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Microencapsulation of Capsanthin by Soybean Protein Isolate-Chitosan Coacervation and Microcapsule Stability Evaluation

Jun-Xia Xiao, Guo-Qing Huang, Shi-Qing Wang, Yan-Ting Sun
College of Food Science and Engineering, Qingdao Agricultural University, Qingdao 266109, Shandong, China
Correspondence to: G.-Q. Huang (E-mail: gqgood@126.com)

ABSTRACT: Although capsanthin possesses excellent coloring performance and healthcare functions, its application in the food industry is limited due to its susceptibility to humidity, heat, and light. The purpose of this research was to microencapsulate capsanthin by soybean protein isolate (SPI)-chitosan coacervation and evaluate whether the microencapsulation improved the stability of capsanthin against the adverse conditions mentioned above. The results indicated that the optimum conditions for capsanthin microencapsulation were emulsification speed 10,000 rpm, emulsification temperature 45°C, wall concentration 15 g/L and core to wall ratio 1:2 (w/w). Under these conditions, the droplets in the emulsion were even in size distribution without agglomeration and the microencapsulation efficiency and microencapsulation yield reached 90.46% and 86.69%, respectively. Microencapsulation increased the stability of capsanthin against low/medium moisture, heat, and especially light, but was less effective in protecting capsanthin microcapsules in high moisture. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39671.

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INTRODUCTION

Capsanthin (3,3′-dihydroxy-β,κ-caroten-6′-one) is the major carotenoid present in paprika (*Capsicum annuum*) and has been approved for the coloring of cheese, juices, sauces, and meats in many countries.[1] Meanwhile, capsanthin has potential applications in functional foods due to its antioxidant and anti-tumor activities.[2] However, capsanthin is susceptible to oxidants, light and heat and can be easily decomposed when exposed to such factors.[3] Because of the commercial importance and considerable role in healthcare, many efforts have been devoted to increase the stability of capsanthin during storage and processing, such as the application of ascorbic acid and reduced glutathione.[3,4]

In addition to the supplementation of antioxidants, microencapsulation is also recognized as a promising way of stabilizing natural colorants.[5] Spray-draying is the most widely used microencapsulation technology in the food industry and has been reported to effectively protect paprika oleoresin from oxidation.[6,7] Furthermore, Santos et al. evaluated the functionalities of microencapsulated paprika oleoresin in Arabic gum and rice starch/gelatin incorporated into cake and gelatin gel. It was found that the encapsulated pigment successfully dyed the cakes without negative effect on taste, flavor or texture of analyzed systems, but reduced the global sensory acceptance of gelatin gel.[7] To overcome the adverse effects of high temperature used in spray-drying, Zilberboim *et al.* proposed an alternative method to spray-drying for microencapsulating paprika oleoresin by cold dehydration with ethanol, but this process was cost-ineffective and was rarely used in the food industry.[8]

Complex coacervation is another important microencapsulation process and has attracted extensive attentions in recent years due to its high loading capacity, ease of controlled release and mild reaction conditions compared with spray-drying.[9] The microencapsulation of carotenoids by complex coacervation and the increase of storage stability have been reported by many authors.[10–13] Complex coacervation is the electrostatic interaction between two oppositely charged polymers. Gum Arabic–gelatin is the most classical complex coacervation system and has gained industrial applications, but new coacervation pairs based on plant-derived proteins and cationic polysaccharides are emerging in recent years.[14,15]

Chitosan is the second most abundant polysaccharide in the world and carries positive charges in acidic solutions. This natural polymer has been widely reported for the microencapsulation of sensitive compounds through complex coacervation.[16,17] Soybean protein isolate (SPI) is amphoteric and is negatively charged in solutions above its isoelectric point (*p*I). The authors of this work have reported a complex coacervation pair based on SPI and chitosan. It was found that the resultant coacervates had a sponge-like structure interspaced by heterogeneously sized

vacuoles, which provided locations for the inclusion of sensitive compounds.[18] Hence, the chitosan-SPI coacervates were expected able to microencapsulate capsanthin and improve the stability of capsanthin against adverse conditions.

The purpose of this study was to optimize the conditions for microencapsulating capsanthin through chitosan-SPI coacervation and the effects of emulsification speed, emulsification temperature, wall concentration, and wall to core ration on the morphology of emulsion droplets, microencapsulation efficiency and microencapsulation yield were explored. Besides, whether the resultant capsanthin microcapsules have improved stability against moisture, heat, and light were also concerned.

