Microencapsulation of Natural Anthocyanin from Purple Rosella Calyces by Freeze Drying

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Abstract. Anthocyanin extract in powder form will improve its use since the powder is easier to store and more applicable. Microencapsulation method is introduced as an efficient way for protecting pigment such as anthocyanin. This research was aimed to characterise anthocyanin encapsulated products prepared from purple Roselle calyces by freeze drying. The liquid anthocyanin extracts from ultrasound-assisted extraction were freeze-dried with and without the addition of 10% w/w maltodextrins as a carrier and coating agents. The quality attributes of the powders were characterised by their colour intensity, water content, and solubility. Analysis of encapsulated material was performed for the powder added by maltodextrin. The stability of the microencapsulated pigment in solution form was determined for 11 days. Total anthocyanin content was observed through pH differential method. The results of the colour intensity analysis confirm that the product with maltodextrin addition has more intense colour with L* value of 29.69 a* value of 54.29 and b* value of 8.39. The result with the addition of maltodextrin has less moisture content and more soluble in water. It is verified that better results were obtained for powder with maltodextrin addition. Anthocyanin in the powder form with maltodextrin addition exhibits higher stability even after 11 days. In conclusion, the microencapsulation of anthocyanin with maltodextrin as a carrier and coating agent presented a potential method to produce anthocyanin powder from purple Roselle.

1. Introduction

Anthocyanins are easily found in many kinds of fruits, vegetables, flower, or leaves. Roselle flower is a source of anthocyanin that is easy to find in Indonesia; it could be used as a good source for producing red colourant for many foods. Anthocyanin has broad colour scheme from bright red, purple, red, dark red and dark blue. It is well known as harmless water-soluble pigments that provide red to the purple shade which makes them an important group of natural colourants [1]. Most colours used in the food industry are artificial from synthetic sources. Due to their harmful effects, there is currently an increasing trend towards applying natural dyes [2]. Anthocyanin also has side properties such as antimicrobial and anticancer that make it suitable as an alternative for synthetic dyes [3],[4].

Produce high-quality food colourant from the natural resource such as Roselle flower as an alternative for synthetic colourant is a new scientific challenge. The stability of anthocyanin is very susceptible to colour degradation. The degradation depends on various factors including the structure of anthocyanin, pH, temperature, oxygen, light, concentration, ascorbic acid, sugar, and water activity [5],[6]. The degradation started from the extraction process or storage [7],[8],[9].

Microencapsulation was offered as an efficient way to introduce natural compounds into food products. In microencapsulation process, anthocyanin as the core material is coated with thin film of...
wall materials that protect it from environmental disturbance. Encapsulated color is easier to handle and offer better stability. Microencapsulation with the addition of coating materials can be done with various techniques, include spray-drying [10],[11],[12], freeze-drying [3],[13],[14],[15], microwave-assisted technique [16]. Freeze drying is considered as the most suitable method to obtain high-quality dry pigment product, compared to other methods [13]. Various materials are used as coatings agent such as maltodextrin, Arabic gum, disaccharides, starches, chitosan-cellulose and many others [4],[13],[17], of which maltodextrin gave better physicochemical properties as colourant powder. The previous study has considering maltodextrin as an appropriate coating agent for food colourant product [5],[18],[19]. However, specific investigation of microencapsulation product of purple Roselle is limited.

The objective of the present study was to characterise anthocyanin encapsulated products prepared from purple Roselle calyces by freeze drying. Compare it with the character of non-encapsulated powder. Specifically, the effect of maltodextrin addition on the product characteristic was addressed. The analysis of powder stability in solution form was also conducted to predict its behaviour when applied as a colourant.

2. Materials and Methods
The study is consist of several phases which is anthocyanin extraction from Roselle calyces, preparation and microencapsulation of the liquid extract with maltodextrin using Freeze-dryer, analysis of encapsulation efficiency, physical properties characterization of the powders, and stability analysis.

2.1. Anthocyanin Extraction
Dry purple Roselle flower petals were bought from the Roselle garden in Blitar, East Java, Indonesia. Dried calyces were ground and sieved until 100 mesh in size then stored in the dry container. Distilled water as a solvent was supplied from Indrasari Chemical Store, Semarang. The ratio of solute and solvent are 1:15 w/w. The anthocyanin from the Roselle calyces was extracted by ultrasonic assisted extraction method, conducted at a frequency of 40 kHz for 30 minutes at room temperature. After extraction, the liquid extract was then filtered through a filter paper and stored in the refrigerator for not more than 6 hours.

