Studies on extraction, characterization and antioxidant activity of biocolour extracted from plum waste

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INTRODUCTION

- Colouring of food is an age old practice
- Colour also indicates quality and condition of food product.
- Food colour and flavour both are closely associated
- Colour determines the acceptability of the product.
- A red apple is expected to be sweet, green mango to be sour and brown ice cream will have a chocolate flavour.

PERMITTED SYNTHETIC COLOURANTS

COLOURING	SHADES
Coal tar dyes:	
Allura red, Amaranth Azorubine, onceau 4R	Red
Sunset Yellow Tartrazine, Yellow 2G	Yellow
Green S	Green
Brilliant Blue FCF Indigotine	Blue
Brown HT	Brown
Brilliant Black BN	Black

TOXIC EFFECTS OF SYNTHETIC COLOURS

Pigments	Health Effects
Brillient blue FCF	Chromosomal damage
Indigo carmine	Brain tumor
Brillient green acid	Brain tumor
Fast Green FCF	Bladder tumor
Tartrazine	Thyroid and lymphatic tumors, Allergy
Yellow Orange S	Kidney tumors, Chromosomal damage

What are Biocolours ?

Natural colours are generally extracted from fruits, vegetables, roots and microorganisms and are often called “biocolours” because of their biological origin.

Benefits of Biocolours

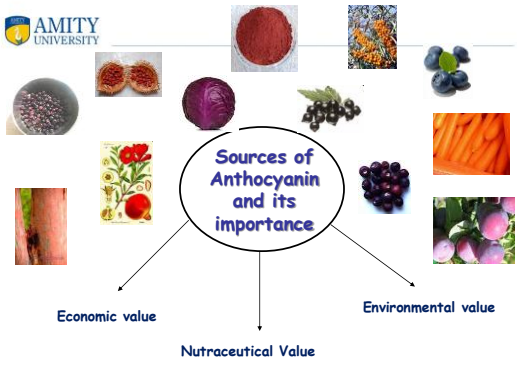
- Biocolours have protective role against lethal photo oxidation.
- Antioxidant activity by protecting cells against oxidative damage which leads to degenerative diseases such as atherosclerosis, cancer, arthritis and macular degeneration.
- Inhibition of mutagenesis.
- Enhancement of immune systems.
- Inhibition of tumour development.

Example of Biocolours and their shades

Annato , Carotenoids Beetroot extract Fruit & vegetable juices/extracts Grape skin extract Paprika, Riboflavin Saffron, Turmeric	Yellow to Red
Caramel	Yellow to brown
Chlorophyll	Green
Vegetable carbon	Black

What Are Anthocyanins?

- ❖Natural, water-soluble plant pigments
- ❖Display a variety of pH dependent colors
- ❖Polyphenolic compounds (flavonoids)
- ❖Used as food colorants
- ❖Numerous "**functional**" components



Common anthocyanin

- Delphinidin
- Cyanidin
- Petunidin
- Pelargonidin
- Peonidin
- Malvidin

Anthocyanins Sources and Applications

Sources

- Grape skin
- Red cabbage
- Elderberry
- Purple carrots
- Purple potatoes
- Red radish
- Jamun, Phalsa, Black Carrot, Kokum, Mulberry, Strawberry, blueberry, blackberry, black current, hibiscus, roselle

Applications

- Soft drinks
- Instant drinks
- Fruit drinks
- Liquors
- Confectionery
- Fruit jellies
- Jams

Plum - a rich source of anthocyanin

- Plum (*Prunus salicinia* Lind.), is one of the most important fruit crop of Himachal Pradesh, INDIA
- Plum is a natural rich source of anthocyanins

- Processing waste of the plum contains sufficient quantity of anthocyanin pigments

Table 1: Physico-chemical characteristics of plum fruit, pulp and pomace (var. Santa Rosa)

Characteristics	Mean± SD	Range	CV
Plum Fruit			
Fruit Weight (g)	33.80±1.62	32.00-36.00	4.79
Pulp (per cent)	52.20±2.15	49.00-55.00	4.12
Pomace (per cent)	5.50±0.20	5.20-5.80	3.64
Total soluble solids (°B)	**15.00±0.52**	13.80-15.40	3.50
Titratable acidity (as % malic acid)	2.04±0.03	1.98-2.08	1.47
pH	3.28±0.04	3.24-3.39	1.22
Total anthocyanins (mg/100g)	34.20±2.15	31.00-37.00	6.29
Plum Pulp			
Total Soluble Solids (°B)	14.56±0.25	14.20-14.80	1.72
Titratable acidity (as % malic acid)	1.98±0.14	1.88-2.10	7.07
pH	3.41±0.06	3.26-3.49	1.80
Total phenolics (mg/ 100g)	77.90±1.20	76.72-80.90	1.55
Total anthocyanins (mg/100g)	48.52±0.38	43.10-57.4	0.78
Plum pomace			
Total soluble solids (°B)	0.82±0.03	0.78-0.89	3.66
Titratable acidity (per cent as malic acid)	1.55±0.02	1.51-1.59	1.29
pH	3.62±0.21	3.19-3.73	5.80
Total phenolics (mg/ 100g)	**215.70±2.06**	212.00-219.00	0.95

