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Improvement of Anthocyanin Encapsulation Efficiency into Yeast Cell by Plasmolysis, Ethanol, and Anthocyanin Concentration Using Response Surface Methodology

Lieu My Dong[1]*, Hoang Thi Thuy Hang[1], Nguyen Huyen Nguyet Tran[1], and Dang Thi Kim Thuy[2]

[1]*Faculty of Food Science and Technology, Ho Chi Minh City University of Food Industry, 140 Le Trong Tan, Tay Thanh Ward, Tan Phu District, Ho Chi Minh City Vietnam*
[2]*Department of Plain Cell Technology, Institute of Tropical Biology, 9/621 Ha Noi highway, Ho Chi Minh City, Vietnam*

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Anthocyanins are antioxidant compounds susceptible to environmental factors. Anthocyanin encapsulation into yeast cells is a viable solution to overcome this problem. In this study, the optimal factors for anthocyanin encapsulation were investigated, including anthocyanin concentration, plasmolysis contraction agent, and ethanol concentration, and response surface methodology was evaluated, for the first time. Anthocyanin from *Hibiscus sabdariffa* L. flowers was encapsulated into *Saccharomyces cerevisiae* using plasmolysis contraction agent (B: 3%–20% w/v), ethanol concentration (C: 3%–20% v/v), and anthocyanin concentration (A: 0.15–0.45 g/ml). The encapsulation yield and anthocyanin loss rate were determined using a spectrometer (520 nm), and color stability evaluation of the capsules was performed at 80℃ for 30 min. The results of the study showed that these factors have a significant impact on the encapsulation of anthocyanin, in which ethanol agents have the highest encapsulation yield compared to other factors in the study. Statistical analysis shows that the independent variables (A, B, C), their squares (A^2, B^2, C^2), and the interaction between B and C have a significant effect on the encapsulation yield. The optimized factors were anthocyanin, 0.25 g/ml; NaCl, 9.5% (w/v); and ethanol, 11% (v/v) with an encapsulation yield of 36.56% ± 0.55% and anthocyanin loss rate of 15.15% ± 0.98%; This is consistent with the expected encapsulation yield of 35.46% and loss rate of 13.2%.

Keywords: **Anthocyanin, encapsulation, ethanol, plasmolysis, *Saccharomyces cerevisiae*, response surface methodology**

Introduction

Currently, the biological function of anthocyanins such as antioxidant capacity, atherosclerosis prevention, and anticancer activity has been shown to be beneficial in treating diseases [1]. Besides, the replacement of natural food colors is receiving a lot of attention due to the health benefits they bring [2]. However, anthocyanin is easily degraded by many factors such as pH, temperature, light, oxygen, etc. [3]. This motivates studies to find methods to protect bioactive compounds from food, in which encapsulation is considered one of the most effective solutions. The main aim of these microcapsules is to protect sensitive compounds from adverse conditions such as light, moisture, oxygen, etc. [4]. Therefore, there have been many studies suggesting encapsulation methods such as using spray drying and freeze-drying methods [5, 6]. However, spray drying controls difficult, heteroge-

***Corresponding author**
Tel: +84-98996-1848
E-mail: lieudong289@gmail.com

neous particle sizes, capsules are easily dissolved in water [5]. Moreover, the process of making products from the spray drying method can affect natural color compounds, leading to the need to search for encapsulation methods more effectively. Recently, there has been considerable interest in the use of encapsulation yeast cells. Because the yeast structure with their presence in human nutrition makes them attractive and a new means of encapsulation for the food industry [7]. Previous studies have shown that yeast cells are used to encapsulate bioactive compounds such as essential oil encapsulated [8], limonene flavor [9], fish oil [4], or enzymes [10]. In the study of Bishop *et al.* (1998), sodium azide was used to inactivate the cell, allowing passive diffusion to incorporate the compound into the cell. Besides, the extraction of water-soluble components (proteins, saccharides, enzymes, amino acids, nucleic acids) from the cell outside may increase intracellular space [11], leading to increase encapsulation performance. To date, the plasmolyzed using sodium chloride to treat yeast cell inactivation before successful encapsulation is reported in previous studies [12–14]. Similarly, treatment by ethanol increasing membrane permeability showed a significant enhancement of encapsulated efficiency [3]. Besides, the concentration of bioactive substances can influence the envelopment of these color compounds in yeast cells. However, studies evaluating the interaction of the concentration of color, NaCl, and ethanol on the encapsulation yield of color compounds have not yet been published. Also, high-temperature treatment, a common technique in food processing, affects the bioactive compounds of anthocyanin. Therefore, evaluating the heat resistance of anthocyanin after the microencapsulation process is necessary. Response surface methodology (RSM) is a popular optimization method commonly used to find out the correlation as well as the optimal values of impact factors that have many works based on application RSM in chemical and biochemical processes [15]. It is necessary to improve the performance of systems and increase the productivity of processes without increasing costs [16]. The main advantage of RSM is to reduce the number of tests needed to evaluate many parameters, and their interactions are more efficient and easier to arrange and explain these tests than others [17]. In this study, the individual effects of the concentrations of plasmolysis, ethanol, and color on the anthocyanin encapsulation yield into yeast cells were investigated. Appropriate jumps are selected and optimized according to the Box-Behnken model to assess the combined effect of factors on encapsulation efficiency and a heat resistance of encapsulated anthocyanin.

