

Phytochemical properties, antioxidant potential and fatty acids profiling of three dragon fruit species grown under sub-tropical climate

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Abstract

The physical, biochemical and antioxidant properties of one white pulped (*Hylocereus undatus*; DG-I) and two red pulped dragon fruit species (*H. polyrhizus*, DG-II; *H. costaricensis*, DG-III) grown under sub-tropical conditions of north-west India were determined. Fruit size, fruit weight, pulp weight and pulp: peel ratio was significantly higher in DG-III, though the fruit numbers and yield per pillar was significantly less than other species. The pH, TSS, acidity, total sugar, reducing sugars and moisture content in all the species varied between 4.78-5.72, 8.63-9.31 °Brix, 0.30-0.56%, 6.64-6.91%, 4.60-4.76% and 83.44-85.82%, respectively. Total phenols and flavanols content in DG-I was 24.04 mg GAE 100 g⁻¹ and 14.54 mg RE 100 g⁻¹, whereas in red pulped it was significantly higher; varying between 49.12-56.40 mg GAE 100 g⁻¹ and 30.41-31.10 mg RE 100 g⁻¹ fruit pulp, respectively. β-carotene values in red pulped species DG-II and DG-III were 47.48 and 43.82 μg 100 g⁻¹, respectively compared to corresponding values of 1.96 μg 100 g⁻¹ in DG-I, a white pulped dragon fruit. Similarly, DPPH-RSC, FRAP, CUPRAC and ABTS values for red pulped dragon fruit ranged between 238.98-262.04 μmol 100 g⁻¹, 358.8-386.40 μmol TE 100 g⁻¹, 830.40-917.0 μmol TE 100 g⁻¹ and 571.4-589.60 μmol 100 g⁻¹, respectively in DG-II and DG-III in comparison to respective values of 108.75 μmol 100 g⁻¹, 192.6 μmol TE 100 g⁻¹, 525.6 μmol TE 100 g⁻¹ and 400.2 μmol 100 g⁻¹ in DG-I. The β-lain, responsible for imparting red colour in DG-II and DG-III was absent in white pulped DG-I. Seed oil content in both groups of dragon fruit varied between 31.90-33.5% with highest proportion of an essential fatty acid, linoleic acid (46.32-47.96%). In conclusion, red pulped dragon fruit has a considerably higher antioxidative potential than white one and these species may play a vital role in ensuring nutritional security for millions of people in developing nations.

Keywords: antioxidants; β-carotene; fatty acids; total sugars; dragon fruit

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Introduction

Dragon fruit, commonly known as Pitaya belongs to Cactaceae family and *Hylocereus* genus. The plants of this genus are perennial herbaceous climbers. The native place of dragon fruit is Mexico, Costa Rica and Guatemala (Mizrahi *et al.*, 1997). This fruit crop is primarily distributed in tropical regions and now spreading to sub-tropical regions at faster pace owing to its wider adaptability to varied soil and climatic conditions. Moreover, high nutritional value, precocious bearing, long plant life and fruiting period, tolerance to various biotic and abiotic stress factors, easy propagation and high market price made it one of the most preferred fruits worldwide. Presently, Vietnam, Taiwan, Thailand, Philippines, Malaysia, Sri Lanka, China, Israel, America, Mexico, Australia and India are mainly cultivating dragon fruit (Waghmare *et al.*, 2021) and it is also gaining momentum in India. On the basis of fruit characteristics, *Hylocereus* have five species; *H. undatus* (pink peel and white pulp fruits), *H. polyrhizus* (pink peel and red pulp fruits), *H. costaricensis* (pink peel and violet-red pulp fruits), *H. guatemalensis* (reddish-orange peel and red pulp fruits) and *H. megalanthus* (yellow peel and white pulp fruits). Small black seeds are scattered throughout the pulp of all dragon fruit species. The fruits of dragon fruit especially with coloured pulp are very rich in antioxidants owing to presence of phytochemicals such as flavonoids, polyphenols, vitamins etc. Most of the investigations have been focused on *H. polyrhizus* (red pulped) and *H. undatus* (white pulped) owing to high commercial potential and wide distribution. According to numerous studies, different plant parts of various dragon fruit species varied in their antioxidant capacity (Choo and Yong, 2011; Nurul and Asmah, 2014; Jerônimo *et al.*, 2015; Moo-Huchin *et al.*, 2017; Mahdi *et al.*, 2018; Zain *et al.*, 2019; Wu *et al.*, 2019).

Dragon fruit comprise high dietary fibres and rich in various vitamins (Karunakaran and Arivalagan, 2019). Minerals such as potassium, calcium, zinc and magnesium and other bioactive compounds are also present in high proportion in dragon fruit pulp (Tran *et al.*, 2015). However, the nutritional contents are extremely variable in dragon fruit species (Jaafar *et al.*, 2009; Nurul and Asmah, 2014; Jerônimo *et al.*, 2015). Moo-Huchin *et al.* (2017) reported two classes of carotenoids viz. carotenes (lycopene and β -carotene) and xanthophylls (lutein and β -cryptoxanthin) in white pulped dragon fruit. Lutein ($30.8 \mu\text{g } 100 \text{ g}^{-1}$) and β -carotene ($209.1 \mu\text{g } 100 \text{ g}^{-1}$ fruit pulp) had higher proportion than others. The demand for natural products has been increasing for prevention of cellular impairment instigated by the free radicals involved in many cancer and other ailments (Young and Woodside, 2001). The disease preventing properties in natural produce might be ascribed to the presence of antioxidants having free radical scavenging characteristics. These include phenolic acids, flavonoids, vitamin C, alkaloids etc (Nyamai *et al.*, 2016; Gan *et al.*, 2017; Pehlivan, 2017; San Miguel-Chávez, 2017). Phenolic compounds are known to offer many health benefits, including prevention of cardiovascular diseases and cancer (Guimarães *et al.*, 2019). Insulin resistance, adipose hypertrophy and hepatic steatosis induced by high-fat diet can be negated with consumption of white pulped dragon fruit (Song *et al.*, 2016). Throughout the world, specifically in India, dragon fruit has drawn attention due to its sweet taste, pleasant flavour, attractive colour and enormous nutritional properties. Growing consumer preference, increasing demand of fresh dragon fruit in domestic and international market and high remuneration has lifted the potential of this fruit crop in India. Although, dragon fruit is a tropical cactus, but the commercial cultivation of this fruit is gaining momentum in sub-tropical areas too. In India, dragon fruit is primarily being cultivated in Southern and Central states, but it is also spreading in Northern and Eastern states. In Punjab and adjoining states in north-west India, which are typical subtropical regions, commercial cultivation of the dragon fruit has gained an impetus in all the districts. Despite the fact that the dragon fruit is rich in nutrients and offers a number of health benefits, information on the complete biochemical and nutritional profile is lacking. Thus, the aim of the present study was to generate the nutrient composition data for three dragon fruit species. The generation of data would facilitate the effective use of dragon fruit in a regular diet and may promote it as a “tropical superfruit” in the future.

