0014 ± 0.0000	495.0 ± 9.9	0.0028 ± 0.0001	243.6 ± 10.6
55 ± 1	0.0036 ± 0.0000	193.0 ± 0.6	0.0027 ± 0.0002	253.9 ± 17.1	0.0058 ± 0.0001	119.1 ± 2.4
60 ± 1	0.0072 ± 0.0001	96.4 ± 1.1	0.0058 ± 0.0006	120.7 ± 12.0	0.0125 ± 0.0008	55.6 ± 3.7
65 ± 1	0.0116 ± 0.0000	59.8 ± 0.1	0.0133 ± 0.0005	52.0 ± 2.0	0.0210 ± 0.0004	33.0 ± 0.7
70 ± 1	0.0146 ± 0.0000	47.5 ± 0.2	0.0178 ± 0.0001	39.0 ± 0.2	0.0235 ± 0.0004	29.4 ± 0.6
75 ± 1	0.0165 ± 0.0002	42.1 ± 0.4	0.0194 ± 0.0011	35.7 ± 2.0	0.0242 ± 0.0012	28.6 ± 1.4

TABLE 2  
*C<sub>R</sub>* values of phycocyanin in phosphate-citrate buffer at pH 5.0, 6.0, and 7.0 at the indicated temperatures for short-term treatment in darkness

Temperature (°C)	<i>C<sub>R</sub></i> (%)											
	pH 5.0				pH 6.0				pH 7.0			
	1 min	2 min	4 min	1 min	2 min	4 min	1 min	2 min	4 min	1 min	2 min	4 min
60 ± 1	97.5 ± 0.6	93.9 ± 0.4	89.4 ± 0.5	98.9 ± 0.8	97.2 ± 0.6	91.6 ± 1.0	96.3 ± 0.3	91.7 ± 0.1	80.7 ± 0.7			
65 ± 1	99.2 ± 0.5	92.7 ± 0.5	84.8 ± 0.5	98.3 ± 0.3	95.2 ± 0.7	87.8 ± 0.0	96.1 ± 0.5	86.8 ± 0.0	74.3 ± 0.6			
60 ± 1	95.9 ± 0.3	88.0 ± 0.1	79.3 ± 0.2	94.2 ± 0.0	87.3 ± 0.2	74.8 ± 0.4	90.4 ± 0.2	76.8 ± 1.0	61.0 ± 0.2			
75 ± 1	92.3 ± 2.4	77.9 ± 0.1	70.6 ± 0.1	92.8 ± 0.4	75.7 ± 0.2	65.3 ± 1.2	81.9 ± 0.6	57.9 ± 0.3	51.4 ± 0.3			

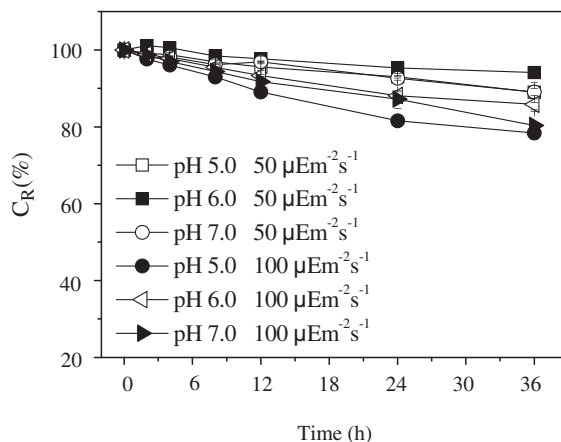


FIGURE 3  $C_R$  values of phycocyanin in phosphate-citrate buffer at pH 5.0, 6.0, and 7.0 exposed to light for 50 and 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at room temperature for indicated durations.