Material and Methods

Plant material

Hibiscus sabdariffa L. (Fig. 1) from Binh Thuan province, located at 10°56′N 108°6′E in the southeast region of Vietnam, was used in this study. Calyces were dried at 45℃ for 60 h, after removing washed and separated seeds. Dried calyces were crushed and sealed until used. Anthocyanin extraction from calyces was performed according to the description of Nguyen *et al.* [3] with slight modifications. Ten-gram of sample was diluted in 100 ml of distilled water for 20 min at room temperature. Collected products was concentrated by evaporating water at 60℃ and at 650 mmHg pressure with a vacuum rotary device. From the color translation of 0.45 g/ml, the solution was diluted to 0.15 g/ml and 0.3 g/ml respectively, and stored at 4℃.

Microorganisms and culture

Saccharomyces cerevisiae was obtained from the strain collection of Faculty of Food Science and Technology, Ho Chi Minh City University of Food Industry. The yeast cells were grown in Hansen agar for 24 h at 30℃. Then, the biomass was harvested by rinsing plates with NaCl 0.9% (w/v) and centrifuged (5000 rpm) for 10 min. The yeast cells were used in the next encapsulation process.

Fig. 1. *Hibiscus sabdariffa* L.

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The effect of anthocyanin concentration on encapsulation efficiency into *S. cerevisiae*

Plasmolysis treatment. The biomass of yeast was plasmolyzed with 5% NaCl, and the solid fraction obtained after centrifugation was mix with anthocyanin (0.15; 0.3; 0.45 g/ml) in 1 h, 120 rpm at 30℃. Biomass after encapsulated and determined encapsulation yield by measure infrared spectrum.

The effect of NaCl concentration on encapsulation efficiency into *S. cerevisiae*. The anthocyanin fixation procedure from yeast cells was carried out according to the description of Paramera *et al.* (2011) with some changes summarized below [11]: Yeast (0.5 gram fresh) was treated plasmolysis with concentrations of NaCl (3; 5; 10; 15; 20% (w/v), incubation 24 h, 120 rpm at 30℃. The biomass obtained after centrifugation mixed with anthocyanin color (0.3 g/ml) for 1 h, 120 rpm at 30℃. Biomass was determined encapsulation yield and thermal stability of anthocyanin.

The effect of ethanol concentration on encapsulation efficiency into *S. cerevisiae*. The effect of ethanol on anthocyanin immobilization ability on yeast cells was performed according to the description of Nguyen *et al.* (2018) with some changes summarized below [3]: yeast biomass (0.5 gram) mixed with anthocyanin color translation (0.3 g/ml) in ethanol solution with concentrations of 3; 10; 20; 30% (v/v) incubated 1 h, 120 rpm at 30℃. Biomass was determined encapsulation yield and thermal stability of anthocyanin.

Influence of the combined effect of color concentration, plasmolysis, and ethanol contraction on encapsulation efficiency into *S. cerevisiae*

Yeast biomass (0.5 gram) after treatment plasmolysis in different NaCl concentrations is mixed with anthocyanin color solution for 1 h, 120 rpm at 30℃. Biomass was determined encapsulation yield and thermal stability of anthocyanin.

Yeast biomass (0.5 gram) treated ethanol at different concentrations was mixed with anthocyanin color solution for 1 h, 120 rpm at 30℃. Biomass was determined encapsulation yield and thermal stability of anthocyanin.

Yeast biomass (0.5 gram) after treatment plasmolysis at different NaCl concentrations was mixed with anthocyanin color solution in ethanol solution at concentrations of 1 h, 120 rpm at 30℃. Biomass was determined encapsulation yield and thermal stability of anthocyanin.

Effect of temperature on the ability of Saccharomyces cerevisiae to retain anthocyanin color

Anthocyanin encapsulated yeast cells were incubated in the water bath at 80℃ for 30 min and evaluated for the thermal stability of anthocyanin.

