



# Microencapsulation of betacyanin from red dragon fruit (*Hylocereus polyrhizus*) peels using pectin by simple coacervation to enhance stability

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**Abstract** Betacyanin is a red natural dye pigment widely used in food products. However, the pigment is also unstable and easily degraded by temperature during storage and food processing. This research aims to increase the stability of betacyanin obtained from dragon fruit peels using pectin as a wall medium via the coacervation method. Due to the efficiency and shell integrity, the coacervation method was selected instead of spray drying to enhance betacyanin's stability. Coacervation was conducted in a three-necked round-bottomed flask fitted with a mercury-sealed stirrer and reflux condenser. An accelerated stability test was conducted at 80 °C and 100 °C for 30 min and considered completed after obtaining a stable absorbance. Two full factorials, three-level design, for 80 °C and 100 °C, were analyzed by Response Surface Methodology using Minitab® 19. The core/wall ratio, agitation speed, and pH were the continuous variables, with temperature as the categorical variables. The models were yielded high R-square and low coefficient of variance on the validation process. Simple coacervation is selected because of a superior method such as simplicity, low-cost, high efficiency, and high shell integrity.

**Keywords** Betacyanin · Coacervation · Natural dye · Microencapsulation · Pectin

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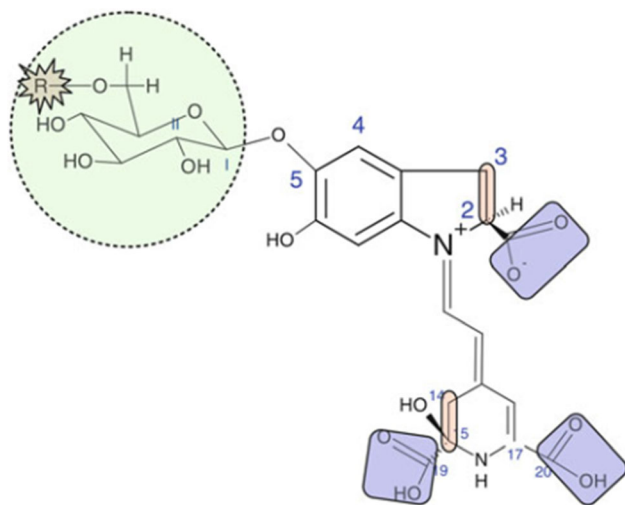
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## Introduction

As a natural dye, betacyanin can be derived from red dragon fruit (*Hylocereus polyrhizus*) peels. Dragon fruit peels as agro-industry waste are abundantly available in Indonesia, with around 150,000 tons/year of dragon fruit (Muas et al. 2019) and can support natural dye production requirements. Betalain consists of two components, namely, betacyanin and betaxanthin. Betacyanin is responsible for the reddish to the peels' violet color, while betaxanthin produces a yellow to orange color. However, compared with synthetic dyes, betacyanin is much easier to degrade by light exposure, temperature, pH, water activity, soluble metals, and enzymatic reactions. During food processing, heating causes betacyanin to break down into betalamic acid and cyclo-DOPA-5-O-β-glucoside (Gengatharan et al. 2015). Degradation is one of the key issues encountered in the use of food colorants. As natural dyes' potential and drawback, some innovation is necessary to minimize natural dyes' disadvantages as food colorants (Rahayuningsih et al. 2019).

Improving the stability of betacyanin could greatly expand its applications, although many schemes of degradation reactions exist, as seen in Fig. 1. The stability of betacyanin may be improved by adding a stabilizer. A stabilizer commonly used in the food industry and betacyanin extraction is ascorbic acid (Esquivel 2016). The addition of 0.01% (w/v) ascorbic acid increases betacyanin's stability and protects the pigment from degradation (Skopińska et al. 2015). However, Woo et al. (2011) revealed that ascorbic acid is only effective under dark storage conditions.

