Optimization of maltodextrin coating for masking to maintain the characteristic of Spirulina powder using the Box-Behnken design: a Response Surface Methodology approach

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Abstract. Masking or encapsulating spirulina has been widely used to overcome unpleasant flavours and aromas. Masking procedures, including rehydration, mixing, and heating, might impact the characteristic of spirulina powder. This study aimed to identify the relationship between variables spirulina masking using maltodextrin and develop the model to optimize the response. Response Surface Methodology (RSM) and Box-Behnken Design (BBD) was employed to evaluate optimum conditions for spirulina masking. The masking variables, such as heating temperature, heating period, concentration of maltodextrin and ratio of maltodextrin and spirulina, were studied extensively. Masking process was evaluated based on colour changes (ΔE), moisture, and crude phycocyanin content (CPC) responses. The results showed a moderate positive relationship between the masking variables, with a coefficient of determination (R^2) value between 0.3-0.7. The response analysis showed a non-significant *lack of fit* ($p>0.05$), indicating that the data response will match with the optimized prediction model. The optimized conditions of spirulina masking were determined in low-temperature heating at 33°C for 60 min, with 7 g/L maltodextrin concentration and a 1:1 ratio of spirulina and maltodextrin (m/v). The optimized masking process condition could reach responses, moisture of 7.23 %, ΔE of 1.68, and CPC of 6.71 mg/g, respectively.

1 Introduction

Spirulina platensis is a filamentous blue-green microalgae that has promoted the functional properties applied in food, pharmaceutical, and cosmeceutical industries. Spirulina contains a high nutritional content and active components such as pigments, vitamins, and peptide

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minerals that have a role in health [1]. The development of spirulina has problems where it is easily deteriorating due to oxidation, acidic condition and heat [2,3]. The biomass of *Spirulina platensis* quickly degrades when under heat and light and indicating the need for attention to preservation.

Phycocyanin is a natural pigment produced by Spirulina, which can indicate deterioration process. The quality of phycocyanin deteriorates when it is exposed to heat, evidenced by decreased antioxidant activity and colour [4]. There were significant correlations in pigment content and colour properties of processed spirulina [5]. Efforts that can be made to reduce the impact of the deterioration of spirulina include coating it using materials such as polysaccharide and their derivatives [6].

Physically method such as masking (encapsulation) using β-cyclodextrin with low temperature heating treatment can removes the odor, protect the color and maintains functional protein of spirulina. The optimal condition β-cyclodextrin masking was achieved by addition 4 g/L, at 30 °C for 40 min [7]. Implementing β-cyclodextrin in pharmaceutical products with a ratio of 1:3 can minimize unpleasant aroma and reduce bitter taste [8]. Maltodextrin is hydrolysate of starch product that can be used to encapsulating and masking agent. Combination treatment preservation using maltodextrin can enhance color, aroma and taste product, improve the solubility, and stability in release system [6]. Spirulina aqueous extract containing with maltodextrin has the ability to preventing thermal degradation and preserving the natural color [9]. Encapsulation of *Chlorella vulgaris* using maltodextrin can maintain the biomass's colour properties, moisture, and pigment content after drying [10].

Several initiatives have been performed to maintain quality and preventing deterioration of spirulina, especially for food and drug purposes. Besides affecting color and phycocyanin content, spirulina deterioration might reduce sensory acceptability, including odor and taste. The soaking method spirulina with basil leaves extract at ratio of 1:4 (m/v) for 15 min and dried at 40°C, could increase the sensory acceptance, maintain color and the bioactive compound [11]. The rehydration and heating process can encourage crystallization and increase the stability of sugar and protein [12]. Dehydrating spirulina at 40°C can minimize the losses of phycocyanin content and bioactive compounds and increase thermal stability [13]. Masking procedures such as rehydrating, mixing, drying, and grinding can be applied in developing food and medicine products to improve organoleptic properties without the consequences of loss of biological activity [14]. Furthermore, there needs to be more information on optimizing spirulina masking conditions, such as heating temperature, heating period, maltodextrin concentration, and the ratio of maltodextrin and spirulina to maintain the quality of spirulina powder.

