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Stability of Betacyanin Pigment in Peel and Pulp of Red Flesh Dragon Druit (*Hylocereus polyrhizus*)

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ABSTRACT

Stability of betacyanin pigment in peel and pulp of red dragon fruit (*Hylocereus polyrhizus*) was studied at three different pH levels (1, 3, and 6) by extracting with different solvents (acetone, methanol and distilled water) for a period of 10 days and absorbance was recorded. Results revealed that distilled water (S3) gave highest pigment extraction in peel (36.05 mg/100 g) at pH 1 on Day-1whereas methanol (S2) recorded highest pigment extraction (43.41 mg/100g) at pH 6 in pulp at Day-1. Betacyanin pigment extracted from peel by using distilled water as a solvent at pH 3 and pulp at pH 6 were found stable at 10 days of storage. The pigment content in pulp was recorded highest than the peel. The pH of the sample had greater effect on stability of the pigment.

Keywords: Betacyanin, stability, red flesh dragon fruit, pH, solvents and natural colorant.

INTRODUCTION

Dragon fruit (*Hylocereus* spp.), a climbing cactus vine native to tropical regions of Mexico, Central and South America[3,9] is an emerging super crop among farmers for its economic value and rich nutrient content. Currently, four types of dragon fruit are contributing to the world market as table fruit i.e., i) red skin, white flesh (*Hylocereus undatus*) (ii) red skin, red flesh (*Hylocereus polyrhizus*) (iii) red skin, purple flesh (*Hylocereus costaricencis*) (iv) yellow skin, white flesh (*Hylocereus megalanthus*). The red or red-violet colour of red flesh dragon fruit is due to a pigment called betacyanin belonging to the betalin group[5] which has a potential to become a game changer for food industry as a natural food colorant.

Betalins are classified into two major groups: betacyanin and betaxanthin, of which only the former is present in dragon fruit and latter is devoid of it[14,19]. Within the *Hylocereus* genus, there are seven identified betacyanin's which have same absorption spectra contributing to deep purple colour of pulp. Betacyanin has previously been exploited from red beet but its earthy odour caused by geosmin and pyrazine derivatives, have created a demand for alternative sources[18]. With the increasing awareness for organic food consumption, there is a growing demand for natural food colorants. However, the instability, easy degradation and heat sensitivity of betalins pose challenges for extraction and storage.Betalins are prone to oxidation due to their antioxidant properties, so preventing oxidation during extraction and storage is crucial[18]. Stintzing and Carle[12] reported that betacyanin are further classified into four types *i.e.,* betanin, gomphrenin, amaranthine and bougainvillea. Betacyanin found in dragon fruit is a polar solvent and exhibits high antioxidant activity[10]. Degradation of pigment is majorly influenced by factors such as heat, light, pH, moisture and oxygen which can affect its storage and stability[8]. These limitations may restrict the use of natural plant pigments in the food industry. To overcome these challenges, an attempt was made to extract the betacyanin pigment by using different solvents (acetone, methanol and distilled water) and assessed the effect of pH and solvents on the stability of the betacyanin pigment in both peel and pulp of red flesh dragon fruit.

METHODS

Sample collection

The red flesh dragon fruit samples were obtained from the Experimental Farm, SAS, Nagaland University**.** The Fruits were carefully cleaned and cut in to small pieces using a knife separating the peel and pulp.

Extraction by using different solvents

Three different solvents- acetone (80%), methanol (80%) and distilled water were used to extract the pigment. Five grams of both peel and pulp were mixed with each solvent to achieve a total volume of 50 ml using a mortar and pestle. The peel and pulp samples were then centrifuged at 3000 g for 10 minutes and the supernatants were collected and filtered. The absorbance of the samples was measured at 538 nm using a UV-Vis spectrophotometer, with the blank reagent being the same solvent used for the sample.

pH treatment

Following the estimation of betacyanin content, the pH of the samples was adjusted to 1, 3 and 6 using 1M HCL and 1M NaOH solutions. A pH meter was used until the desired pH was achieved.

Spectrophotometer analysis

The absorbance of the pH- adjusted samples was measured using UV-Vis spectrophotometer at 538 nm on Day-1 and the samples were stored at room temperature, covered with aluminium foil, and kept in the dark until the pigment's colour diminished. The betacyanin content was assessed every day.

The absorbance readings were used to calculate betacyanin concentration (mg/ 100 g of fresh weight) in sample and was expressed as mg betacyanin content (BCE/100 g fresh weight). It was calculated by using equation:

$$\text{Betacyanin (mg/100 g)}=\frac{A(MW)\times V\times(DF)}{1000/\,LW\times 100}$$

Where, A_{538}=absorbance at 537 nm (λmax), L (path length of cuvette) = 1.0 cm, DF=Dilution factor, V= volume extract (ml), W= weight of extracting material (g). $\wedge$ is the molar extinction coefficient of betanin = 60000 l / mol x cm) and MW (molecular weight) = 550 g/mol.

