Thermal degradation kinetics of phycocyanin encapsulation as an antioxidant agent

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Abstract. Phycocyanin is a blue-light pigment that found in Cyanobacteria and two Eukaryotics algae such as Rhodophyta and Crytophyta. Phycocyanin is soluble in water and has a strong fluorescent properties as an antioxidant and normally used in food industry, cosmetic, biotechnology, and drug. However, Phycocyanin is easily damaged by a heating process. The aim of this study is to obtain the optimal condition of phycocyanin encapsulation with different coating materials, Chitosan and Carrageenan, by the calculation of heat resistance of antioxidant activity (D), range of temperature that increase the rate of degradation (Z), rate constant of degradation (k), and activation energy (Ea). The ratio of phycocyanin and the coating material are 2% (w/v) and 2 % (w/v).
Keyword: Phycocyanin, Antioxidant, Carrageenan, Chitosan, Degradation

1. Introduction

Antioxidants are compounds that can be used to protect food by slowing down the damage, rancidity or change of colour caused by oxidation [9]. Antioxidants are able to act as hydrogen radical contributors or free radical acceptors so as to delay the initiation phase of free radical formation. The presence of natural (such as Phycocyanin compounds) and synthetic antioxidants can inhibit lipid oxidation, prevent damage, change inorganic components in foodstuffs so as to extend shelf life [8]. T-butyl hydroxy anisol (BHA) and di-t-butyl hydroxytoluene (BHT) compounds are used as food antioxidants, but the possibility of adverse side effects is not used for therapeutic agents. The development of natural antioxidants has received considerable attention in recent years. It is intended for the purpose of preventive medicine and for the food industry. The DPPH test is an effective and rapid method for estimating antioxidant activity. The principle of this method is to measure the power of extracting an ingredient against the free radical of DPPH [3].

Development of antioxidant components is widely used in the production of functional foods. The functional properties of functional foods are determined by the bioactive components contained in them, eg dietary fiber, inulin, FOS and antioxidants [6]. Based on the source, antioxidants are divided into endogenous antioxidants and antioxidant enzymes, such as: Superoxide Dismutase (SOD), Catalase (Cat), and Glutathione Peroxidase (Gpx); as well as exogenous antioxidants, which are obtained from outside the body / food. Various natural ingredients native to Indonesia contains many

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antioxidants with various active ingredients, including vitamin C, E, pro vitamin A, organosulfur, α-tocopherol, flavonoid, thymoquinone, statin, niacin, Phycocyanin, and others [2].

One type of antioxidant that comes from outside the human body is Phycocyanin. Phycocyanin is the most important pigment in *Spirulina sp.* Microorganisms [4].Phycocyanin has a significant content as an antioxidant, protector of liver function, and radical compounds remover [10]. Therefore, Phycocyanin is widely used in the field of food colouring and cosmetics. Phycocyanin content in 10 grams of dried spirulina is about 1400 mg or 14%, so it has been recommended to consume 0.25-2.5 grams of Phycocyanin per day to boost the immune system and inhibit cancer growth [4]. There are several methods to protect Phycocyanin from degdradation, microencapsulation is one of them.

Microencapsulation is a process where solid materials, liquids, and even gases can be capsulated (encapsulated) with microscopic particle size, by forming thin wall covering around the material to be capsulated [1]. The techniques used for microencapsulation are spray drying, spray cooling / chilling, fluidization of bed drying, melt extrusion, melt injection, centrifugal extrusion, coacervation, liposome capture, co-crystallization, emulsification, rotational suspension rotation, and molecular inclusion [7]. The active ingredient in the microencapsulation of phycocyanin is sensitive to light, heat and oxygen so as to have a limited shelf life. Through the desired microencapsulation process can minimize the occurrence of quality degradation of phycocyanin. Therefore, thermal degradation kinetics model of the microencapsulation of phycocyanin needs to be further investigated.

2. Material and Methods

2.1. *Materials*

In this study, phycocyanin is used as raw material. In addition, side materials are also used such as: chitosan, carrageenan, DPPH, methanol, acetic acid, STTP, KCl, NaCl and aquadest. Chitosan and carrageenan were used as a coating material. Methanol, acetic acid, NaCl, and aquadest were used as solvent. STTP and KCl were used as a microencapsulation media, and DPPH as a material test. Phycocyanin is powder form of *Spirulina platensis*. While other materials obtained from laboratory in Diponegoro University.