Response surface methodology (RSM) is a statistical technique widely used to optimize various processes that can be applied in industry [15]. Box-Behnken designs (BBD) are an excellent RSM that provides information on the influence of experiment variables and overall experimental error in a minimum number of required runs [16]. Process optimization of spirulina and other microalgae using RSM has been widely carried out, such as maintaining characteristic powder [10], extraction method [17,18], development of food product [19], and biosorption minerals [20]. This research aims to identify the relationship between variables of masking conditions such as temperature and period of heating, concentration, and ratio and develop a model to optimize the characteristics of spirulina powder response.

2 Materials and methods

The research was conducted in the Laboratory of Marine and Fisheries Resource, Universitas Pendidikan Indonesia (UPI), Serang Campus, Banten, Indonesia, for studies on optimizing spirulina masking using maltodextrin. The factors of optimizing can be using a combination of low-temperature heating, heating period, concentration maltodextrin and the ratio of

maltodextrin and spirulina. The spirulina masking process was evaluated based on moisture, colour changes, and crude phycocyanin content (CPC).

2.1 Preparation of spirulina masking

The 5 g of spirulina powder (PT. Algaepark, Indonesia) was mixed with Maltodextrin DE-15-20 (Kimia Mart, Indonesia) in solution with 1, 4, and 7 g/L concentrations and 1:1, 1:3, and 1:5 ratios between spirulina and maltodextrin (m/v). The mixture was treated with low temperatures heating of 10, 30, and 50°C and heating periods of 20, 40, and 60 min. The mixture of spirulina and masking solution was dried with a Food dehydrator (ACE, Kris 8L) at 40°C for ± 8 h. The dried spirulina (flakes) was crushed and sieved with a 100-mesh size. Spirulina powder was analysed for colour changes, moisture, and crude phycocyanin content (CPC).

2.2 Colour changes

The colour changes in spirulina resulting from masking was carried out using a colorimeter (Linshang-171) connected using a computer device. The analysis begins by inserting non-masking spirulina powder into a quartz dish and then scanning as standard. Next, the masking spirulina powder was scanned and set as a sample. The value is expressed in ΔE as the color difference score between the standard and the sample [21].

2.3 Moisture content

Moisture content of spirulina powder was analysed gravimetrically. The empty porcelain dish was dried in an oven at 105 °C for 15 min and then placed in a desiccator. The empty porcelain dish is weighed and recorded. Spirulina was weighed as much as 1 g in a porcelain dish and dried in an oven at 105 °C for 3 hours. Next, the sample was put in a desiccator and weighed again. The percentage difference in weight of the initial and final spirulina was calculated as moisture content.

2.4 Determination of Crude Phycocyanin Content (CPC)

Spirulina was added with PBS solvent in a ratio of 1:20 m/v using a freeze-thawing extraction procedure [22]. The concentration of PC was determined spectrophotometrically [18], with the following equation:

$$\text{CPC} = \frac{(\text{OD } 620)\text{-}0{,}474\ (\text{OD } 652)}{5{,}34} \qquad (1)$$

Where CPC is the concentration (mg/mL), OD620 is the optical density of the sample at 620 nm, OD652 is the optical density of the sample at 652 nm using a spectrophotometer UV-VIS (N271), The yield of the extraction was defined as:

$$\text{Yield PC (mg/g)} = \frac{\text{PC} \times \text{V}}{\text{DB}} \qquad (2)$$

Where, V is the solvent's volume (mL), and DB is the dry-weight biomass (g).

