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# Antioxidant and Physical Characteristics of Anthocyanin Extract from Purple Yam (*Dioscorea alata* L.) Nanoencapsulation: Effect of Maltodextrin and Whey Protein Isolate Ratios

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**Abstract**

Anthocyanins are bioactive compounds with antioxidant, anti-inflammatory, and anti-diabetic activities. This bioactive compound is susceptible to changes in temperature and pH conditions. Its use in food products is also limited. Therefore, protection efforts that can bring anthocyanins into the food system through encapsulation are needed. Encapsulation is a technique for protecting bioactive compounds by coating the mixtures using biomaterials. The spray drying encapsulation process does not require difficult preparation and has good protection capabilities. The goal of this study was to investigate the encapsulating properties of the ratio of whey protein isolate to maltodextrin. This study used maltodextrin : whey protein isolate ratios (MD: WPI) 1:3, 1:1, and 3:1 (w/w). Anthocyanin extract was added 30% (w/w). The mixtures were powdered by drying them with a spray drier. The results showed that MD: WPI = 1:3 can retain more bioactive components than other treatments. The characteristics of nanoencapsulants in antioxidant activity, total phenol content, and anthocyanin were 65.16 ± 2.87 % RSA, 776.25 ± 45.23 mg GAE/100 g, 60.83 ± 1.56 mg/100 g, respectively. The nanoencapsulations had irregular round morphology, particle size and zeta-potential were 301.3 nm and -31.9 mV, respectively. Therefore, anthocyanin encapsulation from purple yam extract was successfully produced with lower-cost material for food and pharmaceutical use.

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Anthocyanin;
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## Introduction

There are various tubers in Indonesia, such as potatoes, sweet potatoes, yams, etc. One example of a tuber that is often used is purple yam or “uwi ungu” in Indonesia. Yam is a tuber that has unique characteristics. At first glance, there are

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many similarities between yam and sweet potato. However, yam is more prominent in size than sweet potato. Purple yam, or (*Dioscorea alata* L.) is a type of yam often used in the food sector. Its predominantly red-purple colour indicates that this fruit is rich in anthocyanins compounds amounting to 31-56.24 mg/100 g (db).[1,2] Therefore, the use of anthocyanins from purple yam is interesting to study. Unfortunately, anthocyanins are prone to damage, influenced by temperature, pH, light, oxygen and chemical structure.[3,4] The number and location of the hydroxyl and methoxy groups of ring B in the chemical structure of anthocyanins can influence the chemical stability of anthocyanins.[5] Anthocyanidin deteriorated by 30% when exposed to sunshine, while only 29.1% remained after 30 minutes at 70 degrees Celsius.[6] High temperatures above 40 °C result in glycosylation, hydration reactions, polymerization, etc. In addition, anthocyanin pigments will fade when exposed to light because they can absorb visible light.[7] Anthocyanins are predominantly red in color with the dominant structure being flavylium cations at pH 1, then fade and turn yellow at alkaline pH. Degradation occurs at alkaline pH due to transformation and hydration of flavylium cations.[8] Combining different wall materials provides better protection for anthocyanin encapsulation than single wall materials. The addition of whey protein to maltodextrin (MD), β-cyclodextrin (CD), and/or gum Arabic improves the thermal stability of blueberry anthocyanins.[9] Anthocyanin from red raspberry encapsulation also showed stability at high temperatures using a combination of wall materials of 2.5% soy protein isolate (SPI) and 2.5% gum arabic compared to soy protein alone.[10] Thus, strategies are needed to protect these compounds to be utilized better and improve anthocyanin stability, bioavailability, and color preservation through encapsulation.[6,8]

