



Preparation of phycocyanin microcapsules and its properties

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ABSTRACT

Phycocyanin was microencapsulated by an extrusion method using alginate and chitosan as coating materials. This work was aimed to optimize the encapsulation process, characterize the physicochemical properties of microcapsules, and evaluate the storage stability and *in vitro* release performance. The optimum process conditions for preparing microcapsule gained from the single factor experiments were as follows: alginate content 2.5%, ratio of phycocyanin to alginate 1.5:1, content of calcium chloride 2.5%, and chitosan content 2.0%. Phycocyanin/alginate/chitosan microcapsules (PACM) were found to have compact spherical shape with mean diameters of 1.03 μm , whereas phycocyanin/alginate microspheres (PAM) were internal porous spherical appearances with mean diameters of 1.81 μm . Storage stability study showed that encapsulation by alginate and chitosan conferred greater ability to phycocyanin against temperature during storage. *In vitro* release study revealed that both PAM and PACM could be resistant against acidic environment, and would rapidly release phycocyanin under mild alkali condition. The sustained-release profile of phycocyanin from PACM was superior to that from PAM.

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Keywords: Microcapsule; Phycocyanin; Alginate; Chitosan

1. Introduction

Phycocyanin is a blue phycobiliprotein, composed of two relatively homologous subunits: the α -chain with one phycocyanobilin attached at cysteine 84 and the β -chain with two phycocyanobilins attached at cysteines 84 and 155. The two subunits form $\alpha\beta$ monomers, which aggregate into $\alpha_3\beta_3$ trimers and further into disc-shaped $\alpha_6\beta_6$ hexamers, the functional unit of phycocyanin (Eriksen, 2008). It has good therapeutic values, such as antioxidative, immunomodulating, anti-cancer, antiviral, anti-allergic, anti-mutagenic, anti-inflammatory, hepatoprotective, blood vessel-relaxing and blood lipid-lowering activities (Thangam et al., 2013), which made it a better active ingredient in functional food. It is also used as natural dyes in food including chewing gum, ice sherbets, soft drinks, and candies, and cosmetics including lipstick and eyeliners, replacing the synthetic colourants (Chaiklahan et al., 2011). Another application of phycocyanin is as phycofluor probes for immunodiagnosis owing to its fluorescence properties. However, its application is often limited by the instability towards moisture, light and temperature due to the degradation of the protein fraction (Chaiklahan

et al., 2012). Studies show that microencapsulation is an effective and economical method for protecting natural colourants against adverse conditions (Rocha et al., 2012). Therefore, it was inferred that the stability of phycocyanin should be improved using microencapsulation technologies. However, up to now, reports were scarce about phycocyanin microencapsulation.

Microencapsulation is a technique by which the sensitive ingredients, called core materials, are entrapped in coating or wall materials. The coating material protects the sensitive ingredients from the external influences, controls the release of the ingredient, and sometimes converts liquids into powders, easy to handle (Frascarelli et al., 2012; Bakowska-Barczak and Kolodziejczyk, 2011). So far, various kinds of microencapsulation techniques, such as emulsification, coacervation, spray drying, spray cooling, freeze drying, fluid bed coating, and extrusion, have been developed (Qv et al., 2011), among which, extrusion is one of the simple and convenient technologies. It denotes feeding the matrix dispersion through a single or a plurality of pathways directly into the continuous extraction phase. In extrusion, the flow is mainly laminar and the droplets are formed directly at the site of introduction

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of the dispersed phase into the continuous phase, so it is considered to allow for more uniform and better-controlled microsphere sizes (Freitas et al., 2005).

The coating materials must retain and protect the encapsulated core material from loss and chemical damage during manufacture, storage and handling, and subsequently release them into the final product during its manufacture or consumption (Kim et al., 2006). Alginate, one of linear anionic polysaccharides, has been considered one of the most suitable biopolymer for microencapsulation. The advantages of using alginate as coating material include: non-toxicity, formation of gentle matrices with calcium chloride to trap sensitive materials, low cost, and an accepted food additive and be safely used in foods (Chávarri et al., 2010). However, alginate beads show poor stability, resulting in the limitation of alginate application in microencapsulation. Previous research reported that coating alginate microcapsules with chitosan had improved the stability of the alginate beads (Krasaekoopt et al., 2004). Chitosan is a hydrophilic, biocompatible and biodegradable polysaccharide with low toxicity. The strong electrostatic interaction of the amino groups of the chitosan with the carboxylic groups of the alginate leads to formation of the complex alginate/chitosan microcapsule (Finotelli et al., 2010). Although alginate-chitosan bead had already been known in the literature, study was few on its application in phycocyanin microencapsulation. Phycocyanin, with a high molecule weight, can evidently affect the viscosity of alginate solution, and thereby influence the preparation process and properties of microcapsules. So it is of great necessity to study the phycocyanin coated by alginate and chitosan.