2.5 Optimizing spirulina masking

Optimization was done using the Response Surface Methodology (RSM) method and Box-Behnken Design (BBD) with four independent variables. The independent variables are

temperature of heating (°C), period of heating (min), concentration of maltodextrin (g/L), and the ratio of maltodextrin and spirulina (v/m). The RSM Box-Behnken Design (BBD) was implementted in the Minitab version 18. The minimum (-1), maximum (1) and centre (0) levels of each variable can be seen in Table 1. The centre of the level (0) used was modified from optimized condition masking using β-cyclodextrin [7,10].

Table 1. Independent variable and levels of optimization of the spirulina masking process using maltodextrin.

Independent variables	**Levels**		
	-1	**0**	**1**
A: temperature of heating (°C)	10	30	50
B: period of heating (min)	20	40	60
C: concentration of maltodextrin (g/L)	1	4	7
D: ratio of maltodextrin and spirulina (v/m)	1:1	3:1	5:1

A multiple linear regression model, as given in equation 3, shows a relationship between response variable (Y) and independent variables A, B, C, and D:

$$Y = \beta_0 + \beta_a A + \beta_b B + \beta_c C + \beta_d D \ldots \ldots \ldots \ldots + \beta_k K \ldots\ldots + \varepsilon \qquad (3)$$

Where, β_0 is an intercept, β_a, β_b, β_c, β_d are regression coefficients of Independent variables *A*, *B*, *C*, *D*, and ε stands for the error term.

The research data is entered in the BBD response column to produce a significance test equation. The significance test using Analysis of Variance (ANOVA) are used to predict the suitability of the model and the relationship between independent variables based on values such as *p-value, lack of fit* and *R-squared*. The optimiser response is obtained, which displays each variable recommended to get the optimum value.

3 Results and discussion

3.1 Box–Behnken Design in spirulina masking and responses analysis

The study used four parameters and three responses to optimize spirulina masking with maltodextrin. Factors and responses are processed with the Minitab version 18 application using Box-Behnken Design, and the resulting 27 is the number of runs. The experimental data design is then tested according to the response selected and the test results data obtained are as follows.

Table 2. Box–Behnken Design and values of the response of spirulina masking.

Run	A	B	C	D	Moisture (%)	Colour changes (Δ*E*)	Yield CPC (mg/g)
1	10	40	7	3:1	9.8	2.95	5.59
2	30	20	4	5:1	6	2.27	5.50
3	30	20	7	3:1	8.2	2.56	6.48
4	30	60	1	3:1	9.2	2.51	5.15
5	50	20	4	3:1	8	2.32	5.39
6	50	40	4	1:1	10.4	7.03	4.47
7	30	40	7	1:1	8	3.45	5.64
8	50	40	1	3:1	9	3.49	5.01
9	30	40	4	3:1	8	2.27	5.36
10	30	60	7	3:1	6.8	2.69	6.19
11	30	20	1	3:1	9.6	4.71	5.36
12	10	40	4	5:1	8.4	1.74	5.41
13	10	40	4	1:1	9.4	7.06	5.46
14	30	40	1	1:1	8	4.88	4.96
15	30	60	4	5:1	9	4.60	5.18
16	30	60	4	1:1	8.4	1.67	6.06
17	50	40	4	5:1	9.8	4.69	5.05
18	10	20	4	3:1	8	2.68	5.26
19	30	40	4	3:1	9.4	6.76	5.55
20	30	20	4	1:1	8.8	7.68	4.42
21	30	40	1	5:1	10	2.49	5.52
22	30	40	4	3:1	9.2	2.82	5.29
23	10	40	1	3:1	9.2	5.16	5.04
24	30	40	7	5:1	9.6	5.42	4.49
25	10	60	4	3:1	10.6	2.46	4.81
26	50	40	7	3:1	8	4.79	5.59
27	50	60	4	3:1	9.8	4.58	6.52

Noted: Independent variable of temperature of heating (A), period of heating (B), concentration of maltodextrin (C), and ratio between maltodextrin and spirulina (D), the measurement of responses was carried out in triplicate.