At least two main factors are essential in making anthocyanin nanoencapsulation. First, the solvent used for extraction of anthocyanins from natural ingredients. The extraction of anthocyanins from purple yam can be done with methanol solvent containing 1% (v/v) tartaric acid.[2] The presence of acid content is known to produce better extracts. Second is the choice of coating material as the encapsulant. Coating ingredients consist of a group of polysaccharides (gum arabic, modified starch, maltodextrin, alginate, pectin, chitosan, carrageenan, cellulose, and its derivatives, and cyclodextrin), proteins (gelatin, whey protein, caseinate, soy protein, gluten casein and zein) and lipids (lecithin, medium-chain triglycerides and glyceryl).[8] Maltodextrin is a cheap encapsulant with a mild flavor that dissolves easily but has low emulsification and protection capabilities. Meanwhile, whey protein is a functional protein with sound capabilities, but it can cause discoloration when used in high amounts.[11] The combination of these two materials is known to be more effective in increasing polyphenol stability and antioxidant capability compared to single materials.[11–13]

Various studies have succeeded in encapsulating anthocyanin extracts. These anthocyanins are extracted from red cabbage, brown rice, blueberries, black soybeans, and grapes. The biopolymers used include a combination of whey protein isolate and pectin, carboxymethyl chitosan, lysozyme, etc. On average, it can produce anthocyanin encapsulation in sizes below 500 nm. Techniques commonly used in making anthocyanin encapsulation include emulsification, coacervation, nanoprecipitation, supercritical fluid, ultrasonication, and spray drying.[8] Spray drying or spray drying produces solid powder from a solution using a drying "chamber". The advantages of this technique are its easy, fast, reproducible application and efficient encapsulation rate. Researchers have widely used this technique to encapsulate bioactive compounds.[12,13] Spray drying remains the most essential approach for bioactive component microencapsulation. It enables the creation of easy-to-handle powdered microcapsules from liquid material in one simple and scalable operation.[14]

Until now, study regarding the encapsulation of anthocyanins from purple yam tubers has not been found. On the other hand, these anthocyanins need to be protected because they are easily damaged. This research will focus on the encapsulation of anthocyanins from purple yam extract by varying the ratio of maltodextrin (MD) and whey protein isolate (WPI) as the encapsulants. Then, the spray drier was chosen to produce the powder. The goal of the study was to explore the MD:WPI ratio affected the antioxidant activity and physical aspects of anthocyanin encapsulation from purple yam extract. The encapsulation results from this study use materials that are easily available and have relatively

lower production costs so that it can be used by the public to improve the quality of life.

## Materials and Methods

### Materials

The materials were purple yam from Sleman, Yogyakarta. The whey protein isolate (WPI) and maltodextrin (MD) from local market. The chemicals were aquadest, citric acid, sodium citrate, ethanol, HCl, KCl, acetic acid, 2,2- Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), sodium carbonate, Folin-Ciocalteu (Merck, Germany), sodium acetate, and gallic acid (Sigma Chemical Co., St Louis, United States).

### Methods

#### Preparation of Purple Yam Flour

The purple yam flour was made in accordance with earlier studies.[15] The purple yam was first peeled, cut into slices, and crushed for eight minutes. It was then dried for ten hours at 50 °C in a cabinet drier until it had a moisture content of 10%. An 80-mesh sieve was used to grind the dry materials into a powder. The acquired purple yam flour was prepared to continue analysis.

#### Anthocyanin Encapsulation

#### Anthocyanin Extract Preparation

Anthocyanin extract was made by weighing 50 grams of the purple yam flour and then dissolving it with 500 ml of 70% ethanol containing 3% citric acid.[16] Ethanol was firstly prepared in water to achieve 70% ethanol. Then, the amount of citric acid was dissolved in 70% ethanol to obtain 70% ethanol containing 3% citric acid. The solution was stirred for up to 30 minutes and protected from the light. The solution was stored for 12-24 hours at 8-10 °C. Following that, Whatman filter paper no. 02 was used to filter it. A rotary evaporator or rotavapor Buchi R II (BÜCHI Labortechnik AG, Flawil, Switzerland) was used to concentrate the anthocyanin extract at a temperature of 50 °C and a speed of 175–250 rpm.[17]