adverse chemical changes, stabilizing agents are often used as preservatives to protect the structural order of the protein chains. Glucose, sucrose, citric acid, sorbic acid, sodium chloride, ascorbic acid, and sodium azide are commonly used as stabilizing agents for phycocyanin.<sup>[23,25,26]</sup> The addition of sodium azide and sorbic acid did not have a significant effect on the stability of phycocyanin extracted from *Spirulina* sp.<sup>[25]</sup> Considering the desired applications and the cost of various stabilizing agents, we selected sodium chloride, sucrose, and glucose as promising stabilizing agents for food-grade phycocyanin derived from *S. platensis*. The effects of these edible stabilizing agents on the  $C_R$  values of phycocyanin solution are shown in Fig. 4A. The addition of sodium chloride, sucrose or glucose at a concentration of 20% (w/v) resulted in a reduction of the  $C_R$  value compared to the control, which did not include stabilizing agents. The addition of sodium chloride had the least effect on the  $C_R$  value, causing a reduction of less than 8% at various pH levels. Nevertheless, after exposure to 65°C for 30 min, the  $C_R$  values of these

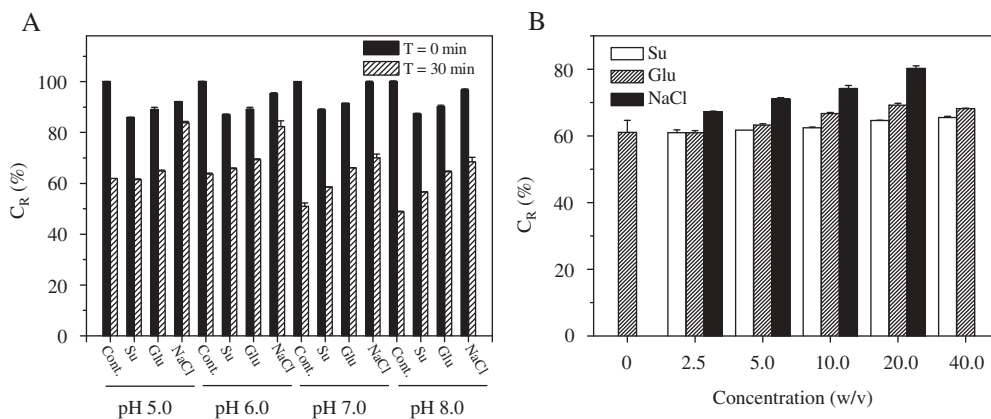


FIGURE 4  $C_R$  values of the phycocyanin solution at pH 5.0, 6.0, 7.0, and 8.0 with 20% sucrose, glucose or NaCl (w/v) after heating at A: 65°C for 30 min.  $C_R$  values of the phycocyanin solution at B: pH 6.0 with sucrose, glucose or NaCl at each concentration after incubation at 65°C for 30 min.

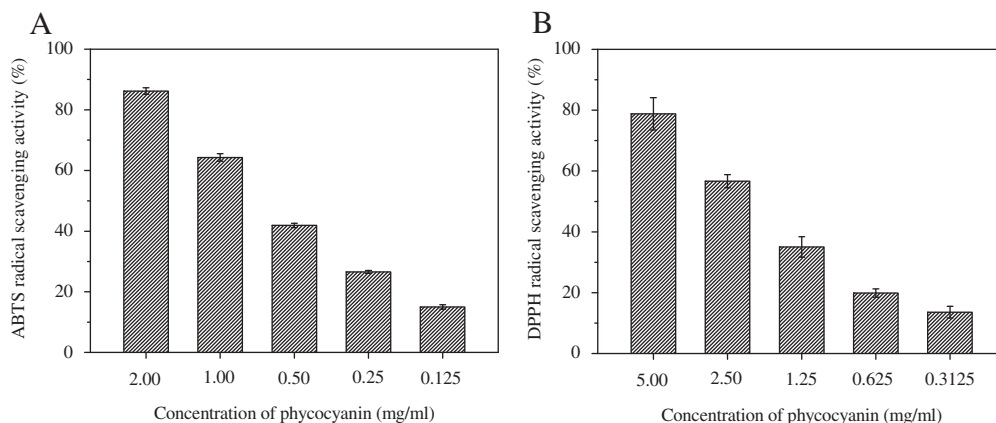


FIGURE 5 Antioxidant activity of phycocyanin as determined by ABTS and DPPH methods.

samples with the addition of stabilizing agents was greater than that of the control sample. The  $C_R$  values of the phycocyanin solutions at pH 5.0, 6.0, 7.0, and 8.0 with the addition of sodium chloride were significantly higher than those of the control and the solutions with other stabilizing agents ( $p < 0.05$ ).