2.2. *Variables*

The controlled variables in this research were heating time during 90 minutes with observation time for every 30 minutes. The response variable in this study was the activity of antioxidant. Independent variables in this study were temperature (40°C, 50°C, 60°C) and coating materials (chitosan 2% and carrageenan 2%).

2.3. *Making Microencapsulation of Phycocyanin*

Making of microencapsulation of Phycocyanin is devided into two methods, they are chitosan coating and carrageenan coating. Once, Coating of chitosan was done by dissolving chitosan into 1% of acetic acid solvent. One percent of acetic acid solution was prepared by dissolving 1 ml glacial acetic acid into 100 ml of aquadest. Then, 2 grams of chitosan was dissolved in 100 ml of 1% acetic acid solution, stirred with magnetic stirrer for ±4 hours until homogenous to obtain chitosan 2% w/v 100 ml. After that, dissolve Phycocyanin in aquadest with ratio of Phycocyanin and aquadest of 1:10. Then, Phycocyanin solution mixed with chitosan solution with ratio of volume of 1:1. After that, stir the solution by using magnetic stirrer until homogeneous. Then, homogeneous solution is inserted into syringe and injected into STTP (Sodium Tripolyphosphate) solution 0.5 M. Wait for about 15 minutes until the matrix perfectly formed. Then, filtered and washed the granules with 1% of NaCl. After that, dried it by freeze drying method (10°C) and wait for about 24 hours. Finally, microencapsulation with chitosan coating is formed. In the other hand, carrageenan coating was done by dissolved 2 grams of carrageenan in 100 ml of 1% NaCl solution and heated with stirring by using magnetic stirrer at temperature about 40-50 °C until gel of carrageenan are formed, and carrageenan 2% w/v 100 ml was obtained. After that, dissolved Phycocyanin in aquadest with ratio of Phycocyanin and aquadest of

1:10. Then Phycocyanin solution mixed with carrageenan solution with ratio of volume of 1:1. After that, stirred the mixture by using magnetic stirrer until homogeneous. The homogeneous solution is inserted into syringe and injected into KCl 0.5 M. Wait for about 15 minutes until the matrix perfectly formed. Then filtered and washed the granules with 1% NaCl. After that, dried by freeze drying method (10°C) and wait for about 24 hours. Finally, microencapsulation by carrageenan coating is formed.

2.4.*Antioxidant Activity Test*

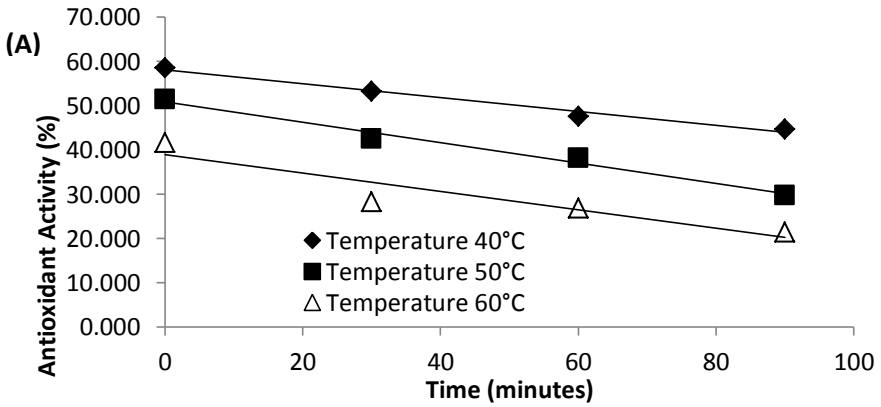
The DPPH test is an effective and rapid method for estimating antioxidant activity. The principle of this method is to measure the power of extracting an ingredient against the free radical of DPPH [3]. The various variables are temperature (40°C, 50°C, and 60°C) and heating time (0, 30, 60, 90 minutes). Antioxidant activity of microencapsulation Phycocyanin analysis was conducted by dissolving 10 mg of microencapsulation into 10 ml of aquadest, then heated according to various temperature (40°C, 50°C, and 60°C). Then, took 1 ml sample for various times (0, 30, 60, 90 minutes). Make a solution of 0.1 mM DPPH by dissolving 2 mg DPPH into 50 ml of methanol. Then 1 ml of heated microencapsulated Phycocyanin, 1 ml DPPH 0.1 mM, and 5 ml of methanol are incubated for about 30 minutes. Then, take the solution that has been incubated to have an antioxidant activity test. And also, prepared blank solution in the same way without using of Phycocyanin encapsulation. Perform absorbance measurement at optimum λ in wavelength of 515 nm. Antioxidant capacity is to inhibit free radicals. To determined the equation of antioxidant activity is:

Antioxidant Activity (100%) = {1- (A sample / A blank)} x 100%.

