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Optimizing Anthocyanin Yield and Stability from Black Rice Bran through Response Surface Methodology and Microencapsulation

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Abstract

Black rice bran is a rich source of bioactive compounds, particularly anthocyanins, which offer significant health benefits and are thus suitable for development into high-value products. However, anthocyanins are prone to degradation due to environmental factors. The objectives of this research were to study the extraction and encapsulation of anthocyanins using spray drying. Anthocyanin extraction from black rice bran was optimized using response surface methodology with a Box-Behnken design. Additionally, the parameters for spray-dried microcapsule production, including wall materials and inlet air, were investigated. Three key factors for the extraction of anthocyanin from black rice bran involved varying citric acid concentrations (1–4%), temperatures (40–90°C) and time (30–180 min). The optimal conditions providing maximum total anthocyanin content emerged as a 4% citric acid concentration, a temperature of 74.66°C and an extraction time of 37.24 min. Under these conditions, the resulting extract exhibited a total anthocyanin content of 70.70 mg/L. Microencapsulation using maltodextrin and gum arabic at air temperatures of 160°C, 170°C and 180°C produced microcapsules with low moisture content (5.37-6.23%), water activity (0.38–0.48) and high encapsulation efficiency (94.25-98.50%). These microcapsules exhibited substantial antiradical properties, with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) values ranging between 6.90 to 10.11% and 20.82 to 35.51 mg Trolox equivalent (TE)/g, respectively. Maltodextrin at 160°C exhibited the greatest solubility and lowest wettability. The findings of this study offer valuable insights into the extraction and future application of anthocyanin extracts derived from black rice bran in powder form.

Introduction

Black rice has significant economic importance in many countries due to its marketability, export potential, and nutritional benefits. It is rich in essential vitamins, minerals, fiber, and amino acids, making it a valuable food for human nutrition. Particularly, it contains high levels of iron, calcium, magnesium, and potassium. Black rice is gluten-free, cholesterol-free, and low in sugar, making it beneficial for managing various health

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conditions, including diabetes, because of its low glycemic index. It is also a rich source of antioxidants, such as anthocyanins, which protect cells from oxidative stress (Panda et al., 2022). However, despite growing interest among health-conscious consumers, black rice remains less popular than expected. This limited popularity is attributed to the unappealing dark color and firm texture that often result from cooking. Polishing black rice can improve its appearance and texture, but it significantly reduces its antioxidant content, which is concentrated in the rice bran. Given this, the extraction of antioxidant compounds from black rice bran to produce high-value extracts, followed by encapsulation to enhance their stability, offers a promising approach to increase the value of black rice. These encapsulated black rice bran extracts could find applications in the food industry, functional foods, dietary supplements, and beyond.

In the study conducted by Hou et al. (2013), an investigation into the extraction of anthocyanins from black rice across different fractions was conducted. Notably, rice bran emerged as the fraction with the highest anthocyanin content. Anthocyanins, characterized as pigments, exhibit dual utility, serving both as a food colorant and as biologically active substances possessing antioxidative properties (Mattioli et al., 2020).

Response Surface Methodology (RSM), a comprehensive framework encompassing mathematical and statistical techniques, serves as a powerful tool for elucidating the intricate interdependencies between multiple variables and response variables (Latha et al., 2017). It facilitates optimization procedures for configuring factorial variables, with the objective of attaining a desired maximum or minimum value of the response variable. Notably, RSM has proven effective in optimizing extraction parameters, leading to increased yields of antioxidants across a range of source materials, as supported by relevant literature. Nekkaa et al. (2021) successfully employed the Box-Behnken design to optimize the extraction process for polyphenols and flavonoids from *Rhamnus alaternus* leaves, considering factors such as solvent-to-solid ratio, stirring speed, and extraction time. Furthermore, Feki et al. (2021) demonstrated the effectiveness of the Box-Behnken design in assessing the impact of independent variables, including solvent-to-cake ratio, ethanol percentage, microwave power and extraction time, on the extraction of polyphenols and simmondsin from defatted jojoba (Simmondsia chinensis) seed cake. To accomplish the extraction of anthocyanins, Abdel-Aal and Hucl (1999) outlined the use of a variety of solvents, including methanol, ethanol, citric acid and water. Citric acid solution can stabilize anthocyanins in a mildly acidic environment while being non-toxic and cost-effective. Studies have demonstrated the use of citric acid solutions for anthocyanin extraction from blueberries (Koh et al., 2018; Lee & Wrolstad, 2004). Mugwagwa and Chimphango (2019) successfully optimized process conditions for anthocyanin extraction from mango peel using RSM, considering factors such as ethanol concentration, acetic acid concentration and extraction time, resulting in higher yields and antioxidant activity compared to conventional extraction methods. RSM has also been utilized to optimize extraction conditions (temperature, extraction time, liquid-to-solid ratio and ethanol concentration) for anthocyanins from purple corn flour (*Zea mays* L) (Ursu et al., 2023). However, the application of RSM to determine optimal conditions for anthocyanin extraction from black rice bran remains relatively limited in the literature. The present investigation aims to elucidate and establish the efficacy of RSM (Box-Behnken design) as a valuable instrument for the precise optimization of antioxidant extraction procedures from black rice bran. This study serves as a pivotal reference, offering comprehensive insights and guidelines for the extraction of anthocyanin from black rice bran.