#### Encapsulation of Anthocyanin Eextract

The encapsulation of anthocyanin extract according to the previous research[18] with some modifications. First, MD and WPI were weighed in the following ratios: 1:3, 1:1, and 3:1 (w/w) (Table 1). The mixtures were dissolved with 250 ml of 0.1 M pH 3 citric buffer and put it in a 500 ml measuring flask, and stir with a stirrer for 15 minutes at a speed of 400 rpm. After that, the concentrated anthocyanin extract was added to the system up to 30% (w/w) followed by stirring for 15 minutes at 400 rpm. The citric buffer pH 3 were poured up to the mark in the 500 ml volumetric flask and stirred again for 15 minutes at 400 rpm. The samples were protected to the light until spray drying process. The spray drier was set up with an inlet temperature of 100 °C, an outlet temperature of 60 to 62 °C, and a drying air flow of 350 mL per hour.

**Table 1: The maltodextrin/whey protein isolate ratio as encapsulants of purple yam flour anthocyanin extract**

| Ratio MD: WPI (w/w) | MD (g) | WPI (g) | Anthocyanin extract (g) | Final volume (ml) |
|---|---|---|---|---|
| 1:3 | 18.75 | 56.25 | 150 | 500 |
| 1:1 | 37.5 | 37.5 | 150 | 500 |
| 3:1 | 56.25 | 18.75 | 150 | 500 |

Note: MD and WPI referred to maltodextrin and whey protein isolate, respectively.

### Analysis Methods

#### Analysis of Total Anthocyanins

Total anthocyanins were measured using the technique suggested by.[19]

#### Analysis of Total Phenolic Content

The Folin-Ciocalteu technique was used to determine total phenolic levels with gallic acid as the standard.[20]

#### Analysis of Antioxidant Activity

For the antioxidant activity test, the DPPH free radical scavenging capacity was determined.[21] The scavenging capacity of free radicals was determined and reported as a percentage (%) RSA = % Radical Scavenging Activity, which was the percentage of DPPH bleaching. The equation was % RSA=1-(absorbance of sample)/(absorbance of control) X 100%.

#### Scanning Electron Microscopy (SEM) Observation

The morphological and surface structures of anthocyanin encapsulations were observed using SEM JEOL JSM-6510LA, auto Coater JEOL JEC-3000FC Auto Fine Coater.

**Particle Size**

A particle size analyzer (Horiba scientific SZ-100) by dynamic light scattering was used to determine the particle size, polydispersity index and zeta-potential of the anthocyanin encapsulations.

**Statistical Analysis**

The research used a completely randomized design with the encapsulants of MD: WPI (1:3; 1:1 and 3:1, (w/w)). The data were analyzed statistically by Duncan New Multiple Range Test (DMRT) at the 95% degree of confidence using IBM SPSS Statistics 24.

**Results**

**Antioxidant Activity And Total Phenol Content**

Table 2 shows the effect of MD and WPI ratios on anthocyanin encapsulation antioxidant activity. The antioxidant activity of anthocyanin encapsulants in this study vary from 35-65 %RSA. Encapsulation with MD:WPI (1:3) showed the highest antioxidant activity compared to other treatments. This result has a similar trend with the anthocyanin content (Table 2). Align with the results, the total phenol concentration was similarly affected by the encapsulant material ratios ($p<0.05$). The MD:WPI (1:1) treatment had a total phenol content of 868.44 mg GAE/100 g wb, the highest compared to the MD:WPI (1:3 and 3:1) treatments.

**Anthocyanin Content**

Table 2 also revealed that the anthocyanin levels differed significantly ($p<0.05$) among different encapsulant ratios. Anthocyanin levels in the MD:WPI treatment (1:3) was the highest (60.83 mg/100 g) compared to the two others. Meanwhile, the other nanoencapsulations containing 21-24 mg anthocyanin/100 g.

**Color Properties**

**Table 2: Antioxidant activity, total phenol content and anthocyanin content at the various ratio of encapsulants**

| MD:WPI ratio (w/w) | Antioxidant activity (%RSA) | Total phenol content (mg GAE/100 g) | Anthocyanin content (mg/100 g) |
|---|---|---|---|
| 1:3 | $65.16 \pm 2.87^{c}$ | $776.25 \pm 45.23^{b}$ | $60.83 \pm 1.56^{b}$ |
| 1:1 | $35.58 \pm 1.19^{b}$ | $868.44 \pm 13.39^{c}$ | $24.33 \pm 2.70^{a}$ |
| 3:1 | $41.09 \pm 1.99^{a}$ | $687.30 \pm 3.78^{a}$ | $21.63 \pm 1.35^{a}$ |

Note: the similar superscript in the identical column showed that there is no substantial difference ($p>0.05$).