Table 3. Significance test results on Box-Behnken Design (BBD) responses using Analysis of Variance (ANOVA)

Response (Y)	*p-value*	*Lack of fit*	*R-squared* R^2	*Second*-order *p-value*
Moisture	0.810	0.295	0.4171	0.178 (BD)
Colour changes	0.195	0.942	0.6583	0.018 (BD)*
CPC	0.288	0.059	0.6180	0.067 (BD)

Noted: *significant ($p<0.05$) for 2-way interaction between independent variable B and D

Analysis of variance (ANOVA) is used to test the feasibility of the model generated from the input response value (Y), which can be seen in Table 3. Analysis of variance in the moisture response using a quadratic model shows *p-value* of 0.810, which means the model is not significant ($p>0.05$) in first-order term (linier models). The *R-squared* value for the moisture response is 0.4171, which is classified as moderate. The *R-squared* values between 0.3 and 0.7 indicate a moderate positive linear relationship through a *fuzzy-firm* linear rule [23]. The *lack of fit* moisture response is 0.295, which is not significant ($p>0.05$). A *lack of fit* value that is not significant is a requirement for a good model because it shows that the response data matches with the model [24]. The following is a quadratic equation for the moisture response model:

$$\begin{aligned}\text{Moisture } (\%) = {} & 8.17 - 0.043\,\text{A} + 0.083\,\text{B} + 0.370\,\text{C} - 0.80\,\text{D} + 0.00133\,\text{A} - 0.00117\,\text{B}^2 \\ & - 0.0102\,\text{C}^2 - 0.010\,\text{D}^2 - 0.00050\,\text{AB} - 0.00667\,\text{AC} + 0.0025\,\text{AD} \\ & - 0.00417\,\text{BC} + 0.0213\,\text{BD}\ \ 0.0167\,\text{C} \qquad (4)\end{aligned}$$

Where, A is the temperature of heating (°C), B period of heating (min), C concentration of maltodextrin (g/L), and D ratio of maltodextrin and spirulina (v/m).

Equation (4) shows that the increase in moisture response is directly proportional to heating period (B), concentration of maltodextrin (C), temperature interaction (A^2), interaction between temperature and ratio (AD), and interaction between heating period and ratio (BD), which is indicated by a positive value. Conversely, the moisture response will decrease with increasing temperature of heating (A), ratio of spirulina and maltodextrin (D), the interaction of period (B^2), the interaction of concentration (C^2), interaction of ratio (D^2), interaction between temperature and period (AB), interaction between temperature and concentration (AC), interaction between period and concentration (BC), and interaction between concentration and ratio (AD), with a negative constant value indicates this. The positive sign of the coefficient in the equation indicates the synergistic influence between the independent variables on the response variable, while the negative sign indicates the antagonistic influence [25]. The amount of amorphous sugar such as trehalose and maltodextrin in encapsulation dry matter can increase water absorbed and relate with the activity of water (a_w) [9]. The amount of water content is related to shelf life, so it must be kept as low as possible. Powdered products with a moisture content of <10% were accepted as microbiologically safe [21].

