Journal of Scientific & Industrial Research
Vol. 73, January 2014, pp. 57-61

Extraction of anthocyanins from plum pomace using XAD-16 and determination of their thermal stability

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Received 10 October 2012; revised 25 September 2013; accepted 07 November 2013

Crude anthocyanins were extracted from plum pomace by passing the pomace-water mixture (1:1) through XAD-16 in a column, followed by desorption with ethanol. Optimum concentrations of XAD-16 for adsorption, adsorption time of anthocyanins and desorbent (ethanol) concentration were standardized. The maximum adsorption (61.70 per cent) of anthocyanin took place with 40 per cent XAD-16 which was almost comparable with 35 per cent XAD-16 (61.50 per cent). The lowest 'a' value (12.52) of the extract was observed at 8 hours showing the highest desorption of anthocyanins. The maximum desorption (94.96 per cent) took place with 60 per cent ethanol while it was minimum (36.06 per cent) with 20 per cent ethanol. On the basis of desorbed anthocyanins content and L, a, b colour values, adsorption with 35% XAD-16 for 8 hours followed by desorption with 60% ethanol was found to be the best method for the extraction of plum anthocyanins. Anthocyanin extract was heated at different temperature viz. 80°, 100° and 120°C for 10 and 20 min to determine its thermal stability. A marked degradation of anthocyanins took place at higher temperatures than lower ones. Stability of anthocyanins was decreased with the increase in temperature and heating time. It is concluded that the adsorption of anthocyanins by using XAD-16 was a suitable method for its extraction. The anthocyanins extract was found to be stable to heating as reflected by 'L', 'a' and 'b' value. Therefore, plum pomace can be utilized for the production of biocolour for the food industry.

Keywords: Adsorption, Anthocyanins, Colour value, Plum, Stability

Introduction

Anthocyanins comprise a diverse group of intensely coloured pigments responsible for the appealing and often spectacular vegetables, flowers, leaves, roots and other plant storage organs. They are water soluble, that facilitates their incorporation into aqueous food systems and have been consumed for centuries without adverse effects. Besides the color attributes, interest in anthocyanins has intensified because of their possible health benefits. These qualities make anthocyanins attractive alternatives to synthetic dyes, though they have some limitations that have restricted the use of natural colorants in food systems. However, there are few technologies with potential suitability at industry scale that can be used for extraction of anthocyanins (Palamidis and Markakis[1], Duangmal *et al*[2]). Many adsorbents have successfully been used for the extraction of anthocyanins as X-5 in mulberry (Liu *et al*[3]), Toyopearl TSK HW-40S and NKA-9 in blood orange (Cao *et al*[4]), AB-8 in sweet potato (Wang *et al*[5]) and XAD-1600 in purple fleshed potato (Liu *et al*[6]). Plum (*Prunus salicinia*) has very attractive red colour (Joshi *et al*[7]; Vyas *et al*[8]). During processing of the plum into juice or other beverages; a large quantity of waste is generated. It has stones and parts of skin which is a waste. Even this waste called pomce has large quantity of anthocyanins that can be extracted. However, there is no report on the extraction of anthocyanins from fruits like plum using adsorbent. To make the application of biocolour successfully at the commercial scale it is necessary that it should be cheaper and stable as it will not only enhance its appeal but also the quality of food. The present study was therefore conducted to standardize the adsorption method for the manufacture of crude anthocyanins from plum processing wastes and to study the effect of different time and temperature conditions on its suitability. The results obtained are discussed in this paper.

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Material and methods

Raw materials

Plum (cv. Santa Rosa) fruits were procured from the local market. Fruits were washed and made into pulp and pomace (skin and stones) by addition of a known volume of water followed by boiling for 15 minutes. All the chemicals and reagents used were from C D H and Loba Chem. Glass wares used were of Borosil make.

Extraction of anthocyanins from plum pomace

Plum pomace was mixed with water in the ratio of 1:1 and boiled for 30 minutes then, filtered through muslin cloth and concentrated. Extraction was done by using column chromatography. Amberlite XAD-16 at ambient temperature was used as an adsorbent (Kammerer *et al,* 2005) which was filled in the coloum having 100 cm length and 40 mm diameter and plum pomace extract was passed through the adsorbent, thereafter, residual plum pomace extract was withdrawn (after keeping for 6 hours) and anthocyanin content in the samples was estimated. Anthocyanins were eluted from XAD-16 using 20-60% ethanol as detailed in the later section of this paper.

