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## Physiological and biochemical changes of red-fleshed dragon fruit (*Hylocereus polyrhizus*) during development and maturation

LE V. TRONG – LE T. THUY – HOANG V. CHINH – BUI B. THINH

### Summary

The red-fleshed dragon fruit (*Hylocereus polyrhizus*) is a popular fruit in tropical countries due to its taste and nutritional value. This study aimed to investigate the physiological and biochemical changes during the development and maturation of red-fleshed dragon fruit grown in Vietnam. Fruits were measured for size and content of chlorophyll, carotenoids, reducing sugars, starch, total organic acids, vitamin C, lipids and proteins from 6 to 34 days after anthesis (DAA). The fruits reached a maximum size at 32 DAA. Chlorophyll content increased gradually from fruit formation to 18 DAA, then rapidly decreased until fruit ripening, whereas carotenoid content increased gradually from fruit formation to ripening. Starch and total organic acids contents gradually increased and reached maximum values at 18 and 22 DAA, respectively, and then declined. The contents of reducing sugars, lipids and vitamin C increased as the fruit proceeded towards ripening, reached maximum values at 32 DAA and then declined once the fruit was overripe. Proteins content gradually increased from 6 to 14 DAA and then decreased as the fruit proceeded towards ripening. These results suggest that red-fleshed dragon fruit should be harvested at 32 DAA to maximize the nutritional value and quality of the fruit.

### Keywords

dragon fruit; *Hylocereus polyrhizus*; fruit development; maturation; post-harvest; fruit ripening

Dragon fruit (*Hylocereus* spp.) is a member of the family Cactaceae, native to the desert regions of Mexico and South America [1]. There are 14 dragon fruit species but only four are widely grown around the world: *H. undatus*, *H. monacanthus* (syn. *H. polyrhizus*), *H. costaricensis* and *H. megalanthus* (syn. *Selenicereus megalanthus*) [1]. As of 2021, Vietnam has the largest dragon fruit production area as well as volume in Asia and leads the world in exports of dragon fruit [2]. Dragon fruit has made a significant contribution to Vietnam's fresh fruit exports. It is widely grown in southern provinces such as Binh Thuan, Long An or Tien Giang. The majority of dragon fruit species grown in Vietnam are *H. undatus* with red or pink peels and white flesh, while the remainder is the red-fleshed *H. polyrhizus* [2].

Red-fleshed dragon fruit cultivation and consumption have increased in many tropical and subtropical regions of the world, including Vietnam, in recent years [2]. It has long been recognized as a nutrient-dense fruit with high medicinal value since it is an excellent source of antioxidants, promotes heart health, improves digestion and helps to prevent cancer and diabetes [3–5]. Because of these nutritional and medicinal benefits, red-fleshed dragon fruit has recently become a popular research topic, with published studies on its nutritional composition, biological properties and yield enhancement measures [6–9]. However, research on the physiological and biochemical changes of the red-fleshed dragon fruit during development and maturation is limited.

Fruit maturity is the first step of post-harvest

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quality management since fruit's quality cannot be improved, only maintained [10]. Harvest maturity is thus an important factor in long-term storage, consumer satisfaction and marketing. Physiological and biochemical changes that occur during fruit development are important criteria for determining fruit maturity and quality [11, 12]. Furthermore, assessing fruit quality throughout the development and ripening is an important aspect of production planning, allowing growers to ensure quality while minimizing losses and maximizing yields. In continuation of our ongoing studies aimed at nutritional composition as well as physiological and biochemical changes at different developmental stages of fruits grown in Vietnam [13–16], here we report information on physiological and biochemical changes during the development and maturation of red-fleshed dragon fruit.

## MATERIALS AND METHODS

### Experimental materials

Red-fleshed dragon fruits (*Hylocereus polyrhizus*) were obtained from four-year-old plants located in an orchard in Yen Dinh District (Thanh Hoa province, Vietnam) from June to July 2021. A total of 50 plants, selected based on visual uniformity of fully opened flowers in the middle part, were marked on a single day at flowering time. The fruit samples from marked inflorescences were harvested at ten development stages, specifically, 6, 10, 14, 18, 22, 25, 28, 31, 32 and 34 days after anthesis (DAA). At each stage, ten of the previously marked trees were sampled and two fruits were collected from each tree according to the method described previously [16]. The fruits were hand-picked in the morning and immediately transported in a refrigerated container to the Laboratory of Biology, Faculty of Natural Sciences, Hong Duc University (Thanh Hoa, Vietnam).

