*Bull. Health Sci. Technol. **2018, 16(1): 01-11**. ISSN 0858-7531*

BETALAIN CAPSULES FROM DRAGON FRUITS PEEL FOR LIP GLOSS

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Abstract: The objectives of this research were to prepare lip gloss containing 0.5% (w/w) betalain capsules (LBC) and to evaluate its stability. The betalain capsules were prepared from the crude ethanolic extract of red dragon fruit peels obtained from Taiwan and encapsulated with alginate/chitosan by using coacervation technique. The average particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (%EE), and loading capacity (%LC) of betalain capsules were studied. The lip gloss was also studied for its stability and DPPH radical scavenging activity via freeze-thaw cycle method in comparison of the lip gloss containing 0.5% (w/w) crude ethanolic extract (LCE). The characteristics of betalain capsules were 1.64 ± 0.03 µm for their average size, 0.415 ± 0.021 for PDI, -41.0 ± 0.5 mV for zeta potential, 32.43% ± 0.06 for %EE, and 0.43 ± 0.01 for %LC, respectively. On the six freeze-thaw cycle examination, chemical and physical changes of LBC were observed to be similar to those of LCE. Moreover, change in DPPH radical scavenging activity of LBC had a similar decreasing tendency; however, it reduces in less extent. The percentages of DPPH radical scavenging per mg betalain were found to be 4.55 ± 0.07 and 4.02 ± 0.08 for LBC and LCE, respectively. Thus, LBC is useful and can be a new alternative coloring agent for the natural cosmetic products.

Keywords: betalain, dragon fruit, encapsulation, lip gloss

บทคัดย่อ: วัตถุประสงค์ของงานวิจัยนี้ต้องการเตรียมลิปกลอสที่ประกอบด้วยแคปซูลบีตาเลน (LBC) ความเข้มข้น 0.5% โดยน้ำหนัก และประเมินความคงตัวของลิปกลอส แคปซูลบีตาเลนเตรียมจากสารสกัดหยาบเอทานอลของเปลือกแก้วมังกรพันธุ์สีแดงจากประเทศไต้หวันและห่อหุ้มด้วยแอลจิเนต/ไคโตซาน โดยใช้เทคนิค coacervation ศึกษาขนาดอนุภาคเฉลี่ย ดัชนีการกระจายตัว (PDI) ศักย์ซีต้า ประสิทธิภาพการห่อหุ้ม (%EE) และประสิทธิภาพการบรรจุ (%LC) ของแคปซูลบีตาเลน นอกจากนั้นยังศึกษาความคงตัวและฤทธิ์ในการกำจัดอนุมูลอิสระ DPPH ของลิปกลอสโดยวิธี Freeze-thaw cycle เทียบกับลิปกลอสที่ประกอบด้วย 0.5% โดยน้ำหนักของสารสกัดหยาบเอทานอลของบีตาเลน (LCE) คุณลักษณะของแคปซูลบีตาเลนประกอบด้วยขนาดอนุภาคเฉลี่ยเท่ากับ 1.64 ± 0.03 µm PDI เท่ากับ 0.415 ± 0.021 ศักย์ซีต้าเท่ากับ -41.0 ± 0.5 mV %EE เท่ากับ 32.43% ± 0.06 และ %LC เท่ากับ 0.43 ± 0.01 ตามลำดับ จากการทดลอง Freeze-thaw cycle 6 ครั้ง พบว่าการเปลี่ยนแปลงทางกายภาพและเคมีของ LBC มีลักษณะใกล้เคียงกับการเปลี่ยนแปลงทางกายภาพและเคมีของ LCE นอกจากนั้นการเปลี่ยนแปลงฤทธิ์ในการกำจัดอนุมูลอิสระ DPPH ของ LBC ยังมีแนวโน้มลดลงเหมือนกับฤทธิ์ในการกำจัดอนุมูลอิสระ DPPH ของ LCE แต่ลดลงช้ากว่า ร้อยละการกำจัดอนุมูลอิสระ DPPH ต่อมิลลิกรัมบีตาเลนของ LBC มีค่าเท่ากับ 4.55 ± 0.07 และของ LCE มีค่าเท่ากับ 4.02 ± 0.08 ด้วยเหตุนี้ LBC จึงมีประโยชน์และสามารถนำไปใช้เป็นสารให้สีที่เป็นทางเลือกใหม่สำหรับผลิตภัณฑ์เครื่องสำอางจากธรรมชาติ