In the present study, alginate and chitosan were used as coating materials for producing microencapsulated phycocyanin by extrusion technique. And particle size, microstructure, storage stability and *in vitro* releasing property of encapsulated phycocyanin were investigated. The encapsulated phycocyanin will mainly be applied in functional food.

2. Materials and methods

2.1. Materials

Phycocyanin was extracted from *Spirulina platensis*, offered by Yunnan Green A Biological Project Co., Ltd, Yunnan, China, in the laboratory according to the method of Chaiklahan et al. (2011) with a slight modification. It has a molecular weight of 240 kDa. Alginate (low viscosity) was supplied by Qingdao Bright Moon Seaweed Group Co., Ltd, Shandong, China. Chitosan, having a molecular weight of 30 kDa, and a degree of deacetylation >90%, was purchased from Zhejiang Yuhuan Ocean Biochemical Co., Ltd, Zhejiang, China. All other reagents used were of analytical grade.

2.2. Microencapsulation of phycocyanin

Microencapsulated phycocyanin was prepared by extrusion technique, using alginate and chitosan as the coating materials. Extrusion process is shown in Fig. 1. Pressure was imposed to make droplets be extruded dropwise. Referring to the previous research (Meng and Chen, 2010), alginate content, ratio of phycocyanin to alginate, content of calcium chloride, and chitosan content had obvious effects on microencapsulation effect. Therefore, influences of these variables were evaluated by single factor experiments. For studying effects of

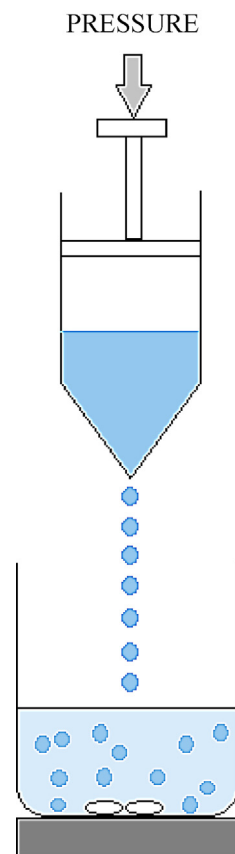


Fig. 1 – Extrusion process for preparing phycocyanin microcapsules.

alginate content on microencapsulation, alginates were prepared into solutions with the content of 1.0% (w/w), 1.5% (w/w), 2.0% (w/w), 2.5% (w/w), 3.0% (w/w), 3.5% (w/w), respectively. Then phycocyanin was added into alginate solutions according to ratio of phycocyanin to alginate 1:1. The mixing solutions were stirred uniformly, and extruded into calcium chloride solutions with the content of 2.0% to immobilize for 45 min. After that, the products were washed with distilled water, and the calcium chloride solution and distilled water were collected for determining the content of not-coated phycocyanin. The products were freeze-dried to get phycocyanin/alginate microcapsules (PAM) using lyophilizer (Christ, Osterode, Germany). Next, the products were shook in chitosan solutions with the content of 2.0% for 2 h, previously dissolved in 1.0% (w/w) acetic acid solution, and then freeze-dried to obtain phycocyanin/alginate/chitosan microcapsules (PACM). The other three single factor tests, ratio of phycocyanin to alginate, content of calcium chloride, and chitosan content, took similar process as the above. Their individual conditions were that (1) alginate content 2.5% (w/w), ratio of phycocyanin to alginate 3:1, 2.5:1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, content of calcium chloride 2.0% (w/w), chitosan content 2.0% (w/w); (2) alginate content 2.5% (w/w), ratio of phycocyanin to alginate 1.5:1, content of calcium chloride 1.0% (w/w), 1.5% (w/w), 2.0% (w/w), 2.5% (w/w), 3.0% (w/w), chitosan content 2.0% (w/w); (3) alginate content 2.5% (w/w), ratio of phycocyanin to alginate 1.5:1, content of calcium chloride 2.5% (w/w), chitosan content 0.5% (w/w), 1.0% (w/w), 1.5% (w/w), 2.0% (w/w), 2.5% (w/w), 3.0% (w/w).

2.3. Determination of encapsulation efficiency and phycocyanin load

The phycocyanin concentration (PC) was determined spectrophotometrically, using the equation of [Bennett and Bogorad \(1973\)](#) as follows:

$$\text{PC (mg mL}^{-1}\text{)} = \frac{A_{615} - 0.474A_{652}}{5.34}$$

The encapsulation efficiency of microcapsule was calculated, following the method of [Ge et al. \(2009\)](#) with some modifications, by measuring the mass of not-coated phycocyanin and the mass of phycocyanin added at the beginning of the microencapsulation process as follows:

$$\text{encapsulation efficiency (\%)} = \frac{\text{mass of phycocyanin added at beginning} - \text{mass of not coated phycocyanin}}{\text{mass of phycocyanin added at beginning}} \times 100$$

Phycocyanin load could be understood as phycocyanin content in the microcapsules, expressed by a ratio of the mass of phycocyanin to the mass of coating material in beads as follows:

$$\text{phycocyanin load (\%)} = \frac{\text{mass of phycocyanin in beads}}{\text{mass of coating material in beads}} \times 100$$

Because the mass of chitosan in microcapsule was difficult to be determined, the mass of coating material in beads was only represented by the mass of alginate.