Materials and Methods

Experimental site and establishment of dragon fruit plantation

The site of experiment is located at Fruit Research Farm, Punjab Agricultural University, Ludhiana, Punjab (India) (Latitude: 30.90° N, Longitude: 75.85° E and Altitude: 247 m above mean sea level). It is a sub-tropical region of north-west India with plain topography having 725 mm average annual rainfall and 11–13 h sunshine in the summers. The soil of the experimental site is sandy loam in nature having pH value about 8.0 and about 0.5% organic carbon. The cutting of white pulped dragon fruit *H. undatus*; DG-I plants were procured from National Institute of Abiotic Stress Management (NIASM), Pune (India) in March, 2017. The species with coloured flesh *H. polyrhizus*; DG-II (pink peel and red pulp fruits), *H. costaricensis*; DG-III (pink peel and violet-red pulp fruits) were procured from private commercial orchards and planted in the same year. The stem cuttings of 25-30 cm were planted in nursery poly-bags of 12 x 30 cm size filled with garden soil, farmyard manure and sand with equal proportion. poly-bag planted cuttings were kept under semi-shade conditions. Light and frequent watering was done to maintain optimum moisture level. The rooted cutting was planted after 5 months (August, 2017) at 8 × 8 feet distance. For training of dragon fruit plants, the concrete pillars of 7'6" length and 5.0" thickness (5.5' outside the soil and 2.0' inside the soil) with circular concrete ring of 2 feet diameter 3 -inch thickness at the top of pillar were used. A raised bed of about 6-inch was made around the fixed pillars and well rotten farm yard manure was mixed in soil. Four plants were planted on each side of pillar and were allowed to grow along with the height of pillar. For proper climbing and training of dragon fruit, the plants were tied with jute rope. The side growth of plants was regularly removed to ensure that the all plant climbs the support and reach the circular ring. Within one year of planting the vine passes through the concrete ring and attained a shape of an umbrella. Light dose of inorganic fertilizers was applied in February, June and August with total application of Urea 100: Single Super Phosphate 200: Muriate of Potash 100 g in first year with 20, 40 and 60% increase in following years. Well rotten farm yard manure at 10 kg per pillar was also applied in the month of February every year. Light and frequent irrigations were done to maintain the optimum moisture level in the root-zone of plants though drip irrigation system. The reproductive phase of plants started after 14-15 months of planting. After flower bud emergence, it takes 55-60 days to fruit harvest. The number of fruit bearing was too less to be analysed for yield and quality analysis for first year. The fruits harvested during 4th year of planting were analysed for physical and biochemical characteristics.

Sample collection and recording physical characteristics

Dragon fruit were harvested by clockwise twisting of fruits from the cladodes when more than 80% portion of the fruit attained a characteristic external colour. Fruits at appropriate maturity stage were harvested to record the fruit yield and other physical characteristics of fruits. The fruit size and weight were measured before taking the measurement of peel and inner pulp. The proportion of pulp and peel in terms of thickness and weight was recorded to calculate pulp:peel ratio.

Determination of moisture, TSS, pH, acidity, total and reducing sugars

Pulp and peel of dragon fruit samples were separated for biochemical analysis. Moisture content was estimated by following the procedure of AOAC (2010). The total soluble solids (TSS) of the fruit juice were measured using a refractometer (Model ATAGO-PAL-1 M/s Atago Co. Ltd. Japan) and expressed as °Brix. The pH of the fruits was measured by taking filtrate of 10 g of finely grounded pulp in 50 ml distilled water using pH meter (Model-361 μ controller-based pH system; M/s SYSTRONICS India Ltd.). The titratable acidity of the fruits was measured as per the standard procedure (AOAC, 2010) and calculated using the following formula.

Acidity % CAE = Volume of NaOH used (ml) × Normality of NaOH × milliequivalent factor × 100 / Weight of the sample (g)

Where, CAE= Citric Acid Equivalent; 0.064 is milliequivalent factor for citric acid.

