Research Article

Optimization of Extraction of Natural Pigment from Purple Sweet Potato by Response Surface Methodology and Its Stability

Jinwei Li,^{1,2} Lianfu Zhang,² and Yuanfa Liu²

¹ State Key Laboratory of Dairy Biotechnology, Technology Center, Bright Dairy & Food Co. Ltd., Shanghai 200436, China

² State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China

Correspondence should be addressed to Yuanfa Liu; ljw6688@hotmail.com

Received 8 November 2012; Revised 19 March 2013; Accepted 2 April 2013

Academic Editor: Mehmet Emin Duru

Copyright © 2013 Jinwei Li et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Purple sweet potato colour (PSPC) was a kind of natural pigment that attracted the general concern in recent years. In this paper, the response surface methodology was employed to optimize the extraction conditions of PSPC. The results showed that the extraction yield of purple colour was 11.6355 mg/g at the optimum extraction conditions of extraction temperature 60°C, extraction time 1 h, the ratio of solid to liquid ratio of 1:30, and acidified ethanol solution concentration 80%. Stability experiment showed that Fe³⁺ and Al³⁺ could increase the stability of PSPC, but Cu²⁺, Zn²⁺, and Pb²⁺ would decrease the stability of PSPC. Ascorbic acidified could significantly increase the stability of PSPC, and Na₂SO₃ would reduce the PSPC's stability.

1. Introduction

The colour of foods could affect the customer's decision on purchasing behavior by causing customer's direct attention on the sensory. However, the synthetic pigment had a negative impact on human's healthy. More and more attentions are paid to the natural pigment, which could be served as a functional component.

Anthocyanin is one of the most important natural pigments. Purple sweet potato is a plant which is rich in anthocyanin. The anthocyanin from purple sweet potato could be not only used as the natural pigment but also used as the functional compound due to its obvious antioxidant, antimutagen, and antineoplastic activities [1–8]. In this paper, the objective was to optimize the extraction condition by response surface methodology and investigate its stability.

2. Materials and Methods

2.1. Materials. Fresh purple sweet potato was purchased from Tianheyuan agricultural incorporation in Suzhou.

2.2. Method

2.2.1. Pretreatment of Purple Sweet Potatoes. Purple sweet potatoes were washed and chopped into pieces, then they were dried in the oven at 50°C for 12 hours. Finally, they were smashed and kept in brown desiccator.

2.2.2. The Maximum Absorbance Wavelength of Purple Sweet Potatoes Pigment. A UV-Vis spectrophotometer (UV-2102PCS) was used to determine the maximum absorbance of purple sweet potato pigment. Purple sweet potato powder was weighted and extracted in acidified ethanol aqueous solution at 60°C for one hour. Then, it was centrifuged at 5000 r/min for 15 min. The pH of supernatant was adjusted to 2, and then the supernatant was scanned from 200 nm to 700 nm.

2.2.3. Optimization of Extraction of Purple Sweet Potato Pigment. A RSM was used to optimize the extraction conditions for purple sweet potato pigment. A multivariate study based on Box-Behnken design was chosen to evaluate effects of extraction parameters. The four independent variables were

TABLE 1: The coding schedule of response surface methodology.

Independent variable/unit	Symbol	Level		
		−1	0	1
Extraction temperature/°C	X_1	55	60	65
Time/min	X_2	50	60	70
Solid-liquid ratio/1 : X	X_3	25	30	35
Acidified ethanol aqueous solution concentration/%	X_4	75	80	85

extraction temperature (X_1), extraction time (X_2), solid-liquid ratio (X_3), and acidified ethanol aqueous solution concentration (X_4), and three levels of each independents were chosen for study. The coded values of the three independent variables were summarized in Table 1. The response value was the anthocyanin yield that was calculated from the absorption measured at 525 nm by a UV-Vis diode array spectrophotometer.

2.2.4. Experiment of Verification. 1.0000 g purple sweet potato powder was weighted and PSPC was extracted according to the conditions which were optimized by RSM, and the results were compared with the modeling value.

