ENCAPSULATION EFFICIENCY OF ROSELLE CALYCES FOOD COLOURANT USING DIFFERENT WALL MATERIALS

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Abstract

Roselle calyces have the intense red colour due to the accumulation of anthocyanins. They could be extracted using distilled water and 2 % citric acid solution. Total anthocyanins content expressed as cyanidin – 3-glycoside in each extract was determined by pH differential method. Total anthocyanins content of 2 % citric acid extract was higher than that of watery extract. Some quantitative tests for anthocyanins extract were carried out by using ferric chloride, dilute hydrochloric acid and dilute sulphuric acid. Powders of anthocyanins were prepared using different wall materials : maltodextrin (MD), gum arabic (GA) and a combination of maltodextrin and gum arabic (MD + GA) with 1 : 1 ratio. Tween – 80 was used as emulsifier. Each of the wall materials was homogenized to the core material with the ratio of 1 : 1 and were micro encapsulated by microwave –assisted drying at 1100 W. The encapsulated powders were analysed for moisture, hygroscopicity, colour density and morphology. The stability of anthocyanins powder was evaluated under different temperature conditions (Refrigerator, room temperature, sunlight). The results indicated that encapsulated powder with the GA and MD combination gave the better quality of powder and it could be applied in colouring of jelly.

Keywords: Anthocyanins, roselle calyces, pH differential method, encapsulated powder

Introduction

Food dye or food colouring is a type of food additive that is added to food or drinks. It is a form of pigment, dye or substance that imparts colour when it is added. Food dye can be found in the form of powder, liquid and gel. In the current market, food dye can be divided into two types: natural food

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dye and synthetic food dye (artificial colourants). Natural colourants have been widely used in food and cosmetic products (Piyarat *et al*., 2014).

Artificial colourants are synthetic dyes derived from petroleum. These chemicals may have harmful side effects and could cause health problems in adults or children. Moreover artificial food dyes do not add nutritional value to the food. Their chemicals may have synthesized colours which are easier and less costly to produce and are superior in colouring properties. In the last 20 years synthetic colourants have been increasingly perceived as undesirable or harmful by consumers (Ni and Gong, 1997). Hence, most countries have limited the use of synthetic colourants. Natural dyes have been used for centuries to colour food. These dyes are produced from plant and animal sources. Natural food colours offer additional health benefits of biologically active compounds like vitamins, minerals, flavonoids, chlorophyll, and other cancer fighting antioxidants. But natural colourants are generally more sensitive to light, temperature, pH and redox agents (Macrae *et al*., 1993).

Roselle (*Hibiscus subdariffa* L.) is a tropical plant (Figures 1 and 2) which belongs to the family Malvaceae and is known in Myanmar as chin-baung. It is probably a native of West Africa and is now widely cultivated throughout the tropics and subtropcis e.g. Sudan, China, Thailand, Egypt, Mexico and the West India (Purseglove, 1974).

Botanical description of *Hibiscus sabdariffa* L. is as follows:

Botanical name	-	*Hibiscus sabdariffa* L.
Family	-	Malvaceae
Common name	-	Roselle
Myanmar name	-	Chin baung thee
Parts used	-	Calyces

Figure 1: Plant of roselle (Chin-baung)

Figure 2: Roselle calyces

Encapsulation is a technique that is used for protection, stabilization and slow release of core materials. There are several techniques and wall materials that are available for encapsulation of natural food colourants to overcome their instability, solubility and handling problems (Khazaei and Jafari, 2014).

In this research, anthocyanin was extracted from roselle calyces by using 2 % citric acid solution and then encapsulated with wall materials such as maltodextrin, gum arabic and combination of maltodextrin and gum arabic. The stability of encapsulated powder was evaluated under different light and temperature conditions.

Materials and Methods

Sample Collection

The roselle calyces were collected from Yat Sauk Township, Southern Shan State during August 2017. The roselle calyces were washed with distilled water and dried at room temperature for about two weeks and then ground into powder.

The encapsulating agents used were maltodextrin (DE 4-17), gum arabic and Tween-80. All reagents were analytical grade.

Phytochemical Investigation on Roselle Calyces

Phytochemical investigation on roselle calyces was carried out according to the reported methods (Harborne, 1984).