คำสำคัญ**:** betalain, dragon fruit, encapsulation, lip gloss

INTRODUCTION

Lip gloss is a cosmetic product that is designed to provide moisture, glossy luster and sometimes subtle color to the lips as well as to conceal dark spot (Miller, 2016). Typical ingredients of a lip gloss are composed of emollients, thickeners, humectants, coloring agents and additives (including flavors, fragrances and preservatives) (Romanowski, 2012). Up to now, the synthetic materials, especially synthetic coloring agents have been the most widely used in cosmetic products such materials give intense color with a variety of shades and enhance color stability at low cost of production. However they may cause the side effects to human body. For examples, heavy metals in synthetic colors can cause diarrhea, anorexia, intestinal inflammation and cancer (Clay, 2016 and Mercola, 2011). Thus, natural colors are excellent attractive options for customers who care for safety and wellness of health (Villett, 2015 and Azwanida *et al.*, 2014). Dragon fruit peels (*Hylocereus costaricensis*) are rich red color and contain betalains that are bioactive compounds beneficial for anti-aging, anticancer, antioxidant and antimicrobial. The fruits are consumed as confectionery for food industry, drugs for pharmaceutical industry, and coloring agent for cosmetic industry (Rebecca *et al.*, 2010 and Lako *et al.*, 2008 and Stintzing *et al.*, 2003). Betalains composed of betacyanin and betaxanthin, are water soluble pigments. The color of betacyanin ranges from reddish to violet, while betaxanthin appears to be yellow to orange color (Stintzing *et al.*, 2005 and Tenore *et al.*, 2011). Nevertheless, betalains are unstable under strong acids, light and high temperatures (Cai and Corke, 1999 and Han *et al.*, 1998) along with some metal ions such as iron (II and III), copper (II), tin (II) and aluminum (III). These metals can speed up the degradation of betalains (Savolainen and Kuusi, 1978). Encapsulation is one of the techniques that is used for enhancing the stability of the active compounds both in liquid and solid states using polymer films (Wilson and Shah, 2007). The average size of capsules is in the range of 1-1000 µm (Neubauer and Poehlmann, 2014). A capsule contains 2 parts; core and shell (Kaimainen *et al.*, 2015). For shell, it could be made either from bio-macromolecules; carbohydrates (starch, maltodextrin, and gum), proteins (whey protein) or soy protein (Arvisenet *et al.*, 2002, Shahidi and Han, 1998, Assaf *et al.*, 2004, Sheu and Rosenberg, 1998 and Duvel *et al.*, 2004). In this research, alginate and chitosan were chosen for coating the crude betalain extract. Alginate is an anionic polysaccharide with non-toxic, biocompatible, biodegradable, and mucoadhesive properties, which can form a reversible gel with multivalent cations (e.g. Ca^{2+}) (Muzzarelli *et al.*, 2014, Sarmento *et al.*, 2007 and Vriqnaud *et al.*, 2011). Chitosan is cationic polysaccharide that is non-toxic, biocompatible and biodegradable and also contains adsorption and mucoadhesive; anti-winkle, antimicrobial, and good skin adhesive properties (Sarmento *et al.*, 2007, Aqnihotri *et al.*, 2004 and Muzzarelli *et al.*, 2014). Due to their excellent features, both alginate and chitosan are widely used for pharmaceutical applications (Azevedo *et al.*, 2014). Here, we prepared betalain capsules from the crude betalain extract encapsulated with alginate/chitosan, applied these capsules as a coloring agent in lip gloss, and studied its stability against 2,2-diphenyl-1-picrylhydrazy (DPPH radical).