EXPERIMENTAL

Materials

Food-grade soybean protein isolate (SPI) with protein content 90.5% was purchased from Qingdao Tianxin Food Additives Co., Ltd. (Qingdao, China). Food-grade chitosan with viscosity average molecular weight 150 kDal and 93.9% degree of deacetylation was purchased from Shandong Lake Crustacean Products Co., Ltd. (Qingdao, China). Capsanthin was a gift from Shandong Tongxing Natural Pigments Co., Ltd. (Qingdao, China). Transglutaminase with specific activity 125 U/g (one unit is defined as the amount required to liberate 1 μmol hydroxamate per min from *N*-carbobenzoxy-L-glutaminylglycine at pH6.0 and 37°C) was purchased from Dongsheng Food Science & Technology Company (Taixing, China). All other reagents were of analytical grade.

Microencapsulation by Complex Coacervation

Because the optimum conditions for SPI-chitosan coacervation has been established in our previous research,[18] only the effects of emulsification speed, emulsification temperature, wall concentration, and core to wall ratio on capsanthin microencapsulation were investigated. After SPI stock solution (26.7 g/L) and chitosan stock solution (20 g/L) were mixed in SPI to chitosan ratio 4:1 (w/w), capsanthin of a certain weight was added to the mixture and emulsified with a dispersing homogenizer (FJ-200, Shanghai Specimen Model Factory, China) for 5 min in water bath of set temperature. Then, the emulsion pH was adjusted to 6.5 with 100 g/L NaOH at 100 rpm stirring to initiate the coacervation between SPI and chitosan. Ten minutes later, the pH of microcapsule suspension was adjusted to 6.0 and subsequently transglutaminase (18.75 U per gram of SPI) was added to harden the microcapsule wall at 60 rpm stirring for 1 h. The microcapsules were filtered through 300-mesh nylon cloth, washed with water and freeze dried (Alpha1-4, Martin Christ GmbH, Germany) for analysis.

Microencapsulation Efficiency (MEE) and Microencapsulation Yield (MEY)

Capsanthin Content. Capsanthin content was determined using a spectrophotometric method.[5,19,20] Briefly, a certain weight of capsanthin microcapsules were suspended in anhydrous ethanol, diluted, and then applied to a UV spectrophotometer (Shanghai Unicom Instrument, China) at 475 nm. Capsanthin content was calculated according to the following equation:

$$x = \frac{Ay}{A^{1\%}_{1\,\mathrm{cm}}\, m} \tag{1}$$

in which, x is the capsanthin content, A is the measured absorbance, y is the dilution factor, $A^{1\%}_{1\,\mathrm{cm}}$ is the specific absorption coefficient of a solution of 1 g capsanthin in 100 mL of solution, and m is the weight of capsanthin microcapsules.

MEE and MEY. MEE was defined as the percentage of capsanthin load that were entrapped inside the microcapsules to the total microencapsulated capsanthin load and MEY was defined as the ratio of microencapsulated capsanthin load to the capsanthin load in the emulsion. The equations for the two indexes were as follows:

$$\mathrm{MEE} = \frac{\text{Microencapsulated capsanthin load} - \text{Capsanthin load on the surface}}{\text{Microencapsulated capsanthin load}} \tag{2}$$

$$\mathrm{MEY} = \frac{\text{Microencapsulated capsanthin load}}{\text{Capsanthin load in the emulsion}} \tag{3}$$

The capsanthin load on the surface was determined in the method mentioned above. The microencapsulated capsanthin load was determined in the same procedure, except that the microcapsules were exposed to ultrasonic (500 W) for 15 min prior to determination.

Morphology of Capsanthin Emulsions

The morphology of emulsions was analyzed by a binocular biological microscope (Nikon YS100, Japan) under an objective magnification of 20 and imaged using a digital camera (Samsung M310W, South Korea).

Stability of Capsanthin Microcapsule

Capsanthin Retention Rate. The capsanthin retention rate was calculated according to the following equation:

$$Y(\%) = \frac{x_t}{x_0} \times 100 \tag{4}$$

in which, Y was the capsanthin retention rate, x_t and x_0 were capsanthin contents of microcapsules after and before a period of storage, respectively.

Stability Against Moisture. A certain weight of free capsanthin and freeze-dried capsanthin microcapsules were placed at 25 °C in constant-temperature incubator under relative humidity (RH) of 33%, 58%, 68%, or 98%, which were produced by saturated solutions of $MgCl_2$, KBr, $CuCl_2$, and Na_2HPO_4 respectively. Samples were taken every two days to examine capsanthin retention rate. The experiment lasted 10 days in total.