To examine the effects of these stabilizing agents at various concentrations within their maximum permissible levels, the  $C_R$  values of treated phycocyanin were measured. After incubation at 65°C for 30 min, the  $C_R$  values of the phycocyanin solution at pH 6.0 with the addition of stabilizing agents of various concentrations increased to different degrees, as shown in Fig. 4B. The  $C_R$  values of the phycocyanin solution with the addition of sodium chloride were significantly higher than those of the control and increased in a concentration-dependent manner. The  $C_R$  values were 67.3, 71.1, 74.2, and 80.2% for 2.5, 5.0, 10.0, and 20.0% (w/v) sodium chloride, respectively, while no significant changes were observed after sucrose addition. It was obvious that sodium chloride was a suitable stabilizing agent for protecting phycocyanin from degradation.

### Antioxidant Activity of Phycocyanin

CPC, the major phycobiliprotein in *S. platensis*, contained high level of glutamic acid (GLU), aspartic acid (ASP), alanine (ALA), leucine (LEU), arginine (ARG), isoleucine (ILE), serine (SER), glycine (GLY), and threonine (THR),<sup>[40]</sup> most of which have been reported to be related to higher antioxidant activity levels.<sup>[41]</sup> The antioxidant activities of the food-grade phycocyanin solution were evaluated by the ABTS and DPPH methods, which are often used in the determination of the antioxidant abilities of natural compounds.<sup>[32,42]</sup> The ABTS radical-scavenging activity of phycocyanin solution at different concentrations is shown in Fig. 5A. As seen, the ABTS radical-scavenging activity increased in a concentration-dependent manner. The  $IC_{50}$  value and the TEAC for ABTS radical scavenging activity were found to be  $0.56 \pm 0.02$  mg/mL and  $0.75 \pm 0.01$  mmol TE/g phycocyanin, respectively (Table 3). The DPPH radical-scavenging activities of the phycocyanin solution at different concentrations are shown in Fig. 5B. The activities increased in a concentration-dependent manner. The  $IC_{50}$  value for the DPPH radical scavenging activity was  $1.86 \pm 0.18$  mg/mL (Table 3). It is known that oxidative reactions and reactive oxygen species (ROS) are involved in various diseases including atherosclerosis, diabetes, and Alzheimer's disease.<sup>[43,44]</sup> Data from both assays clearly demonstrated that food-grade phycocyanin possesses

TABLE 3  
Antioxidant activity of phycocyanin as determined by TEAC and DPPH methods

	TEAC <sup>a</sup>	ABTS (IC <sub>50</sub> ) <sup>b</sup>	DPPH (IC <sub>50</sub> ) <sup>b</sup>
Phycocyanin	0.75 ± 0.01	0.56 ± 0.02	1.86 ± 0.18

<sup>a</sup>TEAC expressed in mmol Trolox equivalent/g phycocyanin; <sup>b</sup>IC<sub>50</sub> expressed in mg/mL.

significant antioxidant properties, suggesting that food-grade phycocyanin may improve human health upon consumption.

## CONCLUSIONS

The present study, demonstrated that the optimal pH range for food-grade phycocyanin obtained from *S. platensis* cultured in seawater-based medium was influenced by the working temperature; the higher working temperature, the lower the optimal pH within a certain range. The food-grade phycocyanin solution remained stable at temperatures up to 45°C but was light-sensitive at room temperature. Stabilizing agents helped maintain the stability of phycocyanin. The optimal conditions for preserving the stability of food-grade phycocyanin obtained from *S. platensis* were found to be a pH of 5.0–6.0, low temperature, darkness, and the addition of edible stabilizing agents, such as sodium chloride. Food-grade phycocyanin also exhibited good antioxidant capacities, making it an excellent health food for human consumption.

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