Jurnal
Teknologi

Full Paper

ANTIOXIDANT ACTIVITIES OF PHYCOCYANIN MICROCAPSULES USING MALTODEXTRIN AND CARRAGEENAN AS COATING MATERIALS

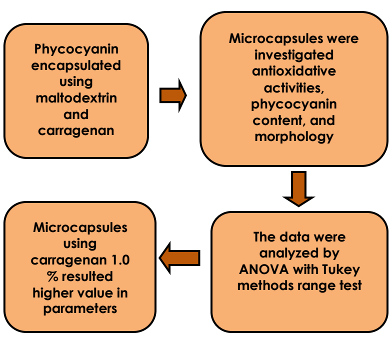
Article history
Received
21 October 2015
Received in revised form
23 February 2016
Accepted
25 February 2016

Eko Nurcahya Dewi*, Lukita Purnamayati, Retno Ayu Kurniasih

Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. Soedarto, SH Tembalang, Semarang – 50275, Indonesia

*Corresponding author
nurdewisatsmoko@yahoo.com

Graphical abstract



Abstract

The aim of this study was to investigate the antioxidant activities of phycocyanin microcapsules from *Spirulina* sp. using maltodextrin (MD) and carrageenan (C) as coating materials. Microcapsules were prepared with five different variations concentration of carrageenan in maltodextrin i.e. 0 %; 0.25 %; 0.5 %; 0.75 %, and 1.0 % (w/w). Results indicated that microcapsule with C 1.0 % of phycocyanin showed higher value in parameters evaluated, i.e. encapsulation yield (12.89 ± 0.289) %, moisture content (8.36 ± 0.059) %, phycocyanin content (2.83 ± 0.072) % and antioxidant activity (49.05 ± 1.017) %. The results of Scanning Electron Microscopy (SEM) microstructures showed that microcapsules with only maltodextrin (C 0 %) as coating material were cracked. It was found that all of microparticles had irregular spherical appearances and various sizes, but the carrageenan had flake appearances.

Keywords: Antioxidant activities, carrageenan, maltodextrin, phycocyanin microcapsules

1.0 INTRODUCTION

Phycocyanin is a blue phycobiliprotein. It has good therapeutic values, such as antioxidant, which made it a better active ingredient in functional food. It is also used as natural colourants in foods, beverages and cosmetics, such as chewing gum, ice sherbets, soft drinks, candies, lipstick, and eyeliner, replacing the synthetic colourants. However, phycocyanin is often limited by the instability towards moisture, light, and temperature due to the degradation of the protein fraction [1–4]. Therefore, it is necessary to develop a method to protects the antioxidant activity of phycocyanin from the external influences. One method is the encapsulation of phycocyanin .

Microencapsulation is an effective and economical method for protecting natural colourants adverse conditions. Microencapsulation is a technique by which the sensitive ingredients are entrapped in coating 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 [5, 6].

Various techniques are used to form microencapsulation including spray drying, spray chilling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, liposome entrapment, centrifugal extrusion and rotational suspension separation. The most common and economical way to carry out microencapsulation is spray drying. Numerous papers have been published about the encapsulation of liquid flavors and oils by spray drying [7–11]. It results in powders with good quality, low water activity, easier handling and storage. Furthermore it also protects the active materials against undesirable reactions.

Coating materials selection can also affect the efficiency process and the microencapsulated product stability. The coating materials must retain and protect the encapsulated core material from loss and chemical damage. Most common coating materials are gum arabic, maltodextrin, emulsifying starches and others. Maltodextrin is one of the most commonly used for encapsulation of bioactive materials. It is water-soluble material and able to protect encapsulated ingredient from oxidation [12]. However, the biggest problem of this coating material is its low emulsifying capacity. Therefore, it is desirable to use maltodextrin in combination with other surface active biopolymers, such as carrageenan. Carrageenan is linear water-soluble sulfated polysaccharides that extracted from red seaweeds. κ-carrageenan is the strongest gels within the carrageenan family. This biopolymer has been studied a lot as a coating material and a carrier for controlled drug release [13,14].

Very little information is available about microencapsulation of phycocyanin [4] and none of the published work reported the antioxidant activity of phycocyanin microcapsules that using maltodextrin and carrageenan as coating materials with spray drying method. Therefore, the objective of this study was to investigate the antioxidant activities of phycocyanin microcapsules from *Spirulina* sp. using maltodextrin and carrageenan as coating materials. In this study, maltodextrin and carrageenan were used as coating materials for producing microencapsulated phycocyanin by spray drying method. The encapsulation yield, moisture content, phycocyanin concentration, antioxidant activity, and morphology of phycocyanin microcapsules were characterized.