Other methods should be developed to optimize the utilization of betacyanin as a natural dye. The encapsulation method has recently gained increased interest on



**Fig. 1** Structure of betacyanin with easily degraded groups. Here green, blue, and roseate groups/bonds can be modified by deglycosylation, decarboxylation, and dehydrogenation, respectively (Stintzing and Carle 2007)

account of its ability to enhance betacyanin's stability. Several encapsulation methods, including emulsification, fluid bed coating, coacervation, extrusion, and coextrusion (Zuidam and Nedovic 2010; da Silva et al. 2016), have been proposed. Coacervation is a microencapsulation method in which the active compound is continuously coated by an encapsulation medium (Wong and Siow 2015). In recent work by Castro-Enríquez et al. (2019), the coacervation method has not been applied in increasing betacyanin stability; with spray drying, and freeze-drying dominated the findings. Although spray-drying technology is commonly applied to achieve microencapsulation because of its low cost, flexibility, good quality, and high yield (Ileana et al. 2019; Gómez et al. 2018), coacervation has some advantages such as more applicable, high encapsulation efficiency, and high shell integrity (Gaonkar et al. 2014).

This research aims to boost the stability of betacyanin from dragon fruit peels using pectin as a wall medium via the coacervation method. Pectin as the wall medium is proposed to achieve the microencapsulation of betacyanin by adsorbing the water formed during coacervation to form a gel. Pectin, guar gum, and locust bean gum are reported to be promising matrices that can enhance betacyanin's stability (Esquivel 2016). Accelerated conditions to test the stability of betacyanin after microencapsulation was also proposed in this study (Aguilar-Tuesta et al. 2018; Gómez et al. 2018; Ileana et al. 2019; Otálora et al. 2015; Vergara et al. 2014).

## Materials and Method

### Materials

The material used in this study was the dragon fruit peels. Dragon fruits were purchased from a market in Yogyakarta City, Indonesia. The flesh and peels were separated using a knife. The acetic acid (glacial) and reagent grade methanol were purchased from Merck. Pectin (food grade) with a high methoxyl content was purchased from a grocery store in Yogyakarta, Indonesia.

### Preparation of dragon fruit peel

Dragon fruit peels were washed with water at room temperature to remove adherent dirt. The peels were cut into small pieces using a knife, dried in an oven at 70 °C until the moisture was reduced by 30%. The drying process was occurred for around 2 h. The dried peels were blended into 0.50 mm accordingly.

### Control solution preparation

The treated dragon fruit peel was weighed out into samples of 1.5, 2.0, and 2.25 g and mixed with 300, 400, and 450 mL of distilled water, respectively. Separately, each mixture was blended for 10 min with the blender rotation indicator on a scale of 3. The mixture was then filtered with filter paper. The resulting filtrate is a solution of betacyanin dye. The mixture has measured the pH using a pH meter, and the absorbance using Vernier—SpectroVis plus SVIS-PL spectrophotometer at 540.2 nm assisted with the Logger Lite program. These three solutions are then used as control solutions. The control sample was used to compare with or without a simple coacervation method on the accelerated test as a preliminary study.

### Sample solution preparation

Betacyanin dye solution was prepared in the same way as the control solution. The variation of the core/wall ratio was determined to be 1:1; 2:1 and 3:1, made by mixing pectin as much as 1.5 g, 1.0 g, and 0.75 g, respectively dissolved into 300 mL, 200 mL, and 150 mL of distilled water in a three-neck flask equipped with a stirrer. To this pectin solution, each of the previously prepared betacyanin solutions was added (1.5 g, 2.0 g, and 2.25 g dragon fruit peel powder in 300 mL, 400 mL, and 450 mL aquadest). Thus, the core/wall ratio variation was 1:1; 2:1 and 3:1 can be obtained. The stirrer rotation speed was varied by 400 rpm, 600 rpm, and 800 rpm in the same methods. Moreover, the betacyanin solution was also added by

prepared acetic acid solution (5 mL 96% acetic acid plus 40 mL distilled water) to get a variation of pH 4, pH 5, pH 6.

### Encapsulation efficiency

The encapsulation efficiency test was conducted by the dissolution method because betacyanin is soluble in methanol. Betacyanin solution was dissolved in methanol with a 1:1 ratio for 10 min. The solution was centrifuged to separate insoluble pectin. The solution, then, was analyzed using a Vernier—SpectroVis plus SVIS-PL spectrophotometer at 540.2 nm assisted with the Logger Lite program. The encapsulation efficiency was calculated using the following equation:

$$\%EE = \frac{A_{final}}{A_{initial}} \times 100\% \quad (1)$$

where %EE is encapsulation efficiency,  $A_{final}$  is the absorbance of the sample after dissolution, and  $A_{initial}$  is the initial absorbance of the sample.

