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Physical Properties of *Spirulina* Phycocyanin Microencapsulated with Maltodextrin and Carrageenan

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Phycocyanin as a source of natural blue dye is unstable to light, temperature, and pH during processing and storage. Microencapsulation is used to protect phycocyanin from external influences where the type and form²³ of coating materials used may affect the characteristics of phycocyanin microcapsules. This study aims to evaluate the physical properties of encapsulated phycocyanin from *Spirulina* and the potential of maltodextrin in combination with κ -carrageenan in its microencapsulation process by spray drying. Microcapsules were prepared with five different concentrations of maltodextrin and κ -carrageenan i.e., 10% : 0%; 9.75% : 0.25%; 9.5% : 0.5%; 9.25% : 0.75%, and 9% : 1% (w/w). Results indicated that microcapsules of phycocyanin with 9% of maltodextrin and 1% of κ -carrageenan as coating material produced the highest bulk density, particle size, and encapsulation efficiency, which were $1,501.27 \text{ kg} \cdot \text{m}^{-3}$, $1,152.33 \text{ nm}$, and 48.87%, respectively. The differential scanning calorimeter thermogram and the Fourier Transform Infrared Spectroscopy measured the presence of phycocyanin, maltodextrin, and κ -carrageenan in microcapsules.

Key words: κ -carrageenan, maltodextrin, phycocyanin, physical properties

INTRODUCTION

Nowadays, there has been an increase in the utilization of pigments from natural plants in the food industry as replacement for artificial coloring agents, in an effort to provide healthier food for consumers. Eriksen (2008) mentioned that *Spirulina* sp. is a microalga that contains large amount of pigments such as phycocyanin and allophycocyanin. The major problem for the pigments is associated with its application in food industries as coloring agent, since natural coloring agents show less resistance and low storage stability when exposed to oxygen, changes in temperature, pH, and other factors.

Microencapsulating phycocyanin would become one of methods to improve qualities of resistance and stability

to protect the pigment. However, the types of coating material used are critical in the encapsulation process. The most common materials used for microencapsulation is alginate (Dong et al. 2013), other supporting materials including carrageenan. As a coating material, κ -carrageenan makes the formation of spherical and smooth-surfaced microparticles more desirable, since it has the strongest gels within the various types of carrageenan (Daniel-da-Silva et al. 2011; Leong et al. 2011). However, the use of carrageenan is limited due to its low physical stability in gelling temperature; hence the coating matrix mixture should be added with other binding material to control the gel texture hardening at room temperature (Doleyres et al. 2002). Maltodextrin is a hydrolyzed starch that can be used as coating material due to low water solubility, high viscosity, and their low cost (Krishnaiah et al. 2012).

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In this study, the phycocyanin (as an active agent) was entrapped in coating materials made from maltodextrin and carrageenan by spray dried process. The coating materials prevented phycocyanin from being released from its core, increased its stability, and improved its functionality; hence, it is easy to be applied to food.

The strong electrostatic interaction of the carboxylic groups of carrageenan with carboxyl groups of the maltodextrin leads to formation of the complex maltodextrin-carrageenan microcapsules (Finotelli et al. 2010). Although maltodextrin and carrageenan have already been known in the food industry, this mixture is rarely used as coating materials in phycocyanin microencapsulation. In high molecular weight composition, phycocyanin can affect microencapsulation preparation, since carrageenan solution has a high viscosity and gelling ability. The aim of this study is to determine the application of spray drying method for the encapsulation of *Spirulina* phycocyanin. The coating materials microcapsules of maltodextrin in combination with κ -carrageenan were characterized in terms of encapsulation physical properties such as particle size, encapsulation efficiency, bulk density, temperature of glass transition, and molecular structure.

