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Encapsulation of anthocyanins from purple yam extract (*Dioscorea alata*, L.) flour using maltodextrin-whey protein isolate

S Tamaroh* and Y P Sari

Department of Agricultural Product Technology, University of Mercu Buana
Yogyakarta, Karanglo 55753, Indonesia.

E-mail: *tamaroh@mercubuana-yogya.ac.id

Abstract. Anthocyanins are antioxidant compounds that can act as anti-inflammatory, anti-viral, and prevention of diabetes. In Indonesia, many foods are rich in anthocyanin compounds, including purple yam tuber (*Dioscorea alata* L.). Anthocyanins are easily damaged by exposure to light changes in pH and temperature. Encapsulation can increase the nutritional value, color, shelf life, and bioavailability and stability of anthocyanin. The encapsulant such as maltodextrin and whey protein isolate were chosen because they were affordable and can protect the anthocyanin. Anthocyanin extract from purple yam flour were prepared to be incorporated in nanoencapsulation. Encapsulation were prepared with a ratio of maltodextrin and whey protein isolate = 1 : 3 (w/w) with anthocyanin extract of 5, 20 and 30%. Each formulas were dried by a spray drier. The results showed that the treatment using 30% anthocyanin extract resulted in the best nanoencapsulation. The encapsulation had anthocyanin content was 77.72 mg/100 g, total phenolic content was 510.07 mg GAE/100 g (db), antioxidant activity was 24.06 % RSA, color $L^* = 79.15$, $a^* = 5.58$, $b^* = -0.39$. Therefore, anthocyanin extract encapsulation can be produced successfully by this method.

1. Introduction

Yam (*Dioscorea* spp.) has become a staple food in countries worldwide, especially in Africa and South America. *Dioscorea alata* L. is one of the two most extensively cultivated species in Asia, apart from *D. polystachya*. The existence of consumer awareness regarding the provision of safe nutrition and the change in the concept of health from "passive treatment" to "active prevention" has made yam increasingly sought after [1]. The ease of cultivating yam and its high yield, even in non-staking conditions with storing fresh tubers for longer, make it superior in sustainable production and the right commodity to overcome hunger. Besides being an energy source, yam has bioactive compounds in traditional medicine and high therapeutic potential [2]. There are natural sources of anthocyanins in Indonesia, including the purple yam tuber (*Dioscorea alata* L.). Anthocyanins are antioxidant compounds that can act as inhibitors of heart damage, prevent diabetes, and have anti-inflammatory effects for bowel disease in the mouse model [3,4]. In comparison to other tropical root and tuber crops, *Dioscorea alata* has the highest anthocyanin content. The tubers contain up to 93.32 mg anthocyanin/100 g of dry matter [5]. The purple yam flour has 56.24 mg of anthocyanin/100 g of dry matter, even after storage under controlled conditions for 10 days [6]. Anthocyanin extraction from purple yam can also be carried out with methanol solvent containing 1% (v/v) tartaric acid [7].



Anthocyanins have limited stability and are easily damaged in certain environments. Temperature, pH, and light are the causes of easy damage to anthocyanins [8–10]. Due to the nature of easily damaged anthocyanins and cannot be directly applied to food products, it is necessary to make them into encapsulation. Encapsulation can increase the nutritional value, color, shelf life, bioavailability, and stability of anthocyanins. Increasing the strength of anthocyanins in encapsulation can expand their application in food systems, maintain the color of food ingredients, and increase absorption. The presence of encapsulation can also prevent interactions between anthocyanins and other food constituents so that they have the potential to be applied in foodstuffs with different matrix compositions [8].

Nanoencapsulation is the formation of a structure by biopolymer to protect active compounds, with a diameter ranging from 1-1000 nm. Nanoencapsulation has long been known to protect and control the release rate of active compounds in the body and increase the bioavailability of various bioactive compounds. There are several things to consider regarding anthocyanin nanoencapsulation. First, the selection a solvent to extract anthocyanin from its natural source. Solvents are not allowed to interfere with anthocyanin's physical and antioxidant properties. In this case, organic solvents are more recommended because of the high permeability of extraction in tissues, their affinity, and the high solubility of anthocyanins in these solvents.