Stability Against Heat. A certain weight of free capsanthin and freeze-dried capsanthin microcapsule were placed in petri dishes

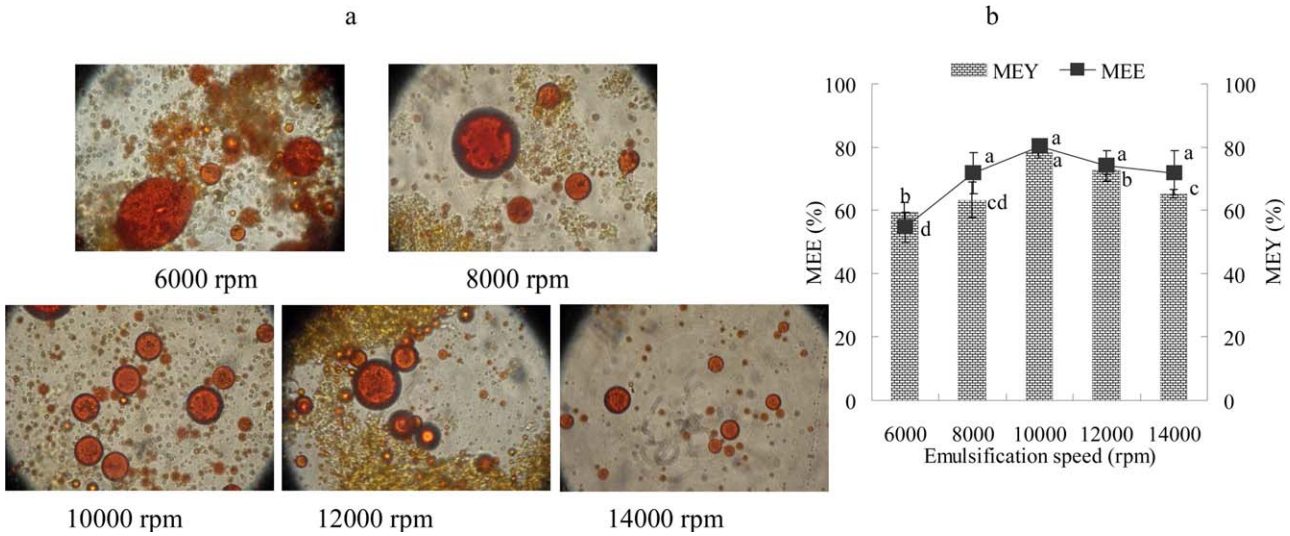

Figure 1. Effect of emulsification speed on the morphology (a), efficiency and yield (b) of capsanthin microcapsules. The microcapsules were prepared at 35°C, total biopolymer concentration 15 g/L and core to wall ratio 1:2 (w/w). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and kept at 20, 40, 60, 80, or 100°C for 30 min in dark. Capsanthin content was then determined to calculate capsanthin retention rate.

Stability Against Light. A certain weight of free capsanthin and freeze-dried capsanthin microcapsule were preserved in brown jars respectively at room temperature and placed in dark to avoid exposure to light, while other equivalent samples were kept in transparent jars and exposed to outdoor light to examine the stability against light. Samples were taken every 2 days to calculate capsanthin retention rate. The experiment lasted 10 days in total.

Statistical Analysis

All the experiments were performed on triplicate samples and values were expressed as mean values ± SD. Differences between mean values were conducted using the one-way analysis of variance (ANOVA) by SAS 8.2 software. Differences were statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effects of Emulsification Speed on Microencapsulation

As shown in Figure 1(a), the emulsification speed markedly influenced the morphology, efficiency and yield of capsanthin microcapsules. Homogenization at 10,000 rpm produced the highest size uniformity and least empty microcapsules. As emulsification speed decreased or increased, the microcapsules became uneven in size distribution. Besides, higher emulsification speed led to smaller particle sizes. Though smaller droplets