Preparation of the Anthocyanin Extracts from Roselle Calyces

Extraction of anthocyanin pigments from roselle calyces was carried out according to the procedure described by Spanga*et al*., (2003). The extracting solvents were: distilled water and 2 % citric acid solution. The extracts were kept in refrigerator until further analysis.

Determination of Total Monomeric Anthocyanins

Before the determination of anthocyanins content, maximum absorption of anthocyanins extract was detected by UV-VIS spectrophotometer. Total anthocyanin content was determined by using the spectrophotometric pH-differential method described by Wang and Xu (2007) using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 mol/L) (125 mL of 0.2 mol/L KCl and 375 mL of 0.2 mol/L HCl) and sodium acetate buffer, pH 4.5 (0.4 mol/L) (400 mL of 1 mol/L sodium acetate, 240 mL of 1 mol/L HCl and 360 mL of distilled water). The absorbance was measured at 520 and 700 nm with distilled water as blank by using a UV–VIS spectrophotometer (Spectro UV 2550). The absorbance difference between the pH 1.0 and pH 4.5 was calculated:

Absorbance= (A_{520nm} pH1.0 – A_{700nm} pH1.0) – (A_{520nm} pH4.5 – A_{700nm} pH4.5).

The total anthocyanin content was calculated as cyanidin-3-glucoside according to the following equation:

$$\text{Total anthocyanin content (mg/100g)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon l}$$

where, MW (molecular weight)

DF = the dilution factor; *l* = path length, A =Absorbance, ε=Molar absorptivity.

Choice of Solvent for Extraction of Anthocyanin

To choose the best solvent, pH and yield percent of both extracts were compared. 2 % citric acid extract with deep colour was chosen for the research work.

Qualitative Tests for Anthocyanins in 2 % Citric Acid Extract

The presence of anthocyanins in 2 % citric acid extract was tested by using three common reagents: (i) sulphuric acid (ii) ferric chloride and (iii) dilute hydrochloric acid (Spanga*et al*., 2003).

Preparation of Anthocyanins Encapsulated Powder

Anthocyanin extract (100 mL) was added to 100 mL of each wall material (gum arabic (GA) 40 % solution, maltodextrin (MD) 40 % solution and combination of maltodextrin (MD) and gum arabic (GA) with 1:1 ratio). This immiscible mixture was homogenized at 1000 rpm for 10 min by using magnetic stirrer until the anthocyanin dispersed completely. Two drops of Tween-80 was added to aid the emulsification process (Krishan *et al*., 2005). The mixture was placed inside crucible and placed in a domestic microwave oven. Each mixture was treated for up to 14 min at microwave power intensities (1100 W). They were ground into powder and immediately placed in brown bottle and stored in refrigerator.

Determination of some Physicochemical Properties of Encapsulated Powders

Some physicochemical properties such as pH, moisture content, hygroscopicity, colour density, dissolution time and solubility were carried out by the reported methods. Colour density was determined by densitometer and the surface morphology of the encapsulated powders was examined by scanning electron microscope (SEM, JEOL-JSM-5610LV, Japan) at Universities' Research Centre (URC). The presence or absence of artificial colours in encapsulated powder was determined at Food Chemical Control Laboratory, Department of Food and Drug Administration, Nay Pyi Taw.

Evaluation and Application of Encapsulated Anthocyanins Powder (III)

The stability of encapsulated powder (MD+G) was evaluated placing under different storage conditions (Refrigerator, Room and Sunlight) and it was applied in colouring of jelly.

Sample Preparation and Analysis of Colour by Paper Chromatography using Ascending Method

The Whatman No.1 paper was spotted with the sample solution. The sample spot was dried with a hair dryer. It was run in a chromatographic chamber containing solvent (I) (2 g trisodium citrate in 20 mL ammonia and 80 mL distilled water) by ascending method. Similarly, the same experiment was run in a chromatographic chamber containing solvent (II) (n-butanol: acetic acid: distilled water 12:3:5). At the end of the running, the paper was removed from the jar and hanged them to be dried. The results were compared with standard artificial colours such as Auramine O, Brilliant Bule (BB), Tartrazine (TT) and Curcumin.

Application of Encapsulated Anthocyanin Powder in Jelly Preparation

About 1 g of jelly powder was added to 50 mL of distilled water, then heated at 70^{o} C, stirred for about 15 min and cooled at room temperature. The boiled encapsulated anthocyanin powder solution (1 g in 20 mL of distilled water) was added to jelly solution. When the mixture was left for about 15 min, the coloured jelly was obtained.

