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Original article

Physical extraction and extrusion entrapment of C-phycocyanin from *Arthrospira platensis*☆

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ABSTRACT

C-phycocyanin (C-PC) is a blue photosynthetic pigment and the major phycobiliprotein in many blue-green algae with high nutritional values and antioxidant, antivirus, anti-cancer, and anti-inflammatory properties. It also boosts the immune system in various organisms and can be used in immunoassays to track target cells because it has fluorescent properties. This study focused on optimizing extraction conditions for C-phycocyanin encapsulation from *Arthrospira platensis* using ultrasonic and extrusion techniques. Parameters affecting C-phycocyanin production were type of biomass, buffer, and biomass-solvent ratio. Results indicated that the highest C-phycocyanin concentration of 6 mg ml^{-1} was obtained from freeze-dried samples extracted by 0.01 M sodium phosphate buffer (pH 7) and biomass-solvent ratio of 1:15, with extract purity and yield of 0.6 and 60 mg g^{-1}, respectively. The optimized conditions of C-phycocyanin ultrasonic encapsulation at 98% were 3% sodium alginate, 2.5% calcium chloride, and 1 mg ml^{-1} initial C-phycocyanin concentration. C-phycocyanin extracted from *A. platensis* can be prepared in encapsulated form and utilized for various applications.

1. Introduction

The microalga *Arthrospira* (formally called *Spirulina*) is produced commercially all over the world (Oliveira et al., 2010). It is a non-toxic cyanobacterium that can produce light-harvesting pigments including chlorophyll, carotenoids, and phycobiliproteins. This microorganism has long been used as a food source and is considered safe for human consumption as it contains protein (60–70%), vitamins, and minerals (Oliveira et al., 2010; Wu et al., 2016), and has been used as a nutritional supplement due to its high essential nutrient content to prevent and cure many diseases including diabetes (Silva et al., 2002), anti-inflammation (Romay et al., 1998), reduction of swelling and resistance to Enterovirus 71 (Shih et al., 2003).

Phycobiliproteins are light-harvesting, water-soluble protein pigments present in cyanobacteria, organized in supramolecular complexes as phycobilisomes and located on the outer surface of the thylakoid membrane (Vali Aftari et al., 2015). They can be divided into three major classes as C-phycocyanin (C-PC), allophycocyanin (APC) and phycoerythrin (PE) (Rezaei et al., 2015). C-phycocyanin is a natural blue pigment widely used as a nutritional ingredient, in natural dyes, as fluorescent markers, and in pharmaceuticals as an anti-oxidant and anti-inflammatory reagent (Kumar et al., 2014; Liao et al., 2011).

C-phycocyanin can be extracted from cyanobacteria by different procedures which combine breakage of the cell walls and extraction of water-soluble phycobiliproteins into aqueous media (Eriksen, 2008). Several factors influence C-phycocyanin extraction; the most important being the cellular disruption method, type of solvent, biomass-solvent ratio, and type of biomass (Silveira et al., 2007). Physical methods include sonication, cavitation, osmotic shock, and repeated freeze-thawing, whereas chemical methods use acids, alkalis, detergents, and enzymes (Kuddus et al., 2013). Combinations of a variety of physical and chemical methods are exploited for cell breakage (Kuddus et al., 2013). For C-phycocyanin extraction by ultrasonic methods, the biomass solution is treated ultrasonically to accelerate cell wall breakage through direct osmosis which effectively shortens the treatment

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time (Liu et al., 2016). The purity of C-phycocyanin is examined based on the ratio between absorbencies from phycocyanobilin at 620 nm and aromatic amino acids in all proteins at 280 nm (Eriksen, 2008). The A_{615}/A_{280} ratio is considered a good indicator of C-phycocyanin purity, especially when other forms of protein contaminants are taken into account (Moraes and Kalil, 2009).

Encapsulation is a process which involves the coating or entrapment of one substance (active agent) into another substance (wall material) (Bernard et al., 1999; Nedovic et al., 2011; Ray et al., 2016). The substance which is encapsulating is called the coating, membrane, shell, capsule, carrier material, external phase, or matrix (Nedovic et al., 2011; Ray et al., 2016). Several techniques are used to form the capsules including spray drying, freeze drying and extrusion (Bernard et al., 1999). A less hazardous encapsulation method involves extrusion technologies (de Vos et al., 2010). The extrusion method consists of adding droplets of an aqueous solution (mixed with 0.6–3.0% (w/v) sodium alginate) into a gelling bath (0.05–1.50 M calcium chloride solution) (Nedovic et al., 2011). Extrusion is the most common encapsulation technique to protect sensitive core material from environmental conditions and extend the shelf life of bioactive compounds. The efficiency of the process depends on the encapsulation conditions such as the type of core material, pH, and the concentration of coating materials (Peanparkdee et al., 2016). Encapsulation is useful for various applications including agriculture, food, pharmaceutical, biotechnology and in the textile industry (Ray et al., 2016). Moreover, several materials have been successfully encapsulated in the food industry including amino acids, vitamins, minerals, antioxidants, colorants, enzymes and sweeteners (Deladino et al., 2008). Limited information exists in the literature regarding the encapsulation of C-phycocyanin.

