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Physicochemical Characteristics of Anthocyanin Extract Powder from Black Rice Based on Maltodextrin and Skimmed Milk Powder Ratio as Encapsulant

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ABSTRACT

Anthocyanins are bioactive components in black rice. Black rice anthocyanins are potent antioxidants, so they have the potential to be developed into functional food products. As a bioactive component, anthocyanins in extracts have low stability to environmental conditions such as light, temperature, and pH. Encapsulation process using the spray drying technique is known to protect and increase the stability of anthocyanin bioactive compounds. Maltodextrin (MDE) is widely used as a coating material in the encapsulation of anthocyanins using the spray drying technique with many advantages. However, skim milk powder (SMP) was found to encapsulate black rice anthocyanins efficiently. This study's general objective was to determine the effect of the ratio of MDE and SMP as an encapsulant on black rice extract powder's physical and chemical characteristics. The research method is an experimental type using a single factor Completely Randomized Design (CRD), which consisted of 6 treatments, namely the MDE:SMP ratio (100:0, 90:10, 80:20, 70:30, 60:40, and 50:50). The results showed that there was a very significant effect of the ratio of MDE and SMP on the physical and chemical characteristics of coated anthocyanin powder. MDE and SMP ratios of 50:50 resulted in coated anthocyanin powder's best physical and chemical characteristics.

1. INTRODUCTION

Black rice (*Oryza sativa* L.) is a pigmented rice that has black bran covering the endosperm. Consumption of pigmented rice, such as black rice is currently starting to increase because people have begun to change their food consumption patterns towards foods that are beneficial to health, namely functional foods. Black rice has potential as a functional food because it contains bioactive components, namely polyphenolic compounds, flavonoids, and anthocyanins that act as antioxidants, anti-inflammatory and have other important health benefits (Kong *et al.*, 2012; Hosoda *et al.*, 2018).

Anthocyanin pigments in black rice can be obtained by extraction using the maceration method. Generally, ethanol solvent is used in the maceration method. Ethanol has advantages over other solvents such as methanol and acetone, namely it is more economical and has low toxicity (Nour *et al*., 2013). Acidified ethanol has been confirmed to increase yield and anthocyanin content in black rice extract (Pedro *et al.*, 2016). Extraction with heat-assisted maceration technique based on a recent study (Nurhidajah *et al*., 2022) was reported to be very effective in preparing black rice anthocyanin extract. Anthocyanin extracts can be degraded during the storage process. This is due to its low chemical stability to environmental conditions including temperature, light intensity, and pH (Cassidy, 2018; Fernandes *et al*., 2014). The anthocyanin decomposition process occurs faster at high temperatures (Patras *et al.*, 2010). Anthocyanins are unstable at high light intensity. The release of the sugar group causes the anthocyanin aglycones that are formed to fade quickly when exposed to light (Janna *et al*., 2006). Encapsulation techniques can overcome this weakness using a freeze dryer, spray dryer, and others (Bao *et al.*, 2019; Fang & Bhandari, 2010; Howard *et al.*, 2013).

Encapsulation using a spray dryer is an effective solution to protect and increase the stability of anthocyanin bioactive compounds (Kalušević *et al*., 2017). This technique has been widely used in the food industry. In addition to being applicable, the spray drying (SD) method for thermosensitive products is due to a short contact time with a heat source (Lourenço *et al*., 2020). The success of SD is influenced by several factors, including temperatures of inlet and outlet, feed flow rate, speed of the atomizer, air flow rate, and type as well as concentration of coating material (Patil-Gadhe & Pokharkar, 2014). One of the elements that will influence the encapsulated powder is the coating substance.

Mahdavi *et al.* (2014) reported maltodextrin (MDE) is extensively used as a coating material in anthocyanin encapsulation using the SD technique. MDE as a coating material with various advantages, including high solubility, low viscosity, and maintaining the stability of bioactive compounds (Suhag & Nanda, 2016). Recent research by Kalušević *et al.* (2017) reported that skimmed milk powder (SMP) reported that SMP coating material could encapsulate anthocyanin components at higher levels than maltodextrin. As a coating material, SMP has excellent encapsulation effectiveness and non-stick characteristics. This ingredient adds extra nutritional value in addition to being a source of protein, lactose, vitamins, and minerals (Bylaitë *et al.*, 2001; Jing & Giusti, 2005; Shamaei *et al*., 2016). However, the use of SMP as a coating material in the encapsulation of black rice extract still has a weakness, namely the coating efficiency of phenolic and flavonoid components is still very low (Kalušević *et al*., 2017).

