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Psychochemical Characteristics of Black Rice Powder Based on Maltodextrin and Skimmed Milk Powder Ratio as Encapsulant

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. This study's general objective was to determine the effect of the ratio of maltodextrin (MDE) and skim milk powder (SMP) as an encapsulant on black rice extract powder's physical and chemical characteristics. The research method is an experimental type using a monofactor Completely Randomized Design (CRD), which consists 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 black rice powder. MDE and SMP ratios of 50:50 resulted in black rice powder's best physical and chemical characteristics.

Keywords: *black rice extract, encapsulation, coating material, physicochemical characteristics.*

ABSTRAK

Antosianin merupakan komponen bioaktif pada beras hitam. Antosianin beras hitam bersifat antikosidan kuat sehingga sangat potensial dikembangkan menjadi produk pangan fungsional. Sebagai komponen bioaktif antosianin dalam bentuk ekstrak memiliki stabilitas yang rendah terhadap kondisi lingkungan seperti cahaya, suhu dan pH. Proses enkapsulasi menggunakan Teknik spray drying diketahui mampu melindungi dan meningkatkan stabilitas senyawa bioaktif antosianin. Tujuan umum penelitian yaitu untuk mengetahui pengaruh rasio maltodekstrin (MDE) dan susu bubuk skim (SMP) sebagai enkapsulan terhadap karakteristik fisik dan kimia serbuk ekstrak beras hitam. Metode penelitian berjenis eksperimen menggunakan Rancangan Acak Lengkap (RAL) monofaktor, yang terdiri dari 6 perlakuan yaitu rasio MDE:SMP (100:0, 90:10, 80:20, 70:30, 60:40 dan 50:50). Hasil penelitian menunjukkan ada pengaruh yang sangat nyata dari rasio MDE dan SMP terhadap karakteristik fisik dan kimia serbuk beras hitam. Rasio MDE dan SMP 50:50 menghasilkan karakteristik fisik dan kimia serbuk beras hitam terbaik.

Kata Kunci: ekstrak beras hitam, enkapsulasi, bahan penyalut, karakteristik fisikokimia.

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1. INTRODUCTION

Black rice is pigmented rice with an endosperm covered with black aleurone. Black rice cultivation in Indonesia is increasing, but its utilization in the food sector is not optimal. However, black rice has anthocyanin bioactive components, potent antioxidants, so it can potentially be developed as an ingredient in functional food products (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. Black rice anthocyanin extract is volatile to light, temperature and pH (Cassidy, 2018). 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 things, including inlet and outlet temperatures, air flow rate, feed flow rate, speed of the atomizer, to the type

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Commented [H5]: Are light and pH influence the volatility od a substance? If so.. please add some more references. “susceptible’ is more appropriate word instead of using “volatile” and concentration of coating material used (Patil-Gadhe & Pokharkar, 2014). The coating material is one of the factors that will affect the encapsulated powder.

Mahdavi et al. (2014) reported maltodextrin (MDE) as the most optimal coating material in anthocyanin encapsulation using the SD technique. MDE is 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) coating material produced higher anthocyanin levels than maltodextrin. As a coating material, SMP has non-stick properties and high encapsulation efficiency. Apart from being a source of protein, lactose, vitamins, and minerals, this ingredient provides additional nutritional value (Bylaitë et al., 2001; Jing & Giusti, 2005; Shamaei et al., 2016). However, using SMP to encapsulate anthocyanin extracts resulted in powders with lower phenolic/flavonoid content (Kalušević et al., 2017).

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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 black rice extract powder with the best physical and chemical characteristics.

2. MATERIALS AND METHODS

2.1. Materials

The research material was black rice of the Jeliteng variety collected from organic rice farmers in the Karanganyar region, Central Java Province, Indonesia, food-grade citric acid from PT Gunacipta Multirasa Indonesia, and aquadest. Pro-analytical chemicals from Merck include ethanol, potassium chloride, sodium acetate, folin-ciocalteu, sodium carbonate, gallic acid (C7H6O5), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH).

2.2. Black rice anthocyanin extraction (Nurhidajah et al., 2022)

Black rice is ground into flour. 100 g of black rice flour was added with ethanol (56% v/v) and citrate (4.5% w/v) solvent in a ratio of 1:10 (w/v). The extraction process was carried out in a thermostatic water bath with a controlled temperature (50 C) for 120 minutes with constant stirring (500 rpm). The following process separates the liquid from the residue using 400 mesh filter paper. The ethanol in the extract was then evaporated using a rotary evaporator at 55 °C. The anthocyanin extract of black rice was stored in a dark glass bottle at 4°C until used.

