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Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM)

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Abstract

Optimization for purple sweet potato (*Ipomoea batatas* (L.) Lam) anthocyanins (PSPAs) extraction was investigated using response surface methodology in this paper. PSPAs were extracted using acid–ethanol at different extraction temperature (40–80 °C), time (60–120 min) and solid–liquid ratio (1:15–1:30). The combined effects of extraction conditions on PSPAs yield and color attributes (expressed as L^*, C^* and H) were studied using a three-level three-factor Box–Behnken design. The results showed that The highest yield (158 mg/100 g dw) of PSPAs were reached at the temperature 80 °C, extraction time 60 min, and solid–liquid ratio 1:32. PSPAs yield indicated a high and significant correlation with L^* ($P<0.05$; $r=-0.961$) and it was significantly affected by extraction temperature ($P<0.01$) and solid–liquid ratio ($P<0.05$).

Keywords: Purple sweet potato; Anthocyanin; Extraction; Response surface methodology; Optimization

1. Introduction

Anthocyanins widely existed in the high plants, are found in the roots, caudexes and leaves as well as flowers and fruits. They were used to substitute synthetic pigments for their attractive color and physiological functionality. Anthocyanins also possess known pharmacological properties and are used by humans for therapeutic purpose. More and more studies show that anthocyanins have demonstrated ability to protect against a myriad of human diseases such as liver dysfunction, hypertension, vision disorders, microbial infections, and diarrhea (Rice-Evans & Packer, 1998; Smith, Marley, Seigler, Singletary, & Meline, 2000; Wang et al., 2000).

Purple sweet potato contains a high level of anthocyanins, compared to white, yellow, and orange ones, and the contents differ depending on varieties (Terahara, Konczak, Ono, Yoshimoto, & Yamakawa, 2004). The stability of PSPAs is dependent on several factors, including structure and concentration of the pigment, pH, and temperature in the extraction process. Purple sweet potato has the majority of its anthocyanins acylated, while purple corn does not (de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2002). The anthocyanins in purple sweet potato are mono- or di-acylated forms of cyaniding and peonidin (Yukihiro et al., 1997). However, they may undergo denaturalization when they are extracted from a natural source. The extraction process involves a loss of color followed by formation of brownish degradation products and insoluble compounds (Castillo-Saánchez, Mejuto, Garrido, & García-Falcón, 2006). The extraction of PSPAs reported is focused on analyzing extraction (Bolívar, & Luis, 2004); however, little information is available on optimizing extraction conditions.

Several extraction methods have been proposed to obtain extracts rich in anthocyanin, usually based on solvents as methanol, ethanol, acetone, water or mixtures. The addition of a small amount of hydrochloric acid or formic acid is recommended to prevent the degradation of the nonacylated compounds (Brigita, Mirko, & Alenka, 2005; Glenda, Julián, José, & Ana, 2006; Gonnet & Fenet, 2000; Kjell & Øyvind, 2005). Current studies on anthocyanins are focused on the stability, structure, and physiological functionality (Ikuo et al., 2003).

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Color assessment of anthocyanin extracts, according to the legal requirements in Spain (Real Decree No. 2107/1996) comprises only the measurement of the color intensity (CI) of 1% extract solution (pH 3.0) in a 1 cm glass cell, at wave length (λ) of 520 nm (CI_{520}), whereas the European Community (EC) Directive 95/45/EC specifies values for λ between 515 and 535 nm and defines the absorption of the pure pigment solution ($E_{1\%}^{1\,cm}$) as 300. The application of colorimetric systems, based on uniform color spaces (CIELUV and CIELAB) and non-uniform color spaces (CIEXYZ), is of great value in the quantification and characterization of the color properties of pigments and foods such as the cochineal pigment or orange juices among others (Montes, Vicario, Raymundo, Fett, & Heredia, 2005).