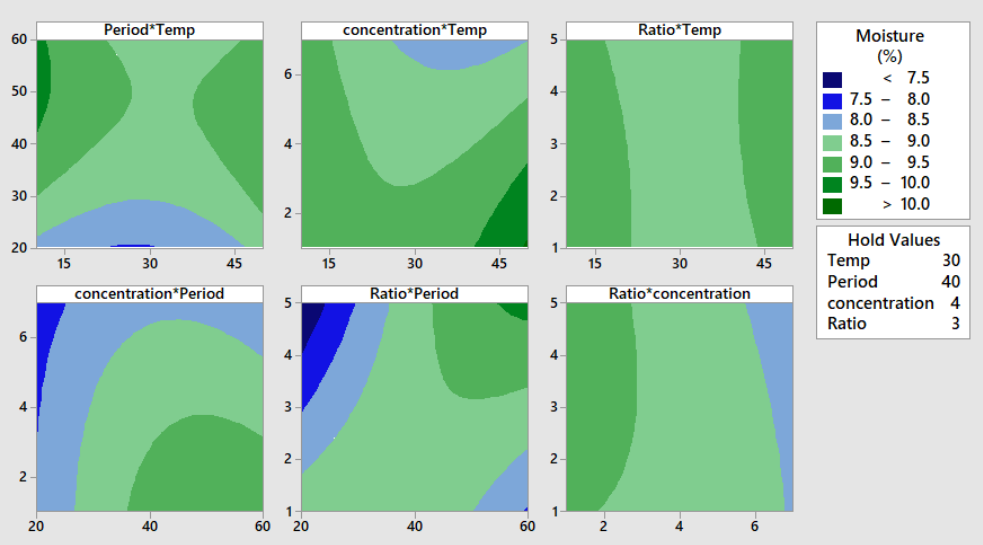

Fig. 1. Contour Plot of Moisture Response of Spirulina masking

The two-dimensional (2-D) contour graphical of the optimal moisture can be seen in Fig. 1, the darker-colour areas indicate optimal conditions. Contour plot of the period of heating vs temperature of heating, the optimum condition between 20-30 minutes and 20-30°C. Time contour plot of optimum concentration of maltodextrin vs temperature, located in the 6-7 g/L concentration and 25-50°C. The contour plot of concentration vs heating period is 4-7 g/L and 20 min. The ratio vs period of heating optimum is 1:3 to 1:5 for 20-30 minutes. The minimum water content can be achieved by optimizing low-temperature masking, short heating period, and medium to high maltodextrin concentrations and ratios. Other hand reported the moisture content decreased with the increase in maltodextrin ratio in spray dried product [21].

Colour is most important quality sensory attributes for the food industry, as it has a significant impact on consumer preferences and pleasure. Spirulina standard (non-masking) has RGB coordinates 87, 95, and 84, included in the dark olive-green colour category. The range of colour changes (ΔE) for masking spirulina using maltodextrin ranged from 1.67 to 7.06 (Table 2). Analysis of variance the colour changes response using a quadratic model shows *p-value* of 0.195, which means the model is not significant ($p>0.05$) model in linier term (first-order term) (Table 3). The smallest significance value found in second-order form (2-way interaction) between period heating (B) and the ratio of spirulina and maltodextrin (D) was 0.018, this means there is a significance ($p<0.05$) corelation in colour changes response (ΔE). The *R-squared* value for the colour changes response is 0.6583, which is classified as moderate. The *R-squared* values between 0.7 and 1, indicate a strong positive linear relationship [22]. The value of *lack of fit* colour changes response is 0.942, which is not significant ($p>0.05$), and the response of colour changes will match with prediction models. The following is a quadratic equation for the colour changes response model:

$$\Delta E = 19.25 - 0.191\,A + 0.098\,B + 1.188\,C - 4.91\,D + 0.00058\,A^2 - 0.00199\,B^2 - 0.0278\,C^2 - 0.183\,D^2 - 0.00155\,AB - 0.0146\,AC + 0.0186\,AD - 0.0097\,BC + 0.0521\,BD - 0.181\,CD \quad (5)$$

Where, ΔE is the color change, A temperature of heating (°C), B period of heating (min), C concentration of maltodextrin (g/L), D ratio of maltodextrin and spirulina (v/m).

The equation 5. shows that the increase in colour changes response is directly proportional to period of heating (B), concentration of maltodextrin (C), temperature interaction (A^2), interaction between temperature and ratio (AD), and interaction between

period of heating and ratio (BD), which is indicated by a positive value. Conversely, the colour changes response will decrease with increasing temperature of heating (A), ratio of maltodextrin and spirulina (D), interaction of period (B^2), interaction of concentration (C^2), interaction of ratio (D^2), interaction between temperature and period (AB), the interaction between temperature and concentration (AC), interaction between period and concentration (BC), and interaction between concentration and ratio (AD), with a negative constant value indicates this. An increase in the ratio of spirulina and maltodextrin correlates with a decrease in the colour change of spirulina masking. The amount of maltodextrin solution which adds interferes with the colour changes of spirulina masking. The soaking and drying spirulina using basil leaf extract with a high ratio addition produces the best colour characteristics and sensory acceptance [11].