Anthocyanins, being pigments of relatively low stability, are susceptible to degradation from many factors, including heat, light, oxygen, pH, concentration and others (Rein, 2005; Cavalcanti et al., 2011). In the realm of the preservation of bioactive compounds and antioxidants, encapsulation has garnered substantial popularity. Among the encapsulation techniques, spray drying stands out as a prevalent method that transmutes products into dry powders that are conducive to storage, exhibit a lightweight nature and are well-suited for both utilization and transportation. The fundamental of this technique involves the encasement of core materials, often susceptible to decomposition, within wall materials, thereby fortifying the core materials against the influence of diverse environmental factors (Mohammed et al., 2020). Consequently, encapsulation through spray drying holds the potential to augment the stability of the core material.

Wall materials commonly employed in anthocyanin encapsulation exhibit diverse characteristics. Gum arabic, for instance, possesses remarkable attributes, such as proficient film formation and emulsification capabilities.

Conversely, maltodextrin excels in forming a protective film (Madene et al., 2006). Gum arabic stands as one of the predominant choices for wall materials in microencapsulation via spray drying due to its high solubility and low viscosity (Pratami et al., 2019). This biopolymer comprises d-glucuronic acid, l-rhamnose, d-galactose and l-arabinose, alongside approximately 2% protein content (Dickinson, 2003). However, the elevated cost and limited availability hinder its utilization within the encapsulation industry. Maltodextrin serves as an effective wall material, conferring stability against oxidation to resulting microcapsules. Furthermore, maltodextrin exhibits low moisture absorbency and is characterized by its safety, colorlessness, lack of odor or taste, widespread availability and cost-effectiveness (Risch &Reineccius, 1988; Kenyon, 1995). In the study conducted by Tonon et al. (2008), an examination of various wall materials, namely maltodextrin, Arabic gum and cassava starch, was undertaken for the purpose of producing anthocyanin microcapsules from acai (*Euterpe oleracea* Mart.). The investigation revealed that microcapsules fabricated using maltodextrin and Arabic gum exhibited notably higher anthocyanin content and superior antioxidant activity when compared to microcapsules generated from cassava starch. The temperature of the inlet air stands out as a crucial determinant influencing the characteristics of microcapsules produced through spray drying. Tonon et al. (2008) observed a notable trend during the spray drying process of acai juice, noting that elevating the inlet air temperature from 138 to 202°C resulted in a reduction of anthocyanin retention from 84.62 to 77.21%. Conversely, Kunapornsujarit and Intipunya (2013) found that the solubility of longan beverage powder exhibited an augmentation with the rise in the inlet air temperature.

Black rice bran is a valuable source of health-promoting anthocyanins. With increasing market demand for functional foods and high-value supplements, extracting anthocyanins from black rice bran and encapsulating them in powder microcapsules is an effective strategy to enhance the value of this agricultural product. This study aimed to determine the optimal conditions for extracting anthocyanin from black rice bran using RSM. Additionally, the research sought to explore the impact of wall materials and inlet air temperature on the microencapsulation of anthocyanin derived from black rice.

Materials and methods

1. Materials

Black rice cv. Hom Nil (*Oryza sativa* L.) was procured from a local rice miller situated in Chiang Mai Province, Thailand. Subsequently, the bran of the rice grain was meticulously separated using a laboratory rice miller. The acquired black rice bran was carefully packaged in metalized bags and preserved at -20°C in a freezer until required for use. Maltodextrin with a dextrose equivalent (DE) of 10, sourced from WGC Co., Ltd., Thailand, along with gum arabic from Union Science Co., Ltd., Thailand, were employed as the designated materials in the study.

2. Study of anthocyanin extraction from black rice bran using response surface methodology

The investigation focused on assessing the impact of three independent variables: citric acid concentration (X_1, ranging from 1 to 4% w/v), temperature (X_2, varying from 40 to 90°C) and time (X_3, spanning 30 to 180 min). Black rice bran (10 g) was extracted with 200 mL of citric acid solution under continuous shaking at various times and temperatures. The evaluation was conducted using RSM within a three-factor Box-Behnken design, as detailed in Table 1. Seventeen experimental runs were generated and the corresponding actual values are presented in Table 2. To model the experimental data, a quadratic polynomial model was applied and the regression coefficients were derived. The generalized quadratic model (eq. 1) employed for the response surface analysis is presented below:

$$Y=\beta_0 + \sum_{i=1}^{3} \beta iXi + \sum_{i=1}^{3} \beta iiXi^2 + \sum_{i=1}^{2} \sum_{i=2}^{3} \beta ijXiXj \quad (1)$$

where β_0 is the constant, βi is the linear coefficient, βii is the quadratic coefficient and βij is the interaction coefficient. *Xi* and *Xj* are levels of the independent variables.