MATERIALS AND METHODS

Microencapsulation of Phycocyanin

The microcapsules containing phycocyanin, maltodextrin, and κ -carrageenan were prepared by spray drying method. Phycocyanin was extracted from *Spirulina* sp. (CV Neoalga, Indonesia) using aquades 1:100 (w/v). The extract was stirred at 300 rpm for 4 h at room temperature and separated using centrifuge at 6000 rpm for 15 min (Wina 505, Indonesia). Coated material was prepared with maltodextrin (CV Multi Kimia Raya, Indonesia) and κ -carrageenan (PT Selalu Lancar Maju Karya, Indonesia). Ninety percent (90%) of phycocyanin extract and 10% of coated material were combined with different concentrations of maltodextrin and κ -carrageenan. Microcapsule coating material was prepared with five different concentrations of maltodextrin and κ -carrageenan i.e., 10% : 0%; 9.75% : 0.25%; 9.5% : 0.5%; 9.25% : 0.75%, and 9% : 1% (w/w). For the next step, the phycocyanin extract and different treatments of coating material were mixed with ratio 9:1 (v/w). The mixture was then homogenized with a homomixer (Ika-Werke T50 basic, Germany) at 10,000 rpm for 2 min. The final homogenous mixture was then spray dried (PlantLab, UK) with 1.3 mm diameter nozzle at inlet air temperature of 80°C and feed flow rate of 5.1 mL · min⁻¹.

Particle Size

The particle size distribution of phycocyanin microcapsules was determined by using a laser scattering particle size distribution analyser (Malven Zetasizer Nano ZS ver 6.20, UK) (Krishnaiah et al. 2012).

Encapsulation Efficiency (EE)

The EE was calculated according to the method from Yan and co-authors (2014) by measuring the mass of non-coated phycocyanin and the mass of phycocyanin added at the beginning of the microencapsulation process.

Bulk Density

Phycocyanin microcapsules were poured into a 10 mL tube and the samples were repeatedly tapped by lifting and dropping the tube under its own weight until negligible difference in volume between succeeding measurement was observed. The bulk density was calculated as m/V (kg · m⁻³) (Caparino et al. 2012).

Differential Scanning Calorimeter (DSC)

Thermogram of phycocyanin microcapsules was measured with DSC (Shimadzu DSC-60 plus, Japan). Maltodextrin and κ -carrageenan compounds as coating materials were also observed for the thermogram before it was encapsulated. The mixture of phycocyanin, maltodextrin, and κ -carrageenan were then sealed in an aluminum pan and heated in the range of 25-300°C with the heating rate was of 10°C min⁻¹ under nitrogen atmosphere with a flow rate of 20 mL/min (Saha & Ray 2013).

Fourier Transform Infrared Spectroscopy (FTIR)

The molecular structure of maltodextrin powder, κ -carrageenan powder, phycocyanin microcapsules with maltodextrin (10%) as a coating material, and phycocyanin microcapsules with maltodextrin (9%) and κ -carrageenan (1%) as coating materials were determined by FTIR. Spectrum 100 series FTIR spectrometer (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 (Krishnaiah et al. 2012).

Statistical Analysis

All experiments were conducted in triplicate and statistical analysis was carried out by using SPSS version 17.0 (International Business Machines Corporation, USA). The data were analyzed with one-way analysis of variance (ANOVA). To find out the differences among the treatments, the Duncan's New Multiple Range Test was applied.

