



Microencapsulation of Betacyanin Extract from Red Dragon Fruit Peel

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Abstract

The aim of this research was evaluated the effect of type and ratio of coating materials on characteristics of betacyanin extract microencapsulated by freeze drying. The combination was consisted of maltodextrin+gum arabic (MD+GA), maltodextrin+carboxymethyl cellulose (MD+CMC), maltodextrin+carrageenan (MD+C), and maltodextrin (MD) with ratio 3:1 and 4:1 (w/v) to the extract. Betacyanin microcapsules was analyzed for its characteristics, including encapsulation efficiency and microstructure. The result showed type and ratio of coating materials significantly influenced moisture content, color, and bulk density of the microcapsules ($p < 0,05$). MD+GA coating material had the highest value of encapsulation efficiency (99.41 %). Microstructure analysis of the microcapsules showed it had amorphous shape. Betacyanin microcapsules from red dragon peel was potential to be natural food colorant.



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Introduction

Natural food colorant becomes one of the important components in the composition of food products. Adding colors to the food will make it look more interesting and may affect the consumer acceptance of a food product. The use of synthetic food colorant that exceeds the value of ADI (Acceptable Daily Intake) may cause side effects for health, such as decrease motoric activity of the brain, induce


free radical formation, and damage the DNA of the colon.^{1, 2, 3} Natural food colorant can be an alternative food colorant that does not cause side effects for health.

Betacyanin pigment is one of natural colorant source that produces red-violet color. This pigment is stable in pH 3-7.⁴ Red dragon fruit (*Hylocereus polyrhizus*) is one kind of plant which is rich in

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betacyanin pigment. Red dragon fruit peel which is rich in betacyanin usually is taken away, but it can be utilized as a natural color. Betacyanin extract is not stable during storage. The stability of it is easily influenced by the increase of water activity (*aw*), light exposure, oxygen, temperature, enzyme activity, and pH.⁵ Therefore, the right method to protect betacyanin from degradation is needed. Microencapsulation method will protect the pigment by coating it with specific coating material. Microencapsulation the pigment will protect it from degradation process and extend the shelf life.⁶ One of the microencapsulation techniques is using freeze drying. Freeze drying uses low temperature which is a good method to treat component that sensitive to high temperature. Freeze drying also produces a product with good sensory characteristic.⁷

Coating material is an important factor in microencapsulation process, as it related to the efficiency of the process.⁷ The type of coating material may affect the physical and chemical characteristics of the final product.⁸ Maltodextrin is a common coating material which has high water solubility, cheap, and can protect the core material from oxidation.^{9, 10} However, maltodextrin has low film-forming capacity.⁸ According to,¹¹ single use of coating material will not raise the characteristics of microencapsulate product as required for encapsulate product. Combination of some coating material with the right proportion may needed to produce a product with high encapsulation efficiency and may has lower cost than single coating material.⁸ This study wasevaluated the effect of several types and ratio of coating material on characteristics of betacyanin microcapsule from red dragon fruit peel.

Materials and Methods

Materials

Red dragon fruit was kindly obtained from the traditional market in Malang City, East Java, Indonesia. Maltodextrin (MD), gum arabic (GA), carboxymethyl cellulose (CMC), κ-carrageenan (C), sodium phosphate dibasic, and citric acid (Merck KgaA, Germany) were purchased from chemical store in Malang City, East Java, Indonesia.

Extraction of Betacyanin

Red dragon fruit peel was separated from the fruit with knife. Peels were washed and steam blanched at (90 ± 2) °C for 5 min. The peel then cooled

andmashed. Puree mixed with ethanol 76,80 % in ratio 1:5 (w/v) thenshaked in shaker waterbath (54 ± 1) °C; 26 min, 100 rpm) (Memmert WNE 14, Memmert GmbH + Co. KG, Germany). The extract solution was filtered and evaporated using rotary evaporator (45 °C) (BÜCHI Rotavapor R-205, Switzerland) until left 20% of the extract volume.