Statistical analysis

The experiment was designed following factorial completely randomized block design (FCRD) with 9 treatments at 5% level of significance. Each treatment was replicated three times. Analysis of variance (ANOVA) was performed using the SPSS software version 16.0 for Windows.

RESULTS AND DISCUSSION

Effect of pH and solvents on peel extracts

The pH and solvents played significant role in the pigment stability in dragon fruit. Solvents like distilled water and methanol resulted in higher extraction of betacyanin pigment than acetone and the it remained stable at pH range 3 and 6 in peel until Day-10. The initial extraction (Day-1) was high with distilled water at pH 1(36.05 mg/100 g) and methanol at pH 6 (35.08 mg/100 g) was found at par with it, while acetone at pH 6 (20.27 mg/100 g)

Table 1: Interaction effect of pH and solvents on betacyanin content of *Hylocereus polyrhizus* peel extracts

Treatments	Day-1			Day-2			Day-3			Day-4			Day-5		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
S1P1	25.16	25.65	25.41	13.26	14.16	13.71	10.99	12.34	11.66	11.28	9.60	10.44	7.56	7.19	7.37
S1 P3	24.52	24.91	24.72	17.75	16.86	17.30	16.62	15.93	16.27	8.17	7.73	7.95	9.95	10.38	10.17
S1 P6	14.89	25.66	20.27	10.22	10.63	10.42	10.00	11.52	10.76	12.00	13.49	12.74	7.56	5.81	6.68
S2 P1	36.98	22.43	29.70	38.90	16.48	27.69	35.30	10.33	22.81	13.13	7.43	10.28	3.14	7.15	5.14
S2 P3	39.37	22.25	30.81	51.22	19.61	35.42	49.16	25.09	37.12	24.71	16.43	20.57	9.43	15.39	12.41
S2 P6	45.61	24.55	35.08	49.42	24.92	37.17	46.42	27.55	36.98	27.44	15.81	21.62	23.33	16.46	19.89
S3 P1	36.26	35.84	36.05	21.28	25.38	23.33	21.34	22.49	21.91	21.32	19.48	20.40	21.42	18.06	19.74
S3 P3	32.48	34.64	33.56	34.11	33.25	33.68	26.54	26.28	26.41	31.81	33.72	32.76	28.90	34.14	31.52
S3 P6	36.25	32.82	34.53	33.14	32.82	32.98	24.30	28.62	26.46	21.25	18.56	19.90	15.46	16.40	15.93
SEm ±	1.28	2.33	1.15	0.70	1.21	0.78	0.91	1.39	0.80	0.65	1.20	0.64	1.02	1.37	0.56
CD at 5%	3.86	NS	3.46	2.12	3.64	2.35	2.72	4.17	2.41	1.96	3.61	1.94	3.07	4.13	2.94

*S-Solvent, P-pH (S1-Acetone, S2-Methanol, S3-Distilled Water; P1-pH 1, P3-pH 3, P6-pH 6)

Table 2: Interaction effect of pH and solvents on betacyanin content of *Hylocereus polyrhizus* peel extracts

Treatments	Day-6			Day-7			Day-8			Day-9			Day-10		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
S_1P_1	5.64	6.01	5.83	5.60	6.15	5.88	4.66	5.64	5.15	5.11	4.61	4.86	5.14	7.56	6.35
$S_1 P_3$	8.71	8.94	8.82	9.90	9.33	9.61	9.98	10.46	10.22	8.04	8.38	8.21	9.32	9.79	9.56
$S_1 P_6$	4.94	5.25	5.09	5.08	5.27	5.17	6.37	6.73	6.55	5.19	3.34	4.26	7.52	8.52	8.02
$S_2 P_1$	14.78	10.47	12.62	11.36	04.04	7.70	9.25	8.36	8.80	7.02	4.59	5.80	9.58	3.52	6.55
$S_2 P_3$	25.86	11.46	18.66	27.47	11.48	19.48	20.11	14.94	17.52	20.94	10.95	15.95	19.76	7.64	13.70
$S_2 P_6$	33.16	11.67	22.41	31.53	10.59	21.06	29.91	15.09	22.50	23.22	11.11	17.16	16.65	10.16	13.41
$S_3 P^1$	12.57	10.69	11.63	23.42	10.66	17.04	17.55	16.53	17.04	14.87	12.50	13.68	6.23	10.93	8.58
$S_3 P_3$	23.27	23.80	23.54	21.21	21.15	21.18	21.61	23.67	22.64	21.24	17.41	19.33	23.57	10.44	17.00
$S_3 P_6$	16.42	10.76	13.59	15.28	12.86	14.07	16.37	16.11	16.24	23.09	13.66	18.38	23.26	9.75	16.50
SEm±	1.16	1.29	1.11	1.20	0.87	0.76	1.15	0.86	0.49	0.94	0.88	0.72	1.20	0.65	0.73
CD at 5%	3.48	3.86	3.34	3.61	2.62	2.28	3.44	2.57	2.55	2.84	2.64	2.18	3.59	1.95	2.19