MATERIALS AND METHODS

Preparation of betalain crude extract

The red dragon fruits were bought from Chiayi Province, Taiwan and were washed with tap water. It skins were peeled off, cut into small pieces and macerated with 95% ethanol for 3 days. The mass to volume ratio of the plant material and solvent for extraction was 1 kg / 2 L. The extract was filtrated by vacuum filtration and the filtrate was concentrated

by rotary evaporator (Ulvac, G-100D, Japan) at 45ºC. The obtained product was kept at -15ºC for 3 hours. After that, the frozen concentrate was dried by freezing drier (Labconco, Free Zone 6, USA) at -50±2ºC under 18 Pa for 3 days. The dried crude extract was kept in refrigerator before use.

Betalain content

The presence of betacyanin and betaxanthin was observed by UV-Vis spectroscopy following the bands with λ_{max} = 538 nm and 480 nm, respectively (Maran et al., 2013 and Moßhammer et al., 2006). Their contents can be determined as follows

$$\text{Betacyanin or betaxanthin content (mg/g crude extract)} = (A \times DF \times V \times MW)/(\varepsilon \times l \times W) \quad (1)$$

where A is the maximum absorbance at λ 538 nm for betacyanin and at λ 480 nm for betaxanthin; DF is the dilution factor; V is the volume of solution (mL); MW is the molecular weights of betacyanin (550 g/mole) and betaxanthin (308 g/mole); ε is the molar extinction coefficients of betacyanin (60,000 L $mole^{-1}cm^{-1}$) and betaxanthin (48,000 L $mole^{-1}cm^{-1}$) (Stintzing *et al.*, 2003); l is the path length of the cuvette (1 cm) and W is the weight of crude extract (g). The betalain content (BC) is calculated as the sum of betacyanin and betaxanthin contents.

Preparation and characterization of betalain capsules

1. Preparation of betalain capsules

The preparation of betalain capsules was performed according to the method of Azevedo *et al.*, 2014. 2 mg/mL of sodium alginate and 18 mM of calcium chloride were prepared in distilled water. 2 mg/mL of chitosan was prepared in 1% (v/v) acetic acid at 70˚C. The pH of the sodium alginate and chitosan solutions were adjusted with 1 M sodium hydroxide or 1 M hydrochloric acid to reach 4.9 and 4.6, respectively. In order to encapsulate betalain in the crude extract, 0.20 g of the crude extract was stirred in 30 mL of 2.0 mg/mL sodium alginate at 1000 rpm for 1 hour. 2 mL of 18 mM calcium chloride was added into the extract solution drop by drop with the flow rate of 0.1 mL/min by using micropipette and stirred with a high speed homogenizer (T 25, Ika-Werke, Germany) at 20000 rpm, and then 6 mL of 2 mg/mL chitosan was sequentially dropped into the extract solution with the flow rate of 0.2 mL/min by using micropipette and stirred with the high speed homogenizer at 10000 rpm. After that, the mixture solution was continually stirred with the high speed homogenizer at 10000 rpm for 5 min to obtain the encapsulated betalain solution. Finally, the betalain capsules were separated from the mixture solution by centrifugation at 12000 rpm for 10 min. The supernatant was separated out and the precipitate was collected and washed by 95% ethanol for several times and then dried in oven at 30˚C until the constant weight of betalain capsules was obtained.

2. Size analysis

The average size, polydispersity index (PDI) and zeta potential of betalain capsules were characterized by using photon correlation spectrometer equipped with Malvern Zetasizer (Malvern Instruments, UK) in folded capillary zeta cell at 25˚C with electrodes at both ends and the detection angle of 173˚.

