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Formulation and Characterization of Phycocyanin Microcapsules within Maltodextrin-Alginate

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ABSTRACT

Phycocyanin is a source of natural blue dye which can be extracted from *Spirulina* sp. The main characteristics of phycocyanin are unstable by temperature and pH during processing and storage. Microencapsulation methods could be proposed to protect the phycocyanin from the external effect, where the types and concentration of encapsulant used may affect the characteristics of the result. The aim of this study was to determine the best formulation and characterization of phycocyanin microcapsules from *Spirulina* sp. with maltodextrin and alginate as an encapsulant. The microcapsules were produced using five different concentrations of alginate in maltodextrin, namely 0%; 0.2%; 0.4%; 0.6%; and 0.8% (w/w). The total encapsulant used was 10% of phycocyanin microparticle solution. The results showed that the increasing concentration of alginate could raise the levels of phycocyanin, moisture content, encapsulation efficiency, bulk density, blue intensity, and particle size, it also improved the morphology of the microcapsules. Phycocyanin microcapsules with alginate concentration of 0.6% and 9.4% maltodextrin had the highest phycocyanin content, encapsulation efficiency, and blue intensity.

Keywords: Maltodextrin-alginate; microcapsules; phycocyanin

INTRODUCTION

Phycocyanin is blue-colored phycobiliprotein contained in *Spirulina* sp. which can be used as alternative natural colorant of food, drink and cosmetics. Several reports have shown that phycocyanin has advantages as compared with blue synthetic colourant are that phycocyanin functions as antioxidant (Zheng *et al*., 2012), anti-coagulant activity (Jensen *et al*., 2016) and anti-cancer (Ravi *et al*., 2015). Phycocyanin will be denaturized at a temperature above 60°C (Martelli *et al*., 2014) and at a pH<5.0 (Duangsee *et al*., 2009), causing it to fade.

Microencapsulation is one of the methods that can be used to protect phycocyanin from external influences and to facilitate distribution process. According to Ozkan and Bilek (2014), microencapsulation of natural food colourants is an active compound coating technology for the protection, stabilization, and the slow release of core material. The final size of the product is related with the term microencapsulation, that is of a micro scale (<1 mm), and nano-encapsulation, that is of a nano scale (< 1 µm). The active compound being coated is called core and the material coating is called encapsulant or wall (Carvajal *et al*., 2010).

Spray drying is a method most commonly used in food industry because it is easy to handle, fast process, lower costs, and produces small-sized products. The process of spray drying is used for microencapsulation of various natural colourants like carotenes (Kha *et al*., 2010), anthocyanin (Fang and Bhandari, 2011; Bakowska-Barczak and Kolodziejczyk, 2011), and betalains (Pitalua *et al*., 2010). However, this method can damage to the active compound being encapsulated due to the use of high temperature during spray drying process. To protect the active compound during spray drying process, a precise type and ratio of the encapsulant are needed.

Encapsulant can be selected from a variety of polymers based on the characteristics of the microcapsules. The type of encapsulant commonly used is maltodextrin, it is starch derivative compounds which has low solubility in water, high viscosity, and relatively low price, thus widely used as an encapsulant (Cakrawati and Handayani, 2017). Due to maltodextrin as an encapsulant has low emulsion stability, made the skin layer weak, so the active compound as core material to be less protected during spray drying (Hermanto *et al*., 2016). Therefore, it needs another biopolymer to combine encapsulants, such as alginate. According to Chavarri *et al*. (2010), alginate is one of the linear anionic polysaccharides, which enables it to be used as the encapsulant. The benefit of using alginate as an encapsulant is that it is non-toxic, can

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develop a strong matrix, is water-soluble, is of low price, and is safe to be consumed. Hadiyanto *et al.* (2017), the phycocyanin microcapsule is produced by using an extrusion method with alginate as the encapsulant.

Based on the above description, research on phycocyanin microencapsulation using maltodextrin and alginate as an encapsulant by spray drying method needs to be conducted. The objective of this research is to determine the best formulation and characterization of phycocyanin microcapsules with maltodextrin and alginate as an encapsulants. The use of encapsulant with a precise formulation is expected to protect phycocyanin during the spray drying process, thus increasing the functional characteristics of phycocyanin microcapsules.