*S-Solvent, P-pH (S1-Acetone, S2-Methanol, S3-Distilled Water; P1-pH 1, P3-pH 3, P6-pH 6)

Table 3: Interaction effect of pH and solvents on betacyanin content of *Hylocereus polyrhizus* pulp extracts

Treatments	Day-1			Day-2			Day-3			Day-4			Day-5		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
S_1P_1	21.00	24.20	22.60	16.17	16.32	16.24	13.38	14.52	13.95	7.33	8.78	8.05	9.83	9.14	9.49
$S_1 P_3$	20.14	20.95	20.55	20.35	20.13	20.24	10.21	10.57	10.39	13.79	11.81	12.80	14.39	11.18	12.78
$S_1 P_6$	17.64	20.35	19.00	18.55	19.87	19.21	16.66	18.05	17.36	4.33	6.52	5.42	5.76	8.37	7.07
$S_2 P_1$	48.33	33.46	40.89	39.97	27.00	33.48	36.18	19.85	28.01	10.18	12.98	11.58	7.64	7.95	7.79
$S_2 P_3$	50.65	35.98	43.33	56.36	32.95	44.65	39.62	18.77	29.19	16.85	19.36	18.10	18.39	15.95	17.17
$S_2 P_6$	47.50	39.33	43.41	45.48	33.31	39.40	34.77	36.00	35.39	33.06	12.73	22.89	20.79	22.27	21.53
$S_3 P_1$	34.98	36.25	35.62	27.37	24.53	25.95	21.87	23.62	22.74	23.27	23.62	23.44	21.61	20.15	20.88
$S_3 P_3$	39.50	40.10	39.80	35.23	39.77	37.50	36.62	40.28	38.45	39.16	40.80	39.98	35.67	37.98	36.83
$S_3 P_6$	32.63	33.24	32.94	26.37	30.78	28.57	40.27	42.10	41.19	40.17	43.76	41.96	38.84	40.84	39.84
SEm±	1.57	1.76	1.21	0.97	0.77	0.49	0.91	1.50	0.90	1.26	1.07	0.93	1.08	0.85	0.55
CD 5%	NS	5.27	3.62	2.92	2.31	1.48	2.74	4.49	2.71	3.79	3.20	2.81	3.25	2.54	1.67

*S-Solvent, P-pH (S1-Acetone, S2-Methanol, S3-Distilled Water; P1-pH 1, P3-pH 3, P6-pH 6)

Table 4: Interaction effect of pH and solvents on betacyanin content of *Hylocereus polyrhizus* pulp extracts

Treatments	Day-6			Day-7			Day-8			Day-9			Day-10		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
S_1P_1	5.29	5.79	5.54	5.48	6.23	5.85	8.03	8.56	8.29	6.11	5.77	5.94	5.05	6.46	5.75
$S_1 P_3$	5.47	6.22	5.84	6.71	6.89	6.80	9.17	10.15	9.66	5.50	6.35	5.93	7.42	7.33	7.38
$S_1 P_6$	4.66	6.78	5.72	5.52	6.72	6.12	7.38	8.49	7.94	5.00	5.17	5.08	5.33	5.38	5.35
$S_2 P_1$	8.81	6.02	7.42	6.89	4.54	5.70	3.12	8.08	5.60	3.28	4.04	3.66	2.67	6.30	4.48
$S_2 P_3$	16.10	8.19	12.18	10.50	8.48	9.49	22.06	11.44	16.75	10.40	7.84	9.12	5.56	7.13	6.35
$S_2 P_6$	34.52	18.10	26.21	31.13	12.98	22.05	27.48	17.21	22.34	18.50	13.15	15.83	14.42	9.57	11.99
$S_3 P_1$	16.89	16.77	16.83	14.75	13.16	13.95	15.00	14.94	14.97	10.43	10.60	10.51	10.61	10.72	10.66
$S_3 P_3$	35.82	32.45	34.14	33.21	31.74	32.48	35.49	35.85	35.67	31.42	31.78	31.60	22.29	26.11	24.20
$S_3 P_6$	36.22	38.27	37.25	37.58	38.43	38.01	36.93	39.81	38.37	36.53	37.17	36.85	30.51	29.49	30.00
SEm±	1.09	1.30	0.74	1.08	1.08	0.68	1.05	0.60	0.52	1.07	0.91	0.60	1.01	0.77	0.49
CD at 5%	3.26	3.89	2.21	3.24	3.23	2.06	3.14	1.81	1.57	3.22	2.74	1.81	3.05	2.31	1.47