2.3. Anthocyanin extract encapsulation (Nurhidajah et al., 2022)

MD and SMP coating materials emulsion ~~solutions~~ 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 laboratory-scale spray dryer with an inlet air temperature of 120 ± 1 C, an outlet air temperature of 80 ± 5 C, and a feed flow rate of 6.0 mL/minute at a pressure of 1.5 bar. The obtained powder was collected and stored at -20°C until analyzed.

2.3. Anthocyanin content (Yamuangmorn et al., 2018)

A differential pH method was used to determine black rice powder's anthocyanin content. First, 1 g of powder was dissolved with 1 mL of ethanol in a test tube, then mixed separately with two buffer solutions (1 mL of potassium chloride buffer pH 1.0 and 1 mL

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2.4. Total phenolics

The Folin-Ciocalteu method (Pedro et al., 2016), with slight modifications, was used to analyze total phenolics content (TPC). 0.5 g of microcapsules were prepared in a dark tube, then 5 mL of Folin-Ciocalteu 10% (v/v) reagent was added. The solution was homogenized for 5 mins and added 4 mL of 7.5% Na2CO3 (w/v). The mixture was incubated for 60 mins at room temperature (25 ± 1°C). Ethanol is used as a blank solution. Gallic acid in ethanol with a concentration of 100-500 ppm is used as a standard solution. Next, the absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 765 nm. TPC of black rice extract was expressed as mg GAE/100g.

2.5. Flavonoid content

Cai et al. (2016) adopted a slight modification method in determining the flavonoid content. A sample of 0.5 ml black rice extract was prepared in a dark tube, to which 1.5 ml of ethanol, 0.1 ml of 10% AlCl3, 0.1 ml of 1 M CH3COOK, and 2.8 ml of distilled water were added. The solution was homogenized and incubated at room temperature (25

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± 1 °C) for 30 minutes. The absorbance of the sample was measured using a UV-Vis spectrophotometer with a wavelength of 415 nm, the blank solution using aquadest. The standard curve uses a quercetin solution in aquadest with a concentration range of 20-100 ppm. Therefore, the flavonoid content of black rice extract was expressed as mg QE/100 g.

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2.6. Antioxidant activities

Antioxidant activity was determined according to Pedro et al. (2016), with slight modifications. A total of 1.5 ml of 0.2 mM DPPH in ethanol was mixed with 0.2 ml of the sample into a test tube, and ethanol was added to a final volume of 3.5 ml. The tubes were tightly closed, homogenized, and incubated at room temperature (25 ± 1 °C) for 60 minutes. The absorbance was measured at a wavelength of 517 nm, and the ability of the extract to scavenge DPPH was obtained by reducing the absorbance of the blank to the sample, then compared with the absorbance of the blank and expressed in % of inhibition.

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2.7. pH value

The pH value of black rice powder was measured with the instrument Hanna HI 2211 bench pH meters (Hanna instruments Ltd.), calibration solution using standard buffer.

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2.8. Yield, moisture content and water activity (a_w)

Yield was obtained from the final weight percentage of black rice powder (g) with the total solids of the sample (g). Determination of the moisture content of the powder using a moisture analyzer (Shimadzu MOC63u, Japan), while the aw of the powder was determined using a water activity analyzer (Rotronic Hygropalm-HP23-Aw-A, Switzerland) at 25 C.

2.9. Color measurement

Color characteristics of black rice powder were measured using a calibrated Minolta CR-310 Chromameter (Konica Minolta Business Solution Asia Pte Ltd). The hue angle, H° [tan-1(b*/a*)] and chroma, C° [(a*2 + b*2)1/2] were also determined. The H° is used to identify colors (red, yellow, green, and blue), whereas C° distinguishes between bright and dull colors (Caparino et al., 2012).

2.10. Statistical analysis

All experiments were carried out in 4 repetitions. Results are presented in terms of mean ± standard deviation. The effect of treatment factors was analyzed using one-way ANOVA, and Duncan's post hoc test was used to detect differences between treatments. The significance level used is $p<0.05$. Statistical analysis was carried out 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 extract powder obtained was 51.22-84.59 mg/100g (Figure 1a). This amount is higher than the research results reported by Pramitasari and 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 generally contains β-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

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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 black rice powder ranged from 2.60-2.95 (Figure 1b). The pH value of the black rice extract powder 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).