**Table 3: Color properties of anthocyanin nanoencapsulation at different ratio of encapsulants**

| MD : WPI ratio (w/w) | L* | a* | b* |
|---|---|---|---|
| 1:3 | $78.13 \pm 0.18^{a}$ | $9.54 \pm 0.12^{c}$ | $-0.39 \pm 0.05^{a}$ |
| 1:1 | $79.76 \pm 0.32^{b}$ | $6.45 \pm 0.11^{b}$ | $-0.82 \pm 0.03^{b}$ |
| 3:1 | $79.72 \pm 0.16^{b}$ | $5.12 \pm 0.12^{a}$ | $-1.85 \pm 0.04^{c}$ |

Note: the similar superscript in the identical column showed that there is no substantial difference ($p>0.05$).

**Morphology Properties**

In line with Figure 1, anthocyanins nanoencapsulation by using MD:WPI (1:3) had irregularly round with several depressions that were not too deep. The MD:WPI (1:1) encapsulant treatment showed an irregular round shape with deeper depressions. Meanwhile, the MD:WPI (3:1) encapsulant treatment showed an irregular round shape with deeper, more

numerous depressions and some torn parts. The desired product morphology is tight, wrinkled and without cracks because it allows fewer compounds or extracts to diffuse and degrade.[22] The result of the encapsulant in this study which had a tight shape and no cracks was the MD:WPI 1:3 encapsulant treatment.