2.0 MATERIAL AND METHOD

2.1 Material

Phycocyanin was extracted from *Spirulina* sp. biomass provided by PT. Neoalga, Indonesia, according to the method of Chaiklahan *et al.* [3] with slight modification. Maltodextrin having dextrose equivalent 10 was purchased from CV. Multi Kimia Raya, Semarang, Indonesia. κ-carrageenan was purchased from PT. Selalu Lancar Maju Karya, Jakarta, Indonesia. All other used reagents were of analytical grade.

2.2 Microencapsulation of Phycocyanin

Microencapsulated phycocyanin was prepared by spray drying method, using maltodextrin and carrageenan as the coating materials. Maltodextrin was added into phycocyanin according to ratio of coating materials 10 % (w/v) to phycocyanin. The mixing solutions were stirred homogenously. Carrageenan was prepared into solutions with the concentration of 0 % (w/w), 0.25 % (w/w), 0.5 % (w/w), 0.75 % (w/w), and 1.0 % (w/w) to coating materials, (respectively) to find out its effects on microencapsulation. Then carrageenan was added into phycocyanin and maltodextrin solution. They were homogenized using homogenizer (Ultraturrax T50 Basic Ika Werke, Germany) at 4 500 rpm for 2 min (1 rpm = 1/60 Hz). Subsequently, the dispersed sample was fed into a spray dryer (PlantLab, England) for drying with a nozzle atomization system 0.7 mm diameter nozzle. The operating conditions were drying inlet air temperature of a 80 °C, and outlet air temperature of a 56 °C. Feed flow rate was set at 5.1 mL $\cdot$ min^{-1}.

2.3 Encapsulation Yield

The encapsulation yield (EY) was calculated based on the percentage ratio of the total mass of microcapsules obtained after encapsulation and the total mass of solids before [8].

2.4 Moisture Content

The moisture content of the microcapsules was determined gravimetrically by drying samples in oven at 105 °C for 24 h. The moisture content (%) was recorded for each sample after stable weight was obtained [11].

2.5 Phycocyanin Concentration

The phycocyanin concentration (PC) was determined spectrophotometrically, using the Equation (1) of Boussiba and Richmond [15]. Microcapsules (40 mg) were mixed with 10 mL of Buffer Phosphat (pH 7). The mixture was vortexed and the absorbance of this solution was read at 620 nm. Phycocyanin concentration was determined by following equation:

$$\% \text{ Phycocyanin} = \frac{A_{620} \times (\text{mL Solvent}) \times 100\ \%}{3.39 \times (\text{mg Sample}) \times (\text{drywt})} \quad (1)$$

in which, 3.39 = coefisien of C-Phycocyanin at 620 nm, drywt = dry weight of sample.

2.6 Antioxidant Activity

The hydorogen donating abilities of phycocyanin microcapsules were determined from the change in absorbance at 515 nm. For free radical scavenging measurements, samples in methanol solution were prepared by a methanolic solution (0.1 mL) in which 2.0 mg of sample was mixed with 2 mL methanol. A methanolic solution of α,α-diphenyl-β-picrylhydrazyl (DPPH) (1 mM, 0.250 mL) was then added. The mixture was vortexed for 30 min and the absorbance of this solution was read at 515 nm [8]. The DPPH radical scavenging activity in terms of percentage was calculated according to the following Equation (2)

$$\text{Antioxidant activity (\%)} = \frac{(A_{control} - A_{sample}) \times 100\ \%}{A_{control}} \quad (2)$$

in which, Acontrol = Absorbance of methanolic solution with DPPH (without sample).

2.7 Morphology

The morphology of the microcapsule was observed under scanning electron microscope (Jeol JSM-6510LA, Japan). The microcapsules were fixed on stubs using copper-conducting adhesive tape and then coated with gold. Observations were made using scanning electron microscope (SEM) at an acceleration voltage of 10 kv [4].

2.8 Statistical Analysis

The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey methods range test. ANOVA data with a $P < 0.05$ was classified as statistically significant SPSS 17 software was used.

