Optimization for the Anthocyanin Extraction from Purple Sweet Potato Roots, Using Response Surface Methodology[1]

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Abstract

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Anthocyanins were successfully extracted from 'TNG 73' purple sweet potato roots by using acidic-ethanol extraction. Three extraction parameters including HCl concentration, extraction time, and temperature were evaluated. Response surface methodology (RSM), an effective statistical technique was applied to establish a regression model. The coefficients of the regression model were calculated and used to predict the extraction performance and determine the optimal operating conditions. In conclusion, the optimal conditions for the 'TNG 73' anthocyanin extraction were determined to be 12% HCl (1.5 N HCl in EtOH), 45 min, and 50°C.

Key words: Anthocyanins, Purple sweet potato, Extraction, Response surface methodology.

Introduction

A newly cultivated purple sweet potato 'Tainung 73' ('TNG 73'), was bred by Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute (TARI), ROC. 'TNG 73' root has reddish-purple flesh and contains significant amount of dietary fibers. It also contains 70% water and 50 to 300 μg/g anthocyanins on a dry basis. 'TNG 73' is the first sweet potato variety developed by a Taiwanese breeder with high anthocyanin content. The average yield is above 30 t/ha.

Purple sweet potatoes are a good source of anthocyanins. The anthocyanin contents of purple sweet potato roots have been compared previously (Shi *et al*. 1992; Goda *et al*. 1997). Other than purple sweet potatoes, some vegetables and fruits, like grape, cherry, carrot, berry, and cabbage, also contain anthocyanins (Gao & Mazza 1996; Ju & Howard 2003; Longo & Vasapollo 2006). Temperature, pH, and the storage condition affect the stability of anthocyanins (Torskangerpoll & Andersen 2005; Kirca *et al*. 2007). Anthocyanins prepared from the purple sweet potatoes are more stable than those from vegetables and fruits (Kano *et al*. 2005). Moreover, anthocyanins and their derivatives are widely used as natural pigments and food additives (Downham & Collins 2000; Giusti & Wrolstad 2003). They show remarkable anti-oxidative, anti-inflammatory, and anti-tumor ability (Bomser 1996; Wang *et al*. 1999). However, their low stability makes them difficult to sustain thermal treatments (Scalzo *et al*. 2008).

Anthocyanins can be extracted from deep-purple plants by using organic solvents (Brigita-Lapornik *et al*. 2005; Kapasakalidis *et al*. 2006). For example, an aqueous solution of acidic-methanol was used to extract anthocyanins from grapes (Revilla *et al*. 1998). Although acidic-methanol has been approved to be a useful and

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feasible extraction method, methanol is less desirable for medical or food applications. Therefore, acidic-ethanol is chosen as an alternative because of its lower toxicity. Meanwhile, some new extraction methods that do not use hazardous solvents have been developed recently. They require more energy; thus, they are economically infeasible sometimes.

Response surface methodology (RSM) is an effective statistical technique of process optimization. The theoretical aspects of RSM have been reviewed (Bas & Boyaci 2007; Myers *et al*. 2009). Most of the empirical methods assume that only individual factors affect the response, and there is no interaction between these factors. RSM is able to evaluate the interactions between various factors. It has been widely used in chemical, biological and agricultural applications to predict the optimal conditions of the systems (Liyana-Pathirana & Shahidi 2005; Cacace & Mazza 2006). If there is a linear relationship between the independent factors and the response, the first-order model can be used.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \varepsilon \qquad (1)$$

Where Y is the response; X_i are input variables; β_0 is the intercept; β_i are linear coefficients; and ε is an error term.

If the relationship between the independent factors and the response is a curve, the second-order model will be introduced to fit the systems. The second-order model includes linear, quadratic, and interactive components.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k}\sum_{i<j}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (2)$$

Where Y is the response; X_i, X_j are input variables; β_0 is the intercept; β_i are linear coefficients; β_{ii} are squared coefficients; β_{ij} are interaction coefficients; ε is an error term.

For three independent variables analysis (X_1, X_2, and X_3), equation 2 can be rearranged as:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 \\ + \beta_{13} X_1 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \qquad (3)$$

The central composite design (CCD) is the most popular design method for second-order model. CCD has 2^k factorial experiments where k represents the number of independent factor. Two parameters, including the central point (n_c) and the distance (α) from the axial point to central point, need to be decided before designing the experiment. Based on the definition, $\alpha = (2^k)^{1/4}$, α is equal to 1.414 and 1.682 for two-factor and three-factor designs respectively. The main goal of this study was to establish a regression model to predict the performance of

anthocyanin extraction process of TNG73. The influence of independent factors on the extraction process was also analyzed by using RSM.