Here, C-phycocyanin extraction from *A. platensis* was investigated using ultrasonic-assisted techniques. Independent variables including the type of sample preparation, buffer concentration and biomass-solvent ratio were considered to determine the optimum C-phycocyanin extraction condition. Moreover, independent variables including C-phycocyanin, alginate, and calcium chloride concentration were examined for efficient C-phycocyanin (C-PC) encapsulation. Results can be used to optimize encapsulated C-phycocyanin from *A. platensis.*

2. Materials and methods

2.1. Algal strain and biomass preparation

Arthrospira platensis microalga was obtained from the Institute of Food Research and Product Development, Kasetsart University, Thailand and was maintained in Zarrouk medium. Biomass production was cultivated in open raceway pond of 200 L under Zarrouk medium culture (Zarrouk, 1996). The biomass of *A. platensis* was grown to a maximum dry cell concentration in the range of 1–2 g l^{-1}, harvested by nylon filtration and washed with tap water to remove the residual media culture. The biomass was then dried by different methods including oven-drying and freeze-drying. Oven-drying was performed using a hot air oven at a temperature of 70 °C for 6 h, whereas freeze-drying involved freezing at −80 °C overnight and then placement in a freeze-dryer (Labconco, USA) at −50 °C and <0.06 mbar pressure for 6 h. The dried *A. platensis* biomass was milled to 0.5 mm particle size in order to reduced and equal size of samples in each experiment.

2.2. C-phycocyanin extraction

C-phycocyanin extraction from *A. platensis* was optimized for three factors including preparation of oven- and freeze-dried samples at sodium phosphate buffer concentrations of 10 and 100 mM, pH 7.0, and biomass-solvent ratio (g solid biomass: ml liquid solvent) at 1:50, 1:25, and 1:15. Physical extraction in each experiment was determined by ultrasonic-assisted techniques using an ultrasonic processor (Sonic, VCX 750, USA) with a 25 mm solid probe. Frequency and power applied were 20 kHz and 750 W, respectively. Samples were sonicated at 50% amplitude with a pulse of 60 s on and 30 s off, for a total extraction time of 5 min. The mixtures were placed on ice during the sonication process to prevent overheating. After extraction, the mixtures were harvested by centrifugation at 4303 RCF for 30 min (Sorvall RC-6 Plus Series, Thermo Scientific). All experiments were carried out in triplicate.

2.3. C-phycocyanin encapsulation

Encapsulation was evaluated under different conditions of C-phycocyanin concentration at 1, 2, and 3 mg ml^{-1}, sodium alginate at 2, 3, and 4%(w/w) and calcium chloride at 2, 2.5, and 3%(w/w) using an extrusion technique combined within sodium alginate. C-phycocyanin was extracted from freeze-dried *A. platensis* at biomass-solvent ratio of 1:15 in 0.01 M phosphate buffer (pH 7.0) using an ultrasonic-assisted physical technique. C-phycocyanin collection after centrifugation was used to study the encapsulation. C-phycocyanin was added to the alginate solution at final contents of 2, 3 and 4%, and also to citric acid at final concentration of 0.4% to improve stability (Pan-utai et al., 2018). The mixtures were stirred continuously and extruded by a hypodermic needle (18G × 1″, 1.2 × 25 mm, Nipro, Japan) into calcium chloride solutions (2, 2.5 and 3%) and immobilized for 30 min. The encapsulated C-phycocyanin was then washed with distilled water and collected for further determination. All experiments were carried out in triplicate.

2.4. Analytical methods

2.4.1. C-phycocyanin

Concentration and purity of C-phycocyanin samples were evaluated using a UV–visible spectrophotometer (Evolution 201, USA) with absorbance at 615, 652, and 280 nm (Bennett and Bogorad, 1973; Silveira et al., 2007). C-phycocyanin concentration (C-PC; mg ml^{-1}) was defined by the following equation:

$$C-PC(\text{mg ml}^{-1}) = [OD_{615} - 0.474OD_{652}]/5.34 \quad (1)$$

Purity of C-phycocyanin (extract purity, EP) was defined as the ratio of absorbance at 615 and 280 nm as the following equation:

$$EP = OD_{615}/OD_{280} \quad (2)$$

Yield of C-phycocyanin extraction (mg g^{-1}) was calculated using C-phycocyanin concentration (C-PC; mg ml^{-1}), volume of solvent extraction (V; ml), and weight of dried biomass (Biomass) defined as the following equation:

$$\text{Yield}(\text{mgg}^{-1}) = (C-PC^{*}V)/\text{Biomass} \quad (3)$$

2.4.2. DPPH radical scavenging activity

To investigate the antioxidant activity of the various C-phycocyanin extraction conditions, DPPH radical scavenging activity was measured following the method of Huang et al. (2007). Two milliliters of sample were mixed with 1 ml of 200 μM DPPH in an ethanol solution and incubated for 30 min at room temperature. The absorbance of the mixture was measured at 517 nm by a UV–vis spectrophotometer (Evolution 201, USA). Percentage of inhibition (%) was calculated by the following equation (Huang et al., 2007):

$$\text{Inhibition}(\%) = (OD_{\text{blank}} - OD_{\text{sample}})/OD_{\text{blank}} \times 100 \quad (4)$$