Total sugars and reducing sugars were quantified from juice through procedure standardized by (AOAC, 2005). Total sugars are expressed in percent and measured by formula given under:

$$\text{Total sugars (\%)} = \frac{\text{Fehling g factor}(0.05) \times \frac{\text{Dilution} \times \text{Final volume} \times 100}{\text{Volume of filtrate}}}{\text{Volume of filtrate} \times \text{weight of sample}}$$

To quantify reducing sugar standard procedure was used and calculated by following equation.

$$\text{Reducing sugars (\%)} = \frac{\text{Fehling factor}(0.05) \times \text{Dilution} \times 100}{\text{Volume of juice} \times \% \text{Titre}}$$

Determination of antioxidants

Total phenols: Dried fruit sample (0.25 g) was homogenized in 10 ml of 80% methanol, which was then refluxed on a water bath of 70-75 °C for 2h. The volume of methanolic extract was made to 10 ml by washing with 80% methanol. Later, the Folin-Ciocalteu reagent (0.5 ml) was added to methanolic extract (0.5 ml, 50 g/ml) and shaken well. Saturated solution of sodium carbonate (1.0 ml) was added after 5 minutes. The absorbance of the blue color was measured on a spectrophotometer at 760 nm against the control after an hour of incubation at room temperature. Only water and reagent were used for preparation of the blank. The standard curve made with gallic acid (10-100 µg) (Thomas Baker Chemicals Private Limited, India) was used to calculate the concentration of the total phenols (mg GAE 100 g⁻¹, GAE = gallic acid equivalents).

Flavanols: Flavanols content was determined using 0.1M aluminium chloride according to Balabaa *et al.* (1974). To 1.0 ml of methanolic extract prepared as above, 1.0 ml of water and 3.0 ml of methanolic aluminium chloride were added and incubated for one hour at room temperature. Absorbance was recorded at 420 nm. Rutin (10-50 µg) was used as a standard and the results were expressed as mg of rutin equivalents (RE) per 100 g of dried fruit sample.

β-lains: β-lains content was determined by measuring the absorbance of aqueous extract at 538 nm using a spectrophotometer, and the concentration was calculated using the formula:

$$\text{Total } \beta\text{-lain content (mg 100 g}^{-1}\text{ fresh weight)} = \frac{A \times MW \times V \times DF \times 1000}{E \times L \times W \times 100}$$

where A- absorbance at 538 nm, MW- molecular weight of β-cyanin (535), V- volume of extract, DF – dilution factor, E- molar extinction coefficient of β-cyanin (60000), l-path length (1 cm) and W- weight of the sample.

β-carotene: About 50g of fresh fruit pulp sample was weighed, crushed and transferred to Erlenmeyer flask covered with aluminium foil to protect from sunlight exposure. To the extract, 50 ml of hexane: acetone: ethanol (2:1:1 v/v) was added and shaken for 30 min. The sample extract was filtered using separating funnel and part of the solvent hexane: acetone: ethanol was taken for quantitative testing. The concentration of the β-carotene (µg 100 g⁻¹) was calculated from the standard curve prepared by using β-carotene (5 µg /ml).

Total antioxidant potential: The potential was assessed in terms of reducing power using the CUPRAC and FRAP methodologies, as well as radical scavenging activity using the DPPH (1, 1'-diphenyl-12-picrylhydrazyl) and ABTS (2,2'-azinobis [3-ethylbenzothiazoline-6-sulphonic acid]) radicals. The method outlined by Brand-Williams *et al.* (1995) and Arnao *et al.* (2001) was used to measure the DPPH and ABTS radical scavenging activity. In both procedures, trolox served as a positive control. The 50% DPPH and ABTS radicals scavenging concentration (SC₅₀) of the sample as well as trolox to scavenge 50% of the DPPH and

ABTS radicals was calculated. Finally, the values of DPPH and ABTS for the samples were computed using the formula below.

$$SC_{50} \text{ of sample } (\mu\text{mol } 100\text{g}^{-1}) = \frac{SC_{50} \text{ concentration of trolox } (\mu\text{mol}) \times 100}{\text{Weight of the sample required for } SC_{50} \text{ (g)}}$$

The FRAP assay was done according to the method specified by Benzie and Strain (1996). The sample extract used for DPPH activity estimation was used for FRAP assay. The reaction mixture comprises of 0.5 ml of extract, 0.5 ml of phosphate buffer and 0.5 ml of potassium ferricyanide solution. The tubes were placed in water bath at 50 °C for 20 minutes followed by addition of 0.5 ml of 10% TCA and centrifugation of the contents to ascertain the precipitate formation. Later, in 1.0 ml of supernatant, 1.0 ml of distilled water and 0.1 ml of ferric chloride solution were added and absorbance was recorded at 700 nm after five minutes. CUPRAC assay was carried out using the method of Apak *et al.* (2004). About 1.0 ml each of alcoholic solution of neocuproine ($7.5 \times 10^{-3} \text{ mol L}^{-1}$), CuCl_2 solution ($1.0 \times 10^{-2} \text{ mol L}^{-1}$) and ammonium acetate (1 mol L^{-1} , pH 7.0) buffer solution was added followed by 100 μl of sample. The tubes were then kept in dark for 30 minutes, and absorbance was measured at 450 nm against the reagent blank. Trolox served as a positive control and results were expressed in $\mu\text{mol TE } 100 \text{ g}^{-1}$ of fresh fruit pulp.

Estimation of oil content and fatty acid composition in dragon fruit seeds

Oil content: Fruits of dragon fruit species harvested at horticultural maturity were wrapped in aluminium foil precisely and autoclaved for one hour. The peel of the fruits was separated from the pulp portion carefully. The sterilized pulp having seeds was washed repeatedly and agitated vigorously in water in a big glass beaker. The separated seeds settled at the bottom of the beaker were decanted from the beaker using a mesh. The seeds were then weighed and dried for overnight in an oven at 60 °C. Finely crushed weighed seed material of 4g was put into a thimble and placed into the extractor of Soxhlet apparatus with petroleum ether solvent in the flask and the oil extraction was carried out for 6-7 h at 40-60 °C (AOAC, 1970).