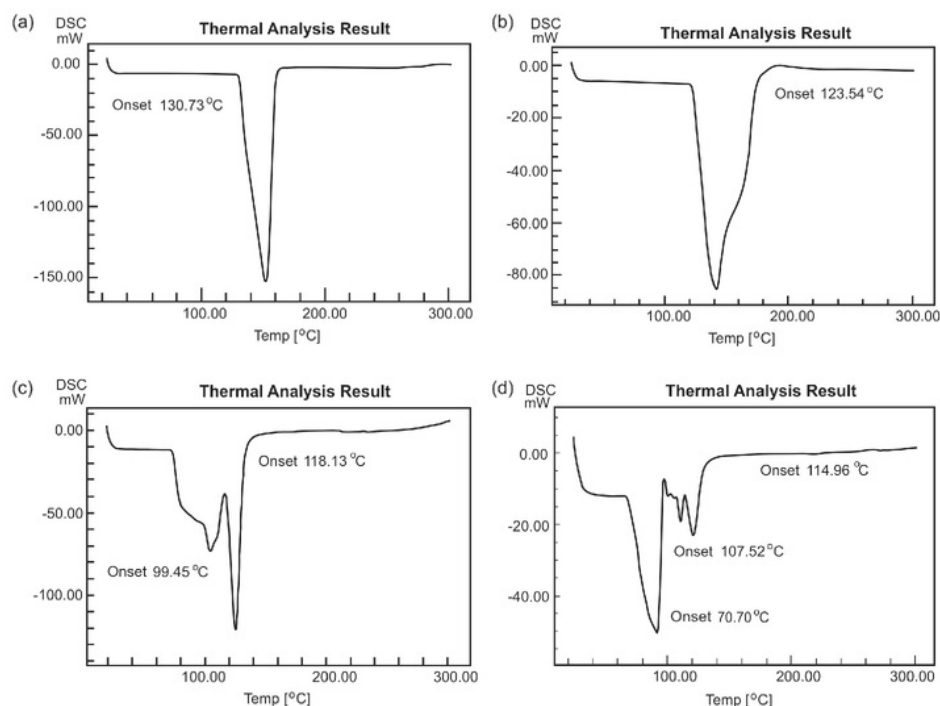


Figure 1. DSC thermogram of (a) maltodextrin powder, (b) κ -carrageenan powder, (c) microcapsules of phycocyanin with maltodextrin (10%) as a coating material, and (d) microcapsules of phycocyanin with maltodextrin (9%) and κ -carrageenan (1%) as coating materials.

Table 1. Particle size, efficiency encapsulation, and bulk density of phycocyanin microcapsules prepared by maltodextrin and κ -carrageenan (combined concentration 10%) with different concentrations of κ -carrageenan.

Concentration of maltodextrin and κ -carrageenan (%)	Particle size (nm)	Encapsulation Efficiency (%)	Bulk density ($\text{kg} \cdot \text{m}^{-3}$)
10 : 0	363.40 ± 6.24^a	13.11 ± 0.10^a	$1\,474.99 \pm 3.42^a$
9.75 : 0.25	414.10 ± 23.52^a	12.44 ± 0.28^a	$1\,500.00 \pm 0.00^b$
9.50 : 0.5	414.83 ± 65.30^a	13.06 ± 0.59^a	$1\,500.86 \pm 0.88^b$
9.25 : 0.75	632.53 ± 17.13^b	27.54 ± 2.18^b	$1\,501.38 \pm 0.45^b$
9 : 1	$1\,152.33 \pm 111.18^c$	48.87 ± 1.10^c	$1\,501.27 \pm 0.36^b$

Note: Values are mean of three replications $\pm$ standard deviation. Means in the same column not sharing the same letters are significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Particle Size

Table 1 shows that the use of carrageenan with concentrations up to 0.75% as coating material had significantly increased the diameter of phycocyanin microcapsules. The particle size of phycocyanin microcapsules prepared with maltodextrin (9%) and κ -carrageenan (1%) was found to be 1,152 nm (mean diameter). The particle size of

microcapsules is affected by the emulsion stability, the inlet air temperature of spray dryer, and the ratio of mass of core to mass of coating material (Zheng et al. 2011; Krishnaiah et al. 2012; Carneiro et al. 2013; Pitchaon et al. 2013; Pasrija et al. 2015).

Bulk Density

Table 1 shows that the use of maltodextrin and κ -carrageenan as coating materials significantly increased

the bulk density of phycocyanin microcapsules. As a coating material, κ -carrageenan can retard the process of evaporation during spray drying, thus affecting the weight of each particle. Mehrad and co-workers (2015) reported that moisture content from fish oil encapsulated with maltodextrin and fish gelatin as coating materials were lower than the fish oil encapsulated with maltodextrin and κ -carrageenan as coating materials. The same result was also reported by Dewi and co-authors (2016) where moisture content of phycocyanin encapsulated with κ -carrageenan and maltodextrin was higher than phycocyanin encapsulated with maltodextrin. The moisture content can result several effects on the formation of droplet surface, consequently transforming a tough leather-like skin and avoiding the moisture evaporation.

Caparino and colleagues (2012) explained that the bulk density increased with decreasing particle size. Those finding was different from the observation, where the bulk density of phycocyanin microcapsules increased with increasing particle size. However, the same result was shown by Painsi and co-workers (2015), where the increasing maltodextrin concentration led to decreasing bulk density of encapsulated olive pomace polyphenol.

1 Encapsulation Efficiency

The encapsulation efficiency (EE) is defined as the percentage relative of core material (phycocyanin) that is encapsulated inside the matrix (the total amount of the core material added during encapsulation process) (Table 1). The highest EE indicated that most of the phycocyanin was encapsulated (Dewi et al. 2016). The EE of phycocyanin microcapsules was significantly influenced by κ -carrageenan as coating material. Efficiency of encapsulation at maltodextrin (9%) and κ -carrageenan (1%) reached almost 49%. Those data were similar to those by Yan and co-authors (2014), where the EE of phycocyanin microcapsules was increased with coating material addition.