RESEARCH METHOD

Materials

Phycocyanin used for this research was extracted from dry *Spirulina platensis* (PT Neoalga, Indonesia) using aquadest. The substance used as an encapsulant is maltodextrin (CV Multi Kimia Raya, Indonesia) and sodium alginate (PT Selalu Lancar Maju Karya, Indonesia)

The instruments used in making phycocyanin microcapsules are homogenizer (WiseTis HG-15D, Germany) and spray dryer (Buchi Mini B-290, Switzerland). While the tools used for analysis are spectrophotometer UV-VIS (Shimadzu UV-1280, Japan), chroma meter (Minolta CR-200, Japan), scanning electron microscope (SEM) (Jeol JSM-6510LA, Japan), and particle size analyzer (Malvern Zetasizer Nanoseries Nano ZS ver 6.20, Malvern Instruments Ltd, Malvern, UK).

Phycocyanin Microencapsulation

Phycocyanin was extracted using fresh water 1:100 (wPhycocyanin was extracted using aquadest 1:100 (w/v) with a homogenization speed of 300 rpm at a room temperature for 4 h. Next, the solution is centrifuged at 4800 *g* for 15 min to separate phycocyanin solution from *Spirulina sp*. Phycocyanin and encapsulant were homogenized at the speed of 10,000 rpm using a homogenizer. Than, phycocyanin microparticle solution was dried using spray dryer with an inlet temperature of 130 °C. Further information about phycocyanin microparticle solution formulation is shown in Table 1.

Phycocyanin Content

Phycocyanin content was measured using the modified method of Boussiba and Richmond (1979). The phycocyanin microcapsules (40 mg) were added with 10 mL of aquadest and then vortexed. The wavelength used to measure phycocyanin content was 620 nm. Phycocyanin content was determined using Equation (1).

$$\text{Phycocyanin content } (\%) = \frac{\text{A620 x v}}{\text{3.39 x w x dw}} \text{ x } 100\% \qquad (1)$$

In which: v = solvent volume (mL); 3.39 = coefficient C-Phycocyanin at 620 nm; w = sample weight (mg); dw = dry weight (mg)

Table 1. The Formulation of phycocyanin microparticle solution (per 200 mL solution)

Sample	Maltodextrin (%)	Alginate (%)	Phycocyanin (%)
FM	10	0	90
FMA2	9,8	0,2	90
FMA4	9,6	0,4	90
FMA6	9,4	0,6	90
FMA8	9,2	0,8	90

Notes:
FM = phycocyanin encapsulated with maltodextrin 10%
FMA2 = phycocyanin encapsulated with maltodextrin 9.8% and alginate 0.2%
FMA4 = phycocyanin encapsulated with maltodextrin 9.6% and alginate 0.4%
FMA6 = phycocyanin encapsulated with maltodextrin 9,4% and alginate 0.6%
FMA8 = phycocyanin encapsulated with maltodextrin 9.2% and alginate 0.8%

Moisture Content

Phycocyanin microcapsules (1 g) were weighed after it was dried in the oven at 105 °C until constant weight. The moisture content (%) was calculated based on the loss of weight before and after drying (Li *et al.*, 2017).

Antioxidant Activity

Free radical scavenging activity of different phycocyanin microcapsules (2.0 mg) were measured by 1 mM DPPH (1-diphenyl-2-picryl hydrazyl) (0.25 mL) in methanol (2 mL). The mixture was read at 517 nm. Antioxidant Activity (%) was determined based on Shekhar & Anju (2014) through Equation (2).

$$\text{Antioxidant Activity } (\%) = \frac{\text{A0 - A1}}{\text{A0}} \text{ x } 100\% \qquad (2)$$

In which: A_0 = Absorbance in control; A_1 = Absorbance in sample

Encapsulation Efficiency (EE)

The percentage EE was calculated as the ratio of active compound (phycocyanin content) in the microcapsule with active compound before microencapsulation (Mirhojati *et al.*, 2017).

