OPTIMIZATION OF SPRAY DRYING CONDITIONS OF *NELUMBO NUCIFERA* LEAVES EXTRACT USING RESPONSE SURFACE METHODOLOGY

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Abstract: The purpose of the present work is to study the effect of inlet drying temperature (140-170^{o}C), feed flow rate (20-30ml/min) and maltodextrin ratio (10-20%) on the total phenolic content (TPC) and total flavonoid content (TFC) of spray-dryed *Nelumbo nucifera* extracts using response surface method/technique (RSM). The optimal conditions for spray drying process from leaves of *N. nucifera* extracts were found to be inlet drying temperature of 141^{o}C, feed flow rate of 30ml/min and maltodextrin ratio of 10%w/v. The experimental values of TPC and TFC were 36.89±0.05mg GAE/g and 9.22±0.02 mgCE/g.

Keywords. *Nelumbo nucifera*; total phenolic content (TPC); total flavonoid TFC; response surface methodology (RSM).

1. Introduction

Nelumbo nucifera Gaertn. (Nymphaeaceae) is a potential aquatic crop grown and consumed throughout Asia. All parts of *N. nucifera* have been used for various medicinal purposes in various systems of medicine including folk medicines, Ayurveda, Chinese traditional medicine, and oriental medicine. Many chemical constituents have been isolated till the date. However, the bioactive constituents of lotus are mainly alkaloids and flavonoids. Traditionally, the whole plant of lotus was used as astringent, emollient, and diuretic. It was used in the treatment of diarrhea, tissue inflammation, and homeostasis. The rhizome extract was used as antidiabetic and anti-inflammatory properties due to the presence of asteroidal triterpenoid. Leaves were used as an effective drug for hematemesis, epistaxis, hemoptysis, hematuria, and metrorrhagia. Flowers were used to treat diarrhea, cholera, fever, and hyperdipsia. In traditional medicine practice, seeds are used in the treatment of tissue inflammation, cancer and skin diseases, leprosy, and antidote [1-3].

Spray drying is a technique of producing a dry powder from pumpable liquid or slurry using rapid drying with a heated gas media. Spray drying technology is widely used in food technology to produce powder continuously [4-5]. It is also applied to dry liquid foods [6-7] and food from natural extracts [8]. The physicochemical properties of powders produced by spray drying depends mainly on significant process variables such as air inlet temperature, maltodextrin ratio, air pressure and feed flow rate. Other less important parameters are ignored in this study due to the limitations of the equipments used (e.g., air outlet temperature, atomizer type and size, air humidity, drying air flow rate). The liquid feed was assumed to be close to water characteristics (e.g., viscosity, solid content, surface tension, solvent volatility).

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Response suface methodology (RSM) is regarded as an effective method to optimize processing parameters for functional food components [9-10]. The objective of the present study is to elucidate the effect of adjuvant in the spray-drying of leaves of *N. nucifera* extract and to find the optimal processing parameters of spray-drying to create powder from leaves of *N. nucifera* extract with the highest total phenolic content, the highest total flavonoid content as well as the best moisture content by applying response surface methodology. As far as the authors know, no spray-drying process of phenolics compounds in *N. nucifera* products extracts has been reported.

2. Material and methods

2.1. Material

Leaves of *N. nucifera* were collected at the Nam Dan District, Nghe An Province, Vietnam. The material is washed, drained and freeze-dried at 50^{o}C for 2 days, then the leaves are crushed and stored in a freezer of -20^{o}C. Proceeding to extract with solvent is ethanol 50% at 57^{o}C in 210 minutes (other study of group). The extract liquid is used to study the spray drying process.

2.2. Methods

2.2.1. Spray drying

Spray drying of *N. nucifera* extract with maltodextrin as an additive (wall material) at different ratios was performed using a Buchi B-290 mini spray dryer. The *N. nucifera* extract was mixed with maltodextrin at the proportion of 10%, 15% and 20% (w/v) respectively, inlet temperature used to optimize the model were 140 ^{o}C, 155 ^{o}C and 170^{o}C. Feed rates used were 20, 25, 30 ml/min. After spray drying processing, the *N. nucifera* powder thus obtained was stored in small glass jars with screw caps to evaluate their ending product quality.

