P_38

Encapsulation of Anthocyanin from Mamao (*Antidesma thwaitesianum* Mull. Arg.) by using Freeze Dry Technology

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Abstract

Introduction: Mamao (*Antidesma thwaitesianum* Mull. Arg.) has the high anthocyanin content which is one of the most important group of water-soluble and vascular pigment in nature. This phytochemical is interested in food and health products, due to the wide range of biological activities including antioxidant, anti-inflammatory and activation of immune system. However, the used of anthocyanin from fruit extract had low stability during processing and storage. The goal of this research was to prepare and characterize the encapsulated anthocyanin from Mamao by freeze dry technology. **Methods:** The fruit extract (1.0 g) was added to 89 g of aqueous solution of 10 % maltodextrin (Wall material I) and 10% combination between maltodextrin and gum arabic (Wall material II). The mixture was homogenized and frozen using freeze dryer. The physicochemical properties were characterized on scanning electron microscope, encapsulation efficiency (EE), encapsulation yield (EY), pH, color, moisture content and solubility in water and ethanol. **Results:** The results demonstrated that the total anthocyanin content could be loaded to the wall materials and EE was in the range of 90 – 100 %. The EY was in the range of 90 – 100 %, indicating the encapsulation process did not influence on the loss of active ingredients. **Conclusion:** The encapsulation processes of fruit extract with suitable wall material could be applied to create a new innovation for modifies the efficacy and stability of fruit extract. The efficacy and stability of encapsulated powders in accelerated condition (40 ^{0}C, 75 % RH. for 6 months) will be further investigated.

Keywords: anthocyanin, encapsulation, freeze dry technique, mamao, health products.

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1. Introduction

Anthocyanin as a natural pigment was obtained from roots, leaves, fruits and flowers of plants. Attractive color and functional properties of anthocyanin make them a good substitute for synthetic pigments in the food and pharmaceutical industries, due to the wide range of biological activities included antioxidant, anti-inflammatory, anti-cancer and activation of immune system (Khazaei, *et al*., 2014; Statos, *et al*., 2013; Wang and Mazza, 2002). Mamao *(Antidesma thwaitesianum* Mull. Arg.) is a tropical fruit in Northeast Thailand and its fruits are used for soft drink and healthy food (Figure 1). Its fruits would be used for artificial colorants, due to their colors varying from red to blue, water solubility and non-toxicity. The fruit extract from mamao had many bioac tivities i.e., antioxidant, anti-inflammation, antican cer, activation of immune system and increased the cardiovascular system. It has been an increased interest in development of food colorants from natural sources as alternatives to synthetic dyes because of legislative action, consumer concern and green technology (Ge, *et al*. 2009; Markasis, 1974; Hummer, *et al*., 2002; Tsai, *et al*., 2002 Mazza, *et al*., 1993; Narayan and Venkataraman, 2000). Possibility of the usage of Mamao as a natural colorant in the production of food, nutraceuticals and pharmaceuticals has been also studied. Nevertheless, the utilization of these pigments in food and health products have been hampered by their poor stability, resulting from the physical and chemical factors such as temperature, pH, light, solvent and the structure of the pigment itself (Malien-Aubert, *et al*., 2001; Khazaei, *et al*., 2014; Santos, *et al*., 2013).

Figure 1 Botanical characteristic of *Antidesma thwaitesianum.*

Encapsulation is a technology that is used for protection, stabilization, and slow release of core materials. There are several techniques and wall materials that are available for encapsu lation of natural food colorants to overcome their instability, solubility, and handling problems. It can be an interesting alternative for the replacement of the artificial colorants by anthocyanin in the food and health products, acting as a protector coat against ambient adverse conditions. It is the advantage method to provide the stability of active ingredients such as: to prolong the degradation of active ingredients during processing and storage, to protect the degradation of color to guard against light induced reactions and/or oxidation, to increase the shelf-life of products. The encapsulation processing of sensitive compounds consists of two steps: the first is often emulsification of a core material with a dense solution of a wall material such as a polysaccharide or a protein and the second step is drying or cooling of the emulsions (Malien-Aubert, *et al*., 2001; Khazaei, *et al*., 2014; Santos, *et al*., 2013; Ersus and Yurdagel, 2007).