Control samples

The control sample (+) is a sample that did not take any treatment steps. Yeast cells (0.5 gram) after were cultured, which was mixed with anthocyanin fluid (0.3 g/ml) for 1 h, 120 rpm at 30℃. The biomass was harvested by centrifugation, evaluated for encapsulation yield, and thermal stability of anthocyanin

Optimizing the factors affecting the fixation of encapsulation efficiency into *S. cerevisiae*

The response surface method uses Box-Behnken design to optimize factors: color concentration (A), NaCl (B), ethanol (C). The experimental design consists of 17 experiments of three variables (A, B, C) at three levels (-1; 0; 1). Independent variables are coded -1 and 1 at low and high levels. The scope of implementation and the value are shown in Table 1. All experiments were performed three times and the average performance was obtained as the dependent variable (Y). The following quadratic polynomial equation is used to study the effect of variables on encapsulation yield and color loss rate:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$

where Y is the encapsulation yield and color loss rate, β_0 is the constant term; β_1, β_2, and β_3 are the coefficient of

Table 1. Experimental range, level, and code of independent variables.

Independent variables	Unit	Symbol coded	Range and levels		
			-1	0	+1
Anthocyanin	g/ml	A	0.15	0.3	0.45
NaCl	%	B	3	11.5	20
Ethanol	%	C	3	11.5	20

linear terms; β_{11}, β_{22} and β_{33} are the coefficient of quadratic terms; and β_{12}, β_{13} and β_{23} are the coefficient of cross-product terms, respectively.

Independent variables are optimized by the desired function criteria available in Design-Expert software (version 7.1.5). The goal is to maximize encapsulation yield and color loss rate while keeping variables in the corresponding test range.

Methods of determining anthocyanin content

The method of determining the content of anthocyanin is based on the description of Nguyen *et al.* [3] that used to determine the anthocyanin content by spectrometers (520 nm) calculated by Beer-Lamber formula:

$$AC = \frac{Abs \times M \times D \times 10^3}{\varepsilon \times l}$$

Abs: Absorbance of the diluted solution (λ_{max} = 520 nm).
M: Molecular weight: 465.2 (g.mol 1).
D: Dilution factor.
ε: (Delphinidin-3-glucosides): 23,700 (L.mole^{-1} cm^{-1})
l: Length of the optical path in the cuvette (1 cm)

Encapsulation yield

$$EY(\%) = \frac{Q_E}{Q_T} \times 100$$

Q_E: was the amount (g) of anthocyanin encapsulated
Q_T: amount (g) of anthocyanin in the original sample

Statistical analysis

All data are expressed in a meaningful form ± standard deviation (SD), at least 3 repetitions for each treatment. The difference between the variables is checked by using the ANOVA test. Design-Expert 7.0 software used for evaluating the effect of factors agents on the anthocyanin encapsulation yield by response surface methods.

Results and Discussion

Influence of anthocyanin concentration factor, plasmolysis and ethanol contraction on encapsulation efficiency into *S. cerevisiae*

Encapsulation yield of anthocyanin into *Saccharomyces cerevisiae* under the influence of color concentration, NaCl and ethanol concentration is shown in Fig. 2. The results show that in samples containing untreated yeast cells, the color was recorded at 10%, the lowest ($p < 0.05$) compared to samples containing treated yeast cells (Fig. 2). In the investigation of the effect of anthocyanin concentration on encapsulation yield, with concentration of 0.15; 0.3 and 0.45 g/ml increase encapsulation yield EY of 11.22 ± 0.28%; 15.05 ± 1.27%, and 18.21 ± 0.35% (Fig. 2A). The results obtained from the treatment of plasmolysis by contraction NaCl showed that the concentration of NaCl affected EY encapsulation yield increased from 11.78% to 23.65% when increasing the corresponding NaCl concentration from 3% to 15%, twice higher than untreated yeast samples (EY 10%). However, when the NaCl concentration was increased to 20%, the encapsulation yield decreased to 17.75 ± 1.08% (Fig. 2B). The impact of ethanol showed that when the ethanol concentration increased from 3 to 30% (w/v), the anthocyanin encapsulation yield from 12.86 ± 0.95% to 26.11 ± 1.12%. However, color loss due to a high temperature significantly increased ($p < 0.05$) when heating at the sample treated by ethanol 30% compared to the remaining samples in the survey (Fig. 2C).

