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Colour Extraction from Dragon Fruit (*Hylocereus spp.*) Peel

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ABSTRACT

Background: The peels of dragon fruits, usually discarded as waste after fruit processing, contains betacyanin, a red-purple pigment that can be utilised to replace synthetic colours in food industry. This can be an efficient waste management system and can provide additional income to the farmers.

Methods: Colour extracts were prepared from peels of both white and purple type dragon fruits by different maceration techniques *viz.*, hydromaceration, maceration in ethyl alcohol, hydromaceration of freeze-dried peel, low temperature hydromaceration and low temperature ethyl alcohol maceration for 24 hours adopting 1:2 peel to solvent ratio. Extracts prepared by the two best extraction methods were subjected to ambient and low temperature (4 to 7°C) storage for three months for analyzing storage stability.

Result: Hydromaceration of purple dragon fruit peel produced extracts with the highest betacyanin content. Highest total antioxidant activity was found in extracts prepared by low temperature hydromaceration of purple dragon fruit peel. Hydromaceration of purple-fleshed dragon fruit peels for 24 hours using 1:2 peel solvent ratio and storage at low temperature of 4 to 7°C can be recommended as the best maceration method for betacyanin pigment extraction.

Key words: Betacyanin, Colour stability, Dragon fruit, *Hylocereus spp.*, Maceration, Natural pigment, Peel utilisation.

INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is an emerging exotic fruit crop in India and the area of production as well as its popularity is increasing nowadays not only because of its attractive colour and economic value, but for its high nutritional value and antioxidant potential (Liaotrakoon, 2013). Dragon fruit tastes mildly sweet and can be eaten as a fresh fruit, where 70-80% of the fruit is edible, filled with tiny black edible seeds. Stems, blooms, peel and pulp of the fruit are only a few among the several parts that contain bioactive substances that are engaged in a wide range of advantageous biological processes (Luu *et al*., 2021). Owing to its nutritional attributes and commercial importance, the significance of dragon fruit in the processing industry cannot be overlooked.

Natural source of food additives plays prominent role in improving human health conditions. However, importance and application of these additives have been widely neglected in food industry. Dragon fruit peel is identified as a potential source of pectin and red purple colour with a moderate antioxidant activity for food and cosmetic preparation. The fruit consists of 36.70% to 37.60% of peel which is normally discarded during processing, which creates environmental problems (Jamilah *et al*., 2011). Developing natural food colorant and its utilization is not only healthy for human body, but also is environment friendly, as it is sourced from a disposable part, peel of the fruit, without affecting the natural benefit of dragon fruit (Lourith and Kanlayavattanakul, 2013). The natural colour extract yield was reported to be higher in peels of grape and pomegranate. Anthocyanin content in grape and pomegranate and betalain content in beetroot were high in peel (Mini *et al*., 2021). Hence an experiment was undertaken at Department of Postharvest Management of College of Agriculture, Vellayani during the time period of 2022-2023, with the objective to standardize the colour extraction procedures from peel of different dragon fruit types and assess its storage stability.

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MATERIALS AND METHODS

Peels of good quality uniform ripe dragon fruits of two types; white flesh with pink skin, *Hylocereus undatus* (F_1) and purple flesh with red skin, *Hylocereus polyrhizus* (F_2) were collected from processing unit of the Department of Postharvest Management and utilized for the study.

Standardization of peel colour extraction

The dragon fruit peels were washed thoroughly in clean water, chopped into small pieces of approximately l cm^3 and utilized for colour extraction. The peel pieces of both types were subjected to five different maceration techniques *viz.*, C_1 - Hydro-maceration, C_2 - Maceration in ethyl alcohol, C_3 - Hydro-maceration of freeze-dried sample, C_4 - Low temperature hydro- maceration (4 to 7°C), C_5 - Low temperature maceration in ethyl alcohol (4 to 7°C) by adopting 1:2 peel to solvent ratio in general for 24 hours (Lourith and Kanlayavattanakul, 2013). In C_1, C_3 and C_4,

distilled water was utilized as the solvent. In C_3, the pieces were subjected to freeze drying till 11% moisture content before hydro maceration. Except C_4 and C_5, all the other treatments were carried out under ambient temperature. After the maceration, solvent was removed using water bath at 60°C to collect the extract. The experiment was conducted in Completely Randomised Design with 10 treatments and replicated twice.