Table 1 Factors and levels of independent variables for Box-Behnken design

Factors	Units	Levels	
		Low	High
Citric acid concentration: X_1	% (w/v)	1	4
Temperature: X_2	°C	40	90
Time: X_3	min	30	180

3. Analysis of total anthocyanin content

The quantification of anthocyanins was conducted in triplicate utilizing a pH differential method as outlined by Giusti and Wrolstad (2001). Sample dilution was carried out employing two buffer solutions with distinct pH values. The first tube received potassium chloride buffer at pH 1.0, while the second tube was treated with sodium acetate buffer at pH 4.5. Following a 15 min incubation period, the absorbances at 510 and 700 nm for both tubes were measured using a UV–vis spectrophotometer (Genesys 10, Thermo Scientific, USA). Total monomeric anthocyanin contents were determined using the provided equation (eq. 2 and 3) and expressed in mg/L.

$$A_{diff} = (A_{510} - A_{700})_{pH1.0} - (A510 - A700)_{pH\ 4.5} \quad (2)$$

$$\text{Total anthocyanin content} = \frac{\text{Adiff x MW x DF x 1000}}{\varepsilon \text{ x } l} \quad (3)$$

where A_{diff} is the difference of absorbance at variant pH, MW is molecular weight (449.2 g/mol) of cyanin-3-glucoside, DF is dilution factor, molar absorptivity (ε) is 26,900 L/mol. cm and path length (*l*) is 1.0 cm.

4. Study of microencapsulation of black rice bran extract

The microencapsulation of black rice bran anthocyanin extract obtained under optimal conditions was carried out utilizing a Buchi Mini Spray Dryer (B-290, Switzerland). This process involved varying the inlet air temperature (160°C, 170°C and 180°C) and utilizing wall materials consisting of maltodextrin and Arabic gum (Fig. 1). The experiment followed a completely randomized design with three replications. To prepare for microencapsulation, 10% of either maltodextrin or Arabic gum was meticulously blended with the anthocyanin extracts, employing continuous stirring via a magnetic stirrer at ambient temperature. Subsequently, these feed mixtures were introduced into the spray dryer at a consistent feed flow rate of 25 mL/min and subjected to spray drying through a pressure nozzle. The inlet hot air was configured to maintain a concurrent flow pattern. Upon completion of the drying process, the resulting black rice bran microcapsules were collected and then hermetically sealed within metalized bags, ensuring preservation at -18°C until subjected to analysis. This procedure was replicated independently three times to produce multiple batches of spray-dried microcapsules.

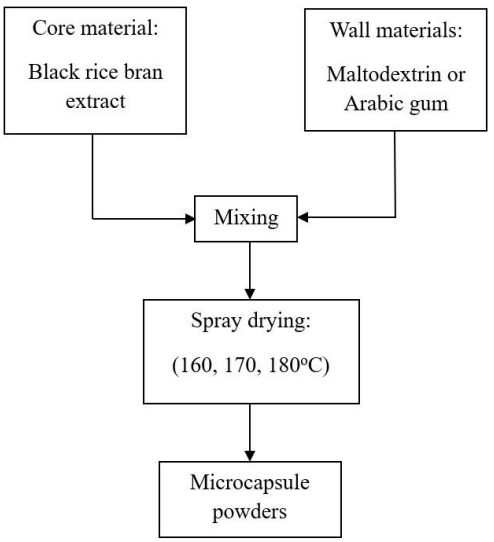

Fig. 1 Microencapsulation process of black rice bran anthocyanin extract

5. Analysis of black rice bran microcapsules

5.1 Color

The color analysis of black rice bran microcapsule samples was conducted within the CIELab system, employing a colorimeter (CR-410, Konica Minolta, Japan) under D65 illuminant conditions. This analysis encompassed several color parameters: The *L** values span a range from 0, denoting darkness, to 100, denoting lightness. The -*a** value corresponds to greenness, while the +*a** value corresponds to redness. The -*b** value denotes blueness min the +b* value signifies yellowness.

5.2 Moisture content and water activity

To assess the moisture content of the black rice microcapsules, a method outlined in AOAC (2000) was employed. Specifically, 2 g of powdered samples were subjected to drying at 105°C for a duration of 3 h using a hot air oven (UN110, Memmert GmbH + Co. KG, Bavaria, Germany). The percentage of moisture content was calculated as the relationship between the mass of evaporated water and the mass of the powder.

Water activity was measured using a water activity analyzer at 25°C (Aqua Lab, Decagon Devices Inc., Pullman, WA, USA).

