Original article

Preparation and stability of butterfly pea color extract loaded in microparticles prepared by spray drying

Angkana Tantituvanont*, Pornpen Werawatganone, Pacharaporn Jiamchaisri and Kasorn Manopakdee

Department of Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

**Corresponding author: Tel: +66(0) 2218 8350 E-mail address: t_tuvanont@hotmail.com*

Abstract:

Butterfly pea is one of the most interesting sources of natural color used in food and cosmetics. Anthocyanins are the main coloring compounds in its petals and could be extracted easily with water. The pH of medium, temperature, and light were found to affect stability of the color aqueous extract from butterfly pea petals. Acidity and alkalinity of the solvent did not only change shade of the color but also affected the color stability. The color presented the most stable in pH 4 solution under darkness and the least stable in pH 7 solution under UV light. The higher the temperature the more the color loss. In an attempt to improve the color stability, microparticulated system prepared by spray drying technique was employed in this study. Hydroxylpropylmethyl cellulose (HPMC) and gelatin were used as carrier polymers. The operating condition providing optimum production yield was determined using 2^3 factorial design. The factors were % solid in the feed solution, inlet temperature, and solution feed rate. The optimized condition was 5% w/w of solid in the feed solution, 130 °C of inlet temperature, and 10 ml/min of solution feed rate for both HPMC and gelatin. Color stability of the microparticulated particles was studied under heat and UV light. Gelatin microparticulated system presented better protection against UV light than HPMC microparticulated system and aqueous color solution. Therefore, polymer type should be carefully selected for preparing the microparticulated particles. However, no protection against thermal degradation was observed in both gelatin and HPMC microparticulated systems.

Keywords: Butterfly pea; *Clitoria Ternatea;* Color stability; Microparticles; Spray drying

Introduction

Public concerns on the safety of synthetic colorants have given rise to demand for natural colorants used in cosmetics and food. Natural color is safe for body contact, unsophisticated, and harmonized with nature. Colors obtained from natural sources include yellow from *Curcuma Longa* [1], purple from *Hibiscus Rosasinensis* [2], red from *Pterocarpus Santalinus* and blue from *Clitoria Ternatea* [3]. *Clitoria Ternatea,* commonly known as "Butterfly Pea" or "Blue Pea", is a perennial climber belonging to the family Leguminosae. It originates in Southeast Asia and is known to accumulate ternatins, a group of (poly) acylated anthocyanins, in its petals. The main anthocyanins in butterfly pea are delphinidin glycoside which attributes to their blue color [3]. Blue dye aqueous extract from the petal of butterfly pea is traditionally used in cosmetics as hair dying to cover grey. It is also used as a confectionary coloring in the food industry and as a natural pH indicator in the pharmaceutical industry. However, the substitution of synthetic colorants is still a problem because natural colorants are more difficult to handle and less stable. Similar to other anthocyanin extracts, blue dye from butterfly pea is susceptible to pH, light, and temperature degradation which has limited its application in food and cosmetics.

Microparticulated system can entrap chemicals such as colors in the microparticles, which may provide the protection against labile factors such as pH, light, heat and oxidation. Several approaches have been used to create and stabilize the particles. Spray drying is a common approach for entrapping the colors into the microparticles matrix to improve the stability of the colors and thus extend their shelf life. Studies have been shown that natural colors entrapped in the microparticulated system have better heat, light, and pH stabilities [4, 5], which allows the use of natural colors in applications where they typically could not have been successfully used.

Hydroxypropyl methylcellulose (HPMC) and gelatin are widely used as the wall material or carrier in spray-dried encapsulation of food and flavor compounds [6]. Gelatin is a natural material isolated from animal skin and bone. It has properties of good solubility and low viscosity at high concentrations which are desirable in an encapsulating agent. HPMC is a cellulose derivative which is petition as an alternative to gelatin due to current concern about the use of animal derived product. HPMC has ability to retain and prevent the active ingredient against the external factor [7, 8]. In addition, both HPMC and gelatin are versatile, safe, cheap, and compatible with many food and flavor ingredients making them the interesting choice for encapsulation [9, 10].

The objectives of this study were; to determine the spray drying conditions that maximizing the microparticle production yield, to evaluate the effect of pH, temperature and light on the color stability of butterfly pea color extract, and to investigate the effectiveness of spray-dried microparticulated systems composed of HPMC and gelatin in preventing color degradation of butterfly pea color extract.

