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Optimization of extraction treatment and concentration of extract on yield and quality of anthocyanins from plum *var*. 'Santa Rosa'

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The leftover material of the plum (*Prunus salicinia* L.) processing, thrown out presently, has been employed for the extraction of anthocyanins. The treatment of plum extract with pectin esterase produced a clear extract without any gel formation. To extract anthocyanins, ethanol (50% and 100%) and citric acid (0.1% and 0.2%) with two levels of concentration of anthocyanin extracts (8:1 and 10:1) were tried. There were significant differences both in yield and colour of the extracts in the treatments employed. Of the various treatments employed, the best results were obtained with 50% ethanol + 0.2% citric acid with 10:1 concentration ratio in terms of maximum anthocyanin content (325 mg/100 mL) with highest sensory evaluation score on hedonic scale. Extraction of anthocyanin reflects the potential of plum waste to serve as a source biocolours.

Keywords: Plum, *Prunus salicinia,* Anthocyanins, Biocolour, Ethanol, Citric acid.

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Introduction

Colour is one of the first characteristics perceived by our senses and helps in determining the acceptability and basic aesthetic values of food[1]. Colour is added either of natural or synthetic origin to the food to the uniformity in appearance but synthetic colours are not totally safe for human consumption[2]. In recent years, increasing consumer awareness for natural products with almost no chemical additive and certified dyes (earlier considered safe) has necessitated the need to explore food colourants of natural origin[3]. Natural colours having biological origin like fruits, vegetables, seeds, roots and microorganisms are called as 'Biocolours'[4-6]. Chlorophyll, carotene, lycopene, anthocyanins, flavonoids and anthoxanthins are all biocolours[7] but anthocyanins are of particular interest to the food industry due to their ability to impart vibrant colors and enhance the health promoting qualities to the food products.

Plum (*Prunus salicinia* Lindl.) is one of the most important fruit crops of mid-hill areas of temperate regions of India, viz., Himachal Pradesh, Jammu and Kashmir, Uttaranchal and also to some extent in Nilgiri Hills of South India[8] and are used both as fresh and in preserved form. Anthocyanins are responsible for the attractive colour of plum fruits and the total amount of anthocyanin in plum ranges from 33 to 173 mg as cyanidin 3-glucoside per 100 g fresh tissue[9]. The waste of the plum after processing is thrown out by the processing industries which contain sufficient quantity of anthocyanin pigment that can be further processed for extraction of anthocyanins. In the literature, various anthocyanin extraction techniques have been employed for different fruits like grapes. Anthocyanins from grapes have been precipitated successfully using lead acetate although it leads to accumulation of lead in the extracted anthocyanins. Extraction of anthocyanins is mostly carried out by acidified ethanol using hydrochloric acid. Since the colour would be used for food purpose, it was thought to use citric acid which is frequently used in food processing and preservation. Mazza and Miniati[10] acidified the water used for extraction as anthocyanins are generally more stable at lower than higher pH therefore, use of 0.01% citric acid mixture has also been employed[11]. Boutaric *et al*[12] reported that the non-Beer's Law phenomenon disappeared when 50 % ethanol was used as the diluting solution. Harborne[13] found that most of the anthocyanins display a

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bathochromic shift of the maximum wavelength by upto 25 nm in acidified ethanol. But there is no documentation of solvent used for extraction of anthocyanin from plum nor further processing methods. Looking into this opportunity, the present study was undertaken to optimize the extraction method of anthocyanins from plum *var*. 'Santa Rosa' and the results are presented here.

Materials and Methods

Extraction and concentration of anthocyanins

The study was conducted in the department of Postharvest Technology (now Food Science and Technology) of Dr. Y. S. Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh. The fully ripened fruits were processed to obtain the pomace. The pomace (skin and stones) of variety 'Santa Rosa' was used to prepare the anthocyanin extract which was preserved by the addition of 2000 ppm of sodium benzoate. Prior to anthocyanin extraction, the plum pomace extract was treated with pectinestrase enzyme (M/S Triton Chemical, Mysore, India). Different ratios of plum pomace extract: ethanol were tried (1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8) before extraction to determine the proper ratio of extract : ethanol. To standardize the anthocyanin extraction procedure, two different extraction methods (E) involving ethanol (50% and 100%) and citric acid (0.1% and 0.2%) with two levels of concentration (C) of anthocyanin extracts (8:1 and 10:1) were tried (Table 1). Anthocyanin extract was concentrated under vacuum at 35-40°C and 950 mbar pressure using rotary evaporator (Buchi type).