3.2. Total phenolics, flavonoid, and antioxidant activities

Total phenolic and flavonoid content of black rice 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 in the ratio of the coating material. 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 underlies 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 black rice 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

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component of SMP due to the high temperature during the drying process (Wang et al., 2011).

3.3. Aw, mouisture content, and yield of microcapsules

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All samples showed an Aw value lower than 0.6 (0.49-0.58), so the black rice extract powder in this study had an excellent aw value (Goyal et al., 2015). A low aw value tends to result in extended shelf life. The water content values ranged from 0.91 to 1.13% (Table 2). 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.

The highest yield was 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 black rice extract 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 SBHs, the coating with a 50:50 ratio had the lowest brightness value (40.58 ± 0.83) and the highest values for a* and C*, 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 hydrophobic interactions and hydrogen bonds 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).

4. CONCLUSIONS

~~Based on the results of the research that has been carried out~~, 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. 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 interaction between~~ 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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5. ACKNOWLEDGMENTS

The authors would like to thank all parties involved and those who contributed to this research, especially for funding by KEMENDIKBUD-RISTEK through the PTUPT research scheme on behalf of Dr Nurhidajah, M.Si.

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Table 1. Total phenolic, total flavonoid and antioxidant activity of black rice extract 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}

Information:

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

Table 2. Physical properties of black rice 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}

Information:

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

Table 3. Color parameters of black rice 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}	Red-violet
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}	Red-violet
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}	Red-violet
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}	Red-violet
60:40	48.06 ± 0.54^{a}	39.90 ± 0.47^{d}	9.71 ± 0.39^{c}	41.06 ± 0.39^{d}	13.68 ± 0.65^{b}	Red-violet
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}	Red-violet

Information:

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

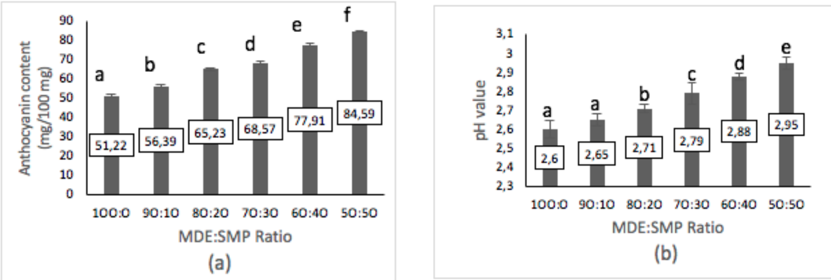

Figure 1. Anthocyanin and pH value black rice powder based on MDE:SMP Ratio

Psychochemical Characteristics of Black Rice Powder 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. This study's general objective was to determine the effect of the ratio of maltodextrin (MDE) and skim milk powder (SMP) as an encapsulant on black rice extract powder's physical and chemical characteristics. The research method is an experimental type using a monofactor Completely Randomized Design (CRD), which consists 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 black rice powder. MDE and SMP ratios of 50:50 resulted in black rice powder's best physical and chemical characteristics.

Keywords: *black rice extract, encapsulation, coating material, physicochemical characteristics.*

ABSTRAK

Antosianin merupakan komponen bioaktif pada beras hitam. Antosianin beras hitam bersifat antikosidan kuat sehingga sangat potensial dikembangkan menjadi produk pangan fungsional. Sebagai komponen bioaktif antosianin dalam bentuk ekstrak memiliki stabilitas yang rendah terhadap kondisi lingkungan seperti cahaya, suhu dan pH. Proses enkapsulasi menggunakan Teknik spray drying diketahui mampu melindungi dan meningkatkan stabilitas senyawa bioaktif antosianin. Tujuan umum penelitian yaitu untuk mengetahui pengaruh rasio maltodekstrin (MDE) dan susu bubuk skim (SMP) sebagai enkapsulan terhadap karakteristik fisik dan kimia serbuk ekstrak beras hitam. Metode penelitian berjenis eksperimen menggunakan Rancangan Acak Lengkap (RAL) monofaktor, yang terdiri dari 6 perlakuan yaitu rasio MDE:SMP (100:0, 90:10, 80:20, 70:30, 60:40 dan 50:50). Hasil penelitian menunjukkan ada pengaruh yang sangat nyata dari rasio MDE dan SMP terhadap karakteristik fisik dan kimia serbuk beras hitam. Rasio MDE dan SMP 50:50 menghasilkan karakteristik fisik dan kimia serbuk beras hitam terbaik.