2.4. Physicochemical properties of phycocyanin microcapsules

2.4.1. Size analysis

The size of the phycocyanin microcapsules was expressed by the average of particle diameter, determined by vernier caliper (530-312/104, Mitutoyo, Japan). In all measurements at least 100 particles were examined.

2.4.2. Morphology

The morphology of the microcapsules was observed under scanning electron microscope (S-4800, Hitachi, Japan). The microparticles were fixed on stubs using copper-conducting adhesive tape, and coated with gold. Observations were made using scanning electron microscope (SEM) at an acceleration voltage of 10 kV.

2.4.3. Infrared atlas analysis of microcapsules

Potassium bromide (KBr) was ground with freeze-dried microcapsules, phycocyanin, alginate and chitosan, respectively, to obtain five powders, and then the prepared powders were made into transparent plates using the presser. The spectra were recorded using infrared spectrophotometer (Jasco-4100, Tokyo, Japan) from 4000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} per point at room temperature.

2.5. Storage stability of phycocyanin microcapsules

The effect of environmental factors including relative humidity (31% and 81% at 25 °C in dark), light (at 25 °C) and temperature (40 °C and 50 °C without light) on the stability of microencapsulated phycocyanin (freeze-dried) during storage was assessed. The preservation rate of phycocyanin was a ratio of the mass of phycocyanin that was retained in the

microcapsules after a specified period of storage to the initial mass of phycocyanin in the microcapsules. Samples were taken every five days to examine the preservation rate.

2.6. In vitro phycocyanin release study

In vitro release profiles of phycocyanin from microcapsules were examined in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4), prepared according to United States Pharmacopoeia (USP), for 10 h under mechanical stirring rotation of 100 rpm at 37 ± 0.5 °C. At scheduled time intervals, agitation was stopped, the sample (4 mL) were withdrawn and replaced with fresh medium. The phycocyanin

content was determined spectrophotometrically according to the equation of [Bennett and Bogorad \(1973\)](#). All the experiments were made in triplicate.

2.7. Statistical analysis

All statistical data were analyzed using Microsoft Excel 2000 and Origin 7.5. Results were presented as mean \pm standard deviation (SD) of replicated determinations. Student's t-test was applied to compare the averages of properties with a level of 95% confidence interval.

3. Results and discussion

3.1. Preparation of phycocyanin microcapsules

Phycocyanin microcapsules were prepared by extrusion technique using alginate and chitosan as coating materials. The variables including alginate content, ratio of phycocyanin to alginate, content of calcium chloride and chitosan content are able to affect the extrusion process together with the properties of the resulted microcapsules including encapsulation efficiency, phycocyanin load and microcapsule shape. The results are shown in [Table 1](#).

There were evident effects of alginate content on the encapsulation efficiency, phycocyanin load and shape of phycocyanin microcapsules. Both encapsulation efficiency and phycocyanin load were increased with alginate content increased ranging from 1.0% to 3.5%, different from the results reported by others ([Lyu et al., 2004](#); [Meng and Chen, 2010](#)). Microcapsule shape could be affected by the viscosity of the alginate solution, and higher viscosity would bring difficulty in extrusion process and easily result in tailing. In short, microcapsule shape was correlated with the extrusion process, generally, regular shape reflecting the extrusion easy to handle. Therefore, microcapsule shape was selected as an index to evaluate the preparation. The resulted phycocyanin microcapsules were oblate spherical after freeze-drying at lower alginate content, the reason might be that there was not enough alginate molecule to form stable structure, while at higher alginate content, they were irregular spherical, attributed to the higher viscosity of alginate solution. When alginate content reached 2.0–2.5%, the microcapsules were regularly spherical. By weighed averages method, the better level of alginate content for preparing phycocyanin microcapsule was 2.5%.

Table 1 – Effect of alginate content, ratio of phycocyanin to alginate, content of calcium chloride and chitosan content on the properties of phycocyanin microcapsule.