Materials and Methods

Materials

Methocel E5 viscosity of 4-6 cps, a gift from Colorcon (West Point, PA, USA), and gelatin from Sigma-Aldrich (St. Louis, MO, USA) were used as carriers and coating polymers for the microparticle preparations. Citric acid and sodium hydroxide for pH adjustment were purchased from Spectrum, Spectrum Quality Products, Inc. (Gardena, CA, USA). Avicel pH 101 as a glidant was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylparaben (MP) and propylparaben (PP) as antimicrobial preservatives were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Spectrophotometric analysis

The λ_{max} and absorbancies of aqueous extract of butterfly pea at pH values of 4.0-10.0 were measured at the visible wavelength (400-800 nm) using a Shimadzu spectrophotometer, Model UV-1601, Shimadzu, Inc. (Japan). The λ_{max} is the wavelength at which the greatest absorbance in the spectrum is observed [11-13].

Preparation of butterfly pea color extract

Butterfly pea flower was collected from the botanical garden at Faculty of Pharmaceutical Sciences, Chulalongkorn University. Fifteen grams of fresh butterfly pea petals were blended (Moulinex®, France) with 600 ml of vehicle solution (1.0% MP, 0.1% PP in Deionized (DI) water) to a uniform consistency and filtered twice through 4-layers of cheesecloth. Filtrate was collected and the pH of the solution was measured and adjusted to 4, 7, and 10 using 1 N sodium hydroxide or 10% citric acid solution. The extract solution was further used in the stability studies.

Stability studies of butterfly pea color aqueous extract

The effect of temperature and light on the stability of butterfly pea color aqueous extract was studied. The extract solution was prepared at three different pH values (pH 4, 7, 10) as aforementioned. Aliquots of the samples were placed in 10 ml capped glass vials and kept under UV light (UVA/B irradiation, (0.36 J/cm^2), in darkness (room temperature), in a refrigerator (4 ˚C), and in an oven (40˚C) for 60 days. Samples were taken right after prepared (day 0) and after day 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, and 60, centrifuged at 3000 rpm (956 ×g) for 5 minutes, and analyzed using UV-VIS spectrophotometer. The absorbance at 573, 627, and 628.5 nm was monitored for the extract solutions at a pH value of 4, 7, and 10, respectively. The samples were performed in triplicate. Color remaining at a particular time was calculated from the change in the absorbance from the original solution in percentage over a period of 60 days.

Preparation of spray-dried microparticles

The feed solution loaded with butterfly pea color aqueous extract was prepared based upon a formulation list in Table 1. Briefly, the polymer (HPMC E5 or gelatin) was dissolved in DI water. An equal volume of butterfly pea color aqueous extract (30% fresh petal in DI water) was added into the polymeric solution with continuous stirring followed by an addition of Avicel pH 101, a glidant. The amount of Avicel added was 1% of total polymer mass. The pH of the solution was adjusted to 4, using 10% citric acid solution, where the solution was the most stable in previous study.

Table 1 Effect of spray-drying conditions on production yield and moisture content of microparticle products

Formulation	Total solid polymer in feed solution (% w/v)		Inlet-temp (˚C)	Feed rate (ml/min)	Production yield (%)	Moisture content (%)
	HPMC	Gelatin				
1	5	-	130	5	31.03	5.50
2	5	-	130	10	35.15	6.17
3	2.5	-	130	5	17.28	7.18
4	2.5	-	130	10	23.79	8.17
5	5	-	160	5	27.44	5.67
6	5	-	160	10	30.23	5.33
7	2.5	-	160	5	19.40	8.33
8	2.5	-	160	10	26.84	8.00
9	-	5	130	5	31.43	7.35
10	-	5	130	10	31.63	7.17
11	-	2.5	130	5	26.18	7.32
12	-	2.5	130	10	27.91	7.83
13	-	5	160	5	30.70	5.83
14	-	5	160	10	29.44	6.67
15	-	2.5	160	5	26.31	5.83
16	-	2.5	160	10	28.31	6.83

A SD-06 Laboratory Scale Spray Dryer (LabPlant, West Yorkshire, England) was employed for preparing spray-dried microparticles. The 2^3 factorial design was used to determine the operating condition that maximized the production yield of the microparticle products. The aspirator rate was 200 m^3/h and the atomizing air pressure was set approximately at 50 bars. The inlet drying temperatures and solution feed rate were varied depending upon the formulation (Table 1). The microparticles were collected for moisture content analysis using Moisture Analyzer, Mettler-Toledo GmbH, Switzerland, particle size determination using Mastersizer, Malvern Instrument Ltd., Malvern, UK and further stability studies.