Kata Kunci: ekstrak beras hitam, enkapsulasi, bahan penyalut, karakteristik fisikokimia.

1. INTRODUCTION

Black rice is pigmented rice with an endosperm covered with black aleurone. Black rice cultivation in Indonesia is increasing, but its utilization in the food sector is not optimal. However, black rice has anthocyanin bioactive components, potent antioxidants, so it can potentially be developed as an ingredient in functional food products (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. Black rice anthocyanin extract is volatile to light, temperature and pH (Cassidy, 2018). 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).

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so the sentence is one of the weaknesses of black rice anthocyanin extract which is unstable and volatile to light, temperature and pH (Cassidy, 2018)

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 things, including inlet and outlet temperatures, air flow rate, feed flow rate, speed of the atomizer, to the type and concentration of coating material used (Patil-Gadhe & Pokharkar, 2014). The coating material is one of the factors that will affect the encapsulated powder.

Mahdavi et al. (2014) reported maltodextrin (MDE) as the most optimal coating material in anthocyanin encapsulation using the SD technique. MDE is 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) coating material produced higher anthocyanin levels than maltodextrin. As a coating material, SMP has non-stick properties and high encapsulation efficiency. Apart from being a source of protein, lactose, vitamins, and minerals, this ingredient provides additional nutritional value (Bylaitë et al., 2001; Jing & Giusti, 2005; Shamaei et al., 2016). However, using SMP to encapsulate anthocyanin extracts resulted in powders with lower phenolic/flavonoid content (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 black rice extract powder with the best physical and chemical characteristics.

2. MATERIALS AND METHODS

2.1. Materials

The research material was black rice of the Jeliteng variety collected from organic rice farmers in the Karanganyar region, Central Java Province, Indonesia, food-grade citric acid from PT Gunacipta Multirasa Indonesia, and aquadest. Pro-analytical chemicals from Merck include ethanol, potassium chloride, sodium acetate, folin-ciocalteu, sodium carbonate, gallic acid (C7H6O5), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH).

2.2. Black rice anthocyanin extraction (Nurhidajah et al., 2022)

Black rice is ground into flour. 100 g of black rice flour was added with ethanol (56% v/v) and citrate (4.5% w/v) solvent in a ratio of 1:10 (w/v). The extraction process was carried out in a thermostatic water bath with a controlled temperature (50 C) for 120 minutes with constant stirring (500 rpm). The following process separates the liquid from the residue using 400 mesh filter paper. The ethanol in the extract was then evaporated using a rotary evaporator at 55 °C. The anthocyanin extract of black rice was stored in a dark glass bottle at 4°C until used.

2.3. Anthocyanin extract encapsulation (Nurhidajah et al., 2022)

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MD and SMP coating materials emulsion solutions 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 laboratory-scale spray dryer with an inlet air temperature of 120 ± 1 C, an outlet air temperature of 80 ± 5 C, and a feed flow rate of 6.0 mL/minute at a pressure of 1.5 bar. The obtained powder was collected and stored at -20°C until analyzed.

2.3. Anthocyanin content (Yamuangmorn et al., 2018)

A differential pH method was used to determine black rice powder's anthocyanin content. First, 1 g of powder was dissolved with 1 mL of ethanol in a test tube, then mixed separately with two buffer solutions (1 mL of potassium chloride buffer pH 1.0 and 1 mL sodium acetate buffer pH 4.5), and incubated at room temperature (± 25 °C) for 15 minutes. The absorbance was measured by spectrophotometer at 520 and 700 nm. The absorbance value was obtained by subtracting difference in absorbance at a wavelength of 520 nm and 700 nm at pH 1.0 with the difference in absorbance at pH 4.5. Anthocyanin content was obtained by multiplying the absorbance value by the molecular weight of cyanidin-3-glucoside (448.8 g/mol) and the amount of dilution, then divided by the coefficient of molar absorptivity of cyanidin-3-glucoside (26900 l/mol cm) and the width of the cuvette (1 cm). The anthocyanin content of black rice extract was expressed in mg/100g.

2.4. Total phenolics

The Folin-Ciocalteu method (Pedro et al., 2016), with slight modifications, was used to analyze total phenolics content (TPC). 0.5 g of microcapsules were prepared in a dark tube, then 5 mL of Folin-Ciocalteu 10% (v/v) reagent was added. The solution was homogenized for 5 mins and added 4 mL of 7.5% Na2CO3 (w/v). The mixture was incubated for 60 mins at room temperature (25 ± 1°C). Ethanol is used as a blank solution. Gallic acid in ethanol with a concentration of 100-500 ppm is used as a standard solution. Next, the absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 765 nm. TPC of black rice extract was expressed as mg GAE/100g.