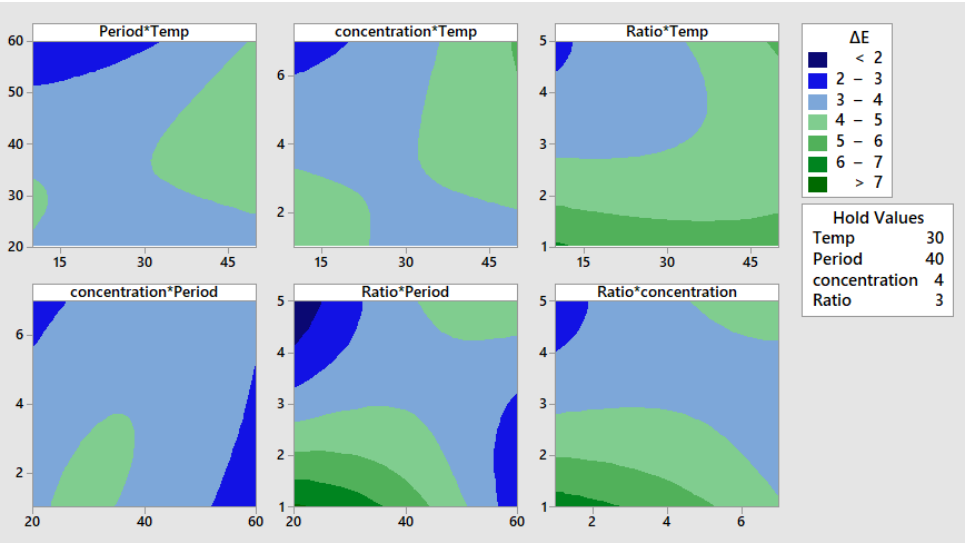

Fig. 2. Contour plot of colour changes (ΔE) response spirulina masking

A two-dimensional (2-D) contour graphical can be seen in Fig. 2., the darker-colour areas indicate where the colour changes response is optimal. Contour plot of heating period vs temperature, optimum condition area in 50-60 minutes and at 10-30°C. The optimum contour concentration vs temperature at 6-7 g/L and 10-20°C. The optimum contour of ratio vs temp is found at 1:5 and 10°C. The contour plot of optimum maltodextrin concentration vs temperature at 6-7 g/L and t 10-20°C. Contour plot of concentrations vs heating period was at 6-7 g/L for 20-25 and 50-60 minutes. The optimum response of ratio vs period is 1:4 to 1:5 for 20 minutes. The contour plot of ratio vs concentration an optimum at 1:5 and 1-2 g/L. The minimum colour changes (ΔE) response can be achieved by optimizing low-temperature masking, medium to large heating period, and high concentrations and medium ratio of maltodextrin. Application of mixture small and high carbohydrate can prevent the colouring ability under thermal stress [9]. Ozyurt et al., (2022) reported use of maltodextrin in unfermented and fermented Spirulina provided more effective drying and also showed positive effects on colour [21].

Phycocyanin (PC) is a water-soluble pigment-protein complex discovered in cyanobacteria. It has antioxidant and high fluorescent capabilities [21]. Spirulina standard contains of CPC of 8.52±0.24 mg/g. Meanwhile, spirulina from masking had a CPC range of 4.36-6.81 mg/g (Table 2). Based on Analysis of variance in the crude phycocyanin content (CPC) response using a quadratic model shows a significance value (*p-value*) of 0.288, which means the model is not significant ($p>0.05$) in linier term (first-order term). The *R-squared*

value for the CPC response is 0.6180, which is classified as moderate positively interaction between independent variables. The *lack of fit* value of colour changes response is 0.059, which is not significant (p>0.05), and the response of CPC will match with prediction models. The following is a quadratic equation for the CPC response model:

$$CPC = 0.206 - 0.00172\ A + 0.00162\ B + 0.0119\ C + 0.0545\ D - 0.000013\ A^2 + 0.000029\ B^2 + 0.00040\ C^2 - 0.00359\ D^2 + 0.000050\ AB + 0.000008\ AC + 0.000197\ AD - 0.000016\ BC - 0.000610\ BD - 0.00355\ CD \quad (6)$$