Micro Encapsulation

Maltodextrin (MD), maltodextrin+gum arabic (MD+GA), maltodextrin+carboxymethyl cellulose (MD+CMC), and maltodextrin+carrageenan (MD+C) were used as coating materials. Each type of coating material was dissolved into water with a concentration of 40 % for maltodextrin and 1 % for gum arabic, CMC, and carrageenan. The coating material solution were stored in the refrigerator for 24 hours to obtain perfect hydration.¹² The coating materials were mixed with betacyanin extract with ratio 3:1 and 4:1 (w/w). The mixture then stirred (600 rpm, 15 min). Then the mixture solution were lyophilized using freeze dryer (Christ LMC-2, Martin Christ, Germany) at -41 °C in vacuum condition. The dried encapsulated extracts were grinded and sieved with 80 mesh sieve.

Determination of Betacyanin Content

Determination of betacyanin content based on the method by the reference.¹³ Samples were diluted with McIlvaine buffer (pH 6.5) until reached maximum absorption value (1.00 ± 0.05). Samples were analyzed using UV-Vis spectrophotometer (UV mini-1240, SHIMADZU CORPORATION, Japan). The McIlvaine buffer was prepared from 0.1 M citric acid (30 mL) and 0.2 M sodium phosphate dibasic (70 mL). Betacyanin content (Bc) was calculated by the following equation (Eq. 1):

$$Bc \text{ (mg /L)} = [(A \times F \times MW \times 1000) / (\epsilon \times L)] \quad \dots(1)$$

where A is absorption value at maximum wavelength (537 nm) corrected by the absorption at 600 nm (correction for impurities), F is dilution factor, MW is molecular weight of betanin (550 g/mol), ε is molar extinction coefficient of betanin (60.000 L/mol cm), and L is path length of the cuvette (1 cm).

Moisture Content

Moisture content was analyzed using method by the reference.¹⁴

Color

Color (L^* , a^* , b^*) parameters were measurement using a colorimeter (CR-10, KONICA MINOLTA, INC., Japan) using method by the reference.¹⁵ Total color difference (ΔE) was calculated to study color changes using the Eq. 2¹⁶ :

$$\Delta E = [(L^*_i - L^*_o)^2 + (a^*_i - a^*_o)^2 + (b^*_i - b^*_o)^2]^{0.5} \quad \dots(2)$$

where L^*_o, a^*_o, b^*_o are the values of untreated sample and L^*_i, a^*_i, b^*_i are the measured values of each sample with treatment.

Bulk Density

Determination of bulk density value was based on method by the reference¹⁶. As much as 1 g sample was placing in 10 mL graduated cylinder. Then it was tapped 10 times from a height of 10 cm. Subsequently the volume was recorded. Bulk density was calculated in terms of g/mL.

Encapsulation Efficiency

Determination of encapsulation efficiency value was based on method by the reference¹⁸ with modification. To determination of total betacyanin (TB), coating material of the sample was destructed. As much as 200 mg of sample were dispersed in 2 ml mixture of methanol:acetic acid:water (50:8:42 v/v/v). Then sample was agitated using vortex for 2 min. Then sample was centrifuged at 112 000 x g for 5 min. Betacyanin content then analyzed using method by the reference.¹³ To determination of surface betacyanin (SB), 100 mg of sample was dispersed in 2 ml mixture of ethanol:methanol (1:1 v/v). Then sample was agitated using vortex for 1 min and centrifuged at 112 000 x g for 5 min. Betacyanin content then measured by the reference¹³ method. The encapsulation efficiency was determined by the following equation (Eq. 3):

$$\% EE = ((TB - SB) / TB) \times 100 \quad \dots(3)$$

where TB is total betacyanin and SB is surface betacyanin

Recovery of Betacyanin

Determination of recovery of betacyanin was based on study by the reference.⁵

$$\text{Recovery (\%)} = (CB_i / CB_o) \times 100 \quad \dots(4)$$

where CB_o is concentration of betacyanin before drying process and CB_i is concentration of betacyanin after drying process.

Solubility

Solubility of the betacyanin microcapsules was analyzed using method by¹² with modifications. As much as 1 g sample was dissolved in 25 ml of distilled water and stirrer (250 rpm) for 5 min. Then solution was centrifuged at 760 x g for 10 min. As much as 20 ml supernatant was transferred to a pre-weighed petri dish. Then petri dish which containing the sample was dried in the oven at 105°C overnight. The solubility (%) was calculated based on the percentage dried supernatant in relation to the initial weight of the sample (1 g).