Bulk Density (BD)

Phycocyanin microcapsules were put in a tube and weighed. BD is calculated as m/V (kg · m^{-3}) (Janiszewska and Wlodarczyk, 2013).

Colour

The colour of phycocyanin microcapsules were measured using chroma meter. L* is brightness, a* and b* show color in which –a* is greenish and +a* is reddish, -b* is bluish and +b* is yellowish (Ravichandran *et al.*, 2014).

Particle Size Distribution

Phycocyanin microcapsules were dispersed in hexane using ultrasonic waves for 2 min and then particle size distribution was directly determined using Dynamic Light Scattering (Parrarud and Pranee, 2010).

Morphological Observation

Phycocyanin microcapsule's morphology was observed using modified method of Venil *et al.* (2016), in which the sample was coated with gold and then observed using an SEM at 5,000 magnification and 20 kV voltage.

Data Analysis

Results were analyzed with SPSS version 17 (International Business Machines Corporation, USA) using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test comparisons among means. Significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Phycocyanin Content

The result shows that the phycocyanin content of FM is lower than FMA (Table 2). There showed that more phycocyanin is trapped using maltodextrin-alginate than using maltodextrin alone. Novianty *et al.* (2015) stated that the use of alginate up to 1% concentration combined with maltodextrin as an encapsulant can protect phenol compound on liquid smoke during spray drying with an inlet temperature of 130 °C. According to Hadiyanto *et al.* (2017), alginate has capabilities of cross-linking formation with polymer bonds, thus increasing its ability to trap a compound.

Phycocyanin microcapsule with maltodextrin-alginate as an encapsulant produced by this research has phycocyanin content between 2.05 ± 0.03 (%) to 2.42 ± 0.10 (%). Earlier research (Dewi *et al.*, 2016) showed that the use of 2 types of encapsulant (maltodextrin-carrageenan) is more effective in trapping and protecting phycocyanin than the use of maltodextrin alone as an encapsulant. Phycocyanin microcapsule with maltodextrin-carrageenan (carrageenan concentration between 0.25% and 1.0%) as an encapsulant has phycocyanin content between 0.71 ± 0.04 (%) to 2.83 ± 0.07 (%).

The increase of alginate concentration of up to 0.6% and combination with maltodextrin cause increase phycocyanin content in microcapsule (Table 2), that is, 2.37 ± 0.05 (%). This is suspected as due to the increase of emulsion stability, correlated with the increase of alginate concentration. The characteristics of microcapsule can be influenced by emulsion stability, amount of solids, viscosity, particle size, encapsulant type, and microencapsulation method (Martins *et al.*, 2014; Wilkowska *et al.*, 2016).

At 0.8% alginate concentration, more of the phycocyanin produced sticks to the chamber and cyclone walls during the spray drying process.This is probably due to the fact that an increase of encapsulant concentration causes an increase of glass transition temperature, thus causing an unsuitable use of the inlet temperature. Sormoli *et al.* (2012) reported that glass transition temperature increases along with the increase of encapsulant concentration, which relates with the increase of polymer bond between the types of encapsulant used in lactose drying using spray dryer. Marcela *et al.* (2016) explained that the increase of alginate ratio as an encapsulant and the use of low inlet temperature cause more particles sticky to the walls of the spray dryer's chamber and the particles cannot be removed.

Moisture Content

Differences in alginate concentration as an encapsulant cause significantly difference the moisture content of phycocyanin microcapsule (Table 2). The moisture content of phycocyanin microcapsule produced in this research is between 2.97±0,07 (%) to 3.35±0.18 (%). The higher of the alginate concentration can increase the moisture content of phycocyanin microcapsule. The moisture content of microcapsule is also influenced by the type and composition of encapsulant which can hinder evaporation. The increase of encapsulant composition that is not accompanied with inlet temperature increase can cause low evaporation rate (Sormoli *et al.*, 2012; Marcela *et al.*, 2016). Different result had been reported by Fazaeli *et al.* (2012), the moisture content of microcapsules was decreased with addition of encapsulant concentration. These results could be explained that additional concentration of encapsulant caused an increase in total solids and a reduction in total moisture for evaporation.