Results and Discussion

Preliminary Phytochemical Tests for Roselle Calyces

The preliminary phytochemical tests on the dry powder sample revealed the presence of alkaloids, amino acids, carbohydrates, glycosides, phenolic compounds saponins, steroids, sugars, tannins, flavonoids, lipophilic group, proteins, polyphenols and starch. However, resin and cyanogenic glycoside were absent.

Extraction of Anthocyanin Pigments from Roselle Calyces

Distilled water and 2 % citric acid solution were used as solvents for extraction of anthocyanins. Material to solvent ratio was 1:25 w/v. The pH

values of red colour of watery extract of anthocyanins and 2 % citric acidextract of anthocyanin (Figure3) were found to be 2.7 and 2.5, respectively.

(a) (b)

Figure 3: Extracts of anthocyanin (a) watery extract (b) 2 % citric acid extract

Total Anthocyanin Pigment

Before determination of total anthocyanin pigment in both extracts, maximum absorption of both anthocyanin extracts were detected by uv-visible spectrophotometer. In both extracts, maximum absorption was found to be 520 nm (Figure 4). Therefore λ_{max} = 520 nm was used for the calculation of anthocyanin content in pH differential method.

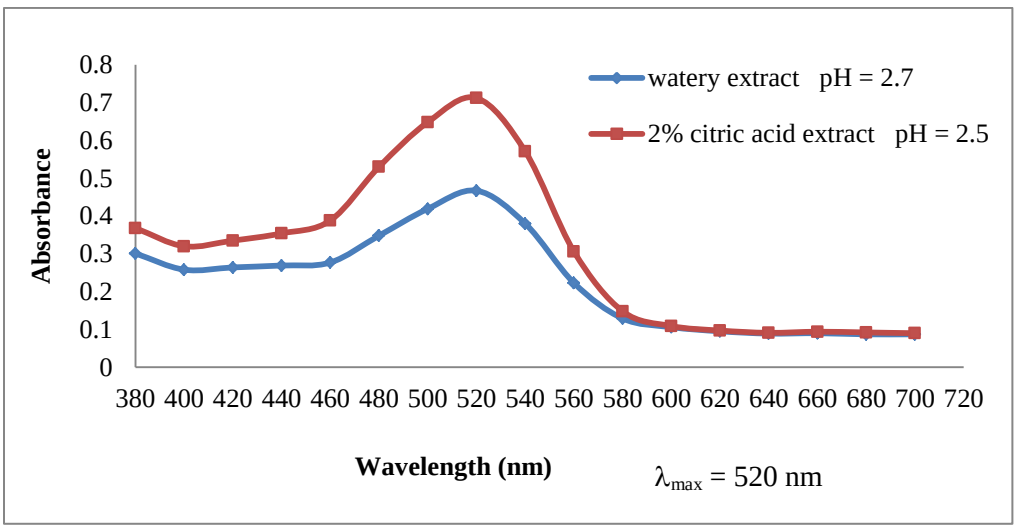


Figure 4: UV- visible absorption spectra of anthocyanin in watery extract and 2 % citric acid extract

Choice of Solvent for Extraction of Anthocyanins

The extraction efficiency of anthocyanin was affected by the type of solvents. The anthocyanin content of 2 % citric acid extract was higher than that of water (Table 1). At low pH value, anthocyanin content was found to be higher and the colour was more stable (Bronnum-Hansen *et al.*, 1985). Therefore, 2 % citric acid was chosen for the extraction of anthocyanins from roselle calyces.

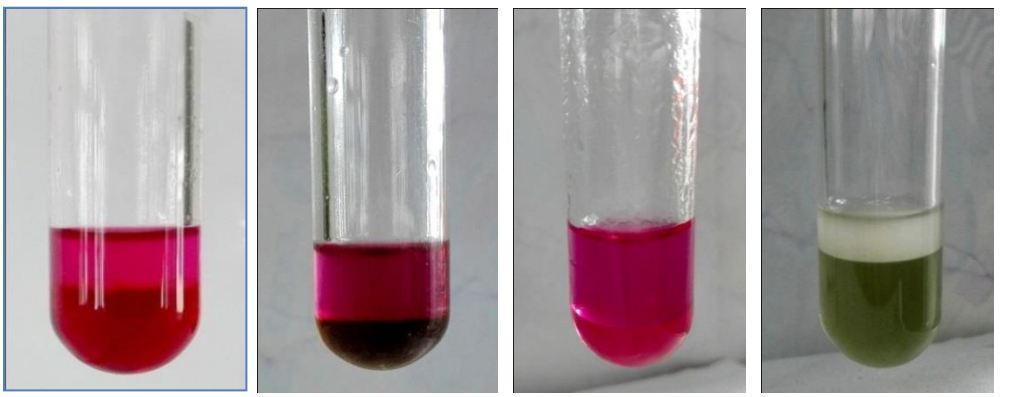
Table 1: Extraction Efficiency of Anthocyanin Affected by the Type of Solvents