3. Morphology

The morphology of betalain capsules was studied by using scanning electron microscopy (SEM) (JEOL JSM-6610, USA) that was operated at the voltage of 10 kV

(Azevedo *et al.*, 2014). Before morphologic examination, betalain capsules were mounted on metal aluminum stubs with adhesive tape, and gold coated under vacuum.

4. Encapsulation efficiency and loading capacity

The encapsulation efficiency (%EE) and loading capacity (%LC) of betalain capsules were determined according to the method of Azevedo *et al.*, 2014. After centrifugation of the encapsulated betalain solution at 12000 xg for 10 min, the precipitate (betalain capsules) and supernatant (free betalain in the solution) were separately collected. Both the precipitate and supernatant were used to determine %EE and %LC as follows:

$$\%EE = ((B_{total} - B_{free})/B_{total}) \times 100 \quad (2)$$

$$\%LC = ((B_{total} - B_{free})/W_{total}) \times 100 \quad (3)$$

where B_{total} was the total amount of betalain in the crude extract used; B_{free} was the amount of free betalain in the supernatant and W_{total} was the total weight of betalain capsules. The B_{total} and B_{free} were calculated according to the determination of the betalain content described above (Stintzing *et al.*, 2003).

Formulation of lip glosses

Typical formula of lip gloss was formulated according to the method of Luigi, 2015 and Harripersad *et al.*, 2010 with certain modifications. It was composed of emollients, humectants, thickeners, emulsifiers, coloring agents and flavors. All ingredients used for this research and their function were tabulated in Table 1. In this formula, 0.5% w/w of betalain was used as a natural coloring agent instead of a mineral coloring agent.

Table 1. Typical formula of lip gloss studied.

Ingredients	Contents (% w/w)	Function
Oil phase		
1. Camellia Oleifera seed oil	73.0	Emollient
2. Beeswax	10.0	Emollient/Thickener
3. Soy lecithin	3.5	Emulsifier
Aqueous phase		
1. Glycerin	10.0	Humectant
2. Betalain crude extracts / Betalain capsules	0.5	Coloring agent
3. Ethyl vanillin	1.0	Flavor
4. Strawberry flavor	2.0	Flavor

Stability of Lip Gloss

The stability of lip gloss containing betalain capsules was evaluated by using freeze/thaw cycle method for 6 cycles (Leelapornpisid *et al.*, 2014). 5 grams of lip gloss was kept in oven at 45±1 ºC for 48 hours and then kept in refrigerator at 4±1 ºC for 48 hours) for each freeze/thaw cycle. For each testing cycle, changes in its physico-chemical properties (color, odor, homogeneity, smoothness, pH, viscosity, moisture and glossy) and its antioxidant activity against DPPH radicals were also determined. Lip gloss containing only the crude extract was also studied as a reference in comparison of the stability of lip gloss containing betalain capsules. Each lip gloss formula was tested in triplicate.

Antioxidant activity

The antioxidant activity of a lip gloss was determined by using DPPH radical scavenging assay, based on the method of Stintzing *et al.*, 2005. To a reaction tube, 2.0 mL of DPPH in methanol (0.1 mM) and 2.0 mL of lip gloss in hexane (2.5% w/v) were added. After incubation for 30 min in dark, the absorbance of the reaction mixture was measured at 517 nm by UV-Vis spectrophotometer. The percentage of DPPH scavenging activity was calculated as follows:

$$\% \text{ Scavenging activity} = ((A_1 - A_2)/A_1) \times 100 \quad (4)$$

where A_1 was the absorbance of the control (without lip gloss) and A_2 was the absorbance of the lip gloss sample.

Statistical analyses

All the experiments were performed in triplicate. The experimental results were expressed as the mean ± standard deviation (SD) of mean. Statistical analysis was performed using Student's t-test and one-way analysis of variance (ANOVA). Results were processed by Excel (Microsoft Office 2010) and SPSS Version 17.