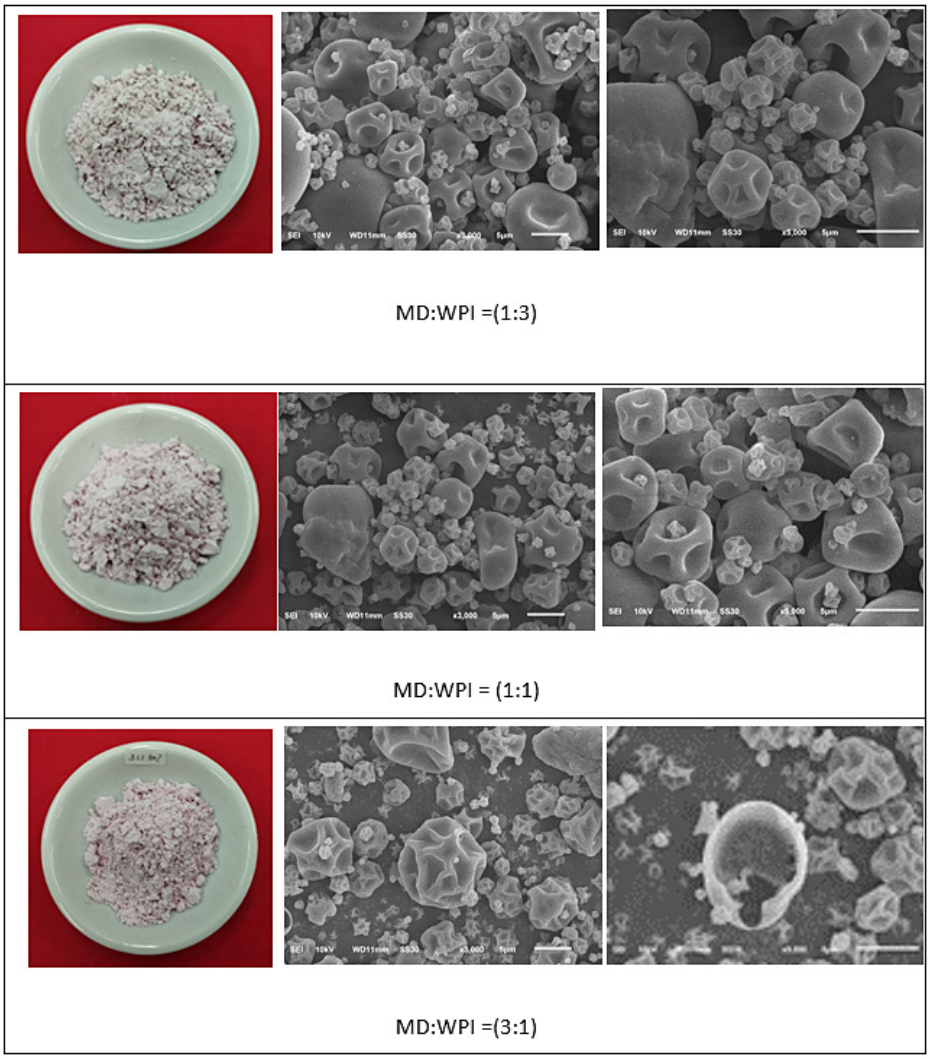


**Fig. 1: Anthocyanin nanoencapsulation (left) and the pictured that captured by the Scanning Electron Microscope of anthocyanin nanoencapsulation at the ratio of MD:WPI (1:3; 1:1 dan 3:1). The pictures were zoomed up to 3000 X (middle) and 5000 X (right)**

**Table 4: The particle size of anthocyanin nanoencapsulation**

| MD : WPI ratio (w/w) | Particle size (nm) | Polydispersity index | Zeta potential (mV) |
|---|---|---|---|
| 1:3 | 301.3 | 0.326 | -31.9 |
| 1:1 | 304.2 | 0.302 | -31.5 |
| 3:1 | 300.5 | 0.342 | -30.4 |

**Particle Size**

Table 4 showed the particle sizes of the samples were between 300.5 to 304.2 nm. The The desired product morphology is tight, wrinkled and without cracks because it allows fewer compounds or extracts to diffuse and degrade.22 size of the anthocyanin extract nanoparticles includes nanoparticles. The results of this research also showed that the zeta potential value ranges from -30.4 to -31.9, which is categorized as a stable nanoparticle.

**Discussion**

Table 2 shows the influence of maltodextrin and whey protein isolate ratios on antioxidant activity of anthocyanin encapsulation. The antioxidant activity of anthocyanin encapsulants in this study were significantly different ($p<0.05$) among the different ratio of encapsulants. Encapsulation with MD:WPI (1:3) showed the highest antioxidant activity compared to other treatments. This result had similar trend with the anthocyanin content (Table 2). Align with the results, the total phenol concentration was similarly affected by the encapsulant material ratios ($p<0.05$). The MD:WPI (1:3) treatment had the highest an antioxidant activity at 65.16 ± 2.87 (%RSA) and anthocyanin content (60.83 ± 1.56 mg/100 g), compared to the MD:WPI (1:1 and 3:1) treatments. Therefore, the more WPI will gave the more protection by these parameters. Total phenol content of encapsulants from MD:WPI (1:3) treatment was relatively high (776.25 ± 45.23 mg GAE/100g). Other researchers found that the encapsulation of grape seed extract phenolic compounds, showed that encapsulation with whey protein isolate and maltodextrin (4:1) will protect antioxidant activity more effectively than using maltodextrin or whey protein isolate itself.[23] Encapsulation of phenolic compounds in grape seed with whey protein–polysaccharide complex results in higher retention of phenolic compounds and antioxidant activity.[23] The use of whey protein isolate in the coating process is more effective in maintaining the bioactive content in the coated material.[24] Whey protein has good protection against oxidation compared to maltodextrin. However, combining whey protein with maltodextrin as a coating becomes more effective in the encapsulation process because maltodextrin can produce an amorphous glass matrix that acts as a barrier against oxidation.[25] Encapsulation treatment of purple grape extract with whey protein isolate and maltodextrin can maintain anthocyanin and antioxidant activity.[12] Protein has a good ability to package bioactive components.[26] Using maltodextrin and whey protein isolates in a 1:3 ratio in the encapsulation process proved a successful combination in encapsulating bioactive substances.