Variable	Level	Encapsulation efficiency (%)	Phycocyanin load (%)	Microcapsule shape
Alginate content [*]	1.0%	30.69 ± 2.34 ^c	8.74 ± 0.67 ^c	Oblate spherical after freeze-drying
	1.5%	36.29 ± 1.20 ^c	9.91 ± 0.33 ^c	Oblate spherical after freeze-drying
	2.0%	44.91 ± 0.84 ^b	12.00 ± 0.22 ^b	Spherical
	2.5%	53.84 ± 0.81 ^a	14.20 ± 0.21 ^a	Spherical
	3.0%	57.48 ± 4.15 ^a	15.03 ± 1.08 ^a	Spherical with tailing
	3.5%	62.66 ± 0.72 ^a	16.28 ± 0.19 ^a	Irregular shape
Ratio of phycocyanin to alginate [*]	3:1	68.48 ± 0.02 ^a	52.28 ± 0.02 ^a	Irregular shape
	2.5:1	58.50 ± 0.11 ^b	37.35 ± 0.07 ^b	Spherical with tailing
	2:1	53.91 ± 0.63 ^e	27.69 ± 0.32 ^c	Spherical with a little tailing
	1.5:1	54.60 ± 0.04 ^e	21.22 ± 0.01 ^d	Spherical
	1:1	55.16 ± 0.11 ^d	14.55 ± 0.03 ^e	Spherical
	1:1.5	53.49 ± 0.06 ^e	9.57 ± 0.01 ^f	Spherical
	1:2	52.39 ± 0.29 ^f	7.28 ± 0.04 ^g	Spherical
	1:2.5	58.55 ± 0.40 ^b	6.67 ± 0.05 ^h	Spherical
	1:3	56.02 ± 0.10 ^c	5.46 ± 0.01 ⁱ	Spherical
Content of calcium chloride [*]	1.0%	56.67 ± 0.23 ^a	22.03 ± 0.09 ^a	Oblate spherical after freeze-drying
	1.5%	56.20 ± 0.11 ^a	21.84 ± 0.04 ^a	Oblate spherical after freeze-drying
	2.0%	56.01 ± 0.07 ^a	21.77 ± 0.03 ^a	Oblate spherical after freeze-drying
	2.5%	55.50 ± 0.25 ^a	21.57 ± 0.10 ^a	Spherical
	3.0%	55.80 ± 0.26 ^a	21.69 ± 0.10 ^a	Spherical
Chitosan content	0.5%	55.91 ± 2.06 ^a	21.73 ± 0.80 ^a	Spherical
	1.0%	55.99 ± 0.52 ^a	21.76 ± 0.20 ^a	Spherical
	1.5%	58.09 ± 0.93 ^a	22.58 ± 0.36 ^a	Spherical
	2.0%	58.95 ± 0.72 ^a	22.91 ± 0.28 ^a	Spherical
	2.5%	58.05 ± 0.56 ^a	22.57 ± 0.22 ^a	Spherical
	3.0%	57.54 ± 0.63 ^a	22.37 ± 0.24 ^a	Spherical

Note: Values are mean ± standard deviation of three determinations.

For each parameter, means in the same column not sharing the same letters are significantly different ($p < 0.05$).

* Effect of the variable on the properties of phycocyanin microcapsule coated by alginate was investigated.

From Table 1, it could be found that higher ratio of phycocyanin to alginate induced the encapsulation efficiency and phycocyanin load of microcapsules higher, but had adverse effect on shape, the reason might be that the viscosity of alginate solution was augmented by phycocyanin with large molecular weight. The microcapsules with lower ratio of phycocyanin to alginate were regularly spherical, although the encapsulation efficiency and phycocyanin load were lower. By comprehensive consideration, ratio of phycocyanin to alginate 1.5:1 was chosen to preparing phycocyanin microcapsules.

Content of calcium chloride had no significant effect on the encapsulation efficiency and phycocyanin load of microcapsules, as shown in Table 1, different from the conclusion obtained by Lyu et al. (2004), Vandenberg et al. (2001) and Gao et al. (2009). Its reason was possibly that high-molecular-weight phycocyanin could not exude from the alginate gel (Yoo et al., 2006) formed even at lower content of calcium chloride. From the microparticle shape, at the content of calcium chloride from 1.0% to 2.0%, the microcapsules were oblate spherical after freeze-drying, suggesting that there was not enough calcium ion to interact with alginate. When the concentration came to 2.5% or above, they were regularly spherical. From the point of saving the materials, content of calcium chloride 2.5% was used for preparing the phycocyanin microcapsules.

Table 1 suggested that chitosan content had no marked impact on the encapsulation efficiency, phycocyanin load

and microcapsule shape. However, it was found that the introduction of chitosan could make the diameters of phycocyanin microcapsules reduced, as shown in Table 2, which might be correlated with the electrostatic interaction between alginate and chitosan to form a stable polyelectrolyte complex (Li et al., 2002). The diameter of the fresh microcapsule decreased from 2.54 ± 0.18 mm to 1.37 ± 0.14 mm evidently as the chitosan content increased from 0 to 2.0%, while as the chitosan content further increased to 3.0%, the diameter tended to level off, suggesting that there was fully interaction between alginate and chitosan in microcapsule when chitosan content reaching or above 2.0%. However, after freeze-drying, the diameter of microcapsule decreased from 1.81 ± 0.22 mm to 1.12 ± 0.13 mm with chitosan content increased from 0 to 1.0%, and then became stable with chitosan content further increased to 3.0%. In this study, the microcapsules coated with chitosan were mainly to stabilize Ca-alginate microparticles (Selina et al., 2008). When chitosan reacted with alginate completely, Ca-alginate microparticles were more stable. So it was determined that chitosan content should be 2.0%.