Quality parameters of peel colour extract

The peel colour extracted by five different maceration methods were analyzed for betacyanin content and total antioxidant activity according to standard procedures.

The betacyanin content of the colour extract was estimated using the method suggested by Stintzing *et al*., 2003. The colour extract was diluted in 10 ml distilled water and maintained at pH 7. Sample was agitated for 10 seconds and then homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was collected after centrifugation and repeated same step twice to ensure maximum betacyanin extraction. The final supernatant was collected and betacyanins in the concentrates were resolved by UV-VIS spectrophotometer at 538 nm, using the formula:

$$\text{Betacyanin content [mg L}^{-1}\text{]} = \frac{[(A \times DF \times MW \times 1000)]}{(\varepsilon \times L)}$$

Where:
A= Absorption at 538 nm.
DF= Dilution factor.
L= Pathlength (1 cm) of the cuvette.
MW= Molecular weight (550 g mol^{-1}).
ε= Molar extinction coefficient (60000 L mol^{-1}).

Total antioxidant activity of peel colour extract was assessed using 2, 2- diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay (Sharma and Bhat, 2009). Based on high betacyanin content and antioxidant activity, the two superior maceration methods were selected for further storage analysis.

Storage stability analysis of peel colour extract

The peel colour extracts prepared using the two superior maceration methods were stored in amber coloured glass bottles under two different *viz*., ambient (S_1) and refrigerated (S_2) conditions and analyzed for betacyanin content and total antioxidant activity, initially and at monthly intervals for a period of three months to assess the storage stability and selection of the best extraction procedure. Storage stability was assessed by calculating the percent reduction in betacyanin content and antioxidant activity. The method which resulted in production of extracts with minimum percent reduction during storage was selected as the best maceration method. The experiment was carried out in CRD with 4 treatments, replicated 4 times.

RESULTS AND DISCUSSION

Significant differences were noticed between the betacyanin content of colour extracts prepared from different fruit types, maceration methods and their interaction (Table 1).

Effect of fruit types and maceration methods on betacyanin content

Between the fruit types, extracts prepared from purple fruits had higher betacyanin content (46.097 mg L^{-1}) (Table 1) which was in accordance with the findings of Herbach *et al*. (2007) who proposed purple pitaya (*Hylocereus polyrhizus*) as a natural source of red-violet colour. Suh *et al*. (2014) reported that *H. polyrhizus* peel contains higher betacyanin content than that of *H.undatus* (white-fleshed pitaya), which can be the reason for superior betacyanin content in the extracts prepared from the peels of purple dragon fruit.

When the maceration techniques were analysed, the highest betacyanin content was recorded in the extracts prepared by hydromaceration (C_1) (38.74 mg L^{-1}). This was in agreement with the findings of Lourith and Kanlayavattanakul (2013), where water was found to be the best solvent for betacyanin extraction from dragon fruit peel among other solvents like ethanol and n-Hexane. Selection of the solvent is always crucial for maceration. Selectivity, solubility, cost and safety should be considered in selection of solvents. When the interaction effect was considered, the highest betacyanin content (67.14 mg L^{-1}) was observed in the colour extract prepared from the hydro-maceration of purple dragon fruit peel which was found to be significantly superior to all other extracts. The betacyanin content of the extract prepared from purple dragon fruit peel by low temperature hydro-maceration method was the next highest, 53.8 mg L^{-1}, which was on par with the one prepared from the same fruit type by ethyl alcohol maceration (46.88 mg L^{-1}).

Table 1: Effect of fruit types and maceration methods on betacyanin content (mg L^{-1}).

Fruit types (F)	Betacyanin content (mg L^{-1})					
	Maceration methods (C)					Mean (F)
	C_1	C_2	C_3	C_4	C_5	
White ($F_{1)}$	10.35	6.64	4.03	5.04	8.24	6.86
Purple ($F_{2)}$	67.14	46.88	29.33	53.8	33.32	46.09
Mean (C)	38.74	26.76	16.68	29.42	20.78	
S.Em±		F - 1.09,	C- 1.73,	F × C - 2.44		
CD at 5%		F - 3.22,	C - 5.09,	F × C - 7.21		
CV %		F- 15.977,	C - 15.977,	F × C - 15.977		

Hydromaceration of freeze-dried white dragon fruit peel resulted in extracts with least betacyanin content (4.03 mg L^{-1}), which was on par with all the extracts prepared from white fruit types irrespective of extraction methods.