2.4.3. Encapsulated C-phycocyanin

Encapsulation efficiency (EE;%) of C-phycocyanin (C-PC) was calculated by the following equation:

$$EE(\%) = [(\text{initial concentration of C} - \text{phycocyanin} - \text{concentration of uncoated C} - \text{phycocyanin})/\text{initial concentration of C} - \text{phycocyanin}] \times 100 \quad (5)$$

2.4.4. Size of encapsulated C-phycocyanin

The size of the encapsulated C-PC was expressed as the average particle diameter determined by a vernier caliper (Macoh, Thailand). At least 50 particles were examined for all measurements.

2.5. Statistical analysis

Statistical data were analyzed using SPSS version 10 (SPSS, Inc., Chicago, IL, USA). Results were presented as mean ± standard deviation (SD) of replicated determinations. C-phycocyanin extraction and influence of the three parameters as biomass preparation, solvent concentration, and biomass-solvent ratio were evaluated for C-phycocyanin concentration, purity, yield, and antioxidant activity using a one way ANOVA. Multiple comparisons were conducted by Tukey's method with significance set at 0.05 ($p < 0.05$). The effects of the three parameters as initial C-phycocyanin concentration, alginate content, and calcium chloride content were evaluated on C-phycocyanin encapsulation efficiency using a factorial design with multiple comparisons conducted by Tukey's method and significance set at 0.05 ($p < 0.05$).

3. Results

The influences of different conditions including sample preparation, solvent concentration, and biomass concentration on C-phycocyanin extraction from *A. platensis* were investigated using ultrasonic-assisted techniques. C-phycocyanin extraction was tested using the extrusion technique.

3.1. C-phycocyanin concentration

C-phycocyanin concentrations obtained from *A. platensis* under various conditions combined with ultrasonic-assisted techniques are shown in Fig. 1. C-phycocyanin concentration was higher in all experiments compared to the control without ultrasound-assisted techniques (Fig. S1). Under various biomass-solvent reaction ratios, reduction in solvent ratio resulted in increased C-phycocyanin concentration. Oven-dried biomass under 100 mM buffer gave higher C-phycocyanin concentration than 10 mM buffer. In contrast, C-phycocyanin concentration from 10 mM buffer with freeze-dried biomass was higher than 100 mM. As the biomass-solvent ratio increased for all different conditions, C-phycocyanin concentration also increased ranging from 1.0 to 6.1 mg ml^{-1}. Highest C-phycocyanin concentration was obtained with biomass-solvent ratio at 1:15 and 10 mM buffer concentration of freeze-dried biomass. C-phycocyanin concentration from biomass-solvent ratio at 1:15 of oven-dried biomass under 100 mM buffer showed insignificant difference ($p \geq 0.05$).

3.2. Extract purity of C-phycocyanin

The extract purity (EP) of C-phycocyanin extracted from *A. platensis* is significant regarding its application. Fig. 2 shows the extract purity of C-phycocyanin under different conditions of ultrasonic-assisted extraction. Oven-dried biomass conditions gave higher EP, with statistically significant difference from the freeze-dried biomass. Results indicated purity in the range of 0.52–0.67. The highest purity was obtained from oven-dried biomass at a ratio of 1:50 with 10 mM buffer concentration, while conditions for oven-dried biomass at 10 mM buffer concentration did not show a significant difference in extract purity ($p \geq 0.05$).

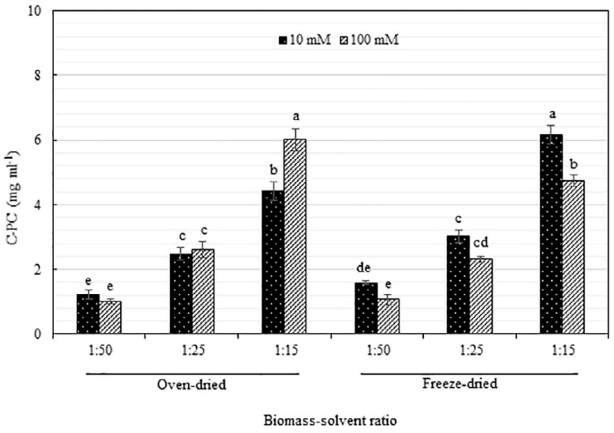

Fig. 1. C-phycocyanin concentration from *A. platensis* under different sample preparation conditions (oven- and freeze-dried), buffer concentration (10 and 100 mM), and biomass-solvent ratio (1:50, 1:25 and 1:15). Data were calculated from triplicate experiments with standard deviation shown by error bars. [a,b] Different letters are significantly different ($p < 0.05$).