### Preliminary study of accelerated stability

Prior to the main research, screening research was conducted to determine the betacyanin solution's behavior on heating time. The control and sample solutions were treated at 70 °C to determine the effective time of the accelerated stability. The sample solutions used in the preliminary study was varied at 400, 600, and 800 rpm with a 1:1 core/wall ratio and neutral pH. The samples' absorbance value was analyzed using a Vernier—SpectroVis plus SVIS-PL spectrophotometer at 540.2 nm assisted with the Logger Lite program every 5 min for 60 min. The effective time was obtained by stable absorbance value within time. The effective time would be used as the duration of stability test on a simple coacervation method. Moreover, the effectiveness of simple coacervation was observed accordingly to compare control and sample solutions.

### Acceleration of betacyanin stability

The sample solutions were treated at 80 °C and 100 °C to accelerate betacyanin degradation. The samples' absorbance was measured at the initial condition and final condition at an effective time from the preliminary study. The analysis was conducted by a Vernier—SpectroVis plus SVIS-PL spectrophotometer at 540.2 nm, assisted with the Logger Lite program. The retention percentage was calculated using the following equation:

$$\%retention = \frac{A_t}{A_0} \times 100\% \quad (2)$$

where % retention is the ability to deal with temperature in betacyanin stability,  $A_t$  is the absorbance of the sample at the effective time, and  $A_0$  is the initial absorbance of the sample. This formula, which relates the absorbance to the concentration of betacyanin in solution, was described by Cai and Corke (1999).

$$BC = \frac{A \times DF \times MW \times 1000}{\epsilon \times L} \quad (3)$$

where  $BC$  is the amount of betacyanin equivalents in mg/L,  $A$  is the absorption at 540.2 nm,  $DF$  is the dilution factor,  $MW$  is the molecular weight of betacyanin (550 g/mol),  $\epsilon$  is the molar extinction coefficient (60,000 Lmol<sup>-1</sup> cm<sup>-1</sup>), and  $L$  is the length of the cuvette (1.0 cm).

### Data analysis

In this work, the independent variables were core/wall ratio (1:1, 2:1, and 3:1), agitation speed (400, 600, and 800 rpm), and pH (4, 5, and 6). The dependent variable was betacyanin retention of the sample solution. Meanwhile, the temperature, 80 °C and 100 °C, became the categorical variable in this study. All samples were conducted three times to ensure data repeatability. All variables, three continuous and two categorical variables, were analyzed using Minitab® 19 optimization software via the response surface methodology (RSM). Thus, a three-level full factorial design was used to analyze the correlation of variables for each categorical variable. RSM provides a means to explain the effect of each parameter studied on the retention percentage. Indeed, this method has been used in many previous research studies (Rahayuningsih et al. 2018, 2019, 2020). The validation was also conducted using specified independent variables to generate the response at 80 °C and 100 °C. The retention value was compared with the model, and the Coefficient of Variance (CV) was calculated accordingly. The morphology analysis was also conducted to strengthen the coacervation process using Transmission Electron Microscopy (TEM).