2.5. Flavonoid content

Cai et al. (2016) adopted a slight modification method in determining the flavonoid content. A sample of 0.5 ml black rice extract was prepared in a dark tube, to which 1.5 ml of ethanol, 0.1 ml of 10% AlCl3, 0.1 ml of 1 M CH3COOK, and 2.8 ml of distilled water were added. The solution was homogenized and incubated at room temperature (25

± 1 °C) for 30 minutes. The absorbance of the sample was measured using a UV-Vis spectrophotometer with a wavelength of 415 nm, the blank solution using aquadest. The standard curve uses a quercetin solution in aquadest with a concentration range of 20-100 ppm. Therefore, the flavonoid content of black rice extract was expressed as mg QE/100 g.

2.6. Antioxidant activities

Antioxidant activity was determined according to Pedro et al. (2016), with slight modifications. A total of 1.5 ml of 0.2 mM DPPH in ethanol was mixed with 0.2 ml of the sample into a test tube, and ethanol was added to a final volume of 3.5 ml. The tubes were tightly closed, homogenized, and incubated at room temperature (25 ± 1 °C) for 60 minutes. The absorbance was measured at a wavelength of 517 nm, and the ability of the extract to scavenge DPPH was obtained by reducing the absorbance of the blank to the sample, then compared with the absorbance of the blank and expressed in % of inhibition.

2.7. pH value

The pH value of black rice powder was measured with the instrument Hanna HI 2211 bench pH meters (Hanna instruments Ltd.), calibration solution using standard buffer.

2.8. Yield, moisture content and water activity (a_w)

Yield was obtained from the final weight percentage of black rice powder (g) with the total solids of the sample (g). Determination of the moisture content of the powder using a moisture analyzer (Shimadzu MOC63u, Japan), while the aw of the powder was determined using a water activity analyzer (Rotronic Hygropalm-HP23-Aw-A, Switzerland) at 25 C.

2.9. Color measurement

Color characteristics of black rice powder were measured using a calibrated Minolta CR-310 Chromameter (Konica Minolta Business Solution Asia Pte Ltd). The hue angle, H° [tan-1(b*/a*)] and chroma, C° [(a*2 + b*2)1/2] were also determined. The H° is used to identify colors (red, yellow, green, and blue), whereas C° distinguishes between bright and dull colors (Caparino et al., 2012).

2.10. Statistical analysis

All experiments were carried out in 4 repetitions. Results are presented in terms of mean ± standard deviation. The effect of treatment factors was analyzed using one-way ANOVA, and Duncan's post hoc test was used to detect differences between treatments. The significance level used is $p<0.05$. Statistical analysis was carried out with the help of SPSS 22.0 software.

3. RESULTS AND DISCUSSION

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3.1. Anthocyanin and pH value of capsules

The mean anthocyanin content of black rice extract powder obtained was 51.22-84.59 mg/100g (Figure 1a). This amount is higher than the research results reported by Pramitasari and 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 generally contains β-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 black rice powder ranged from 2.60-2.95 (Figure 1b). The pH value of the black rice extract powder 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).

3.2. Total phenolics, flavonoid, and antioxidant activities

Total phenolic and flavonoid content of black rice 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 in the ratio of the coating material. 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 underlies 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 black rice 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).

3.3. Aw, mouisture content, and yield of microcapsules

All samples showed an Aw value lower than 0.6 (0.49-0.58), so the black rice extract powder in this study had an excellent aw value (Goyal et al., 2015). A low aw value tends to result in extended shelf life. The water content values ranged from 0.91 to 1.13% (Table 2). 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.

The highest yield was 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 black rice extract 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 SBHs, the coating with a 50:50 ratio had the lowest brightness value (40.58 ± 0.83) and the highest values for a* and C*, 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 hydrophobic interactions and hydrogen bonds 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).

4. CONCLUSIONS

Based on the results of the research that has been carried out, 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. 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 interaction between 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.