3.0 RESULTS AND DISCUSSION

3.1 Encapsulation Yield

Phycocyanin microcapsules were prepared by spraydrying method using maltodextrin and carrageenan as coating materials. The different variable concentration of carrageenan affects the spray drying process together with the properties of the resulted microcapsules including encapsulation yield, water content, phycocyanin concentration, antioxidant activity, and microcapsule shapes. The results of encapsulation yield, moisture content, phycocyanin concentration and antioxidant activity are shown in Table 1.

Table 1 shows that higher concentration of carrageenan decreases the encapsulation yield of microcapsules. Encapsulation yield decreased significantly with the increase of carrageenan concentration from 0.25 % to 1.0 %.

The higher content of carrageenan led to agglomeration on the microparticles. As a result, there was a caking process during spray drying. Some of microcapsules were sticked on spray dryer chamber which was similar to previous study on microencapsules of α-tocopherol using inlet spray drying at 120 °C and 5 mL · min^{-1} of air speed [16].

The result indicated that the insufficient addition of maltodextrin concentration caused agglomeration and caking product. It could be likely to be influenced by delaying of permeable layer during drying process. Furthermore the lower yields were also influenced by unbalanced inlet temperature and air speed [17].

3.2 Moisture Content

Moisture content is an important attribute since it is directly related to food stability. Dried products are more stable than liquid formulations, on the subject of the physicochemical aspects and microbiological spoilage. Moisture content of microcapsules varied from a minimum value of (6.80 ± 0.046) % to a maximum value of (8.36 ± 0.059) %. There was significant difference in the moisture content of microcapsules as affected by ratio of carrageenan and maltodextrin concentration. The moisture is mainly related to the drying conditions and the composition of the coating materials. Because of drying could promote changes in water binding and dissociation, that will affect the properties of the dried product [11].

Table 1 Phycocyanin concentration and antioxidant activity of phycocyanin microcapsules

Concentration of carrageenan (%)	Encapsulation Yield (%)	Moisture content (%)	Phycocyanin concentration (%)	Antioxidant activity (%)
0	27.96 ± 0.905^{a}	6.80 ± 0.046^{a}	0.76 ± 0.017^{a}	15.02 ± 0.808^{a}
0.25	27.48 ± 0.389^{a}	7.71 ± 0.082^{ab}	0.71 ± 0.044^{a}	16.10 ± 0.280^{a}
0.5	19.31 ± 1.223^{b}	7.78 ± 0.556^{ab}	0.73 ± 0.035^{a}	18.39 ± 0.610^{b}
0.75	17.03 ± 0.792^{b}	7.87 ± 0.026^{b}	1.56 ± 0.139^{b}	31.51 ± 0.628^{c}
1.0	12.89 ± 0.289^{c}	8.36 ± 0.059^{b}	2.83 ± 0.072^{c}	49.05 ± 1.017^{d}

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

Table 1 shows that higher concentration of carrageenan increases the moisture content of microcapsules. Similar findings had been reported that the moisture content of the spray dried powder decreased when the amount of maltodextrin added increased. The addition of maltodextrin increased the total solid content and reduce the amount of water available for evaporation. The microcapsules influenced the moisture content as follow type and encapsulan concentration [8, 18]. In spray dried microencapsulation, the values of low moisture content from encapsulated of fish oil with maltodextrin and fish gelatin as a coating materials were lower in comparison to the encapsulated of fish oil with maltodextrin and κ-carrageenan as coating materials. The different moisture content probably can be resulted from different effects of the formation on droplet surface, consequently transformed a tough leather-like skin and avoids the moisture evaporation. Crust acts as a barrier againts moisture evaporation and thus powders with higher moisture content were produced [11].

3.3 Phycocyanin Concentration

Table 1 shows that higher concentration of carrageenan induced the increase of phycocyanin concentration of microcapsules. There were evident effects of carrageenan concentration on the phycocyanin concentration. Phycocyanin concentration increased significantly with the increase of carrageenan concentration from 0.75 % to 1.0 %. However, at concentration higher than 1 %, the solution could not be dried due to higher viscosity. This result was similar to the earlier reports [4] in which phycocyanin load was increased with alginat content increased ranging from 1.0 % to 3.5 %.

In this study, it can be demonstrated that higher phycocyanin concentration can be achieved by using carrageenan reinforced maltodextrin particles, compared to plain maltodextrin microcapsules. Carrageenan is highly effective to trap the phycocyanin and to protect the phycocyanin from thermal effect during spray drying. Using 0.75 % carrageenan as coating material increased the phycocyanin concentration up to 50 %. For the microencapsulation by spray drying, carrageenan is a good choice as the coating material due to its pseudoplastic properties. These properties allow it to act as a plasticiser and enhancing the adhesion force between the coating and the core materials [19].