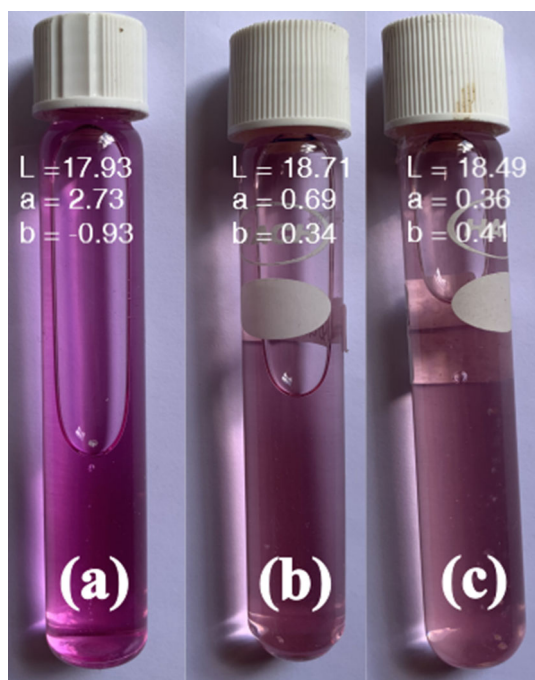
### Results and discussion

In preliminary research, the results demonstrated that the solutions' absorbance shows a relatively constant value after 30 min. Moreover, the trend is typical at various agitation speeds, while the microencapsulation process yielded good results compared with the control. The control had a 23% retention value, while the sample had a

$66 \pm 5\%$  retention value. The retention percentage was calculated using Eq. (2).

The color degradation could be analyzed by colorimeter and UV–Visible spectrophotometry. In general, a colorimeter can be used to describe the color degradation, but the calculation should be conducted further with a specified formula. In addition, UV–Visible spectrophotometry offers more advantages such as higher sensitivity, flexibility, and versatility compared with colorimeters (Lee et al. 2020). Thus the color difference could be calculated easily and precisely, and the retention value would represent the phenomena. To give a perspective of color degradation, Fig. 2 represents the color difference of the study. The encapsulation process was evaluated by encapsulation efficiency using the dissolution method. The encapsulation efficiency showed a significant result as much as  $93.2 \pm 3.1\%$ . The absorbance value of the encapsulation efficiency is presented in Table 1. The simple coacervation process was also confirmed by morphology analysis, as seen in Fig. 3. The morphology image showed that the betacyanin size was increased with a simple coacervation process, from Fig. 3a to Fig. 3b. In other words, pectin as the wall media has successfully encapsulated betacyanin. Pectin has a large molecule, which eventually enlarges the betacyanin particle size.

Prior to RSM, a residual analysis was carried out to check the data distributions. The residual data versus percentage are close to the reference line. These results



**Fig. 2** Visual perception of the samples at **a** initial condition, **b** 30 min at 80 °C, and **c** 30 min at 100 °C with Hunter lab colorimeter result

**Table 1** Encapsulation efficiency test result

No	Sample	Absorbance	%EE
1	EE1	0.0766	87.8
2	EE2	0.0793	90.9
3	EE3	0.0791	90.7
4	EE4	0.0811	93.0
5	EE5	0.0827	94.8
6	EE6	0.0840	96.3
7	EE7	0.0825	94.6
8	EE8	0.0852	97.7
9	EE9	0.0807	92.6
10	Initial condition	0.0872	

confirmed that the data obtained in this research could be used to determine correlations among the independent and dependent variables. The data were analyzed using RSM for both temperatures, which are shown in Table 2. The *F*-value and *P*-value describe the fitness and significance of the RSM models. The *F*-values should be greater than the *F*-statistic values, 25.025 and 28.771 for experiments conducted at 80 °C and 100 °C, respectively. A comparison of *F*-values indicated that the model fit the data well. Moreover, the *P*-value of the models obtained at 80 °C and 100 °C revealed statistical significance. *R*<sup>2</sup> results also gave high values of 95.74% and 96.72% for both models.

All of the parameters examined revealed good results; thus, the models could be used to analyze the relationships between the independent and dependent variables. The models obtained for the data collected at 80 °C and 100 °C are presented below:

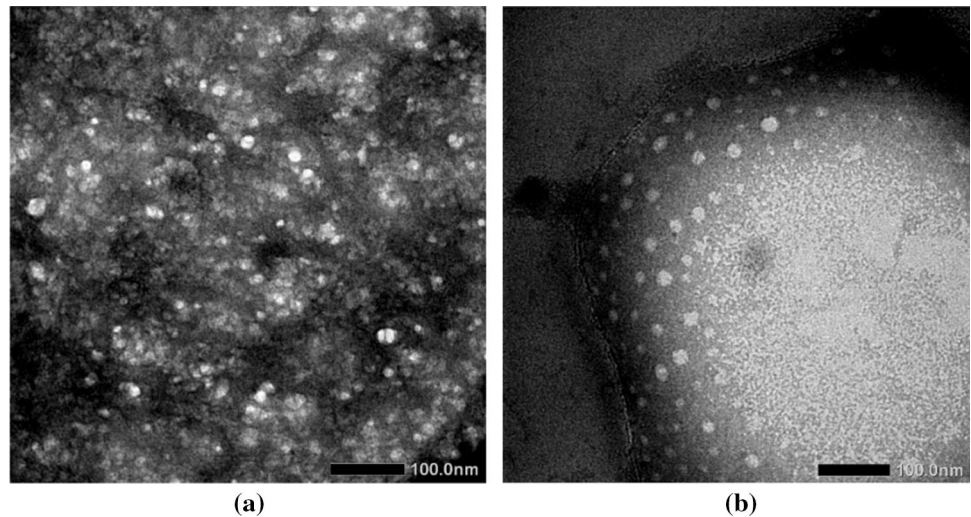
$$\begin{aligned} \%Retention = & -76.6 - 16.19X_1 + 0.1803X_2 \\ & + 40.4X_3 + 2.60X_1^2 - 0.000112X_2^2 \\ & - 2.99X_3^2 - 0.00129X_1X_2 \\ & - 2.36X_1X_3 - 0.00549X_2X_3 \end{aligned} \quad (4)$$

$$\begin{aligned} \%Retention = & -10.4 - 15.05X_1 + 0.0229X_2 \\ & + 32.7X_3 + 1.07X_1^2 - 0.000021X_2^2 \\ & - 2.66X_3^2 + 0.00307X_1X_2 \\ & - 1.78X_1X_3 + 0.00101X_2X_3 \end{aligned} \quad (5)$$

where *X*<sub>1</sub> is the core/wall ratio (i.e., 1, 2, and 3), *X*<sub>2</sub> is the agitation speed (i.e., 400, 600, and 800 rpm), and *X*<sub>3</sub> is the pH (i.e., 4, 5, and 6). Linear plots of the retention models versus the experimental retentions obtained are provided in Fig. 4. Overall, the models showed high *R*<sup>2</sup> values (> 0.95) and good value of slopes ( $\approx 1$ ).

Furthermore, the validation had been conducted accordingly with fixed *X*<sub>1</sub>, *X*<sub>2</sub>, and *X*<sub>3</sub>. The resulted percent

**Fig. 3** TEM analysis result of betacyanin **a** before simple coacervation process and **b** after simple coacervation process

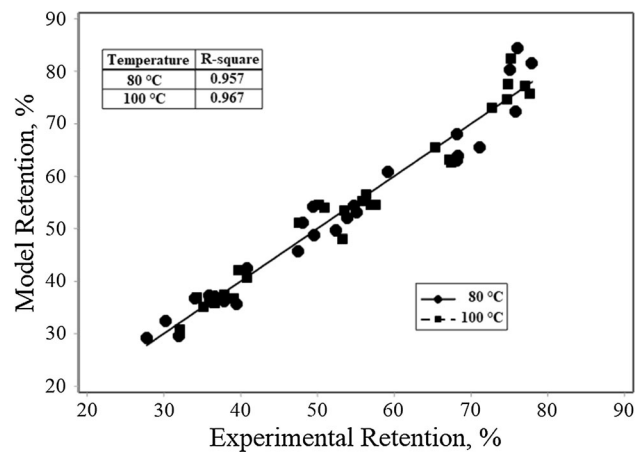


**Table 2** Analysis of variance results

Source	80 °C		100 °C	
	F-value	P value	F-value	P value
Model	42.490	0.000	55.730	0.000
Linear	121.810	0.000	164.510	0.000
$X_1$	348.030	0.000	473.980	0.000
$X_2$	10.750	0.004	5.120	0.037
$X_3$	6.650	0.020	14.430	0.001
Square	4.100	0.023	1.480	0.256
$X_1^2$	2.330	0.145	0.570	0.459
$X_2^2$	6.900	0.018	0.340	0.568
$X_3^2$	3.080	0.097	3.520	0.078
2-Way Interaction	1.570	0.233	1.190	0.343
$X_1 \times X_2$	0.050	0.833	0.370	0.549
$X_1 \times X_3$	3.830	0.067	3.160	0.093
$X_2 \times X_3$	0.830	0.374	0.040	0.843
$R^2$	95.74%	96.72%		

retention was compared with the model. The result showed that each model’s Coefficient of Variance (CV) generates satisfied results with 0.7% and 1.6% for 80 °C and 100 °C, respectively. Thus, the models represent the phenomenon, correctly, of the simple coacervation method of betacyanin from the red dragon (*Hylocereus polyrhizus*) peels using pectin.