RESULTS AND DISCUSSION

Crude ethanolic extract of betalain

The extraction of dragon fruit peels with 95% ethanol resulted in a crude betalain extract as a viscous and red-violet liquid. Its percent yield was 1.62% (w/w) of the extract material. Based on spectrophotometric analysis, it showed that the crude extract was consisted of 274.8 mg betacyanin and 148.6 mg betaxanthin per 1 gram of the crude extract. Thus, the betalain content which was the sum of the betaxanthin and betacyanin contents was 423.4 mg/g of the crude extract.

Characteristics of betalain capsules

The betalain capsule was obtained as a red-violet powder with 34.13% ± 0.03 encapsulation efficiency and 0.30% ± 0.02 loading capacity. On particle size analysis, it demonstrated that the betalain particles in solution (0.053% (w/v)) show the average size of 1.64 ± 0.03 µm and the particle size distribution of 0.415 ± 0.021. The electrostatic charge (zeta potential) at the particle surface determined by dynamic light scattering (DLS) technique, was found to be -41.0 ± 0.5 mV at pH 4.6. The negative value of the zeta potential is due to the high ratio of negatively carboxylate groups on the alginate molecules, which exceeds the amount of the amino groups in chitosan. The particle size is in the range of microns because the overall charge on the surface is reduced (Azevedo *et al.*, 2014). The morphology of the betalain capsules was also studied using SEM technique at the suspension concentration of 0.530% (w/v) the results were shown in Figure 1. It was found that the betalain capsules showed various shapes and have the unimodal distribution of the particle sizes.

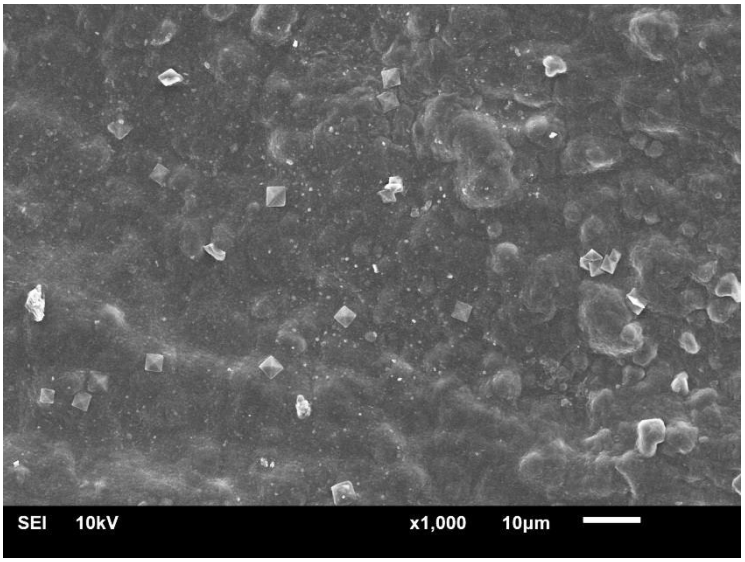

Figure 1. SEM image of betalain capsules by alginate/chitosan at the suspension concentration of 2 mg/mL (scale bar = 10 µm and magnification = 1000×).

Formulation of lip gloss

To formulate lip gloss, we first tasted the betalain capsules as well as the crude betalain extract. The result suggested that substances are slightly bitter and odorless. Consequently, it was necessary to improve both favor and taste of the capsules and the crude for custom acceptance when they are used to prepare lip gloss. Thus, strawberry and vanilla flavors were added into the formulation process. From Table 2, a series of lip glosses prepared by 0.5% (w/w) betalain concentration from various combinations of the betalain capsules and crude betalain extract were noted. Shown in Figure 2, the products revealed dark pink color, decent odor, good homogeneity and smoothness in use. Based on the betalain content determination as described above, the betalain quantity appeared to be 8.53 mg/g and 10.58 mg/g respectively.