Taken together, the phycocyanin microcapsules prepared with 2.5% alginate, 1.5:1 ratio of phycocyanin to alginate, 2.5% calcium chloride, and 2.0% chitosan were chosen for further investigation. Next, its physicochemical properties, including particle size, morphology, and infrared spectra, storage stability and *in vitro* release property were studied in detail.

Table 2 – Diameter of phycocyanin/alginate/chitosan microcapsule.

Chitosan content (%)	Diameter (mm)	
	Fresh microcapsule (before freeze-drying)	Microcapsule after freeze-drying
0	2.54 ± 0.18 ^a	1.81 ± 0.22 ^a
0.5	1.89 ± 0.14 ^b	1.25 ± 0.13 ^b
1.0	1.65 ± 0.08 ^c	1.12 ± 0.13 ^c
1.5	1.47 ± 0.08 ^d	1.05 ± 0.09 ^c
2.0	1.37 ± 0.14 ^e	1.03 ± 0.05 ^c
2.5	1.41 ± 0.07 ^e	1.05 ± 0.11 ^c
3.0	1.36 ± 0.07 ^e	1.08 ± 0.06 ^c

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

3.2. Physicochemical properties of phycocyanin microcapsules

Generally speaking, the assessment of physicochemical properties of microcapsules is a basic method to appraise product quality, an auxiliary means for technique optimization as well as a significant theoretical foundation for choice of storage conditions (Qv et al., 2011).

3.2.1. Particle size of phycocyanin microcapsules

The particle size is an important parameter representing the feature of the microcapsules (Zheng et al., 2011). Table 2 shows results of diameters of phycocyanin microcapsules coated by alginate with or without chitosan. The mean diameters of fresh microparticles were reduced from 2.54 ± 0.18 mm to 1.37 ± 0.14 mm after 2.0% chitosan entered, mainly resulting

from the interaction between alginate and chitosan (Li et al., 2002) as mentioned above. Freeze-drying also induced the mean diameter lessened significantly, those of PAM and PACM decreasing by 28.74% and 24.82%, respectively, which was correlated with the shrinkage of microcapsule due to the loss of water content. In the production process, phycocyanin microcapsules with different particle size could be obtained by the extrusion method according to the requirement.

3.2.2. Morphology of phycocyanin microcapsules

The SEM images of phycocyanin microcapsules are illustrated in Fig. 2. It could be found that the microparticles all had spherical appearances. The surface of PAM was accented, attributed to rapid evaporation of water during the freeze-drying process (Rosenberg et al., 1985) and its internal structure. Nevertheless the PACM showed a smooth outer surface after the introduction of chitosan in preparation. Similar image was observed by Finotelli et al. (2010) in alginate/chitosan containing magnetic microcapsules obtained by extrusion method. As shown in Fig. 2(c) and (d), the cross-section of PAM and PACM also varied. The PAM showed porous structure, while the PACM were compact, which would lead to the characteristics diversity of phycocyanin microcapsule.

3.2.3. Infrared spectra analysis of phycocyanin microcapsules

The infrared spectra of phycocyanin, alginate, chitosan, and phycocyanin-loaded microcapsules are shown in Fig. 3. The spectrum of PAM was similar to that of alginate, but different from that of phycocyanin, suggesting that phycocyanin was embedded well. After microencapsulation, the absorption band at 1627 cm^{-1} and 1415 cm^{-1} of alginate both shifted to higher frequency, near 1639 cm^{-1} and 1423 cm^{-1} , respectively, indicating that there was electrostatic interaction