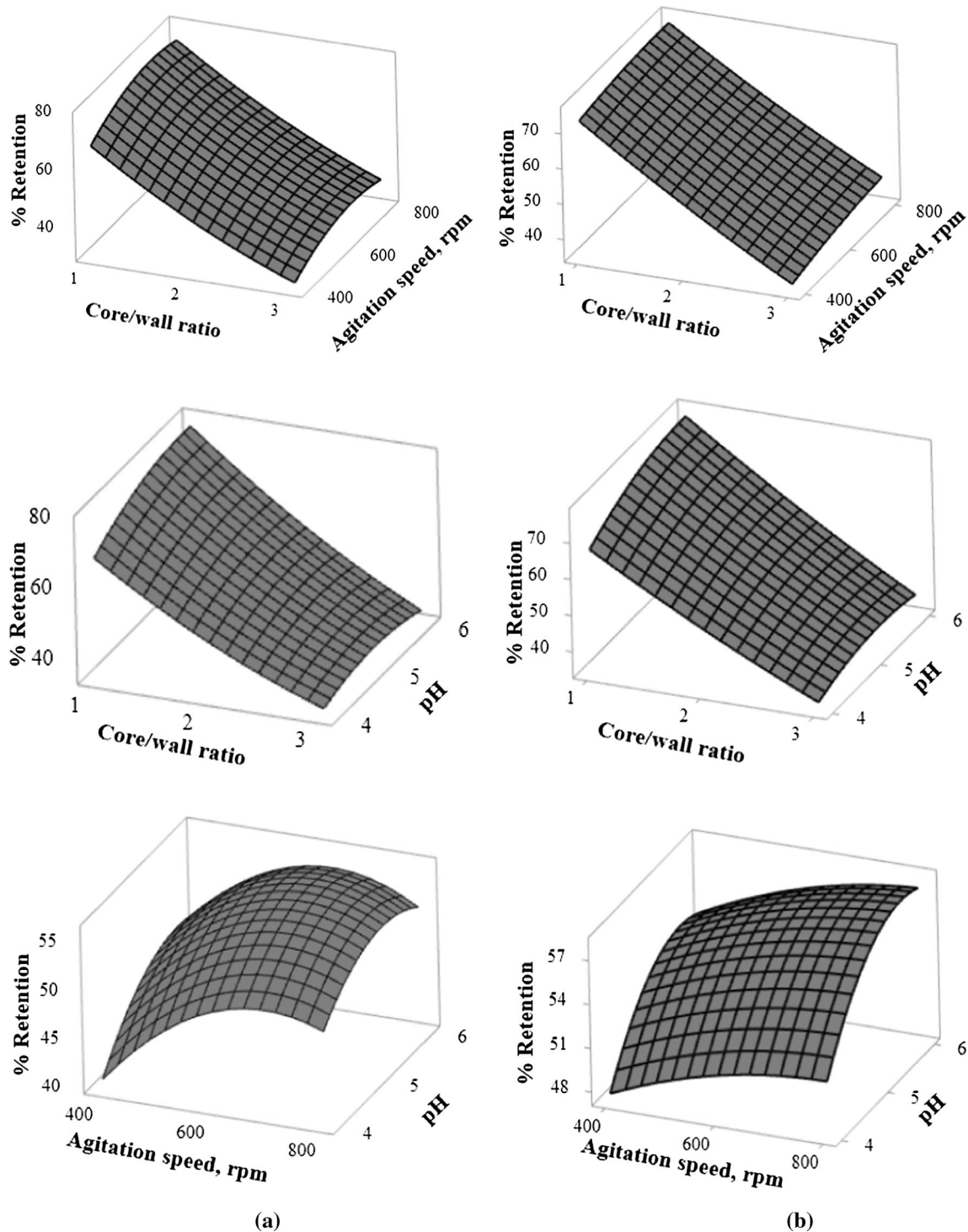
The effects of each parameter on the response variable were evaluated in turn, and the resulting surface plots are presented in Fig. 5. In general, the most significant effects for both temperature conditions were obtained by varying the core/wall ratio, followed by pH and agitation speed. The results revealed that higher core/wall ratios yield smaller retention percentages. The minimum and