Response surface methodology (RSM) is an affective statistical technique for optimizing complex processes. It is wide used in optimizing the process variables. The basic theoretical and fundamental aspects of RSM have been reviewed (Chandrika & Fereidoon, 2005; Farooq Anjum, Imran, & Khaled, 1997). In this study, the main factors (extraction temperature, time and solid–liquid ratio) related with the extraction of anthocyanins from a starchy material, the purple sweet potato, were investigated. The color properties of the anthocyanins extracts were established by Tritimulus Colorimetry. The aim was to choose the best conditions for a quantitative and qualitative (related to color properties) extraction of anthocyanins from purple sweet potato in order to be used as food colorant.

2. Material and methods

2.1. Sample preparation

Purple sweet potato were kindly supplied by National Sweet Potato Research Institute, in Xuzhou (Xuzhou City, Jiangsu Province, China) and were washed in running tap water, cut into pieces of approximately 0.5 cm, dried in a heated air drier (50 °C) (ZT-3, Jiangdu City, Jiangsu Province, China), and then pulverized by the disintegrator (FSD-100A, Taizhou city, Zhejiang Province, China) and sifted through a 100 mesh sieve. Samples were kept at 4 °C.

2.2. Methods

2.2.1. Extraction of purple sweet potato anthocyanins

Purple sweet potato powder were put into a 50 ml conical flask, then added in acid–ethanol (HCl, 1.5 mol/l) with different solid–liquid ratio (1:15–1:30) and put in thermostatic water bath at selected temperatures (40–80 °C) for various duration periods of time (1:15–1:30), then, centrifuged at 4000 rpm for 15 min. The supernatant was collected and transferred into 50 ml volumetric flask for the determination of anthocyanins yield. About 1 g of the samples was used for each treatment.

2.3. Experimental design

RSM was used to determine the optimum condition for anthocyanin extraction of purple sweet potato. The experimental design and statistical analysis were performed using Stat-Ease software (Design-Expert 6.0.10 Trial, Delaware, USA Echip, 1993). A three-level three-factor Box–Behnken design was chosen to evaluate the combined effect of three independent variables extraction temperature, time and solid–liquid ratio, coded as X_1, X_2 and X_3, respectively. The minimum and maximum values for extraction temperature were set at 40 and 80 °C, extraction time between 60 and 120 min and solid–liquid ratio 1:15 and 1:30 (Table 1). The response values were anthocyanin pigment yield, L^*, C^* and H. The complete design consisted of 17 combinations including five replicates of the center point (Table 2) (Myers & Montgomery, 2002). The responses function (Y) was partitioned into linear, quadratic and interactive components:

$$Y = \beta_0 + \sum_{i=1}^{k} B_i X_1 + \sum_{i=1}^{k} B_{ii} X^2 + \sum_{i>j}^{k} B_{ij} X_i X_j,$$

where β_0 is defined as the constant, B_i the linear coefficient, B_{ii} the quadratic coefficient and B_{ij} the cross-product coefficient. X_i and X_j are levels of the independent variables while k equals to the number of the tested factors ($k = 3$). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the F-value at a probability (P) of 0.001, 0.01 or 0.05. The regression coefficients were then used to make statistical calculations to generate contour maps from the regression models.

2.4. Quantitative analysis

The anthocyanins were quantified following the spectrophotometric method proposed by Francis (1989). The concentration of anthocyanins was determined applying the Lambert–Beer law. The factor 98.2 is the molar absorptivity value for the acid–ethanol solvent and it refers to the absorption of a mixture of cranberry anthocyanins in acid–ethanol, measured in a 1-cm cell at 535 nm, at a concentration of 1% (w/v).

Table 1
Independent variables and their coded and actual values used for optimization

Independent variable	Units	Symbol	Code levels		
			−1	0	1
Extraction temperature	°C	X_1	40	60	80
Time	min	X_2	60	90	120
Solid–liquid ratio	1:X	X_3	15	25	35

Table 2
The Box–Behnken design and experiment data for anthocyanins extraction from purple sweet potato