Standardization of adsorbent concentration

For the optimization of concentration of adsorbent, different concentrations of Amberlite XAD-16 as adsorbent were used for adsorption of anthocyanins. Five different concentrations of XAD were taken *viz.* 15 per cent, 20 per cent 25 per cent, 30 per cent, 35 per cent and 40 per cent and were kept at room temperature for 6 hours for adsorption process to take place.

Standardization of adsorption time

Standardization of time for anthocyanin adsorption was carried out by passing plum pomace extract in standardised adsorbent (through column) and measuring the optical density of sample at different time intervals (0, 4, 8, 12, 16, 20, 24 and 44 hours). Anthocyanin content of withdrawn sample was estimated and adsorbed anthocyanins were determined.

Standardization of desorbent concentration

Adsorbed anthocyanins were eluted from XAD-16 using different concentrations of ethanol in order to standardize the concentration of desorbent. For desorption of anthocyanin, from adsorbent, different volumes of ethanol (20%, 40%, 60%, 80% and 100% ethanol) were used at room temperature and their concentration was standardized for the maximum elution of crude anthocyanins extract. The concentrations of ethanol were maintained by distilled water and used for elution of anthocyanins. Elute was estimated for anthocyanin recovery and colour value ('L', 'a' and 'b') with the help of UV-vis spectrophotometer (Shimadzu UV spectrophotometer).

Determination of stability of anthocyanins

Effect of heating on anthocyanin degradation

Effect of heating at different temperature for different time intervals on anthocyanin stability was determined by using the different temperature and time viz. 80°C for 10 minutes, 80°C for 20 minutes, 100°C for 10 minutes, 100°C for 20 minutes, 121°C for 10 minutes and 121°C for 20 minutes. Anthocyanin extracts were heated at mentioned temperatures and time. Anthocyanin content was determined by the method described by Ranganna[9].

Total Anthocyanins

Total anthocyanins present in all the samples were determined by the method given by Ranganna[9]. The procedure involved extraction of the anthocyanin with ethanolic-HCl and measurement of colour at a wavelength of 535 nm against blank of ethanolic-HCl using a UV spectrophotometer.

Statistical analysis

Completely Randomized Design (CRD) was used for data analysis in different experiments except colour analysis where Randomized Block Design was used for analysis. All the experiments were performed in triplicate. The mean values and standard deviation were obtained using SPSS (Chicago, JAV) statistical software.

Colour analysis (L, a and b Values)

Colour analysis of the product was conducted with the help of spectrophotometer (Shimadzu). Colour graph was plotted with the help of colour plotter. 'L' denotes the lightness, 'a' denotes redness and 'b' denotes greenness in the samples. It was done using the software supplied with UV-spectrophotometer.

Results and discussion

Standardization of adsorbent concentration

Total 6 different concentrations of XAD-16 (15 per cent, 20 per cent, 25 per cent, 30 per cent,

35 per cent and 40 per cent) were tried for adsorption of anthocyanins from plum pomace extract. XAD-16 is a non-ionic acrylic polymer adsorbent which has been reported to be one of the most suitable adsorbents among 16 different solid phase extraction (SPE) phases for anthocyanin purification from fruit juice (Kraemer-Schafhalter *et al*[10]) All the treatments showed the significant differences among each other. The highest 'L' value (39.35) and the lowest 'a' value (11.44) was recorded with 35 per cent XAD-16 (Fig. 1). 'L' value denotes the lightness of the colour while 'a' value denotes the the redness of colour. The data showed that maximum adsorption (61.70 per cent) of anthocyanin took place with 40 per cent XAD-16 that was almost comparable with 35 per cent XAD-16 (Fig. 2). The highest 'a' value along with the lowest 'L' value indicated that 35 per cent XAD-16 was the optimum concentration for significant adsorption of anthocyanins, among all the treatments and was selected for the further study. Data clearly showed that adsorption of anthocyanins increased with the increase in XAD-16 upto 35% of concentration after that a marginal decrease was recorded.