Fatty acid profiling: The fatty acid composition was determined by gas chromatography (GC) of fatty acid methyl esters (FAME). FAME were prepared from oil samples as per the procedure developed by Appelqvist (1968) and further 1 μl was injected at a split ratio of 90:1 onto the FAME column CP-Sil 88 with dimensions 25 m \times 0.25 mm \times 0.20 mm fitted in the GC model 7820A series (Agilent technologies) attached to a flame ionization detector and using nitrogen as a carrier gas. The separation was carried out at oven temperature programmed at 180-210 °C (rate 5 °C min^{-1}). Temperatures of detector and injector were maintained at 240 and 230 °C, respectively. The flow rate of nitrogen, hydrogen and air were 60-, 30- and 30- ml min^{-1} , respectively. Retention time (R_T) of standard fatty acyl esters were used for identification of peaks. The relative concentration of each fatty acid was calculated using EZ Chrome elite software. Individual fatty acids were expressed as percentages of total fatty acids.

Statistical analysis

All experiments were conducted on five replications and the values were expressed as means \pm standard deviation (SD). Variability between dragon fruit species for fruit and yield characteristics, biochemical composition, mineral constituents and fatty acid profile of dragon fruit seed oil was evaluated by ANOVA using RStudio version 2022.07.1 (www.rstudio.com). For the traits, where assumptions of ANOVA were not met, non-parametric equivalent, Kruskal-Wallis rank sum test was used to evaluate the variability in three dragon fruit species. Pearson correlation coefficients were also calculated using R studio.

Results

Plant and fruit characteristics

Statistically significant differences were observed for plant characteristics such as length of cladodes, cladode width, distance between areoles, length of areoles and number of spines among the tested dragon fruit species. Moreover, three dragon fruit species significantly differed for all the fruit characteristics except pulp (%) and pulp: peel ratio as depicted in Table 1. The variation in three dragon fruit species based on fruit characteristics and yield parameters is shown in Figure 1. The flowering and fruiting in all dragon fruit species started after 14-15 months of planting in the field, although the fruiting was asynchronous. In 2nd year, all the plants started fruit bearing. However, the fruit yield and physio-chemical properties were recorded during the 4th year after planting as sufficient and even fruit bearing initiated on all plants. Flowering in all species commenced after 32-36 days of flower bud appearance and after fruit set fruits become mature after 30-40 days.

Table 1. Variation for plant, fruit, biochemical and nutritional characteristics of three dragon fruit species

Traits	White pulp	Red pulp		ANOVA (<i>p</i> -value)
	<i>H. undatus</i> (DG-I)	<i>H. polyrhizus</i> (DG-II)	<i>H. costaricensis</i> (DG-III)	
	Mean ± SD	Mean ± SD	Mean ± SD	
Plant characteristics				
Length of cladodes (cm)	73.52 ± 1.69	66.48 ± 2.74	64.78 ± 3.44	0.000626*
Cladode width (cm)	3.49 ± 0.04	3.62 ± 0.06	3.54 ± 0.04	0.00311*
Distance between areoles (cm)	3.56 ± 0.10	3.86 ± 0.10	3.71 ± 0.08	0.000744*
Length of areoles (mm)	3.71 ± 0.09	3.10 ± 0.07	3.12 ± 0.03	6.95e-09*
Number of spines	4.00 ± 0.00	4.00 ± 0.00	3.60 ± 0.55	0.116 ^{kw}
Fruit characteristics and yield parameters				
Fruit numbers/pillar	25.6 ± 4.28	23 ± 2.92	15.4 ± 2.97	0.00145*
Fruit yield (kg/ pillar)	7.93 ± 1.45	6.96 ± 0.71	5.83 ± 1.00	0.0302*
Fruit length (cm)	12.96 ± 0.67	12.12 ± 0.45	13.35 ± 0.54	0.0141*
Fruit breadth (cm)	9.14 ± 0.27	8.98 ± 0.13	9.52 ± 0.34	0.0194*
Fruit weight (g)	309.86 ± 18.42	302.76 ± 9.03	378.52 ± 15.19	4.73e-06*
Peel weight (g)	72.52 ± 4.33	58.06 ± 2.99	65.88 ± 3.44	0.000156*
Pulp weight (g)	237.34 ± 17.21	244.70 ± 8.40	312.64 ± 11.85	1.37e-06*
Pulp (%)	76.60 ± 1.56	80.82 ± 0.95	82.60 ± 0.28	0.00193 ^{kw}
Yield (t/ha)	8.73 ± 1.59	7.66 ± 0.78	6.41 ± 1.10	0.0299*
Pulp: peel ratio	4.12 ± 0.09	4.02 ± 0.13	4.75 ± 0.09	0.006685 ^{kw}
Fruit set (%)	77.28 ± 3.42	86.16 ± 2.21	82.12 ± 1.56	0.000465
Peel colour	Pink	Reddish pink	Dark pink	-
Pulp colour	White	Dark red	Violet-red	-
Biochemical characteristics				
Moisture (%)	83.92 ± 0.59	85.82 ± 1.10	83.44 ± 0.86	0.00241*
TSS (°Brix)	8.99 ± 0.22	8.63 ± 0.13	9.31 ± 0.40	0.00706*
pH	4.78 ± 0.28	5.72 ± 0.29	5.38 ± 0.13	0.000178*
Acidity (%)	0.56 ± 0.04	0.30 ± 0.04	0.40 ± 0.04	4.91e-07*
TSS: acid ratio	30.33 ± 3.71	15.35 ± 0.90	23.43 ± 2.19	3.17e-06*
Total Sugar (%)	6.81 ± 0.17	6.91 ± 0.14	6.64 ± 0.32	0.222
Reducing sugars (%)	4.65 ± 0.28	4.60 ± 0.14	4.76 ± 0.12	0.442
Total phenols (mg GAE 100g ⁻¹)	24.04 ± 2.03	56.40 ± 3.59	49.12 ± 4.68	1.81e-08*
Flavanols (mg RE 100g ⁻¹)	14.54 ± 0.91	31.10 ± 2.32	30.41 ± 4.83	0.009005 ^{kw*}
β-lain (mg 100g ⁻¹)	0.00 ± 0.00	21.45 ± 1.67	17.97 ± 1.05	0.001678 ^{kw*}
β-carotene (μg 100g ⁻¹)	1.96 ± 0.24	47.48 ± 3.38	43.82 ± 1.97	7.55e-13*