2.2.2. Total phenolic content (TPC)

The TPC was measured according to the Folin - Ciocalteu's method [11] with some modifications. This method is based on measuring color change caused by reagent by phenolates in the presence of sodium carbonate. 1ml of sample was mixed 5ml of Folin - Ciocalteu's solution. After 3 min, 4ml of 7.5% sodium carbonate solution was added to mixture and turned into 10ml with deionized water. The mixture was allowed to stand at room temperature in the dark environment for 60 min. The color change was determined by scanning the wavelength at 765nm (Agilent 8453 UV - Visible Spectrophotometer) when maximum absorbance was obtained. TPC of the *Nelumbo nucifera* extract was determined as mg gallic acid equivalent using the standard curve prepared at different concentrations of gallic acid and reported as mgGAE/g dry weight (DW).

2.2.3. Total flavonoid content (TFC)

The TFC of the *N. nucifera* extract was estimated according $AlCl_3$ method [12]. An aliquot (1 mL) of extracts or standard solution of catechin (0.01 ÷ 0.07 mg/mL) was added to 10 volumetric flask containing 4 mL of H_2O. To the flask was added 0.3 ml 5%

$NaNO_2$. After 5 min, 0.3 mL 10% $AlCl_3$ was added. At 6th min, 2 mL 1M NaOH was added and the total volume was made up to 10ml with H_2O. The solution was mixed well and the absorbance was measured againtst prepared reagent blank at 510 nm. Total flavonoid content in *N. nucifera* leaves was expressed as mg catechin equivalents (mgCE/g DW).

2.2.4. Experimental design

RSM was used to determine the optimum levels of maltodextrin ratio (% w/v), feed flow rate (ml/min) and inlet drying temperature (^{o}C) as drying on two responses namely TPC and TFC in the *N. nucifera* extracts spray drying. These three factors, namely inlet drying temperature (X_1), feed flow rate (X_2) and maltodextrin ratio (X_3), were coded into three levels (-1, 0, +1). The coded independent variables used in the RSM design are shown in table 1. Ranges of maltodextrin ratio, inlet drying temperature and feed flow rate and the central point were selected based on preliminary experimental results. Statistical analysis on the means of triplicate experiments was carried out using the ANOVA procedure of the design expert software, version 7.0.

3. Results and discussion

3.1. Model fitting

The responses consisting of TPC and TFC for spray drying from *N. nucifera* extracts were optimized based on the Box-Behnken. The Box-Behnken with three independent variables was used as follows: maltodextrin ratio, inlet drying temperature and feed flow rate. This design consisted of 15 experimental points with three replicates at the central point. The input range of the selected variables was determined by the preliminary experiments (Table 1).

Two dependent variables including total phenolic content and moisture content were determined following spray drying under optimal conditions. These experimental values were compared with those of the predicted values to check the validity of the model.

Table 1: *Coded level of independent variables used in the RSM design*

Independent variables	Coded symbols	Coded variable levels		
		-1	0	+1
Inlet drying temperature (^{o}C)	X_1	140	155	170
Feed flow rate (ml/min)	X_2	20	25	30
Maltodextrin ratio (%)	X_3	10	15	20

The effects of the extraction parameters were evaluated using the program Design- Expert®, version 7.0.0. The response variable was fitted be a second- order polynomial model as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j$$

