*Journal of Crop and Weed, **8**(2):7-11(2012)*

Effect of different extraction methods and concentration of extracts on yield and quality of anthocyanin from plum var. *Santa rosa*

M. P. DEVI AND V. K. JOSHI

Department of Postharvest Technology
Dr Y.S. Parmar University of Horticulture and Forestry, Solan, H.P., India

Received: 21-1-2012, Revised: 8-11-2012, Accepted: 15-11-2012

ABSTRACT

Plum (Prunus salicinia L.) is one of the most important fruit crops of mid-hill areas of temperate regions which are used both as fresh and in preserved form. Anthocyanins are responsible for the colour in plum fruits. The waste part of the plum after processing is thrown out by the processing industries which contain sufficient quantity of anthocyanin pigment which can be further processed for extraction of anthocyanins. Present investigation was carried out to standardize the extraction and concentration of anthocyanin from plum var Santa Rosa. Four different extraction methods involving 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. Result shows that with different treatment combinations tried there were significant differences in terms of yield and colour of the extracts. The best result was obtained with 50% Ethanol + 0.2% Citric acid with 10:1 concentration ratio in terms of maximum anthocyanin content (325 mg/100 ml) and highest sensory evaluation (hedonic) score (8).

***Key words*:** Anthocyanin, extraction, pigment, standardization.

Colour is one of the first characteristics perceived by senses and help in determining acceptability, judging the quality and basic aesthetic values of food. Addition of colour to the food ensures the uniformity and intensifies the naturally occurring pigments in our food. Unfortunately, synthetic colours are not totally safe for human consumption; for example high levels of erythrosine intake causes thyroid tumors, while Ponceau 4R, tartrazine and sunset yellow were found allergic (Clydesdale *et al*., 1978). Many dyes, used at present as colourant, have been proved to be carcinogenic. In recent years, increasing consumer awareness for natural product with almost no chemical additive and certified dyes (which was earlier considered safe) has necessitated the need to exploit food colourants of natural origin (Prasad *et al.,* 1993). Presently the volume of natural food colorants in the global market has reached up to 1 billion US $ with 4% annual growth rate. In a nutshell, it seems that consumers makes a close link between "health" and "natural" and believe that "natural is better". Due to this reason, it is expected that the demand for natural pigment will increase in future (Kishor *et al.,* 2004). Natural colour having biological origin like fruits, vegetables, seeds, roots and microorganisms are called as 'Biocolours' (Pattnaik *et al.*, 1999 and Joshi and Attri, 2005). Naturally occurring food colours are chlorophyll, carotene, lycopene, anthocyanins, flavonoids and anthoxanthins (Downham and Collins, 2000).

In an endeavor to identify the active health-promoting ingredients, many researchers have focused on the properties of the flavonoids, a large class of phenolic compounds that is abundant in fruits and vegetables (Wang *et al*., 1997). Most prominent among the flavonoids are the anthocyanins, represented by over 600 molecular structures. 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 product.

Plum (*Prunus salicinia* Lind.) is one of the most important fruit crops of mid-hill areas of temperate regions which are used both as fresh and in preserved form. In India, plum is predominantly grown in H.P., Jammu and Kashmir, Uttaranchal and also to some extend in Nilgiri Hills of South India (Jindal and Chandel, 2002). Santa Rosa is one of the leading commercial cultivars of plum for mid-hills of Himachal Pradesh. Anthocyanins are responsible for the colour in plum fruits. The total amount of anthocyanin in plum ranges from 33 to 173 mg as cyanidin 3-glucoside per 100g fresh tissue (Cevallos-Cavals *et al.,* 2006). The waste part of the plum after processing is thrown out by the processing industries which contains sufficient quantity of anthocyanin pigment and can be further processed for extraction of anthocyanins. There is no documentation of method of extraction of anthocyanin from plum. Looking in to this opportunity, the present study was undertaken to standardize the extraction method of anthocyanins from plum var. Santa Rosa.

MATERIALS AND METHODS

The study was conducted in the department of Post Harvest Technology of Dr. Y. S. Parmar University of Horticulture and Forestry, Solan, H.P. in the year 2008. Plum pomace (skin and stones) of variety Santa Rosa was used to prepare the anthocyanin extract. The fully ripe fruits were processed to obtain the pomace, followed by extraction of pomace material. The extract

Email: preema.horti@gmail.com

was preserved by the addition of 2000 ppm of sodium benzoate. Prior to anthocyanin extraction the plum pomace extract were treated with pectinestrase enzyme (Triton Chemical, Mysore, India) to reduce gel formation. Different ratios of plum pomace extract: ethanol was tried (1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8) before extraction in order to determine the proper ratio of extract : ethanol. For standardizing the method of anthocyanin extraction, four different extraction method (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. The eight different treatment combinations were as given below.