Therefore, the purposes of this study were production of freeze dried anthocyanin extract from Mamao *(A. thwaitesianum* Mull. Arg.) with different compounds of wall materials and to characterize the physicochemical properties of encapsulated products such as encapsulation efficiency, encapsulation yield, pH, color, moisture content, solubility in water and ethanol and scanning electron microscope (SEM).

2. Materials and Methods

2.1 Materials

Maltodextrin (10 DE) was obtained from A.E. Staley Manufacturing Company, USA while gum arabic was obtained from Sigma-Aldrich (USA). Mamao was obtained from agricultural department Thailand Institute of Scientific and Technological Research, Prathumthani, Thailand.

2.2 Encapsulation of Mamao *(A. thwaitesianum* Mull. Arg.)

The encapsulation of Mamao was included the mixture process and freeze drying. Mixture: The fruit extract of Mamao (1.0 g.) was added to 89 % of an aqueous solution of 10 % maltodextrin (Wall material I) and 10% combination between maltodextrin and gum arabic (Wall material II) as showed in Table 1 The mixture was homogenized at 2500 rpm for 10 min. Freeze drying: The mixture was frozen at -20 °C. The freeze dryer Dura-top (FTS system, Store Ridge, NY) was operated at a pressure of 50 mtorr. The temperature was increased stepwise as follow: 24 hr. at -35^{0}C, 18 hr. at -5 ^{0}C, 18 hr. at 0 ^{0}C and finally 12 hr. at 5 ^{0}C. A dried mixture was obtained and ground as previously to provide a powder.

Table 1 Compositions of encapsulated anthocyanin with different wall materials.

Types of encapsulating agents	Ingredients	grams
I	Fruit extract	1
	Moltodextrin	10
	H_2O	89
II	Fruit extract	1
	Moltodextrin	6
	Gum Arabic	4
	H_2O	89

2.3 Solubility in water and ethanol

The solubility in water and ethanol of encapsulated powders were determined by a method adapted from Bertan, et al. (2005) and Tonon, et al. (2008). The samples were accurately weighed (5.0 g) to give the dried powder (W_0). The powder put into test beakers with 100 ml distilled water or ethanol. The samples were dissolved and shaken under constant agitation at 180 rpm for 24 h at 25 ^{0}C. The remained of the powder were then filtered and dried in a hot air oven at 70 ^{0}C until a final constant weight was obtained (W_1). All samples were performed in triplicate. The percentage of solubility was calculated from the weight difference of the filter paper that was dried at 70 ^{0}C before and after filtration using Eq. (1):

$$\text{Percentage of solubility} = \frac{(W_0 - W_1)}{(W_0)} \times 100 \quad \text{.....(1)}$$

where W_0 and W_1 were the accurate dried weights before and after the test, respectively.

2.4 Moisture content

The samples were accurately weighed (1.0 g, W0) and dried by loss on drying measurement (W1) as adapted from Soradech et al., (2012). The temperature of drying was 105 0C. The weights of the sample before and after drying were calculated for the water contents. All samples were performed in triplicate. The water content was determined as the percentage of water in the powder using the following Eq. (2)

$$\text{Water content (WC)} = 100 \times (W_0 - W_1)/W_0 \quad \text{... (2)}$$

2.5 pH: The pH of samples (1% solution) was measured by using pH meter (Ph 700, Germany).

2.6 Color measurement

For the qualification of the color a La hunter system was used, which consists of a rectangular coordinates system for the definition of color in terms of luminosity (L*), red versus green (a*) and yellow versus blue (b*). The qualification of the color of the samples was carried out by the direct reading of the reflectance of the coordinates L*, a* and b*, using a Jobin-Yvon U 1000 double monochromator coupled to a GaAs photomultiplicator and to a conventional photon counter, As a stamdard, the standard illuminant A, incandescent light, was used. All samples were performed in triplicate.

