

Research Article

Extraction and Purification of Pigment from Purple Sweet Potato Wine Vinasse

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Abstract: Purple sweet potato pigment is a natural food pigment with bright color and multiplies biological functions such as antioxidant activity etc. There is a large amount of unused pigment in the vinasse of purple sweet potato wine. Therefore, in this study, the extraction processes of purple sweet potato pigment from purple sweet potato wine vinasse, as well as its purification conditions were investigated. As the results, 0.9% citric acid-95% ethanol (2/3, v/v) was a suitable extraction solvent to obtain the higher yield of purple sweet potato pigment from vinasse. AB-8 column chromatography showed that the loading ratio of 1/10 (w/v) of resin and pigment with the solvent of 40% ethanol at the flow rate of 2 mL/min were the optimal conditions for the purification of purple sweet potato pigment.

Keywords: Anthocyanin, macroporous resin, vinasse

INTRODUCTION

The purple sweet potato contains rich anthocyanins pigment. The purple sweet potato pigment is in bright color, non-toxic and soluble in water which has the certain nutritional and pharmacological effects. It also plays an important role in antioxidant (Furuta *et al.*, 1998), anti-inflammatory (Suda *et al.*, 1997), anti-mutation (Yoshimoto *et al.*, 2001), antitumor (Lim *et al.*, 2013) and prevention and treatment of cardiovascular diseases (Park *et al.*, 2010). Such, it has a great potential applications in food, cosmetic and pharmaceutical and so on. Purple sweet potato pigment also has good heat and light resistance (Li *et al.*, 2011; Xu *et al.*, 2002). Because of the characteristic of high and stable yield, developing the purple sweet potato pigment will have the abundant resource advantage. With regard to the processing and utilization of purple sweet potato, purple sweet potato wine is one of the its important deep processing product. Previously, we have successfully developed the technology and processing products of purple sweet potato wine (Fu and Li, 2012). However, we found that there has a lot of purple sweet potato pigment in the lees which is the processing by-products of Purple sweet potato wine. Therefore, to developing the extraction method of pigment from purple sweet potato lees well has positive significance in improving the deep processing and utilization efficiency of purple sweet potato.

Purple sweet potato pigment was a kind of water-soluble natural pigment which is more stable in low pH. At the present, acidic solvent like hydrochloric acid aqueous solution or organic solvent are widely used to

extract the Purple sweet potato pigment (Nishama and Muroi, 1996; Shi *et al.*, 1992). However, extracting by using the hydrochloric acid will increase the excessive contamination risk of chloride ion. Also, the extraction by using organic solvents has the defective like solvent residue and the high requirements for production operation, equipment and manufacture places. Therefore, in this experiment, the feasibility and technical parameters of extracting the Purple sweet potato pigment with citric acid are investigated.

MATERIALS AND METHODS

Experimental materials: Purple sweet potato wine vinasse was obtained after brewing purple sweet potato wine and was dried and crushed to use. Macroporous adsorption resin (AB-8, D3520) were purchased from the chemical factory of Nankai University (China).

Extraction of purple sweet potato pigment: A certain proportion of citric acid and ethanol mixture was used to extract the purple sweet potato pigment from purple sweet potato wine vinasse powder by oscillation extraction for 90 min at 60°C. The supernatant was recovered by centrifugation (5000 rpm, 5 min). The extraction was repeated for 2 times and the supernatant was merged as crude extract of purple sweet potato pigment.

Purification of purple sweet potato pigment: Completely purified resins of AB-8 and D3520 are added into the purple sweet potato pigment extract as the ratio of 1:10 (resin/extract, m/v), to shake and

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absorb pigment for 1h at 30°C. In previous study (Suda *et al.*, 1997), we have proved that purple sweet potato pigment appeared highest absorbance peak at 530 nm. Thus, the absorbance of supernatant after adsorbed by resin at 530 nm was measured, in order to investigate the absorbing efficiency of resin to pigment and then to determine the optimum resin for purification of purple sweet potato pigment.

The resin confirmed above are added with different proportion (1:5~1:30, *m/v*) into the pigment extract of purple sweet potato, shacked and absorbed for 1h at 30°C, then measured the absorbance of supernatant at 530nm to determine the optimum ratio of resin and purple sweet potato pigment extract. After that determination, same volume but different concentration ethanol solutions were used to desorb the pigment from resin which saturatedly adsorbed the pigment. The optimum elution concentration of ethanol solution was determined by judging the content of pigment in eluted solution which was represented with absorbance of elution solution at 530 nm.