Materials and Methods

Purple sweet potatoes TNG73 were grown in the field of Chiayi Agricultural Experiment Branch following standard commercial cultural practices. After harvest, roots were cut into cubes (approximately 2 cm × 2 cm), dried in a vacuum oven at 50℃ for 24 h, then pulverized and sieved with a 100 mesh screen. The collected powder was then transferred into bottles, and stored in a refrigerator at 4℃ for further use.

For extraction parameter study, the dried powder was extracted by HCl/EtOH (1.5 N HCl in ethanol) solutions with solid/solvent ratio of 10 g/100 mL. A water-bath shaker (Model No. B601D, Firstek[R] Co. Ltd., Taiwan) was used to maintain the temperature. Each sample was initially diluted 5× with 0.1 N HCl/EtOH [15 : 85 (V/V)] before analysis. The absorbance of anthocyanin was measured at 532 nm wavelength at 25℃ using UV/VIS spectrophotometer (Jasco V530, International Co. Ltd., Tokyo, Japan).

Statistical analysis

The statistical evaluation was performed by running analysis of variance (ANOVA) and regression calculation using SAS[R] (version 9.0, SAS institute, Cary, NC, USA). A 3^3 full factorial design with three independent factors (HCl concentration, extraction time, and extraction temperature) was employed to fit a second-order extraction model. Each factor had three levels which were coded as -1, 0, and 1. The central points (n_c, coded as 0) for each extraction factor were 9% HCl, 30 min, and 40℃ respectively. The solid/solvent ration was 10 g/100 mL. The significance of regression coefficients, appeared as associated probabilities, was also evaluated. For significant values, means were separated by the least significant difference (LSD) test at $p \leqq 0.05$ to generate a mathematical model to predict system responses. The 3-D mesh plots and contour maps were used to demonstrate the results.

Results and Discussion

The results indicated that the extraction performance was mainly dependent on HCl concentration, extraction time, and extraction temperature. The central composite design (CCD) was used to optimize the extraction conditions and investigate effects of three independent factors on anthocyanin extraction. In addition, analysis of variance (ANOVA) was applied to highlight the most significant factors in the extraction process. For extraction parameter study, the results of 20 experimental

runs with completed design were shown in Table 1, and the range of absorbance was from 0.370 to 0.588. The central points (n_c), upper, and lower levels of three independent factors were chosen based on the practicability of scaling-up.

Table 2 shows the ANOVA of three independent factors (HCl concentration, extraction time, and extraction temperature) for anthocyanin extraction. Since all factors were all significant for determining the extraction performance, any change of these factors would have a significant effect on anthocyanin content of extracts. The regression coefficients of intercept, linear, quadratic, and interaction terms in the experimental model and their levels of significance were determined by using regression sub-routine function of SAS[R] (Table 3). All linear components in the experimental model were significant.

Table 1. Experimental data and the observed response values (absorbance, with 10 g/100 mL solid/solvent ratio) with three independent factors, X_1 (% HCl, 1.5 N HCl in EtOH), X_2 (extraction time, min), and X_3 (extraction temperature, ℃)

Run No.	Factors			Response values Absorbance
	X_1 (% HCl)	X_2 (min)	X_3 (℃)	
1	6.0 (-1) [z]	15.0 (-1)	30.0 (-1)	0.392 [y]
2	6.0 (-1)	15.0 (-1)	50.0 (1)	0.451
3	6.0 (-1)	45.0 (1)	30.0 (-1)	0.426
4	6.0 (-1)	45.0 (1)	50.0 (1)	0.512
5	12.0 (1)	15.0 (-1)	30.0 (-1)	0.415
6	12.0 (1)	15.0 (-1)	50.0 (1)	0.460
7	12.0 (1)	45.0 (1)	30.0 (-1)	0.490
8	12.0 (1)	45.0 (1)	50.0 (1)	0.588
9	4.0 (-1.68)	30.0 (0)	40.0 (0)	0.396
10	14.0 (1.68)	30.0 (0)	40.0 (0)	0.538
11	9.0 (0)	4.8 (-1.68)	40.0 (0)	0.370
12	9.0 (0)	55.2 (1.68)	40.0 (0)	0.517
13	9.0 (0)	30.0 (0)	23.2 (-1.68)	0.425
14	9.0 (0)	30.0 (0)	56.8 (1.68)	0.515
15	9.0 (0)	30.0 (0)	40.0 (0)	0.474
16	9.0 (0)	30.0 (0)	40.0 (0)	0.464
17	9.0 (0)	30.0 (0)	40.0 (0)	0.484
18	9.0 (0)	30.0 (0)	40.0 (0)	0.479
19	9.0 (0)	30.0 (0)	40.0 (0)	0.487
20	9.0 (0)	30.0 (0)	40.0 (0)	0.477

[z] (-1), (0), and (1) were coded for levels of independent factors.
[y] Each value was mean of 3 replication.