Treatments (T)	Notations (EC)	Extraction Method (E)	Concentration (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

Total anthocyanin content present in the sample and colour measurement of anthocyanin were determined by spectroscopy method as given by Ranganna (1997). The procedure involved extraction of the anthocyanin with Ethanolic-HCl and measurement of colour at the wavelength of 535 nm against blank of Ethanolic-HCl using a UV spectrophotometer. The anthocyanin were calculated and expressed as mg per 100 ml using the formula given below:

$$\text{Total O.D./100 ml} = \frac{\text{O.D.} \times \text{Volume made up of the extracts used for colour measurement} \times \text{Total Volume}}{\text{Volume of extract used} \times \text{Volume of sample taken}} \times 100$$

$$= X \text{ (say)}$$

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

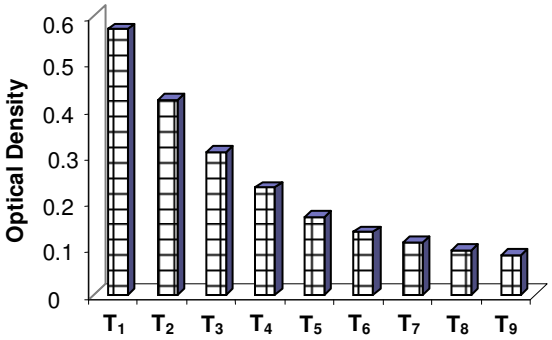
where, E = Extinction coefficient

The *E* value for 1% solution of cyaniding-3-glucoside (*i.e.* 10 mg ml^{-1}) at 535 nm is equal to 982 (Ranganna, 1997). Therefore, the absorbance of a solution containing 1 mg per ml is equal to 98.2. Anthocyanin extract was concentrated under vacuum at 35-40^{0}C and 950 mbar pressure using rotary evaporator (Buchi type). Colour of the anthocyanin extract was measured with Hunter Colour and Colour Difference scale (Ranganna, 1997), where the L, a, b values were recorded using Shimadzu U.V. Spectrophotometer at a wavelength between 780-380 nm. Tintometer colour evaluations of the pigment dissolved in ethanol were measured with Lovibond Tintometer (Model E). The colour was expressed as red (R) and yellow (Y) units as per the standard procedure (Ranganna, 1997). The sensory evaluation of the anthocyanin extract and the concentration of the extract were conducted by using 9 point hedonic scale for each attributes as per the method described by earlier (Joshi, 2006). All the treatments were replicated thrice to increase the precession of the experiment. The data for quantitative estimation of various physico-chemical characteristics were analyzed by Completely Randomized Design (CRD) while the data on sensory analysis were analyzed by Randomized Block Design (RBD) as described by Mahony (1985).

RESULTS AND DISCUSSION

The perusal of the data revealed some important information on different extraction methods and concentration of extracts on yield and quality of anthocyanin from plum var. Santa Rosa. 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.

Figure 1: Optical density (535 nm) of different ratios of extracts: ethanol



Different treatment combinations tried for anthocyanin extraction and concentration showed

significant differences in terms of yield and colour of the extracts (Table 1). The maximum anthocyanin content (325 $mg100^{-1}$ 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 associated 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.,* 2006. Irrespective of concentration of extracts, extraction with 50% Ethanol and 0.2% Citric acid was found to be most suitable in terms of anthocyanin content (267.50 mg 100^{-1} ml); whereas, 10:1 concentration ratio of the extract was found better (282.30 $mg100^{-1}$ ml) than 8:1 (187.50 $mg100^{-1}$ ml). Acidified ethanol solvents are effective in extracting anthocyanins from the plant materials (Pifferi and Vaccari, 1983), but 50% ethanol shows better efficiency, although it is relatively costly (Gao and Mazza, 1996). Mazza and Miniati (1993) acidified the water used for extraction as anthocyanins are generally more stable at lower pH than at high pH. In an earlier study, use of 100% ethanol along with 0.01% citric acid mixture was employed (Main *et al.,* 1978). Boutaric *et al.* (1937) showed that the non-Beer's Law phenomenon disappeared when 50% ethanol was used as the diluting solution. Harborne (1958) found that most anthocyanin display a bathochromic shift of the maximum wavelength by up to 25 nm in acidified ethanol. Cacace and Mazza (2003) obtained a 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 was affected by both the concentration and the type of the solvent. A non linear relationship between the increase of extracted mass and acid concentrations was indicated by Xavier *et al.,* 2008. As the acetic acid concentration increased 10-fold, the dye concentration increased only 50%, showing that slightly acidified solutions were more efficient for the extraction. (Xavier *et al*., 2008). Citric acid is less corrosive than HCl and would still act to stabilize the anthocyanin structure in the cationic form by maintaining a low acid pH (Heidari *et al.,* 2006).

