The effect of different temperature on the stability of phycocyanin on microcapsule *Spirulina platensis*

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**Abstract:** Phycocyanin as antioxidants is a special pigment in *Spirulina platensis* that belongs to the temperature-sensitive. Microencapsulation of *S. platensis* is needed to maintain the phycocyanin content. The aimed of this study was to determine the effect of microencapsulation and different temperatures (40 °C and 100 °C) process to phycocyanin degradation, the relative concentration (C_R) of phycocyanin, the antioxidant degradation and total color different (TCD) and its interaction. The result shown the microencapsulation process could increase the stability of phycocyanin against temperature. The higher heating processes cause an increase in phycocyanin degradation. The interaction between high temperature treatment and microencapsulation produced the highest value of phycocyanin degradation (57.23±0.16%) and antioxidant degradation (46.85±0.04%), namely *S. platensis* powder (without microencapsulation) with a temperature of 100 °C, while the highest of phycocyanin C_R (95.72±0.13%) at *S. platensis* microcapsules with a temperature of 40 °C. However, microencapsulation gave a higher TCD value of *S. platensis* caused by the Maillard reaction.

1. **Introduction**

*Spirulina platensis* is one type of spiral cyanobacteria microalgae which is found in Indonesian saline waters and fresh waters. The active biopigment compounds contained in *S. platensis* were chlorophyll (147.43 µg/mL), phycocyanin (55.4 µg/mL), allophycocyanin (51 µg/mL) and phycoerythrin (39.6-44.1 µg/mL) [1]. Phycocyanin is a special pigment in *S. platensis* which can provide blue-green colors that can dissolve in polar solvents such as water. Phycocyanin was a protein compound that belongs to the group of phycobiliproteins and had the ability as an antioxidant compound [2].

Phycocyanin was sensitive to heating so that in its application as a functional food material, it was necessary to do a microencapsulation to reduce the loss of active compounds in the material during the processing. The method commonly used in the microencapsulation of compounds which are sensitive to high temperatures is freeze drying, which is a microencapsulation method that uses very low temperatures for drying. Thus, thermal degradation can be prevented [3,4].

The microencapsulation process could increase the potential of *S. platensis*, as well as maintain the stability of phycocyanin content in the use of processing temperatures in its application as functional food material. Research on phycocyanin stability has been carried out by Chaiklahan *et al.* [5], which is...
phycocyanin was not stable against heat, pH, light and humidity. Phycocyanin would decrease its stability at temperatures above 47 °C. Dewi et al. [6] has also carried out studies of phycocyanin microencapsulation with spray drying method using maltodextrin and carrageenan as an encapsulant. However, no one has reported microencapsulation of S. platensis treated basil leaf extract with freeze drying method using maltodextrin. Thus, the aimed of this research was to determine the effect of microencapsulation and heating treatment and the interaction of both of them on the stability of phycocyanin in S. platensis.

2. Methodology

2.1. Sample preparation
S. platensis powder (PT. Neoalga Indonesia Makmur, Indonesia) was mixed with basil leaf extract (1:4 w/v) to eliminate the muddy and earthy odor in spirulina powder. The basil leaf that was obtained from local market in Semarang (Central Java Province, Indonesia) was extracted with aquadest. The mixture was dried at 40 °C for 17 h then crushed using a ball mill (Planetary Ball Mill PM 400, Germany) to obtain powder-like texture.

2.2. S. Platensis microencapsulation
Microencapsulation process was done by adding S. platensis powder, 15% maltodextrin DE 10 (CV. Multi Kimia Raya, Indonesia) (b/b) into distilled water, the homogenized using Homogenizer ultraturrax (WiseTis HG-15A, Germany) at 10,000 rpm for 3 min. The homogenized solution was frozen at -35 °C for 24 h. Then, the sample was dried using freeze dryer (Powerdry LL 1500 SYSTEM 240 V, Germany) at -100°C with vacuum pressure for about 48 h. The dried sample is mashed using a ball mill to powder and is called S. platensis microcapsule.

2.3. Stability against temperature
Microencapsulated S. platensis and unencapsulated S. platensis were heated at temperature 40 °C and 100 °C using an oven (Binder ED 53, Germany) for 30 min to determine the stability of phycocyanin in S. platensis. Phycocyanin degradation, C_R of phycocyanin, antioxidant degradation, and TCD in all samples were calculated.