3.4 Antioxidant Activity

As shown in Table 1, all samples of microcapsules had strong DPPH free radical scavenging activity. Antioxidant activity increased significantly by the increase of the carrageenan concentration (ranging between from 0.25 % to 1.0 %). The addition of carrageenan (up to 1.0 %) increase the phycocyanin protection from the heat influence during the spray drying process. The antioxidant stability during spray drying process was strongly influenced by the combination coating materials. In this study, it was discovered that the mixture of maltodextrin and carrageenan was more effective in the protection againts lipid oxidation than only maltodextrin as coating material. A positive correlation was observed where the antioxidant activity of flaxseed oil microencapsulated was more effective in the protection againts lipid oxidation during spray drying process by mixing of maltodextrin and whey protein concentrate than plain maltodextrin as coating material [9].

The highest antioxidant activity was observed in phycocyanin microcapsules obtained using maltodextrin 9.0 % and carrageenan 1.0 % as coating materials. The antioxidant activity of phycocyanin microcapsules was well correlated with the phycocyanin concentration. Similar findings had been reported that the higher concentration of phycocyanin showed the increase antioxidant activity. The phycocyanin was isolated from *Spirulina platensis* [20].

3.5 Morphology

The SEM images of phycocyanin microcapsules are illustrated in Figure 1. It can be seen that all microparticles had irregular spherical appearances, in contrast to the carrageenan which had a flake appearances. Microcapsule shape could affect the viscosity and it would bring difficulty in spray drying process. Therefore, microcapsule shape was selected as an index to evaluate the preparation. The result of phycocyanin microcapsules at higher carrageenan concentration suggested that they had irregular spherical, attributed to the higher viscosity of carrageenan solution. Similar findings was reported that encapsulation of phycocyanin using alginate and chitosan as coating materials with extrusion method [4].

The phycocyanin microcapsules with maltodextrin 10.0 % as coating material showed that the particles were cracked. While the phycocyanin microcapsules with maltodextrin 9.0 % and carrageenan 1.0 % as coating materials did not show cracks or fissures, as shown in Figure 1. It might be correlated with the electrostatic interaction between maltodextrin and carrageenan that form a stable polyelectrolyte complex. This is an advantage since it implies that capsules have lower permeability to gases, increasing protection and retention of the active material. Moreover, the size variety is a typical characteristic of particles produced by spray drying. Similar morphological characteristic were found by other studies [7, 9]. The mixtures of different coating materials influenced on microparticles morphology.