DPPH-RSC ($\mu\text{mol } 100\text{g}^{-1}$)	108.75 \pm 3.62	238.98 \pm 7.71	262.04 \pm 14.15	1.51e-11*
ABTS ($\mu\text{mol } 100\text{g}^{-1}$)	400.20 \pm 15.69	589.60 \pm 23.14	571.40 \pm 32.88	6.8e-08*
FRAP ($\mu\text{mol TE}100\text{g}^{-1}$)	192.60 \pm 13.24	386.40 \pm 21.34	358.80 \pm 14.04	6.26e-10*
CUPRAC ($\mu\text{mol TE}100\text{g}^{-1}$)	525.60 \pm 13.83	917.00 \pm 18.99	830.40 \pm 20.45	5.67e-13*
Seed oil content (%) and fatty acid profile (%)				
Seed oil content	33.50 \pm 1.30	32.46 \pm 0.75	31.90 \pm 0.95	0.0797
Myristic acid (C _{14:0})	0.75 \pm 0.03	0.65 \pm 0.03	0.65 \pm 0.03	5.91e-05*
Palmitic acid (C _{16:0})	14.40 \pm 0.54	14.33 \pm 0.50	15.04 \pm 0.23	0.0514*
Stearic acid (C _{18:0})	4.60 \pm 0.07	4.60 \pm 0.12	4.67 \pm 0.06	0.329
Oleic acid (C _{18:1})	31.65 \pm 1.08	33.36 \pm 1.84	32.82 \pm 1.87	0.279
Linoleic acid (C _{18:2})	47.77 \pm 2.48	46.32 \pm 2.25	47.96 \pm 2.99	0.567
Linolenic acid (C _{18:3})	1.02 \pm 0.05	0.79 \pm 0.04	0.95 \pm 0.08	0.000163*

SD = Standard Deviation, **= Significant values and ^{kw} = p-value from Kruskal-Wallis rank sum test.

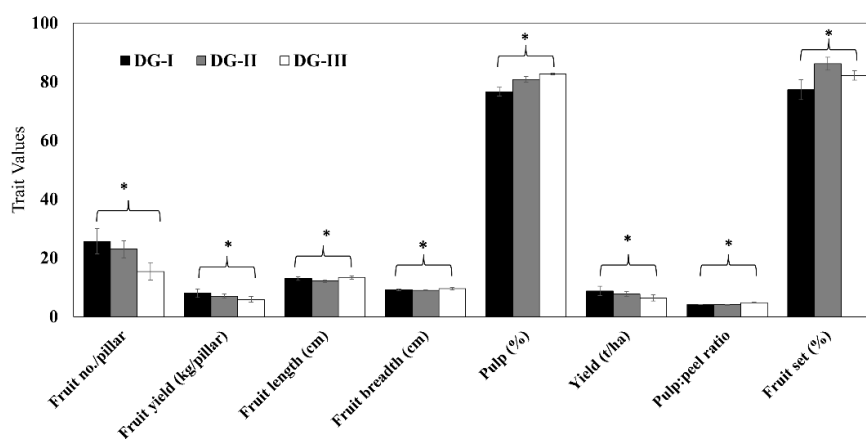


Figure 1. Comparison of average values of fruit characteristics and yield parameters among three dragon fruit species. Error bars represent standard deviation. x-axis represents average of various fruit and yield traits while y-axis depicts the values of these traits. Three vertical bars represent three individual dragon fruit species: DG-I = *H. undatus* (white pulped), DG-II = *H. polyrhizus* (red pulped) and DG-III = *H. costaricensis* (red pulped); * represents the significant difference.

The number of fruits differ significantly ($p < 0.05$) in all species. In white pulped species (DG-I); and red pulped dragon fruit (DG-II and DG-III), an average number of fruits per pole were 25.6, 23 and 15.4, respectively. Whereas, the fruit size (b: 9.52 cm and l:13.35 cm) and weight (378.52 g) was highest in DG-III followed by DG-II and DG-I. Yield per pole ranged between 5.46-9.16 kg, 5.9-7.53 kg and 4.45-7.20 kg per pole in DG-I, DG-II and DG-III, respectively. Likewise, the respective mean fruit yield per hectare (1100 pillars at 10×10 feet distance) was recorded 8.73-, 7.66- and 6.41-ton ha^{-1} . The fruit size varied in all species, elliptical fruits with narrow edge on styler end in DG-I, elliptical fruits with broad edges on both sides in DG-II, elliptical-round fruit in DG-III were noted as depicted in Figure 2.