**Fig. 4** Linear plot of experimental retentions versus the retention models

maximum retention percentages were approximately 40% and 75%, respectively, at 80 °C and 100 °C. These findings are in line with the hypothesis; the higher core/wall ratio gives lower retention because higher core/wall ratios provide smaller amounts of coacervation medium in the solution to maintain betacyanin stability. Dong et al. (2007) stated that increasing the core/wall ratio would increase particles’ size and cause them to develop irregular forms. The stability of a particle is difficult to maintain when it is large and irregular in form. Thus, a low core/wall ratio provides a large amount of the wall medium to the solution and, in turn, a larger surface area for encapsulation. Consequently, cores with small particles have a larger surface area. The effects of the core/wall ratio on the encapsulation process show similar tendencies at 80 °C and 100 °C.

Agitation speed and pH did not have a significant effect on the retention percentage. Variations in agitation speed and pH resulted in only a 5% (from 50 to 55%) change in retention percentage. The pH curves in Fig. 4a and Fig. 4b



**Fig. 5** Surface plot of the dependent variables at **a** 80 °C and **b** 100 °C

revealed the same tendency reported in previous works (Herbach et al. 2006). Betacyanin is mainly applied to food with low acidity (e.g., pH 3–7) on account of its weak stability (Herbach et al. 2006). While previous research reported different pH ranges of betacyanin stability, the range cited most often is between 4 and 7.

Comparison of Fig. 5a (curved plot) and Fig. 5b (plain plot) indicated that temperature increases would gradually affect the agitation speed. In general, the higher the operating temperature, the lower the solution's viscosity, and the smaller and more stable the resulting particle sizes (Lokhande et al. 2013). Moreover, variations in agitation

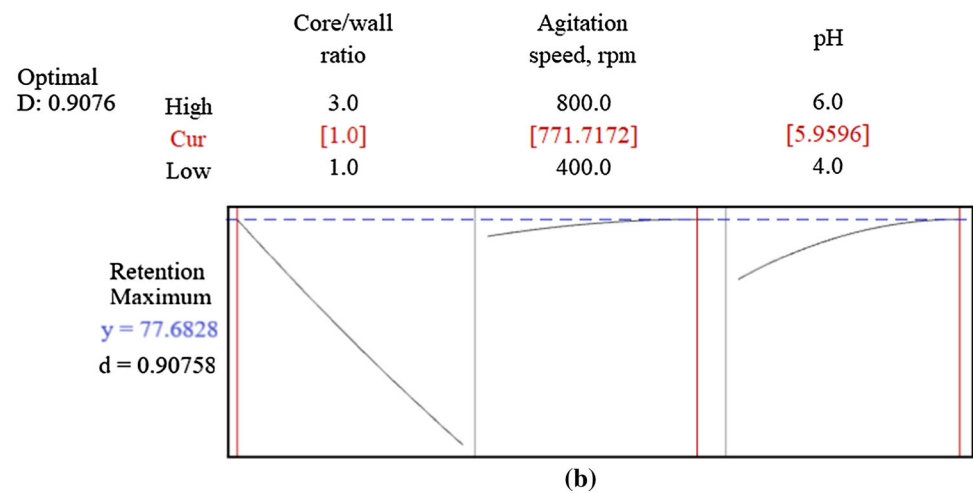
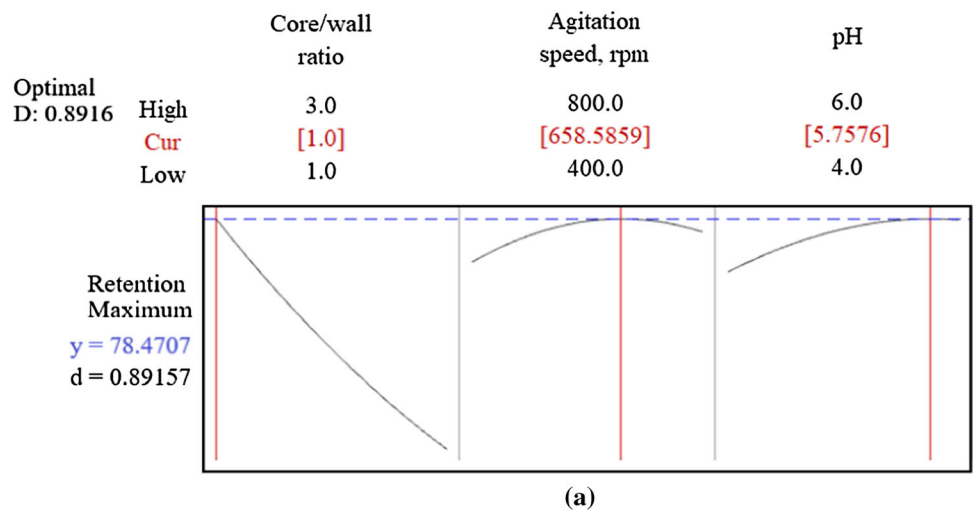
speed affect particle collision probability, and higher agitation speeds are observed at higher temperatures.