Stability studies of spray dried microparticles

The spray-dried microparticles and the ground dried petal powder (0.1 g weight) were transferred to 10 ml capped glass vials, kept, and taken under the same conditions as the aqueous color solution stability study. The taken samples were dissolved in DI water, adjusted pH to 4 with 10% citric acid solution and analyzed at 573 nm using UV-VIS spectrophotometer. The sample of each condition was performed in triplicate. The percentage of color remaining was calculated in the same manner as the stability study of the aqueous color solution and compared to that from the ground dried petal powder, which served as a control.

Results and Discussion

Effect of pH on the color of butterfly pea color extract

The color of butterfly pea solution altered upon the exposure at various pH. In acidic condition (pH 4), the solution color was red. However, at higher pH values the solution had a bruised purple color. The change in the color of butterfly pea solution depends on the change in equilibrium of four anthocyanin species according to the prevailing pH [14]. At low pH, anthocyanins present as the red flavylium cation. Increasing the pH results in decreasing the color intensity and the concentration of the flavylium cation, as it is transformed into blue quinonoidal base or colourless carbinol pseudobase, and into yellow chalcone species [14]. The effect of pH on the color of butterfly pea solution was confirmed by spectrophotometric analysis. Scan spectrum showed different λ_{max} values at various pH as described in Table 2.

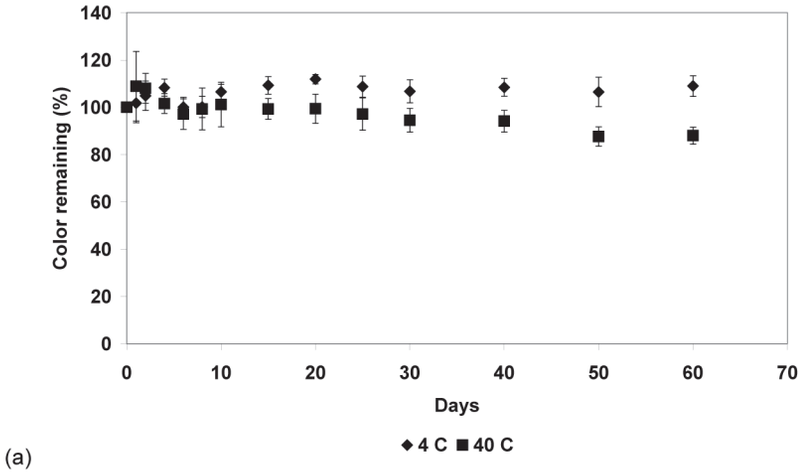
Color stability of butterfly pea aqueous color solution

Thermal stability

Temperature affected the color stability of the extract solution at various pH. The butterfly pea solution stored at 4 °C showed higher color stability than the solution stored at 40 °C (Figure 1, Table 3). The effect of rising temperature on color loss was more predominant on neutral solution (pH 7) than the acidic and alkaline solutions (pH 4 and 10, respectively); color remaining of 90% was observed in 15 days at pH 4 and 10 while it was in 1 day at pH 7. Increasing temperature induces the loss of glycosyl moieties of anthocyanin by the hydrolysis of glycosidic bond [15]. The resulting aglycones are less stable and lead to the loss of anthocyanin color. In general, anthocyanins are more stable in the acidic medium at low pH than in the alkaline medium at high pH. However, several studies demonstrated that some anthocyanins showed an improvement of color stability in the alkaline region around pH 8-9 [16, 17]. The color extract was the most stable in acidic solution at 4 °C and the least stable in neutral solution at 40 °C.