Until now, there has been no research report on the combination of MDE and SMP as a coating material in the encapsulation of black rice extract. Based on this, it is necessary to review further the effect of the MDE-SMP combination as a coating material for black rice extract, the right combination is expected to produce coated anthocyanin powder with the best physical and chemical characteristics.

2. MATERIALS AND METHODS

2.1. Materials

The main ingredient used is black rice of the jellyteng variety obtained from organic rice farmers in Central Java. While the chemicals used are citric acid, gallic acid (C7H6O5), ethanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium chloride, folin-ciocalteu, sodium acetate, and sodium carbonate are some of the pro-analytical substances used. manufactured by Merck.

2.2. Black Rice Anthocyanin Extraction (Nurhidajah *et al.*, 2022)

Flour was made from pulverized black rice. Black rice flour sample (100 g) was mixed in a 1:10 (w/v) ratio with the solvents ethanol (56% v/v) and citrate (4.5% w/v). The extraction was carried out for 120 minutes with constant agitation (500 rpm) in waterbath at 50 °C. Using 100 mesh filter paper, the following procedure separates the liquid from the residue. The extracted ethanol was then evaporated at 55 °C using a rotary evaporator. The evaporation procedure was repeated until the ethanol solvent ceased pouring, yielding a final volume of 44% black rice extract. The black rice anthocyanin extract was kept at 4 °C in a dark glass bottle.

2.3. Anthocyanin Extract Encapsulation (Nurhidajah *et al.*, 2022)

MD and SMP coating materials emulsion with different ratios (100:0, 90:10, 80:20, 70:30, 60:40 and 50:50) were prepared at a concentration of 20% (w/v) with deionized water at room temperature. Anthocyanin extract was mixed with each coating material in a ratio of 1:1 (v/v). Each mixture was homogenized separately for 15 min at 3000 rpm. It was dried using a lab-scale spray drier with 120 ± 1 °C inlet air temperatures, and 80 ± 5 °C outlet air temperatures, 6.0 mL/min feed flow rate, and 1.5 bar feed pressure. The obtained coated anthocyanin powder was gathered and kept at -20°C until analyzed.

2.4. Anthocyanin Content (Yamuangmorn *et al.*, 2018)

To measure covered anthocyanin powder, a differential pH technique was employed. First, the sample (1 g) was dissolved in 1 mL of ethanol in a test tube. Next, two buffer solutions 1 mL of potassium chloride buffer, pH 1.0, and 1 mL of sodium acetate buffer, pH 4.5 were combined independently, and the test tube was incubated at room temperature (±25 °C) for 15 min. The absorbance was read by spectrophotometer at 520 and 700 nm. The following equation was used to determine the extract sample's absorption value (A):

$$A = [(A_{520} - A_{700})_{pH1} - (A_{520} - A_{700})_{pH4.5}] \quad (1)$$

Then anthocyanin content (AC) of black rice extract was calculated according to Equation (2) and was expressed in mg/100g:

$$AC\ (mg/100\ g) = \frac{A \times MW \times DF \times V \times 100}{E \times 1 \times W} \quad (2)$$

where MW = 448.8 g/mol is molecular weight of cyanidin-3-glucoside, DF is dilution factor, V is volume of the sample mother liquor, E = 26900 l/mol cm is the measure of molar absorptivity of cyanidin-3-glucoside, and W is sample weight (g).

2.5. Total Phenolics

The Folin-Ciocalteu method, which was modified slightly from Pedro et al. (2016), was used to measure the total phenolic content (TPC). Sample (0.5 g) was mixed with 5 ml of 10% Folin-Ciocalteu reagent. The addition of 7.5% Na_2CO_3 as much as 4 mL then homogenized. The solution was incubated for 60 minutes at 25 ± 1 °C. Reference solution used gallic acid at a strength of 100–500 ppm. The absorbance was measured using a UV-Vis spectrometer set at 765 nm. TPC was calculated as mg GAE/100g of black rice.