Table 2: *Experimental Design and Response Values*

RUN	Inlet drying temperature X_1 (^oC)	Feed flow rate X_2 (ml/min)	Maltodextrin ratio X_3 (%w/v)	TPC Y_1 (mgGAE/g)	TFC Y_2 (mgCE/g)
1	+	0	+	36.98	7.08
2	0	-	-	30.29	3.98
3	0	0	0	35.05	5.74
4	0	0	0	34.03	5.72
5	-	+	0	33.11	6.82
6	0	-	+	37.75	8.34
7	0	+	+	35.47	7.16
8	-	-	0	30.72	4.01
9	0	0	0	34.27	5.95
10	-	0	+	33.38	5.37
11	+	-	0	29.09	3.08
12	-	0	-	35.86	6.85
13	0	+	-	36.33	8.22
14	+	0	-	29.75	3.14
15	+	+	0	31.85	4.54

The values of the two evaluation indices for each spray drying condition were listed in Table 2. The maximal TPC was 37.75 mgGAE/g at maltodextrin ration 20%w/v, Inlet drying temperature 155^oC and feed flow rate 20ml/min; The maximal TFC was 8.34 mgCE/g at the same condition. From the multiple linear regression analyses of the 15 data entries, the final empirical regression model of their relationship between responses and the three tested variables for phenolic and favonoid contents could be expressed by the following quadratic polynomial equation [Eqs. (1-2)]:

$$Y_1 = 34.45 - 0.68X_1 + 1.36X_2 + 1.17X_3 + 2.43X_1X_3 - 1.58X_2X_3 - 1.86X_1^2 - 1.39X_2^2 + 1.40X_3 \quad (1)$$

$$Y_2 = 5.80 - 0.65X_1 + 0.92X_2 + 0.72X_3 + 1.35X_1X_3 - 1.36X_2X_3 - 1.25X_1^2 + 1.06X_3^2 \quad (2)$$

Where Y_1 is total phenolic content, Y_2 is the total flavonoid content, X_1 is the Inlet drying temperature, X_2 is the Feed flow rate and X_3 is the Maltodextrin ratio.

ANOVA results for multiple regression analysis and response surface quadratic model of $Y_{1,}$ Y_2 were evaluated using the corresponding F, p and R^2 values (Table 3). F values of Y_1 and Y_2 were calculated to be 80.06 and 45.87, both lead to a p-value < 0.05, suggesting the both the models were statistically extremely significant. The models'coefficient of determination (R^2) were 0.9931 and 0.9880, indicating that more than 99.31% and 98.80% of the response variability were explained, and supporting a good accuracy and ability of the established model within the range limits used. The F-values of Lack of Fit of $Y_{1,}$ Y_2 were 0.079; 9.33, respectively implies that the Lack of Fit was not significantly relative to the pure error. This indicated that the accuracy of the polynomial model was adequate.

Table 3: *Analysis of variance (ANOVA) for the model*

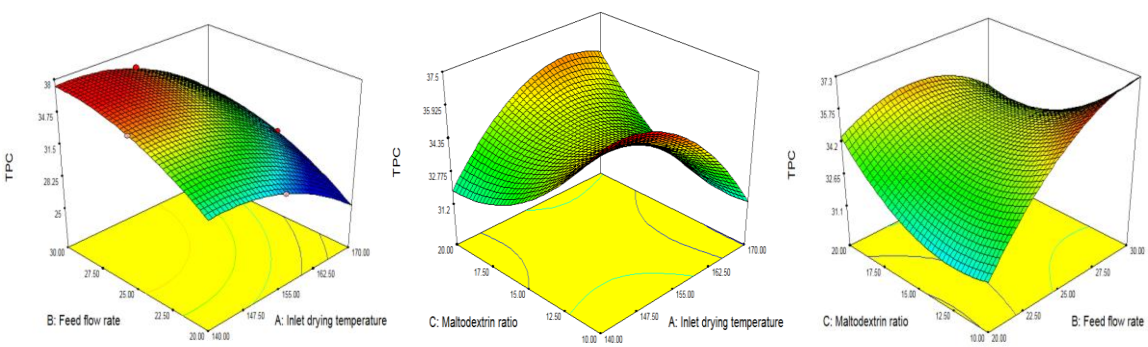
Source	Y_1 - TPC		Y_2 - TFC	
	F- value	p- value	F- value	p- value
Model	80.06	<0.0001***	45.87	0.0003***
A	28.65	0.0031**	34.86	0.0020**
B	116.94	0.0001***	69.00	0.0004***
C	85.89	0.0002***	42.61	0.0013**
AB	0.27	0.6261^{NS}	4.68	0.0828^{NS}
AC	185.26	<0.0001***	75.45	0.0003***
BC	78.48	0.0003***	75.45	0.0003***
A^2	100.67	0.0002***	59.55	0.0006***
B^2	56.47	0.0007***	0.15	0.7179^{NS}
C^2	57.29	0.0006***	42.59	0.0013**
Lack of Fit	0.079	0.9655	9.33	0.0984
R^2	0.9931		0.9880	