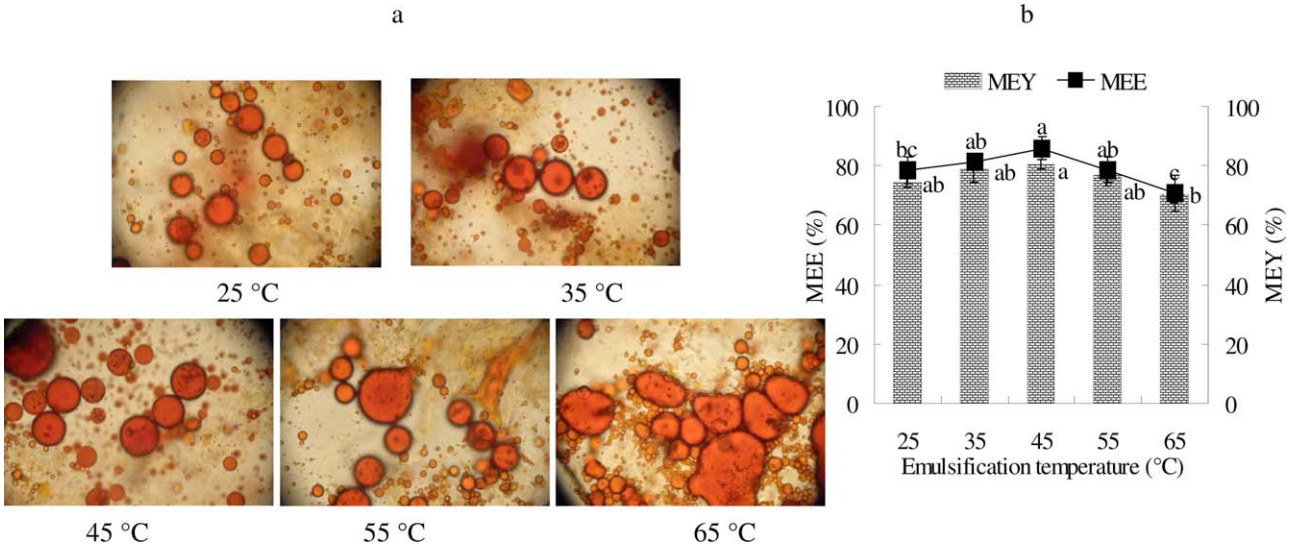

Figure 2. Effect of emulsification temperature on the morphology (a), efficiency and yield (b) of capsanthin microcapsules. The microcapsules were prepared at emulsification speed 10,000 rpm, core to wall ratio 1:2 (w/w), and wall concentration 15 g/L. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

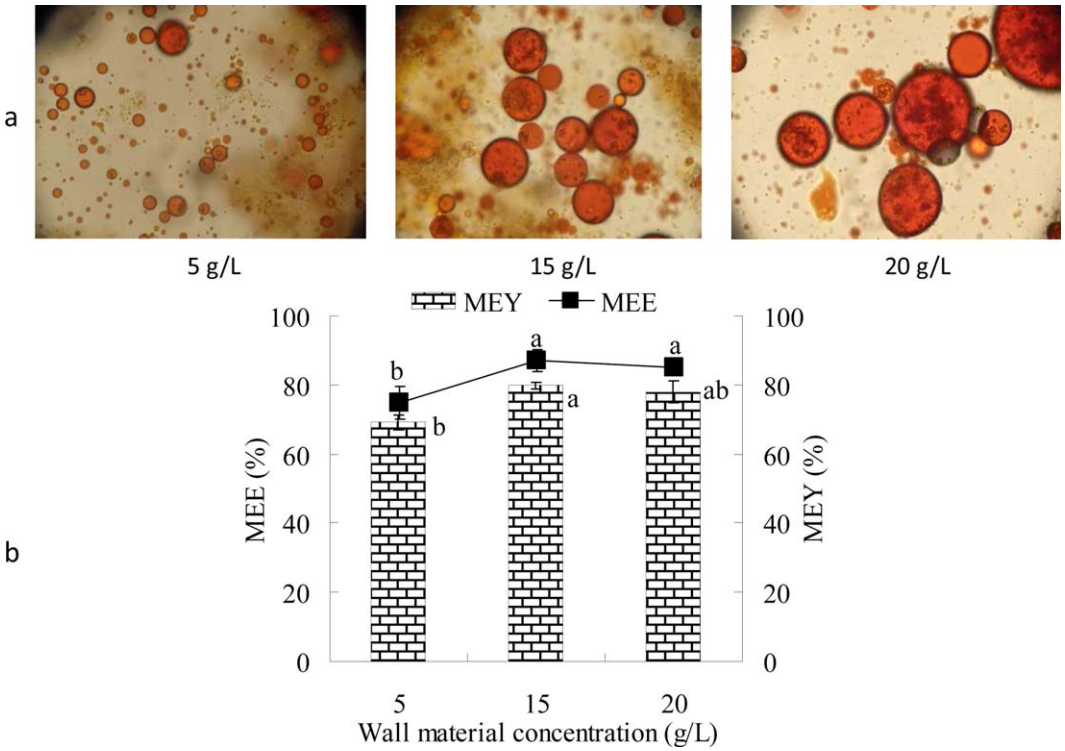

Figure 3. Effect of wall concentration on the morphology (a), efficiency and yield (b) of capsanthin microcapsules. The capsanthin microcapsules were prepared at 45°C, emulsification speed 10,000 rpm and core to wall ratio 1:2 (w/w). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

had a higher surface area compared to bigger ones, the encapsulating film around the droplets was thinner and further the protection ability of the microcapsule against oil oxidation was poorer than that of the microcapsule prepared from emulsion with the bigger droplets.[21] Meanwhile, both MEE and MEY peaked at emulsification speed 10,000 rpm and MEY was significantly higher than those at other emulsification speeds ($P < 0.05$) [Figure 1(b)]. Hence, the emulsification speed 10,000 rpm was selected in subsequent experiments.