The important factors that affect the encapsulation efficiency and the stability of microcapsules are the type of coating material, the core materials, the properties of the emulsion (viscosity, total solids, drop size), and the conditions of the drying process (da Rosa et al. 2014; Jafari et al. 2008; Martins et al. 2014). Mehrad and co-workers (2015) and Betz and co-authors (2012) explained that a successful microencapsulation must result in a powder with maximum retention of the active material. Even all the parameters showed still increasing trend on EE, the addition of more than 1% κ -carrageenan seem to be difficult to examine since the coating mixture was on the gel form.

19 Differential Scanning Calorimetry (DSC)

The DSC thermogram of maltodextrin powder and

κ -carrageenan powder (Fig. 1) showed that there was a strong endothermal peak at around 10-150°C. The onset of maltodextrin occurs at 130.73°C and the onset of carrageenan occurs at 123.54°C. The microcapsules of phycocyanin with maltodextrin as coating material has two onsets, which are 90.45°C and 125.09°C. The results indicated that microcapsules are composed of two compounds, namely phycocyanin and maltodextrin. Meanwhile, microcapsules of phycocyanin with maltodextrin (9%) and κ -carrageenan (1%) as coating material have three onsets, which are 70.70°C, 110.57°C, and 120.87°C. The results demonstrated that microcapsules are composed of three compounds, namely phycocyanin, maltodextrin, and κ -carrageenan. The layering compounds on microcapsules are effected by rapid evaporation of a solvent in which the coating material is dissolved.

Kotzsi and co-workers (2012) stated that the first onsets or the second glass transition temperature (T_g) indicated two polymeric layers around the droplet. T_g is related to the physical stability of amorphous formulations. Over T_g , the stability of amorphous foods and pharmaceuticals is reduced because of an increase in molecular mobility of component molecules.

The Fourier Transform Infrared Spectroscopy (FTIR) spectra of maltodextrin are usually determined as a strong broad band between 856 cm^{-1} and 1,200 cm^{-1} and it is the most characteristic band for a polysaccharide. The peaks at 1,154 cm^{-1} and 1,079 cm^{-1} were assigned to C-O stretching (Fig. 2) (Namazkar & Ahmad 2013).

The band of κ -carrageenan was characterized by band of 848.68 cm^{-1} , which comprised galactose-4-sulfate. The band of 925.83 cm^{-1} comprised 3,6-anhydrogalactose, 1,072.42 cm^{-1} comprised glycoside linkage, the band of 1,257.59 cm^{-1} indicated the presence of ester sulfate, and the band of 3,448.72 comprised O-H stretching, respectively. The bands identified in this research are similar to those found by Freile-Pelegrin and colleagues (2011), Dewi and co-authors (2012), and Namazkar & Ahmad (2013) who also worked on carrageenan research.

Based on FTIR analysis, it can be seen from phycocyanin maltodextrin microcapsules and phycocyanin maltodextrin κ -carrageenan microcapsules (Fig. 2) that C-O on the same peak on 1,026.13 cm^{-1} and 1,149.57 cm^{-1} were attributed to phycocyanin, respectively. After microencapsulation of phycocyanin, maltodextrin, and κ -carrageenan, there were some peaks which, according to other researchers (Venkatesan et al. 2012; Jerley & Prabu 2015), are as follows: carboxylic bonding (1,419.61 cm^{-1}), amine bonding (1,635.54 cm^{-1}), and alkene groups (1,020.01 cm^{-1}).

Those spectra were characterized and it can be inferred that the blue pigment phycocyanin exists in the coating material. The absorption band of 1,026.13 cm^{-1}