**p< 0.05; **p< 0.01; ***p< 0.001; NS: non-significant.*

3.2. Optimization and model verification

Based on the empirical second-order polynomial model, the experimental data was analyzed by RSM using the Design-Expert 7.0 software. The X- and Y- axes of the three-dimensional response surfaces represented two factors, for maltodextrin ratio and inlet drying temperature (feed flow rate 25ml/min), inlet drying temperatureand feed flow rate (maltodextrin ratio 15%), maltodextrin ratio and feed flow rate (inlet drying temperature 155°C). The Z- axes represented one of the four evaluation indices (TPC, TFC). Two dimensional response surfaces were constructed as depicted in Fig. 1 and Fig. 2.

3.2.1. Response surface analysis of total phenolic content



(A) (B) (C)

Fig. 1: *Response surface of TPC*

Figure 1(A) is a response surface plot showing the effect of feed flow rate and inlet drying temperature on the total phenolic content. Feed flow rate showed a negative linear effect on the total phenolic content ($p < 0.0001$). Inlet drying temperature showed a negative linear effect on the total phenolic content ($p < 0.05$). The TPC increased gradually when feed flow rate increased from 20ml/min to 30ml/min and the maximum amount of phenolic can be achieved at the highest feed flow rate of 27.5ml/min to 30ml/min at the lowest inlet drying temperature at 140°C. We could observe that the yields of total phenolic content decreased with the increase of inlet drying temperature from 140°C to 170°C. These results are similar to a study reported by of Krishnaiah *et al*. [13].

The surface plot in Fig. 1(B) shows the function of maltodextrin ratio versus inlet drying temperature effect on TPC at feed flow rate (25ml/min). The yields of TPC increased with the decrease of maltodextrin ratio from 20%w/v to 10%w/v and the maximum phenolic content can be achieved at lowest inlet drying temperature at 140°C.

Figure 1(C) is a response surface plot showing the effect of feed flow rate and maltodextrin ratio on the total phenolic content. The TPC increased when feed flow rate increased from 20ml/min to 30ml/min and the maximum amount of phenolic can be achieved at the highest feed flow rate of 27.5ml/min to 30ml/min at the highest maltodextrin ratio at 20%w/v.