5.3 Solubility

Solubility assessment was conducted following the methodology outlined by Shittu and Lawal (2007), with slight modifications. Specifically, 1 g of powdered microcapsules was dissolved in 10 mL of distilled water and the resulting mixture was stirred continuously for a duration of 30 min. Subsequently, the suspension was

carefully transferred to a tube and subjected to centrifugation at 6,000 rpm (Magafuge 1.0R, Heraeus, Germany) for a period of 20 min. The supernatant obtained from the centrifugation was completely transferred to an aluminum can and subjected to a drying process at 105°C for a duration of 24 h. After the drying procedure, the weight of the dried soluble solid was measured and this measurement was employed to calculate the solubility as a percentage.

5.4 Wettability

Wettability assessment followed the procedure outlined by Jinapong et al. (2008), with slight modifications. Specifically, 0.1 g of powdered microcapsules was introduced into a 250 mL beaker containing 100 mL of distilled water. The duration of time needed for the powder to achieve complete wetting was recorded, with the visual observation of all powder particles penetrating the water's surface serving as the criterion for determining complete wetness.

5.5 Encapsulation efficiency

The assessment of encapsulation efficiency was conducted in triplicate, involving the analysis of anthocyanin content extracted from two distinct regions of the microcapsules: Total anthocyanin content (TAC) and surface anthocyanin content (SAC), employing the method detailed by Idham et al. (2012). For TAC determination, 100 mg of samples were reconstituted with 1.0 mL of distilled water for 30 sec to disrupt the microcapsule wall structure. Subsequently, 9.0 mL of a 95% (v/v) ethanol solution was introduced to the sample and vigorously mixed using a vortex for 3 min. The resulting anthocyanin extracts were then filtered through Whatman No: 1 filter paper and quantified via the pH differential method, as described previously. To ascertain SAC, 100 mg of samples were rapidly extracted with 10 mL of a 95% (v/v) ethanol solution. The extraction process entailed continuous agitation with a vortex for 30 sec. Following extraction, the anthocyanin extracts were similarly filtered through Whatman No: 1 filter paper and quantified using the pH differential method. The calculation of encapsulation efficiency (%) was executed as follows (eq. 4):

$$\text{Encapsulation efficiency (\%)} = (1 - (\text{SAC}/\text{TAC})) \times 100 \quad (4)$$

5.6 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was assessed following the method described by Gülçin et al. (2006). Briefly, 1 g of the powder was extracted with 20 mL of distilled water and the extract was filtered through Whatman No. 1 paper. One mL of 0.1 mM DPPH (Sigma-Aldrich, Germany) in ethanol solution was added to 3 mL of the extract and the mixtures were incubated in the dark at 30°C for 30 min. The absorbances of the sample (A1) and control (A0) were measured at 517 nm against a blank using a UV-visible spectrophotometer. The DPPH radical scavenging activity was calculated using the following equation (eq. 5):

$$\text{DPPH radical scavenging activity (\%)} = (1 - (A1/A0)) \times 100\% \quad (5)$$

where A1 and A0 are the absorbances of the sample and control, respectively, at 517 nm.

5.7 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) of the microcapsule samples was determined following the method described by Foo et al. (2020), with some modifications. The FRAP reagent was prepared by mixing 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM ferric chloride in a 10:1:1 ratio (v:v:v). FRAP reagent (150 µL) and ddH_2O (100 µL) were added to 50 µL of the microcapsule methanol extracts. Absorbance readings were taken immediately after the addition of the sample and measured at 593 nm. The final absorbance of each sample was compared to a standard curve prepared with Trolox and the antioxidant activity was expressed as mg TE/g microcapsule.

6. Statistical analysis

The design and analysis for the Box-Behnken design experiments, including the calculation of statistically relevant parameters and the generation of three-dimensional graphs, were conducted using Design-Expert software version 13.0.5.0 (Stat-Ease Inc., Minneapolis, MN, USA). All other analyses were carried out in triplicate.

For the analysis of variance (one-way ANOVA) and the determination of significant differences among means, Duncan's multiple range test (DMRT) was applied. These statistical procedures were executed using the Statistical Package for the Social Sciences (Version 24, SPSS Inc., Chicago, IL, USA), with a predetermined significance level set at $P<0.05$.

Results and discussion

1. Model fitting and statistical analysis

The total anthocyanin content resulting from 17 combination treatments across the examined variables is presented in Table 2. The model exhibited a significant fit ($P < 0.05$) with a model F-value of 4.86. Notably, the linear terms (X_1, X_2, X_3), quadratic terms of citric acid concentration (X_1^2) and temperature (X_2^2), as well as the interactions of citric acid concentration and temperature (X_1X_2) and citric acid concentration and time (X_1X_3), exerted significant influences on the response value. The determination coefficient (R^2) for total anthocyanin content reached 0.791, signifying that 79.1% of the variance in the response value can be elucidated by the fitted model. Meanwhile, the lack of fit value was insignificant ($P \geq 0.05$), demonstrating that the model adequately represents the real relationships among the parameters chosen. The regression equation for total anthocyanin content is expressed as follows (eq. 6):

$$Y=-171.58+11.68X_1+5.60X_2+0.35X_3-3.61X_1^2-0.05X_2^2+0.37X_1X_2-0.14X_1X_3 \quad (6)$$

where X_1 is citric acid concentration (%), X_2 is temperature (°C) and X_3 is time (min)