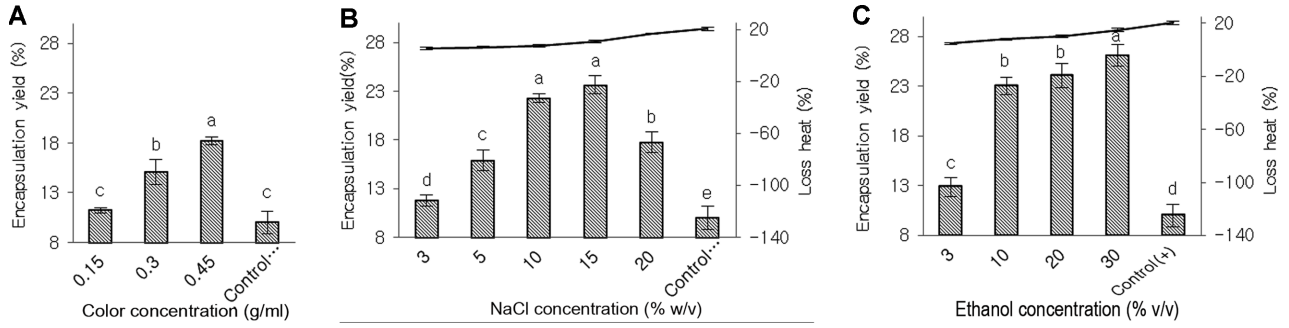

Fig. 2. Encapsulation yield and color loss rate under the influence of color concentration (A), NaCl concentration (B), ethanol concentration (C). ^{abcde} is the average value of the column and the difference of characters is statistically significant ($p < 0.05$).

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The concentration of encapsulation-compounds has significant effects on encapsulation yield. A high concentration of encapsulation-compound increases encapsulation yield (Fig. 2A). However, the high concentration of encapsulation compounds is also a factor in the reduction of cell loading, leading to do not increase in encapsulation yield %EY [11]. High anthocyanin concentrations (0.45 g/ml) increase the viscosity of color fluids. This makes it possible to increase the color to 0.45 g/ml by 1.5 times 0.3 g/ml, but the encapsulation yield does not increase accordingly (Fig. 2A).

The effect of plasmolysis on the encapsulation yield of encapsulation compounds into yeast cells has been published in previous studies. Plasmolysis is the phenomenon of draining water out of cells, in which cells are incubated in high saline or sugar solution [18]. Plasmolysis has the ability to increase intracellular space or rate of packaging and reduce protein, nucleic acid in cellular components [7]. Paramera *et al*. (2011) suggested that the process of plasmolysis in curcumin encapsulated is not effective [11]. However, the results from this study (Fig. 2B) concur with Shi *et al*. (2007) that the effect of treating plasmolysis with NaCl 5% concentration (w/v) for chlorogenic encapsulation yield doubled compared to the control sample [13]. Similarly, encapsulated drugs containing biological compound treated NaCl 5% for 24 h helps increase encapsulation yield by eliminating intracellular components of yeast cells and higher encapsulation yield than spray drying, freeze-drying [19]. Results from this study showed that NaCl 10% concentration for anthocyanin encapsulation yield was significantly higher ($p < 0.05$) than 5% concentration (Fig. 2B). This shows that the encapsulated of compounds into yeast cells depends on the type of compound encapsulated and the concentration of the plasmolysis agent.

Besides NaCl, ethanol has also been shown to play a role in the physicochemical and physiological functions of widely studied cell membranes [20]. Under the action of ethanol, the double lipid layer undergoes a phase transition that significantly reduces its thickness [3]. Thus, ethanol alters the area and thickness of the lipid layer resulting in changes in the mechanical properties of permeability and diffusion of cell membranes [21]. In addition, ethanol forms a layer on the membrane, compounds that can be used to increase fluidity. These effects make anthocyanin encapsulated into yeast cells more effective than not treated by ethanol (Fig. 2C). However, soluble molecules in membranes do not always increase but can reduce fluidity (due to increased viscosity) [18]. In previous studies, a high concentration of ethanol (50% v/v) caused curcumin encapsulation yield decreased double from 33.8% to 16.6% [1], the encapsulated resveratrol only achieved 4.52% efficiency [14]. The results of this study show that increasing the concentration of ethanol increases encapsulation yield (Fig. 2C). However, when the concentration increases to 30%, the encapsulation yield increases but when affected by high temperatures, the color molecules are easily dispersed leading to a large color loss rate compared to the remaining concentrations (Fig. 2C). This can be explained by the large concentration of ethanol which completely removes the permeability of cell membranes. The results from the study show that the factors in the survey have a significant impact on the anthocyanin encapsulation yield of yeast cells (Fig. 2). As a result, finding the correlation between factors is necessary to determine the interactions for the highest encapsulation yield with the lowest color loss rate.

Table 2. Box Behnken design for independent variables encapsulation yield, and color loss rate.