2.4. Phycocyanin degradation and relative concentration (C_R) of phycocyanin
Phycocyanin content were determined using the absorbance of spectrophotometry [7]. The sample (40 mg) was added to 1 mL of phosphate buffer pH 7.0 and distilled water 1:1 (v/v) and stored for 24 h at 4 °C. Then the sample was centrifuge (Hettich EBA 20, Germany) with 4000 rpm and took the supernatant, read the absorbance in UV-Vis Spectrophotometer (Shimadzu, Japan) at 620 nm and calculated with the formula (1).

\[
\text{Phycocyanin content (\%) = \frac{Absorbance \times mL \ of \ solvent}{3.39 \times mg \ of \ sample \times dw}} \times 100\% \tag{1}
\]

In which, 3.39 = C-Phycocyanin coefisien at 620 nm, dw = dry weight of sample (mg).

The phycocyanin relative concentration (C_R) referred to Chaiklahan et al. [5] the remaining phycocyanin content as percentage of the initial content. The phycocyanin degradation referred to Irwan et al. [8] the percentage of phycocyanin occurred because of the temperature (2).

\[
D (\%) = \frac{(C_o - C_i)}{C_o} \times 100\% \tag{2}
\]

In which, C_o = phycocyanin content before heating treatment, C_i = phycocyanin content after heating treatment.
2.5. **Antioxidant activity degradation**

Radical cations were made by mixing 7 mM ABTS (2,2-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid) stock with 2.45 mM potassium persulfate (1:1 v/v) and incubated for 4 to 16 h at ambient temperature until the reaction was completed and stable. The methanol was used to dilute the ABTS solution. The photometric test was carried out on 0.9 mL ABTS + solutions and 0.1 mL sample (100 and 200 µg/mL) and mixed for 45 s. The measurement was carried out immediately at 734 nm after 15 min. The inhibition of ABTS was calculated by the formula (3) [9]. Then, antioxidant degradation was calculated based on the percentage between the difference of inhibition before and after the heating treatment with the inhibition before heating.

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of the sample}}{\text{Absorbance of blank}} \times 100\% \tag{3}
\]

2.6. **Total color difference (TCD)**

The TCD was calculated based on color value with Colorimeter (Color meter NH310, China). The color value consists of lightness (L), redness to greenness (a), and yellowness to blueness (b) [10]. The TCD represented as \(\Delta E\), which can be calculated using formulas (4).

\[
\Delta E = (\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2 \frac{1}{2} \tag{4}
\]

2.7. **Statistical Analysis**

The data with three replications were tested of analysis of variance (Anova) and then followed by the tukey test to determine differences in treatment. Statistical Package for the Social Science (SPSS) version 17 was used for statistical analysis.

3. **Results and Discussion**

3.1. **Phycocyanin Degradation**

Spirulina powder experienced phycocyanin degradation of 9.37% at 40°C and 57.23% at 100°C, while microencapsulated of spirulina experienced phycocyanin degradation of 4.28% at 40°C and 17.40% at 100°C (Fig. 1). These results indicated that the microencapsulation of spirulina can reduce the degradation of phycocyanin during heating. The same research results were also shown by Lin et al. [11] that the content of astaxanthin pigment was encapsulated with alginate encapsulation higher than the control of astaxanthin pigment without encapsulation, so it could be explained that encapsulation protected pigment from degradation by temperature.

Based on Fig. 1, it appears that the degradation of phycocyanin was influenced by the temperature when heating. i.e. the higher of heating temperature, causes more phycocyanin in spirulina to be degraded. It is because phycocyanin was a group of pigments bound to the phycobiliprotein protein, consisting of 20% cellular protein. The content of chromoprotein (polypeptide α and β) in phycocyanin was sensitive to temperature. The occurrence of denaturation process was seen from changes in physical structure, i.e. denaturation of proteins will usually experience unfolding in certain parts [12,13]. Fig. 1 also shown that spirulina powder experienced phycocyanin degradation more than 50% at 100°C. This result could be concluded that phycocyanin was denatured faster at high temperature [14].
3.2. Phycocyanin relative concentration (C_R)

The C_R of phycocyanin in S. platensis microcapsules with different temperature was relatively higher than S. platensis powder (Fig. 2). The result showed that the microencapsulation process was considered to maintain the stability of phycocyanin in S. platensis during heating. Kurniasih et al. [15] reported that active compounds in the form of pigments can be protected from environmental influences by microencapsulation. Pigments can be trapped by coating material through the microencapsulation process.