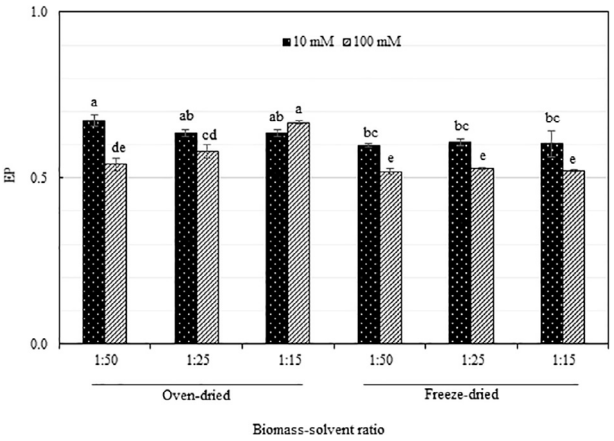

Fig. 2. Extract purity of C-phycocyanin from *A. platensis* under different condition; type of sample (oven- and freeze-dried), buffer concentration (10 and 100 mM) and biomass-solvent ratio (1:50, 1:25 and 1:15). Data were calculated from triplicate experiments with standard deviation shown by error bars. [a,b] Different letters are significantly different ($p < 0.05$).

3.3. Extraction yield

Yield of C-phycocyanin from *A. platensis* using ultrasonic-assisted extraction is shown in Fig. 3. Yield increased with increasing biomass-solvent ratio in all different conditions. Yield of C-phycocyanin extracted ranged from 10 to 60 mg g^{-1}. Highest biomass-solvent ratio at 1:15 gave the highest extraction yield. However, biomass-solvent ratio in terms of oven- and freeze-dried biomass showed an insignificant difference in extraction yield. Moreover, yield of oven-dried biomass at 100 mM buffer concentration was no statistically significant difference to yield of freeze-dried biomass at 10 mM buffer concentration.

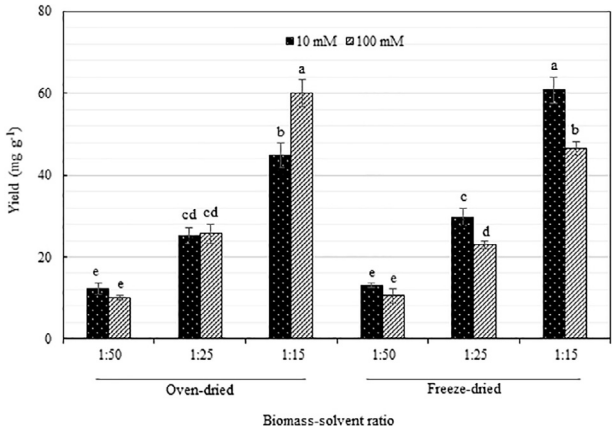

Fig. 3. Yield of C-phycocyanin extracted from *A. platensis* under different condition; type of sample (oven- and freeze-dried), buffer concentration (10 and 100 mM) and biomass-solvent ratio (1:50, 1:25 and 1:15). Data were calculated from triplicate experiments with standard deviation shown by error bars. [a,b] Different letters are significantly different ($p < 0.05$).

3.4. Antioxidant activity

Antioxidant activity of C-phycocyanin extracted from *A. platensis* under different ultrasonic-assisted conditions was evaluated using DPPH assay. The DPPH radical scavenging activities of C-phycocyanin extracted at 0.1 mg ml^{-1} are shown in Fig. 4. C-phycocyanin from *A. platensis* using ultrasonic-assisted extraction inhibited DPPH radicals in the range of 6.2–46.0%. Buffer concentration at 10 mM resulted in higher antioxidant activity than 100 mM in all experiments. Oven-dried biomass showed increased DPPH radical scavenging activity with increasing biomass-solvent ratio. Moreover, freeze-dried biomass at 10 mM buffer concentration recorded increased DPPH radical scavenging activity with increasing biomass-solvent ratio, whereas at 100 mM buffer concentration these results were reversed. Highest DPPH radical scavenging activity was 46% from freeze-dried biomass at 1:15 biomass-solvent ratio under 10 mM buffer concentration.