Biopolymer, as an encapsulant material, has low toxicity and a relatively affordable price. This biopolymer can produce encapsulation in liposomes, nanospheres, nanocapsules, and nanoemulsions [11]. Maltodextrin is a low-cost encapsulant that dissolves quickly but has poor emulsification and protective properties. On the other hand, whey protein is a functional protein with outstanding qualities, but excessive use may result in discolouration [12]. The main factors related to the formation of this nanostructure include pH, temperature, stirring time, and the composition of the formula between the capsule and the bioactive compounds to be added. The other researcher discovered that successfully encapsulating Moringa leaf extract with maltodextrin and whey protein isolate (WPI) covering materials at a ratio of 1:3 resulted in significant total phenols [13].

The amount of encapsulated anthocyanin extract affects the components that make up the encapsulant, for example, phenol content and antioxidant activity. The encapsulation of anthocyanins from black onion extract was found that the more extract, the greater the antioxidant activity [14]. In this study, purple yam flour anthocyanin extract nanoencapsulants were prepared using encapsulant ingredients maltodextrin and whey protein isolate at a 1:3 w/w ratio, and concentrations of purple yam flour anthocyanin extract of 5%, 20%, and 30%. Thus, the purpose of this study was to assess the anthocyanin from purple yam flour's antioxidant activity when it was encapsulated by whey protein isolate and maltodextrin. The combination of maltodextrin and whey protein isolate can better protect the bioactive components and become a source of protein with the presence of whey protein so that the product can be used to improve the quality of life at an affordable cost.

2. Materials and methods

2.1. Materials

Purple yam from Sleman, Yogyakarta, served as the raw material. Whey protein isolate and maltodextrin from a local market. Aquadest, diethyl ether, DPPH (2,2-Diphenyl-1-picrylhydrazyl), ethanol, KCl, sodium nitric, sodium-acetic acid buffer, methanol, sodium hydroxide, Follin-Ciocalteu (Merck, Germany), sodium acetic, HCl, gallic acid (Sigma Chemical Co., St Louis, United States) were the chemicals.

2.2. Methods

2.2.1. Preparation of purple yam flour. Purple yam flour were produced according to [6]. The flour was stored in freezer before the analysis.

2.2.2. Anthocyanin encapsulation. Anthocyanin extract from purple yam flour was made by weighing 50 grams of purple yam flour and then dissolving it with 500 ml of 3% citric acid and 70% ethanol in a 500 ml beaker glass. The solutions were stirred for up to 30 minutes in the dark. Then, the solutions were stored in a cold temperature for up to 12-24 hours. After that, the solutions were filtered with Whatman filter paper no.02. The anthocyanin extracts were then concentrated using an evaporator Buchi R II (BÜCHI Labortechnik AG, Flawil, Switzerland) at a temperature of 50°C at a speed of 175 – 250 rpm.

The preparation of the purple yam anthocyanin sample followed [15]. The encapsulant materials were weighed with ratio maltodextrin:whey protein isolate (MD:WPI) 1:3 (w/w). Then the materials were dissolved with 250 ml of 0.1 M pH 3 citrate buffer. The solutions were stirred for 15 minutes at a speed of 400 rpm. After that, the concentrated anthocyanin extracts were poured at 5, 20 and 30% (w/w) and stir again for 15 minutes at 400 rpm. The citrate buffer pH 3 was added up to the mark in the 500 ml volumetric flask and stir for 15 minutes at 400 rpm. The samples were stored in the dark. The samples were ready to be dried into a mini spray dryer B-290 (Büchi Labortechnik AG, Switzerland), with an inlet temperature of 100 °C, an outlet temperature of 60-62 °C and a drying air speed (air flow) of 350ml/hour. The treatments or formulas in this study can be seen in Table 1.

