Utilization of carrageenan and chitosan as coating material in phycocyanin encapsulation

Hadiyanto1,2, H Sutanto3, M Suzery4, N P Adetya1*, A M Nilamsari1, A Yunanda1
1Department of Chemical Engineering Faculty of Engineering, Diponegoro University, Jl. Prof Soedarto, SH Tembalang, Semarang, Central Java, Indonesia
2Master Program of Environmental Science, School of Postgraduate Studies, Diponegoro University, Jl. Imam Bardjo No 3-5, Semarang, Central Java, Indonesia
3Physic Department, Faculty of Science and Mathematic, Diponegoro University. Jl. Prof. Soedarto, SH-Tembalang Semarang
4Departement of Chemistry, Diponegoro University, Jl. Prof.Soedharto SH, Tembalang, Semarang
Email: naispinta26@gmail.com

Abstract. Phycocyanin is one of the pigments found in Spirulina platensis. Phycocyanin has potential as natural blue dye and antioxidant. The application of phycocyanin as food ingredient is still limited due to its vulnerability to environment. Microencapsulation can keep phycocyanin from environmental influences such as temperature, light and pH. This research aimed to determine the antioxidant activity of phycocyanin microencapsulation with carrageenan and chitosan coating material caused by the effect of thermal degradation overtime and its influence on the physical characteristics of the microencapsulation. The highest antioxidant activity of 61.48% was found in chitosan coating material that occurred at temperature of 40℃ with 30 minutes heat treatment. Phycocyanin microcapsule with chitosan has more vibrant blue color compared to carrageenan. Phycocyanin microencapsulation either by using carrageenan or chitosan have functional groups such as alkane, alcohol, ether and ester.

1. Introduction
Phycocyanin is a natural pigment which has high economic value, Spirulina platensis may contain this compound up to 20% of its dry mass [1]. The blue-green pigment can be used as natural coloring for foods. This substance can substitute the synthetic coloring because it can produce a bright blue color [2]. Besides, it can also be developed as additive, functional nutrition, and non-toxic. Phycocyanin has ability as antioxidant which inhibits peroxidation of lipid in rats [3].

The use of natural antioxidant has been increasingly demanded by society because it is considered saver than synthetic antioxidant. One of the chemical compounds in Spirulina sp is phycocyanin which is known to have the ability to boost immune system, contains antioxidant, anti-inflammation, also neuroprotective [4, 2]. However, phycocyanin is very susceptible to degradation when it is exposed to light and high temperature thus it has limited storage life [5]. One of the alternatives to overcome the problem is microencapsulation. Microencapsulation can be literally translated as a process of coating a core particle with another material in order to obtain the expected physical and chemical characteristics. Microencapsulation is a process which transform solid or liquid substance into micro (0.2 – 5000 μm) capsule form. This process is intended to protect and preserve the active component from environmental influence [6].
The coating material used in the process of microencapsulation should have emulsifier property so that it can be added into food ingredient. The commonly used materials for coating are carrageenan and chitosan. Besides used as chemicals in various industries, chitosan also have antibacterial property [7]. The use of carrageenan in food products is able to improve emulsion stability [8]. Therefore, carrageenan and chitosan can be used as edible coating material which inhibit the growth of bacteria on food product so that the storage life can be extended [7]. This research is aimed to determine the antioxidant activity of phycocyanin microcapsules with carrageenan and chitosan coating materials caused by thermal degradation overtime. Moreover it also aimed to find out the influence of the use of coating materials on phycocyanin microcapsules’ physical characteristics.

2. Materials and methods

2.1. Materials
Phycocyanin powder from Spirulina platensis microalgae was used in this study. Carrageenan and chitosan are used as coating materials. It also used other materials in the process of microencapsulation and measurement of antioxidant activity such as acetic acid, NaCl, methanol, DPPH, STTP, KCl and aquades.

2.2. Phycocyanin microcapsule with carrageenan coating material
Carrageenan is dissolved in NaCl 1% solvent and heated while being stirred at 40°C-50°C. The stirring process is stopped after 100ml of 2% w/v carrageenan is obtained. Phycocyanin is dissolved in distilled water (1:10) and then combined with carrageenan and stirred. The solution is put into a syringe and injected into KCl 0.5 M solution. The formed granules are then washed and dried by using freeze drying method.

2.3. Phycocyanin microcapsule with chitosan coating material
2 gram of chitosan is dissolved with 100ml of acetic acid 1% solution and then stirred for 4 hours. The stirring process is stopped after 100ml of 2% w/v chitosan is obtained. Meanwhile, phycocyanin is dissolved in distilled water with 1:10 ratio. The solution then mixed with chitosan solution with 1:1 ratio and then stirred. After it turns homogenous, the solution is put into syringe and injected into STTP 0.5 M. The formed granules are washed with NaCl 1% and then dried with freeze drying at 10°C [9].