Std	Run	Color (g/ml)	NaCl (%)	Ethanol (%)	EY (%)	Color loss (%)
1	14	0.15	3	11.5	24.45	10.11
3	11	0.15	20	11.5	28.15	15.45
5	7	0.15	11.5	3	21.15	11.65
7	13	0.15	11.5	20	27.98	13.88
9	12	0.3	3	3	18.85	7.5
10	1	0.3	20	3	28.55	16.22
11	2	0.3	3	20	26.65	15
12	16	0.3	20	20	25.34	25.05
13	6	0.3	11.5	11.5	38.75	13.88
14	15	0.3	11.5	11.5	37.45	13.21
15	5	0.3	11.5	11.5	37.45	14.52
16	4	0.3	11.5	11.5	35.5	12
17	10	0.3	11.5	11.5	36.99	12.78
2	3	0.45	3	11.5	27.87	15.41
4	8	0.45	20	11.5	29.68	20.45
6	9	0.45	11.5	3	29.11	17.68
8	17	0.45	11.5	20	31.14	19.88

Optimizing the factors affecting the encapsulation efficiency into *S. cerevisiae*

Table 2 shows the process variables and test data 17 runs containing 5 iterations at the central point. By applying the analysis of test data, the model for the variable is represented by the following quadratic equation in the form of encoded values:

$$Y_1 = 37.23 + 2.01A + 1.74B + 1.68C - 0.47AB - 1.2AC - 2.75BC - 3.6A^2 - 6.09B^2 - 6.29C^2$$

$$Y_2 = 14.98 + 2.79A + 3.64B + 2.6C$$

In which, Y_1 is the encapsulation yield, Y_2 is the color loss rate, the values A, B, C are color, NaCl and ethanol respectively.

ANOVA variance analysis for the model is presented in Table 3. For the objective function Y_1, the coefficient of determination ($R^2 = 0.9753$) indicates that 2.47% of the total variation is not explained by the model. For a good statistical model, the adjusted coefficient of determination R^2_{adj} must be close to R^2. As adjusted Table 3 R^2_{adj} (0.9436) near R^2. Furthermore, R^2_{pred} (0.7487) matches R^2_{adj} and confirms this model significantly. Similarly, the objective function Y2 coefficient determines R^2_{adj} (0.7653) close to R^2 (0.8093), R^2_{pred} (0.6651) in accordance with R^2_{adj} and confirms this model significantly. The "Lack of fit" $0.2602 > 0.05$ and $0.0546 > 0.05$, with no significance compared to pure error and indicates a suitable model to describe the test data. Inconsistent accuracy is a measure of signal/noise ratio, a ratio greater than 4 is desirable. The value of the appropriate accuracy is 17,397 and 13,216 indicates an adequate signal. Therefore, the full prediction model is within the scope of experimental variables. The importance of each coefficient is measured by the p-value and the F value is listed in Table 3. The p-value of the model is less than 0.0001 which indicates that the model is significant and can be used for darkening optimization of variables.

3D response surface and 2D contour plot lines are graphical representations of the regression equation

Table 3. Analysis of variance for the fitted quadratic model.

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F	
Encapsulation yield (%EY)						
Model	534.43	9	59.38	30.72	< 0.0001	significant
A-Color	32.28	1	32.28	16.70	0.0047	
B-Nacl	24.15	1	24.15	12.49	0.0095	
C-Ethanol	22.61	1	22.61	11.70	0.0111	
AB	0.89	1	0.89	0.46	0.5186	
AC	5.76	1	5.76	2.98	0.1280	
BC	30.31	1	30.31	15.68	0.0055	
A^2	54.46	1	54.46	28.17	0.0011	
B^2	156.37	1	156.37	80.89	< 0.0001	
C^2	166.40	1	166.40	86.08	< 0.0001	
Residual	13.53	7	1.93			
Lack of Fit	8.07	3	2.69	1.97	0.2602	not significant
Color loss (%)						
Model	222.42	3	74.14	18.4	< 0.0001	significant
A-Color	62.33	1	62.33	15.4	0.0017	
B-Nacl	106.22	1	106.22	26.35	0.0002	
C-Ethanol	53.87	1	53.87	13.37	0.0029	
Residual	52.39	13	4.03			
Lack of Fit	48.60	9	5.4	5.7	0.0546	not significant
Y1: = $R^2 = 0.9753$	$R^2_{adj} = 0.9436$	$R^2_{pred} = 0.7487$	Adeq Precision = 17.397			
Y2: = $R^2 = 0.8093$	$R^2_{adj} = 0.7653$	$R^2_{pred} = 0.6651$	Adeq Precision = 13.218			

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which are useful for adjusting the relationship between independent variables and dependent variables. Different shapes of contour lines indicate whether the interaction between variables is significant. The 3D response surface and 2D contour plots created by the model are shown in Fig. 3. In these three variables, when the two variables are described in a three-dimensional surface cell, the third variable is tried set at 0.