Table 2. Analysis of variance for the overall effect of factors on the response

Factors	DF	SS	MS	F	Pr > F
X_1 (% HCl)	4	0.0138	0.0035	14.21	0.0004[*z]
X_2 (min)	4	0.0256	0.0064	26.19	< 0.0001[*]
X_3 (℃)	4	0.0150	0.0037	15.30	0.0003[*]

[z,*] Significant at 5% level of probability.

Table 3. Regression coefficients for the acidic-ethanol anthocyanin extraction process

Regression coefficient	DF	Estimate	t value	Pr > | t |
Intercept β_0	1	0.4772	74.87	< 0.0001
β_1	1	0.0301	7.11	< 0.0001
β_2	1	0.0399	9.44	< 0.0001
β_3	1	0.0322	7.61	< 0.0001
β_{11}	1	-0.0021	-0.51	0.6207
β_{12}	1	0.0135	2.44	0.0347
β_{22}	1	-0.0104	-2.53	0.0299
β_{13}	1	-0.0003	-0.05	0.9648
β_{23}	1	0.0100	1.81	0.1005
β_{33}	1	-0.0010	-0.25	0.8054

For the quadratic components, the extraction time (square component) and the interaction between HCl concentration and extraction time (interactive component) were significant. The regression coefficients were calculated, and the regression model was shown in the coded factors as:

$$Y = 0.4772 + 0.0301\,X_1 + 0.0399\,X_2 + 0.0322\,X_3 +$$
$$0.0135\,X_1X_2 + 0.01\,X_2X_3 - 0.0003\,X_1X_3 - 0.0021$$
$$X_1^2 - 0.0104\,X_2^2 - 0.001\,X_3^2 \qquad (4)$$

Where Y is the observed absorbance of extracts; X_1 is %HCl; X_2 is extraction time; X_3 is extraction temperature; X_1, X_2, and X_3 factors were all coded as -1, 0, and +1, and $R^2 = 0.9552$.

The predicted response values were calculated by applying the polynomial response surface model equation (equation 4), and the regression model with low dispersion ($R^2 = 0.9552$) was observed, suggesting that this model was successful to predict the anthocyanin extraction. If one factor in equation 4 was fixed at a specific value, effects of other two factors on anthocyanin extraction could be showed by plotting the 3-D mesh graph and contour curves. For example, when the extraction temperature (X_3) was set at 50℃ (coded as 1), the effects of HCl concentration and extraction time on absorbance of extracts were shown in Fig. 1 (A) (3-D mesh plots) and Fig. 1 (B) (contour plots). The HCl concentration effect was more significant at longer extraction time. As a result, more HCl and longer extraction time achieved better extraction results. The maximum absorbance value was obtained at 12% HCl (coded as 1) and 45 min (coded as 1). Fig. 2 shows the effect of HCl concentration and extrac-

tion temperature on the absorbance of extracts under 45 min extraction. The maximum absorbance value was obtained at 12% HCl (coded as 1) and 50°C (coded as 1). According to Fig. 2 (B), the increase of absorbance caused by HCl concentration and extraction time was almost linear. Therefore, increasing HCl concentration was as good as increasing extraction temperature. The effect of extraction time and extraction temperature on the absorbance of extracts with 12% HCl (1.5 N HCl in EtOH) was shown in Fig. 3. The maximum absorbance value was obtained at 45 min (coded as 1) and 50℃ (coded as 1). The absorbance of verification test with 12% HCl, 45 min, and 50℃ was 0.588. This value was very close to the predicted value of 0.589 calculated by equation 4. However, scaling-up process requires a consideration of economical aspects to determine the optimal operating conditions.