2.7 Encapsulation efficiency (EE) and Encapsulation yield (EY)

The EE and EY of samples were calculated according to Eqs (3) and (4), respectively based on the total anthocyanin content (TA) and surface anthocyanin content (SA). Determination of TA was followed from Ersus and Yurdagel, (2007) and Robert, et al., (2010). Initially, dried powder samples (1.0 g.) were dissolved in 80 ml of distilled water and then sonicated for 5 min. After solubilization, the sample was also filtered by using Whatman No. 1 and was extracted with 50 ml of dichloromethane. The supernatant was evaporated with the rotary evaporator and then adjusted the volume to 25 ml with 0.01 % hydrochloric acid. The samples was determined the TA

by measuring absorbance at 530 nm with UV-Vis spectrophotometer. All samples were performed in triplicate. The determination of SA was adap ted from Ersus and Yurdagel, (2007) and Robert, *et al*., (2010): At initial, 5.0 g of samples were dispersed in 80 ml of methanol. The dispersions were agitated in a Vortex at room temperature for 5 min and then filtered (Whatman No.1). The supernatants were evaporated with the rotary evaporator and then were extracted with 50 ml of dichloromethane. The samples were adjusted the volume to 25 ml with 0.01 % hydrochloric acid. The samples were determined the SA by using spectrophotometer at the 530 nm. All samples were performed in triplicate.

$$\% \text{ EE} = \frac{(\text{TA} - \text{SA}) \times 100}{\text{TA}}$$

$$\% \text{ EY} = \frac{(\text{TA}) \times 100}{\text{TC}}$$

where TA was the total anthocyanin content of encapsulate product, SA was the surface anthocyanin content of encapsulate product, while TC was the total anthocyanin content of fruit extract.

2.8 Scanning electron microscopy

The morphology structures of the encap sulated powders were evaluated by S-360 scan ning electron microscope (Cambridge, England). Powders were attached to SEM stubs using a 2-slided adhesive tape and left in desiccators containing phosphorous pentoxide for 48 hr. The samples were coated with gold under vacuum before examination. SEM was operated at an accelerating voltage of 8.80 kV.

2.9 Statistical analysis

One-way ANOVA test was used for determination of differences between process with SPSS 12 package program. A probability level of $P < 0.05$ was considered to be significant for all statical procedures.

Results and Disscussion

The encapsulated powders of antho cyanin extracts were showed in Figure 2 The results indicated that the purple powders were obtained after freeze dried process. Results of color indicates (L*, a*, b*) in encapsulated powders immediately after production were revealed in Table 2 Both powders had a high value of color parameter a* which was attributed to high anthocyanin contents. The result was in agreement with author reports (khazaei *et al*., 2014). The color measurements of both encap sulated powders were nearby range between both wall materials (L* = 45.52, 38.92, a* = 9.57, 6.49, b* = -4.32, -4.28 between wall material I and II, respectively).

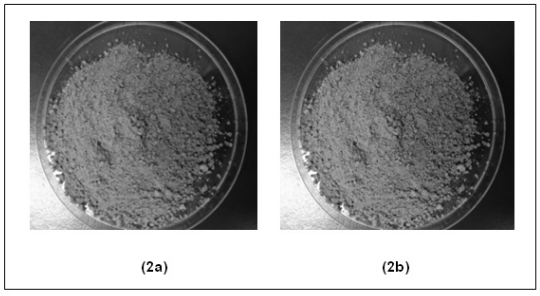


Figure 2 The appearance of encapsulated powders with different compounds of wall materials: Encapsulated powder I (2a) and Encapsulated powder II (2b).