After evaluating the effect of the independent variables in the dependent variable, optimization was conducted to obtain the best values for each variable. Here, maximum retention is the target of the optimization process. The optimum parameters for both temperature conditions are presented in Fig. 6. Moreover, Fig. 6a reveals that the optimum core/wall ratio, agitation speed, and pH at 80 °C were 1:1, 660 rpm, and 5.76, respectively. By comparison, Fig. 5b illustrates that the optimum core/wall ratio, agitation speed, and pH at 100 °C were 1:1, 770 rpm, and 5.96, respectively. Encapsulation under the optimum conditions at 80 °C and 100 °C resulted in response values of as high as 78.47% and 77.68%, respectively. Thus, encapsulation at 80 °C is more effective than encapsulation at 100 °C. Nemzer et al. (2011) and Wybraniec (2005) reported that high temperatures could produce new compounds from betacyanin that may cause bathochromic and hypochromic

shifts. The changes cause a bathochromic shift and hypochromic shift, which are the shifts of an absorbance peak.

As mentioned briefly, the application of the coacervation method for microencapsulation of betacyanin has not been developed. Thus, many researchers mostly applied spray dryer (Aguilar-Tuesta et al. 2018; Antigo et al. 2018; do Carmo et al. 2018; García-Lucas et al. 2017; Kumar and Giridhar 2016; Shaaruddin et al. 2017; Vargas-Campos et al. 2018) and freeze dryer (Antigo et al. 2018; Mohamed et al. 2018; Rodriguez et al. 2016; Tumbas Šaponjac et al. 2016) with different variables of their works. In general, spray and freeze dryer applied physical phenomena (temperature) to conduct microencapsulation, but coacervation preserves the chemical process to enhance the protection. The coacervation method’s main advantages are a simple method and low-cost method due to the energy and solvent used. Hence, microencapsulation can still be applied in the industrial sector for up-scaling processes. Moreover, the coacervation method can be kept in ready used phase, a liquid form, rather than the solid phase.

**Fig. 6** Optimal values of the dependent variables at **a** 80 °C and **b** 100 °C



## Conclusion

In summary, the coacervation method of betacyanin microencapsulation was successfully conducted to improve the stability of red dragon fruit peels using pectin (*Hylocereus polyrhizus*). The result showed that a simple coacervation method enhances the betacyanin stability around 78% for 80 °C and 100 °C. The models generated by Response Surface Methodology (RSM) represent the coacervation phenomena due to the high R-square value of the models and low Coefficient of Variance (CV) value of the validation process. The simple coacervation process, in this study, offered  $93.2 \pm 3.1\%$  encapsulation efficiency. Simple coacervation is a superior method because of simplicity, low-cost, high efficiency, and high shell integrity.

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