Table 1. The formulas of purple yam flour anthocyanin extract encapsulation

Treatment	Composition
Treatment 1	Maltodextrin-WPI (1:3) + 5 % anthocyanin extract
Treatment 2	Maltodextrin-WPI (1:3) + 20 % anthocyanin extract
Treatment 3	Maltodextrin-WPI (1:3) + 30 % anthocyanin extract

2.2.3. Determination of moisture content. After oven drying at 105 °C until a consistent weight is attained, the weight loss of the powder was calculated to determine the moisture content of spray-dried encapsulations [16]

2.2.4. Determination of total anthocyanins. The technique suggested by [16] was used to analyze the total anthocyanins in the samples.

2.2.5. Determination of total phenolic content. Gallic acid was used as the standard in the Folin-Ciocalteu technique method [17] to determine the total phenolic contents.

2.2.6. DPPH Method of free radical scavenging test. The ability of DPPH free radical scavenging was calculated in order to perform the antioxidant activity test. Approximately 0.2 ml of the sample and 3.8 ml of 0.1 mM DPPH solution were vortexed for three minutes. The absorbance was measured at 30 minutes using a spectrophotometer UV-VIS GENESYS 10uv (Thermo Fisher Scientific Inc., Göteborg, Sweden) at a wavelength of 517 nm [18]. Free radical scavenging ability was calculated and reported as a percentage (%) using the formula $\text{RSA} = \% \text{ Radical Scavenging Activity}$, which corresponded to the percentage of DPPH bleaching.

$$\% \text{ RSA} = 1 - (\text{absorbance of sample}) / (\text{absorbance of control}) \times 100\% \quad (1)$$

2.2.7. Color Measurement. The anthocyanin powder was analyzed for color using a NH310 High-Quality Portable Colorimeter (Shenzhen Threneh Technology Co., Ltd, Guangzhou, China) with CIELab scale (L, a, and b).

2.2.8. Statistical analysis. A fully randomized design was employed in this study. The factor was the addition of anthocyanin extracted from purple yam flour (5, 20 and 30%, w/w). Five replicates of the data were examined. Using IBM SPSS Statistics 24, the data were statistically tested with a 95% degree of confidence using the Duncan Multiple Range Test.

3. Results and discussion

3.1. Moisture content

Comparing the concentration of the other extracts, the moisture content of encapsulation in the 30% anthocyanin extract had the greatest, reaching 9.95%. Meanwhile, by using 5 and 20% extract, the encapsulation powder had 8.72 and 7.33%, respectively. This result was still higher than the other study, which was only 4.48% in paprika and cinnamon oleoresin encapsulation using a ratio of 1:3 maltodextrin and whey protein isolate [19]. The moisture content of food powder is <6% with water activity below 0.3 could give the excellent stability [20]. The increase in moisture content along the anthocyanin concentration occurs because the carrier agent's solid composition decreases when the anthocyanin concentration rises. As a result, total humidity increases even after spray drying [21]. The high moisture content at a 5% anthocyanin concentration of 8.72 mg/100 g dry weight was possible due to water absorption from the environment.

Table 2. The moisture content of anthocyanin extract encapsulation

Anthocyanin extract (%)	Moisture content (%)
5	8.72 ± 0.10^b
20	7.33 ± 0.08^a
30	9.95 ± 0.20^c

Note: A significant difference is indicated by a distinct superscript letter in the same column ($p < 0.05$)

3.2. Anthocyanin content

The results of the anthocyanin levels in preparing encapsulation of anthocyanin extract from purple yam flour with the encapsulant material maltodextrin: whey protein isolate (1:3) as shown in Table 3. In this study, the addition of 30% anthocyanin extract produced the highest anthocyanin levels (77.72 mg/100 g db), which was more significant than other treatments ($p < 0.05$). The anthocyanin content of 5 and 20% extract in encapsulation were 16.56 and 48.99 mg/100g, respectively. It showed that the greater the anthocyanin extract added, the greater the anthocyanin content.