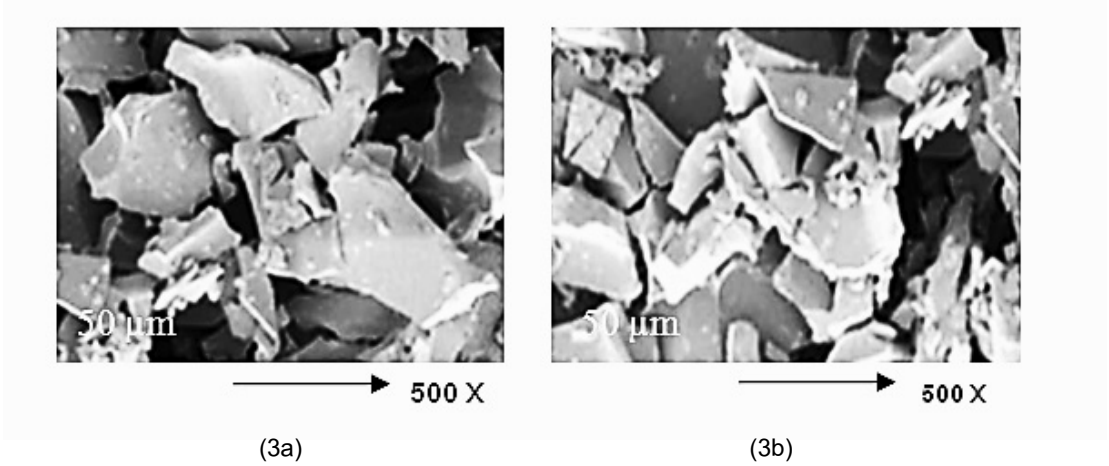
The physicochemical properties of encapsulated anthocyanin with different compounds of wall materials were shown in Table 1. The pH of encapsulated powders were in the range of 4.0 – 5.0 and the moisture contents were less than 3%. The lower moisture content of encapsulated powders was due to the lower hygroscopicity of maltodextrin. The hygroscopicities of maltodextrin and gum arabic were previous report (Khunthawad and Sripui, 2013). The solubility in the water of both encapsulated powders was more than 95% because of the hydrophilic properties of encapsulating wall material. The solubility in the water was in accordance with Khunthawad and Sripui, (2013) and Tonon, *et al*., (2008) which found that the solubility in water was increased with the increased the concentrations of maltodextrin. The solubility in ethanol of powders was less than 1%, indicating the encapsulated powder was lower dissolved in ethanol.

The TA and SA were used to calculate the EE and EY. The results showed that TA could be loaded into different wall materials and the EE was in the range of 90 - 100 %. The EY of powders was in the range of 90 – 100%, indicating the encapsulation process did not influence on the loss of anthocyanin content during freeze drying process.

Table 1 Physicochemical properties of encapsulated anthocyanin with different compounds of wall materials

Physicochemical properties	Encapsulated powder I	Encapsulated powder II
pH (1% solution)	4.75 ± 0.01	4.85 ± 0.01
Moisture content (%)	2.61 ± 0.01	2.76 ± 0.01
Solubility in water (%)	99.66 ± 0.30	99.23 ± 0.03
Solubility in ethanol (%)	0.45 ± 0.06	0.51± 0.05
Color		
L*	45.52 ± 2.74	38.92 ± 0.70
a*	9.57 ± 0.07	6.49 ± 0.11
b*	-4.32 ± 0.02	-4.28 ± 0.06
Encapsulation efficiency (EE, %)	98.85 ± 0.15	99.71± 0.21
Encapsulation yield (EY, %)	100 ± 0.00	91.09 ± 0.24

The EE of anthocyanin was confirmed by the morphological structures of powders as showed in Figure 3 The results indicated that the amorphous glassy shapes of powder were also reported during freeze dried solid materials, indicating the TA was entrapped in to the amorphous structure of both wall materials. The result was in agreement with TA of encapsulate powders when compared with TA of crude extract.



(3a) (3b)

Figure 3 Scanning electron microscope of encapsulated powders with different compounds of wall materials: Encapsulated powder I (3a) and Encapsulated powder II (3b)

4. Concusions

The encapsulation of anthocyanin extract obtained from *A. thwaitesianum* with freeze-drying technique and difference wall materials was successfully accomplished. The encapsulation efficiency and encapsulation yield of anthocyanin content into the different compounds of wall materials were in the range of 90 -100%. Different compounds of wall materials did not show any significant differences the total anthocyanin content of the powders. The efficacy and stability of active ingredients of both encapsulated powders in the accelerated condition at 40 ^{0}C and 75 % RH for 6 months will be further investigated.

5. Acknowledgements

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