Table 2 Absorbance and λ_{max} of butterfly pea solution at different pH values

pH	λ_{max} (nm)
4.0 ± 0.02	573
7.0 ± 0.02	627
10.0 ± 0.02	628.5



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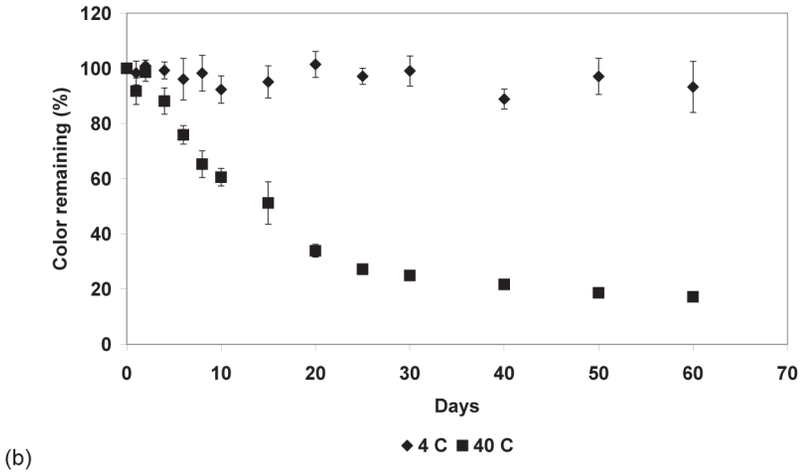

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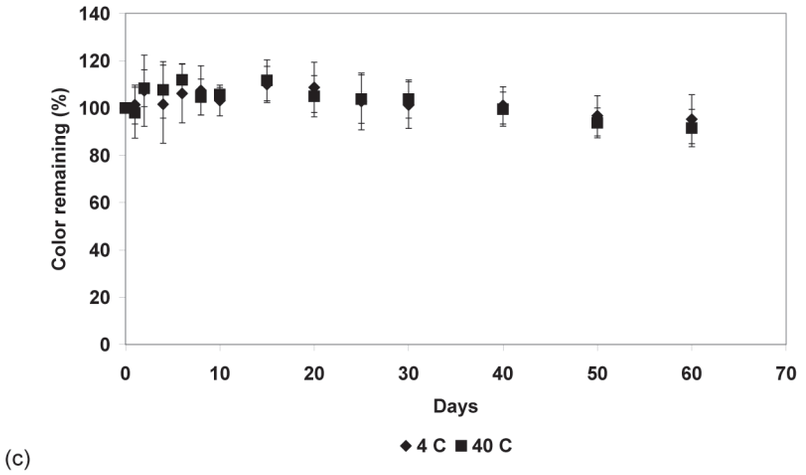

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Figure 1 Effect of temperature on % color remaining of aqueous butterfly pea extract at (a) pH 4 (b) pH 7 and (c) pH 10 (n = 3)

Table 3 Effect of light and temperature on the degradation of butterfly pea color extract in aqueous solution at various pH

Storage condition	Degradation of color extract (%) at 60 day period[a]		
	Acidic (pH 4)[b]	Neutral (pH 7)[b]	Alkaline (pH 10)[b]
4 °C	0 ± 4.36	6.72 ± 9.25	4.73 ±10.33
40 °C*	12.04 ± 3.60	82.85 ± 0.15	8.45 ± 7.90
Darkness*	8.85 ± 5.28	45.62 ± 7.29	0 ± 8.04
UV*	93.69 ± 0.39	95.30 ± 0.33	87.54 ± 3.11

[a]Degradation (%) = (original absorbance-determined absorbance)/original absorbance x 100

[b]Mean ± SD (n=3)