2.2. Microencapsulation of Powders
Maltodextrin obtained from Multikimia Raya Chemical Store, Semarang, were used as wall material. Maltodextrin was dissolved in liquid anthocyanin extract at ambient temperature (25°C) to obtain 10% total solid concentration. The solutions were frozen in the freezer for 24 hours before drying by freeze dryer. The solutions were dried in a freeze dryer (Heto Powerdry LL 1500) for the next 24 hours (minus 100°C vacuum pressure). Dried materials were ground using a pestle and mortar stored in dark container in a freezer (−18°C) until usage. A blank sample was also prepared by the liquid extract without any addition of wall materials and then freeze-dried for 24 hours in similar conditions (-100°C vacuum pressure).

2.3. Materials Encapsulation Efficiency
The efficiency of microencapsulating process was determined by the Equation 1 [20],

$$\% \text{ EE} = \frac{\text{Total Mass} - \text{Surface Mass}}{\text{Total Mass}} \times 100\%$$ (1)

Where total mass is the total anthocyanin in sample and surface mass is the mass of anthocyanin in powder surface. Total mass of anthocyanin in the sample was obtained by dissolving 0.1 grammes of microencapsulated powder in 1 mL of aquadest. Ethanol anhydrate was added to extract it for 5 minutes before filtration [16]. Surface mass was calculated by washing 0.1 gramme of sample in 45
mL of ethanol anhydrate. After filtration, one mL aquadest was added into the filtrate and calculate the mass of the surface materials.

As for process yield, the equation state by Battista (2015) was used.

\[
\text{% EY} = \frac{\text{total anthocyanins in solid materials}}{\text{total anthocyanins extracted}} \times 100\% \tag{2}
\]

Both total anthocyanin extracted and total anthocyanin in solid materials was analysed by spectrophotometer using pH methods [21]. To find the amount of total anthocyanin Equation 3 was used.

\[
\text{Total Antosianin (mg/L)} = \frac{\text{Absorbance} \times 1000}{55.9 \times 1} \tag{3}
\]

2.4. Physical Properties Characterization of Powders

Anthocyanin powder with and without wall material is characterised by its physical properties (moisture content, solubility, and colour intensity) then to be compared. The moisture content of the powders was determined by AOAC (1995) oven method, and the solubility analysis was done by Kainuma method [22].

To investigate the effect of wall material addition on the colour of encapsulated powders, colour intensity analysis was conducted for both samples [23]. Colour intensity was analysed by Chromameter (Color Reader CR-400/410) based on Hunter’s Lab Colorimetric System. L* represent lightness (100=white, 0=black), a* represent the color of red (+) until green (-), b* represent the color of yellow (+) until blue (-). The other parameter considered as Chroma, Hue, and \(\Delta E_{ab}^{*}\), and it can be calculated by the equation,

\[
H_{ab} = \tan^{-1} \frac{b^*}{a^*} \tag{4}
\]

\[
C_{ab} = (a^{*2} + b^{*2})^{\frac{1}{2}} \tag{5}
\]

\[
\Delta E_{ab}^{*} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{\frac{1}{2}} \tag{6}
\]

By taking the value of L*, a*, and b* the colour can be visualised using Colour Converter from Nixsensor. The value will take a calculation to convert it into RGB (Red Green Blue), and the colour can be visualised to see with bare eyes.

2.5. Stability analysis

The stability analysis was done for the solution state of the powder. The powder sample is dissolved into aquadest by 10% of volume. The solution is stored in the closed dark bottle in the same room condition (25-30°C, 1 atm) for 11 days. Total anthocyanin is analysed for a period of time by pH methods using Spectrophotometry UV-Vis (UV Mini 1240 Shimadzu) [21]. KCl buffer solution with pH 1.0 and Na-Acetate buffer solution with pH 4.5 was used to dissolve the Roselle extract. The absorbance test was done at λvis-max and 700 nm wavelength from each solution. The visible-maximum wavelength for cyanidin-3-glucoside is 510 nm. The concentration (mg/l) anthocyanins were calculated according to the following Equation 7:

\[
\left(\frac{(A_{\lambda_{\text{max}}}-A_{700})_{\text{pH }1.0} - (A_{\lambda_{\text{max}}}-A_{700})_{\text{pH }4.5}}{\text{MW} \cdot 1000}\right) \cdot \frac{1}{\varepsilon \cdot l} \tag{7}
\]