No.	Extraction Solvent	pH	Anthocyanin content (mg/100g)
1	Distilled Water	2.7	195
2	2 % citric acid	2.5	254

* Based on dry weight

Qualitative Tests for Anthocyanins in 2 % Citric Acid Extract

According to qualitative tests pelargonidin, cyanidin and malvidin were found to be present in anthocyanin extracts (Figure 5).



Anthocyanins Malvidin Pelargonidin Cyanindin

(i) Sulphuric acid test **(ii) Ferric chloride test** **(iii) Dilute hydrochloric acid test**

Figure 5: Qualitative tests for anthocyanins

Encapsulation of 2 % Citric Acid Anthocyanin Powder

In general, the coating agent alone does not offer all the properties required to ensure a good microencapsulation and a mixture of one or more components is frequently employed to enhance the encapsulation (Turchiuli and Fuchs, 2005).

To increase the stability of anthocyanins and protection from light, high temperature, pH and oxidation, encapsulation was carried out. In this research, anthocyanins pigment of 2 % citric acid solution extract for core material, a mixture of gum arabic and maltodextrin for wall materials and Tween-80 as emulsifier were used. The core to wall materials ratio was 1:1. The powders obtained from different wall materials are shown in Figure 6.

Powder I usingMaltodextrin (MD)

Powder II using Gum Arabic (GA)

Powder III usingMaltodextrin and Gum Arabic (MD+GA)

Figure 6: Encapsulated anthocyanins powders

Some Physicochemical Properties of Encapsulated Anthocyanin Powders

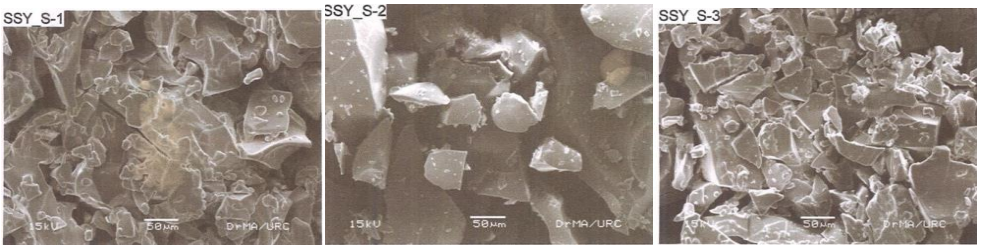
According to Table 2, powder encapsulated with a combination of gum arabic and maltodextrin has medium pH, moisture content, hygroscopicity, colour density and solubility in water. Moreover it showed the highest dissolution time, indicating that the time taken for the powder to reconstitute in water was longer than the others.

Table 2: Some Physicochemical Properties of Encapsulated Anthocyanin Powders with Different Wall Materials

Powder	Coating material	pH	Moisture (%)	Hygroscopicity (%)	Colour density (D)	Dissolution time (min)	Solubility in water (%)
I	Maltodextrin	2.9	4	11.9	0.36	1	96
II	Gum Arabic	3.4	7	15.2	0.53	4	93
III	Maltodextrin and Gum Arabic	3.0	5	13.8	0.42	8	95

SEM Analysis of the Encapsulated Anthocyanin Powder

The encapsulation efficiency of anthocyanin was evaluated by scanning electron microscope (SEM). The morphological structures of powder (I, II, III) are shown in Figure 7.