*S-Solvent, P-pH (S1-Acetone, S2-Methanol, S3-Distilled Water; P1-pH 1, P3-pH 3, P6-pH 6)

yielded the lowest extraction (Table 1). But, methanol at pH 6 and pH 3 gave the highest extraction on Day-2 and Day-3. However, from Day-4 to Day-10 distilled water at pH 3 recorded highest extraction (Table 2). Acetone consistently showed the least extraction when compared to the other two solvents.

Betacyanin extracted with either distilled water or methanol gave similar results initially (with slight variation), but pigment remained stable for longer period when distilled water was used as the solvent (Table 1). Tang and Norziah[16] reported similar results in *Hylocereus polyrhizus* pulp and Castellar *et al.* (2006) also stated that the better extraction of betalin in opuntia was found when water was used as solvent. This might be due to the fact that the water is considered as high polar solvent followed by methanol and acetone. Due to the high molar absorbency index betacyanin is more soluble in water compared to non-polar solvents hence making water as a suitable solvent for extraction[15]. Similarly, the pigment remained stable at pH range of 3 and 6 till the tenth day. This is in line with the findings of Woo *et al.*[18] who stated that betacyanin exhibited stability at the pH range of 3, 5 and 7. Valliant *et al.*[17] also reported that pH 4 to 6 is the optimal pH range for betanin pigment stability. The degradation of the pigment might be due to the influence of different pH ranges which undergoes dehydrogenation, C_{15} isomerisation[2,13] and might also be due to the cleavage in the bond of nitrogen atom, dehydrogenation in carboxyl group and deglycosylation[6].

Effect of pH and solvents on pulp extracts

The interaction effect of betacyanin concentration in the dragon fruit pulp stored for 10 days revealed that on Day-1methanol recorded highest pigment content at pH 6 (43.41 mg/100 g) and pH 3 (43.33 mg/100 g) was found at par with it. Meanwhile acetone at pH 6 (19.00 mg/100 g) recorded the least extraction. On Day-2 methanol at pH 3 gave the highest extraction. However, from Day-4 to Day-10, distilled water at pH 6 recorded highest extraction while acetone recorded the lowest extraction when compared with other solvents (Table 3,4).

Betacyanin extracted with methanol gave best results initially but the pigment remained more stable in the sample's where distilled water was used as solvent. This might be due to the fact that water has high polarity compared to methanol and acetone due to its molecular structure and properties. Water having high polarity and strong hydrogen bonding capability makes an excellent solvent for betacyanin pigment extraction. Similarly, the pigment remained stable at pH 3 and pH 6 till the tenth day. The betacyanin content in pulp recorded highest values compared to peel. The results are in conformity with Abdul Razak *et al.*[1] and Tang *et al.* [16].

After a storage period of ten days the degradation in the colour of samples occurred. Woo et al. 18 reported that there was 80 to 90% change in colour after one week of storage. Degradation of colour also occurred after two days of storage in red beet11. Peel samples extracted with acetone were found completely degraded at pH 1 and pH 6 and samples extracted with methanol also showed loss in colour but left with a tinge of orange. Contrary to the above solvents distilled water at pH 1 and pH 3 retained purple colour even after ten days of storage and complete change in colour was observed at pH 6. Pulp Top of Form samples extracted with acetone were found completely degraded at pH 1, 3 and 6 and those with methanol showed loss in colour with a tinge of orange. Contrary to the above solvents distilled water at pH 1, 3 and 6 retained purple colour even after ten days of storage. The colour retention might be due to the fact that betacyanin is water soluble due to its chemical structure where interaction occurs between polar groups and water molecules through hydrogen bonding thus making water as a suitable solvent for pigment extraction. Lim et al.[7] stated that though betacyanin displays broad pH range from 3 to 7 it was found that pH 6 was considered as the optimum pH for stability of betacyanin pigment. It can be concluded that the betacyanin content extracted from red flesh dragon fruit with distilled water as a solvent resulted in high pigment extraction compared to other solvents and it also revealed that pH of the sample had greater effect on stability of the pigment. Distilled water as a solvent at pH 3 for peel and pH 6 for pulp may be recommended for extraction and stability of betacyanin pigment for industrial use as natural pigment.

Conflict of interest

The authors confirms that there is no conflict of interest related to the manuscript

Authors contribution: Kosgi Mounika was a major contributor in writing the manuscript and conducting the experiment. Pauline Alila and C.S. Maiti designed the experiment, read and approved the final manuscript.

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