Treat	Independent variables			Dependent variables			
	Temp (°C)	Time (min)	Solid–liquid ratio	Anthocyanins yield (mg/100 g)	L^*	C^*	H°
1	−1	−1	0	112	20.80	7.50	17.35
2	1	−1	0	150	20.60	7.12	20.90
3	−1	1	0	124	20.79	7.46	17.31
4	1	1	0	147	20.57	7.05	21.69
5	−1	0	−1	111	20.83	7.46	16.41
6	1	1	−1	142	20.60	7.12	21.48
7	−1	0	1	101	20.93	7.77	15.07
8	1	0	1	152	20.61	7.06	21.42
9	0	−1	−1	127	20.65	7.30	19.49
10	0	1	−1	137	20.71	7.23	19.58
11	1	0	−1	143	20.68	7.25	19.39
12	0	1	1	129	20.73	7.30	18.89
13	0	0	0	142	20.59	7.13	20.06
14	0	0	0	149	20.65	7.23	20.06
15	0	0	0	149	20.59	7.13	20.06
16	0	0	0	139	20.65	7.23	20.06
17	0	0	0	149	20.65	7.13	20.06

The spectra recorded in a UV-2802 diode array spectrophotometer (UNIC, USA) were measured at 25 °C and 530 nm, against the solvent. For that purpose 10-mm quartz cells were used. The anthocyanins yield (g/100 g) was calculated using the following equation:

$$\text{Total anthocyanins (TA)} = A_{530} \times \text{dilution factor}/98.2.$$

2.5. Color coordinates

To obtain the tristimulus values, the weighted-ordinated method (constant intervals, $\Delta\lambda = 2$ nm) was applied, using as references the CIE Standard Illuminant D_{65}, the CIE 1964 Standard Observer, and water as reference blank. Following the most recent recommendations made by the CIE, CIELAB System (the variables related with psysichometric color attributes: L^*, C^* and H for color specification was applied (Bolívar & Luis, 2004).

2.6. Statistics

All trials were carried out in triplicate and all the data were reported as means ± SD (standard deviation). The statistics significance was evaluated using Student's t-test and $P<0.05$ or 0.01 was taken as significant.

3. Results and discussion

3.1. Statistical analysis

Table 3 summarizes the results of each dependent variable with their coefficients of determination (R^2) (Sin, Yusof, Sheikh Abdul Hamid, & Abdul Rahman, 2006). The statistical analysis indicated that the proposed model was adequate, possessing no significant lack of fit and with very satisfactory values of the R^2 for all the responses. The R^2 values for anthocyanins yield, L^*, C^* and H° were 0.9527, 0.9631, 0.9514 and 0.9657, respectively. The closer the value of R^2 to unity, the better the empirical models fits the actual data. On the other hand, the smaller the value of R^2 the less relevance the dependent variables in the model have in explaining the behavior of variations (Ravikumar, Ramalingam, Krishnan, & Balu, 2006). The probability (P) values of all regression models were less than 0.000, with no lack-of fit.

3.2. Effects of extraction temperature, time and solid–liquid ratio

The effects of extraction temperature, time and solid–liquid ratio on anthocyanins yields, L^*, C^* and H are reported (Table 3) by the coefficient of the second-order polynomials. Response surface were used to illustrate the effect of extraction temperature, extraction time and solid–liquid ratio on the responses. Response surfaces for anthocyanins yield is shown in Figs. 1–3.

Fig. 1 shows the contour map for the effect of the independent variables on the anthocyanins yield. As shown in Table 3, anthocyanins yield mainly depend on the extraction temperature as its linear ($P<0.01$) and quadratic effects ($P<0.01$) were significant giving an overall curvilinear effect. Fig. 1 shows that the anthocyanins yield can be increased with the increase of extraction temperature. The linear effect ($P<0.01$) was positive, whereas the quadratic effect ($P<0.01$) was negative, which resulted in a curvilinear increase in anthocyanins yield for all the extraction times. It can be seen that the extraction temperature is the principal effect on the

Table 3
Regression coefficients, R^2, and P or probability values for four dependent variables for anthocyanins extraction of purple sweet potato

Regression coefficient	Anthocyanins yield	L^*	C^*	h_{ab}
β_0 (intercept)	146.128	20.638	7.170	20.212
b_1	17.948**	−0.109**	−0.230*	2.418*
b_2	0.638	0.008	−0.016	0.042
b_3	1.018*	0.001**	0.0338	−0.275
b_{11}	−9.925**	0.035*	0.098*	−0.821*
b_{12}	−3.818	0.005	−0.008	0.208
b_{22}	−2.290	−0.008	0.015	−0.077
b_{13}	5.093	−0.003	−0.090*	0.322
b_{23}	−5.853	−2.671E−17	0.030	−0.149
b_{33}	−9.165**	0.0648**	0.085*	−0.796*
R^2	0.953	0.963	0.951	0.966
P or probability	0.0008***	0.0003***	0.0008***	0.0003***

*Significant at 0.05 level; **significant at 0.01 level; ***significant at 0.001 level.