Powder I MD | Powder II GA | Powder III MD+GA

Figure 7: SEM images of the encapsulated powder with different wall materials(at 500 X magnification)

It was found that all particles produced had smooth surface and flake like structure. SEM image of combination of maltodextrin and gum arabic showed more dents surrounding the core materials whereas few dents appeared in micrograph of encapsulated maltodextrin alone and gum Arabic alone. According to a study carried out by Cano-Chauca *et al*., (2005), the addition of gum arabic to maltodextrin allows particles with better distribution and uniformity to be obtained. Therefore, combination of wall materials had higher encapsulation efficiency than the others.

Evaluation and Application of the Encapsulated Anthocyanins Powder(III)

Evaluation of encapsulated powder (III) (MD +GA)

Stability of anthocyanins in encapsulated powder III (MD +GA) was evaluated under different storage conditions (Refrigerator, Room and Sunlight). The decrease in absorbance implies the degradation of anthocyanins in storage conditions. The resultant data of absorbance and their related concentrations are tabulated in Table 3 and the degradation curves for various storage conditions are shown in Figure 8.

Table 3: The Absorbance and Concentration of the Encapsulated powder (III) in Various Storage Conditions

No.	Time (Days)	At 4 ± 1°C in the absence of light (Refrigerator)		At 25 ± 1°C in the of light		Sun light	
		Absorbance	Conc: (M)	Absorbance	Conc: (M)	Absorbance	Conc: (M)
1	0	0.562	2.089×10^{-5}	0.562	2.089×10^{-5}	0.560	2.082×10^{-5}
2	2	0.562	2.089×10^{-5}	0.562	2.089×10^{-5}	0.556	2.067×10^{-5}
3	4	0.562	2.089×10^{-5}	0.562	2.089×10^{-5}	0.550	2.045×10^{-5}
4	6	0.562	2.089×10^{-5}	0.560	2.082×10^{-5}	0.548	2.037×10^{-5}
5	8	0.562	2.089×10^{-5}	0.559	2.078×10^{-5}	0.540	2.007×10^{-5}

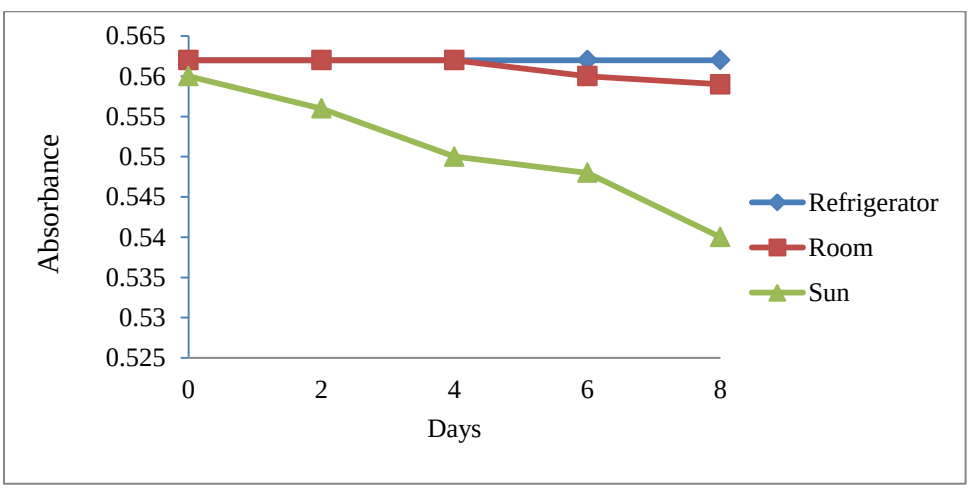


Figure 8: Degradation of the encapsulated anthocyanin powder with time

The anthocyanins were affected by the ambient conditions (light and temperature). Falcao *et al*., (2004) pointed out that light is an important accelerating factor in the degradation of anthocyanins. According to the Table 3 and Figure 8, the samples stored in refrigerator showed lower degradation than the samples stored at 25° C in room temperature and under sunlight. It was noted that when exposed to sunlight at ambient temperature, the sample showed a significant decrease of the absorbance on third day of exposure. These findings are in agreement with Janna *et al*. (2007). The visual colour changes of model samples in various storage conditions for 12 days period are shown in Figure 9. Thus, samples stored in refrigerator and at room temperature showed lower degradation of anthocyanins than the sample placed in sunlight.