*Statistical significant $p < 0.02$, ANOVA

Photo stability

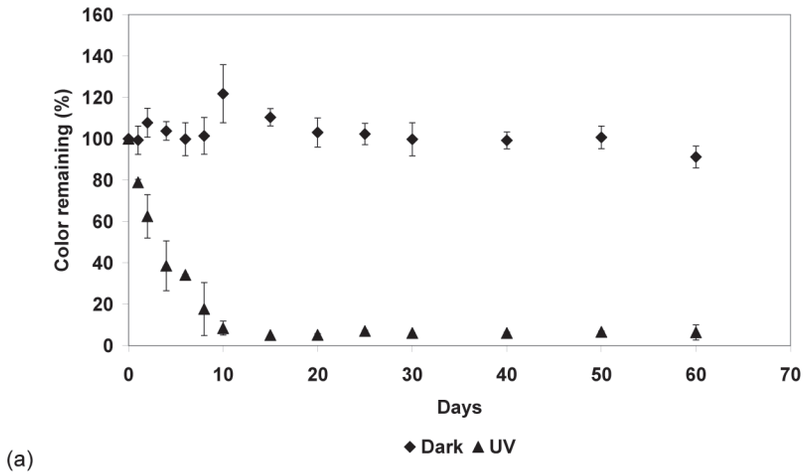
Light was found to accelerate the color loss (Figure 2, Table 3). The samples kept under darkness preserved their color much better than those exposed to UV light. The samples in the acidic solution maintained their color for a period of 60 days when kept in the dark at room temperature while % color remaining in neutral solution decreased linearly to 60% within 30 days and maintained at this value until the end of the study period (day 60). In addition, when kept in darkness, % color remaining in the alkaline solution increased approximately 20% from its original absorbance and maintained at this value until day 60. The increased in % color remaining in the alkaline solution remained speculative. It could possibly due to an improvement of color stability in the alkaline region [16, 17] or an increasing in the color intensity as it is transformed to the blue quinonoidal base [14]. Vigorous color loss was observed under UV light at all pH values; color remaining of 20% was observed in 8 days (pH 4), 6 days (pH 7), and 25 days (pH 10). Photodegradation products of anthocyanins were reported to be the same as those observed for the thermal reaction. However, the different kinetic pathways were proposed for the photodegradation and the reaction rate of chalcone and product formation was increased by light [18].

Preparation of spray-dried microparticles

The operating condition for the microparticle production was a solution feed rate of 10 ml/min, an atomized air pressure of 50 bar, an aspirator rate of 200 m^3/hr, and an inlet drying temperature set at 130°C. The total solid polymer content of 5% w/v provided the maximum yield in both HPMC-microparticles and gelatin-microparticles (Table 1). The spray-dried microparticles loaded with the color extract provided an irregular spherical shape with their size ranging from 10 to 20 μm for both HPMC-microparticles and gelatin-microparticles (Figure 3).

Color stability of butterfly pea color extract loaded in spray-dried microparticles

The color aqueous extract at pH 4.0 was selected for the preparation of feed solution due to its good stability and ease for further development in the cosmetic formulation. The stability of color loaded in the spray-dried microparticles was expressed as a percentage loss of its original absorbance (Table 4). Plots of % color remaining versus time are shown in Figures 4-5. Neither HPMC nor gelatin microparticulated systems protected the degradation of color from the thermal and UV light better than the ground dried petal powder. Especially, the color loaded in HPMC microparticulated system was much less stable than that of ground dried petal powder. When compared to the nonentrapped system (aqueous extract solution), both HPMC and gelatin microparticulated systems and the ground dried petal powder, however, showed protection against photo degradation by the UV light but not the thermal degradation. All preparations showed unstable under UV light exposure. However, color remaining was about 48% (ground dried petal powder), about 38% (gelatin microparticles), and about 18% (HPMC microparticles) on day 60 under UV light which was great higher than that of the extract solution (color remaining of 6%, pH 4).



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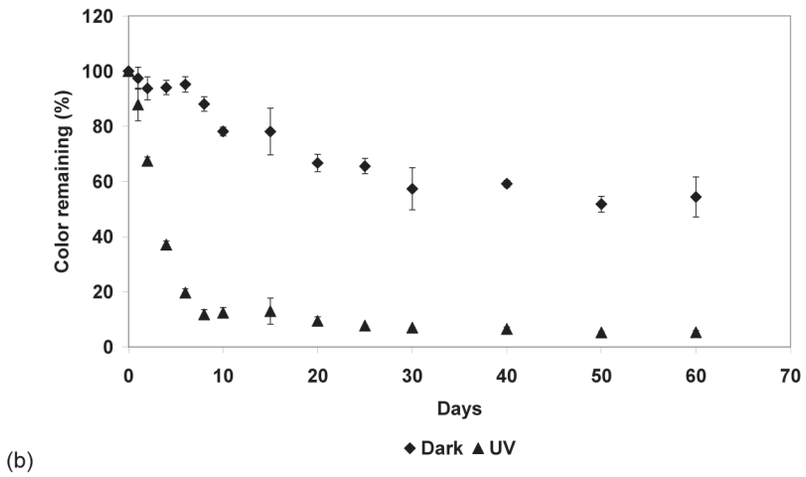