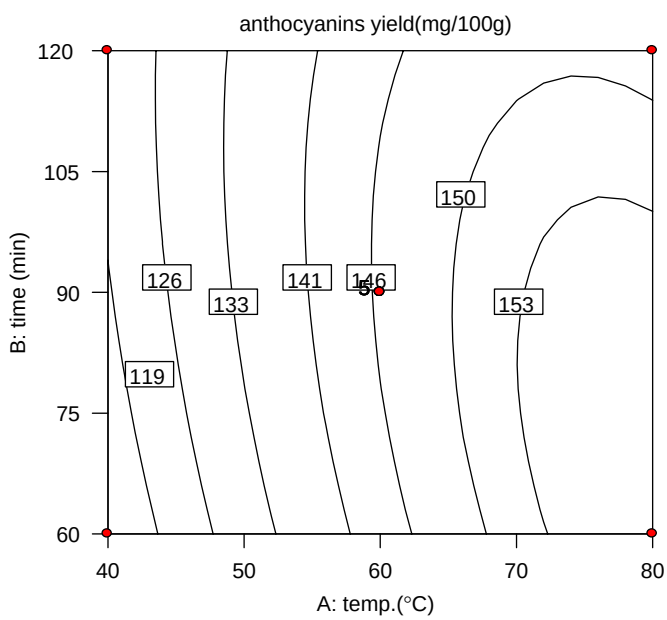

Fig. 1. Contour plots for the effects of extraction temperature and time at a constant solid–liquid ratio of 1:25 on the yield of PSPAs.

pigments yield. In this case, the extraction time has only a slight influence, especially when the extraction temperature is low.

Fig. 2 shows the contour map of the effect of extraction temperature and solid–liquid ratio on anthocyanins yield. The anthocyanins yield depends on solid–liquid ratio where its linear ($P<0.01$) and quadratic ($P<0.01$) effects were significantly affected by pigments concentration. Hence, the overall effect was curvilinear in nature. At the lowest level of temperature, anthocyanins yield of purple sweet potato was found to increase rapidly at the beginning but with a slower rate towards the end. It can be seen in Table 3 that the positive linear and negative quadratic effects of solid–liquid ratio and temperature explained the observed nature of the curve shown in Fig. 2.

Fig. 3 shows the contour map of the effect of extraction time and solid–liquid ratio on anthocyanins yield. The anthocyanins yield depends on solid–liquid ratio where it's linear ($P<0.05$) and quadratic ($P<0.01$) effects were significantly affected by pigments concentration. At the lowest level of time, the anthocyanins yield of purple sweet potato was found to increase rapidly at the beginning but with a slower rate towards the end. It can be seen in Table 3 that the positive linear and negative quadratic effects of time and solid–liquid ratio explained the observed nature of the curve shown in Fig. 1.

3.3. Optimization

The contour plots show the optimum conditions of the extraction process to anthocyanins yield, respectively. It was noted that the optimum conditions for anthocyanins yield were slightly different. There are a number of combinations of variables that could give maximum levels of anthocyanins yield. Since the optimum response for each dependent variable did not fall exactly in the same region, the superimposition of all the contour plots obtained was done. Fig. 1 shows the superimposed contour plot for optimization of anthocyanins yield. The zone of optimization, as shown in the superimposed contour plot, depicts temperature to be over 72.5 °C and solid–liquid ratio between 1:22 and 1:30; keeping the time constant at the central point (Fig. 2), as shown in the superimposed contour plot, depicts temperature to be over 72.5 °C and extraction time between 60 and 80 min. Here, keeping the temperature constant as determined from Fig. 3, the best combination of response function can been determined.