In addition, purple sweet potato pigment was purified by AB-8 column chromatography (2.5×10 cm), with the optimum loading ratio ascertained above. Ethanol solution was used as eluent. The flow rate was 1 mL/min and 2mL/min, respectively. Each fraction was 5 mL. Absorbance of elution was measured at 530 nm.

Determination of anthocyanin content: Purple sweet potato anthocyanins were determined using a modified version of Zhu *et al.* (2006). Pigment samples (0.1 ml) were mixed with 4 mL of acidified ethanol (95% ethanol: 1mol/L HCl = 85:15). After 10 min, the absorbance of the mixture was measured at maximum absorption wavelength ($A_{\lambda_{max}}$), 700 nm and then at 430 nm. The anthocyanins content was calculated as follows:

$$\text{Content of Anthocyanin (mg/100g)} = \frac{(A_{\lambda_{max}} - A_{700}) + (A_{430} - A_{700})}{98.2} \times \text{Dilution Factor} \times 100$$

In which, 98.2 was the extinction coefficient of anthocyanin (Karl, 1978).

RESULTS AND DISCUSSION

Extraction of pigment from the vinasse of purple sweet potato wine: The extraction efficiency of purple sweet potato pigment from vinasse powder by different concentrations of extraction solvents was demonstrated in Table 1. Under the same concentration of citric acid, with the increase of the proportion of ethanol, the content of purple sweet potato pigment in the extraction solution presented a gradually decrease. When the ratio of citric acid to ethanol was 4:6, the content of purple sweet potato pigment in the extraction solution was relatively higher. Under this ratio, along with the increase of citric acid concentration in the extraction

Table 1: Effects of mixture ratios of citric acid and ethanol on the extraction yield (mg/100 g) of pigment from vinasse powder of purple sweet potato

Citric acid (%)	Citric acid/95% ethanol (v/v)			
	4:6	3:7	2:8	1:9
0.1	0.620	0.453	0.378	0.267
0.3	0.775	0.524	0.398	0.312
0.6	1.006	0.863	0.548	0.358
0.9	1.119	0.926	0.734	0.404

Table 2: The adsorption efficiency of purple sweet potato pigment with different resins

	A_{530} (1 st adsorption)	A_{530} (2 nd adsorption)
AB-8	1.004	0.147
D3520	1.907	0.439

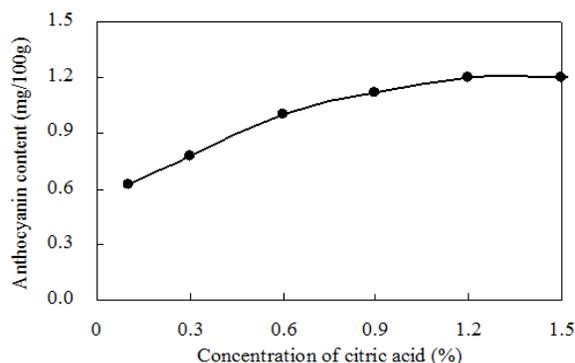


Fig. 1: Effect of concentrations of citric acid on the extracting efficiency of pigment from purple sweet potato vinasse

solution, the extraction efficiency of pigment represented a rising trend. While when the concentration of citric acid reached more than 0.9%, the content of total anthocyanins in extract solution no longer increased (Fig. 1). From the above, the mixed solvent, with the ratio of 0.9% citric acid to 95% ethanol was 4:6, was a better solvent for the extraction of purple sweet potato pigment.

Screening of macroporous resin for the purification of purple sweet potato pigment: The adsorption results (Table 2) of two different type resins of AB-8 and D3520 for purple sweet potato pigment indicates that there were significant differences of residual pigment content in the supernatant after absorption, between two kinds of resins. The absorbance of AB-8 supernatant at 530 nm, which reflect the amount of purple sweet potato pigment, was significantly lower than that of D3520, indicated that the adsorption ability of AB-8 resin for purple sweet potato pigment was stronger than that of D3520. Further, the first adsorbed pigment solutions were treated once more by same proportion of the same resins and the adsorption tendency was similar to the first absorption experimental results (Table 2). Meanwhile, from the results in Table 2 indicated that purple sweet potato pigment in the extract solvent could be completely adsorbed by twice absorption or twice amount of the resin of AB-8.