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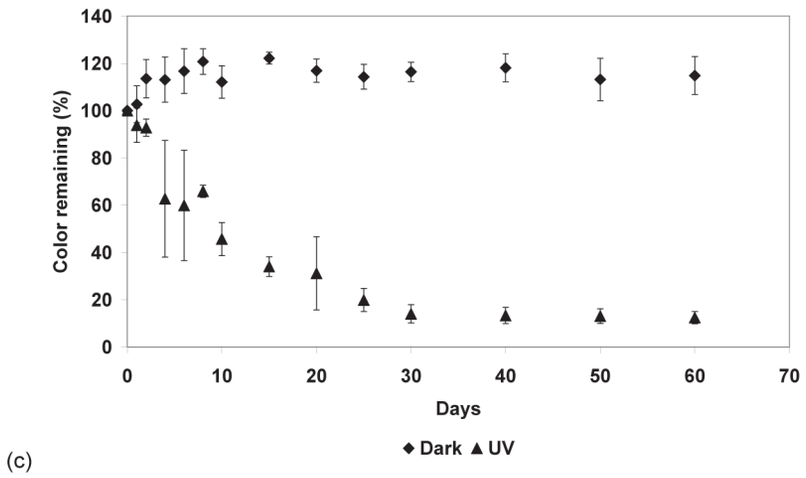

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Figure 2 Effect of light on % color remaining of aqueous butterfly pea extract at (a) pH 4 (b) pH 7 and (c) pH 10 (n = 3)

Table 4 Effect of light and temperature on the degradation of butterfly pea color extract in aqueous solution, HPMC, and gelatin microparticles

Storage condition	Degradation of color extracts (%) at 60 day period[a]			
	Nonencapsulated extracts[b]	Gelatin microparticles[b]	HPMC microparticles[b]	Ground dried petal powder[b]
4 °C*	0 ± 4.36	15.16 ± 5.23	30.39 ± 3.13	18.92 ± 1.64
40 °C*	12.04 ± 3.60	55.63 ± 3.15	63.39 ± 1.79	44.32 ± 4.62
Darkness*	8.85 ± 5.28	13.92 ± 3.05	43.24 ± 7.85	10.35 ± 2.44
UV*	93.69 ± 0.39	61.98 ± 3.43	81.16 ± 4.51	51.76 ± 5.99

[a]Degradation (%) = (original absorbance-determined absorbance)/original absorbance x 100

[b]Mean ± SD (n=3)

*Statistical significant $p < 0.001$, ANOVA

Amorphous form is usually generated during spray drying process and entrapped in the microparticles. In the ground dried petal powder, the compound could be in crystal form which is more stable than amorphous form in the microparticles. HPMC chemical formula consists of hydroxyl groups on its structure and gelatin (pH 4) provides the positive charge on its molecule. The color stability loaded in gelatin-microparticles overcame that of HPMC-microparticles possibly because of the positive charge on gelatin likely protected the flavylium system towards nucleophillic addition of water while the hydroxyl groups on HPMC could induce water molecule and, consequently, enhance the degradation of the color.

The stability of butterfly pea color depended on pH. The color was the most stable in the acidic environment and the least stable in the alkaline environment. The color of the ground dried petal powder was blue which implied that this color existed in the alkaline environment. Therefore, the nonentrapped system (acidic aqueous solution) showed better color stability than the ground dried petal powder. Additionally, citric acid is a weak organic acid generally used as a pH adjuster. The strength of acid is based on the concentration of H^+ ions in the solution. In other words, the more H^+ the stronger the acid will be. During the spray-drying process, the water evaporated away while the molecule of citric was left behind with the color and polymer molecules creating lesser acidic environment in the microparticles than in the acidic aqueous solution. The change of pH in the microparticles towards the alkaline region could be observed from the blue color of the microparticles. As a result, the color in microparticles was found to be less stable than that of the acidic aqueous solution.

The color of butterfly pea was more stable in the ground dried petal powder and in gelatin-microparticles than in HPMC-microparticles. The stability of color in the ground dried petal powder and the microparticulated systems was significantly increased under UV light when compared to that of color aqueous solution. Further studies on the effect of polymer charge and viscosity on the stability of butterfly pea color extract are of interest.