The heating treatment at 100°C affected the value of C_R obtained was smaller than at 40°C (Fig. 2). High temperature had a enormous effect on the stability of phycocyanin obtained because phycocyanin was one of a group of phycobiliprotein that were easily degraded when exposed to high temperature. The result of C_R obtained were (MS100°C) 82.60±0.05% and (S100°C) 42.77±0.16%. Matsuura et al. [16] stated that high protein content was suspected because of the absence of denaturation. Temperatures above 100°C cause irreversible denatured proteins, because under these conditions proteins generally aggregate after heat denaturation.

3.3. Antioxidant activity degradation

S. platensis powder was treated at 40°C and 100°C resulted in antioxidant degradation value was relatively higher than the microcapsules of S. platensis with the same treatment (Fig. 3). This result indicated that microencapsulation maintained the stability of antioxidant in S. platensis. A significantly decrease occurred in the spirulina powder due to directly contact with heat, which in a high amount may
accelerate antioxidant degradation. Dewi et al. [17] reported that phycocyanin can be protected from the effects of heat through microencapsulation. The higher of phycocyanin content caused greater antioxidant activity.

The heating temperature can affect the stability of antioxidant activity in S. platensis. Heating of spirulina powder and microcapsules at 100°C produced an increase in the degradation of antioxidant activity compared to 40°C. Park et al. [18] explained that antioxidants can be naturally contained in food. If the raw materials were cooked at a high temperature, the antioxidant will be reduced because of chemical and physical reactions. Dejsungkranont et al. [19] added that S. platensis has a pigment that function as an antioxidant, namely phycocyanin. Antioxidant will decrease due to high temperatures. The factor that most influences the antioxidant activity of c-phycocyanin is temperature.

Based on Fig. 3, can be seen that S. platensis which is microencapsulated and given lower heating (40°C) causes the least degradation of antioxidant activity. Heat resistance is a key requirement that must be possessed by antioxidants. Most food processing uses high temperatures. The antioxidant activity of microcapsule shaped samples is more resistant to heat than its activity in extract form. Microencapsulation is proven to protect core material from damage [20].

3.4. Total colour different (TCD)
Microencapsulation could protected the phycocyanin contained in S. platensis. However, microencapsulation of spirulina using maltodextrin as a coating material causes the color change of spirulina from green to brownish when given heating treatment. The amount of color change in spirulina can be seen from the TCD value, i.e. the higher TCD value indicated that there is a greater of color change or difference. The results showed that microencapsulated spirulina had a higher TCD value than spirulina without microencapsulation (Fig. 4). The Maillard reaction was responsible for the discoloration of spirulina, where maltodextrin which is a polysaccharide reacts with the protein contained in material in the presence of heating. The concentration of maltodextrin is very important in influencing discoloration. Carbohydrates will turn dark brown when heated [21,22].

The heating treatment at 40°C and 100°C on S. platensis powder and microcapsules showed an increase in the value of TCD at higher temperatures (Fig. 4). The Maillard reaction was non enzimatic browning food caused by heating process, usually caused by a chemical reaction between reducing sugar, especially D-glucose with free amino acids or free amino groups from an amino acid that was part of a protein chain [23]. Increasing temperature can cause an increase in discoloration of the material [24,25].
4. Conclusion
Microencapsulation could protect phycocyanin which contained in S. platensis, so that it can increase the stability of phycocyanin against temperature. The higher heating processes cause an increase in phycocyanin degradation. The interaction between microencapsulation and the temperature of heating treatment produced the highest value of phycocyanin degradation (57.23±0.16%) and antioxidant degradation (46.85±0.04%), namely S. platensis powder (without microencapsulation) with a temperature of 100 °C, while the highest of phycocyanin Cb (95.72±0.13%), the lowest of phycocyanin degradation (4.28±0.13 %) and antioxidant activity degradation (19.26±0.11 %), namely S. platensis microcapsules at 40 °C.

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