In terms of colour measurement, the L, a and b values of the anthocyanin concentrates also varies significantly between the treatments. The mean value for colour especially 'a' shows the depth of redness ranging from 2.7 to 26.1. The highest score (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 score for 'L' (31.5) and 'b' (20) were observed 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 also among the four extraction methods. 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 value is higher in 8:1 concentration, higher L value which is the measure of lightness, where values range from completely opaque (0) to completely transparent (100) indicates an increased in lightness in the colour. Similarly, the high value of b also indicates the increase in the colour intensity towards yellowness.

The tintometer readings also revealed significant differences in the red, yellow and blue units. Maximum mean value for red (20), yellow (14.40) was 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 blue unit, maximum mean value (2) was associated 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 among 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 (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. In a nutshell, the 'L', 'a' and 'b' value was found to be on higher side with 8:1 concentration ratio. It is completely opposite with tintometer reading where, red, yellow and blue values increased with increase in the concentration ratio (10:1). Main *et al.* (1978) reported that concentration up to 10:1 yielded deep red liquid concentrate, which were only slightly degraded due to comparatively low temperature. Similar results have been reported by Heidari *et al.* (2006); Clydesdale *et al.* (1978); Sarni *et al.* (1996) and Wiesenborn *et al*. (1995).

The sensory evaluation of the color of the anthocyanin extracts based on 9 point hedonic score did not show any significant differences among the treatments. However, one of the maximum scores (8) was recorded with 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^{-1} ml).

Table 1: Effect of different extraction methods and concentration of extracts on anthocyanin

Treatments	Anthocyanin content (mg 100^{-1} ml)	Colour measurement			Tintometer			Sensory evaluation (for colour)
		L	a	b	Red	Yellow	Blue	
E_1	225.00	16.30	11.15	10.00	8.40	8.75	1.00	6.00
E_2	235.50	11.30	19.40	7.00	16.35	12.30	0.70	8.00
E_3	211.50	2.50	5.30	1.00	9.90	11.60	1.25	7.25
E_4	267.50	7.45	12.60	5.00	11.65	9.10	0.50	8.00
LSD (0.05)	**1.50**	**0.36**	**0.04**	**1.03**	**0.40**	**0.45**	**0.55**	**NS**
C_1	187.50	15.00	17.00	10.00	10.00	9.00	0.00	7.00
C_2	282.30	3.00	7.00	2.00	13.00	12.00	2.00	8.00
LSD (0.05)	**2.13**	**0.25**	**0.03**	**0.72**	**0.30**	**0.32**	**0.39**	**0.51**
E_1C_1	180.00	31.50	19.60	20.00	6.40	7.30	0.00	5.00
E_2C_1	191.00	15.50	26.10	10.00	20.00	12.60	0.40	8.00
E_3C_1	169.00	2.20	4.50	1.00	5.10	8.80	0.50	7.50
E_4C_1	210.00	12.00	19.10	8.00	9.90	5.70	0.00	8.00
E_1C_2	270.00	1.10	2.70	0.00	10.40	10.20	2.00	7.00
E_2C_2	280.00	7.10	12.70	4.00	12.70	12.00	1.00	8.00
E_3C_2	254.00	2.80	6.10	1.00	14.70	14.40	2.00	7.00
E_4C_2	325.00	2.90	6.10	2.00	13.40	12.50	1.00	8.00
LSD (0.05)	**3.01**	**0.51**	**0.06**	**1.45**	**0.60**	**0.63**	**0.78**	**NS**

In the present investigation, an attempt was made to standardize the method for extraction and concentration of anthocyanin from plum. Among the different treatment combinations, extraction with 50% ethanol+0.2% Citric acid with 10:1 concentration of extracts was found to be most suitable in terms of maximum anthocyanin content (325 mg 100^{-1} ml) and highest sensory evaluation (hedonic) score (8). As the anthocyanin content is relatively very high in the concentrated plum pomace extract, it can be commercially used as natural source of attractive food colour. As anthocyanin is rich in bioactive properties, its use in food items will ensure the added value to the consumers in terms of "improving health" and results in "quality of life".

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