Effects of Emulsification Temperature on Microencapsulation

As shown in Figure 2(a), emulsification temperature markedly influenced the morphology and size of capsanthin microcapsules. Emulsification at 45°C yielded microcapsules with

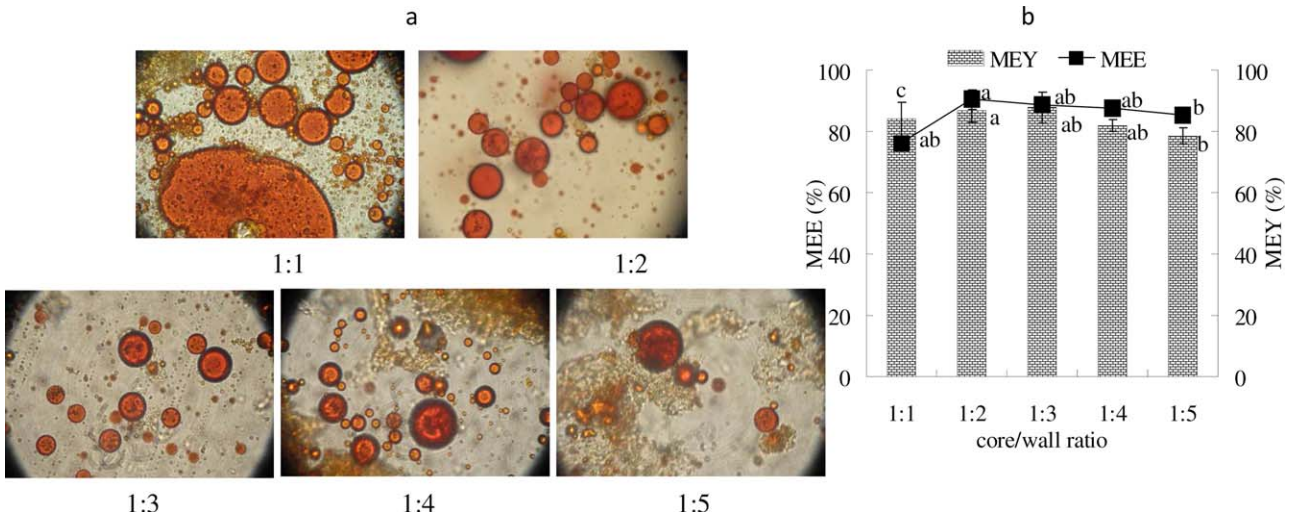

Figure 4. Effect of core to wall ratio on the morphology (a), efficiency and yield (b) of capsanthin microcapsules. The microcapsules were prepared at 45°C, emulsification speed 10,000 rpm and wall concentration 15 g/L. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