Antioxidant Activity

The research results show that the higher the concentration of alginate used as an encapsulant along with maltodextrin can increase antioxidant activity of phycocyanin microcapsules (Table 2). The increase of antioxidant activity is correlated with the amount of active compound that can be encapsulated (Bakowska-Barczak and Kolodziejczyk, 2011). Similar findings result had been reported by Dewi *et al.* (2016), that the high level of phycocyanin content causes an increase in antioxidant activity of phycocyanin microcapsules.

The increase of alginate concentration used as an encapsulant can increase antioxidant activity of phycocyanin microcapsules. The same result is also shown by Hadiyanto *et al.* (2017) that the use of alginate with a higher concentration can increase antioxidant activity and reduce IC_{50} value of phycocyanin microcapsules.

Encapsulation Efficiency (EE)

EE is defined the amount of phycocyanin that can be encapsulated. The lowest EE value is found in FM, that is, 29.74±0.30 (%) and the highest EE value is found in FMA6, that is, 40.74±0.86 (%) (Table 2). This is closely related with phycocyanin content found in phycocyanin microcapsule, in which the higher content of phycocyanin is found in phycocyanin encapsulated using maltodextrin-alginate as contrasted to maltodextrin alone. The type of encapsulant play role in effectively protecting the active compound from the effect of high temperature during spray drying. The type and concentration of the encapsulant can affect the encapsulation efficiency of microcapsules (Murali *et al.*, 2014; Mirhojati *et al.*, 2017). Shinde and Nagarsenker (2011) explained that microencapsulation is considered successful if the powder produced contains the active compound with maximum retention.

Based on the research, the increase of alginate concentration of up to 0.6% can cause an increase of the EE of phycocyanin microcapsules. The increase of alginate concentration also causes the thickening of phycocyanin microparticle solution. According to Pitchaon *et al.* (2013), the high viscosity and thickness of the solution contribute to the reduced microparticle porosity. The high microparticle solution viscosity can also prevent active solution diffusion to microparticle surface. But in this research, the alginate concentration increase of 0.8% produces phycocyanin microcapsule with EE value that is not significantly different with an alginate concentration of 0.6%. However, a certain alginate concentration (maximum) can cause the formation of a more solid network with smaller pores, thus less active compound is trapped in the pores (Sevda and Rodrigues, 2011; Soliman *et al.*, 2013).

Bulk Density

Table 3 shows that an increase of alginate concentration as an encapsulant can cause significantly increase the bulk density of phycocyanin microcapsules. There is between 0.56±0.01 kg · m^{-3} and 0.86±0.02 kg · m^{-3}. According to the observed by Janiszewska and Wlodarczyk (2013), the bulk density value is closely related with the moisture content of phycocyanin microcapsules. The type and composition of the encapsulant can influence evaporation rates, which influences the weight of each phycocyanin microcapsule particles (Fazaeli *et al.*, 2012).

Bulk density is also influenced by the particle size of phycocyanin microcapsules. Caparino *et al.* (2012) explained that bulk density can increase due to the decrease of particle size. Small size particles provide wider surface contact area per volume unit. Based on this research, phycocyanin microcapsule encapsulated using maltodextrin-alginate has more nanometer-sized particles than phycocyanin microcapsule encapsulated using maltodextrin alone.

Colour

The use of alginate with maltodextrin as an encapsulant can cause a decrease in L value and an increase of (-)b value (Table 3). Thus, the phycocyanin encapsulated using maltodextrin-alginate has more dark and blue colour. The blue colour produced from phycocyanin microcapsule is related with the phycocyanin content. The intensity of blue colour increased with increasing phycocyanin content (Sedjati *et al*, 2012). The color of phycocyanin microcapsule produced is also influenced by the type of encapsulant and the drying condition. Based on the previous research (Dewi *et al.*, 2017), phycocyanin microcapsule with different encapsulant types (maltodextrin, maltodextrin-alginate, and maltodextrin-carrageenan) have different brightness (L) values. The (L) value from the highest to the lowest is as follows, phycocyanin microcapsule with maltodextrin-carrageenan, with maltodextrin-alginate, and with maltodextrin alone as an encapsulant. This is probably caused by the browning reaction of maltodextrin during the drying process, which influences the final color of the sproduct.