3.2.2. Response surface analysis of total flavonoid content

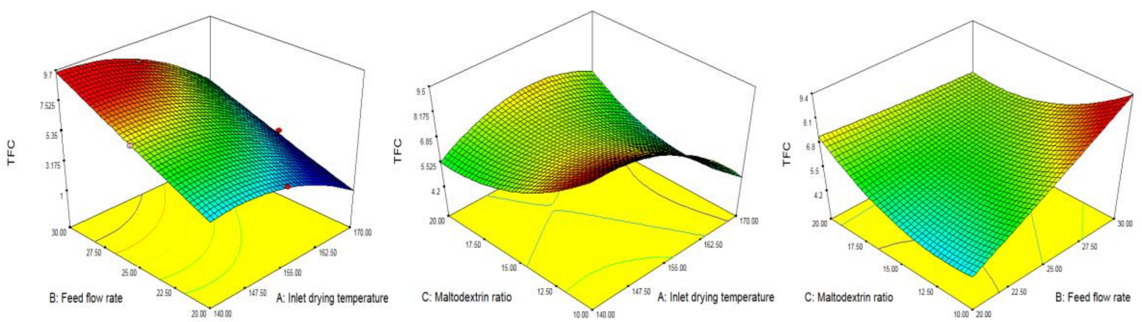


Fig. 2: *Response surface of TFC*

Figure 2(A) is a response surface plot showing the effect of feed flow rate and inlet drying temperature on the total flavonoid content. Feed flow rate showed a negative linear effect on the TFC ($p < 0.0001$). Inlet drying temperature showed a negative linear effect on the TFC ($p < 0.05$). Like TPC, the TFC increased gradually when feed flow rate increased from 20ml/min to 30ml/min and the maximum amount of flavonoid can be achieved at the highest feed flow rate of 27.5ml/min to 30ml/min at the lowest inlet drying temperature at 140°C.

Figure 2(B) show the function of maltodextrin ratio versus inlet drying temperature effect on TFC at feed flow rate (25ml/min) and Figure 2(C) is a response surface plot showing the effect of feed flow rate and maltodextrin ratio on the TFC. The effects of these pairs of factors on TFC are similar to TPC.

3.3. Optimization of process parameters and verification of the model

The optimal values of the independent variables were obtained by solving second order regression equations using a numerical optimization method. Experimental data suggested the existence of optimization of total phenolic content and total phenolic content occurred with feed flow rate 30 ml/min, maltodextrin ratio 10% at 141°C.

Table 4: *Optimum conditions, predicted and experimental values of responses on spray drying of N. nucifera extract*

Independent variables			Dependent variables (Response)	Optimum value		% Difference (CV)
A	B	C		Experimental[a]	Predicted	
141°C	30,0 ml/min	10,0 %	Y_1	36.89±0.05	37.0866	0.53
			Y_2	9.22±0.02	9.27289	0.54

[a]*Mean ± standard deviation (SD) of four determinations (n= 4) from two crude extract replications*.

4. Conclusion

Use response surface methodology were successfully developed to determine the optimum process parameters and the second order polynomial models for predicting responses were obtained. The best combination of inlet drying temperature, feed flow rate and maltodextrin ratio of spray-drying process were found at 141°C with 30ml/min and 10% maltodextrin ratio which rendered a mean phenolic content of 36.89±0.05 mgGAE/g and 9.22±0.02 mgCE/g of total favonoid content from experimental run and thus indicated good antioxidant activities from the leaves of *N. nucifera*.

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TÓM TẮT

TỐI ƯU HOÁ CÁC ĐIỀU KIỆN SẤY PHUN DỊCH CHIẾT LÁ SEN (*Nelumbo nucifera)* SỬ DỤNG PHƯƠNG PHÁP ĐÁP ỨNG BỀ MẶT

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Mục đích của bài báo là nghiên cứu ảnh hưởng các điều kiện sấy phun như nhiệt độ đầu vào (140-170^{o}C), tốc độ dòng (20-30ml/ phút) và tỷ lệ maltodextrin (10-20%) đến hàm lượng tổng phenolic (TPC) và hàm lượng tổng flavonoid (TFC) của dịch chiết lá sen (*N. nucifera*) sử dụng phương pháp đáp ứng bề mặt (RSM). Các điều kiện tối ưu cho quá trình sấy phun dịch chiết lá sen được xác định là nhiệt độ đầu vào 141^{o}C, tốc độ dòng 30ml/phút và tỷ lệ maltodextrin là 10%w/v. Giá trị thực nghiệm của TPC và TFC tương ứng là 36,89±0,05mg GAE/g và 9,22±0,02 mgCE/g.

Từ khoá: Cây sen (*Nelumbo nucifera*); hàm lượng tổng phenolic; hàm lượng tổng flavonoid; phương pháp đáp ứng bề mặt.