2.6. Flavonoid Content

Flavonoid content was determined according to Cai *et al*., (2016), with modifications. Black rice extract was combined with 2.8 milliliters of purified water, 0.1 milliliters of 10% AlCl3, 1.5 milliliters of ethanol, and 0.1 milliliters of 1 M CH3COOK in a dark tube. The mixture was homogenized and kept for 30 minutes at room temperature (25 ± 1 °C). The blank solution was aquadest, and the sample's absorbance was measured with a UV-Vis spectrometer at 415 nm. The reference curve uses a quercetin solution in aquadest with a concentration range of 20-100 ppm. Thus, mg QE/100 g was stated as the black rice extract's flavonoid content.

2.7. Antioxidant Activities

Antioxidant activity was determined according to Pedro et al. (2016), with slight modifications. The sample (0,2 ml) was mixed with 1.5 ml DPPH (0.2 mM), then diluted by adding 3.5 ml of ethanol. The solution was incubated at 25 ± 1 °C for 60 minutes, then read at 517 nm. The following equation was used to determine it:

$$\% \text{ RSA} = \frac{\text{Abs blanko x Abs test}}{\text{Abs blanko}} \text{ x } 100\% \quad (3)$$

2.8. pH value

The pH value was measuring according to Park & Kim (2016). Coated anthocyanin powder sample (4g) was mixed with 16 mL of distilled water in a vortex mixer for 1 min. The pH was recorded using instrument Hanna HI 2211 bench pH meters (Hanna instruments Ltd.), calibration solution using standard buffer.

2.9. Yield, Moisture Content and Water Activity (a_w)

Yield was obtained from the final weight percentage of coated anthocyanin powder (g) with the total solids of the sample (g). Determination of water content and water activity using analysis tools, each of which is a moisture analyzer (Shimadzu MOC63u) and a water activity analyzer (Rotronic Hygropalm-HP23-Aw-A) at room temperature.

2.10. Color Measurement

An appropriately adjusted Minolta CR-310 Chromameter was used to test the coated anthocyanin powder's color characteristics (Konica Minolta Business Solution Asia Pte Ltd). Additionally, the chroma, C° = [(a*2 + b*2)1/2], and the hue angle, H° = [tan-1(b*/a*)], were calculated. Red, yellow, green, and blue are the four primary hues, while the C° differentiates between bright and dull colors (Caparino *et al*., 2012).

2.11. Statistical Analysis

All experiments were performed in 4 repetitions. Results were presented in terms of average value ± standard deviation. The effect of treatment factors was analyzed using one-way ANOVA, and differences among treatments was detected using Duncan's post hoc test. The significance level used is $p < 0.05$. Statistical analysis was conducted with the help of SPSS 22.0 software.

3. RESULTS AND DISCUSSION

3.1. Anthocyanin and pH Value of Capsules

The mean anthocyanin content of black rice coated anthocyanin powder obtained was 51.22-84.59 mg/100g (Figure 1a). This amount is higher than the research results reported by Pramitasari & Angelica (2020), which only ranged from 2.00–12.00 mg/100g. The highest anthocyanin levels were obtained at a coating ratio of 50:50 (84.59 mg/100g). There was an increase in anthocyanin levels of 65% (51.22mg/100g) compared to a ratio of 100:0. SMP 50% as a coating material was able to trap more anthocyanins, resulting in a powder with the highest anthocyanin content, significantly different from all treatments. SMP generally contains b-casein components and has been reported to interact with Cyanidin-3-glucoside compounds from black soybean extract through reversible hydrogen bonds supported by an optimum pH (Kalušević *et al*., 2017). Previous studies also reported that casein protein interacts with malvidin-3-glucoside from grape extract through complex hydrogen bonds, which then form a hydrophobic reaction and effectively increase thermal stability so that the rate of anthocyanin degradation during drying can be minimized (He *et al*., 2016). The pH value of coated anthocyanin powder ranged from 2.60-2.95 (Figure 1b). The pH value increased with the addition of SMP in the coating material ratio because SMP had a neutral pH value. However, the increase in pH value is still in the anthocyanin stability range, at a value of 2-3 (Sipahli *et al*., 2017). Anthocyanins are more stable under acidic conditions in the form of a flavylium cation structure (Khoo *et al*., 2017).