2.2.5. Stability of PSPC.

(1) *Effect of Metal Ion on PSPC Stability.* CuSO_4 , FeCl_3 , AlCl_3 , PbCl_2 , and ZnSO_4 were weighted and added in PSPC solutions with the Fe^{3+} , Cu^{2+} , Al^{3+} , Pb^{2+} , and Zn^{2+} concentration of 100 mg/L. The pH value was controlled at 2 and was kept away from light at room temperature. Measure its absorption at 0 h, 24 h, and 48 h.

(2) *Effect of Food Additives on PSPC Stability.* Na_2SO_3 , ascorbic acid were weighted and added in PSPC solutions with the different concentration. The solutions were kept away from light at room temperature for 2 h and then measured its absorption at 525 nm.

3. Results and Discussion

3.1. Measurement of the Maximum Absorption Wavelength. As shown in Figure 1, there are three peaks at 280~290 nm, 320~340 nm, and 520~530 nm. The peak at 280~290 nm is the characteristic absorption peak of polyphenol, the peak at 320~340 nm is the characteristic absorption peak of organic acid, and the peak located at 520~530 nm is the characteristic absorption peak of anthocyanins. The peak at 520~530 nm is chosen as testing wavelength.

3.2. Effect of Extractant on the PSPC Yield. As shown in Figure 2, compared with the other extractant, acidified methanol has the highest PSPC yield, followed by acidified ethanol aqueous solution, 5% HCl. Considering its use on food, safety, and cost, the acidified ethanol aqueous solution is chosen as the appropriate extractant.

FIGURE 1: Full wavelength scan of PSPC.

FIGURE 2: Effect of the kind of extractant on extraction ratio of PSPC.

3.3. Results of RSM Experiment. The results of RSM analysis of the variation of PSPC yield with extraction temperature (X_1), extraction time (X_2), solid-liquid ratio (X_3), and acidified ethanol aqueous solution concentration (X_4) are shown in Table 2. SAS multivariate regression program was used to analyze the data, and quadratic regression model of extraction temperature, time, solid-liquid ratio, and acidified ethanol aqueous solution concentration is as follows:

$$\begin{aligned}
 Y = & 24.93767 - 0.113417X_1 - 0.106833X_2 - 0.071X_3 \\
 & - 0.50075X_4 - 1.490042X_1^2 - 0.0465X_1X_2 \\
 & - 0.2835X_1X_3 + 0.64975X_1X_4 - 1.090667X_2^2 \\
 & - 0.19725X_2X_3 - 0.09475X_2X_4 - 1.173917X_3^2 \\
 & + 0.44675X_3X_4 - 0.902542X_4^2.
 \end{aligned} \quad (1)$$

3.4. Analysis of Variance (ANOVA) of Quadratic Regression Model. ANOVA of quadratic regression model (Table 3) demonstrated that the variables were adequately fitted to the regression equation (1), which were statistically acceptable at $P < 0.05$ level and adequate with satisfactory determination coefficients (R^2 of 0.9768 and R_{Adj}^2 of 0.9468). The PSPC yield was significantly affected by linear term X_4 , quadratic terms X_1^2 , X_2^2 , X_3^2 , and X_4^2 , and interactions terms of X_1 and X_3 , X_1 and X_4 , X_3 , and X_4 . According to the F value, the significance

FIGURE 3: Contour plot and response surface diagram of effect between extraction temperature and time on PSPC.

FIGURE 4: Contour plot and response surface diagram on effect between extraction temperature and concentration of acidified ethanol on PSPC.

orders of the parameters for the PSPC yield in the prediction model were $X_4 > X_1 > X_2 > X_3$.

3.5. Optimum Extraction Condition for PSPC. Three-dimensional diagram and contour plot made by full model of (1) were used to predict the relationships between the extraction temperature, extraction time, and PSPC yield (Figure 3). When the extraction temperature and extraction

time increased, the PSPC yield increases firstly and then decreases. At the medium value of extraction condition of temperature and time, the PSPC has the higher extraction yield (Figure 3). The contour plot showed that the optimum extraction temperature is between 57.1 and 62.55°C and the optimum extraction time is between 56.6 and 62.95 min.

Three-dimensional diagram and contour plot were used to predict the relationships between the extraction

TABLE 2: Results of Box-Behnken of response surface methodology.