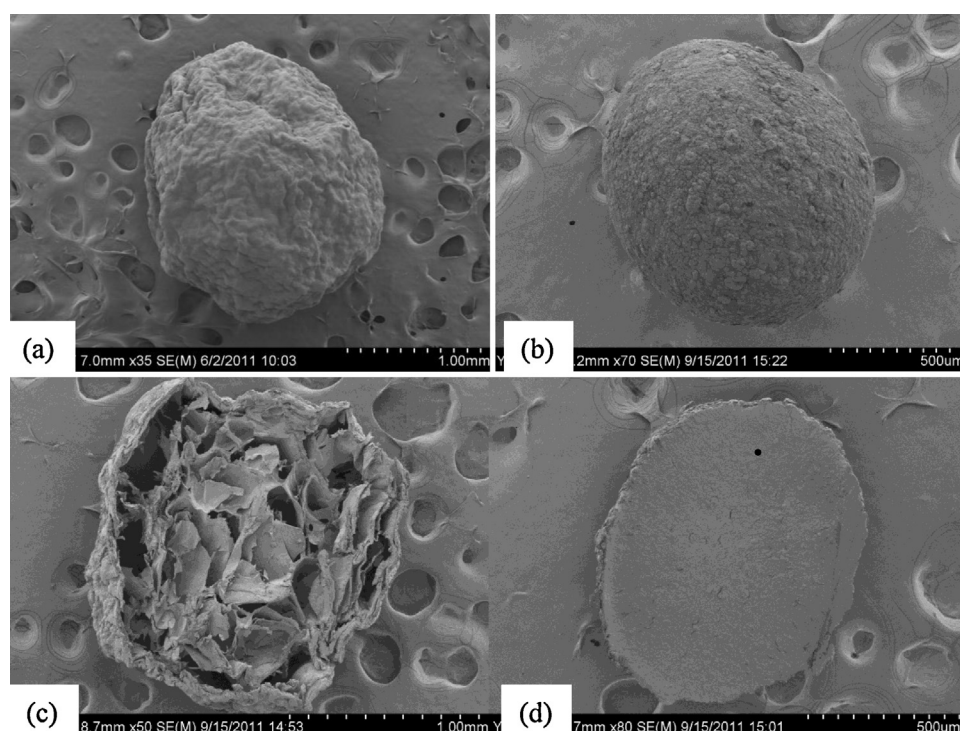


Fig. 2 – SEM images of phycocyanin microcapsules. (a, c) Surface and cross-section of phycocyanin microcapsule coated by alginate, respectively; (b, d) surface and cross-section of phycocyanin microcapsule coated by alginate and chitosan, respectively.

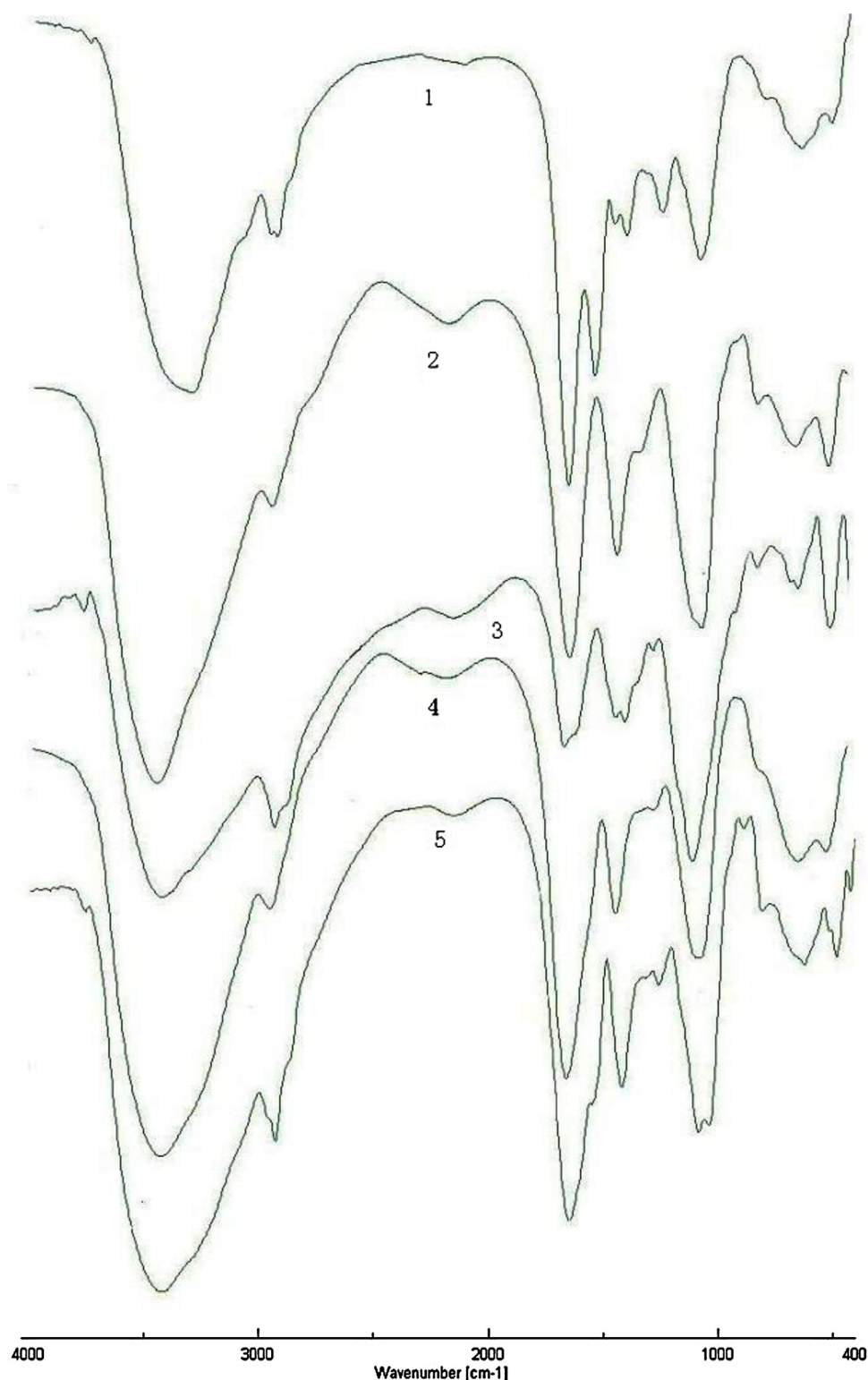


Fig. 3 – Comparison analysis of the infrared spectroscopy of (1) phycocyanin, (2) alginate, (3) chitosan, (4) phycocyanin microcapsule coated by alginate, and (5) phycocyanin microcapsule coated by alginate and chitosan.

between alginate and phycocyanin in microcapsule (Lee, 2000). It could also be observed that no new absorption band appeared among spectra of PACM and wall materials (alginate and chitosan), showing that the formation of PACM was promoted by physical interaction such as electrostatic interaction rather than chemical reactions (Qv et al., 2011; Yan et al., 2007). The results suggested that whether PAM or PACM, they were formed by electrostatic interaction, which was beneficial to sustained release of phycocyanin in microcapsules.