Where A is the absorbance at each wavelength and pH, MW is the molecule weight (449.2 g/mol for cyanidin-3-glucoside, \(\varepsilon\) is the extinction coefficient (26900), and 1 is the path length of the cuvette in cm [24].
3. Results and Discussion

3.1. Encapsulated materials efficiency
Maltodextrin was used as wall material for anthocyanin extract from Roselle calyxes. By adding maltodextrin, it is expected that the pigment material will be protected from damage. Therefore the effectiveness of the micro-encapsulation process needs to be calculated. Encapsulation materials efficiency analysis shows the amount of anthocyanin encapsulated in the powder [11],[17],[20]. Encapsulation methods were said to be successful if it can achieve high retention of the core materials [4]. The physical characteristic of encapsulated materials should be better than the non-encapsulated ones.

The encapsulated powder with 10% of maltodextrin was analysed for its encapsulated materials efficiency and encapsulation process yield. From the calculation, we get that the encapsulated materials efficiency is 33.3% and encapsulation process yield of 56.9%. Showing that there are certainly anthocyanin materials encapsulated in the wall material. The previous research indicates that the addition of wall material will increase its encapsulation efficiency in microencapsulation of procyandin [15]. Wall materials like Arabic gum and maltodextrin also help to gives high encapsulation efficiency in microencapsulation of phytosterols by spray drying [11]. Other previous study states that encapsulation efficiency will be increased if greater carrier concentration was added, giving additional protection [18],[19].

3.2. The effect of maltodextrin to the water content and solubility of encapsulated anthocyanin
The correlation between the addition of maltodextrin with solubility and water content in the encapsulation material can be seen in the Table 1.

| No  | Powder                                | Water content | Solubility |
|-----|---------------------------------------|---------------|------------|
| 1.  | Rosella Powder with addition of 10% maltodextrin | 11.40%        | 24%        |
| 2.  | Rosella Powder without addition of maltodextrin | 18.20%        | 15%        |

Table 1 shows that powder with addition of maltodextrin has lower water content than the powder without any addition of maltodextrin. A prominent characteristic of freeze drying product is that it is very hygroscopic. The powder tends to absorb water from the environment either through absorption or adsorption [25]. This character is the result of drying methods in freeze-drying where the process of water content removal uses the subliming principle. The water in solid form is converted to gas at a vacuum pressure condition [26]. Microencapsulation process can protect sensitive materials such as pigment from moisture, heat, light or oxidation [27]. This is the reason why the encapsulated powder has lower water content. Maltodextrin has been used as wall materials in microencapsulation of anthocyanin from purple corn [8], blackberry by-product [14], saffron petal [28], Jaboticaba pomace [29], and encapsulation of phenolic from grape skin [18].

The powder used as wall materials must exhibit good solubility which is a device factor for the quality of the product. The solubility in water at 25°C was showing that the encapsulate powder has a higher solubility than non-encapsulate powder. This behaviour is because anthocyanin in the extract will show interaction with the carriers during the encapsulation process. The solubility of a freeze drying material is strongly influenced by the wall material. Maltodextrin is a polysaccharide with high polarity, so it is soluble in the water [29]. The interactions between anthoacynins and their carrier make anthocyanins powder also have the same properties as maltodextrin [30].
3.3. The effect of maltodextrin to the colour intensity of encapsulated anthocyanin powder

After freeze-drying, colour intensity of the samples were characterised by using Chromameter. The value of L*, a*, b*, C_ab, H_ab, and ΔE_ab from both powder sample are represented in Table 2.

| Parameter Value | 10% maltodextrin | without maltodextrin |
|-----------------|------------------|----------------------|
| L*              | 29.696           | 23.121               |
| a*              | 54.296           | 47.879               |
| b*              | 8.393            | 12.999               |
| C_ab            | 54.941           | 49.612               |
| H_ab            | 0.153            | 0.265                |

A high value of parameter a* is due to a significant amount of anthocyanin in the extract of Roselle calyxes [3]. Table 2 also shows a higher value of colour parameter a* in encapsulated powder than non-encapsulated powder. This result shows that encapsulated powder is redder than the other one. Maltodextrin as wall materials gives protection to the anthocyanin encapsulated in it. The previous study shows that maltodextrin has great protecting effect for anthocyanin stability [19] and reduce degradation of anthocyanin rather than naked anthocyanin in solution state [16]. The L* notation of the powder shows that powder encapsulated in maltodextrin is lighter than non-encapsulated powder. Maltodextrin is white flourish materials which have L* value of 98.1±0.15 with a really low value of a* and b* [32]. Since the anthocyanin is encapsulated inside the maltodextrin, it is natural that the colour of the powder is slightly lighter.

