Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

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 mm, whereas phycocyanin/alginate microspheres (PAM) were internal porous spherical appearances with mean diameters of 1.81 mm. 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.

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 αβ 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 (Frascareli 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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Received 21 January 2013; Received in revised form 11 July 2013; Accepted 22 July 2013
0960-3085/$ – see front matter
http://dx.doi.org/10.1016/j.fbp.2013.07.008

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

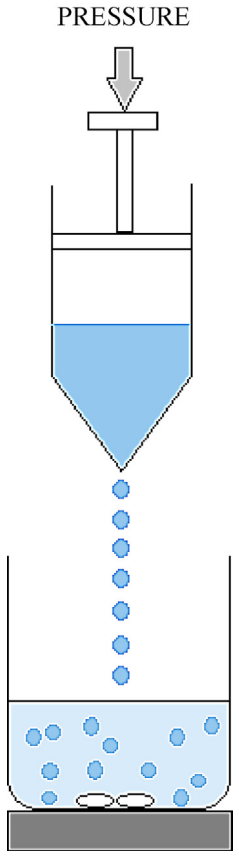

Fig. 1 – Extrusion process for preparing phycocyanin microcapsules.

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).