Table 2 Experimental runs from Box-Behnken design and total anthocyanin content of black rice bran extracts

Runs	Factors			Total anthocyanin content (mg/L)
	Citric acid (X_1; %)	Temperature (X_2; °C)	Time (X_3; min)	
1	2.5	90	30	15.81±5.99
2	1	40	105	20.26±1.39
3	2.5	65	105	62.12±3.53
4	2.5	65	105	67.91±8.59
5	2.5	40	30	20.93±1.02
6	4	65	30	48.54±0.77
7	2.5	65	105	71.03±2.78
8	2.5	65	105	66.80±3.47
9	1	65	30	64.57±5.01
10	1	65	180	76.37±7.74
11	1	90	105	13.14±1.62
12	2.5	40	180	23.82±3.68
13	2.5	90	180	18.04±1.34
14	2.5	65	105	56.33±7.02
15	4	90	105	16.03±4.17
16	4	65	180	57.44±6.78
17	4	40	105	62.71±4.00
Optimal	3.59	74.66	37.24	76.60
Experimental value				70.70±4.10

Remark: Values of anthocyanin content represent mean±SD.

2. Influence of independent factors on the total anthocyanin content

The response surface plots depicting anthocyanin extraction are illustrated in Fig. 2. Specifically, Fig. 2(a) delineates the impact of citric acid concentration and extraction time on the total anthocyanin content, with the temperature held constant at 65°C. The findings suggest that the total anthocyanin content exhibited an upward trend with an increase in citric acid concentration, while extraction time demonstrated a comparatively lower effect, as evident from the low coefficient of X_3 in equation 5. The heightened citric acid concentration enhanced solvent polarity, promoting increased solubility of anthocyanin. Grujic et al. (2012) noted that increasing solvent polarity could enhance the total flavonoid content in the extracted material. In cereals, phenolic acids and flavonoids exist in free and bound forms, primarily concentrated in the cell wall and vacuole of the aleurone layer (Shibuya, 1984; Naczk and Shahidi, 2006; Agati et al. 2012). Laokuldilok et al. (2011) reported that in black rice brans, a significant proportion of phenolic acids (70.8 to 76.4%) existed in a bound form. Increased citric acid concentration negatively impacted the cell membrane, intensifying its disruption and releasing larger amounts of anthocyanin. Additionally, the acidity of the acidified solvent (low pH) enhanced the stability of free anthocyanins in the extracts (Abdel-Aal and Hucl 2003; Naczk and Shahidi 2006). However, the anthocyanin extraction yield reaches its peak at a 4% citric acid concentration and potentially remains constant or declines thereafter. This suggests that an excess amount (> 4%) may lead to the partial hydrolysis of glycosidic bonds or the disruption of linkages with metals or co-pigments (Oancea et al., 2012).

Fig. 2(b) presents the total anthocyanin content under varying temperature and time conditions, maintaining a fixed citric acid concentration of 2.5%. It is evident that an increase in extraction temperature correlates with an elevation in total anthocyanin content. However, beyond 75°C, a notable decline in total anthocyanin content is observed. High temperatures enhance solute solubility and the diffusion coefficient. Nevertheless, as reported by Markakis et al. (1957) anthocyanins undergo rapid decomposition at temperatures exceeding 70°C. The acid hydrolysis of sugar residues, accelerated by heat, results in the production of furfural and hydroxymethylfurfural, a phenomenon that has been shown to accelerate pigment deterioration. At a fixed extraction time of 105 min the augmentation in extraction efficiency due to elevated

temperatures showed a more pronounced effect with increasing acid concentration, indicating a synergistic influence between these two factors (Fig. 2(c)). This finding is consistent with the research conducted by Serea et al. (2023), wherein they optimized the extraction parameters for anthocyanin compounds and antioxidant properties from red grape (*Băbească neagră*) peels.