Effect of fruit types and maceration methods on total antioxidant activity

Significant difference was observed between the total antioxidant activity of colour extracts prepared by different maceration methods, fruit types and their interaction (Table 2).

Betacyanins are nutrient-dense pigments that have both colouring and antioxidant properties. Comparing the fruit types, extracts from purple type (F_2) showed superior antioxidant activity (70.56%) compared to that from white type with 54.24% activity. Suh *et al*. (2014) also reported that peels of *H. polyrhizus* (purple pitaya) exhibited higher antioxidant activity than peels of white pitaya. Comparing the maceration methods, low temperature ethyl alcohol maceration resulted in extracts with highest antioxidant activity (72.37%) and lowest activity was observed in extracts prepared by hydromaceration of freeze-dried peel (53.02%). Considering the interaction, the extracts prepared by low temperature hydromaceration of purple dragon fruit peel had the highest antioxidant activity (83.61%). This was on par with the extract prepared by low temperature maceration of purple fruit peel in ethyl alcohol (82.97%). Higher antioxidant activity can be accounted to the reduced oxidative and enzymatic reactions of the peel when kept in an inert environment. This was in agreement with the study conducted by Zainoldin and Baba (2009) in the antioxidant activity of purple dragon fruit seed extracts. Also the antioxidant activity of the extract is contributed not only by betacyanins, but also by phenols, flavonoids and polyphenols (Pandey *et al*., 2018). The lowest antioxidant activity (40.3%) was observed in the extract prepared by low temperature hydro-maceration of the white type fruit peel, which was significantly different from all other treatments.

Among the five maceration techniques, since betacyanins are thermolabile compounds the hydromaceration of the freeze-dried peel (C_3) was expected to yield extracts with higher betacyanins. On the contrary, it recorded the lowest betacyanin content of 4.03 mg L^{-1} and 29.33 mg L^{-1} in the extracts obtained from the peels of white and purple dragon fruits respectively. This may be due to the lack of acidification process before freezing the peel. Acidification of the solvent medium increased the extraction efficiency of anthocyanin pigment from mangosteen pericarp (Aparna and Lekshmi 2023). However, freeze drying allows production of good quality colour even without addition of colour stabilizers (Dziki *et al*., 2015). This drying technique is reported to be most suitable for materials sensitive to high temperature (Dong *et al*., 2017). The accepted protocol for obtaining natural food colours by freeze drying is extraction, filtration, concentration followed by freeze drying. But in the present experiment freeze drying was adopted as a pre-treatment to maceration, thus the procedure adopted was also different, hence the difference in coloue extraction efficiency. For conversion of betacyanin extracts into powder form, freeze drying can be the most appropriate method, as it does not involve any heat treatment and will also help in keeping the colour at optimum pH range and other physico-chemical properties intact. But it is time consuming and expensive method (Shishehgarha *et al*. 2002).

In general, hydro-maceration of the purple type peel had the highest betacyanin content (67.14 mg L^{-1}), high total antioxidant activity (62.49%). Low temperature hydro-maceration of purple fruit peel had a high betacyanin content of 53.8 mg L^{-1} and highest total antioxidant activity of 83.61%. Hence these extraction methods *viz*., hydro-maceration and low temperature hydro-maceration of purple fruit peel were selected as the two superior colour extraction procedures for storage stability analysis.

Storage stability analysis of peel colour extract

Though betacyanins have properties like high water solubility and colour intensity. However one of the main problems that limit application of betacyanin is their instability (Castellar *et al*., 2003). For studying the extraction method, it is not enough to consider only the extraction medium; it is necessary to have knowledge on how to maintain its stability and how a specific pigment will behave with the temperature variation, exposure to light, pH and storage. Hence analysing the storage stability of the extract is very important to assess its suitability as food colourant. The stability of betacyanin is influenced by various factors including internal factors like pH and water activity and

Table 2: Effect of fruit types and maceration methods on total antioxidant activity (%).