Microstructure of Microcapsules

Microstructure of microcapsules were observed using the method described by the reference.¹⁹ Samples were placed in stubs. The sample was coated with gold under vacuum condition. The particle morphology was analyzed using Scanning Electron Microscope (SEM) (FEI Inspect S50, FEI Technologies Inc., United States).

Statistical Analysis

Data was analyzed using Minitab 16 (Minitab Inc., United States) for variance and paired t-test and further tested by Tukey test.

Results and Discussion**Bulk Density, Moisture Content, and Color of Betacyanin Microcapsules**

The interaction between type and ratio of coating materials were significantly influenced bulk density, moisture content, and color of the betacyanin microcapsules ($p < 0.05$). The bulk density values of the microcapsules ranged from 0.67 g/mL to 0.74 g/mL (Table 1). MD+GA with ratio 3:1 had the highest value of bulk density (0.74 g/mL) while MD+CMC with ratio 4:1 had the lowest value of bulk density (0.67 g/mL). Bulk density is influenced by moisture content and molecular weight of coating material⁸. According to¹⁷, product with high moisture content will have high bulk density as well. It is due to the presence of water, which is much denser than dry material. Their report was agreement with these results. MD+GA with ratio 3:1 had higher moisture content (7.15 %) than MD+CMC with ratio 4:1 (6.47 %).

According to,²⁰ higher molecular weight will make it easier for the material to enter and occupy the space among particles and thus results in higher values of bulk density. Gum arabic has the highest molecular weight (47 000 – 3 000 000 g/mol) and the lowest

is carboxymethyl cellulose/CMC (262.19 g/mol). While the molecular weight of maltodextrin is 1800 g/mol and κ-carrageenan is 1507.37 g/mol.^{8, i} This difference is one factor of affecting MD+GA with ratio 3:1 had the highest value of bulk density.

Table 1: Effect of Various Type and Ratio of Coating Materials to Bulk Density, Moisture Content, L(Lightness), a(Redness), and b(Yellowness) of Betacyanin Microcapsules.

Type of Coating Materials	Ratio of Coating Materials	Bulk Density (g/mL)	Moisture Content (%)	Parameters		
				L	a (+)	b (-)
MD	3:1	0.71 ± 0.003 ^{bc}	7.04 ± 0.07 ^{abc}	61.40 ± 0.46 ^{cd}	32.60 ± 0.61 ^b	3.63 ± 0.12 ^{ab}
MD+GA		0.74 ± 0.006 ^a	7.15 ± 0.34 ^{ab}	60.63 ± 0.15 ^{cd}	33.27 ± 0.23 ^{ab}	3.90 ± 0.17 ^{bc}
MD+CMC		0.72 ± 0.003 ^{ab}	7.57 ± 0.09 ^a	60.27 ± 0.38 ^d	33.50 ± 0.20 ^{ab}	3.27 ± 0.15 ^a
MD+C		0.71 ± 0.008 ^{bc}	6.34 ± 0.03 ^d	61.73 ± 0.35 ^c	34.23 ± 0.25 ^a	4.13 ± 0.15 ^c
MD	4:1	0.71 ± 0.003 ^{bc}	6.64 ± 0.21 ^{bcd}	65.53 ± 0.12 ^b	30.80 ± 0.78 ^c	3.97 ± 0.12 ^{bc}
MD+GA		0.70 ± 0.003 ^{cd}	6.50 ± 0.27 ^{bcd}	67.13 ± 0.76 ^a	29.37 ± 0.21 ^d	4.03 ± 0.32 ^{bc}
MD+CMC		0.67 ± 0.013 ^e	6.47 ± 0.40 ^{cd}	66.40 ± 0.20 ^{ab}	29.77 ± 0.35 ^{cd}	3.70 ± 0.10 ^{abc}
MD+C		0.68 ± 0.010 ^{de}	6.91 ± 0.20 ^{bcd}	66.17 ± 0.21 ^{ab}	29.80 ± 0.35 ^{cd}	3.70 ± 0.17 ^{abc}

The displayed value was mean ± standard deviation (n=3)

The values followed by different letters showed a significant difference (p<0.05)

MD: Maltodextrin; GA: Gum Arabic; CMC: Carboxymethyl Cellulose; C: Carrageenan

The type of coating materials with ratio 3:1 had lower lightness values and higher redness values than ratio 4:1 (Table 1). This was related to the encapsulation formulation. As the ratio of coating material decreased, the proportion of betacyanin extract in formulation increased. In the case of

betacyanin extract from red dragon fruit peel, the amount of betacyanin extract was support redness value. The betacyanin content in powders will influence the redness value of the sample.⁷ The higher of redness value resulted in lower lightness value of the microcapsules.