From the above model and from the results of variance analysis in Table 3, it was found that the three factors of color, NaCl and ethanol have a significant influence on the objective function Y_1 as the encapsulation yield ($p < 0.05$). However, different technological factors have a different effect. The results from the model show that the individual factors of color (A), NaCl (B), ethanol (C) all affect the objective function Y_1 ($p < 0.05$), and their square also affects intentionally meaning to the objective function Y_1 ($p < 0.05$). Similar to the objective function Y_2, three factors color, NaCl and ethanol have significant effects on the objective function Y_2 as color loss rate ($p < 0.05$). Positive values of regression coefficients indicate that the objective function value Y_2 is higher when

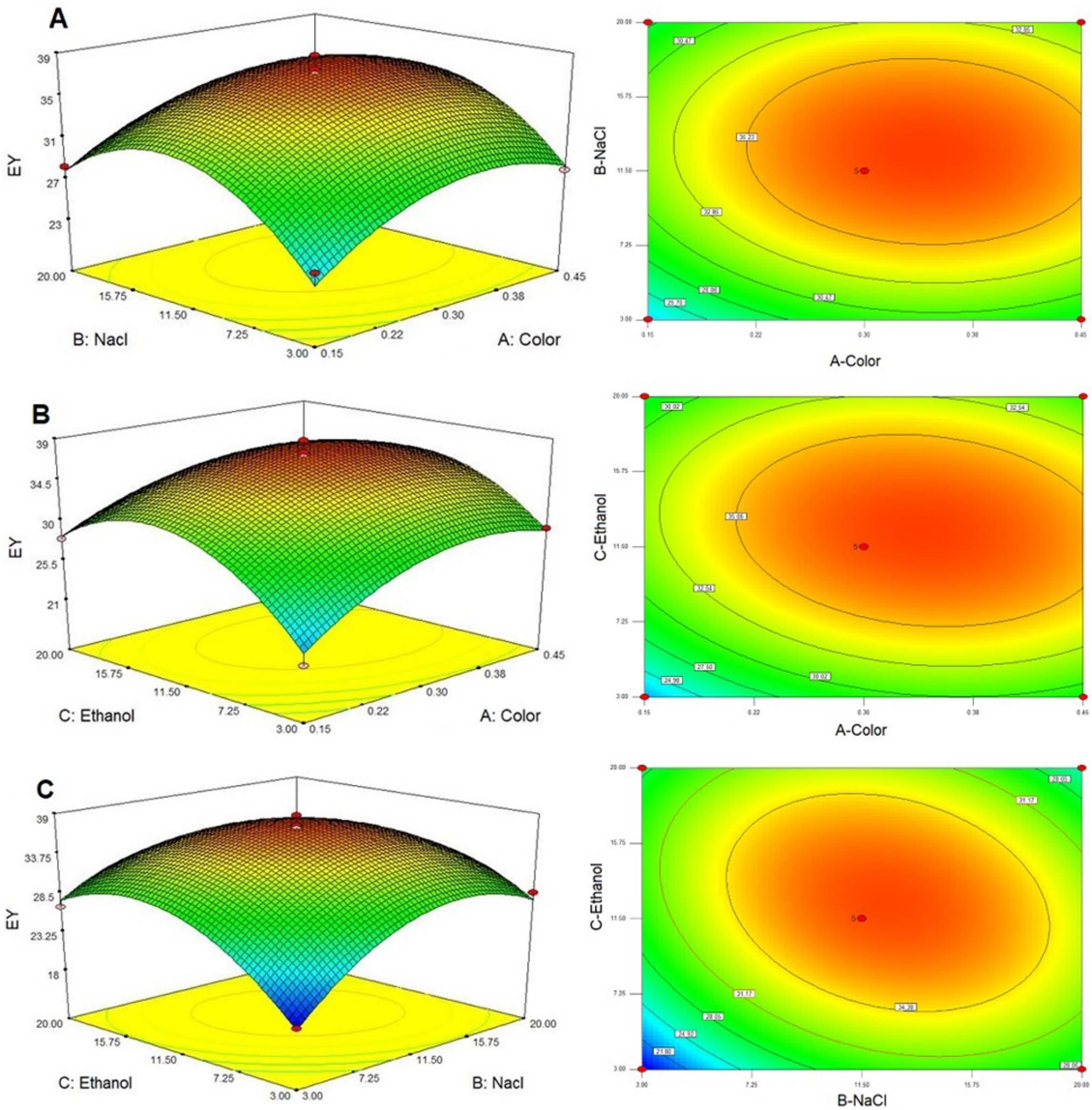

Fig. 3. Response surface and contour plots. (A) the effect of color concentration and NaCl concentration; (B) the effect of color concentration and ethanol concentration; (C) the effect of NaCl concentration and ethanol concentration.

increasing the influencing factors.