Results concluded in the present study are similar to previous studies (Shi *et al*. 1992; Goda *et al*. 1997). They demonstrate important implications for the food ingredient industry. Meanwhile, the change of HCl concentration, extraction time, and temperature had a significant influence on the anthocyanin extraction (Table 2). Similar results have been reported by Torskangerpoll & Andersen (2005) and Kirca *et al*. (2007). Fig. 1 shows that more HCl and longer extraction time achieved better extraction results. However, when scale-up a process, the optimal operating conditions are usually determined by combing the operating parameters with the economical aspects. Thus, the optimal conditions for the TNG73 anthocyanin extraction were determined to be 12% HCl (1.5 N HCl in EtOH), 45 min, and 50℃. The regression model developed by the response surface methodology will help manufacturers optimize the extraction process.

(A)

(B)
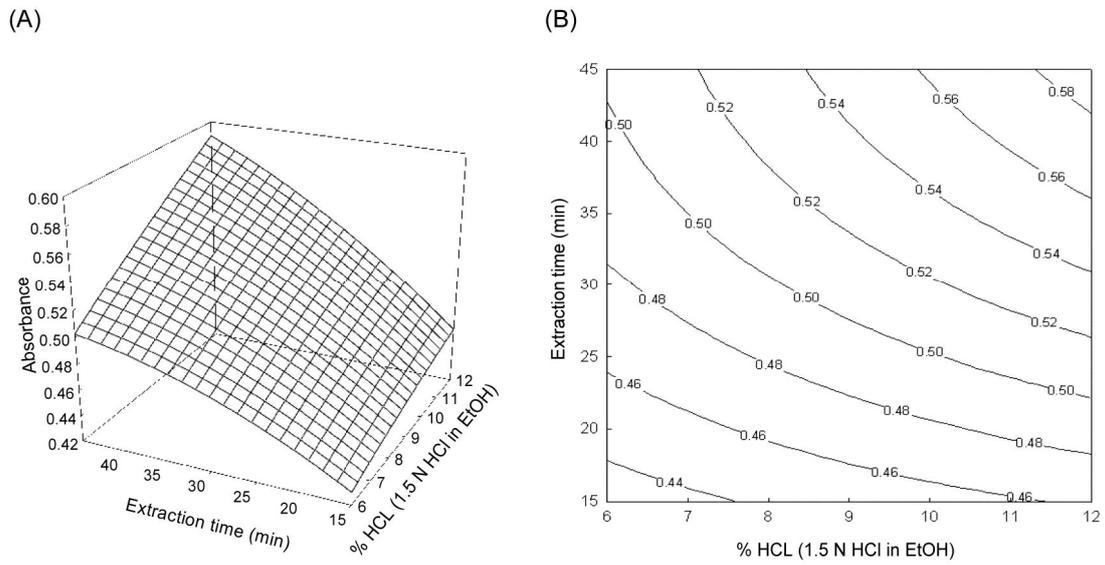
Fig. 1. 3-D mesh plot (A) and contour plot (B) of the effect of HCl concentration and extraction time on absorbance of extracts at 50℃.

(A)

(B)
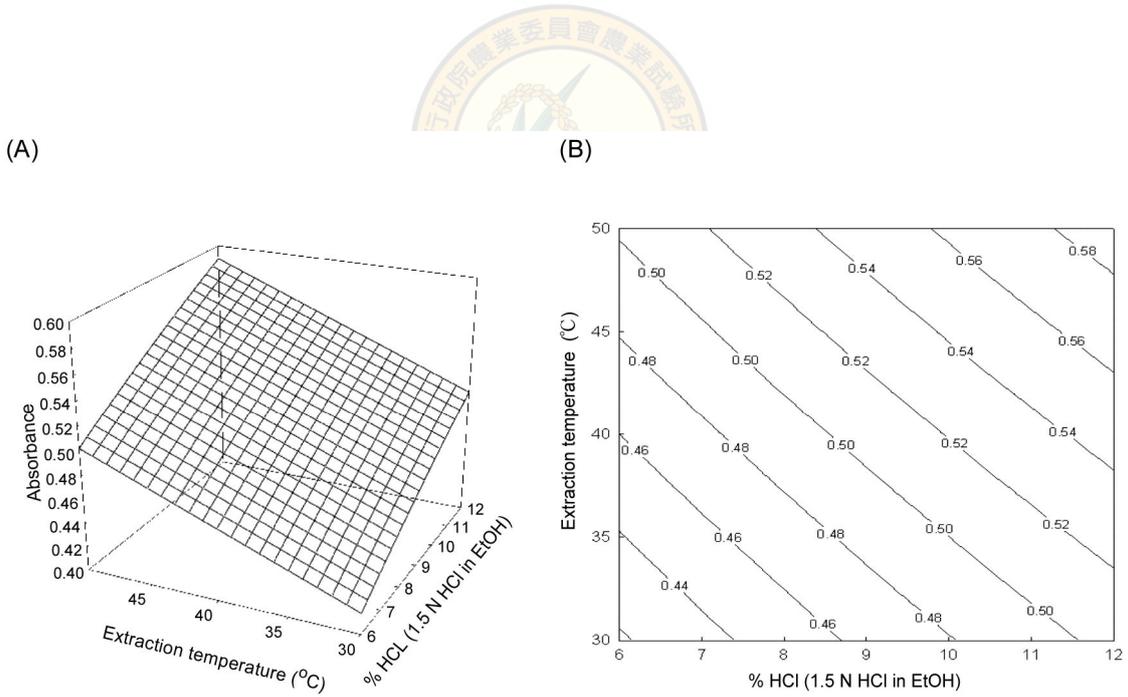
Fig. 2. 3-D mesh plot (A) and contour plot (B) of the effect of HCl concentration and extraction temperature on absorbance of extracts for 45 min extraction.