Table 1—Details of treatment used for anthocyanin extraction and concentration in plum

Treatments (T)	Notations (EC)	Extraction method (E)	Conc (C)
T_1	E_1C_1	E_1 = Ethanol (100%) + Citric acid (0.1%)	C_1 = 8 : 1
T_2	E_2C_1	E_2 = Ethanol (100%) + Citric acid (0.2%)	C_1 = 8 : 1
T_3	E_3C_1	E_3 = Ethanol (50%) + Citric acid (0.1%)	C_1 = 8 : 1
T_4	E_4C_1	E_4 = Ethanol (50%) + Citric acid (0.2%)	C_1 = 8 : 1
T_5	E_1C_2	E_1 = Ethanol (100%) + Citric acid (0.1%)	C_2 = 10 : 1
T_6	E_2C_2	E_2 = Ethanol (100%) + Citric acid (0.2%)	C_2 = 10 : 1
T_7	E_3C_2	E_3 = Ethanol (50%) + Citric acid (0.1%)	C_2 = 10 : 1
T_8	E_4C_2	E_4 = Ethanol (50%) + Citric acid (0.2%)	C_2 = 10 : 1

Estimation of anthocyanins

Total anthocyanin content present in the sample and colour measurement of anthocyanin was carried out by spectrophotometric method[14]. 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. The total anthocyanins were calculated and expressed as mg per 100 mL using the formula given below:

$$\text{Total O.D./100 mL} = \frac{\text{O.D.} \times \text{Volume made up of the extracts used for colour measurement} \times \text{Total Volume}}{\text{Volume of extracts used} \times \text{Volume of sample taken}} \times 100 = X \text{ (say)}$$

$$\text{Total anthocyanin content (mg/100 mL)} = \frac{X}{E}$$

where, E = Extinction coefficient, O.D = Optical density

The *E* value for 1% solution of cyaniding-3-glucoside (i.e. 10 mg per 1 mL) at 535 nm equals to 982[14]. Therefore, the absorbance of a solution containing 1 mg per mL is equal to 98.2.

Colour of the anthocyanin extract was measured with spectrophotometer, using software for measuring L, a, b values[14], where the L, a, b values were recorded using Shimadzu U.V. Spectrophotometer at a wavelength between 380-780 nm. Tintometer colour evaluations of the pigment dissolved in ethanol were carried out with Lovibond Tintometer (Model E). The colour was expressed as red (R) and yellow (Y) units as per the standard procedure.[14]

Sensory evaluation

The sensory evaluation of the anthocyanin extract and the concentrated extract were conducted by using 9 point hedonic scale for each attributes as per the method described earlier.[15]

Statistical Analysis

All the treatments were replicated thrice to increase the precison of the experiment. The data for quantitative estimation of various physico-chemical characteristics was analyzed by Completely Randomized Design (CRD) while the data of sensory analysis were analyzed by Randomized Block Design (RBD), as described by Mahony.[16]

Results and Discussion

Among the different ratio of pomace extract: ethanol (1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8) tried, pomace extract: ethanol @ 1:0.5 gave the highest optical density at 535nm without any gel formation (Fig. 1). Hence, 1:0.5 ratio was selected for succeeding steps ***w.r.t.*** extraction of anthocyanin from plum.