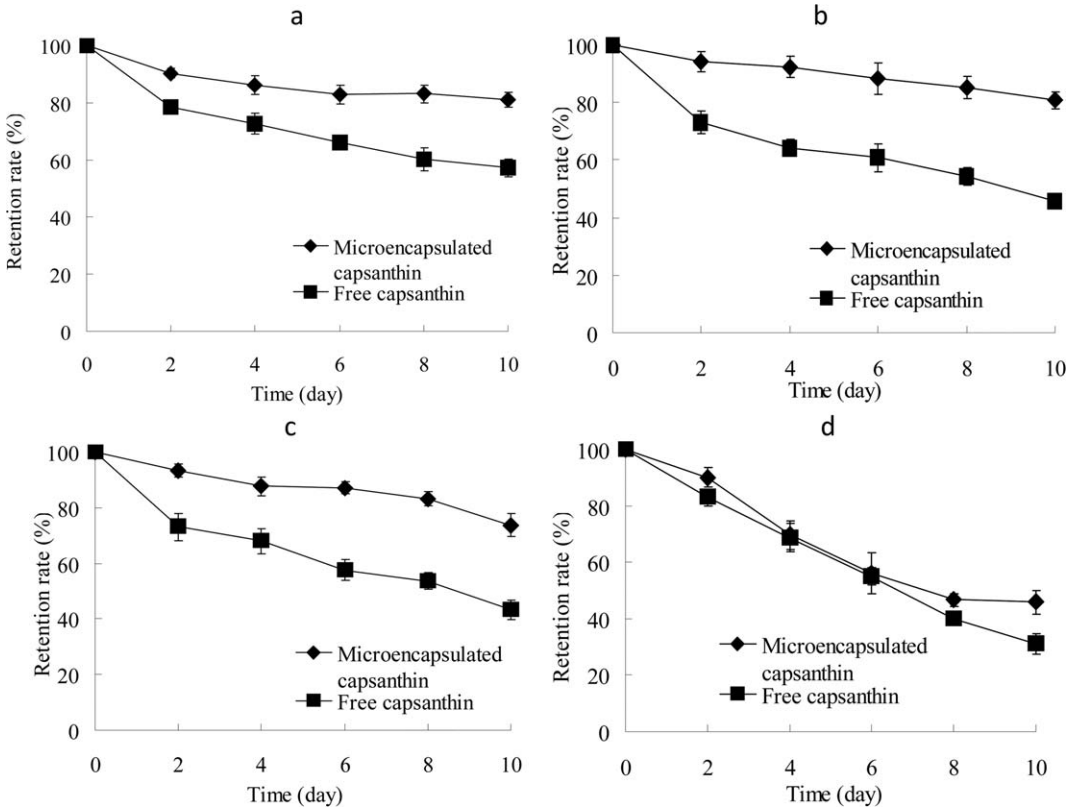

Figure 5. Stability of capsanthin in microcapsules in relative humidity 33% (a), 58% (b), 68% (c) and 98% (d).

uniform size distribution. As temperature increased, the capsanthin microcapsules became uneven in size distribution and poor in film integrity. Besides, the degree of aggregation and capsule size also increased along with emulsification temperature elevation. This was possibly related to the gelation of certain protein components in SPI. When the temperature was above the gelling temperature of the protein components, the shell around oil droplet was fluid. If two oil droplets coated with this fluid layer came in contact for a sufficient amount of time, the two fluid polymer shells will merge together and form a bridge between two capsules, leading to increased microcapsule size and aggregation.[22] The variations of MEE and MEY corresponding to temperature change [Figure 2(b)] were consistent with morphological observation and emulsification in 45°C produced the highest MEE and MEY. As the temperatures increased to 65°C, the MEE decreased significantly ($P < 0.05$).

Effects of Wall Concentration on Microencapsulation

As shown in Figure 3(a), the microcapsule size grew obviously when the wall concentration increased from 5 g/L to 20 g/L This was consistent with the conclusion of Alexander et al., who observed that the mean size of microcapsules grew with the increase of wall concentration.[23] Furthermore, both MEE and MEY were the highest at wall concentration 15 g/L [Figure 3(b)]. Therefore, wall concentration 15 g/L was proper to prepare capsanthin microcapsules.

Effects of Core to Wall Ratio on Microencapsulation

It was been reported that greater core to wall ratio resulted in poorer core retention[24] and the formation of larger dispersed oil droplets.[25] The same trend was observed in this work. As can be seen in Figure 4(a), coacervates obtained in the ratio 1:1 has the largest size than those produced in other ratios and the retention of capsanthin was poor as evidenced by the existence of agglomerated microcapsules. Decreasing the ratio from 1:1 to 1:5 resulted in great changes in MEE and MEY [Figure 4(b)]. The highest MEE occurred in 1:2 and the highest MEY in 1:3. As the ratio further increased, both MEE and MEY declined. This decrease in MEE and MEY were attributed to insufficient SPI-chitosan coacervation available to form films around capsanthin beyond a core to wall ratio of 1:2. Because the index MEE was more convictive to microencapsulation effect, the core

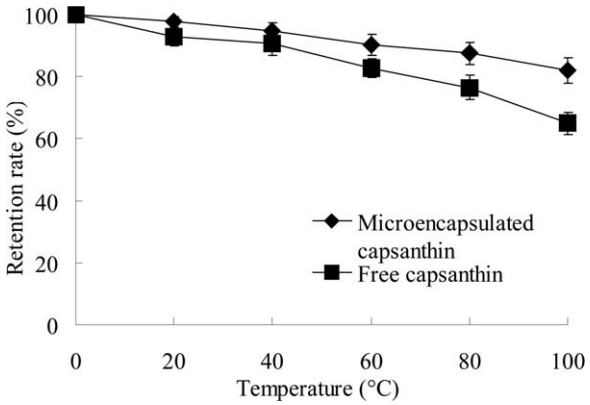

Figure 6. Effect of temperature on the stability of capsanthin in microcapsules.