Table 3. The anthocyanin contents of anthocyanin extract encapsulation

Anthocyanin extract (%)	Anthocyanin content (mg/100 g (db))
5	16.56 ± 0.67^a
20	48.99 ± 7.27^b
30	77.72 ± 9.55^c

Note: A significant difference is indicated by a distinct superscript letter in the same column ($p < 0.05$)

This result was similar by [14], who carried out anthocyanin encapsulation in black garlic, which stated that with the chitosan-alginate encapsulant material with 15 mg/ml anthocyanin extract, anthocyanin levels of 78.82 mg/100 would be obtained. The other study also found that encapsulated anthocyanins from black rice with chitosan alginate and 30 mg of anthocyanin extract which was more effective than 10 and 20 mg of anthocyanin extract [22]. Therefore, the encapsulation was widely potential to store the anthocyanin. The quite high anthocyanin levels in the encapsulated extract of 30%, even though it has gone through spray drying, can occur because the initial anthocyanin levels of fresh yam are already high due to pretreatment. The steamed yam in this study was similar to the treatment of the other study who carried out steam blanching increased anthocyanin levels from 36.09 to 57.28 mg/100 g dry weight [23]

3.3. Antioxidant activity and total phenolic content

Table 4 demonstrates that the overall phenol concentration increases with the amount of anthocyanin extract applied. Compared to the addition of 5% and 20% anthocyanin extract, the total phenol concentration in the anthocyanin extract was 30% w/w (510 mg GAE/100 g dry weight). The amount of total phenolic compounds increases with increasing antioxidant activity [24]. Based on the results shown in Table 4, the total phenolics in the encapsulated samples with 5, 20 and 30% anthocyanin extract were 422.96; 478.66; and 510.07 mg GAE/100g. These results have the same trend as the antioxidant activity in the three samples. This antioxidant activity analysis was based on the free radical scavenging test, the results of which are expressed as % Radical Scavenging Activity (RSA). The respective antioxidant activity values are 4.84; 20.40 and 24.06%RSA. The amount of anthocyanin extract utilized increased along with the test findings for antioxidant activity and total phenolics. There is a strong 98% association between total phenolics and antioxidant activity. The second study discovered that there was a 75% association between total phenolics and antioxidant activity. Therefore, phenolic chemicals affect the evaluated materials' antioxidant activity [25].

Table 4. Total phenolic content and antioxidant activity of anthocyanin encapsulation

Extract anthocyanin (%)	Total phenolic content (mg GAE/100 g (db))	Antioxidant activity (% RSA)
5	422.96 ± 8.94 ^a	4.84 ± 0.69 ^a
20	478.66 ± 15.81 ^b	20.40 ± 2.19 ^b
30	510.07 ± 6.62 ^c	24.06 ± 0.72 ^c

Note: A significant difference is indicated by a distinct superscript letter in the same column (p<0.05).

3.4. Color characteristics of anthocyanin encapsulation

A food's degree of consumer acceptance is significantly influenced by its color. Figure 1 displayed the appearance of the powder sample that was encapsulated. Values of L, a, and b are the parameters that are employed. The value for the letter L* indicates a change in brightness or lightness [26]. The treatment with the addition of greater anthocyanin extract from purple yam flour resulted in a lower L* value, meaning the nanoencapsulant became darker in color. The darker the color, the greater the anthocyanin and phenol compound components. At a glance, the greater the amount of anthocyanin extract added, the color of the sample will have an increasingly red intensity. This result was in line with the trend of the resulting parameter a values. Samples with 20 and 30% anthocyanin extract had significantly greater a values (p<0.05) than samples with 5% extract. Red is shown as having a chromatic color by the a* value. The 20% and 30% extract treatments in this investigation had a* values higher than the 5% extract treatment. The increasing in red color is due to an increase in anthocyanin levels [27]. The degree of yellowness or blueness is indicated by the b* value. A degree of yellowness is indicated by a positive b* value, while a degree of bluishness is indicated by a negative b value. In addition to purple and blue, anthocyanins can also generate red. Spray-dried jussara extract produced similar outcomes, with a high value for the a* parameter that was connected to anthocyanin concentration [28]