Particle Size Distribution

Based on particle size distribution analysis, the particle size of phycocyanin microcapsules with maltodextrin as an encapsulant has the following distribution: 82.8% sized 1,853 nm and 17.2% sized 181.4 nm. While phycocyanin microcapsules with

Table 2. Characterization of phycocyanin microcapsule with different alginate concentrations

Sample	Phycocyanin content (%)	Moisture content (%)	Antioxidant activity (%)	Encapsulation Efficiency (%)
FM	1.74±0.02^{a}	2.97±0.07^{a}	53.46±2.73^{a}	29.74±0.30^{a}
FMA2	2.05±0.03^{b}	3.20±0.17^{ab}	56.88±0.79^{b}	35.08±0.52^{b}
FMA4	2.20±0.06^{c}	3.25±0.16^{ab}	59.19±1.16^{bc}	37.69±1.03^{b}
FMA6	2.37±0.05^{d}	3.35±0.18^{b}	60.94±0.32^{cd}	40.74±0.86^{c}
FMA8	2.42±0.10^{d}	3.41±0.20^{b}	62.12±0.79^{d}	41.42±1.73^{c}

Notes:
Different superscripts in the same columns show significantly different value at level of α=0.05

maltodextrin and alginate 0.6% as an encapsulant has 52.5% sized 3,104 nm, 41.9% sized 688.1 nm, and 5.7% sized 104.2 nm (Fig. 1). The different sizes of phycocyanin microcapsules are influenced by encapsulation method, type of encapsulant, and ratio between encapsulant and active compound (Mirhojati *et al.*, 2017).

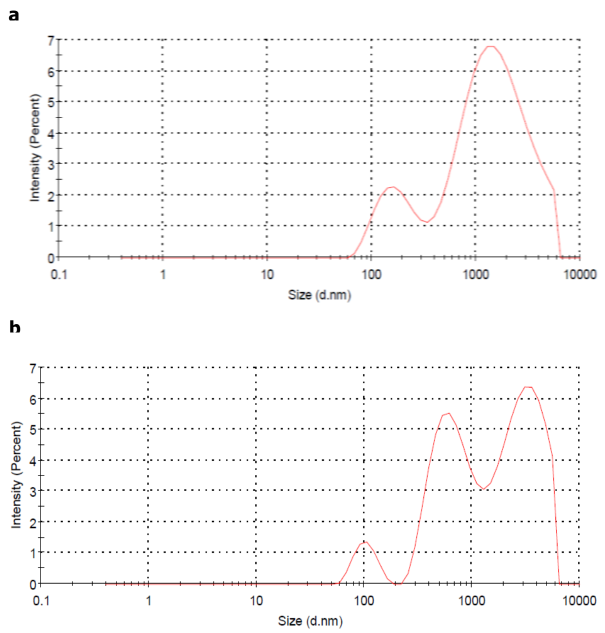

Figure 1. Distribution of phycocyanin microcapsule particle size a) Maltodextrin as encapsulant. b) Maltodextrin-alginate 0.6% as encapsulant

Use of maltodextrin-alginate as an encapsulant can produce phycocyanin microcapsules with bigger size than phycocyanin microcapsules encapsulated using maltodextrin alone. Briones and Sato (2010) explained that the increase of microcapsule particle size is related to the number of active compounds that can be encapsulated. Carvalho *et al.* (2014) added that bigger microcapsule particle size is also caused by the use of more than one types of the encapsulant, thus creating two or more layers that cover the active compound.

Morphological Observation

Figure 2 shows that the phycocyanin microcapsules with maltodextrin-alginate as an encapsulant have a spherical shape than phycocyanin microcapsules with maltodextrin as an encapsulant, which has a rather irregular shape, wrinkled and with the wavy surface (Fig. 2). According to Harris *et al.* (2011), microcapsules with the wrinkled and wavy surface are caused by the rapid solvent evaporation during the spray drying process. Najafi *et al.* (2011) added that the differences in microcapsule morphology are influenced by the atomization and the drying condition.