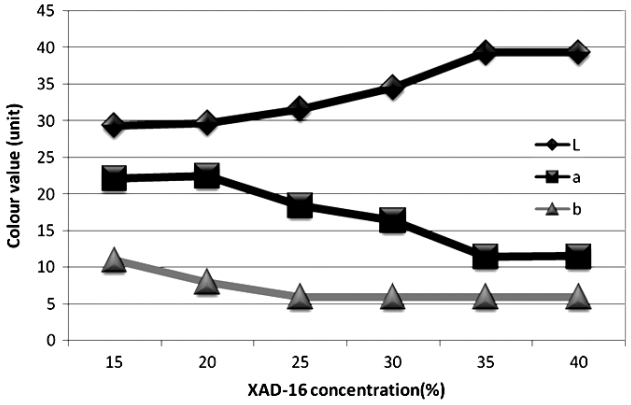

Fig. 1—Effect of adsorbent concentration on colour values of treated plum pomace extract

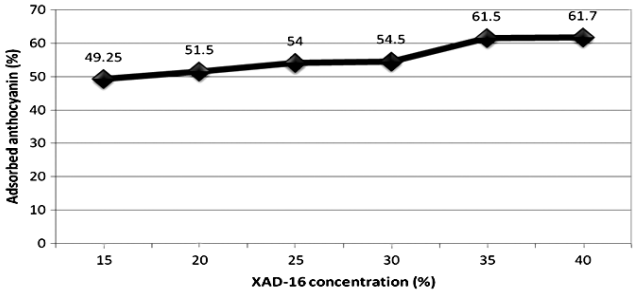

Fig. 2—Effect of XAD-16 concentration on anthocyanin content of treated plum pomace extract

Effect of contact time of adsorbent on the adsorption of anthocyanin and colour

To standardize the adsorption time, 35 per cent XAD-16 was used and colour values (Fig. 3) and adsorbed anthocyanins (per cent) (Fig 4) were recorded at 0, 4, 8, 12, 16, 20, 24 and 44 hours. The lowest 'a' value (12.52) was observed at 8 hours. The range of 'L' value ranged 26.59 to 40.80. The maximum adsorption of anthocyanins was recorded at 8 hours (95.25 per cent) followed by 95.00 per cent (at 44 hours). Due to maximum adsorption, 8 hours was found as the optimum time for adsorption of anthocyanins from plum pomace. The decrease in adsorption per cent after 8 hours may be due to desorption of anthocyanins with increase in the time interval. It was also supported by the a colour values that indicated that the depth of redness was the lowest at 8 hours and after that it increased up to 24 hours. The lowest value of redness in the extract showed the highest adsorption of anthocyanins from the extract.

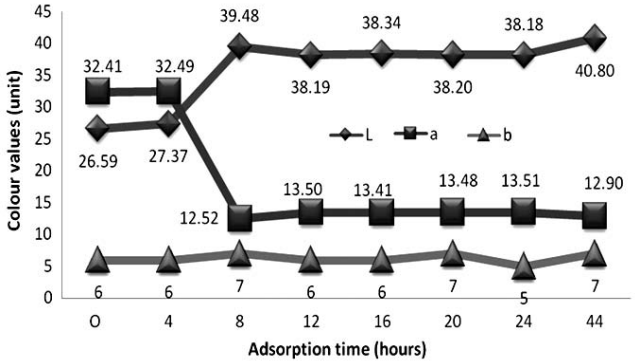

Fig. 3—Effect of adsorption time on colour values of the treated plum pomace extract

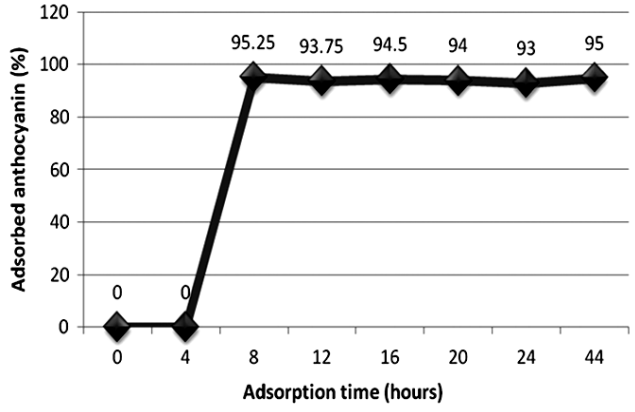

Fig. 4—Effect of adsorption time (hours) on the adsorption of anthocyanin from plum pomace extract

Effect of ethanol concentration on desorption of anthocyanin

Considerable changes were recorded in 'L' and 'a' values while 'b' value did not show any significant change. It is clear from Fig. 5 that 'L' value ranged from 43.50-68.60 and lowest 'L' value was recorded with 60 per cent of ethanol. Highest 'a' value was recorded as 27.80 with 60 per cent of the ethanol. Figure 6 shows the effects of ethanol concentrations on desorption of anthocyanin. Desorption of anthocyanins ranged from 36.06-94.96 per cent. Maximum desorption (94.96 per cent) was recorded with 60 per cent ethanol while it was minimum (36.06 per cent) with 20 per cent ethanol (Fig. 6). Data clearly showed that 60 per cent ethanol gave the best results for desorption of anthocyanin which might be due to reduced amount of water in 80 per cent and 100 per cent of ethanol because mixture of ethanol and water acts better as solvent for anthocyanins. It was in accordance with Liu *et al*[3], who reported that ethanol could effectively elute anthocyanin from the resin at concentration higher than 30% (V/v). It was also supported by the findings of Kammerer *et al*[11] used a styrene divinyl copolymerisate, to extract anthocyanins from grape pomace and found that recovery rate was 86 to 96 per cent with ethanol.