3.3. Storage stability of phycocyanin microcapsules

3.3.1. Effect of relative humidity

Relative humidity is taken as a significant factor influencing product storage (Qv et al., 2011). Fig. 4(a) shows effect of relative humidity on the stability of free and microencapsulated phycocyanin. After 40 days storage at 31% relative humidity, the preservation rate of phycocyanin in PAM and PACM was 92.56% and 97.05%, respectively, close to 95.59% of free phycocyanin. When preservation in 81% relative humidity for

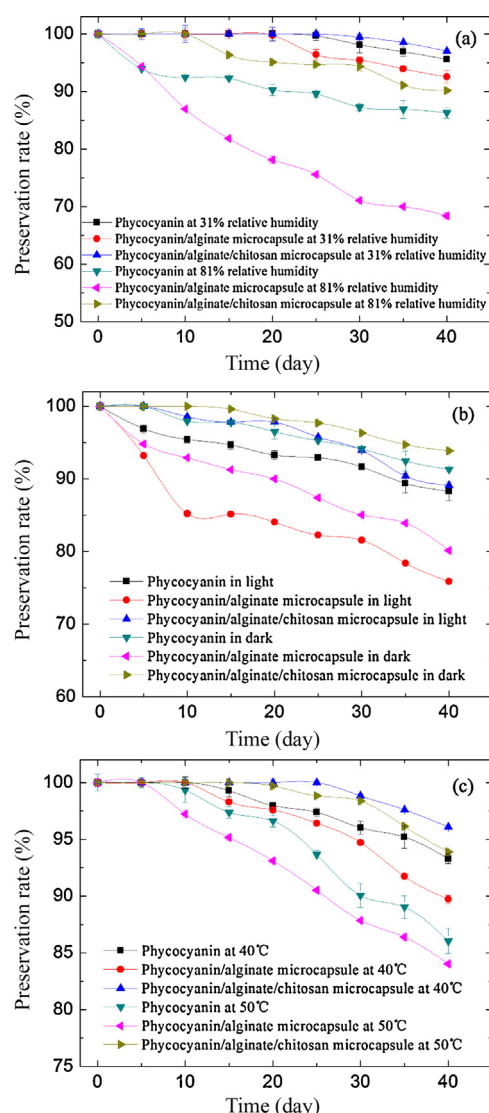


Fig. 4 – Storage stability of free and microencapsulated phycocyanin under different storage conditions. (a) Effect of relative humidity on the storage stability of free and microencapsulated phycocyanin. (b) Effect of light on the storage stability of free and microencapsulated phycocyanin. (c) Effect of temperature on the storage stability of free and microencapsulated phycocyanin.

40 days, the proportion of phycocyanin in PACM was 90.16%, not significantly higher than 86.30% of free phycocyanin ($p > 0.05$), while that in PAM was only 68.40%, much lower than that of free phycocyanin ($p < 0.05$). It could be found that the encapsulated and non-encapsulated phycocyanin should be preserved in low relative humidity conditions. PACM was more stable than PAM whether in low or high humidity environment, revealing that chitosan coating plays a vital role in preventing the entry of water, which might be related to the compact structure of PACM formed by the interaction of alginate and chitosan. However, no evidently higher value was seen in the preservation rate of phycocyanin in PACM compared with that of free phycocyanin, possibly attributed to the hygroscopicity of chitosan. The stability of phycocyanin in PAM was worse than that of free phycocyanin, which might be correlated with its porous structure and alginate's hygroscopicity. So it could be deduced that water insoluble wall materials were more suitable to phycocyanin embedment.

3.3.2. Effect of light

Effect of light on the stability of free and microencapsulated phycocyanin is displayed in Fig. 4(b). When storage in dark for 40 days, the preservation rate of phycocyanin in PAM and PACM was 80.16% and 93.87%, respectively, and that of non-encapsulated phycocyanin was 91.30%, and when preservation with light for 40 days, they were 75.87%, 89.11%, and 88.30%, respectively. The results demonstrated that phycocyanin should be preserved in dark, and whether in light or not, the storage stability of phycocyanin in PACM was similar to that of free phycocyanin, which might be correlated with the too large size of microcapsules, while the preservation rate of phycocyanin in PAM was much lower than that of non-encapsulated phycocyanin ($p < 0.05$), the reason might be that the size of PAM was too large and its internal structure was porous. From the results, it could be inferred that phycocyanin microcapsules with small particle size should have better photostability.