After collecting the fruits, they were washed with water, dried with a paper towel and measured for length and diameter using a Palme calliper to the nearest millimeter. For other analyses, the peel and pulp of fruit samples were separated, placed in labelled bags and stored at  $-20^{\circ}\text{C}$  until analysis for a maximum period of one week.

### Chlorophyll and carotenoids contents

Chlorophyll and carotenoids contents were determined by the spectroscopic method described by TRONG et al. [16]. Five grams of peel were homogenized in 10 ml of 80% acetone, followed by centrifugation at  $10\,000 \times g$  for 20 min. The super-

natant was collected and absorbances were read at 662.0 nm and 644.0 nm for chlorophyll content and at 440.5 nm for carotenoids.

### Reducing sugars content

Reducing sugars content was determined by the Bertrand method. Five grams of pulp were homogenized using a mortar and pestle with 25 ml distilled water and then the volume was increased to 100 ml with distilled water. The solution was then filtered through a filter paper and the filtrate was obtained for analysis. Ten millilitres of Fehling's solution (5 ml Fehling's A + 5 ml Fehling's B) and 10 ml of the test solution was placed in a conical flask, heated to boil and titrated with  $\text{KMnO}_4$   $0.01 \text{ mol}\cdot\text{l}^{-1}$  until a persistent pale pink colour appeared. Reducing sugars content was calculated as previously described [17].

### Starch content

Starch content was determined based on complete acid hydrolysis of starch to glucose. Five grams of crushed pulp were placed in an Erlenmeyer flask with 50 ml of distilled water, shaken, left for 30 min and then filtered through a filter paper. The resulting powder was transferred to an Erlenmeyer flask containing 25 ml of 5% HCl and boiled in a water bath for 3 h. It was then cooled, neutralized with 0.5% NaOH solution to pH 5.6–6.0 and transferred to a 250 ml Erlenmeyer flask. Then, 10 ml of 30%  $\text{Pb}(\text{CH}_3\text{COO})_2$  and 20 ml of saturated  $\text{Na}_2\text{SO}_4$  were used to remove the protein precipitate and excess lead salt, respectively. The solution volume was increased to 100 ml using distilled water, shaken and filtered. The amount of glucose in the solution was quantified via the Bertrand method and used to calculate the starch content as described by MITCHELL [18].

### Total organic acids content

Five grams of pulp were crushed in a ceramic mortar and the sample was transferred into a 250 ml Erlenmeyer flask. Distilled water was added to increase the volume to approximately 150 ml and the flask was heated in a water bath at  $80^{\circ}\text{C}$  for 15 min. The solution was cooled and then it was transferred to a 250 ml volumetric flask, topped with water, shaken vigorously and allowed to settle. The solution was filtered into a beaker. A volume of 25 ml of the filtrate was pipetted into a 100 ml Erlenmeyer flask, 3 drops of 0.1% phenolphthalein were added and the solution was titrated with  $0.1 \text{ mol}\cdot\text{l}^{-1}$  NaOH until a faint pink colour persisted for 30 s. The total organic acids content was determined by the titration value and calculated as previously described [19].

### Vitamin C content

Vitamin C content was determined using the titration method described by SUNTORNSUK et al. [20]. Five grams of crushed pulp were blended with 5 ml of 5% HCl to create a homogenous slurry. This was diluted to 100 ml by distilled water in a volumetric flask. The diluted sample was then filtered and 10 ml aliquots of the filtrate were pipetted into a 125 ml Erlenmeyer flask. The filtrate was immediately titrated with iodine solution until a blue colour appeared.

### Proteins content

Proteins content was determined using the Lowry method [21]. An amount of 5 mg of crushed pulp were blended with 5 ml of lysis buffer in a tube with conical bottom for 20 min to facilitate the extraction of proteins. A volume of 1 ml of this sample solution was placed in a microtube and 0.5 ml of sodium dodecyl sulfate solution was added. Complex-forming reagent (5 ml) was added to the microtube and vortex-mixed. After 10 min, 0.5 ml of Folin 1 mol·l<sup>-1</sup> reagent was added. This solution was immediately followed by vortex mixing and let at room temperature (25 °C) for 30 min. Absorbance at 750 nm was used to calculate protein concentration according to standard graphs. The complex-forming reagent was prepared by mixing solutions of 2% Na<sub>2</sub>CO<sub>3</sub>, 1% CuSO<sub>4</sub>·5H<sub>2</sub>O and 2% sodium potassium tartrate in the proportion of 100:1:1 (by volume).