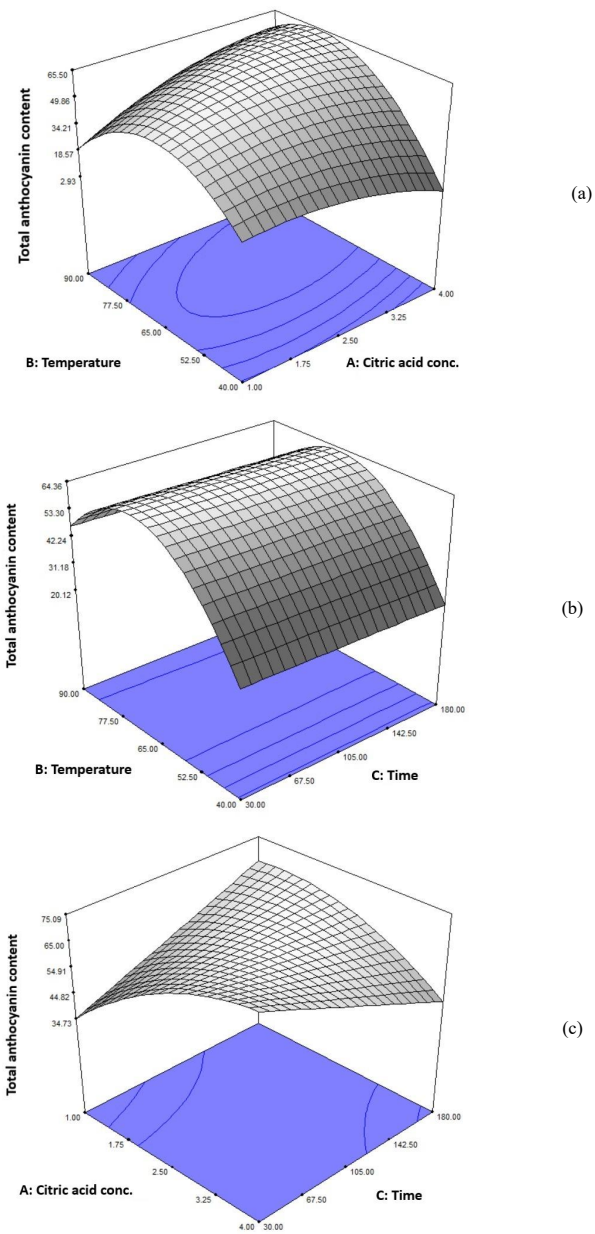

Fig. 2 The response surface plots of independent variables on total anthocyanin content; citric acid concentration and time (a), temperature and time (b), temperature and citric concentration (c)

3. Optimization and validation of extraction conditions

Utilizing Equation 5, the optimal extraction conditions for achieving the maximum anthocyanin content were determined to be a 4% citric acid concentration, a temperature of 74.66°C and an extraction time of 37.24 min projecting a total anthocyanin content of 76.60 mg/L with 1.00 desirability. The optimal conditions included the highest citric acid concentration (4%) and the highest temperature just before anthocyanin decomposition (74.66°C), requiring only 37.24 min to extract the maximum anthocyanin from black rice bran. Subsequent validation experiments conducted under these optimized conditions yielded an extract with a total anthocyanin content of 70.70 mg/L, representing only a 7.70% deviation from the predicted value. This outcome substantiates the reliability of the equation. The extract was spray-dried to investigate microencapsulation in the next study.

4. Encapsulation efficiency and antioxidant activities

The encapsulation efficiency across all samples exhibited no significant differences ($P \geq 0.05$) and fell within the range of 94.25-98.50%. This range surpassed those reported in earlier studies by Rosa et al. (2019), where encapsulation efficiency for anthocyanin compounds extracted from blueberry (*Vaccinium* spp.) microcapsules by spray drying with DE20 maltodextrin as the wall material ranged from 82.53% to 84.79%. Interestingly, this range is comparable to the encapsulation efficiency reported by He et al. (2020) for royal jelly sieve residue microcapsules, which utilized Arabic gum and gelatin as wall materials, with an efficiency of 92.34%. Both inlet air temperature and wall materials significantly impacted antioxidant activity, as determined by DPPH and FRAP assays. Anthocyanins are considered relatively good electron donors. Laokuldilok and Kanha (2017) reported that the antioxidant activity of spray-dried microcapsules is closely related to their total anthocyanin content. Concerning the influence of inlet air temperature, higher temperatures corresponded to lower DPPH and FRAP values, likely due to the thermal degradation of anthocyanin pigments. Ersus and Yurdagel (2007) emphasized that elevated drying temperatures (above 180ºC) are unsuitable for the spray-drying process of anthocyanin pigments. The results suggest that the conditions for producing anthocyanin microcapsules from black rice bran with high antioxidant activity involve using maltodextrin or Arabic gum as wall materials and an air inlet temperature of 160°C.

Table 3 Encapsulation efficiency and antioxidant activities of black rice bran microcapsules

Wall materials	Inlet temperature (C°)	Encapsulation efficiency^{ns} (%)	DPPH (%inhibition)	FRAP (mg TE/g)
Maltodextrin	160	94.2±0.50	10.74±0.73^{a}	35.46±2.09^{a}
Maltodextrin	170	97.42±0.92	10.11±1.38^{ab}	32.85±0.44^{b}
Maltodextrin	180	97.26±0.12	9.87±0.53^{abc}	31.96±0.44^{bc}
Arabic gum	160	98.50±0.60	9.82±0.15^{abc}	35.25±0.33^{a}
Arabic gum	170	97.72±0.96	8.51±0.51^{c}	31.39±1.02^{bc}
Arabic gum	180	96.76±0.17	8.76±0.17^{bc}	30.78±0.72^{c}

Remark: Statistical significance among data sets (mean±SD) is denoted by vertically distinct letters (P≤0.05); ns = not significant (P≥0.05).