3.5. C-phycocyanin encapsulation

C-phycocyanin extracted from *A. platensis* was encapsulated using extrusion techniques. The main effects and interactions between different factors on encapsulation efficiency were dependent variables including C-phycocyanin concentration, sodium alginate content, and calcium chloride content (Table 1). C-phycocyanin encapsulation efficiency showed highly significant differences for C-phycocyanin concentration and alginate content ($p < 0.05$). Percentages of encapsulation efficiency, size and shape of C-phycocyanin alginate beads under different conditions are shown in Fig. 5 and Table 2, respectively. Results indicated that encapsulation efficiency increased with decreasing C-phycocyanin concentration in all experiments, and C-phycocyanin encapsulated at 1 mg ml^{-1} C-phycocyanin concentration showed no significant difference in efficiency (Fig. 5). Moreover, the size of encapsulated C-phycocyanin showed non-significant differences among the various conditions ($p < 0.05$) with diameter of around 2.6 mm (Table 2). Diverse shapes were recorded under different conditions of C-phycocyanin encapsulation including spherical, spherical with tailing, oblate, oblate with tailing and

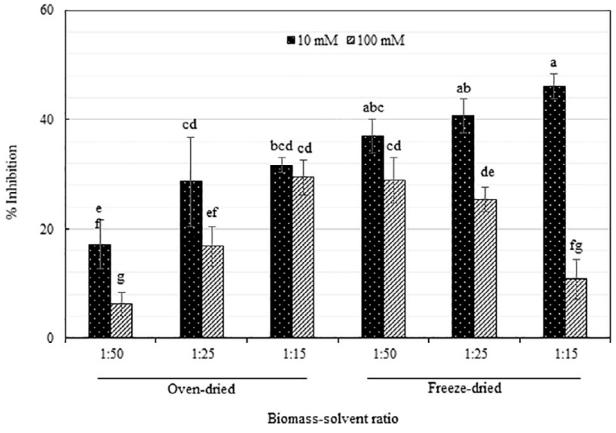

Fig. 4. Antioxidant activity of C-phycocyanin extracted from *A. platensis* under different condition; type of sample (oven- and freeze-dried), buffer concentration (10 and 100 mM) and biomass-solvent ratio (1:50, 1:25 and 1:15). Data were calculated from triplicate experiments with standard deviation shown by error bars. [a,b] Different letters are significantly different ($p < 0.05$).

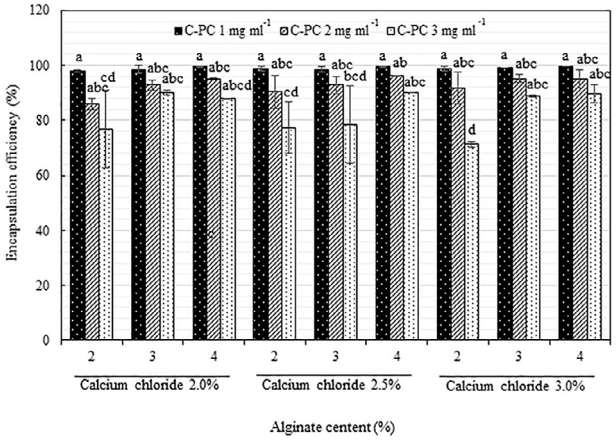

Fig. 5. Encapsulation efficiency of C-phycocyanin entrapment under different conditions. Data were calculated from triplicate experiments with standard deviation shown by error bars. [a,b] Different letters are significantly different ($p < 0.05$).

Table 1
Factors affecting C-phycocyanin encapsulation efficiency.

Factor	SS	df	MS	F-value	*p*-value
C-phycocyanin concentration	2179.205	2	1089.602	49.522	0.000[a]
Alginate	472.658	2	236.329	10.741	0.000[a]
Calcium chloride	6.274	2	3.137	0.143	0.868
Alginate * Calcium chloride	79.083	4	19.771	0.899	0.479
Alginate * C-phycocyanin	290.751	4	72.688	3.304	0.025
Calcium chloride * C-phycocyanin	44.261	4	11.065	0.503	0.734
Alginate * Calcium chloride * C-phycocyanin	131.749	8	16.469	0.748	0.649

SS: sum of squares; df: degree of freedom; MS: mean square.
[a] Significant factors ($p < 0.05$).

Table 2
Effect of alginate content, calcium chloride content and initial C-PC (C-phycocyanin) concentration on encapsulated particle size and shape.