Table 5. Color characteristics of anthocyanin encapsulation

Extract anthocyanin (%)	L*	a*	b*
5	83.09 $\pm$ 0.15 ^a	2.36 $\pm$ 0.01 ^a	2.32 $\pm$ 0.02 ^a
20	78.93 $\pm$ 0.20 ^b	5.53 $\pm$ 0.04 ^b	-0.73 $\pm$ 0.04 ^b
30	79.15 $\pm$ 0.20 ^b	5.58 $\pm$ 0.13 ^b	-0.39 $\pm$ 0.02 ^c

Note: A significant difference is indicated by a distinct superscript letter in the same column ($p < 0.05$)

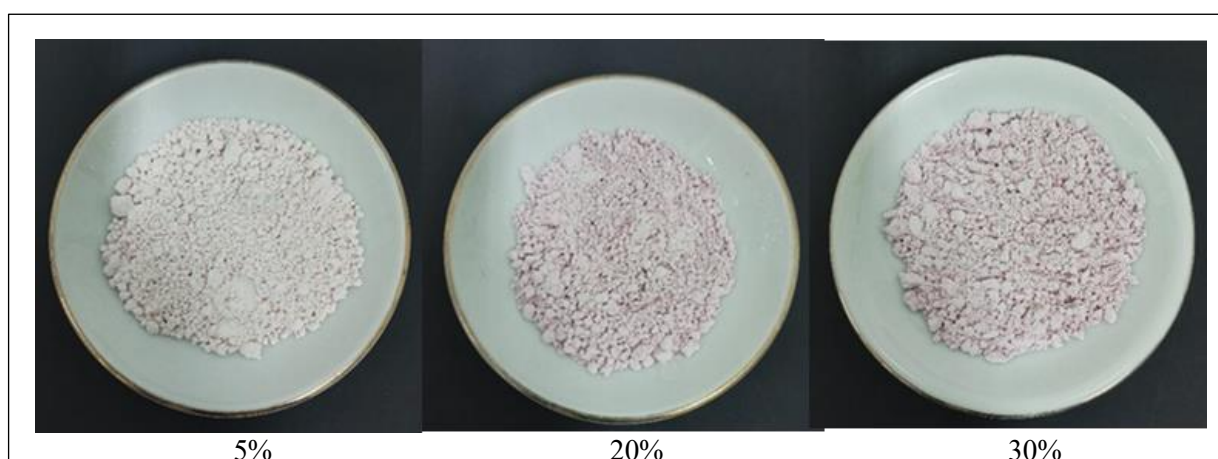


Figure 1. The encapsulation powder of anthocyanin extract from purple yam flour. The encapsulation containing 5, 20 and 30% of extract, respectively (from left to right). The maltodextrin and whey protein isolate at ratio 1:3 (w/w) was used as the encapsulants. The powder was produced by using spray-drier.

4. Conclusions

The primary innovation in the study was the anthocyanin-containing encapsulation made from flour made from purple yams (*Dioscorea alata* L.). Purple yam, or “uwi ungu” was a local tuber in Indonesia rich in anthocyanin. The results showed that the treatment using 30% anthocyanin extract resulted in the greatest anthocyanin content 77.72 mg/100 g (db), total phenolic content 510.07 mg GAE/100 g (db), antioxidant activity 24.06 % RSA, color L* = 79.15, a* = 5.58, b* = -0.39. Using maltodextrin:whey protein isolate (1:3, w/w) as the encapsulants, anthocyanin compounds from purple yam flour were successfully encapsulated. Further studies are needed regarding the composition of maltodextrin and whey protein isolate so that the powder has longer shelf life.

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