Table 3: Effect of material-liquid ratio on the adsorption of purple sweet potato pigment

Resin/extract (w/v)	1:5	1:10	1:15	1:20	1:25	1:30
A ₅₃₀	0.056	0.253	1.113	1.832	1.947	1.955

* Original extracting solution of A₅₃₀ was 6.84

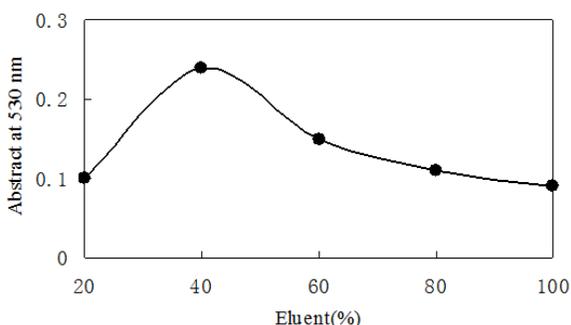


Fig. 2: Effect of ethanol concentration of on the desorption of purple sweet potato pigment

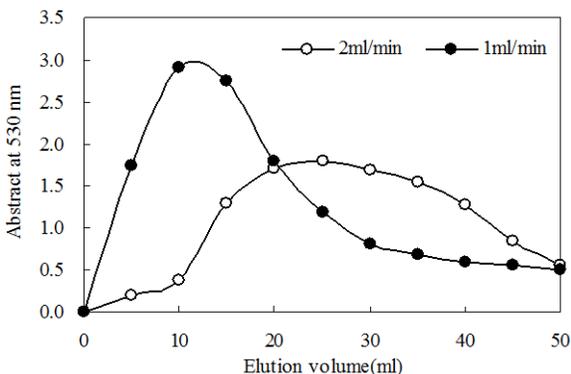


Fig. 3: Elution profile of purple sweet potato pigment on the column of AB-8

Optimization of purification conditions of purple sweet potato pigment: The effects of proportion of AB-8 resin and pigment solution on the adsorption efficiency of purple sweet potato pigment was demonstrated in Table 3. Along with the increase of adding dosage of pigment, which also means the increase of the proportion of pigment/resin, the absorbance of the supernatant increased, that is the adsorption efficiency of AB-8 resin on pigment adsorption decreased. Table 3 indicated that when the proportions of AB-8 resin/pigment extract were in 1:5~1:10, adsorption efficiency of AB-8 for purple sweet potato pigment was as high as 96.3~99.2%. While when a material-liquid ratio was higher than 1:15 (AB-8 resin/purple sweet potato pigment solvent), the adsorption efficiency of pigment on resin was dramatically decreased. Considering the cost and the dosage of resin, a material-liquid ratio of 1:10 (AB-8 resin/purple sweet potato pigment solvent) was considered to be the optimum proportion in the static adsorption of AB-8 resin adsorbing purple sweet potato pigment. Figure 2 showed the desorption profile of pigment from AB-8 resin which saturatedly adsorbed

purple sweet potato pigment eluted by 20~95% ethanol. When 40% ethanol was used, the maximum absorbance of eluent was observed, which means that the best elution efficiency was obtained.

AB-8 column chromatography of purple sweet potato pigment: Elution profile of AB-8 column chromatography under the above optimized sample loading ratio (1/10, AB-8 resin/purple sweet potato pigment solvent), with 40% ethanol solution as the elution solvent, was illustrated in Fig. 3. A preferable elution and enrichment efficiency of purple sweet potato pigment was obtained under the flow rate of 2 mL/min.

CONCLUSION

In this research, the purple sweet potato vinasse was used as raw material to extract the natural pigment. The extraction and purification technologies of pigment were analyzed. As the results, citric acid-ethanol solution was a preferable solvent for the extraction of purple sweet potato pigment. And the extraction efficiency was higher at the conditions of 0.9% citric acid mixed with 95% ethanol at the mixture ratio of 2/3(v/v). The refining result of purple sweet potato pigment showed that the purple sweet potato pigment was well purified by AB-8 macroporous adsorption resin with 40% ethanol as the solvent. The result provided the technical support and evidence in the practice and succinct production of purple sweet potato pigment and also had a great realistic guiding significance in the deep processing and utilization of purple sweet potatoes.

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