Table 2: Effect of Type of Coating Materials on Encapsulation Efficiency of Microcapsules

Coating Materials	Encapsulation Efficiency (%)
MD	97.91 ± 1.08 ^b
MD+GA	99.41 ± 0.48 ^a
MD+CMC	99.08 ± 0.64 ^a
MD+C	99.26 ± 0.46 ^a

The displayed value was mean ± standard deviation (n=3)

The values followed by different letters showed a significant difference (p<0.05)

MD: Maltodextrin; GA: Gum Arabic; CMC: Carboxymethyl Cellulose; C: Carrageenan

Encapsulation Efficiency of Betacyanin Microcapsules

The type of coating materials significantly affects encapsulation efficiency of the betacyanin microcapsules ($p < 0.05$). The encapsulation efficiency values of the microcapsules ranged from 97.91 % to 99.41 % (Table 2). MD+GA had the highest value of encapsulation efficiency (99.41 %) while MD had the lowest value of encapsulation efficiency (97.91 %). The encapsulation efficiency values affected by characteristic of the coating materials. The single use of maltodextrin as single coating material resulted lower encapsulation efficiency value than combination with other coating material. This possibly due to low film-forming capacity of maltodextrin⁸. Combination maltodextrin with other type of coating material resulted in higher encapsulation efficiency value than single use of maltodextrin. This may correlated to the chemical structure of the coating materials.

Gum arabic is composed of branched polysaccharides (D-glucuronic acid, L-rhamnose, D-galactose and L-arabinose) and contains small amounts of protein. The protein (serine and hydroxyproline) binds covalently to the carbohydrate chain (arabinogalactan). These proteins play a role in the better film formation and can also trap molecules of the core material in a better way.⁹ MD+CMC resulted in higher encapsulation efficiency value than the single use of maltodextrin. This may related to the interaction between CMC and betacyanin structure. CMC is an anionic polysaccharide composed of a hydrophobic polysaccharide side chain and has many hydrophilic carboxyl groups.²¹ Betacyanin structure contains cations,⁹ while CMC is an

anionic polysaccharide, which induce electrostatic interaction or produce hydrogen bond among the components. MD+C also resulted in higher encapsulation efficiency value than the single use of maltodextrin. Kappa-carrageenan has pseudoplastic property so it can act as a plasticizer. The addition of kappa-carrageenan may increase the adhesion force between walls and core materials.²²

Recovery of Betacyanin and Solubility of The Microcapsules

The ratio of coating materials significantly affects recovery of betacyanin and solubility of the microcapsules ($p < 0.05$). Betacyanin microcapsules with ratio of coating materials to extract 3:1 had higher recovery value (84.84 %) than ratio 4:1 (79.33 %) (Table 3). It was possibly related to the structure damage (collapse) during the drying process. Microcapsules with ratio of coating materials 4:1 had higher amount of bounding water which may caused higher amount of the non-frozen solution. Structure damage can happen during the first stage of drying when the drying temperature is higher than the glass temperature. At that time, viscosity of non-frozen solution decrease and causing deformation, destroy the matrices, and damage the structure.²³ Recovery of betacyanin value of the freeze dried microcapsules higher than spray dried microcapsules. The recovery of betacyanin value of the spray dried microcapsules ranged from 54 % to 70 %⁹, while other reference¹⁸ reported that recovery of betacyanin value ranged from 70.9 % to 72.4 %. Freeze drying is a process using low temperature. It better for the stability of thermo sensitive substances like betacyanin.²⁴ While spray drying using high temperature during process which caused degradation of betacyanin.

