AIP Conference Proceedings

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Citation: AIP Conference Proceedings **1699**, 030011 (2015); doi: 10.1063/1.4938296
View online: http://dx.doi.org/10.1063/1.4938296
View Table of Contents: http://scitation.aip.org/content/aip/proceeding/aipcp/1699?ver=pdfcov
Published by the AIP Publishing

The Improvement of Phycocyanin Stability Extracted from *Spirulina sp* using Extrusion Encapsulation Technique

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Abstract. The stability of phycocyanin extracted from microalgae Spirulina has been evaluated and it showed that the stability of this antioxidant was affected by temperature and pH changes. The encapsulation technique was of the alternatives to overcome this stability changes. The objective of this paper was to investigate the effects of coating materials (alginate and chitosan) during encapsulation by using extrusion technique. The experiments were conducted with variation of alginate as coating materials. The size of each microcapsules was evaluated by using SEM/XRD for its size and homogeneity.

Keywords: Phycocyanin, encapsulation, alginate, anti-oxidant, stability

INTRODUCTION

Phycocyanin is a blue phycobiliprotein which has been considered as good therapeutic values, such as antioxidative, immunomodulating, anti-cancer, antiviral, anti-allergic, anti-mutagenic, anti-inflammatory, and blood lipid-lowering activities[1]. This compound is also frequently used as natural dyes in food including chewing gum, soft drinks, and candies, and cosmetics[2]. However, its application is eventually facing instability problems towards light exposures, moisture, and high temperature[2]. Due to high content of protein, the phycocyanin is a highly unsaturated molecule which can degrade and lose its bioactive properties during processing of the food products. Therefore, attempting to utilize this compound for fortify foods or coloring agents with phycocyaninn may lead to only limited success. Spriulina has been identified as the most abundant source of phycocyanin besides β-carotene and protein[3].

Microencapsulation is an effective method to protect the stability of bioactive compounds from environmental factors by shielding the compounds from the environment with thin edible coating materials[4]. Several techniques, including spray drying, spray cooling, fluidized bed drying, extrusion, and centrifugal extrusion, can be used to produce microcapsules[5]. Among them, extrusion encapsulation process has been considered as the economics and easy to operate[6]. The coating material used in the encapsulation must protect the active substances from the chemical damage during processing and keep its properties stable for consumption. Alginate has been considered as the most coating material for microencapsulation due to its advantages such as non-toxicity, low cost, and safe for food additive [7,8]. Phycocyanin, with a high molecule weight, can eventually influence the viscosity of alginate solution, and thereby the interaction of alginate and phycocyanin is interesting subject to be explored and for its effects to properties of microcapsules[7]. Therefore,it is of great necessity to study the phycocyanin coated by alginate and how the efficiency of encapsulation by alginate could be increased. The objective of this study was to investigate the stability of phycocyanin after its encapsulated by using alginate as coating material.

International Conference of Chemical and Material Engineering(ICCME) 2015
AIP Conf. Proc. 1699, 030011-1–030011-4; doi: 10.1063/1.4938296

MATERIAL AND METHODS

Microencapsulation of phycocyanin

Microencapsulated phycocyanin has been prepared by extrusion technique and used alginate as the coating material. For studying effect of alginate on microencapsulation, alginates were dissolved into solutions with the content of 2% (w/w) and 2,5 % (w/w). Then solution phycocyanin was added into alginate solution according to ratio of phycocyanin to alginate (1:1) and (1:2). The mixing solution were stirred uniformly and extruded to calcium chloride solution (2,5%) to immobilize for 45 min. After that the product were washed with distilled water and the separated water were collected for determining the content of uncoated phycocyanin (phycocyanin on the surface). The first evaluation was done for the beads drying by incubating the beads on air room temperature (without light), hot air-dryed (43^0C) and on freezer (-2^0C) for making PAM (phycocyanin alginate microsphere) and then drying again by incubating the beads on air room temperature (without light), hot air-dryed (43^0C) and on freezer (-2^0C) to get PACM. The size of needles was evaluated to study the encapsulation efficiency.