Where, CPC is the crude phycocyanin content (mg/g), A temperature of heating (°C), B period of heating (min), C concentration of maltodextrin (g/L), and D ratio of maltodextrin and spirulina (v/m).

The equation 6. shows that the increase in CPC response is directly proportional to period of heating (B), concentration of maltodextrin (C), ratio of spirulina and maltodextrin, period of heating interaction (B^2), concentration interaction (C^2), interaction between temperature and period of heating (AB), interaction of temperature and concentration (AC), interaction of temperature and ratio (AD), which is indicated by a positive value. Conversely, the colour changes response will decrease with increasing temperature of heating (A), interaction of temperature (A^2), interaction of ratio spirulina and maltodextrin (D^2), interaction between heating period and concentration (BC), interaction between heating period and ratio spirulina and maltodextrin (BD), and interaction between concentration of maltodextrin and ratio of spirulina and maltodextrin (CD), with a negative constant value indicates.

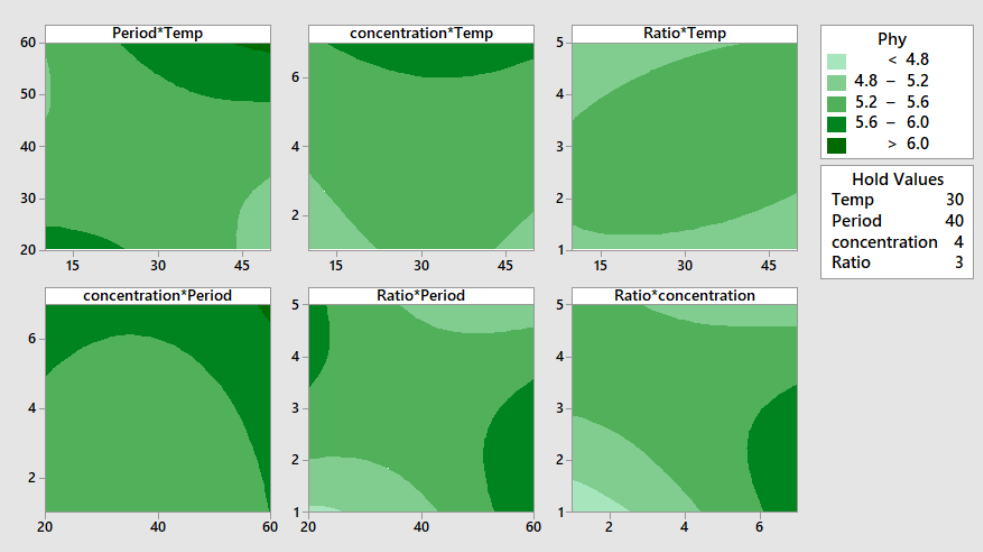

Fig. 3. The contour plot of crude phycocyanin content (CPC) response.

The 2-D contour graph can be seen in Fig. 3, the darker-colour areas indicate areas where the CPC response is optimal. Contour plot of heating period vs heating temperature, optimum at 60 min and 45-50°C. The optimum contour concentration vs temperature is found at 6-7 g/L and 20-50°C. The optimum contour plot of maltodextrin concentration vs period heating at 7 g/L and 60 min. The optimum ratio vs period is 1:4 to 1:5 for 20 minutes. Based on the contour plot of ratio vs concentration an optimum at 1:5 and 1-2 g/L. The maximum CPC can be achieved by optimizing mid-high temperature masking, large the heating period, and medium to high maltodextrin concentrations and ratios. In natural the phycocyanin content of spirulina has achieved of 42.02 mg/g, but there is a decrease due to the heating or drying

process [15]. The right temperature, optimization conditions, and coating material composition can reduce the decrease in phycocyanin content [9].