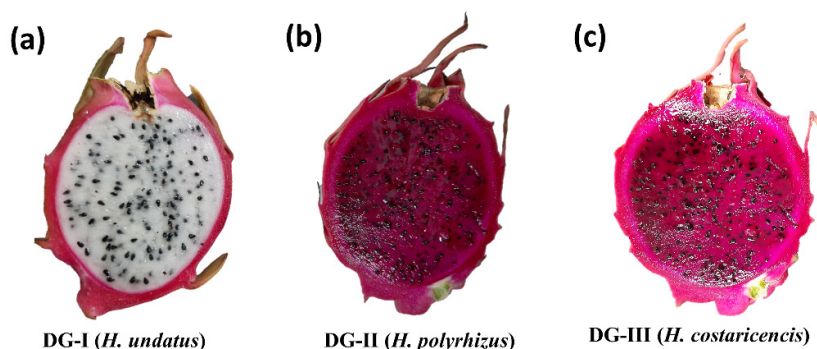


Figure 2. Cross-sectional view of three dragon fruit varieties characterized in present study (a) DG-I (*H. undatus*), white pulp variety (b) DG-II (*H. polyrhizus*), red pulp variety (c) DG-III (*H. costaricensis*), violet-red pulp variety

Biochemical constituents in fruits

Sugars, starch and acidity

The biochemical constituents varied significantly among all species and the contents of these parameters are given in Table 1. Moisture, Total soluble solids (TSS), pH, acidity and TSS: acid ratio were significantly different among three dragon fruit species as depicted by their significant p-values in Table 1. TSS also proved to be significantly different among three species and the content varied between 8.63 to 9.31 °Brix. Red pulped species DG-III had highest TSS content (9.31 °Brix) followed by 8.99 °Brix in white pulped (DG-I) species. The white pulped dragon fruit were comparatively more acidic than red pulped species as the highest titratable acidity (0.56%) and least pH (4.78) was obtained in DG-I. Total and reducing sugars ranged between 6.64 to 6.91% and 4.60 to 4.76%, respectively. No significant differences were observed among the total sugars and reducing sugar contents in the studied dragon fruit genotypes.

Antioxidative compounds and total antioxidant potential

The content of total phenols, flavanols, β -lains, β -carotene along with the antioxidant potential of white and red pulped dragon fruits are presented Table 1. The total phenols and flavanols content varied between 49.12 to 56.40 mg GAE 100 g⁻¹ and 30.41 to 31.10 mg RE 100 g⁻¹ fruit in red pulped species, while in white pulped dragon fruit, the respective contents of these biochemical constituents were 24.04 mg GAE 100 g⁻¹ and 14.54 mg RE 100 g⁻¹ only. The variation in phenolic content in white and red pulped dragon fruit may be credited to protection mechanism against biotic and abiotic stress conditions of sub-tropic climate. In our investigations, total β -laine content in red pulped dragon fruit DG-II and DG-III was 21.45 and 17.97 mg 100⁻¹ g pulp, respectively.

For all the anti-oxidant parameters, three dragon fruit genotypes varied significantly from each other as depicted from their ANOVA in Table 1. Their variation is also displayed in Figure 3a and b. Red pulped species had 20-25 times more β -carotene content than white pulped species. In red pulped it varied between 43.82 to 47.48 μ g 100 g⁻¹, whereas, in white pulped species it was only 1.96 μ g 100 g⁻¹. The antioxidant potential of dragon fruit species was assessed by recording radical scavenging (DPPH and ABTS) activity and measurement of reducing power (FRAP and CUPRAC) (Table 1). Red pulped dragon fruit species exhibited significantly higher radical scavenging potential than white pulped. The DPPH radical scavenging potential was 108.75 μ mol 100 g⁻¹ in white pulped (DG-I) and in red pulped it was 238.98 and 262.04 μ mol 100 g⁻¹ in DG-II and DG-III, respectively. Likewise, ABTS radical scavenging potential in DG-I was 400.2 μ mol 100 g⁻¹ whereas in red pulped DG-II and DG-III, it was to 589.6 and 571.4 μ mol 100 g⁻¹, respectively. The ABTS based

scavenging activity was more than DPPH based scavenging activity and it was found highest in the fruit pulp of DG-II, followed by DG-III and least in DG-I. The DPPH scavenging activity is based on H⁺ transfer only, whereas in ABTS is based on both H⁺ and electron transfer (ET). Higher sensitivity of ABTS radicals to phenolic-containing compounds than DPPH radicals also led to same effect.