Determination of encapsulation efficiency

The phycocyanin content was determined spectrophotometrically using equation:

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

The encapsulation efficiency of microcapsule was calculated according to equation:

$$\text{The encapsulation efficiency (\%)} = \frac{mass\ phycocyani\ \ added\ at\ beginning - m\ \ \ of\ not\ coate\ \ phycocyani}{mass\ phycocyani\ \ added\ at\ beginning} \quad (2)$$

Solubility test

Prior to phycocyanin release study, the solubility of microcapsules were examined. The dried encapsulated products dissolved into water in room temperature at acidic condition (HCl solution (pH 3-4), and alkaline base condition (pH 8,2). The beads soaked and being stirred at 300 rpm in water and the phycocyanin dissolve on each solution being calculated by Equation 3. Mass of phycocyanin completely dissolved interpret the mass of phycocyanin in beads.

$$\text{Phycocyanin Load (\%)} = \frac{mass\ of\ phycocyani\ \ in\ beads}{mass\ of\ phycocyani\ \ added\ at\ beginning} \quad (3)$$

FTIR analysis

Interaction between alginate and microcapsule were also examined by infrared spectroscopy. Firstly ground the grinded microcapsule, phycocyanin and alginate with pottasium bromide (KBr), and the prepared powder were spread into transparant plates using presser. The spectra were recorded by infrared spectrophotometer from 4000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} per point at room temperature.

RESULT AND DISCUSSION

Efficiency of Phycocyanin Microcapsule

Microcapsule phycocyanin coated by alginate were prepared based on the method of[9]. The efficiency was calculated in respected to alginate content and phycocyanin alginate.

Table 2 shows that the efficiency of encapsulation did not depend on the bead sizes, nor the concentration of alginate in the solution. Or result shows that the efficiency of encapsulation was in the range of 68.5-70%. The problem in the encapsulation process was the shape of the bead which have tailed shapes. This is due to higher molecular weight of alginate used in the experiment.

Table 1. Effect of Alginate Content, Phycocyanin to Alginate ratio and Calcium Chloride concentration on encapsulation efficiency

Alginate Content	Phycocyanin to Alginate Ratio	Encapsulation Efficiency (%)		
		1 mm	**3 mm**	**5 mm**
+	+	68,346	68,446	68,723
+	-	68,987	68,497	68,995
-	+	68,557	68,572	68,496
-	-	69,977	69,445	70,556

Alginate content (+) on means 2,5%, (-) means 2%, Phycocyanin to Alginate ratio (+) means 1,5:1; (-) means 1:1

Psycochemical Properties of Phycocyanin Microcapsule

Scanning Electron Microscopy

Microparticle of encapsulated phycocyanin illustrated in Figure 2. Morphology of the microcapsule is spheres, but having a little rough surface with cavities, due to water content loss when the microcapsule is incubated either on air dryer, hot air dryer or on freezer. Microbeads made from extrusion having a larger size than phycocyanin itself since alginate coated it. The compactness and the shape of microcapsule based on research before showing great impacts on the stability of that microcapsule. A larger and porous microcapsule having lower stability against humidity, light and temperature as different storage condition.

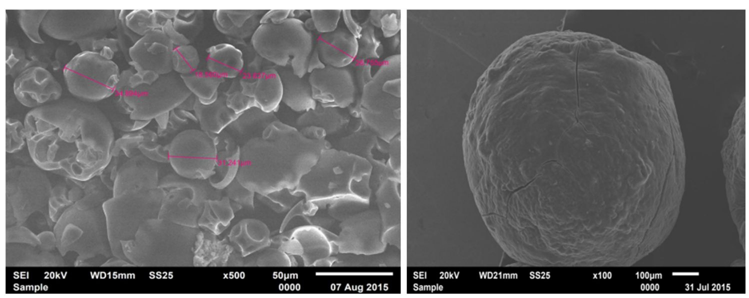

Figure 1- SEM Images of surface phycocyanin (a) before encapsulation (b) after encapsulated with alginate.

Infrared Spectrometry Analysis

Infrared Spectrometry was done to clarify if there was chemical reaction during microencapsulation using extrusion methods. Both phycocyanin (as raw material) and encapsulated phycocyanin (finished product) samples were analyzed in solid state. The FTIR Spectra on Fig 2 shown that there was no new absorption band, indicating that in this process, electrostatic interaction was a major cause of this process which is appropriate for maintained of phycocyanin in microbeads.