5. Power property of black rice bran microcapsules

The moisture content in the black rice bran microcapsule powders ranged from 5.37 to 6.23%, while the water activity of all microcapsules remained low, ranging between 0.35 and 0.40 (Table 4). Water activity functions as a metric for assessing the presence of free water within a food system. As noted by Rahman (2009), a pivotal water activity level below 0.6 is inhibitory to microorganism growth, indicating that microcapsules with lower water activity levels demonstrate microbiological stability.

Table 4 Water activity, moisture content, solubility and wettability of black rice bran microcapsules

Wall materials	Inlet temperature (C°)	Water activity (a_w)	Moisture content (%)	Solubility (%)	Wettability (min)
Maltodextrin	160	0.39±0.01^{a}	6.23±0.02^{a}	99.42±0.03^{a}	2.37±0.03^{f}
Maltodextrin	170	0.38±0.00^{b}	5.88±0.03^{c}	97.13±0.04^{b}	3.00±0.05^{e}
Maltodextrin	180	0.36±0.01^{b}	5.61±0.02^{e}	96.97±0.04^{c}	4.58±0.04^{c}
Arabic gum	160	0.40±0.01^{a}	6.15±0.02^{b}	96.71±0.03^{c}	4.43±0.03^{d}
Arabic gum	170	0.37±0.01^{b}	5.70±0.03^{d}	93.72±0.03^{d}	5.40±0.03^{b}
Arabic gum	180	0.35±0.01^{c}	5.37±0.01^{f}	92.31±0.03^{e}	6.13±0.04^{a}

Remark: Statistical significance among data sets (mean±SD) is denoted by vertically distinct letters (P≤0.05).

Solubility plays a crucial role in defining the characteristics of the powder, particularly its ability to dissolve in water. Table 4 illustrates that the solubility of black rice bran microcapsules ranged from 92.31 to 99.42%. The findings indicate a significant decrease in powder solubility (P≤0.05) with an increase in the inlet temperature. This aligns with the observations of Chegini and Ghobadian (2005) as well as Quek et al. (2007), who noted that elevated inlet air temperature leads to a surface layer hardening, hindering water diffusion through the particles in spray-dried powders of orange and watermelon juices. In contrast to maltodextrin, the use of Arabic gum in microencapsulation led to a decrease in the solubility of black rice bran microcapsules. This finding aligns with the research conducted by Wei and Sulaiman (2022), where the solubility of beet juice skim milk powder was explored using both maltodextrin and Arabic gum. The higher hydrophobic content in Arabic gum compared to maltodextrin contributes to the reduced solubility of the microcapsules. Nevertheless, all samples demonstrated high water solubility, surpassing 92%.

Wettability, the primary property influencing powder reconstitution, determines the time required for a powder to penetrate the water surface and achieve full wetness. A shorter average time signifies superior wettability. Table 4 demonstrates that the wettability of black rice bran microcapsules ranged from 2.37 to 6.13 min.

Interestingly, an increase in inlet temperature from 160 to 180°C enhanced the wettability (P ≤ 0.05) of the powders. This finding aligns with Nguyen et al. (2018) study, where an increase in inlet air temperature led to an elevated wettability index in spray-dried soymilk powder. They proposed that the higher temperature reduced particle agglomeration in the drying chamber, resulting in a larger surface area/mass ratio. While individual particles may not be wet, they could clump together, forming a wet surface layer that reduces the rate of water penetration into the particle clump (Ortega-Rivas, 2009). Microcapsules produced from Arabic gum exhibited significantly lower wettability compared to those produced from maltodextrin (P ≤ 0.05). This difference may be attributed to the higher hydrophobicity of Arabic gum, which hinders particle wetting. Inlet air temperature can influence both particle size and porosity of microcapsules, as observed in studies by Tonon et al. (2011) and Lee et al. (2017), which reported that higher inlet air temperatures result in larger, more porous particles, likely due to increased swelling with rising drying temperatures. At elevated drying rates, rapid moisture evaporation promotes the formation of a rigid outer crust, preventing particle shrinkage during spray drying. Conversely, lower inlet air temperatures prolong moisture retention, allowing gradual shrinkage and reducing particle size. Further investigation into other properties, such as reconstitution behavior, powder characteristics, release profiles and storage stability, may be needed to comprehensively assess product quality.