Chroma, representing colour intensity, which is the distance of a colour from the origin (a*=b*=0) in the a*, b* plane. Hue angle expresses in degrees form (0° to 360°), 0° being a location on the +a* axis which represent red colour. Then the value rotates anti-clockwise to 90° for the +b* axis to indicates yellowness, axis, 180° (green) for -a*, 270° (blue) for -b*, and back to 360° = 0°[12]. Table 2 indicates that the chroma value of encapsulated powder is greater than non-encapsulate powder. On the other hand, the colour of powder is more vivid. As for H_ab parameter value shows that the Hue is not significantly different. The H_ab values are less than 10° which indicates that the powder is considered red colour [33]. CIE total colour difference between the two powders was also calculated to have the number of the difference value. The calculation gives a result of 10.277 ΔE_ab this value is considered to be significantly different in colour [23].

The visualisation of the colour was considered by the colour converter from NixColorSensor. The value of L*, a*, and b* are recalculated to obtain the value of RGB (Red Green Blue). The visualisation of colour using RGB value is easy to find by many graphic programs such as CorelDraw, Photoshop, and many others. The value of RGB for the encapsulated powder is 141 0 60, as for non-encapsulated powder are 116 0 39. The visual colour presented in Figure 1

![Figure 1](image.png)

**Figure 1.** The colour visualisation of the powder, a. Encapsulated powder, b. Non-encapsulated powder
3.4. The effect of maltodextrin to the stability of encapsulated anthocyanin powder

The anthocyanin powder obtained from encapsulation process was dissolved in aquadest to obtained anthocyanin solution. The solution was left for 12 days and being observed in a certain time. The results were provided in Figure 2.

![Figure 2. The relation between storage time and total anthocyanin](image)

Figure 2 shows that anthocyanin solution obtained from powder without maltodextrin have fewer anthocyanin from the very beginning. The amount of total anthocyanin from both samples are getting lower every single day. The solution from encapsulated powder shows a stable trend of total anthocyanins degradation each day. In contrast, the solution from non-encapsulated powder shows a dramatic decline after nine days. After being stored for 11 days, both samples showed a significant decrease in a total of anthocyanins. The solution from non-encapsulated powder exhibits 91.61% of anthocyanin lost from day one until day 11, While the solution from encapsulated powder showed a fewer decrease of 82.53%. This result indicates that addition of maltodextrin as wall materials affected the stability of anthocyanin in dissolved powder as the encapsulated anthocyanin gives more stable condition compared to non-encapsulated anthocyanin.

Degradation of anthocyanin pigment causes the phenomena of decline in the total anthocyanin content. Degradation often occurs in almost all kind of pigment, such as anthocyanin. The previous research shows that degradation of pigment also happens in red pigment from Roselle [5],[34], anthocyanins from red onion [35], and colour degradation in vitamin C fortified cranberry juice [36]. The degradation often occurs starts from the extraction and processing of anthocyanin was done [9],[35]. Maltodextrin as the wall materials could indicate to have the capability to protect the structure and the function of molecules activity thru the drying and storing process [37]. That can be the reason why total anthocyanin of solution from non-encapsulated powder is fewer than the encapsulated one from the beginning. In solution state, maltodextrin reduces the degradation of anthocyanin rather than the non-encapsulate one. The same result also shows in previous research in Roselle extract colourant in a drink [23].

4. Conclusion

The addition of maltodextrin in encapsulation process provided better results than encapsulation process without maltodextrin. The encapsulated anthocyanin powder shows better physical properties with lower water content and higher solubility. It also gives more vivid red colour with higher Chroma value. The stability analysis also shows that encapsulated anthocyanin powder is more stable in solution form, indicate that the powder has better capability to apply as a food colourant. Finally, the study shows that microencapsulation of anthocyanin with maltodextrin as a carrier and coating agent presented a potential method to produce anthocyanin powder from purple Roselle.
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