5. ACKNOWLEDGMENTS

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Table 1. Total phenolic, total flavonoid and antioxidant activity of black rice extract 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}

Information:

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

Table 2. Physical properties of black rice 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}

Information:

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

Table 3. Color parameters of black rice 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}	Red-violet
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}	Red-violet
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}	Red-violet
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}	Red-violet
60:40	48.06 ± 0.54^{a}	39.90 ± 0.47^{d}	9.71 ± 0.39^{c}	41.06 ± 0.39^{d}	13.68 ± 0.65^{b}	Red-violet
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}	Red-violet

Information:

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

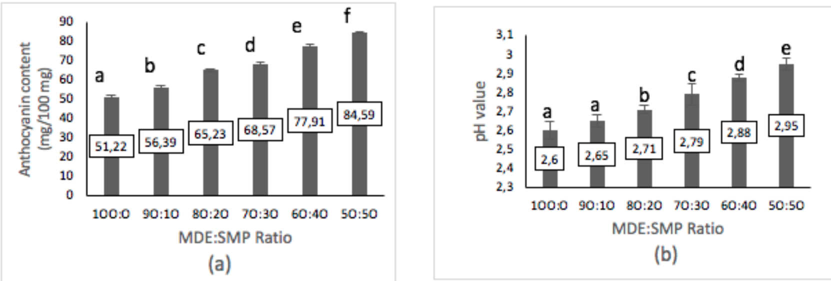

Figure 1. Anthocyanin and pH value black rice powder based on MDE:SMP Ratio

3. Revised version received (14-11-2022)

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

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.

Keywords: *black rice extract, encapsulation, coating material, physicochemical characteristics.*

ABSTRAK

Antosianin merupakan komponen bioaktif pada beras hitam. Antosianin beras hitam bersifat antikosidan kuat sehingga sangat potensial dikembangkan menjadi produk pangan fungsional. Sebagai komponen bioaktif antosianin dalam bentuk ekstrak memiliki stabilitas yang rendah terhadap kondisi lingkungan seperti cahaya, suhu dan pH. Proses enkapsulasi menggunakan Teknik spray drying diketahui mampu melindungi dan meningkatkan stabilitas senyawa bioaktif antosianin. Maltodekstrin (MDE) banyak digunakan sebagai bahan pelapis dalam enkapsulasi antosianin menggunakan teknik spray drying dengan banyak keunggulan. Namun, susu bubuk skim (SMP) ditemukan mampu mengenkapsulasi antosianin beras hitam secara efisien. Tujuan umum penelitian yaitu untuk mengetahui pengaruh rasio MDE dan susu SMP sebagai enkapsulan terhadap karakteristik fisik dan kimia serbuk ekstrak beras hitam. Metode penelitian berjenis eksperimen menggunakan Rancangan Acak Lengkap (RAL) monofaktor, yang terdiri dari 6 perlakuan yaitu rasio MDE:SMP (100:0, 90:10, 80:20, 70:30, 60:40 dan 50:50). Hasil penelitian menunjukkan ada pengaruh yang sangat nyata dari rasio MDE dan SMP terhadap karakteristik fisik dan kimia serbuk beras hitam. Rasio MDE dan SMP 50:50 menghasilkan karakteristik fisik dan kimia serbuk beras hitam terbaik.

Kata Kunci: ekstrak beras hitam, enkapsulasi, bahan penyalut, karakteristik fisikokimia.

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, 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 things, including inlet and outlet temperatures, air flow rate, feed flow rate, speed of the atomizer, to the type and concentration of coating material used (Patil-Gadhe & Pokharkar, 2014). The coating material is one of the factors that will affect the encapsulated powder.

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 non-stick properties and high encapsulation efficiency. Apart from being a source of protein, lactose, vitamins, and minerals, this ingredient provides additional nutritional value (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 research material was black rice of the Jeliteng variety collected from organic rice farmers in the Karanganyar region, Central Java Province, Indonesia, food-grade citric acid from PT Gunacipta Multirasa Indonesia, and aquadest. Pro-analytical chemicals from Merck include ethanol, potassium chloride, sodium acetate, folin-ciocalteu, sodium carbonate, gallic acid (C7H6O5), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH).

2.2. Black rice anthocyanin extraction (Nurhidajah et al., 2022)

Black rice was ground into flour. Black rice powder sample (100 g) was added with ethanol (56% v/v) and citrate (4.5% w/v) solvent in a ratio of 1:10 (w/v). The extraction was carried out for 120 minutes in a thermostatic water bath at a regulated temperature (50 C) with steady stirring (500 rpm). Using 100 mesh filter paper, the following procedure separates the liquid from the residue. The extract's ethanol was then evaporated using a rotary evaporator set at 55 °C. 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 laboratory-scale spray dryer with an inlet air temperature of 120 ± 1 C, an outlet air temperature of 80 ± 5 C, and a feed flow rate of 6.0 mL/minute at a pressure of 1.5 bar. The obtained coated anthocyanin powder was collected and stored at -20°C until analyzed.