Table 2 also revealed that the anthocyanin levels differed significantly ($p<0.05$) among different encapsulant ratios. Anthocyanin levels in the MD:WPI treatment (1:3) was the highest (60.83 mg/100 g) compared to the two others. This research was in accordance with what was carried out by other researcher, encapsulating purple corn anthocyanins with a combination of maltodextrin and gum arabic encapsulants was better able to retain the anthocyanins compared to using maltodextrin encapsulation alone.[14] The other researchers also stated that encapsulating anthocyanin extract from jaboticaba pomace with a combination of maltodextrin, pectin and soy protein biopolymers would increase the stability of anthocyanins due to light exposure.[27]

In color parameters, the value for color L* shows changes in brightness or lightness. The results of statistical tests on lightness parameter (L*) in the research showed that it was significantly different. Encapsulation with maltodextrin:WPI (1:3) had the smallest value (78.13) (Table 3), which showed a darker color compared to other treatments. The dark color indicates the presence of a higher anthocyanin component compared to other treatments. This was in line with researcher who stated that on red onion ethanol extract with a low L* color test indicating high anthocyanin levels.[28] The chromatic color for red is shown by the a* value. In this investigation, the a* color value was statistically significantly different ($p<0.05$). The highest a* value was obtained when using the maltodextrin:WPI ratio (1:3). A high red a* color indicates a higher presence of anthocyanins. The b* value represents the blue chromatic color, in this study the b* color value also showed statistically different ($p<0.05$). In the maltodextrin:WPI (1:3) shows the highest b* color value. A decline in the b* color value indicates an increase in the blueness color value. This showed the presence of anthocyanin in the encapsulant of the results of this study. Therefore, the various ratios of encapsulants had effect on color parameter of anthocyanin

nanoencapsulation. The lower of lightness and the higher of a parameter indicated that the higher anthocyanin content in the sample.

Table 4 showed the particle sizes of the samples were between 300.5 to 304.2 nm. All samples were also in monodisperse. The size of the anthocyanin extract nanoparticles includes nanoparticles. Particle size is the most important characteristic in a nanoparticle system because it directly influences the unique properties of the nanoparticle. Nano particles are included if the particle size is less than 1000 nm.[29] Some researchers have been successfully produce nanoencapsulation. Encapsulation of black rice anthocyanins (*Oryza sativa* L.) with chitosan and alginate resulted in nanoparticles measuring 358.5 – 467.9 nm.[29] Other researcher found that the encapsulation of juwet seed extract (*Syzygium cumini*) showed a particle size of 395.9 – 820.67 nm.[30] Therefore, the encapsulation of anthocyanin extract from purple yam can be successfully produced into the nano-sizes by this method.

The zeta-potential of nanoencapsulations was assessed in addition to particle size and polydispersity index. The zeta potential is an electric charge parameter between colloidal particles. The higher the zeta potential value, the better it prevents flocculation.[30] A zeta potential value of less than −30 mV or more than 30 mV indicates particle stability.[31] The results of this research show that the zeta potential value ranges from -30.4 to -31.9, which was a stable nanoparticle.

## Conclusion

The best ratio of encapsulant for anthocyanin extract from purple yam flour nanoencapsulation was MDI : WPI (1:3). By using this ration, it can retain more bioactive components than two others. Therefore, the nanoencapsulation was potential to apply in food and beverage systems as the carrier of anthocyanin extract from purple yam flour.

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## Conflict of Interest

The authors do not have any conflict of interest.

## Authors' Contribution

**Siti Tamaroh:** extracting anthocyanin, making encapsulation with a spray drier, anthocyanin test, color test, Scanning Electron Microscope test of anthocyanin nan encapsulation, Compiling the manuscript.

**Yuli Perwita Sari:** evaporation of anthocyanin extract, total phenol test, antioxidant activity test, particle size test, data analysis.

## Data Availability Statement

Yes, the manuscript incorporates all datasets produced or examined throughout this research study.

## Ethics Approval Statement

This research did not involve animals or humans for testing

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