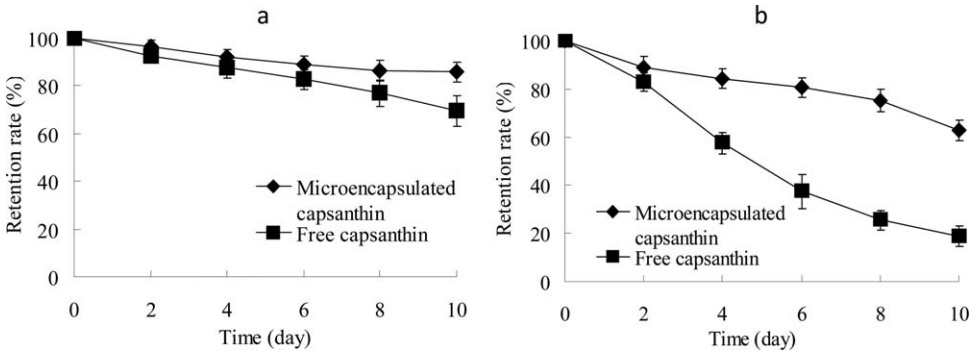

Figure 7. Stability of capsanthin in microcapsule in dark (a) and outdoor light (b).

to wall ratio 1:2 (w/w) along with emulsification speed 10,000 rpm, emulsification temperature 45 °C, and wall concentration 15 g/L were selected as the optimum conditions for the microencapsulation of capsanthin by SPI-chitosan coacervation.

Stability of Capsanthin Microcapsules

Stability Against Moisture. Figure 5 presents the stability of incorporated capsanthin in different RHs. It could be seen that free capsanthin was extremely susceptible to moisture and the percentages of lost capsanthin reached 42.77%, 54.37%, 56.69%, and 68.99% respectively after 10 days' storage in RHs 33%, 58%, 68%, and 98%. Microencapsulation significantly increased the stability of capsanthin in low and medium RHs and the retention rates were 81.01%, 80.71%, and 73.79% respectively in RHs 33%, 58%, and 68%. However, the microencapsulation was less effective in protecting capsanthin in RH 98% and the retention rate of incorporated capsanthin was only 45.81%.

The sensitivity of incorporated capsanthin to high moisture was possibly related to the high water-binding capacity of SPI,[26] which increased the water content in the microcapsules in high RH environment. This result is in line with the storage stability study of Qv et al.,[11] which reported that microencapsulation by gelatin-gum Arabic coacervation failed to protect lutein in high moisture due to the strong hygroscopicity of the two biomolecules. Nevertheless, the coacervation with chitosan was believed to effectively increase the water barrier property of the SPI-chitosan film and the enhanced but not significantly differed retention rates in low and medium RHs supported this view. Hence, capsanthin microcapsules prepared by SPI-chitosan coacervation should be preserved in low and medium RH environments.

Stability Against Heat. As shown in Figure 6, the retention rate of capsanthin decreased with the rise of temperature. No significantly differences in retention rates were observed between microencapsulated and free capsanthin at 20 and 40°C. As the temperature further increased, capsanthin in the microcapsules displayed significantly enhanced stability. That is, the retention rates of incorporated and free capsanthin were 90.18% and 82.70% at 60°C, 87.47% and 76.46% at 80°C, 81.97% and 64.98% at 100°C, respectively. The main effect of food thermal processing on carotenoids is isomerization as well as degradation[27] and the presence of oxygen accelerates the degradation.[28] Though the film around capsanthin droplet formed by SPI-chitosan coacervation did not provide heat insulation, the oxygen-barrier property of the film was believed to reduce the oxygen-induced degradation.

Stability Against Light. As can be seen in Figure 7, capsanthin is extremely sensitive to light. Exposure to outdoor light for 10 days led to up to 81.27% loss of free capsanthin, while storage in dark caused only 30.58% loss. Microencapsulation by SPI-chitosan coacervation tremendously increased the stability of capsanthin against light and the retention rates of incorporated capsanthin were up to 85.84% and 62.91% after 10 days' exposure to dark and outdoor light. This indicated that the film produced by SPI-chitosan coacervation effectively reduced the contact between incorporated capsanthin and light.

CONCLUSIONS

Capsanthin can be successfully microencapsulated by SPI-chitosan coacervation and the microencapsulated capsanthin displayed enhanced stability against low and medium moisture, heat and especially light. Because this is the first report on the microencapsulation of capsanthin by SPI-chitosan coacervation, more researches on cross-linking and dehydration methods are necessary to further improve the stability of capsanthin microcapsules during a long-term storage.