3.3.3. Effect of temperature

Fig. 4(c) presents effect of temperature on the stability of free and microencapsulated phycocyanin. At the end of preservation for 40 days at 40 °C, the preservation rate of phycocyanin in PAM and PACM were 89.73% and 96.08%, respectively, and that of free phycocyanin was 93.29%. When temperature was increased to 50 °C, after storage for 40 days, the rate in PAM and PACM was 84.03% and 93.88%, respectively, and that of free phycocyanin was 86.04%. There was an obvious decline in stability of free phycocyanin and phycocyanin in PAM when temperature rising from 40 °C to 50 °C ($p < 0.05$), whereas that in PACM did not decrease significantly ($p > 0.05$), similar results observed by Zheng et al. (2011) and Wang et al. (2009). And the preservation rate of phycocyanin in PACM was much higher than that of free phycocyanin ($p < 0.05$). The results suggested that phycocyanin in PACM showed greater storage stability against temperature.

3.4. In vitro phycocyanin releasing property of microcapsules

The desirable microcapsule should protect core material from acid-damaged destruction. When it reached small intestine, it should release the core material rapidly (Yoo et al., 2006). The *in vitro* release profiles of phycocyanin from the microcapsules prepared with alginate and alginate-chitosan were investigated in simulated gastric (SGF pH 1.2) and intestinal fluid (SIF pH 7.4) for 10-h period. Generally, human gastric emptying time was 1–2 h. Therefore, the microspheres were in SGF for 2 h, and then they were transferred into SIF in the experiment. PAM and PACM both showed a slower release rate in the acidic medium, as depicted in Fig. 5, similar to the results reported by others (Manconi et al., 2010; Yoo et al., 2006). When they were transferred into SIF, dramatic increases were observed in release percentage of phycocyanin initially, thereafter slight decreases were found, which was ascribed to proteases in SGF and SIF. The highest release percentage of phycocyanin from PAM was 74.14% observed at 3 h, while that from PACM was 95.20% at 4 h. It could be found that when chitosan was included in the wall of the microcapsules, the release speed of phycocyanin became slow, which might be related to the compact structure of microspheres. But the release level of phycocyanin from PACM was much higher than that from PAM,

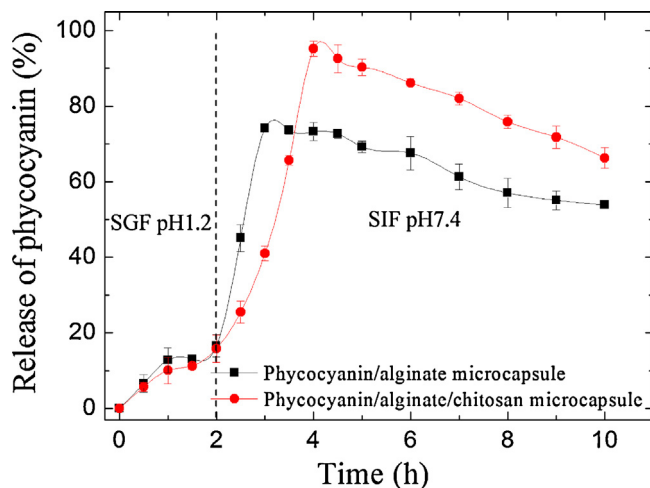


Fig. 5 – Release profiles of phycocyanin from microcapsules in simulated gastric fluid (SGF pH 1.2) and simulated intestinal fluid (SIF pH 7.4).

suggesting that the sustained-release profile of phycocyanin from PACM was superior to that from PAM.

4. Conclusion

Phycocyanin microcapsules were successfully prepared by extrusion technique using alginate and chitosan as coating materials. The optimum process conditions determined by single factor experiments were as follows: alginate content 2.5%, ratio of phycocyanin to alginate 1.5:1, content of calcium chloride 2.5%, and chitosan content 2.0%. The mean diameters of fresh PAM and PACM were 2.54 ± 0.18 mm and 1.37 ± 0.14 mm, respectively, whereas after freeze-drying, they were 1.81 ± 0.22 mm and 1.03 ± 0.05 mm, respectively. PAM and PACM were both spherical appearance. PAM showed an internal porous structure, while PACM were compact. Infrared spectra analysis suggested that PAM and PACM were formed by electrostatic interaction. The encapsulation by alginate and chitosan improved the ability of phycocyanin to resist the adverse impact of temperature on its storage, whereas encapsulation only by alginate did not. *In vitro* release study revealed that both PAM and PACM could be resistant against acidic environment, and they would rapidly release phycocyanin under mild alkali condition. But the sustained-release profile of phycocyanin from PACM was superior to that from PAM. It could be concluded that chitosan-coated alginate microspheres could be a good way to enhance the stability and control sustained-release of phycocyanin. This study provides the basis for the application of phycocyanin in functional food.

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