As seen in Fig. 3A, the color interaction pairs (A) and NaCl (B) have no significant effect on the target function Y_1. In this case, the color (A) and NaCl (B) do not have much interaction in accordance with the contour plots in Fig. 3A and Table 3 results when the coefficient AB is not significant ($p > 0.05\%$). Similar to Fig. 3B color interactions (A) and ethanol (C) when increasing both A (color) and C (ethanol), encapsulation yield increases slowly. Ethanol continues to increase to 20%, the encapsulation yield begins to decrease. The concentration of color continues to increase encapsulation yield still increased slightly. Demonstrate the interaction of two negligible variables, consistent with contour plots Fig. 3B and Table 3 when the AC coefficient is not significant ($p>0.05\%$). In the case of Fig. 3C, both NaCl (B) and ethanol (C) both affect EY encapsulation yield and have a quadratic effect on encapsulation yield which increases efficiency. Ethanol and NaCl act on cell membranes to increase permeability to facilitate easy anthocyanin color molecules to spread into yeast cells. The encapsulation yield increases rapidly when ethanol and NaCl increase, but when NaCl exceeds 11.5–17%, the encapsulation yield starts to decrease due to the high concentration of NaCl negatively affecting the cell membrane. This is consistent with the contour's plots Fig. 3C and Table 3 when the BC coefficient is significant ($p < 0.05$).

Through 3D models and their respective contour plots, the fit of the model equation to predict the optimal response values has been checked by the selected optimal conditions. Results Table 4 shows the optimum color conditions of 0.26 g/ml, NaCl 9.38%, and ethanol 10.82%. Under such conditions, packaging efficiency was 35.46%, and the color loss rate was 13.20%. However, considering the ability to operate in actual production, the optimal conditions can be modified as follows: anthocyanin concentration 0.25 g/ml, NaCl 9.5%, ethanol 11%. Under actual modification conditions, packaging performance reached $36.56 \pm 0.55\%$ and the color loss rate was $15.15 \pm 0.98\%$ close to the predicted value (Table 4). Previous studies have shown that the concentration of color compounds, NaCl, and ethanol significantly affect encapsulation yield in yeast cells (Paramera *et al*., 2011; Shi *et al*., 2008; Nguyen *et al*., 2018). However, studying the correlation as well as the interaction between these factors has not been published. The results from this study indicate the correlation between the factors as well as micro-conditions for the highest encapsulation yield with the lowest color loss rate (Fig. 4, Table 4). Microencapsulation efficiency, as well as heat resistant ability promising application potential of natural color compounds in food which high-temperature requesting such as cookies, etc. Subsequent studies need to assess the application ability in food processing as well as the release of anthocyanin after the encapsulation process.

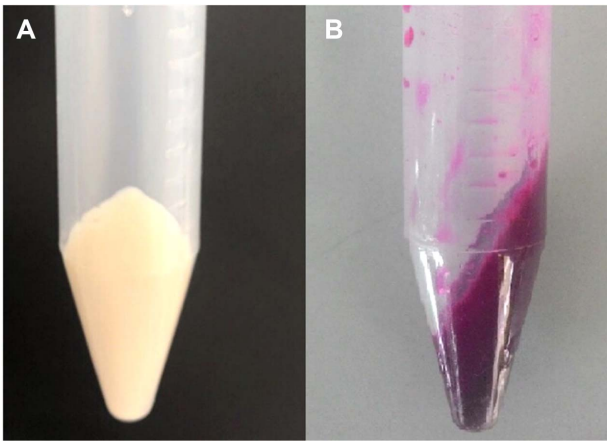

Fig. 4. The biomass of yeast cell before (A) and after (B) encapsulation process.

Conclusion

Results from the study showed that anthocyanin, NaCl and ethanol concentrations both significantly affected anthocyanin encapsulation yield into yeast cells. The interaction of anthocyanin concentration and NaCl con-

Table 4. Optimal conditions, predictive values and tests at optimal conditions.

	Anthocyanin color g/ml	NaCl % (w/v)	Ethanol % (v/v)	Encapsulation yield (%)	Color loss rate (%)
Optimal conditions (predictions)	0.26	9.38	10.82	35.46	13.20
Actual conditions (revised)	0.25	9.5	11	36.56 ± 0.55	15.15 ± 0.98