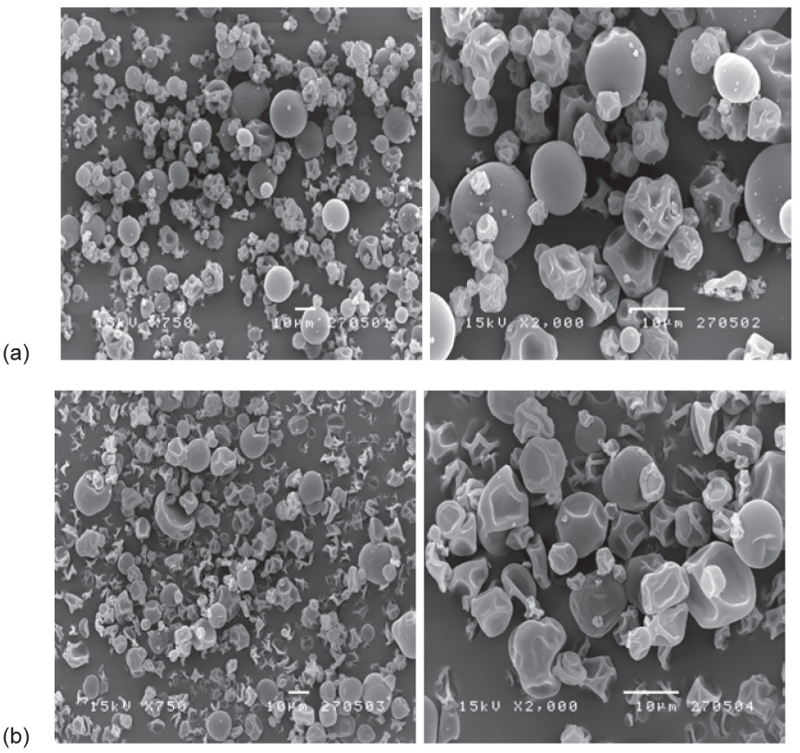


Figure 3 Scanning electron micrograph of (a) spray-dried gelatin microparticles and (b) spray-dried HPMC microparticles

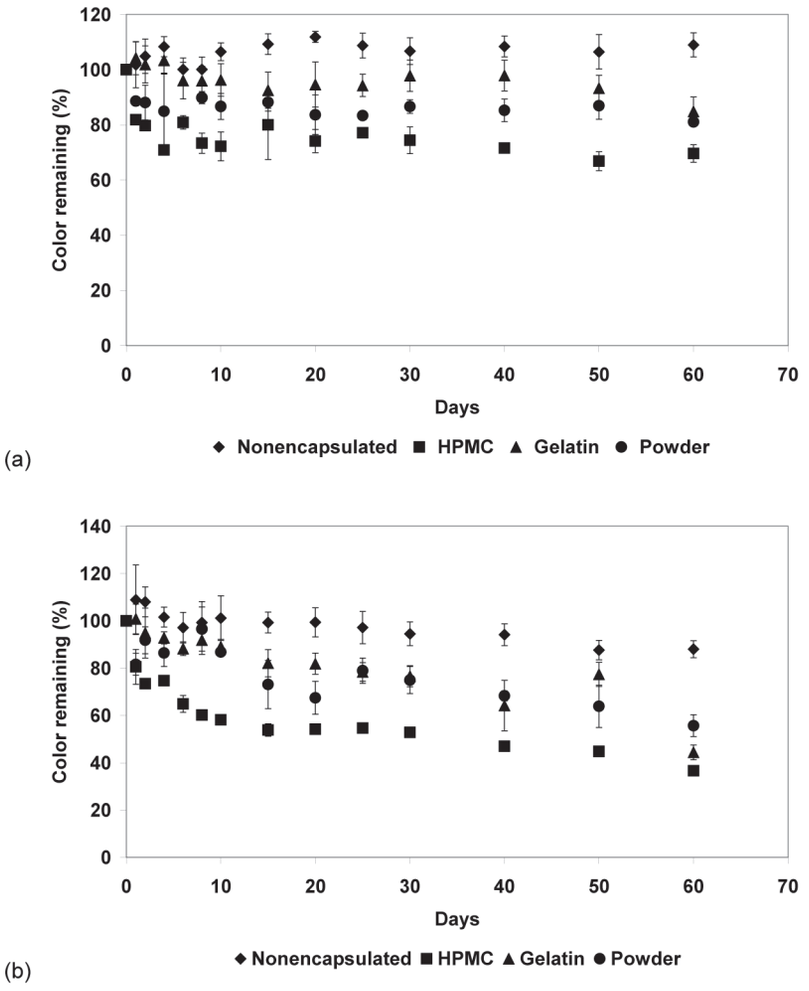
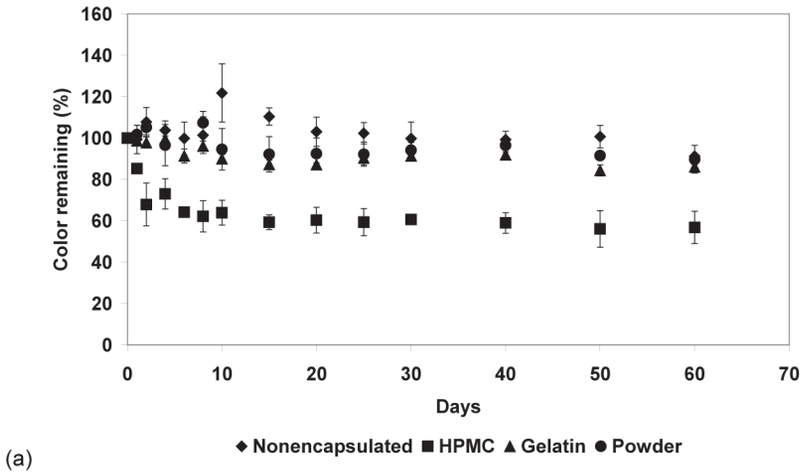