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REFERENCES

1. Aizawa, K.; Inakuma, T. *Br. J. Nutr.* **2009**, *102*, 1760.
2. Fernández-García, E.; Carvajal-Lérida, I.; Jarén-Galán, M.; Garrido-Fernández, J.; Pérez-Gálvez, A.; Hornero-Méndez, D. *Food Res. Int.* **2012**, *46*, 438.
3. Morais, H.; Rodrigues, P.; Ramos, C.; Forgacs, E.; Cserhati, T.; Oliveira, J. *Nahrung* **2002**, *46*, 308.

4. Cserháti, T.; Forgács, E.; Darwish, Y.; Morais, H.; Mota, T.; Ramos, A. C. *J. Chromatogr. A* **2002**, *949*, 269.
5. Rodríduez-Huezo, M. E.; Pedroza-Islas, R.; Prado-Barragán, L. A.; Beristain, C. I.; Vernon-Carter, E. J. *J. Food Sci.* **2004**, *69*, 351.
6. Raziel, A.; Rosenberg, M.; Kopelman, I. J. *Lebenson. Wiss. Technol.* **1985**, *18*, 28.
7. Santos, A. B.; Favaro-Trindade, C. S.; Grosso, C. R. F. *Pesqui. Agropecu. Bras.* **2006**, *41*, 351.
8. Zilberboim, R.; Kopelman, I. J.; Talmon, Y. *J. Food Sci.* **1986**, *51*, 1301.
9. Gouin, S. *Trends Food Sci. Tech.* **2004**, *15*, 330.
10. Nakagawa, K.; Nagao, H. *Colloids Surf. A Physicochem. Eng. Asp.* **2012**, *411*, 129.
11. Qv, X. Y.; Zeng, Z. P.; Jiang, J. G. *Food Hydrocolloid.* **2011**, *25*, 1596.
12. Silva, D. F.; Favaro-Trindade, C. S.; Rocha, G. A.; Thomazini, M. *J. Food Process. Pres.* **2012**, *36*, 185.
13. Tajdaran, R.; Ardjmand, M. *J. Biotech.* **2007**, *131*, S266.
14. Elmer, C.; Karaca, A. C.; Low, N. H.; Nickerson, M. T. *Food Res. Int.* **2011**, *44*, 1441.
15. Nesterenko, A.; Alric, I.; Silvestre, F.; Durrieu, V. *Ind. Crops. Prod.* **2013**, *42*, 469.
16. Maji, T. K.; Hussain, M. R. *J. Appl. Polym. Sci.* **2009**, *111*, 779.
17. Basal, G.; Sirin Deveci, S.; Yalcin, D.; Bayraktar, O. *Appl. Polym. Sci.* **2011**, *121*, 1885.
18. Huang, G. Q.; Sun, Y. T.; Xiao, J. X.; Yang, J. *Food Chem.* **2012**, *135*, 534.
19. Schierle, J.; Bretzel, W.; Bühler, I.; Faccin, N.; Hess, D.; Steiner, K.; Schüep, W. *Food Chem.* **1997**, *59*, 459.
20. Shu, B.; Yu, W.; Zhao, Y.; Liu, X. *J. Food Eng.* **2006**, *76*, 664.
21. Laine, P.; Lampi, A. M.; Peura, M.; Kansikas, J.; Mikkonen, K.; Willfor, S.; Tenkanen, M.; Jouppila, K. *J. Agric. Food Chem.* **2010**, *58*, 981.
22. Lemetter, C. Y. G.; Meeuse, F. M.; Zuidam, N. J. *AIChE J.* **2009**, *55*, 1487.
23. Andrianov, A. K.; Chen, J.; Payne, L. G. *Biomaterials* **1998**, *19*, 109.
24. Beristain, C. I.; Garcĺa, H. S.; Vernon-Carter, E. J. *LWT - Food Sci. Techn.* **2001**, *34*, 398.
25. Leimann, F. V.; Gonçalves, O. H.; Machado, R. A. F.; Bolzan, A. *Mater. Sci. Eng. C* **2009**, *29*, 430.
26. Rhim, J. W.; Lee, J. H.; Ng, P. K. W. *LWT - Food Sci. Technol.* **2007**, *40*, 232.
27. van den Berg, H.; Faulks, R.; Granado, H. F.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S.; Stahl, W. *J. Sci. Food Agr.* **2000**, *80*, 880.
28. Kanasawud, P.; Crouzet, J. C. *J. Agr. Food Chem.* **1990**, *38*, 1238.