### Lipids content

Five grams of crushed pulp were placed in a porous thimble and extracted with 100 ml of *n*-hexane for 4 h of heating by the Soxhlet extraction method. After the extraction process finished, *n*-hexane was removed by a rotary evaporator at 40 °C for 3 h. The lipids were then weighed and calculated as described by SHAHIDI [22].

### Statistical analysis

All analyses were performed in triplicate. Analysis of variance (ANOVA) was used to analyse significance of differences between treatments using IRRISTAT software version 5.0 (International Rice Research Institute, Manila, Philippines), applying the statistical significance level of  $P < 0.05$ .

## RESULTS AND DISCUSSION

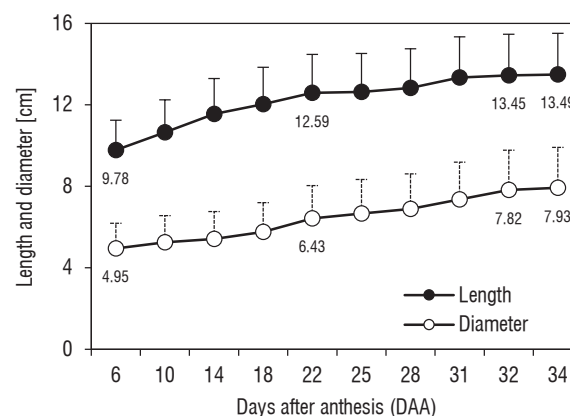
### Fruit length and diameter

The length and diameter of a fruit, indicative of its size and shape, are useful criteria to deter-

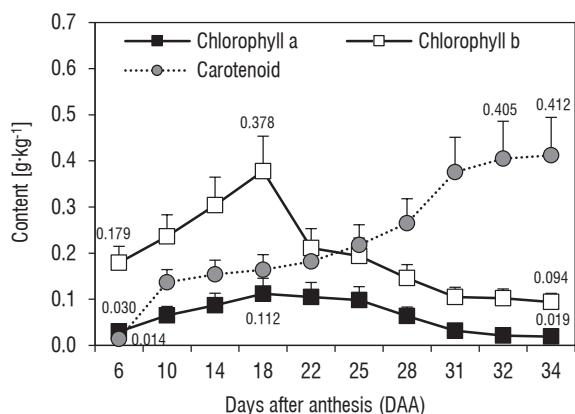
mine optimal harvest time and assess crop yield and quality [23]. Changes in length and diameter of red-fleshed dragon fruit are shown in Fig. 1. At 6 DAA, the fruit length was 9.78 cm, while the fruit diameter was 4.95 cm. From 6 to 22 DAA, the fruit length and diameter increased rapidly due to strong cell division and expansion [24, 25]. Thereafter, fruit length and diameter continued to increase but at a slower rate. At 32 DAA, the fruit's length and diameter were 13.45 cm and 7.82 cm, respectively, and then the fruit's size was almost unchanged. Fruit lengths and diameter increased at 34 DAA, but not significantly. The lack of growth observed after 32 DAA was similar to observations in other fruits that the size of the fruit tends to stabilize or increase insignificantly as the fruit matures [13, 14, 26, 27]. The increase in fruit length and diameter as the fruit matures is controlled by metabolic processes, by regulation and control of the endogenous hormone complex in cells [23].

### Chlorophyll and carotenoid content

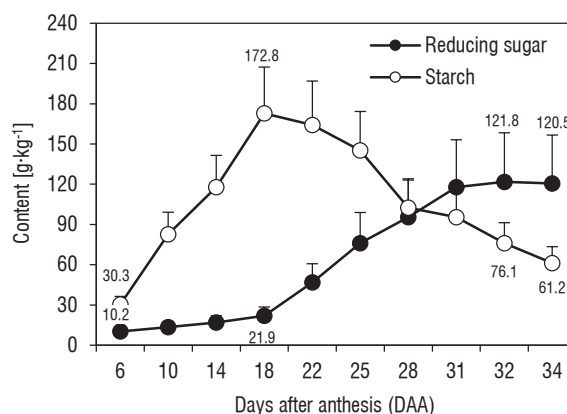
Chlorophyll and carotenoids content, and their ratio, determine the colour and shape of a fruit and serve as quality markers during ripening [28, 29]. Fig. 2 depicts the changes in chlorophyll a, chlorophyll b and carotenoids content in the peel of the red-fleshed dragon fruit. The chlorophyll content was high in the early stages, with chlorophyll a and chlorophyll b levels of 0.030 g·kg<sup>-1</sup> and 0.179 g·kg<sup>-1</sup> at 6 DAA, respectively. The chlorophyll content gradually increased and reached a maximum at 18 DAA (0.112 g·kg<sup>-1</sup> of chlorophyll a and 0.378 g·kg<sup>-1</sup> of chlorophyll b). The chlorophyll content gradually decreased after 18 DAA and reached a minimum at 34 DAA, which was similar to reports in previous studies



**Fig. 1.** Changes in length and diameter of red-fleshed dragon fruit during development and maturation.



**Fig. 2.** Changes in the content of chlorophyll and carotenoids of red-fleshed dragon fruit during development and maturation.