Figure 4 Percent color remaining of acidic aqueous extract, dried ground petal powder, and butterfly pea color extract microencapsulated in HPMC and gelatin during storage at (a) 4˚C (b) 40˚C (n = 3)



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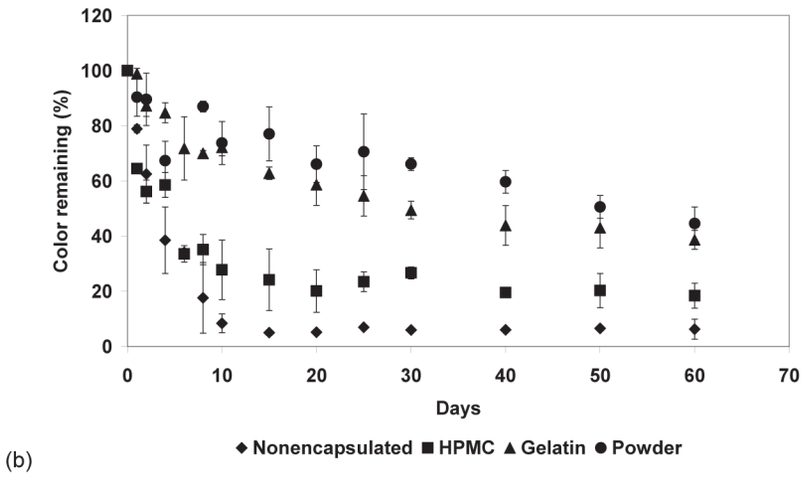

(b)

Figure 5 Percent color remaining of acidic aqueous extract, dried ground petal powder, and butterfly pea color extract microencapsulated in HPMC and gelatin during storage under (a) darkness (b) UV light (n = 3)

Conclusion

The color of butterfly pea aqueous solution varied upon the exposure at various pH. In acidic pH solution, the color displayed a red color; but in alkaline pH solution, the color changed to deep blue. The stability of color aqueous extract depended on pH, temperature, and light. Light found to provide more predominant effect on the color stability than temperature at all pH studies. The color in ground dried petal powder demonstrated better stability than the color loaded in spray-dried microparticulated systems composed of either HPMC or gelatin. Gelatin microparticulated system showed better protection against photo degradation compared to HPMC microparticulated system and the aqueous color solution. Both gelatin and HPMC microparticulated systems showed no protection against the thermal degradation. The results suggested that the polymer type affects the stability of loaded color and should be carefully selected.

Acknowledgements

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