2.4. Anthocyanin content (Yamuangmorn et al., 2018)

A differential pH method was used to determine coated anthocyanin powder. First, sample (1 g) was dissolved with 1 mL of ethanol in a test tube, then mixed separately with two buffer solutions (1 mL of potassium chloride buffer pH 1.0 and 1 mL sodium acetate buffer pH 4.5), and incubated at room temperature (± 25 °C) for 15 minutes. The absorbance was read by spectrophotometer at 520 and 700 nm. The absorbance value was obtained by subtracting difference in absorbance at a wavelength of 520 nm and 700 nm at pH 1.0 with the difference in absorbance at pH 4.5. The absorbance value of the extract sample was calculated using the equation:

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

Then anthocyanin content of black rice extract was expressed in mg/100g:

$$\text{Anthocyanin content (mg/100 g)} = \frac{A \times MW \times DF \times V \times 100}{E \times 1 \times W}$$

A = absorbance

MW = molecular weight of cyanidin-3-glucoside (448.8 g/mol)

DF = dilution factor

V = volume of the sample mother liquor

E = coefficient of molar absorptivity of cyanidin-3-glucoside (26900 l/mol cm)

W = sample weight (g)

2.5. Total phenolics

Total phenolics content (TPC) used the Folin-Ciocalteu technique based on (Pedro et al., 2016) with minor modifications. In a dark tube, coated anthocyanin powder (0.5 g) was made, and 5 mL of Folin-Ciocalteu 10% (v/v) reagent was added. After homogenizing the solution for 5 minutes, 4 mL of 7.5% Na_2CO_3 (w/v) was added. The mixture was incubated at room temperature (25 1C) for 60 minutes. As a blank solution, ethanol is employed. A standard solution of gallic acid in ethanol with a concentration of 100-500 ppm is utilized. The absorbance was read with a UV-Vis spectrophotometer set at 765 nm. TPC was calculated as mg GAE/100g of black rice extract.

2.6. Flavonoid content

Flavonoid content was determined according to Cai et al., (2016), with modifications. In a dark tube, 0.5 ml of black rice extract was mixed with 1.5 ml of ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M CH_3COOK, and 2.8 ml of distilled water. The solution was homogenized and incubated for 30 minutes at room temperature (25 ± 1 °C). The absorbance of the sample was read with a UV-Vis spectrophotometer at 415 nm, and the blank solution was aquadest. A quercetin solution in aquadest with a concentration range of 20-100 ppm is used in the standard curve. The flavonoid concentration of black rice extract was therefore reported as mg QE/100 g.

2.7. Antioxidant activities

Antioxidant activity was determined according to Pedro et al. (2016), with slight modifications. A total of 1.5 ml of 0.2 mM DPPH in ethanol was mixed with 0.2 ml of the sample into a test tube, and ethanol was added to a final volume of 3.5 ml. The tubes were tightly closed, homogenized, and incubated at room temperature (25 ± 1 °C) for 60 minutes. The absorbance was read at a wavelength of 517 nm and calculated using the equation:

$$\% \text{ RSA} = \frac{\text{Abs blanko x Abs test}}{\text{Abs blanko}} \text{ x } 100\%$$

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2.8. pH value

The pH value was measuring according to Park et al., (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 a 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 the moisture content of the powder using a moisture analyzer (Shimadzu MOC63u, Japan), while the aw of the powder was determined using a water activity analyzer (Rotronic Hygropalm-HP23-Aw-A, Switzerland) at 25 C.

2.10. Color measurement

Color characteristics of coated anthocyanin powder were measured using a calibrated Minolta CR-310 Chromameter (Konica Minolta Business Solution Asia Pte Ltd). The hue angle, H° [tan-1(b*/a*)] and chroma, C° [(a*2 + b*2)1/2] were also determined. The H°

is used to identify colors (red, yellow, green, and blue), whereas C° distinguishes between bright and dull colors (Caparino et al., 2012).

2.11. Statistical analysis

All experiments were carried out in 4 repetitions. Results are presented in terms of mean ± standard deviation. The effect of treatment factors was analyzed using one-way ANOVA, and Duncan's post hoc test was used to detect differences between treatments. The significance level used is $p<0.05$. Statistical analysis was carried out 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 and 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 β-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).