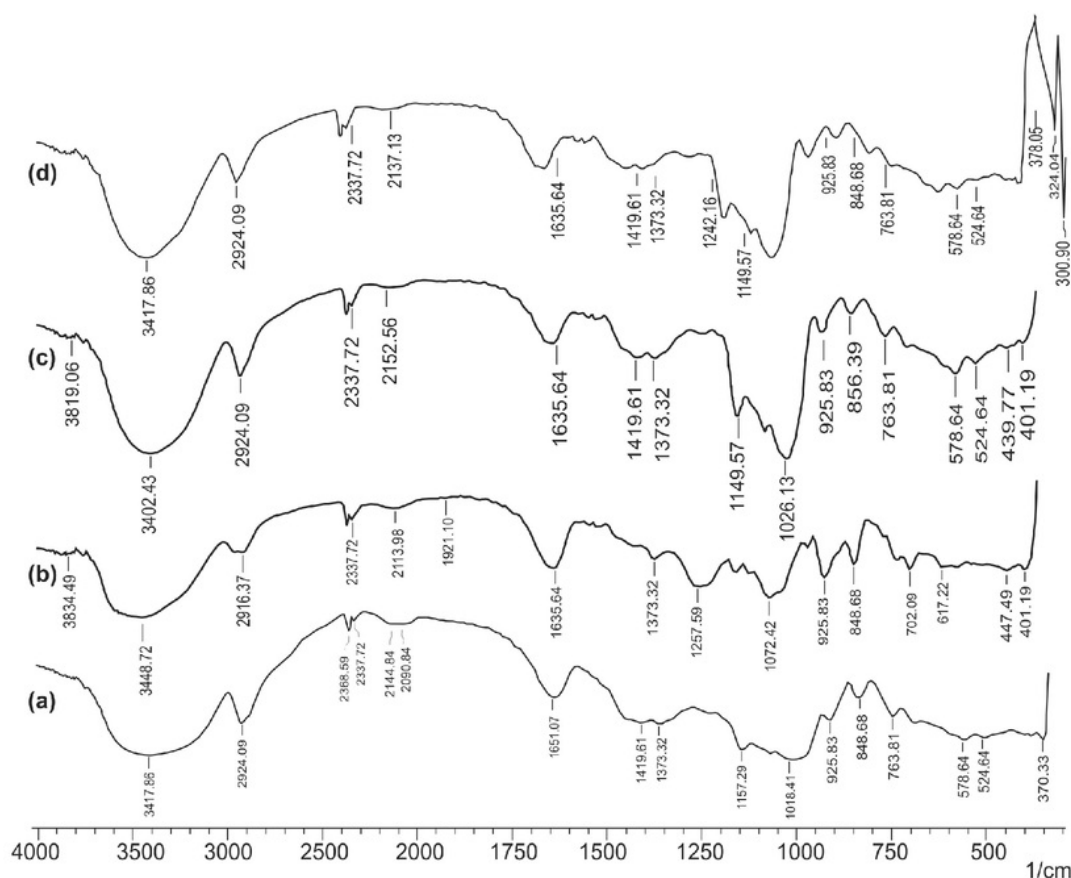


Figure 2. FTIR spectrum of (a) maltodextrin powder, (b) κ -carrageenan powder, (c) microcapsules of phycocyanin with maltodextrin (10%) as a coating material, and (d) microcapsules of phycocyanin with maltodextrin (9%) and κ -carrageenan (1%) as coating materials.

and $1,149.57 \text{ cm}^{-1}$ (these two were characterized as phycocyanin). For maltodextrin and κ -carrageenan, both shifted and existed to higher frequency near $1,635.54 \text{ cm}^{-1}$ and $1,419.61 \text{ cm}^{-1}$, respectively, indicating that there was an electrostatic interaction between carrageenan and phycocyanin in microcapsules. FTIR has been used to access the interaction between the bioactive compound and the polymers (Tadudari et al. 2014; Yuan et al. 2015). The results demonstrated that since the functional groups of phycocyanin, maltodextrin, and κ -carrageenan are still present in the microcapsules, it can be assumed there was no chemical bonding reaction between those compounds.

CONCLUSIONS

Microencapsulation of phycocyanin using maltodextrin (9%) and κ -carrageenan (1%) was successfully prepared by spray drying method. The microcapsules showed the

highest bulk density, particle size, and encapsulation efficiency, which were $1,501.27 \text{ kg} \cdot \text{m}^{-3}$, $1,152.33 \text{ nm}$, and 48.87% , respectively. There were three glass transition temperatures (T_g), namely 70.70°C , 110.57°C , and 120.87°C . This indicates that the microcapsules are composed of three compounds, namely phycocyanin, maltodextrin, and κ -carrageenan. In addition, the FTIR spectra also identified the presence of phycocyanin, maltodextrin, and carrageenan in the microcapsules.

12 ACKNOWLEDGMENTS

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