(A)

(B)
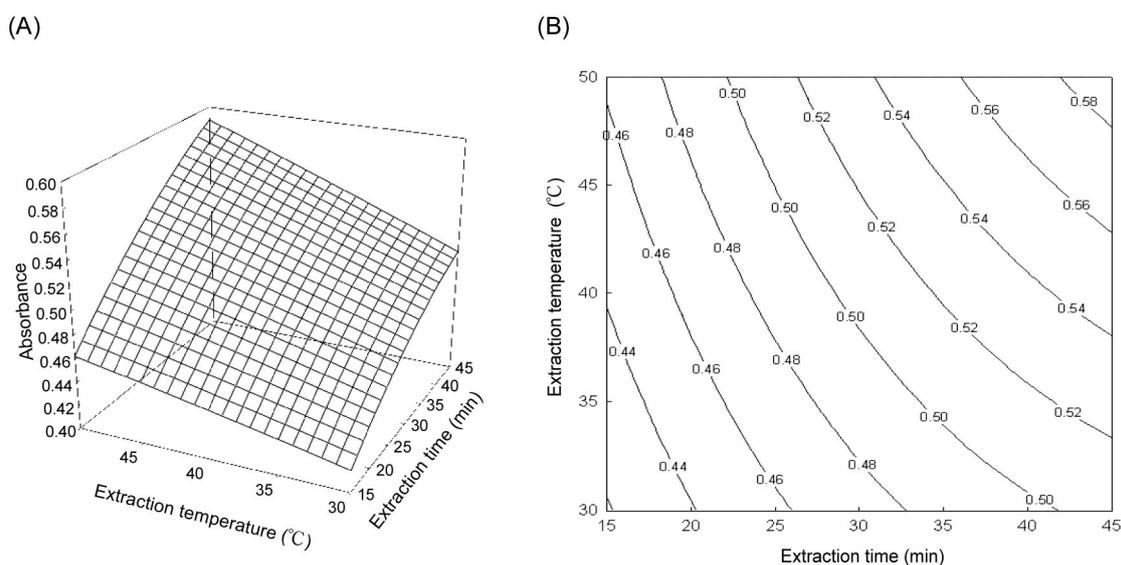
Fig. 3. 3-D mesh plot (A) and contour plot (B) of the effect of extraction time and temperature on absorbance of extracts with 12% HCl (1.5 N HCl in EtOH).

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使用反應曲面法決定紫甘藷塊根最佳花青素萃取參數 [1]

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摘　　要

黃哲倫、廖文昌、詹錦豐、賴永昌。2010。使用反應曲面法決定紫甘藷塊根最佳花青素萃取參數。台灣農業研究 59:143–150。

　　使用酸化乙醇萃取法成功地從'TNG 73'紫甘藷中萃取出花青素，三個萃取參數包括 HCl 濃度、萃取時間、以及萃取溫度都在本研究中被仔細評估，反應曲面法 (Response surface methodology, RSM) 是一種很有效的統計工具，可用來建立廻歸模組，而此廻歸模組的係數經計算後，可用來預測萃取製程的表現及決定最佳的萃取操作條件，本研究結果顯示，12% HCl (1.5 N HCl 和乙醇的混合物)、45 分鐘萃取時間、以及 50°C 萃取溫度是'TNG 73'紫甘藷花青素的最佳萃取條件。

關鍵詞：花青素、紫甘藷、萃取、反應曲面法。