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 underlies 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).

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.

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 hydrophobic interactions and hydrogen bonds 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).

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. 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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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}

Information:

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

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}

Information:

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

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}	Red-violet
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}	Red-violet
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}	Red-violet
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}	Red-violet
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}	Red-violet
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}	Red-violet

Information:

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

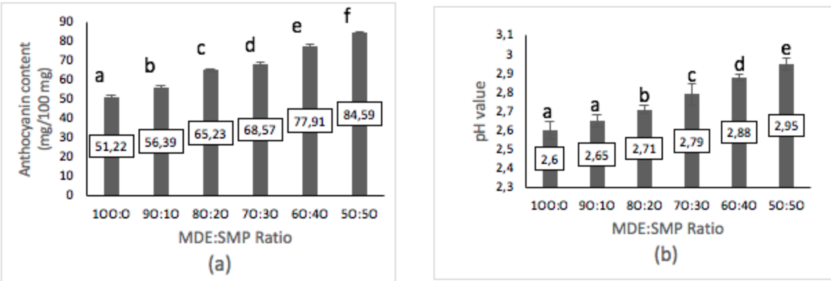

Figure 1. Anthocyanin and pH value coated anthocyanin powder based on MDE:SMP Ratio

4. Paper Accepted (15-11-2022)

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Yth Penulis,

Berdasarkan revisi yang kami terima, dengan gembira kami sampaikan bahwa naskah Saudara yang berjudul: **Psychochemical Characteristics of Black Rice Powder Based on Maltodextrin and Skimmed Milk Powder Ratio as Encapsulant** dinyatakan ACCEPTED dan **dapat dipublikasikan di JTEP**.

Selain itu kami sampaikan kabar gembira bahwa JTEP telah naik peringkatnya menjadi **SINTA 2** (https://sinta.kemdikbud.go.id/journals ketik Teknik Pertanian pada Source) yang berlaku mulai Vol. 10, No. 2, September 2021. Tidak hanya itu, manajemen JTEP akan meningkatkan lagi ke arah jurnal terindeks scopus, sehingga mulai Vol. 11 No. 1, Maret 2022, JTEP akan mempublikasikan paper dalam Bahasa Inggris.

Sehubungan dengan ini semua kami sampaikan bahwa biaya APC (Article Processing Charge) JTEP naik menjadi 750.000. Biaya agar ditransfer ke rekening BNI, No. 0698202763 a/n Elhamida Rezkia Amien selambat-lambatnya Tanggal 21 November 2022.

Demikian surat ini disampaikan, atas perhatiannya kami ucapkan terima kasih.

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Thank you for revising the manuscript, **Psychochemical Characteristics of Black Rice Powder Based on Maltodextrin and Skimmed Milk Powder Ratio as Encapsulant .** We have carefully checked the revised version and we are pleased to inform you that your manuscript is **Accepted** for publication in JTEP. The manuscript is being handled for future process.

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5. Final paper for publication (19-12-2022)

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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 ($C_7H_6O_5$), 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% Na2CO3 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).

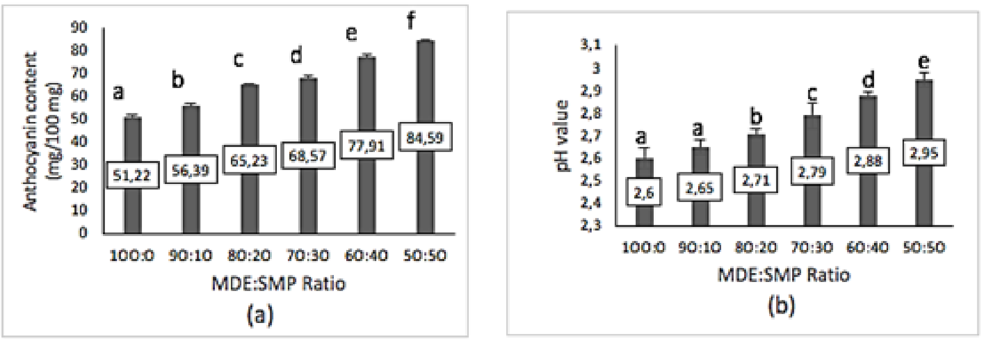

Figure 1. Anthocyanin and pH value coated anthocyanin powder based on MDE:SMP Ratio

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 underlies 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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