Table 2. The quantities of betacyanin, betaxanthin and betalain in lip glosses prepared from the betalain capsules and crude betalain extract.

Contents (mg/g)	Lip glosses	
	Betalain capsules	Crude betalain extract
Betacyanin	5.54	6.87
Betaxanthin	2.99	3.71
Betalain	8.53	10.58

Figure 2. The appearance of lip glosses prepared from **(a)** betalain capsules and **(b)** crude betalain extract.

Stability of lip gloss

The examination of stability efficiency of our lip glosses was performed on the basis of the freeze-thaw cycle test for 6 cycles. The changes in physico-chemical properties and antioxidant activity of lip glosses were examined for each testing cycle. The stability results were shown in Table 3. Only changes in color and odor of both lip glosses prepared from the betalain capsules and crude betalain extract were found as tests at the fifty freeze-thaw cycle. It is well known that the DPPH antioxidant activity of a substance is dependent upon the solution concentration studied. To compare the reduction in the DPPH antioxidant capacity of the lip glosses prepared from the betalain capsules and crude betalain extract, the betalain content present in both lip glosses needed to be considered. Table 4 showed the antioxidant efficiency of betalain capsules and betalain crude extract in term of % DPPH scavenging per mg betalain with the number of cycles tested and this was clarified as shown in Figure 3. Both of the lip glosses had a decreasing tendency in DPPH scavenging capacity against the increasing number of cycles tested in similar pattern. However, the betalain in capsules exhibited significantly higher DPPH scavenging capacity than the crude betalain extract ($p<0.05$). This may be because the encapsulation of betalain with alginate/chitosan could prolong betalain and herein its antioxidant efficiency (Azevedo *et al.*, 2014).

Table 3. Evaluation of lip gloss from betalain capsules and betalain crude extract.

Time	Organoleptic characteristic								pH		Viscosity (cP)		Moisturizer (%)		Glossy (%)		Antioxidant activity (% Scavenging)	
	Color		Odor		Homogeneity		Smoothness											
	Extract	Capsules	Extract	Capsules	Extract	Capsules	Extract	Capsules	Extract	Capsules	Extract	Capsules	Extract	Capsules	Extract	Capsules	Extract	Capsules
Cycle 1	N	N	N	N	N	N	N	N	4.36	5.45	1102.67±11.15^{a}	1019.70±2.65^{a}	40.06±0.81^{a}	43.99±0.30^{a}	229.55±7.89^{a}	160.76±1.77^{a}	42.52±0.83^{a}	38.82±0.56^{a}
Cycle 2	N	N	N	N	N	N	N	N	4.35	5.49	1102.33±6.11^{a}	1011.33±2.08^{b}	39.55±0.36^{a}	44.19±1.14^{a}	224.45±2.51^{a}	159.00±3.35^{a}	40.11±0.89^{b}	37.61±1.32^{ab}
Cycle 3	N	N	N	N	N	N	N	N	4.35	5.44	1091.33±3.21^{a}	1006.00±4.00^{bc}	39.96±0.94^{a}	43.29±0.46^{a}	223.25±3.96^{a}	158.52±2.64^{a}	36.54±0.79^{c}	35.79±0.87^{b}
Cycle 4	N	N	N	N	N	N	N	N	4.34	5.46	1078.00±8.00^{b}	1001.00±3.61^{c}	40.06±0.53^{a}	43.09±0.52^{a}	221.79±2.03^{a}	157.83±1.84^{a}	33.99±0.58^{d}	33.69±0.77^{c}
Cycle 5	C	C	C	C	N	N	N	N	4.35	5.44	1052.67±4.16^{c}	993.33±4.93^{d}	39.96±0.77^{a}	42.99±0.35^{a}	228.38±6.00^{a}	149.99±4.96^{b}	28.65±0.51^{f}	27.81±1.53^{d}
Cycle 6	C	C	C	C	N	N	N	N	4.35	5.44	1002.33±3.51^{d}	951.33±3.21^{f}	39.55±0.36^{a}	43.19±0.87^{a}	222.08±4.57^{a}	147.02±0.09^{b}	25.16±1.81^{g}	23.19±1.75^{f}

Different letters in the same column indicate a statistical difference ($p < 0.05$).
Appearance: N – no change, C - change

Table 4. The antioxidant efficiency of betalain capsule and betalain crude extract.