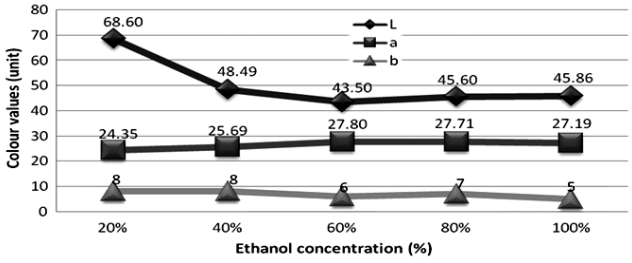

Fig. 5—Effect of ethanol concentration on colour value of elute

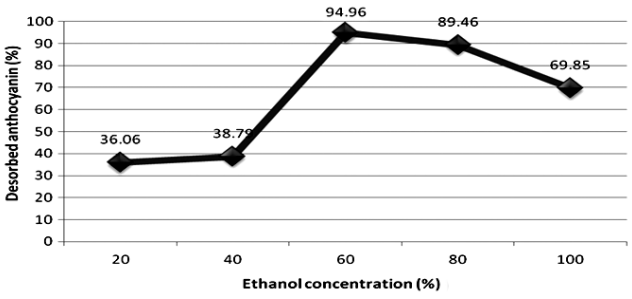

Fig. 6—Effect of ethanol concentration on desorption of anthocyanin

Effect of temperature and time on colour value and stability of extracted plum anthocyanin

Anthocyanin content in plum pomace extract degraded with increase in temperature and time (Table 1). The 'L' value ranged from 25.17-41.33. The highest 'L' value was recorded in T_2 (for 20 minutes) and lowest was receded in T_1. The 'a' value was recorded as the highest in T_1 (for 10 minutes) while lowest value was recorded as 42.25 in T_3 (for 20 minutes). The 'b' value did not show any significant change among all the treatments. The 'a' value denotes redness of the colour. The higher 'a' value in T_1 may be attributed to the maximum stability of anthocyanins at lower temperature for minimum time period. A marked degradation of anthocyanins was recorded at higher temperatures. The maximum degradation (28.71 per cent) was recorded in T_3 (for 20 minutes). The minimum degradation took place in T_1 (heating for 10 minutes) at all the temperatures. This may be ascribed to the degradation of anthocyanins at higher temperature for a longer duration. The higher loss of anthocyanins at elevated temperature as compared to lower temperature has been reported in the case of grapes (Morais *et al*[12]). In an earlier study, it was reported that storage temperature had a clear effect on the pigment degradation kinetics of coloured model juices. At 25°C, higher stability was obtained for juices coloured with radish extract (22 weeks half-life) than with potato extract (11 weeks half-life). Refrigerated temperatures drastically decreased the rate of anthocyanin degradation with estimated half-life of over a year. The degradation kinetics of acylated anthocyanins have been reported to follow linear (Baublis *et al*[13]) or non-linear rates, probably due to the folding of the acyl moiety protecting the aglycon (Baublis *et al*[13]; Shi *et al*[14])

Table 1—Effect of temperature on colour value of pomace extract

Time and Temperature		Colour values (units)		
		L	a	b
C_1: Control		25.17	48.49	8
T_1: Temp 80°C	for 10 min	38.48	44.43	11
	for 20 min	40.03	43.37	12
T_2: Temp 100°C	for 10 min	40.15	43.28	12
	for 20 min	41.33	43.07	13
T_3: Temp 121°C	for 10 min	36.93	42.40	13
	for 20 min	35.46	42.25	13
$CD_{0.05}$		0.06	0.50	NS

Conclusion

It is concluded that plum pomace is a suitable source for pigment production with optimum condition of extraction using adsorption with XAD-16. The pigment yield after adsorption revealed that extraction by adsorption with XAD was the best method. The evaluation of pigment supported the hypothesis of the use of anthocyanin as natural but attractive source of colour or biocolour. As anthocyanin is water soluble, the plum anthocyanin can be used commercially in food products where water is the main solvent.

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