Test code	X_1	X_2	X_3	X_4	Y (mg/g)
1	-1	-1	0	0	11.3175
2	-1	1	0	0	11.1425
3	1	-1	0	0	11.3365
4	1	1	0	0	11.0685
5	0	0	-1	-1	11.9935
6	0	0	-1	1	10.9165
7	0	0	1	-1	11.5735
8	0	0	1	1	11.3900
9	-1	0	0	-1	11.8120
10	-1	0	0	1	10.7630
11	1	0	0	-1	11.1080
12	1	0	0	1	11.3585
13	0	-1	-1	0	11.3780
14	0	-1	1	0	11.3700
15	0	1	-1	0	11.4760
16	0	1	1	0	11.0735
17	-1	0	-1	0	11.3995
18	-1	0	1	0	11.0815
19	1	0	-1	0	10.8575
20	1	0	1	0	11.1065
21	0	-1	0	-1	11.6355
22	0	-1	0	1	11.2575
23	0	1	0	-1	11.7305
24	0	1	0	1	11.1630
25	0	0	0	0	12.4465
26	0	0	0	0	12.4800
27	0	0	0	0	12.4800

temperature and concentration of acidified ethanol solution and PSPC yield (Figure 4). When the extraction temperature and the concentration of acidified ethanol aqueous solution increased, the PSPC yield increased before the medium level and then decreases. The contour plot showed that the optimum extraction temperature is between 55 and 62°C and the optimum concentration of acidified ethanol aqueous solution is between 76.7% and 82.25%.

According to the results of the analysis of regression model, the optimum conditions for the extraction of PSPC are at the extraction temperature of 60°C, time of 1 h, solid-liquid ratio of 1:30, acidified ethanol aqueous solution concentration of 80%, and the extraction yield could reach 11.6355 mg/g.

3.6. Verification Experiment. The extraction experiments were carried out under the optimum conditions of extraction temperature 60°C, time 1 h, solid-liquid ratio 1:30, and acidified ethanol aqueous solution concentration of 80%. The real PSPC yield was 11.5276 mg/g, which was adjacent to the modeling value of 11.6355 mg/g with standard error of 0.93%. This result indicates that this regression model could be used to indicate the relationship between extraction condition and PSPC yield and to predict the extraction yield of PSPC.

TABLE 3: Analysis of variance of regression model.

Source	DF	SS	MS	F	Pr > F
X_1	1	0.15436	0.15436	3.509213	0.085582
X_2	1	0.13696	0.13696	3.113648	0.103052
X_3	1	0.060492	0.060492	1.375222	0.263671
X_4	1	3.009007	3.009007	68.40659	0.0001
$X_1 \times X_1$	1	11.8412	11.8412	269.1971	0.0001
$X_1 \times X_2$	1	0.008649	0.008649	0.196626	0.665347
$X_1 \times X_3$	1	0.321489	0.321489	7.308713	0.019186
$X_1 \times X_4$	1	1.6887	1.6887	38.39082	0.0001
$X_2 \times X_2$	1	6.344287	6.344287	144.2307	0.0001
$X_2 \times X_3$	1	0.15563	0.15563	3.538089	0.084454
$X_2 \times X_4$	1	0.03591	0.03591	0.816382	0.38402
$X_3 \times X_3$	1	7.349762	7.349762	167.0891	0.0001
$X_3 \times X_4$	1	0.798342	0.798342	18.14947	0.001107
$X_4 \times X_4$	1	4.344434	4.344434	98.76612	0.0001
Model	14	22.27012	1.590723	36.16341	0.0001
Error	12	0.527845	0.043987		
Total	26	22.79797			

$$R^2 = 97.68\% \quad R_{Adj}^2 = 94.68\%.$$

TABLE 4: Effects of metallic ion on PSPC stability.

Time (h)	Absorbance value (A)					
	Fe^{3+}	Zn^{2+}	Cu^{2+}	Pb^{2+}	Al^{3+}	Blank
0	0.752	0.752	0.752	0.752	0.752	0.752
24	0.750	0.721	0.745	0.744	0.749	0.746
48	0.748	0.710	0.742	0.743	0.748	0.745

3.7. Effect of Metal Ion on PSPC Stability. Effects of metal ion on PSPC stability is shown in Table 4. Compared with blank solution, the solution with Fe^{3+} or Al^{3+} has the higher absorbance value at 525 nm for 24 h and 48 h, which meant that Fe^{3+} or Al^{3+} contributed to the PSPC stability. However, the solution with Cu^{2+} , Zn^{2+} , or Pb^{2+} has the lower absorbance value at 525 nm for 24 h and 48 h, which meant that Cu^{2+} , Zn^{2+} or Pb^{2+} was against the PSPC stability.