The perusal of the data revealed the effects of different extraction treatments and concentration of extracts on yield and quality of anthocyanins from plum ***var.*** Santa Rosa. Different treatment combinations tried for anthocyanin extraction and concentration showed significant differences in terms of yield and colour of the extracts (Table 2). The maximum anthocyanin content (325 mg/100 mL) was recorded with E_4C_2 (50% ethanol + 0.2% citric acid with 10:1 concentration ratio); whereas minimum anthocyanin (169 mg/100 mL) was obtained with E_3C_1 (50% ethanol+0.1% citric acid with 8:1 concentration ratio). These amounts are higher than those reported by Cevallos-Cavals ***et al***[9]. Irrespective of concentration of extracts, extraction with 50% ethanol and 0.2% citric acid was found to be the most suitable in terms of anthocyanin content (267.50 mg/100 mL); whereas, 10:1 concentration ratio of the extract was found better (282.30 mg/100 mL) than 8:1 (187.50 mg/100 mL). Earlier, acidified ethanol solvents have been found effective in extracting anthocyanins from the plant materials[17], but 50% ethanol showed better efficiency, although it is relatively costlier[18]. Cacace and Mazza[19] obtained maximum anthocyanin extraction using ethanol-water mixture. Higher ethanol concentrations extracted less anthocyanin, regardless of the solvent/solid ratio used because the diffusivity of the anthocyanin in a plant matrix might has been affected by both the concentration and the type of the solvent. However, a non-linear relationship between the increase of extracted mass and acid concentrations was indicated by Xavier ***et al***[20]. As the acetic acid concentration increased to 10-fold, the dye concentration increased by 50%, showing that slightly acidified solutions were more efficient for the extraction than those without it.[20]

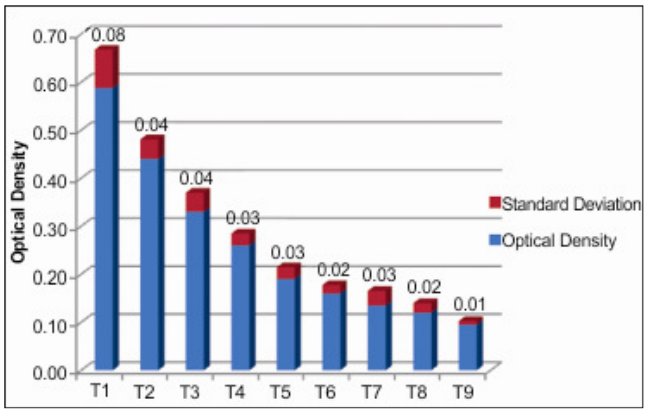

Fig.1—Effect of different ratio of extract: ethanol on the optical density at (535 nm)

It is known that citric acid is comparatively less corrosive than HCl but would still act to stabilize the anthocyanin structure in the cationic form by maintaining a low acidic pH[21], as is obtained from our findings. In terms of colour measurement, the L, a and b values of the anthocyanin concentrates also varied significantly between the treatments. The mean value for colour especially 'a' value showed the depth of redness that ranged from 2.7 to 26.1. The highest value (26.1) for red (a) was found with E_2C_1 (100% ethanol + 0.2% citric acid with 8:1 concentration ratio); whereas, the highest value for 'L' (31.5) and 'b' (20) were obtained with E_1C_1 (100% ethanol+0.1% citric acid with 8:1 concentration ratio). Further, there were significant differences between the two types of concentrations and two types of extraction procedure treatments. Higher mean values for 'L', 'a' and 'b' of the anthocyanin concentrates were obtained with 8:1 concentration than with 10:1 concentration. Even though the 'a' value is higher in 8:1 concentration, higher L value (measure of lightness), ranged from completely opaque (0) to completely transparent (100) indicates an increase in lightness in the colour. Similarly, the high value of 'b' also indicates an increase in the colour intensity towards yellowness.

The tintometer readings also revealed significant differences in the red, yellow and blue units. Maximum mean values for red (20) and yellow (14.40) were recorded with E_2C_1 (100% ethanol+0.2% citric acid with 8:1 concentration ratio) and E_3C_2 (50% ethanol+0.1% citric acid with 10:1 concentration ratio), respectively. However, for the blue unit, maximum mean value (2) was given by treatment with E_1C_2 (100% ethanol+0.1% citric acid with 10:1 concentration ratio) and E_3C_2 (50% ethanol+0.1% citric acid with 8:1 concentration ratio). There were significant differences amongst the two concentrations and also among the extraction methods. Concentration ratio of 10:1 yielded better results (Red: 13, Yellow: 12 and Blue: 2) over 8:1

Table 2—Effect of different extraction methods and concentration of extracts on anthocyanin