Fruit type (F)	Total antioxidant activity (%)					
	Maceration methods (C)					Mean (F)
	C1	C2	C3	C4	C5	
White (F_1)	54.04	57.13	57.95	40.3	61.78	54.24
Purple (F_2)	62.49	75.64	48.1	83.61	82.97	70.56
Mean (C)	58.26	66.38	53.02	61.95	72.37	
S.Em±		F - 0.897,	C - 1.418,	F × C - 2.005		
CD at 5%		F - 2.645,	C - 4.182,	F × C - 5.915		
CV %		F- 5.565,	C- 5.565,	F × C - 5.565		

external factors like light and temperature (Herbach *et al*., 2006). The storage stability of two superior colour extracts prepared by hydromaceration and low temperature hydromaceration of purple dragon fruit peel were analysed at both ambient and low temperature (4 to 7°C) storage conditions.

Effect of storage on betacyanin content in colour extract

When the extracts were stored for testing the stability, there was significant difference between the betacyanin content of extracts prepared by the two maceration methods (Table 3). The extracts prepared by hydromaceration and low temperature hydromaceration methods had a betacyanin content of 67.34 and 53.79 mg L^{-1} betacyanin content respectively at the time of storage.

There was significant difference between betacyanin content of the extracts prepared by two maceration methods as well as their storage conditions through out the storage period. Between the maceration techniques, higher betacyanin content (41.73 and 10.54 mg L^{-1}) was in the extract prepared by hydromaceration compared to the one prepared by low temperature hydromaceration (27.06 and 3.09 mg L^{-1}) at 1st and 3rd month of storage. Considering the storage conditions, the extract stored at low temperature had a higher betacyanin content of 38.41 and 10.59 mg L^{-1}, which were significantly higher than those stored at ambient storage condition (30.38 and 3.04 mg L^{-1}) at 1st and 3rd month of storage respectively. Generally extraction under ambient temperature increases the solubility and diffusion, thereby improving the extraction efficiency. Interaction effect of extraction methods and storage condition was non-significant at one month of storage. When the interaction effect was considered after three months of storage, the extract prepared by hydromaceration and stored at low temperature had the highest betacyanin content of 17.63 mg L^{-1}, which was significantly superior to all other treatments. When stability of pigment was analysed by calculating the percent reduction in betacyanin content during storage, the reduction in betacyanin content of hydromacerated extract of purple dragon fruit peel at one month after storage was 44.56% and 31.5% at ambient and low temperature storage respectively. The reduction was 94.88% and 73.81% at ambient and low temperature storage after a period of three months. Betacyanin content of the colour extract prepared by low temperature hydromaceration had a reduction of 56.44% and 42.94% at ambient and low temperature conditions respectively after one month of storage and this was increased to 95.11% and 93.40% after three months of storage. Factors such as high temperature, light and oxygen pressure can reduce the stability of betacyanins. Besides, the degree of glycosylation or acylation of betacyanins can also influence its stability. The chemical nature of betacyanins can change, depending on the source of the plant used for the extraction (Khan, 2016). Thus, stability is an important aspect to consider before using these natural compounds as colourants and antioxidants in foods (Otálora *et al*., 2016). Prior to the extraction step, pre-treatment of plant materials to enhance the stability of natural pigments is another important step that must be carefully taken care of (Ngamwonglumlert *et al*., 2017). It has been reported that betacyanins are still not widely used by the industry due to their low stability during processing and storage (Celli and Brooks, 2017).

Effect of storage on total antioxidant activity of colour extract

Antioxidant activity of the extracts prepared by low temperature hydromaceration and hydromaceration were 83.61 and 62.50% respectively at the time of storage (Table 4).

The antioxidant activity of the colour extracts after one month of storage showed significant difference with respect to the extraction methods and its interaction with storage conditions. Extract prepared by hydromaceration had total antioxidant activity of 58.54%, which was significantly different and superior to the extract prepared by low temperature hydromaceration (51.25%). The colour extract prepared by hydromaceration and stored at low temperature had higher antioxidant activity of 60.03%, which was on par

Table 3: Effect of storage on betacyanin content (mg L^{-1}) of the colour extracts.