3. Result and discussions

3.1. Determine Thermal Degradation Kinetic of Phycocyanin Encapsulation with Carrageenan

Thermal degradation kinetics of Phycocyanin encapsulation with carragenan are detemined by various parameters, including parameters of k (1/minute), D (minutes), Z (°C) and Ea (kJ/mol). By Arrhenius equation, the order of the reaction can be determined. After a suitable order has been determined, the rate constant of reaction (k) is known by slope of the order equation.



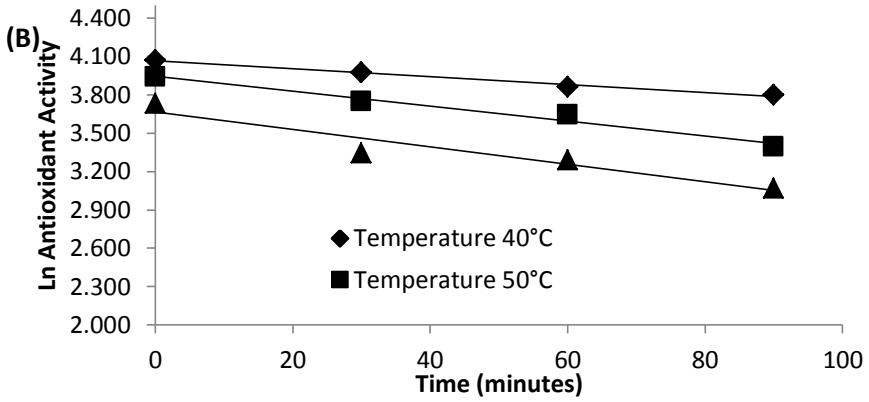


Figure 3.1. Thermal degradation kinetics of antioxidant activity (A) 0 order & (B) 1st order

Based on Figure 3.1.,maximum regression is 1st order. So, thermal degradation kinetics of antioxidant activity of carrageenan are using 1st order equation with R^2 equal to 0.9161-0.9899. The results are according to [5] that the kinetics model of antioxidant capacity by DPPH method of 1st order equation with R^2 is equal to 0.95-0.97.