4 ± 1°C Room Temperature Sunlight

Figure 9: Colour changes of the encapsulated powder (iii) under different conditions

Chromatographic Analysis of Artificial Colours

According to the chromatograms, the powder did not contain artificial colours such as Auramine O, Brilliant Blue (BB), Tartrazine (TT) and Curcumin (Figures 10 and 11). It confirms that the encapsulated powder is natural food colourant. Therefore it can be eaten safely for consumers.

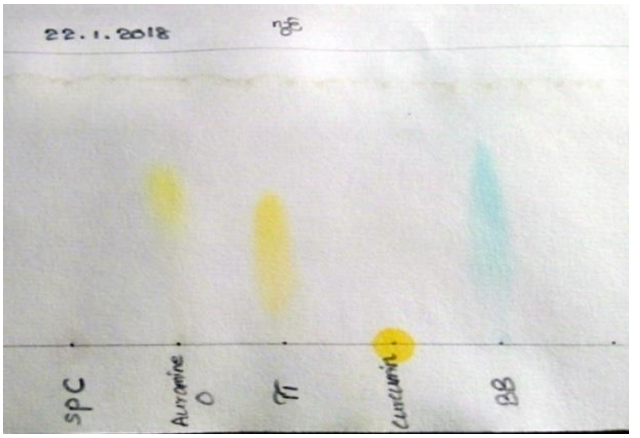


Figure 10: One Dimensional ascending paper chromatogram of the encapsulated powder (iii) in solvent I

Solvent I = Trisodium citrate + ammonia solution
SPC = Encapsulated powder sample Artificial colours
Auramine O
Tartrazine (TT)
Curcumin

Brilliant Blue (BB)

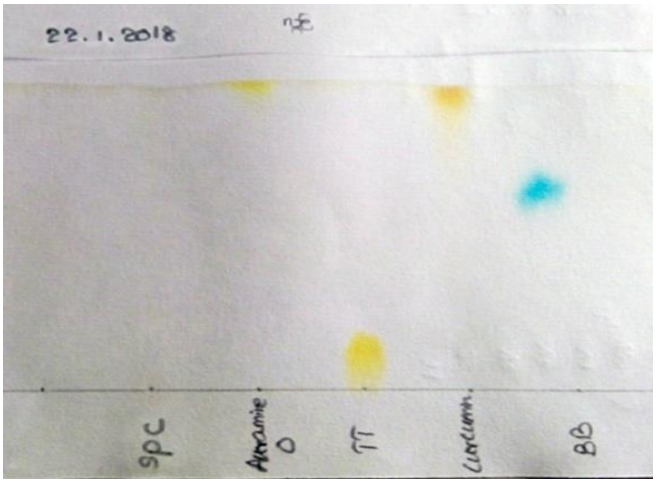


Figure 11: One dimensional ascending paper chromatogram of the encapsulated powder (iii) in solvent II

Solvent II = n-Butanol + acetic acid
SPC = Encapsulated powder sample Artificial colours
Auramine O
Tartrazine (TT)
Curcumin
Brilliant Blue (BB)

Application of encapsulated powder (III) (MD +GA)

Encapsulated anthocyanins powder (GA + MD) was used for colouring jelly. Material to ready made jelly powder ratio was 1:1. The resultant jelly is shown in Figure 12. The stickiness of the products could be reduced by using encapsulated powder.

Figure12: Colouring of jelly

Conclusion

An attempt has been made to extract anthocyanins as natural food colourant from Roselle calyces using 2 % citric acid solution and to prepare encapsulated powders (I, II, III) and to be applied in colouring of jelly. From the results of some physicochemical properties, the encapsulated powder III of (MD + GA) had medium moisture content, hygroscopicity value, solubility in water and colour density compared to other encapsulated powders of sample I and sample II. Moreover, SEM micrograph of MD + GA indicated the better quality microcapsules, which means higher encapsulation efficiency. It also had a longer dissolution time compared to others. According to FDA result, powder III (MD + GA) did not contain synthetic dyes. Due to perceived safety, this powder can be a good substitute for artificial colours causing harmful effects for consumers. Storage of the encapsulated powder (III) in

refrigerator in the absence of light was the best condition for its stability whereas sunlight was found to be unsuitable condition. Therefore the storage temperature and light conditions are very important for natural food colourant.

With regard to natural sources of the raw material for natural colourants, Myanmar is rich with coloured plants which are available year-round. It is believed that the simple and effective technology of this research work may initiate to produce the commercial availability of natural food dye in Myanmar.

Acknowledgements

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