6. Appearance and color parameters of black rice bran microcapsules

Fig. 3 displays the appearance and visible color of anthocyanin microcapsules derived from black rice bran, prepared under varying conditions of inlet air temperature

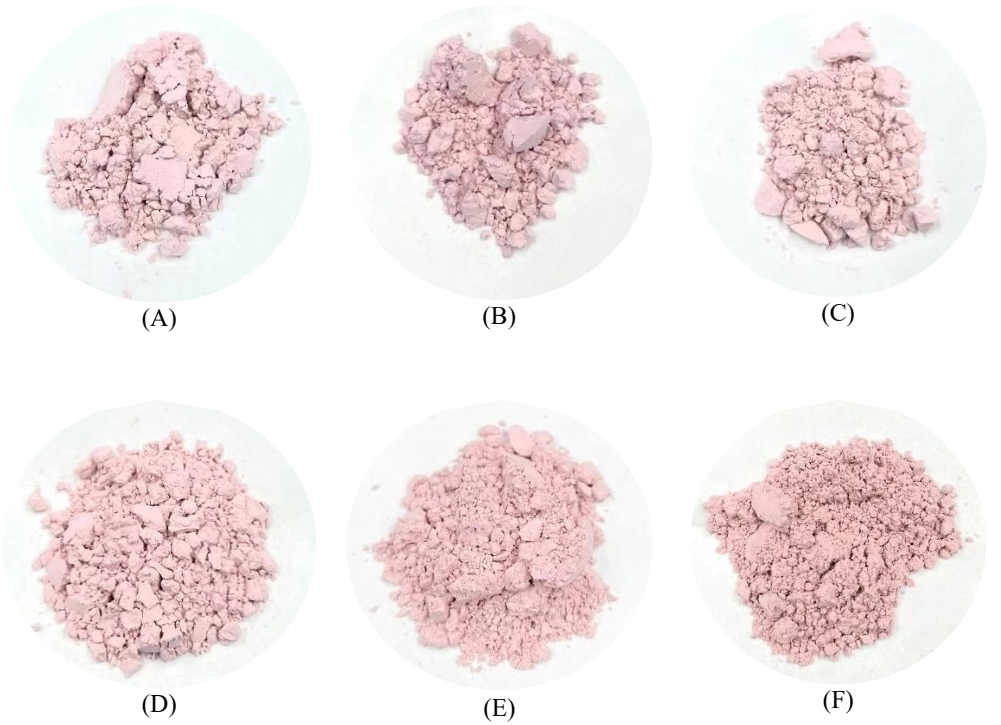

Fig. 3 Black rice bran microcapsules from various wall materials and inlet air temperature
A : Maltodextrin at 160°C inlet temperature
B : Maltodextrin at 170°C inlet temperature
C : Maltodextrin at 180°C inlet temperature
D : Gum arabic at 160°C inlet temperature
E : Gum arabic at 170°C inlet temperature
F : Gum arabic at 180°C inlet temperature

and wall materials. All powder samples exhibited a comparable fine powder texture and their visible color, appearing as a shade of pink, remained indistinguishable to the naked eye. The color parameters, including *L**, *a** and *b** values (Table 5), showed no statistically significant differences (P>0.05). Since maltodextrin and Arabic gum are both white, they had minimal effect on the color of the microcapsules, so the observed color was primarily due to the anthocyanin core. Higher inlet air temperatures led to a decrease in antioxidant activity, suggesting thermal degradation of some anthocyanins. However, this reduction was too small to be detected by colorimetric measurements of the microcapsules. The *L**, *b** and *a** values of the microcapsules ranged between 80.25–81.62, 14.06–14.71 and 3.17–3.38, respectively, indicating a bright red hue with a slight yellow tint across all microcapsules.

Table 5 Color parameters of black rice bran microcapsules

Wall materials	Inlet temperature (C°)	Color parameter		
		L^{*ns}	a^{*ns}	b^{*ns}
Maltodextrin	160	81.62±3.50	14.17±0.75	3.26±0.15
Maltodextrin	170	81.25±3.28	14.46±0.77	3.17±0.20
Maltodextrin	180	80.85±4.17	14.35±0.56	3.38±0.15
Arabic gum	160	80.81±4.43	14.71±0.85	3.27±0.13
Arabic gum	170	81.56±2.22	14.06±0.59	3.27±0.15
Arabic gum	180	80.52±3.12	14.64±0.62	3.36±0.16

Remark: ns = not significantly different (P ≥ 0.05) in vertical data (mean±SD).

Conclusion

The Box-Behnken design employed in this study encompassed all factors–citric acid concentration, temperature and time. These factors were fitted with a quadratic model, yielding optimal conditions: 4% citric acid concentration, a temperature of 74.66°C and an extraction time of 37.24 min resulting in a maximum anthocyanin content of 70.70 mg/L, closely aligned with

the predicted values. Successful production of black rice bran anthocyanin microcapsules in powder form was achieved using maltodextrin and gum arabic as wall materials. Our findings highlight the superiority of microcapsules from maltodextrin over Arabic gum, exhibiting higher solubility, lower wettability and cost-effectiveness. An increase in the inlet air temperature had a negative impact on antioxidant activities, solubility and wettability. For the desired properties, microencapsulation using maltodextrin at 160°C is recommended for producing black rice bran anthocyanin powder. The study suggests that the Box-Behnken design is an effective tool for optimizing anthocyanin extraction conditions from black rice bran and may have potential applications in the extraction of bioactive compounds from other sources. Additionally, the production conditions for anthocyanin microcapsules proposed in this study provide valuable guidance for future applications, including functional foods, supplements and personal care products.

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