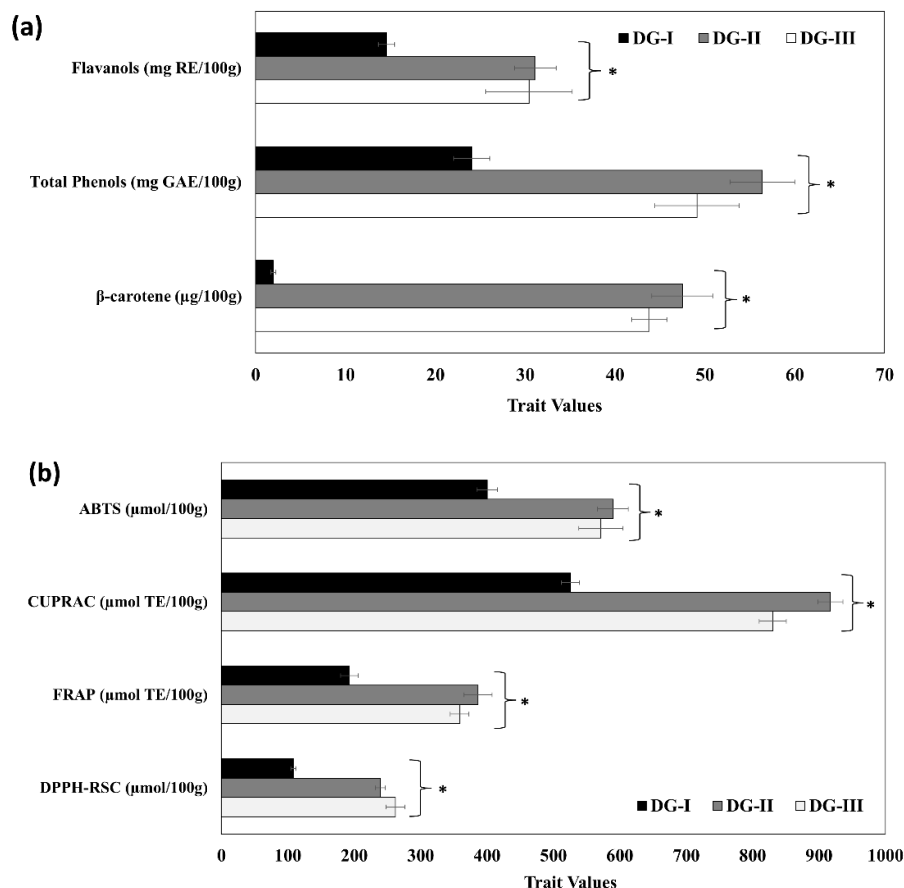


Figure 3. Comparison of average values of antioxidant activities among three dragon fruit species. Error bars represent standard deviation. x-axis represents values of the traits while y-axis depicts the respective trait with three horizontal bars representing three different dragon fruit species. Dragon fruit species: DG-I = *H. undatus* (white pulped), DG-II = *H. polyrbizus* (red pulped) and DG-III = *H. costaricensis* (red pulped); * represents the significant difference.

To confirm the antioxidant potential the measurements of reducing power, FRAP and CUPRAC methods were done. The measured values for DG-I, II and III species were 192.6, 386.4 and 358.8 μ mol TE/100 g, respectively by FRAP method. In CUPRAC method, the reducing power determined 525.6 (DG-I), 917.0 (DG-II) to 830.4 μ mol TE/100 g (DG-III). β -carotene was observed to be positively correlated to flavanols, FRAP and ABTS. Flavanols and ABTS also had positive correlation. Furthermore, phenols and CUPRAC had high positive correlation. Lastly, FRAP was positively correlated to phenols and ABTS. Pearson correlation coefficients are depicted in Table 2.

Table 2. Correlations between various antioxidative parameters

Traits	β -carotene	Phenols	Flavanols	DPPH-RSC	FRAP	CUPRAC	ABTS
β -carotene	1						
Phenols	0.99	1					
Flavanols	0.999*	0.984	1				
DPPH-RSC	0.978	0.937	0.984	1			
FRAP	0.998*	0.996*	0.995	0.963	1		
CUPRAC	0.99	1.000*	0.985	0.939	0.997	1	
ABTS	1.000*	0.992	0.999*	0.974	0.999*	0.992	1

*Significant values

Oil and fatty acid profiling in dragon fruit seeds

The average oil percentage of dragon fruit seeds extracted from DG-I, DG-II and DG-III was 33.50%, 32.46% and 31.90%, respectively (Table 1). No significant differences were observed for the oil content among three dragon fruit species. The dragon fruit seeds also have oil as that of other oilseed crops such as flaxseed, rapeseed-mustard, sesame seed etc. However, the proportion of seeds in fruit pulp is 1:99 ratio. GC results showed that the common plant fatty acids, namely myristic (C_{14:0}), palmitic (C_{16:0}), stearic (C_{18:0}), oleic (C_{18:1}), linoleic (C_{18:2}), and linolenic (C_{18:3}) are present in all the dragon fruit species. Linoleic (C_{18:2}) and oleic acid (C_{18:1}) were present predominantly in seed oil. A substantial proportion of the polyunsaturated fatty acids (PUFA) of the dragon fruit seed oil extract is essential fatty acid, linoleic (C_{18:2}, ω 6) acid. Linoleic and linolenic acid varied between 46.32 to 47.96% and 0.79 to 1.02%, respectively in all dragon fruit species. All dragon fruit species comprise about half the proportion of essential fatty acids (C_{18:2} = 46 to 48% and C_{18:3} = 0.8 to 1.2 %). The monounsaturated fatty acid (MUFA) namely oleic acid has highest proportion after linoleic acid and ranged between 31.65 to 33.36% in all the species. The saturated fatty acids (SFA) *viz.*, palmitic acid and stearic acid range between 14.33 to 15.04% and 4.60 to 4.67%, respectively. For the individual fatty acids, significant differences were observed only for fatty acids for myristic acid (C_{14:0}), palmitic acid (C_{16:0}) and linolenic acid (C_{18:3}) among three dragon fruit genotypes as summarized in Table 1.

This study provides a first look at the biochemical comparison of red-fleshed and white-fleshed dragon fruit species introduced and cultivated under sub-tropical zone of north-west India. The results also showed, for the first time, the biochemical content and the potential health benefit of *Hylocereus* grown in different agro-ecological conditions, providing important information for dragon fruit researchers and application perspective.

Discussion

Owing to richness in antioxidants and low calorific value dragon fruit is becoming one of the most preferred fruits. Consumption of dragon fruit is reported to be beneficial to cure many human ailments due to substantial reduction in triglycerides, LDL cholesterol, total cholesterol levels and increase in HDL cholesterol level (AbdHadi *et al.*, 2012; Omidzadeh *et al.*, 2014). Hence, the biochemical analysis of dragon fruit species was carried out and the results are discussed here in detail.