Samples	Antioxidant efficiency (% Scavenging/mg betalain)					
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Betalain capsules	4.55±0.07[a]	4.41±0.15[a]	4.20±0.10[a]	3.95±0.09[a]	3.26±0.18[a]	2.72±0.21[a]
Betalain crude extracts	4.02±0.08[b]	3.79±0.08[b]	3.45±0.08[b]	3.21±0.05[b]	2.71±0.05[b]	2.38±0.17[b]

Different letters in the same column indicate a statistical difference ($p < 0.05$).

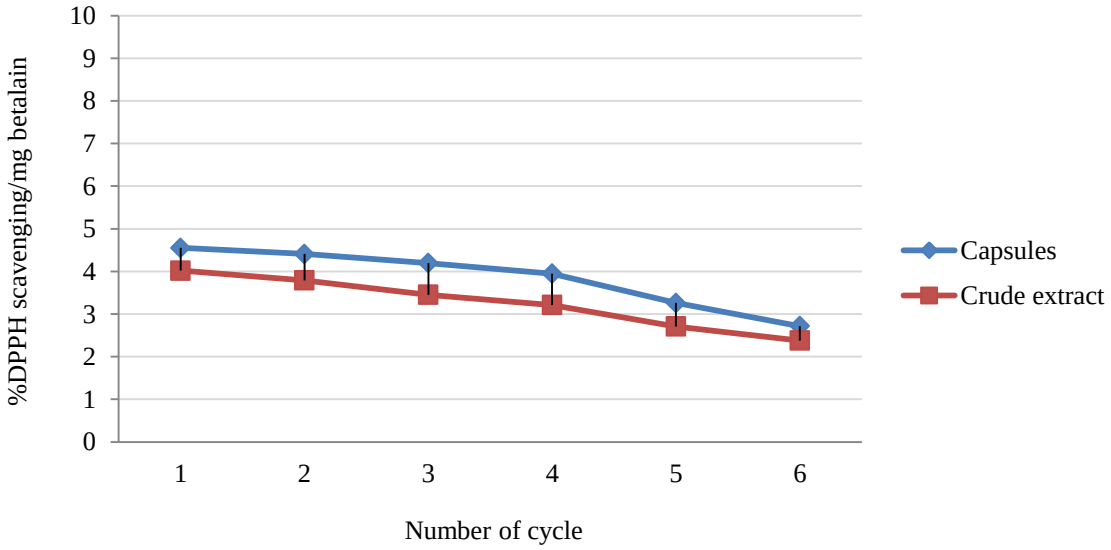

Figure 3. The relation between % DPPH scavenging per mg betalain as function of the number of cycles tested in lip glosses prepared from the betalain capsules and crude betalain extract.

CONCLUSION

In this work, the betalain substances in the crude betalain extract can be successfully encapsulated with alginate/chitosan using coacervation technique, obtaining 34.13% ± 0.03 and 0.30% ± 0.02 values of encapsulation efficiency and loading capacity, respectively. In addition, we succeeded in introducing betalain capsules into lip gloss formula as a coloring agent and prolonging the lip gloss life with altering the important physio-chemical properties in less levels.

ACKNOWLEDGEMENTS

W.S. would like to gratefully acknowledge Faculty of Science, King Mongkut's University of Technology Thonburi, Thailand for Graduated scholarships and Dual Degree Program (DDP) scholarships for financial support to study abroad at Faculty of Cosmetic Science, Chia Nan University of Pharmacy and Science, Taiwan.

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