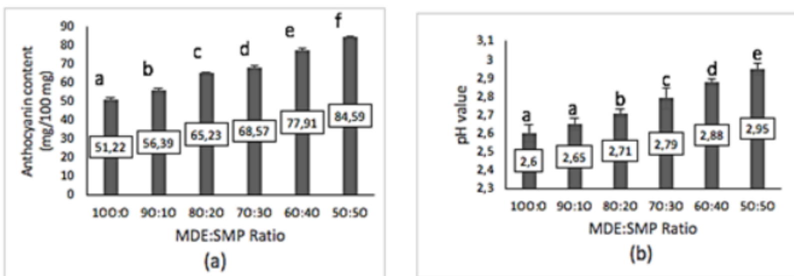

Figure 1. Anthocyanin and pH value coated anthocyanin powder based on MDE:SMP Ratio

3.2. Total Phenolics, Flavonoid, and Antioxidant Activities

Total phenolic and flavonoid content of coated anthocyanin powder is presented in Table 1. From the data, it is shown that the total phenolic and flavonoid content increases with the addition of SMP 50% in the ratio of the coating material, and significanly different with all treatments. B-casein in protein-derived encapsulation forms a more substantial complex with polyphenols, and this is due to the more hydrophobic nature of B-casein. (Hasni *et al*., 2011). The increased flavonoids are associated with the structure and bonds formed from proteins with flavonoid compounds. This result has previously been described by Bohin *et al*. (2012) on the flavonoid encapsulation of tea drinks. This underlines the increase in flavonoids when the ratio of SMP to coating material increases. In a previous study, Kalušević *et al*. (2017) reported the results of high antioxidant activity in coating black rice extract using SMP. Therefore, the antioxidant activity of coated anthocyanin powder is thought to be derived from the encapsulated anthocyanin, phenolic, and flavonoid components. In addition, the increase in antioxidant activity is also potential from the Maillard reaction, which produces melanoidin compounds with antioxidant activity. This reaction occurs in the protein component of SMP due to the high temperature during the drying process (Wang *et al*., 2011).

Table 1. Total phenolic, total flavonoid and antioxidant activity of coated anthocyanin powder based on MD and SMP ratio

MD:SMP Ratio	Total Phenolic (mg GAE/g)	Total Flavonoid (mg QE/g)	Antioxidant Activity (%)
100:0	0.27 ± 0.01^{a}	0.14 ± 0.02^{a}	25.07 ± 0.39^{a}
90:10	0.30 ± 0.01^{b}	0.16 ± 0.01^{ab}	26.52 ± 0.35^{b}
80:20	0.33 ± 0.01^{c}	0.17 ± 0.01^{bc}	29.23 ± 0.22^{c}
70:30	0.37 ± 0.01^{d}	0.18 ± 0.03^{bcd}	30.37 ± 0.38^{d}
60:40	0.38 ± 0.01^{d}	0.19 ± 0.02^{cd}	31.18 ± 0.48^{e}
50:50	0.41 ± 0.05^{e}	0.21 ± 0.02^{d}	31.71 ± 0.94^{e}

Note: All values are mean ± standard deviation 4 times repetition. Different superscript values showed significant differences with a 95% confidence level based on the ANOVA difference test and the LSD post hoc test.

3.3. AW, Moisture Content, and Yield of Coated Anthocyanin Powder

All samples showed an Aw value lower than 0.6 (0.49-0.58), so the coated anthocyanin powder in this study had an excellent aw value (Goyal *et al.*, 2015). A low aw value tends to result in extended shelf life. There is a positive tendency to increase the ratio of SMP as a coating material, SMP 50% is able to increase the aw of the product significantly. The water content values ranged from 0.91 to 1.13% (Table 2), the use of SMP 50% significantly made anthocyanin powder with the lowest water content. On the other hand, coatings with high MDE content produce high water content due to the nature of MDE, which quickly releases water (Kustyawati *et al.*, 2022). The product moisture content was low to prevent particle agglomeration, avoid the caking process, and improve powder dispersion and flowability (Daza *et al.*, 2016). Therefore, the powder from spray drying obtained both the aw level and the moisture content in this study met the requirements and was suitable for inclusion in the dry food matrix.

Table 2. Physical properties of coated anthocyanin powder based on MD and SMP ratio

MD:SMP Ratio	Yield (%)	Moisture content (%)	a_w (%)
100:0	33.51 ± 0.82^{a}	1.13 ± 0.03^{e}	0.58 ± 0.58^{c}
90:10	35.11 ± 0.75^{a}	1.08 ± 0.01^{de}	0.55 ± 0.55^{bc}
80:20	31.14 ± 0.68^{a}	1.04 ± 0.09^{cd}	0.54 ± 0.54^{bc}
70:30	34.06 ± 0.82^{a}	0.99 ± 0.01^{bc}	0.52 ± 0.52^{ab}
60:40	47.64 ± 0.73^{b}	0.96 ± 0.02^{cb}	0.51 ± 0.51^{ab}
50:50	51.29 ± 0.49^{b}	0.91 ± 0.05^{a}	0.49 ± 0.49^{a}

Note: All values are mean ± standard deviation 4 times repetition. Different superscript values showed significant differences with a 95% confidence level based on the ANOVA difference test and the LSD post hoc test.