2.4. Antioxidant activity, color stability test and FTIR test
The chemical for testing antioxidant activity by using DPPH method is 1,1-diphenyl-2-picrylhydrazyl (DPPH) and methanol. The variables used were heating temperature (40°C, 50°C, and 60°C) and heating time (0, 30, 60, and 90 minutes). 10 mg of microcapsules were dissolved into 10 ml of distilled water, and then heated according to the temperature variable. After that 1 ml sample was taken based on heating time variable. 1 ml of DPPH 0.1mM was added into 1ml of pre-heated phycocyanin microcapsules and 5ml of methanol, then incubated for 1 hour. The absorbency measurement was conducted in 515-520nm wavelength [10]. Antioxidant activity is determined by the following equation (1):

Antioxidant activity (100%) = \[1 - \left(\frac{A_{\text{sample}}}{A_{\text{blank}}}\right)\] \times 100\%

(1)

Color stability test was conducted by using digital colorimeter. 5 ml sample of each variables was dripped right under the lens of the camera which was connected to Digital Colorimeter device. Then the camera is directed on the part of which the color was analyzied to obtain the value of L, a and b on the device’s screen. While FTIR test is used to determine the vibration of the functional groups of the compound. From the FTIR spectrum will be known by the function of functional vibrations in the
Phycocyanin microencapsulation. Shimadzu FTIR 8400 Japan was used with a spectral resolution of 1 cm\(^{-1}\) and the spectral profiles were collected in region between 4,000 cm\(^{-1}\) and 400 cm\(^{-1}\).

3. Results and discussion

3.1. The effect of coating material on antioxidant activity

![Figure 1. Effect of heating temperature to antioxidant activity at 60 minutes heating time](image)

Based on Figure 1, it shows that the longer the heat treatment, the graphic tends to decrease. This phenomenon happened to both phycocyanin microcapsule with carrageenan and chitosan coating material. It is because phycocyanin is biliprotein. Biliproteins just like globular protein in general can have structural damage caused by heat [11]. This structural damage is called denaturation, which is the systematic change of organic structure become random three dimensional configuration [12]. Therefore the longer the heat treatment the more the number of damaged phycocyanin. The decrease in the number of active phycocyanin the decrease in antioxidant activity.

Besides, based on the test that had been conducted, antioxidant activity with chitosan coating material is higher than carrageenan coating material. The highest antioxidant activity is 61.48% which is found in chitosan coating material at 40\(^\circ\)C temperature and 30 minutes heat treatment. This happened because the process of phycocyanin microencapsulation was conducted at temperature of 40-50\(^\circ\)C which make some phycocyanin become damaged because phycocyanin is very vulnerable to heat [5].

3.2. The color stability test comparison between carrageenan and chitosan coating material

![Figure 2. The color stability of carrageenan and chitosan coating agent](image)
Phycocyanin microencapsulation with both chitosan and carrageenan coating material have L(-) color which indicate that the color of phycocyanin solution is getting darker. The value of a(-) indicates greenish color and the value of b(-) indicates bluish color, while ∆E (Figure 2) indicate how big the color change that happens [13].

Phycocyanin microcapsule with chitosan has more vibrant blue color compared to phycocyanin microcapsule with carrageenan. The blue color indicates abundant of phycocyanin content. Phycocyanin is a natural blue pigment which can be found in Spirulina sp. which has antioxidant property. However, phycocyanin is vulnerable to temperature [2, 14]. If treated with high temperature, then the phycocyanin extract will be damaged.

3.3. FTIR test of phycocyanin microencapsulation with carrageenan and chitosan

![FTIR test result of (a) phycocyanin+carrageenan; (b) phycocyanin+chitosan](image)

**Figure 3.** FTIR test result of (a) phycocyanin+carrageenan; (b) phycocyanin+chitosan
Figure 3a shows the result of phycocyanin+carrageenan FTIR test have functional group namely alcohol, alkane, ester and ether. The absorption band on a surface area around 3439 cm\(^{-1}\) shows –OH linkage (alcohol), while on the 2930 cm\(^{-1}\) area is C-H linkage (alkane). The absorption band on 1649 cm\(^{-1}\) indicates ester linkage and 1068 cm\(^{-1}\) absorption is ether linkage [15]. This is in accordance with the reference that algae biomass absorb metal ion through complex formation interaction between metal ion and functional group such as –COOH the main constituent of polysaccharide and peptide group (-CO, -NH\(_2\) and –CONH\(_2\)) as the constituent of pectin and protein which act as electron pair donor [16].

Figure 3b shows the result of phycocyanin+chitosan have functional group namely alkane, ester, ether and alcohol. Absorption band on around 3404 cm\(^{-1}\) area shows O-H linkage (alcohol) while on 2928 cm\(^{-1}\) is C-H linkage (alkane). The absorption band on 1650 cm\(^{-1}\) C=O linkage (ester). The absorption band on 1157 cm\(^{-1}\) and 1215 cm\(^{-1}\) indicates ether linkage [17].

4. Conclusion

The highest antioxidant activity is 61.48% which is found in chitosan coating material at 40°C temperature and 30 minutes heat treatment. Phycocyanin microencapsulation with both chitosan and carrageenan coating material have L(\(-\)) color which indicate that the color of phycocyanin solution is getting darker. The value of a(\(-\)) indicates greenish color and the value of b(\(-\)) indicates bluish color. Phycocyanin microcapsule with chitosan has more vibrant blue color compared to phycocyanin microcapsule with carrageenan. Phycocyanin microencapsulation either by using carrageenan or chitosan have functional groups such as alkane, alcohol, ether and ester.

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