Table 2: Effect of different extraction methods on λ-max, optical density and tintometer colour units of plum extract

Extraction methods	λ-max (nm)	Optical Density (at 535 nm)	Tintometer colour unit		
			Red	Yellow	Blue
(T_1) : Pomace + Water (1:1)	301	4.00	**4.50**	1.50	0.30
(T_2) : Pomace+ 20% Ethanol (1:1)	298	4.00	4.40	2.10	0.04
(T_3) : Pomace + 20% Acetone (1:1)	304	4.00	4.30	1.50	0. 08
(T_4): Plum pomace fermented with brewer's yeast	305	4.00	4.50	2.00	0.10
(T_5): Plum pulp fermented with brewer's yeast	343	4.00	3.50	2.20	0.10
CD $_{(0.05)}$	--	--	0.27	0.30	0.14

Table 3: Effect of extraction methods on types of anthocyanins in plum pomace extract using thin layer chromatography

Method	Spot No.	Rf value	Visibility spot	Rf value in literature	Anthocyanin identified
(T_1) : Pomace +Water (1:1)	1	0.203	Ultra-violet	0.20	Cyanidin-3,5-diglucoside
	2	0.222	Day Light	0.22	Malvidin-3-monoglucoside
	3	0.685	Ultra-violet	0.68	Cyanidin-3-monoglucoside
(T_2) : Pomace+ 20% Ethanol (1:1)	1	0.260	Ultra-violet	0.26	Delphinidin-3-monoglucoside
	2	0.552	Ultra-violet	NI	NI
(T_3) : Pomace + 20% Acetone (1:1	1	0.200	Ultra-violet	0.20	Cyanidin-3,5-diglucoside
	2	0.247	Ultra-violet	NI	NI
(T_4) : Plum pomace fermented with wine yeast	1	0.197	Ultra-violet	0.20	Cyanidin-3,5-diglucoside
	2	0.292	Day Light	0.30	Malvidin-3,5-diglucoside
	3	0.583	Ultra-violet	NI	NI
	4	0.708	Ultra-violet	NI	NI
(T_5): Plum pulp fermented with wine yeast	1	0.304	Day light	0.30	Malvidin-3,5-diglucoside
	2	0.473	Ultra-violet	NI	NI

Extraction of anthocyanins from pomace extract

- Crude anthocyanins were extracted by column chromatography using amberlite XAD-16 resin as adsorbent.
- 35% XAD-16 was used for the best adsorption of anthocyanins
- Elution was done by using 60% ethanol.

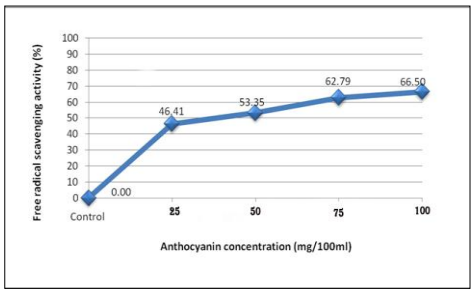
Table 4: Effect of temperature on anthocyanins

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

Table 5: Effect of pH on tintometer colour unit and stability of crude anthocyanin

pH	Tintometer colour unit			Anthocyanin degradation (%)
	Red	Yellow	Blue	
C_1 : Control	**12.50**	7.40	0.80	0
(T_1) : 2.0	**12.40**	**7.50**	0.80	0
(T_2) : 2.5	12.40	**7.50**	0.80	0.95
(T_3) : 3.0	12.20	7.30	0.60	0.95
(T_4) : 3.5	12.00	7.30	0.60	0.97
(T_5) : 4.0	12.00	7.20	0.60	5.72
(T_6) : 4.5	11.80	7.20	0.50	10.48
(T_7) : 5.0	11.60	6.80	0.50	10.48
(T_8): 5.5	11.60	6.90	0.60	13.34
(T_9) : 6.0	11.60	7.00	1.50	14.29
(T_{10}) : 7.0	11.20	6.40	1.70	15.24
(T_{11}) : 8.0	11.00	6.20	2.10	18.10
(T_{12}) :9.0	10.80	5.80	1.60	20.00
(T_{13}) : 10.0	10.80	6.00	**2.20**	**21.43**
$CD_{0.05}$	0.44	0.64	0.36	0.04

Fig. 1: Effect of crude anthocyanins on DPPH free radical scavenging activity



CONCLUSION

- The anthocyanin yield indicates that plum pomace is a suitable source for anthocyanins production with optimum condition of extraction.
- Anthocyanins are potential antioxidants agent for use in food products which will improve the appearance as well as nutritional quality of the food.
- The evaluation of pigment thus supports the hypothesis of the using anthocyanins as natural and attractive source of colour or biocolour.

- Low pH stability of anthocyanins indicates the best possible use of anthocyanins as a colorant in acidic foods like beverages, jam, jellies etc.
- Since anthocyanin is water soluble, the plum anthocyanin can be used commercially in food products where water is the main solvent.
- Thus it can be concluded that plum pomace can be utilized for the production of biocolour that can replace the synthetic food colours.

THANK YOU