**Fig. 3.** Changes in the content of reducing sugars and starch of red-fleshed dragon fruit during development and maturation.

[13, 30]. These findings support the concept that chlorophyll is broken down and carotenoids are synthesized as the fruit ripens [31].

Carotenoids play a role in light capture and are powerful antioxidants [32]. The carotenoids content gradually increased throughout the development period of red-fleshed dragon fruit (Fig. 2). The minimum carotenoids content of 0.014 g·kg<sup>-1</sup> was observed at 6 DAA and then, the carotenoid content increased slowly. After 22 DAA, the carotenoids content increased rapidly with fruit ripening and reached 0.412 g·kg<sup>-1</sup> at 34 DAA. Similar results were observed in other fruits, such as tomato [32], longan [14], litchi [13] or sunberry [33]. The decrease in chlorophyll content and increase in carotenoids as the fruit develops is consistent with the colour of the fruit when ripe [34, 35].

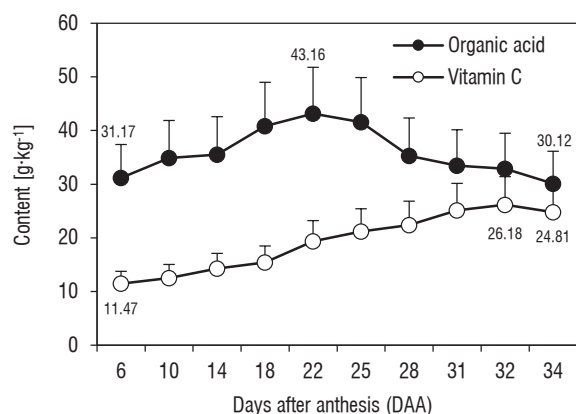
### Reducing sugars and starch contents

Reducing sugars and starch contents are closely related during fruit development and maturation [33]. The reducing sugars content significantly increased as the fruit ripened (Fig. 3). Specifically, the reducing sugars content in the fruit was 10.2 g·kg<sup>-1</sup> at 6 DAA and increased slowly from 6 to 18 DAA. However, from 18 to 32 DAA, the reducing sugars content increased rapidly and reached a maximum of 121.8 g·kg<sup>-1</sup> at 32 DAA. The fruit enters the ripening stage at this point and a large quantity of organic acids and starch are converted into sugar [36]. At 34 DAA, the reducing sugars content decreased to 120.5 g·kg<sup>-1</sup>. This finding is consistent with previous studies that reported reducing sugars content increasing rapidly as the fruit progressed towards ripening and then decreasing [13, 14].

At 6 DAA, the starch content in the fruit was only 30.3 g·kg<sup>-1</sup> (Fig. 3). After 6 DAA, the products of photosynthesis from the leaves and pods are transferred to the fruit [37], providing raw materials for the synthesis of starch, so the starch content in the fruit gradually increases. The starch content was greatest at 172.8 g·kg<sup>-1</sup> at 18 DAA and after 18 DAA, the starch content in the fruit decreased. Under the action of the enzyme  $\alpha$ -amylase, starch is broken down into monosaccharides, which are a direct substrate for respiration [38]. At 34 DAA, the starch content in the fruit was only 61.2 g·kg<sup>-1</sup>. A decrease in starch content during ripening was also reported in longan [14], litchi [13] and sunberry [33].

### Total organic acids and vitamin C contents

Organic acids are of increasing interest because of their role in plant physiology as co-factors, buffers and mediators of the most important metabolic pathways of carbohydrates, lipids and proteins [39]. Therefore, organic acids play a role in maintaining the quality and nutritional value of the fruit. At 6 DAA, fruit accumulated a large amount of total organic acids of 31.17 g·kg<sup>-1</sup> (Fig. 4). From 6 to 22 DAA, the total organic acids content gradually increased, reaching a maximum of 43.16 g·kg<sup>-1</sup> at 22 DAA. From 22 to 34 DAA, the total organic acids content decreased, presumably because organic acids are used in respiration to provide energy for starch synthesis [40]. During ripening, energy continues to be needed for the biosynthesis of substances specific to the ripening period of the fruit, such as hydrolytic enzymes that give the fruit a sweet taste, leading to a gradual decrease in the content of total organic acids [13, 40].