During the anthocyanin extraction, the extraction temperature and solid–liquid ratio are important. Therefore, the best combinations of process variables for response functions are found. The process variables for best combination of response function are extraction temperature 80 °C, time 60 min, and solid–liquid ratio 1:32. The response functions were calculated from the final

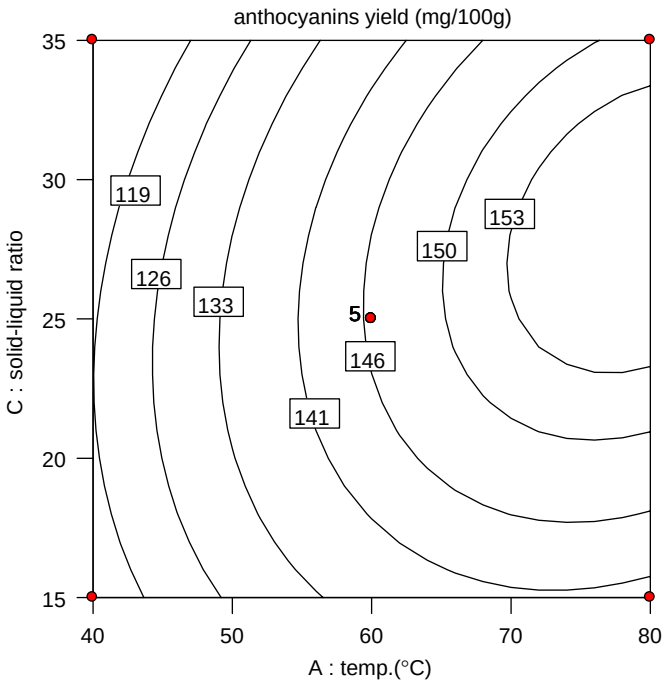

Fig. 2. Contour plots for the effects of extraction temperature and solid–liquid ratio at a constant time of 90 min on the yield of PSPAs.

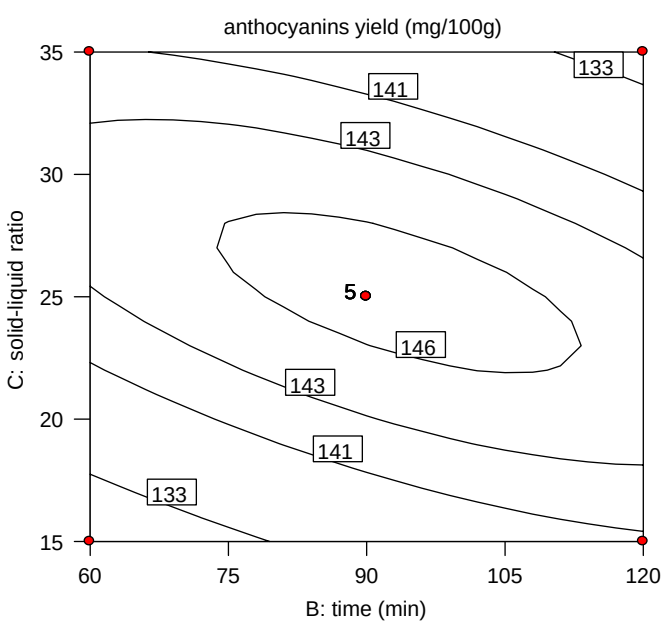

Fig. 3. Contour plots for the effects of time and solid–liquid ratio at a constant extraction temperature of 60 °C on the yield of PSPAs.

polynomial, and the response were anthocyanins yield (158 mg/100g), L^* 20.59, C^* 7.05 and H 21.27.

4. Conclusions

The different conditions (extraction temperature, time and solid–liquid ratio) for anthocyanins extraction revealed that extraction temperature and solid–liquid ratio markedly affect the anthocyanins yield, while extraction time is insignificant. These can be related to the anthocyanins extraction conditions by second-order polynomials. Using the contour plots, the optimum set of the operating variables are obtained graphically in order to obtain the desired levels of these properties of the purple sweet potato anthocyanins, which is suitable for the subsequent analysis based clarification processes. The best combination of response function are extraction temperature 80 °C, time 60 min, and solid–liquid ratio 1:32.

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