Alginate (%)	$CaCl_2$ (%)	C-PC (mg ml^{-1})	Particle diameter (mm)	Particle shape
2.0	2.0	1	$2.50^a \pm 0.00$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		2	$2.63^a \pm 0.14$	Spherical, Spherical with tailing, Oblate
		3	$2.51^a \pm 0.44$	Spherical, Oblate, Oblate with tailing
	2.5	1	$2.63^a \pm 0.14$	Spherical, Oblate
		2	$2.36^a \pm 0.30$	Spherical, Oblate
		3	$2.66^a \pm 0.43$	Spherical, Oblate
	3.0	1	$2.41^a \pm 0.19$	Spherical, Oblate, Oblate with tailing, Irregular shape
		2	$2.50^a \pm 0.00$	Spherical, Spherical with tailing, Oblate, Oblate with tailing
		3	$2.44^a \pm 0.13$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
3.0	2.0	1	$2.41^a \pm 0.19$	Spherical, Spherical with tailing, Oblate, Oblate with tailing
		2	$2.72^a \pm 0.41$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		3	$2.50^a \pm 0.00$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
	2.5	1	$2.79^a \pm 0.28$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		2	$2.69^a \pm 0.13$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		3	$2.54^a \pm 0.15$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
	3.0	1	$2.53^a \pm 0.30$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		2	$2.82^a \pm 0.13$	Spherical, Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		3	$2.75^a \pm 0.00$	Spherical, Oblate, Oblate with tailing, Irregular shape
4.0	2.0	1	$2.94^a \pm 0.13$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		2	$2.94^a \pm 0.13$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		3	$2.63^a \pm 0.14$	Spherical with tailing, Oblate with tailing, Irregular shape
	2.5	1	$2.63^a \pm 0.14$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		2	$2.69^a \pm 0.15$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		3	$2.63^a \pm 0.14$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
	3.0	1	$2.75^a \pm 0.00$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		2	$2.61^a \pm 0.18$	Spherical with tailing, Oblate, Oblate with tailing, Irregular shape
		3	$2.54^a \pm 0.25$	Spherical with tailing, Oblate with tailing, Irregular shape

irregular, with the ideal shape as spherical. A spherical shape of encapsulated C-phycocyanin was obtained from 2% alginate content and 2.5% calcium chloride in all C-phycocyanin concentration experiments.

4. Discussion

Several procedures can be employed for C-phycocyanin extraction from microalgae based on chemical or physical cell disruption which combine the breakage of cell walls and extraction of water-soluble C-phycocyanin into aqueous media (Eriksen, 2008; Vali Aftari et al., 2015). Factors with significant roles in C-phycocyanin extraction include the cellular disruption method, type of solvent, and biomass-solvent ratio (Silveira et al., 2007). Moreover, different techniques can be employed in the extraction of intracellular proteins which depend on the physical strength of the microalgal cell wall, stability, and intended use for the compound of interest (Moraes et al., 2011; Ores et al., 2016). Ultrasound is a novel technology to improve the extraction process of hydrophobic compounds from microorganisms by disrupting the cell wall of the different bio-tissues to facilitate the release of extractable compounds and enhance mass transport of solvent from a continuous phase into the cells (Vali Aftari et al., 2015). C-phycocyanin concentration, extract purity, yield of extract and antioxidant activity from *A. platensis* were studied using ultrasonic-assisted techniques. Freeze-dried biomass gave the highest C-phycocyanin concentration in the presence of 10 mM phosphate buffer, whereas oven-dried biomass gave the highest C-phycocyanin concentration with 100 mM buffer; furthermore, when using dilute phosphate buffer for extraction, the resulting osmotic shock can cause breakage of the cell wall (Sekar and Chandramohan, 2008). Freeze-dried biomass maintains the C-phycocyanin pigment structure of protein in *A. platensis*, whereas oven-dried biomass incurs lower cost for the drying process but causes denaturation of the protein cell structure and composition. Therefore, lower buffer concentration and ionic strength at 10 mM were used to obtain the highest C-phycocyanin concentration. In addition, oven-dried biomass requires higher buffer concentration and ionic strength.

Highest purity of extracted C-phycocyanin was obtained from oven-dried biomass at biomass-solvent ratio of 1:50 with 10 mM buffer, with a non-significant difference among various biomass concentrations at 10 mM buffer. Moreover, oven-dried biomass had higher purity than freeze-dried biomass in the same condition. The A_{615}/A_{280} ratio is considered a good indicator of the purity of C-phycocyanin preparation, especially when other forms of protein contaminants are taken into account (Moraes and Kalil, 2009). The highest purity of crude C-phycocyanin from *A. platensis* using ultrasonic-assisted extraction was similar to 0.7 as food grade. This result showed higher C-phycocyanin extraction and similar purity when compared with C-phycocyanin extracted from cyanobacterium *Nostoc* sp. strain HKAR-2 using sonication 130 W, 20 kHz, followed by repeated freezing (−20 °C) and thawing (4 °C) as 0.088 mg ml^{-1} and 0.601, respectively (Kannaujiya and Sinha, 2016). Our results indicated C-phycocyanin concentration and purity at 0.26 mg ml^{-1} and 0.07, respectively; both less than values reported by Kamble et al. (2013) who observed that C-phycocyanin isolated in distilled water may not be suitable for long extraction periods with degradation due to heat accumulation during cell disruption. Patel et al. (2005) reported that C-phycocyanin from freeze-dried *Spirulina* sp., *Phormidium* sp., and *Lyngbya* sp. cyanobacteria at 0.01 g ml^{-1} in 0.1 M phosphate buffer (pH 7.0) were disrupted by sonication for 60 s, followed by repeated freezing at −20 °C and thawing at room temperature in dark condition with purity values of 0.80, 0.69, and 0.67, respectively. The extract purity of our experiment was similar to Patel et al. (2005) using one step of the short 60 s period of the extraction.