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centration as well as anthocyanin concentration and ethanol concentration are not significant. On the other hand, the interaction between NaCl and ethanol was significant and had a positive impact on encapsulation yield. Pretreatment encapsulation as plasmolysis with NaCl significantly increased encapsulation yield. In addition to combining the effects of ethanol with increased permeability, anthocyanin color molecules easily diffuse into yeast cells. The use of response surface methodology has been shown to be effective in finding concentrations of encapsulating conditions to improve encapsulation yield while keeping color loss rate at a lowest level. The conditions of the impact factors for optimal encapsulation yield and color loss rate were as follows: Color concentration 0.25 g/ml, NaCl 9.5%, ethanol 11%. Under these conditions, the test encapsulation yield was $36.56 \pm 0.55\%$ and the color loss rate was $15.15 \pm 0.98\%$, which is close to the expected value of encapsulation yield of 35.46% and color loss rate of 13.2%. Moreover, the encapsulation stabilizes color degradation by high temperatures. Yeast cells are capable of promising applications for color compound encapsulation in food products and the application potential in food products that high-temperature requesting.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Tsai PJ, McIntosh J, Pearce P, Camden B, Jordan BR. 2002. Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. *Food Res. Int.* **35**: 351-356.
2. Muhamad II, Jusoh YM, Nawi NM, Aziz AA, Padzil AM, Lian HL. 2018. *Advanced natural food colorant encapsulation methods: Anthocyanin plant pigment.* pp. 495-526. *Natural and Artificial Flavoring Agents and Food Dyes*.
3. Nguyen TT, Phan-Thi H, Pham-Hoang BN, Ho PT, Tran TTT, Waché Y. 2018. Encapsulation of Hibiscus sabdariffa L. anthocyanins as natural colors in yeast. *Food Res. Int.* **107**: 275-280.
4. Czerniak A, Kubiak P, Białas W, Jankowski T. 2015. Improvement of oxidative stability of menhaden fish oil by microencapsulation within biocapsules formed of yeast cells. *J. Food Eng.* **167**: 2-11.
5. Mahdavi SA, Jafari SM, Ghorbani M, Assadpoor E. 2014. Spray-drying microencapsulation of anthocyanins by natural biopolymers: A review. *Dry. Technol.* **32**: 509-518.
6. Sahat NS, Zaidel DNA, Muhamad I I, Alam MNHZ. 2014. Stability study of water-in-oil emulsion containing anthocyanins from red cabbage. *J. Teknol.* **69**. DOI:10.11113/JT.V69.3163
7. Paramera EI, Karathanos VT, Konteles SJ. 2014. Yeast cells and yeast-based materials for microencapsulation. *Microencapsulation Food Industry* 267-281.
8. Bishop JRP, Nelson G, Lamb J. 1998. Microencapsulation in yeast cells. *J. Microencapsul.* **15**: 761-773.
9. Normand V, Dardelle G, Bouquerand PE, Nicolas L, Johnston DJ. 2005. Flavor encapsulation in yeasts: limonene used as a model system for characterization of the release mechanism. *J. Agric. Food Chem.* **53**: 7532-7543.
10. Chow CK, Palecek SP. 2004. Enzyme encapsulation in permeabilized *Saccharomyces cerevisiae* cells. *Biotechnol. Progress* **20**: 449-456.
11. Paramera EI, Konteles SJ, Karathanos VT. 2011. Microencapsulation of curcumin in cells of *Saccharomyces cerevisiae*. *Food Chem.* **125**: 892-902.
12. Dardelle G, Normand V, Steenhoudt M, Bouquerand PE, Chevalier M, Baumgartner P. 2007. Flavour-encapsulation and flavour-release performances of a commercial yeast-based delivery system. *Food Hydrocoll.* **21**: 953-960.
13. Shi G, Rao L, Yu H, Xiang H, Pen G, Long S, Yang C. 2007. Yeast-cell-based microencapsulation of chlorogenic acid as a water-soluble antioxidant. *J. Food Eng.* **80**: 1060-1067.
14. Shi G, Rao L, Yu H, Xiang H, Yang H, Ji R. 2008. Stabilization and encapsulation of photosensitive resveratrol within yeast cell. *Int. J. Pharm.* **349**: 83-93.
15. Baş D, Boyac IH. 2005. Modeling and optimization I: Usability of response surface methodology. *J. Food Eng.* **78**: 836-845.
16. Garai D, Kumar V. 2013. Response surface optimization for xylanase with high volumetric productivity by indigenous alkali tolerant *Aspergillus candidus* under submerged cultivation. *3 Biotech.* **3**: 127-136.
17. Chen W, Wang WP, Zhang HS, Huang Q. 2012. Optimization of ultrasonic-assisted extraction of water-soluble polysaccharides from Boletus edulis mycelia using response surface methodology. *Carbohydr. Polym.* **87**: 614-619.
18. Pham-Hoang BN, Romero-Guido C, Phan-Thi H, Waché Y. 2013. Encapsulation in a natural, preformed, multi-component and complex capsule: yeast cells. *Appl. Microbiol. Biotechnol.* **97**: 6635-6645.
19. Sangwai MB, Vavia PR. 2011. Effect of decisive formulation variables on bioencapsulation efficiency and integrity of yeast biocapsules for oral itraconazole delivery. *J. Microencapsul.* **28**: 311-322.
20. Da Silveira MG, Golovina EA, Hoekstra FA, Rombouts FM, Abee T. 2003. Membrane fluidity adjustments in ethanol-stressed Oenococcus oeni cells. *Appl. Environ. Microbiol.* **69**: 5826-5832.
21. Patra M, Salonen E, Terama E, Vattulainen I, Faller R, Lee BW. *et al.* 2006. Under the influence of alcohol: the effect of ethanol and methanol on lipid bilayers. *Biophys. J.* **90**: 1121-1135.