Table 3: Effect of Coating Materials Ratio on Solubility and Recovery of Betacyanin

Ratio of Coating Materials:Extract	Recovery of Betacyanin (%)	Solubility (%)
3:1	84.84 ± 6.57a	71.22 ± 2.85b
4:1	79.33 ± 9.54b	74.52 ± 4.45a

The displayed value was mean ± standard deviation (n=3)

The values followed by different letters showed a significant difference ($p < 0.05$)

The ratio of 4:1 had a higher solubility value (74.52 %) than the ratio of 3:1 (71.22 %) (Table 3). This may

due to the characteristics and the amount of coating materials in the encapsulation formulation. According

to,¹⁵ high solubility of microcapsules possibly due to the water-soluble type of coating materials. Maltodextrin is a polymer of α -D-glucose with 1,4 and 1,6 glycosidic bonds, and composed of amylose (linear) and amylopectin (branched) structures.²⁵ Gum arabic is a polysaccharide composed of D-glucuronic acid, L-rhamnose, D-galactose, L-arabinose, and contains about 2% protein.^{26, 27} Gum arabic can dissolve well in cold or hot water

(concentration up to 50%). CMC structure consists of hydrophobic polysaccharide side chains and has many hydrophilic carboxyl groups.²¹ CMC can dissolve quickly in cold water and produce a clear solution.²⁸ Carrageenan is composed of D-galactose and 3,6-anhydro-galactose (3,6-AG) binding to α -1,3 and β -1,4-glycosidic. Carrageenan is water-soluble and has a high viscosity when dissolved in water.²⁹

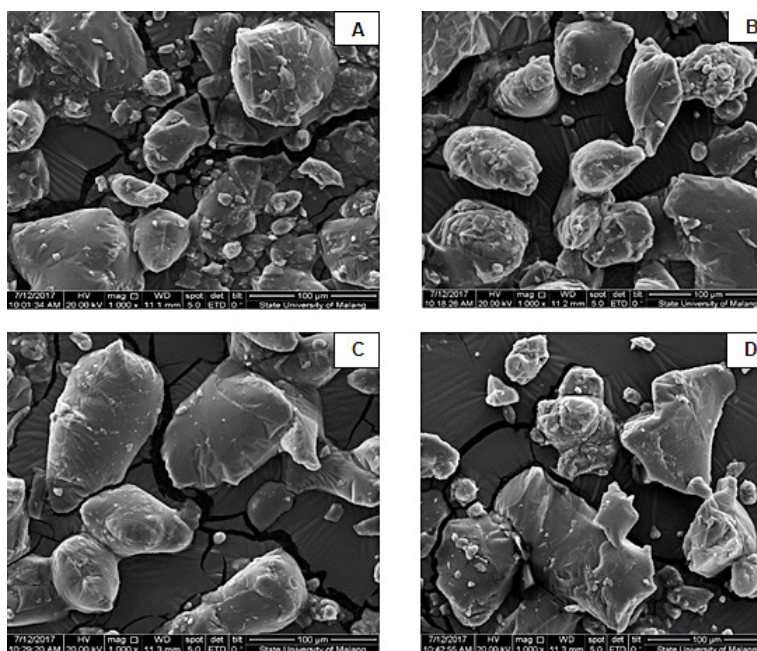


Fig.1: Microstructures of Betacyanin Microcapsules with Different Types of Coating Material: a. Maltodextrin; b. Maltodextrin - Gum Arabic, c. Maltodextrin - CMC, d. Maltodextrin - Carrageenan (Magnification 1000x)

Microstructure of Betacyanin Microcapsules

Betacyanin microcapsules of four different coating materials showed they had amorphous structure (Fig. 1). Microcapsules resulted from freeze drying process has amorphous structure.³⁰ Amorphous structure with glassy shape was formed during the drying, grinding, and sieving process.¹² The shape indicated the betacyanin pigment was trapped by the coating materials and protected from oxidation and heat exposure.^{7, 12} They also reported the SEM micrograph of the particles which resulted amorphous glassy and plate-like shapes. While this study resulted amorphous glassy and rather round shapes. This may due to different encapsulation formulation and type of coating materials.

Conclusion

Based on the results of this study, maltodextrin combined with gum arabic coating material has highest encapsulation efficiency value. The ratio of coating materials to the extract 4:1 had a higher solubility value than the ratio of 3:1. This result indicated betacyanin microcapsules from red dragon fruit peel is a potential natural color to be applied on food product.

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Conflict of Interest

The authors have no conflict of interest to declare.

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