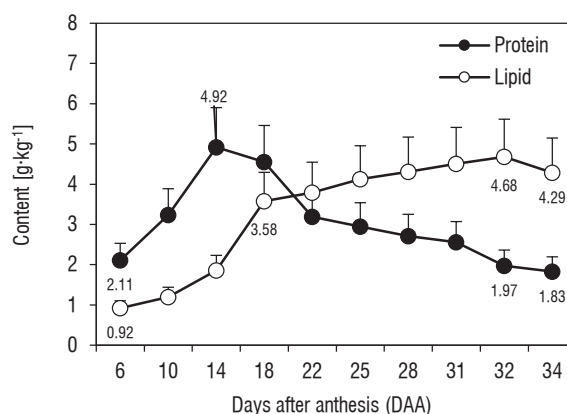


**Fig. 4.** Changes in the content of total organic acids and vitamin C of red-fleshed dragon fruit during development and maturation.

Because it plays an important role in the growth and development of the human body, vitamin C content is a criterion of fruit quality at harvest [41]. Vitamin C content reached 11.47 g·kg<sup>-1</sup> at 6 DAA, then gradually increased from 6 to 32 DAA when it reached a maximum of 26.18 g·kg<sup>-1</sup> (Fig. 4). At 34 DAA, the vitamin C content decreased to 24.81 g·kg<sup>-1</sup>. The decrease in vitamin C content is related to the activity of some groups of enzymes involved in the breakdown of ascorbic acid such as ascorbate oxidase, cytochrome oxidase and ascorbate peroxidase [42]. This result is consistent with the study by RESENDE et al. [43], who found that ascorbate peroxidase enzyme activity in fruit pulp increased continuously during fruit ripening.

#### Proteins and lipids contents

The proteins content of red-fleshed dragon fruit increased steadily from 6 to 14 DAA and then decreased (Fig. 5). Specifically, at 6 DAA, the proteins content in the fruit was relatively high at 2.11 g·kg<sup>-1</sup>, and then gradually increased reaching a maximum of 4.92 g·kg<sup>-1</sup> at 14 DAA. At this stage, cell division and cell elongation accelerate to promote fruit growth, resulting in increased proteins biosynthesis [24, 25]. The proteins content in the fruit gradually decreased after 14 DAA until the fruit was fully ripe, with the fastest period of decrease occurring between 14 and 22 DAA. During this stage, the fruit's protease enzyme increase proteins breakdown to provide energy for respiration [13, 43]. At 34 DAA, the proteins content of the fruit reached a minimum of 1.83 g·kg<sup>-1</sup>. Similar results were reported during the ripening of guava fruit [44].



**Fig. 5.** Changes in the content of proteins and lipids of red-fleshed dragon fruit during development and maturation.

The lipids content of red-fleshed dragon fruit increased rapidly from 6 to 18 DAA, then gradually increased to a maximum of 4.68 g·kg<sup>-1</sup> at 32 DAA (Fig. 5). From 6 to 18 DAA, the accumulation of organic compounds and lipids in the fruit increased rapidly, accompanying growth. After 32 DAA, the lipids content in the fruit decreased to 4.29 g·kg<sup>-1</sup> at 34 DAA. The decrease in lipids content at this stage is caused by the fruit entering the ripening stage, where respiration in the fruit accelerates and the lipids participate in reactions to provide substrates and energy for respiration [45].

#### CONCLUSIONS

The red-fleshed dragon fruit reached its maximum size, in both length and diameter, at 32 DAA. The fruit turned red at 32 DAA due to a decrease in chlorophyll content and an increase in carotenoid content. At 32 DAA, the fruit had the maximum content of reducing sugars, vitamin C and lipids, while other components such as starch, proteins and total organic acids changed throughout the development of the fruit. After 32 DAA, some of the main components of the fruit, such as reducing sugars, lipids and vitamin C, decreased. Therefore, 32 DAA is the optimal time to harvest red-fleshed dragon fruit. If it is picked earlier or later, the quality of the fruit will suffer. However, further research on the physiological and biochemical changes of other dragon fruit varieties during development is needed to be able to determine the optimal harvest time for each variety.

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