Liao et al. (2011) determined the yield of C-phycocyanin extraction from dried *A. platensis* powder using ultrasonic methods at 60 W for 10 s and stopping for 10 s, 30 cycles in total, with deionized water at 18.2 mg g^{-1} and purity 0.65. C-phycocyanin extracted from *A. platensis* using the hexane separation method and high-pressure process gave a lower yield of 10.2% than our study (Seo et al., 2013). Moreover, yield of C-phycocyanin extracted from wet *A. platensis* biomass using an ultrasonic bath, 50 kHz with glass

pearls and 5–12 M HCl for 40 min ranged from 0.57 to 43.75 mg g^{-1}, similar to our results (Moraes et al., 2011). Fresh biomass may produce higher yields of C-phycocyanin extraction. A yield of C-phycocyanin at 90 mg g^{-1} from fresh *Arthrospira* sp. LEB 18 was obtained from an ultrasonic homogenizer with a frequency of 20 kHz for 10 min, also showing a higher yield than our results (Ores et al., 2016). Factors affecting phycobiliprotein extraction include abiotic conditions such as biomass concentration, temperature, and pH (Manirafasha et al., 2016). Different strains of *Arthrospira* sp. depending on the origin and system of C-phycocyanin production such as photoautotrophic, mixotrophic and heterotrophic can also affect extraction (Eriksen, 2008). The effect of pH on the biomass concentration in the buffer solution is crucial because the solubility of bio-compounds and apparent kinetic constants are directly dependent on pH variation. C-phycocyanin extraction from lyophilized *A. platensis* under various pH levels determined that pH at 7.0 gave the maximum equilibrium concentration (Su et al., 2014). Therefore, buffer concentration at pH 7.0 was used as a suitable pH level for C-phycocyanin extraction. Selection of a suitable buffer for phycobiliprotein extraction is also a crucial factor for obtaining high yield and quality of phycobiliproteins. Moreover, among the different buffers evaluated to maximize phycobiliproteins extraction, sodium phosphate was found to be the best (Manirafasha et al., 2016). The cell disruption mechanism of ultrasound is associated with cavitation phenomena which manifest as shear stress developed by viscous dissipative eddies arising from shock waves produced by the implosion of cavitation bubbles (Ores et al., 2016). Therefore, increasing the biomass-solvent ratio produces greater disruption. Phycobiliproteins are highly conservative proteins in cyanobacteria; however, the aggregation state of these pigment proteins shows great variation in solution as dimeric, trimeric, and hexameric forms which are strongly affected by pH, ionic strength, and protein concentration. At neutral pH, moderate ionic strength and a moderate concentration of protein, the highest possible aggregation state is the trimer. In this state, the conformation and spectral properties of phycobiliproteins are well maintained (Niu et al., 2007).

The stable radical DPPH has been widely used for the determination of antioxidant activities and abilities of compounds, plants, fruit, and natural pigment extracts. The assay is based on the reduction of DPPH radicals in methanol, which causes a decrease in absorbance at 517 nm. The potency of a molecule to scavenge DPPH radicals results from the number of hydrogens available for donation by the hydroxyl groups (Chen and Wong, 2008). Oxidative reactions and reactive oxygen species are involved in various diseases including atherosclerosis, diabetes, and Alzheimer's disease. C-PC extracted from *A. platensis* cultured in seawater-based medium and purified by ammonium sulfate precipitation gave DPPH radical scavenging activity around 80% for C-PC at 5.00 mg ml^{-1}, with DPPH radical scavenging activity of less than 20% at C-PC content 0.3125 mg ml^{-1} (Wu et al., 2016). Increasing C-phycocyanin concentration gave higher DPPH radical scavenging activity. Our results indicated higher DPPH activity than previous findings at 0.1 mg ml^{-1} C-phycocyanin concentration. C-phycocyanin extraction from *A. platensis* powder by chemical method was undertaken by mixing with distilled water at a concentration of 0.04 g ml^{-1} for 24 h at 4 °C, giving percentage inhibition and purity at 38.12% and 0.52%, respectively (Piñero Estrada et al., 2001). Our results showed higher values of purity and percentage inhibition than Piñero Estrada et al. (2001) and were also achieved in a shorter time using ultrasound-assisted C-phycocyanin extraction. C-phycocyanin extracted from freeze-dried biomass at 0.06 g ml^{-1} in 10 mM buffer gave percentage inhibition similar to C-phycocyanin reactive grade (46.40%), while the purity of C-phycocyanin extracted differed at 0.603 to C-phycocyanin reactive grade 3.9 (Piñero Estrada et al., 2001). In addition, freeze-dried biomass preparation maintains C-phycocyanin and other components within the cell well better than oven-dried biomass preparation. Although freeze-dried biomass with increasing biomass-solvent ratio gave increasing C-phycocyanin concentration, DPPH radical scavenging activity decreased, maybe as a result of other components contaminating C-phycocyanin extraction.