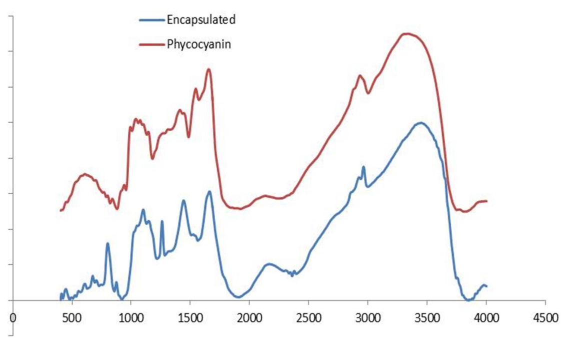

Figure 2- Infrared Spectrum of (red) Phycocyanin (blue) Phycocyanin encapsulated by alginate

Solubility Test

Solubility test was also studied and taken as a fundamental factor influencing application of these microcapsules as release properties and its storage handling. Furthermore, before calculating the phycocyanin load using eq. 3 above, microcapsule need to be diluted. Here the solven used are aquades, acid solution (pH 1.2), base solution (pH 8.2), etanol and methanol as non polar solvent. The desirable phycocyanin microcapsule as antioxidant can maintain itself from acid-damaged destruction (gastric fluid) and immediately release on weak base (intestinal fluid).

The drying beads methods after sinked to cross link solution can actually influence hardness and solubility of the beads. In all solven, incubated beads either on air or on the oven (43^0C) increasing hardness and lowering solubility of the microspheres. Beads incubated on freezer (-8^0C) showing better release effect since it can be diluted on aquades, but can not be diluted using other solven.

It could also be observed that in all methods of drying (air, hot air, and freezer), the beads can not be diluted on methanol, ethanol, on pH 1.2 and on pH 8.2. Its shows that the microcapsule has a high stability, but it is not an appropriate. The desirable microbeads as a functional food containing antioxidant agent could not damage over acid condition wich is simulated by gastric simulated fluid (pH=1.2), but rapidly release on instestinal fluid (pH=8.2). Capsule cristalized and there were a changing color from fresh blue to blackened blue suggesting that, the drying methods should be changed using freeze drying.

CONCLUSIONS

Extrusion technology is an effective way for encapsulating phycocyanin. The optimum process for gaining greater amount of phycocyanin encapsulated (efficiency) is: alginate content 2%, phycocyanin to alginate ratio 1:1, calcium chloride content 2,5%, nozzle syring and incubated on freezer as drying methods. There is no actually great impact of using different size nozzle. Further research using another matrix polysaccharides and freeze drying as drying methods.

ACKNOWLEDGEMENT

We acknowledged Diponegoro University through PNBP Scheme research grant.

REFERENCES

[1] **R.** Thangam, V. Suresh, W.A.Princy, M. Rajkumar, N. SenthilKumar, P. Gunasekaran, R. Rengasamy, C.Anbazhagan, K. Kaveri, S.Kannan, Food Chem. **140,** 262–272(2013).

[2] R. Chaiklahan, N. Chirasuwan, V. Loha, S. Tia, B. Bunnag, Bioresource Technology, **102**, 7159–7164 (2011).

[3] H. Hadiyanto, M.A.Marsya, P. Fatkhiyatul, Jurnal Teknologi, **77 (1)**, 219-222(2015)

[4] S. Sathivel and D. Kramer, *Microencapsulation, edible film and coatings applications in seafood processing. In U. Wanasundara, F. Shahidi, C., Alasalvar and K. Miyashita (Eds.) Handbook of Seafood Quality, Safety and Health Applications*. Wiley-Blackwell Publishers (2010).

[5] S. Krishnan, R. Bhosale and R.S.Singhal, Carbohydrate Polymer, **61(1)**,95-102(2005)

[6] S.Freitas, H.P.Merkle, and B. Gander, J. Control.Release, **102**, 313–332(2005).

[7] M.Yan, B. Liu, X. Jiao and S. Qin, Food and Bioproducts Processing, **9(2)**, 89–97(2014)

[8] M**.** Chávarri, I. Mara˜nón, R. Ares, F.C.Ibá˜nez, F. Marzo, and M.C.Villarán, Int. J. Food Microbiol**. 142,**185–189(2010).

[9] M. Yan, B. Liu, X. Jiao, and S. Qin, Food. Bioprod. Process. **92**, 89-97 (2014).