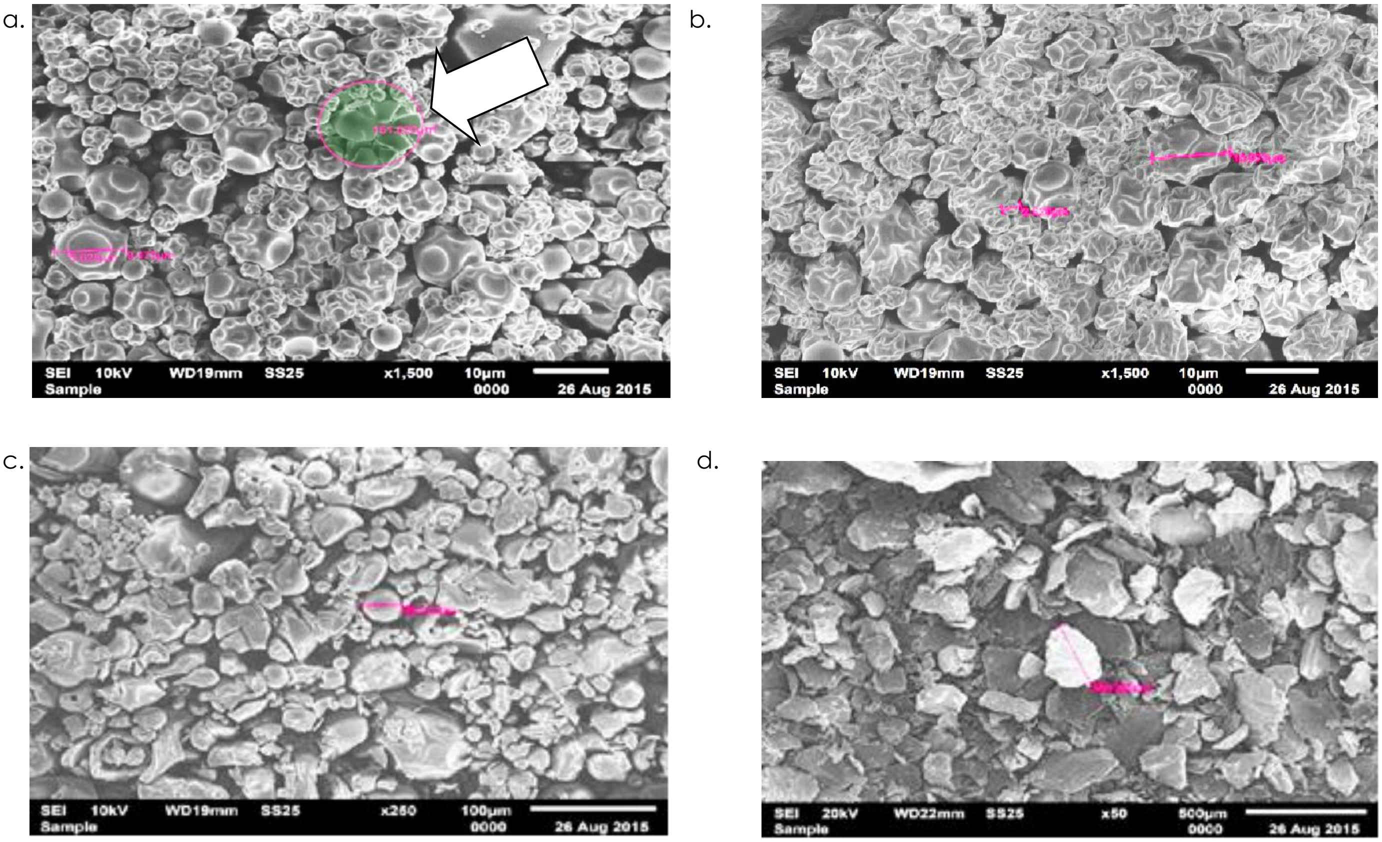


Figure 1 SEM images of phycocyanin microcapsules. (a) Surface of phycocyanin coated by maltodextrin; (b) surface of phycocyanin coated by maltodextrin 9.0 % and carrageenan 1.0 %; (c) surface of maltodextrin; (d) surface of carrageenan

4.0 CONCLUSION

Phycocyanin microcapsules were succesfully prepared by spray drying method using maltodextrin and carrageenan as coating materials. The optimum process conditions were determined by a carrageenan concentration of 1.0 % which showed the highest of the phycocyanin concentration and antioxidant activity. The encapsulation by maltodextrin and carrageenan improved the ability of phycocyanin to resist the adverse impacts of temperature on its spray drying process and protects the antioxidant activity during spray drying process. The particles of phycocyanin microcapsules with 1.0 % carrageenan had a spherical shape and various sizes with no apparent cracks or fissures. It could be concluded that the mixture of maltodextrin and carrageenan coated phycocyanin could be a good method to enhance the stability of phycocyanin. This study provides the basis for the application of phycocyanin in functional food.

Acknowledgement

This work was supported by Diponegoro University and Ministry of Research, Technology, and Higher Education Indonesia, for the financial support in 2015.

References

[1] Eriksen, N. T. 2008. Production of Phycocyanin – A Pigment with Applications in Biology, Biotechnology, Foods, and Medicine. *Journal of Application Microbiology Biotechnology*. 80: 1–14.

[2] Thangam, R., V. Suresh, W. A. Princy, M. Rajkumar, N. Senthilkumar, P. Gunasekaran, *et al*. 2013. C-Phycocyanin from *Oscillatoria tenuis* Exhibited an Antioxidant and *In Vitro* Antiproliferative Activity Through Induction of Apoptosis and G0/G1 Cell Cycle Arrest. *Food Chemistry*. 140: 262–272.

[3] Chaiklahan, R., N. Chirasuwan, and B. Bunnag. 2011. Stability of Phycocyanin Extracted from *Spirulina* sp.: Influence of Temperature, pH and Preservatives. *Journal of Process Biochemical*. 47: 659–664.

[4] Yan, M., B. Liu, X. Jiao, and S. Qin. 2014. Preparation of Phycocyanin Microcapsules and Its Properties. *Food and Bioproducts Processing*. 92: 89–97.