The dragon fruit, a climbing cactus require training system for its proper growth and development. Its cladodes are generally 2-3 feet long having areoles and small spines on each areole. Though, the fruit bearing starts after 15 months of planting but the commercial bearing commenced after 3 years. The higher fruit number in DG-I may be due to longer cladode length, but the fruit size and weight was relatively less than DG-III owing to higher number of fruits per unit cladode length. As far as quality of fruits is concerned, generally, the blend of TSS and acid in fruits gives better taste and flavour (Dasenaki and Thomaidis, 2019) and in dragon

fruit their combination makes high acceptability of fruits among consumers (Karunakaran and Arivalagan, 2019). Arivalagan *et al.* (2021) also found variability in moisture (82-85%), TSS (8-12%), total sugar (5.13-7.06%), and pH (4.8 and 5.4) content in different dragon fruit types. However, Betancur *et al.* (2020) reported higher range (11.2 to 15.6 °Brix) of TSS in red pulped dragon fruit. The moisture content obtained from all species is comparable with most of the reports. Since the sugar content in the dragon fruit is lesser than the commonly consumed fruits, and it provides fewer calories, it is considered as an ideal fruit for weight reduction, and also good for diabetic patients due to less sugar. Phenolic contents and other antioxidant properties are significantly less in white pulped dragon fruit. In a similar study, Attar *et al.* (2022) also found considerably high content of total phenols and antioxidant potential in red-pulped dragon fruit than white pulped ones. Ramli *et al.* (2014a) reported similar observations on phenol (73.8 mg GAE 100 g⁻¹) and flavonoid (145.9 mg rutin 100g⁻¹) content in dragon fruit pulp. High phenolic contents in red pulped fruits were due to presence of β -lains; a red violet ' β -cyanin' water soluble pigments that are responsible for colours in flowers and fruits (Tsai, 2019). Moo-Huchin *et al.* (2017) determined the carotenoid composition and antioxidant activities of carotenoid extracts of dragon fruit (*H. undatus*) and found only 0.86 mg 100 g⁻¹ β -carotene. Carotenoids are considered as very powerful antioxidants (Palace *et al.*, 1999; Wu *et al.*, 2019). Antioxidant activity recorded in red pulped species was significantly higher than white pulped species. Generally, fruits and vegetables contain large number of compounds having antioxidant properties and the antioxidant activity varies with the nature of compounds present in the produce. Hence; a single way to assess the antioxidant potential may not give precise value for a given sample (Arivalagan *et al.*, 2018).

The low phenolic content and consequent low radical scavenging activity in white pulped fruits might be due to absence of these β -lain pigments, whereas in red pulped species, high antioxidant capacity was due to presence of β -lains (Esquivel *et al.*, 2007). Similarly, higher β -carotene concentration in DG-II and DG-III might be due to biosynthesis of high amount of chlorophyll in chloroplast as plant pigment that is responsible for colour development (Cazzaniga *et al.*, 2016). Moo-Huchin *et al.* (2017) also found 42.4 mg GAE phenolic acid, 7.21 mg flavonoids (catechin equivalents) and 10.3 mg β -cyanin equivalents 100 g⁻¹ of fresh pulp in red pulp dragon fruit (*H. polyrhizus*). In a similar study, Arivalagan *et al.* (2021) also reported substantial variability in total phenolics 25 to 55 mg GAE per 100 g⁻¹ and flavonoids 15 to 35 mg CE per 100 g⁻¹ fruit pulp among dragon fruit genotypes with significantly higher quantities in red pulped dragon fruit as compared to white pulped one. However, Attar *et al.* (2022) determined higher amount of total phenolic content in white pulped dragon fruit as compared to red pulped dragon fruit.

The ABTS radicals can react with lipophilic as well as hydrophilic antioxidants, where the DPPH reacts only with lipophilic antioxidants (Cerretani and Bendini, 2010; Alam *et al.*, 2013; Arivalagan *et al.*, 2018; Bibi Sadeer *et al.*, 2020). The DPPH assay performed by Choo and Yong (2011) also showed the values of 9.93 and 11.34 mg ml⁻¹ for the pulp and fruit of *H. polyrhizus*, respectively, and of 9.91 and 14.61 mg ml⁻¹ for the pulp and fruit of *H. undatus*, respectively. The higher capacity of reacting power of Cu with reducing agents than Fe/ FRAP it has lower redox potential than FRAP resulted higher values as in CUPRAC technique (Prior *et al.*, 2005). Moreover, at lower pH the protonation of broad-spectrum phenols and other compounds further suppresses the antioxidant potential of the compounds (Apak *et al.*, 2004). In all the methods of antioxidant potential determination, the red pulped species exhibited significantly higher values than the white pulped species. Variation in antioxidant activity may be due to presence of diverse groups of polyphenols that have different response to ABTS activity in fruit pulp (Re *et al.*, 1999). High content reducing agents viz., ascorbic acid, carotenoids and minerals led to variability in antioxidant activity in red and white pulped species (George *et al.*, 2005; Deepa *et al.*, 2006).

Ariffin *et al.* (2009) also reported significant amount of EFAs (Essential Fatty Acids) and unsaturated fatty acids in dragon fruit seed oil extract. Red as well as white pulped species contain about 50% essential fatty acids C_{18:2} (48%) and C_{18:3} (1.5%). The essential fatty acids are vital acids that are indispensable substrates in

animal metabolism and their *in vivo* synthesis cannot take place. They are also helpful to eliminate eczema, psoriasis and dandruff and help prevent hair loss (Cunnane and Anderson, 1997). The seed oil of dragon fruit is rich in linoleic acid in comparison to rapeseed (canola), sesame seed, flaxseed and grape seed oils (Ariffin *et al.*, 2009).

Conclusions

The present investigations revealed that under sub-tropical climate, the initiation of fruiting in dragon fruit started at 14-15 months of planting, and a commercial yield obtained after 3 years of planting. The yield and fruit quality parameters varied significantly in different species. The red pulped fruits are better for phenolics with high antioxidant potential, whereas white pulped fruits are superiors in terms of yield. Among the red pulped dragon fruit, DG-II is found to be superior in terms of yield and biochemical constituents. A substantial amount of essential biochemical constituents makes the dragon fruit a 'super fruit'. The comparative analysis of major dragon fruit species will aid in selection of variety for its promotion as one of the super fruits in the region.

Authors' Contributions

JSB conceived and supervised the study. SS performed the phenotyping experiments. HK, HS and EKN compiled the results and performed the statistical analysis. JSB, HS and EKN wrote the paper. JSB, SS, HK and TA reviewed the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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