Treatment Details	Anthocyanin content (mg/100 mL)	Colour measurement			Tintometer Colour (units)			Sensory Evaluation (for colour) On hedonic scale (Max. score = 9.0)
		L	a	b	Red	Yellow	Blue	
E_1 [Ethanol (100%) + Citric acid (0.1%)]	225.00	16.30	11.15	10.00	8.40	8.75	1.00	6.00
E_2 [Ethanol (100%) + Citric acid (0.2%)]	235.50	11.30	19.40	7.00	16.35	12.30	0.70	8.00
E_3 [Ethanol (50%) + Citric acid (0.1%)]	211.50	2.50	5.30	1.00	9.90	11.60	1.25	7.25
E_4 [Ethanol (100%) + Citric acid (0.2%)]	267.50	7.45	12.60	5.00	11.65	9.10	0.50	8.00
C.D. (P=0.05)	1.50	0.36	0.04	1.03	0.40	0.45	0.55	**NS**
c_1 (concentration)	187.50	15.00	17.00	10.00	10.00	9.00	0.00	7.00
c_2 (concentration)	282.30	3.00	7.00	2.00	13.00	12.00	2.00	8.00
C.D. (P=0.05)	2.13	0.25	0.03	0.72	0.30	0.32	0.39	**0.51**
E_1C_1 [Ethanol (100%) + Citric acid (0.1%) (8:1)]	180.00	31.50	19.60	20.00	6.40	7.30	0.00	5.00
E_2C_1 [Ethanol (100%) + Citric acid (0.2%) (8:1)]	191.00	15.50	26.10	10.00	20.00	12.60	0.40	8.00
E_3C_1 [Ethanol (50%) + Citric acid (0.1%) (8:1)]	169.00	2.20	4.50	1.00	5.10	8.80	0.50	7.50
E_4C_1 [Ethanol (50%) + Citric acid (0.2%) (8:1)]	210.00	12.00	19.10	8.00	9.90	5.70	0.00	8.00
E_1C_2 [Ethanol (100%) + Citric acid (0.1%) (10:1)]	270.00	1.10	2.70	0.00	10.40	10.20	2.00	7.00
E_2C_2 [Ethanol (100%) + Citric acid (0.2%) (10:1)]	280.00	7.10	12.70	4.00	12.70	12.00	1.00	8.00
E_3C_2 [Ethanol (50%) + Citric acid (0.1%)(10:1)]	254.00	2.80	6.10	1.00	14.70	14.40	2.00	7.00
E_4C_2 [Ethanol (50%) + Citric acid (0.2%) (10:1)]	325.00	2.90	6.10	2.00	13.40	12.50	1.00	8.00
C.D. (P=0.05)	3.01	0.51	0.06	1.45	0.60	0.63	0.78	**NS**

(Red: 10, Yellow: 9 and Blue: 0). Similarly, extraction with 100% ethanol and 0.2% citric acid was found to be better for red (16.35) and yellow (12.30) unit; whereas, maximum value for blue unit (1.25) was associated with 50% ethanol and 0.1% citric acid. Thus, the 'L', 'a' and 'b' values were found to be on higher side with 8:1 concentration ratio. It was completely opposite with tintometer reading where, red, yellow and blue values increased with increase in the concentration ratio (10:1). Main *et al*[11] reported that concentration up to 10:1 yielded deep red liquid concentrate, which was only slightly degraded due to comparatively low temperature. Similar results have been reported by Heidari *et al*[21]; Clydesdale *et al*[2]; Sarni *et al*[22] and Wiesenborn *et al*[23]. Though the concentration of anthocyanin was carried out 8 to 10 times, the content of anthocyanins were not increased in the linear manner as was expected. This could be attributed to the degradation of anthocyanins during concentration of the extract.

The sensory evaluation of the anthocyanin extracts based on 9 point hedonic score for colour did not

show any significant differences among the treatments. However, the maximum score (8) was awarded to the treatment E_4C_2 (50% ethanol+0.2% citric acid with 10:1 concentration ratio). The same treatment also yielded maximum anthocyanin content (325 mg/100 mL).

Conclusion

Among the different treatment combinations tried, extraction with 50% ethanol+0.2% citric acid with 10:1 concentration of extracts was found to be the most suitable treatment to get maximum anthocyanin content (325 mg/100 mL) and highest sensory evaluation (hedonic) score of 8. As the anthocyanin content was relatively very high in the concentrated plum pomace extract, it can be commercially used as a natural source of attractive food colour.

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