[5] Rocha, G. A., C. S. Favaro-Trindade, C. Raimundo, and F. Grosso. 2012. Microencapsulation of Lycopene by Spray Drying Characterization, Stability, and Application of Microcapsules. *Food and Bioproducts Processing*. 90: 37–42.

[6] Frascareli, E. C., E. M. Silva, R. V. Tonon, and M. D. Hubinger. 2012. Effect of Process Conditions on the Microencapsulation of Coffee Oil by Spray Drying. *Food and Bioproducts Processing*. 90: 413–424.

[7] Tonon, R. V., C. R. F. Grosso, and M. D. Hubinger. 2011. Influence of Emulsion Composition and Inlet Air Temperature on the Microencapsulation of Flaxseed Oil by Spray Drying. *Food Research International*. 44(1): 282–289.

[8] Krishnaiah, D., R. Sarbatly, and R. Nithayanandam. 2012. Microencapsulation of *Morinda citrifolia* L. Extract by Spray-drying. *Chemical Engineering Research and Design*. 90: 622–632.

[9] Carneiro, H. C. F., R. V. Tonon, C. R. F. Grosso, and M. D. Hubinger. 2013. Encapsulation Efficiency and Aoxidative Stability of Flaxeseed Oil Microencapsulated by Spray Drying Using Different Combinations of Wall Materials. *Journal of Food Engineering*. 115: 443–451.

[10] Carvalho, A.G.S., Silva, V.M., Hubinger, M.D. 2014. Microencapsulation by Spray Drying Of Emulsified Green Coffe Oil With Two-Layered Membranes. *Food Research International*. 61: 236-245.

[11] Mehrad, B., B. Shabanpour, S. M. Jafari, and P. Pourashouri. 2015. Characterization of Dried Fish Oil from Menhaden Encapsulated by Spray Drying. *International Journal of The Bioflux Society*. 8(1): 57–69.

[12] Saloko, S., Darmadji, P., Setiaji, B., Pranoto, Y., Anal, A.K. 2013. Encapsulation of Coconut Shell Liquid Smoke in Chitosan-Maltodextrin Based Nanoparticles. *International Food Research Journal*. 20(3): 1269–1276.

[13] Daniel-da-Silva, A. L., L. Ferreira, A. M. Gil, T. Trindade. 2011. Synthesis and Swelling Behavior of Temperature Responsive κ-Carrageenan Nanogels. *Journal of Colloid and Interface Science*. 355(2): 512–517.

[14] Leong, K. H., L. Y. Chung, M. I. Noordin, K. Mohamad, M. Nishikawa, Y. Onuki, *et al*. 2011. Carboxymethylation of Kappa-carrageenan for Intestinal-targeted Delivery of Bioactive Macromolecules. *Carbohydrate Polymers.* 83: 1507–1515.

[15] Boussiba, S., and A. E. Richmond. 1979. Isolation and Characterization of Phycocyanins from the Blue-Green Alga Spirulina platensis. *Archives of Microbiology*. 120(2): 155–159.

[16] Pierucci, A. P., Andrade, L. R., Farina, M., Pedrosa, C., and Rocha-Lehao, M. H. M. 2007. Comparison of a-Tocopherol Microparticles Produced with Different Wall Materials: Pea Protein a New Interesting Alternative. *Journal of Microencapsulation*. 24(3): 201–213.

[17] Wang, Y., H. Ye, C. Zhou, F. Lv, X. Bie, and Z. Lu. 2012. Study on The Spray-drying Encapsulation of Lutein in the Porous Starch and Gelatin Mixture. *European Food Research Technology*. 234: 157–163.

[18] Sormoli, M. E., M. I. Islam, and T. A. G. Langrish. 2012. The Effect of Chitosan Hydrogen Bonding on Lactose Crytallinity During Spray Drying. *Journal of Food Engineering*. 108(4): 541–548.

[19] Su, Y. L., Z. Y. Fu, J. Y. Zhang, W. M. Wang, H. Wang, Y. C. Wang, *et al*. 2008. Microencapsulation of *Radix salvia miltiorrhiza* Nanoparticles by Spray-drying. *Powder Technology*. 184: 114–121.

[20] Seo, Y. C., W. S. Choi, J. H. Park, K. H. Jung, and H. Y. Lee. 2013. Stable Isolation of Phycocyanin from *Spirulina platensis* Associated with High-Pressure Extraction Process.

[21] *International Journal of Molecular Science*. 14: 1778.