The highest yield was significantly obtained from powders with a 50:50 coating material, which is in line with the results reported in a study comparing milk protein-based microparticles with other carriers (Belščak-Cvitanovic *et al.*, 2015). A combination of MDE and SMP coating materials resulted in good physical characteristics so that the coating material can be proposed as a coating material for the bioactive component of black rice extract.

3.4. Color Characteristic

Color is one of the indicators in assessing the quality of food products. During the production process, the color of black rice extract is expected to be maintained until a powder is obtained from drying. Therefore, observing the color measurement of coated anthocyanin powder after the encapsulation process is necessary. The results of chromatic color measurements are shown in Table 3. Based on the parameters a*, b*, and hue angle, it can be concluded that the color of the MD-SMP-based powder is red-violet with varying brightness levels. Among all coated anthocyanin powders, coating with a ratio of 50:50 differed significantly by having the lowest brightness value (40.58 ± 0.83) and the highest value for a* and C* with significant differences with other treatments. Indicating that this ratio produced the darkest powder with the highest portion of red color. Many reports have investigated the molecular level of the bond formed between casein-based coating materials and black rice anthocyanins on their stability from a thermodynamic point of view. The casein-anthocyanin bond is mainly stabilized through hydrogen bonds and hydrophobic interactions involving the aglycone and glucosyl moieties of the encapsulated molecule so that the color can be maintained during the drying process (Aprodu *et al.*, 2019).

Table 3. Color parameters of coated anthocyanin powder based on MD and SMP ratio

MD:SMP Ratio	L*	a*	b*	C*	H°	Color[#]
100:0	56.06 ± 0.79^{d}	26.83 ± 0.66^{a}	4.43 ± 0.31^{a}	27.20 ± 0.62^{a}	9.39 ± 0.81^{a}	R-V
90:10	55.46 ± 1.46^{c}	28.48 ± 0.12^{b}	4.30 ± 0.51^{a}	28.80 ± 0.16^{b}	8.58 ± 0.99^{a}	R-V
80:20	52.91 ± 0.61^{c}	28.71 ± 0.39^{b}	5.34 ± 0.50^{ab}	29.20 ± 0.35^{b}	10.55 ± 1.06^{a}	R-V
70:30	49.07 ± 0.82^{b}	35.78 ± 1.45^{c}	6.36 ± 1.56^{b}	36.36 ± 1.65^{c}	10.03 ± 2.13^{a}	R-V
60:40	48.06 ± 0.54^{b}	39.90 ± 0.47^{d}	9.71 ± 0.39^{c}	41.06 ± 0.39^{d}	13.68 ± 0.65^{b}	R-V
50:50	40.58 ± 0.83^{a}	43.64 ± 1.43^{e}	12.75 ± 0.83^{d}	45.47 ± 1.16^{e}	16.31 ± 1.50^{c}	R-V

Note: #) R-V = red-violet.
All values are mean ± standard deviation 4 times repetition. Different superscript values showed significant differences with a 95% confidence level based on the ANOVA difference test and the LSD post hoc test.

4. CONCLUSIONS

It can be concluded that the best combination of coating materials in black rice encapsulation was obtained at MDE: SMP ratio of 50:50 with total anthocyanin levels obtained 84.59 mg/100g, with total phenol 0.41 mg GAE/g, total flavonoid 0.21 mg QE/g and antioxidant activity 31.71% RSA. The encapsulated product has an average value of L* 40.5; a* 43.64; b* 12.75; Chroma 45.47; and Hue 16.31 with red-purple color types. Anthocyanin stability with a pH value of 2.95. Furthermore, it has an aw of 0.49%, moisture content of 0.91%, and a yield of 51.29%. The combination of MDE and SMP as a coating material in the encapsulation process of black rice extract had a very significant effect on total anthocyanin levels, phenolics, total flavonoids, antioxidant activity, pH, color, aw, water content, and yield.

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