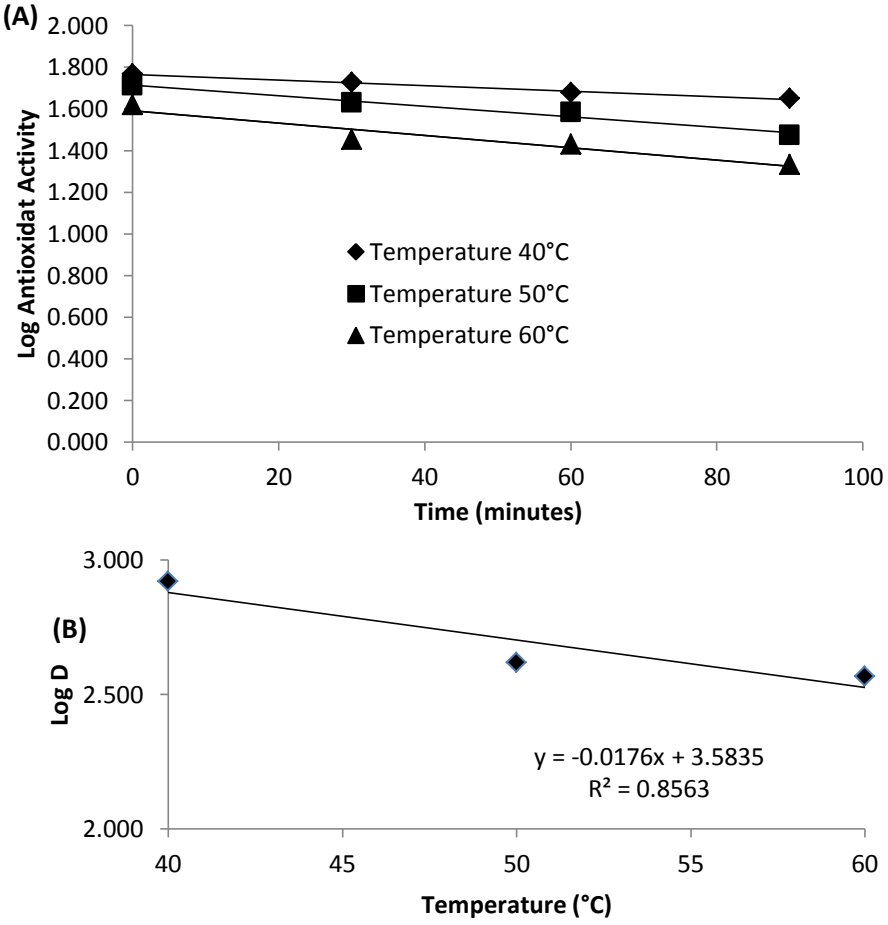


Figure 3.2. Graph (A) value D and (B) value Z

According to the data from Figure 3.2 (A) and Table 3.1, there is a degdradation process in antioxidant during heating process. The value of D, are 769 minutes for $D_{40°C}$, 400 minutes for $D_{50°C}$, and 333 minutes for $D_{60°C}$. Thus, the differences in temperature and heating time also make a different effect of degdradation kinetics of antioxidant activity in Phycocyanin encapsulation. Based on Figure 3.2 (B), it showed that regression equation is y=-0.0176x+3.5835 with R^2=0.8563.

Table 3.1 Calculation of values D, k, Z, and Ea

Temperature (°C)	D (minute)	k (minute^{-1})	Z (°C)	Ea (kJ/mol)
40	769	0.0031		
50	400	0.0058	54.945	804
60	333	0.0068		

From Table 3.1., it has been known that the reaction rate constant (k) at 40°C, 50°C, and 60°C are 0.0031 min $^{-1}$, 0.0058 min $^{-1}$, 0.0068 min $^{-1}$.

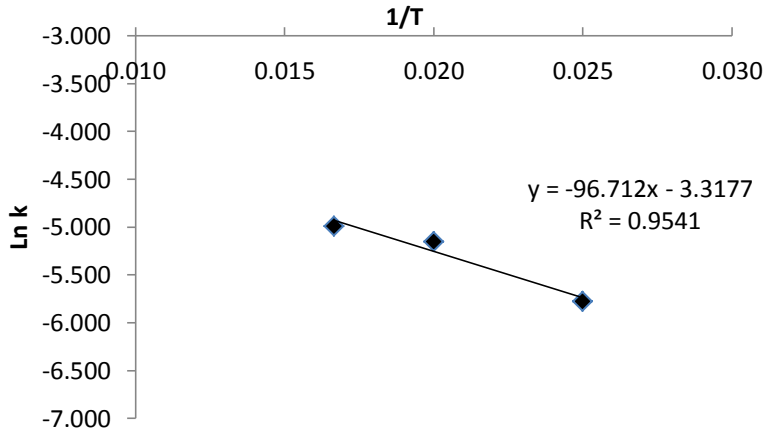


Figure 3.3. Energy activation degradation of antioxidant activity

Based on Figure 3.3., the activation energy (Ea) is determined by using linear regression between 1/T (x-axis) and Ln k (y-axis), then the slope is multiplied by R constant (8.314 J/mol K). With linear regression equation of y=-96.712x-3.3177, R^2 = 0.9541, so we obtained activation energy for 804 kJ/mol. The figure shows the minimum energy is required to initiate the degradation of antioxidant activity in Phycocyanin microencapsulation. The lower of Ea value will be easier to be decreased.

4. Conclusions

Based on the results, have been concluded that D value of carrageenan coating material for $D_{40°C,}$ $D_{50°C}$, and $D_{60°C}$ are 769 minutes, 400 minutes, and 333 minutes. While D value of chitosan coating material material for $D_{40°C,}$ $D_{50°C}$, and $D_{60°C}$ are 833 minutes, 417 minutes, and 370 minutes. Z value of carrageenan coating material is 54.945°C. While Z value of chitosan coating material is 56.818°C. The reaction rate constants (k) of carrageenan coating material for 40°C, 50°C, and 60°C are 0.0031 min^{-1}, 0.0058 min^{-1}, and 0.0068 min^{-1}. While the reaction rate constant (k) of chitosan coating material for 40°C, 50°C, and 60°C are 0.0027 min^{-1}, 0.0055 min^{-1}, and 0.0062 min^{-1}. Activation energy (Ea) of

carrageenan coating material is 804 kJ/mol. While activation energy (Ea) of chitosan coating material is 857.3 kJ /mol.

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