Treatments	Initial	1 MAS			2 MAS			3 MAS		
		Betalain content	P value	T value	Betalain content	P value	T value	Betalain content	P value	T value
T_1 (C_1+ S_1)	67.34	37.33	0.00	18.46	10.11	0.0002	20.95	3.45	0.00	22.23
T_2 (C_1+ S_2)		46.13	0.00	36.42	27.67	0.0003	18.57	17.63	0.00	20.63
T_3 (C_4+ S_1)	53.79	23.43	0.00	24.32	7.38	0.00	101.15	2.63	0.00	133.94
T_4 (C_4+ S_2)		30.69	0.00	30.91	7.30	0.00	40.54	3.55	0.00	129.52

P value <0.05, the null hypothesis is rejected, there is significant difference between the betalain contents of extracts in different months of storage.

T_1 - Extract prepared by hydromaceration and stored under ambient storage condition.

T_2 - Extract prepared by hydromaceration and stored under low temperature storage condition.

T_3 - Extract prepared by low temperature hydromaceration and stored under ambient storage condition.

T_4 - Extract prepared by low temperature hydromaceration and stored under low temperature storage condition.

Table 4: Effect of storage on total antioxidant activity (%) of the colour extracts.

Treatments	Initial	1 MAS			2 MAS			3 MAS		
		Betalain content	P value	T value	Betalain content	P value	T value	Betalain content	P value	T value
T_1 (C_1+ S_1)	62.50	57.04	0.02	3.97	21.39	0.00	239.07	19.34	0.00	61.37
T_2 (C_1+ S_2)		60.03	0.00	46.54	27.63	0.00	47.86	24.54	0.00	99.33
T_3 (C_4+ S_1)	83.61	52.71	0.00	42.46	20.57	0.00	223.75	17.59	0.00	121.63
T_4 (C_4+ S_2)		49.80	0.00	18.95	21.47	0.00	39.58	21.25	0.00	39.56

P value <0.05, the null hypothesis is rejected, there is significant difference between the total antioxidant activity of extracts in different months of storage.

with the extract prepared by hydromaceration and stored at ambient storage conditions (57.04%). The extract prepared by low temperature hydromaceration and stored at low temperature had the lowest antioxidant activity (49.80%) which was on par with the extract prepared by the same method, but stored under ambient temperature.

Significant difference in the total antioxidant activity of extracts was observed with respect to maceration methods and storage conditions after three months of storage. The extract prepared by hydromaceration had higher antioxidant activity (21.94%), which was significantly different from the extract prepared by low temperature hydromaceration (19.42%). Comparing the storage conditions, the extract stored at low temperature had an antioxidant activity of 22.90%, which was significantly different and superior to extract stored at ambient storage condition (18.46%). Total antioxidant activity of extracts reduced within a storage period of three months. When the stability was analysed in the first month of study, there was a reduction of 3.9% and 8.7 % in the antioxidant activity of the extract prepared by hydromaceration and stored at low temperature at ambient and low temperature storage respectively. The reductions were 69.06 % and 60.74% at after a period of three months. Hence it was concluded that the hydromaceration of purple dragon fruit peel and storage at low temperature of 4-7°C retains better antioxidant activity at one month of storage period. The changes in the antioxidant activity can be traced by evaluating each compound that holds antioxidant potential as followed by Kevers *et al*. (2007). Antioxidant activity in dragon fruit is contributed by various food components like Vitamin C, Vitamin E, phenolic compounds and their interaction (Patthamakanokporn *et al*., 2008).

In general, storage of colour extract resulted in reduction in betacyanin content and antioxidant activity. But the reduction was comparatively less in hydromacerated samples stored under low temperature (4 to 7°C) condition exhibiting better stability. This can be due to isolation from temperature and oxygen exposure compared to the ambient storage conditions. There was significant reduction with in a period of one month itself, proving that the colour extract from dragon fruit prepared by maceration cannot be stored even under low temperature storage condition. Storage of betacyanin colour caused degradation due to the water activity of the extract obtained, which was in agreement with Cai *et al*., 2001. The stability can be enhanced by converting the betacyanin colour extracts into powder form. Process involving heat should be avoided, as the betacyanin pigment is thermolabile and can be easily degraded at a temperature above 40°C (Herbach *et al*., 2006).

CONCLUSION

The colour extracts prepared by hydromaceration of l cm^3 purple dragon fruit peel pieces for 24 hours adopting 1:2 peel to solvent ratio and storage of extracts in amber coloured glass bottles under low temperature (4 to 7°C) exhibited better stability in betacyanin content and total antioxidant activity.

Conflict of interest

All authors declare that they have no conflicts of interest.

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