3.8. Effect of Ascorbic Acid and Na_2SO_3 on PSPC Stability. Effects of ascorbic acid and Na_2SO_3 on PSPC stability is shown in Table 5. The absorbance value at 525 nm increased with the increase in the ascorbic acid concentration, which means that ascorbic acid contributed to the PSPC stability and could be used as copigment. The absorption value at 525 nm decreases with the increase in concentration of Na_2SO_3 , which indicates that Na_2SO_3 has a bad effect on PSPC stability.

4. Conclusion

In the basis of RSM experiment, the optimum extraction conditions of PSPC are the extraction temperature of 60°C, extraction time of 1 h, solid-liquid ratio of 1:30, and acidified ethanol aqueous solution concentration of 80%, and the

TABLE 5: Effects of ascorbic acid and Na_2SO_3 on PSPC stability.

		Ascorbic acid concentration (mg/L)			
A	0	0.2	0.4	0.6	0.8
	0.721	0.732	0.758	0.792	0.799
		Concentration of Na_2SO_3 (mg/L)			
A	0	0.01	0.02	0.04	0.06
	0.698	0.657	0.642	0.638	0.632

extraction yield of PSPC is 11.6355 mg/g. Fe^{3+} and Al^{3+} can contribute the stability of PSPC, but Cu^{2+} , Zn^{2+} , and Pb^{2+} would decrease the stability of PSPC. Ascorbic acid can significantly increase the stability of PSPC which can be used as copigment, and Na_2SO_3 would have bad effect on the stability of PSPC.

Acknowledgments

This work was supported by the Research Fund of National 12th Five-Year Plan of China (2011AA100806-3), the National Natural Science Foundation of China (31171703 and 31101361), Industry-Academia Cooperation Innovation Fund Projects of Jiangsu Province (BY20120460), Fundamental Research Funds for the Central Universities (JUSRP211A30), and the Open Project Program of State Key Laboratory of Dairy Biotechnology, Bright Dairy & Food Co. Ltd. (SKLDB2011-002).

References

- [1] Z. Yang, Y. Wang, and L. Gao, "Research progress on purple sweet potato colore," *Journal of Qingdao University (E&T)*, vol. 19, no. 2, pp. 32–36, 2004.
- [2] Z. Fang and Y. Ni, "Research progress on physiological functions of anthocyanins," *Guangzhou Food Science and Technology*, vol. 17, no. 3, pp. 60–62, 2001.
- [3] G. Lu, X. Lan, X. Lou et al., "Study on physical and chemical properties of red pigments from purple sweet potato (PSP)," *Journal of Zhejiang Agricultural University*, vol. 22, no. 3, pp. 308–311, 1996.
- [4] Y. Liu, L. Zhu, Z. Han et al., "Anti-oxidative effect of Ipomoea Batatas Poir Cv anthocyanins on mice irradiated by 60Co gamma ray," *Acta Acadimae Qingdao Umvesitatis*, vol. 41, no. 1, pp. 46–51, 2005.
- [5] X. Meng, X. Cu, F. Wang et al., "In vivo antioxidant activity of sweet -potato anthocyanins in aged mice," *Medical Journal of Qilu*, vol. 20, no. 5, pp. 394–399, 2005.
- [6] W. Han, *Extraction, Composition Analasis, Stability and Antioxidant Activity of Anthocyanins from Purple Sweet Potato*, Nanjing Agriculture University, 2008.
- [7] Z. Wang, *Study on the Technology Conditions of Extraction and Purification and Property of Purple Sweetpotato Color*, Southwest University, 2010.
- [8] J. Li, *Study of the Extraction, Purification by Membrane Technology and Stability of Anthocyanin Pigment from Purple Sweet Potato*, Nanchang University, 2007.