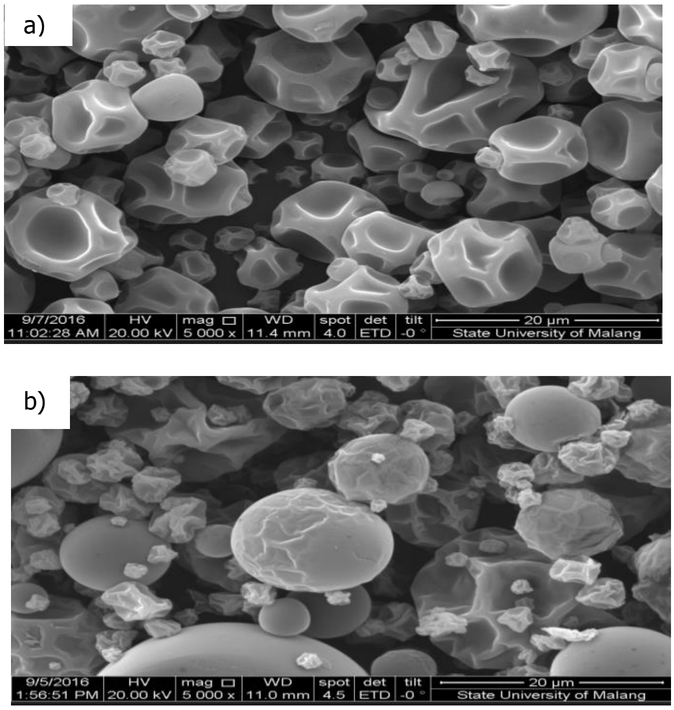

Figure 2. Morphological observation of phycocyan microcapsules. a) Maltodextrin as an encapsulant. b) Maltodextrin and alginate 0.6% as an encapsulant.

Phycocyanin encapsulated using maltodextrin alone produces microcapsules that are more fragile (cracked) (Fig. 2a). Meanwhile, no cracks are found on phycocyanin microcapsule with 0.6% maltodextrin-alginate as an encapsulant (Fig. 2b). This is because of the mannuronic acid and guluronic acid content found in alginate which can influence the ability to form a gel. Fertah *et al.* (2017) stated that alginate with a higher ratio of mannuronic and guluronic acids can increase its ability to develop stable and strong gel. The same result was also shown by Dewi *et al.* (2016), phycocyanin encapsulated using maltodextrin-carrageenan produce whole microcapsules without

Table 3. Results of bulk density measurement and phycocyanin microcapsule colors with different alginate concentration

Sample	*Bulk density* (kg · m^{-3})		a*	b*
FM	0.56±0.01[a]	70.18±0.14[a]	–4.47±0.13[ac]	–8.91±0.03[a]
FMA2	0.64±0.01[b]	72.44±0.29[b]	–4.24±0.07[b]	–9.38±0.20[b]
FMA4	0.67±0.02[b]	77.30±0.40[c]	–4.36±0.02[ab]	–10.47±0.02[c]
FMA6	0.70±0.01[c]	68.64±0.19[d]	–4.22±0.03[b]	–11.22±0.03[d]
FMA8	0.86±0.02[d]	66.73±0.25[e]	–4.54±0.07[c]	–12.30±0.02[e]

Remarks:
Different superscripts in the same columns show significantly different value at $p < 0.05$

cracks as the result of interaction between maltodextrin and carrageenan which forms a stable complex polyelectrolyte. This is an advantage because microcapsules have low permeability against gas, and can increase protection and retention towards the active compound being encapsulated.

CONCULUSION

The higher concentration of alginate combined with maltodextrin as an encapsulant will increase moisture content, bulk density, and particle size of phycocyanin microcapsules. With a formulation of 9.4% maltodextrin, 0.6% alginate, and 90% phycocyanin extract, we can produce phycocyanin microcapsules with the best characteristics, namely phycocyanin content, the efficiency of encapsulation, the highest bluish colour, a rounder shape and solidity without cracks of particles. This shows that the use of maltodextrin combine with alginate can protect phycocyanin during spray drying process.

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