Extrusion involves producing droplets of encapsulation materials by forcing the solution through nozzles or small openings in droplet-generating devices (de Vos et al., 2010). Natural pigment should be protected from the surrounding medium or from processing during food production. C-phycocyanin was extracted from *A. platensis* using ultrasonic-assisted techniques and encapsulated in sodium alginate beads for use in food applications using extrusion techniques. The most commonly applied extrusion technique involves alginate. Alginates are polysaccharides which exist as linear polymers with 1–4 linked-β-D mannuronic acid (M) and α-L-guluronic acid (G) residues arranged as blocks of either type of unit or as a random distribution of each type. Alginates can form gels to allow for a time-controlled release of bioactive molecules (de Vos et al., 2010). Alginate polysaccharides are generally used as delivery agents because they are structure stable, abundant in nature and inexpensive (Mohan et al., 2015). Alginate-based capsules are normally applied to facilitate the release of bioactive compounds in the ileum or colon (de Vos et al., 2010). The main effects and interactions between different factors on encapsulation efficiency were dependent variables including C-phycocyanin concentration, sodium alginate content, and calcium chloride content.

Encapsulated beads prepared from low alginate levels have large pores which rapidly release the substance droplets, while beads prepared at high alginate levels have small pores with slow release of substance droplets (McClements, 2017). Therefore, C-phycocyanin encapsulation efficiency is significantly affected by both C-phycocyanin and alginate content. C-phycocyanin encapsulation shape is affected by the viscosity of the alginate solution; higher viscosity inhibits the extrusion process and encourages tailing. C-phycocyanin concentration increased in low alginate levels causing high C-phycocyanin release. Optimum C-phycocyanin encapsulation efficiency was obtained using 3% alginate, 2.5% calcium chloride and 1 mg ml^{-1} C-phycocyanin from *A. platensis*. Phycocyanin encapsulation using extrusion techniques has been scantily studied under different optimum conditions. Phycocyanin encapsulation with alginate content at 1.5% and 2.0% in spherical shape (Hadiyanto et al., 2017) gave similar results to our paper. Calcium chloride content affected C-PC particle shape. Suzery et al. (2015) determined that C-phycocyanin encapsulation efficiency was higher than phycocyanin encapsulation at optimum conditions with efficiency ranging at 68.5–70.0%. Coating materials can provide improvements for appropriate applications. For example, phycocyanin encapsulated with alginate and chitosan gave high stability during storage (Yan et al., 2014). Moreover, Ilter et al. (2017) used maltodextrin and whey protein isolate at 34.4% and 65.6%, respectively as the coating material for phycocyanin encapsulation via the spray drying method. Encapsulation of color extracted from peels of eggplants obtained optimum shelf life condition using 4.35% sodium alginate, 2% calcium chloride and 12.5 g l^{-1} color concentration (Chatterjee and Bhattacharjee, 2015). Moreover, whey protein isolate as protein encapsulation in alginate using an extrusion technique was suitable with pH-triggered release of protein (Zhang et al., 2016b). A strong electrostatic attraction was found between cationic proteins and anionic alginate molecules in capsules at pH 3 causing protein retention, whereas, at neutral pH, a strong electrostatic repulsion between the anionic proteins and anionic polysaccharide molecules caused

protein release (McClements, 2017). However, pH during the encapsulation system was suitable for further application. C-phycocyanin encapsulated with alginate supported calcium ions and improved stability from the environment. Utilization of alginate to encapsulate astaxanthin with the acidic calcium ions significantly improved the thermal stability of astaxanthin. Moreover, calcium ions play functional roles as crosslinkers between alginate chains, leading to the formation of an alginate matrix layer which prevents oxygen exposure and forms a complex with astaxanthin to enhance its stability (Lin et al., 2016). Chemical stability of carotenoid-enriched lipid droplets can also be improved by encapsulation within calcium alginate beads (Zhang et al., 2016a). This extrusion procedure is not only suitable for laboratory scale processes but can also be used to scale-up of encapsulation processes using extrusion technology. Large-scale extrusion production can be achieved by multiple-nozzle systems, spinning disc atomizers, or jet-cutter technology (de Vos et al., 2010).

5. Conclusions

Physical methods were investigated for C-phycocyanin extraction from *Arthrospira platensis* using ultrasonic-assisted techniques. Optimum conditions for C-phycocyanin extraction were obtained from freeze-dried *A. platensis* at biomass-solvent ratio of 1:15 in 0.01 M phosphate buffer (pH 7.0) performed using ultrasonic techniques. C-phycocyanin encapsulation was optimized using 3% alginate content, 2.5% calcium chloride and 1 mg ml^{-1} C-phycocyanin giving high encapsulation efficiency. Our results can be used as basic information for further development of encapsulated food grade C-phycocyanin from *A. platensis.*

Declaration of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jksus.2018.05.026.

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