3.2 The optimizer response condition

The independent variables are optimized using the Minitab response optimizer. Response optimizer is used to identify the combination of input variable settings that optimize a single response or a set of responses. The response is formed from each optimizer into an optimization model to predict the combination of variables that can produce the optimal response. According to this model, the measured quality characteristics of every predicted response are transformed to a Composite desirability value (D), Fig. 4.

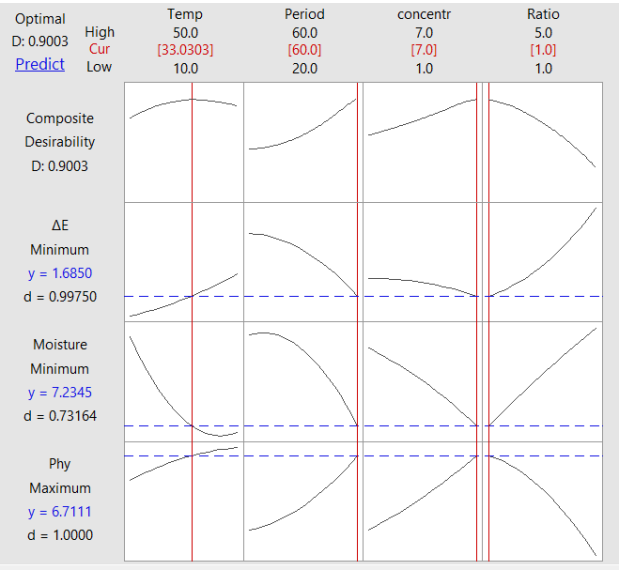

Fig. 4. The plot of Box-Behnken Design (BBD) of the optimiser response

Fig. 4. shows the optimum conditions with response targets of minimizing colour changes (ΔE), moisture content, and maximizing crude phycocyanin content (CPC). The optimum response that can be achieved by optimizing spirulina masking is a colour change of 1.685, a moisture content of 7.23%, and crude phycocyanin content of 6.711 mg/g. These results can be achieved with optimal conditions at a heating temperature of 33.3°C, heating period of 60 min, and maltodextrin concentration of 7 g/L with a spirulina and maltodextrin ratio of 1:1 m/v. The spirulina rehydration process with basil leaf extract has been reported to be successful; soaking for 15 minutes at room temperature, followed by drying at 40°C, can improve spirulina's colour sensory attributes and biological activity [15].

4 Conclusion

The optimization of spirulina maltodextrin masking with different conditions, such as heating temperature, period of heating, maltodextrin concentration, and the ratio of maltodextrin and spirulina, has been studied. Responses that have been observed include moisture content, colour changes (ΔE), and crude phycocyanin content (CPC). The p-value of significant test ANOVA showed the model is not significant ($p>0.05$) in the first-order term (linier models) of the three responses. The second-order term (2-way interaction) between the heating period and maltodextrin concentration is significant ($p<0.05$) to the ΔE

response. The R-squared values for the responses were relatively moderate positive relationships (0.3-0.7), 0.4171 for moisture, 0.6583 for ΔE, and 0.618 for CPC. While these p-values show some correlation, they suggest that the Linear model does not strongly predict the outcomes, particularly for moisture and CPC response. Typically, R-squared values closer to 1 indicate a better fit, so these moderate values might indicate that the model could be improved. All quadratic equation models show that it matches the data response because the lack of fit values is not significant ($p>0.05$). The optimized result can be achieved at a heating temperature of 33.3°C, a heating period of 60 min, and a maltodextrin concentration of 7 g/L with a spirulina and maltodextrin ratio of 1:1 m/v.

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