

IMPROVING POSTHARVEST STORAGE LIFE OF RED-FLESHED PITAYA
(*Hylocereus costaricensis*) FRUIT BY OPTIMIZING THE HARVEST MATURITY,
COATING APPLICATION AND LOW TEMPERATURE STORAGE

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2017

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To my husband, my parents and my children. With love.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Dr. Steven A. Sargent for his invaluable guidance, support, and encouragement through all these years.

I would like to thank the members of my thesis supervisory committee, Dr. Jeffrey K. Brecht, Dr. Bala Rathinasabapathi and Dr. Charles A. Sims for their help and support through this work.

I would like to thank the postharvest family, Adrian Berry, Kim Cordasco, Merce Teodora Aguil Santana, Zilfina Rubio, Yurui Xie, Sonya Stahl, Mildred Makani, Ramadhani Majubwa, and Ferdous Chowdhury for all their help and support.

Special thanks to my husband Mohd Fairuz Ahmad for his patience and sacrifice, my parents Razali Othman and Zainab Hashim for their help and support, my sons Muhammad Adam Firdaus, Faiz Aiman and Aydan Fiqri for their love, as well as the rest of my families and friends for their continuous support.

Finally, I owe my thanks to my sponsor Malaysian Agriculture Research and Development Institute (MARDI) and Ministry of Agriculture Malaysia for the trust, opportunity and financial support throughout my education at the University of Florida.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
C ₂ H ₄	Ethylene
CI	Chilling injury
CO ₂	Carbon dioxide
D	Day(s)
h	Hour(s)
ha	Hectare(s)
MAP	Modified Atmosphere Packaging
min	Minute(s)
RH	Relative Humidity
s	Second(s)
wk	Week(s)

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

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August 2017

Chair: Steven A. Sargent
Major: Horticultural Sciences

Increasing demand for red-fleshed pitaya is driving increased production in southern Florida and other production areas as market prices reach \$55 per 4.5-kg carton. Harvest maturity is a critical component in marketing the pitaya fruit, however its effect on postharvest quality has not been studied when grown under humid, subtropical climate in Florida. Furthermore, the main problems during storage are chilling injury, decay and water loss, the latter reducing freshness and decreasing market price. Postharvest coatings improve shelf life for many crops by retarding water loss and increasing resistance to fruit decay. Three studies were conducted with pitaya (*Hylocereus costaricensis*, cv. Lisa). Fruit were harvested at ½-red, ¾-red and full-red (commercial maturity) stages and stored at 20 °C; pitayas at the ¾-red stage had the longest shelf life (10 d) with compositional quality equivalent to those harvested at commercial maturity. Carnauba-based and vegetable oil-based coatings best maintained freshness of pitaya harvested at full-red stage during storage at 7 °C for 15 d by preventing shriveling and retaining firmness; there was no effect on sensory characteristics. Fruit at the full-red stage were also stored at 1, 4 or 7 °C and those

stored at 1 °C had acceptable quality up to 20 d, no chilling injury symptoms, and suppressed anthracnose and stem-end fungal decay.

CHAPTER 1 INTRODUCTION

Pitaya or dragon fruit (*Hylocereus* spp.) originated in Central America and is now grown worldwide. Even though many species exist, only five species have been commercially cultivated to produce fruit. These are white-fleshed *H. undatus*, red-fleshed *H. costaricensis*, red-fleshed *H. polyrhizus*, red-fleshed *H. ocamponis* and yellow skin *H. megalanthus* (Le Bellec and Vailant, 2011). It is a crop with high adaptability in tropical and sub-tropical climates and has a great potential as a water efficient crop. In the United States, pitaya is produced especially in South Florida, California and Hawaii and the acreage is expanding, with Florida being the main producer in The United States. As of March 2017, the market price of pitaya in a 4.5-kg (10-lb.) box was as high as \$55 per carton (USDA, 2017). This high market price coincides with increased demand for products with antioxidant and beneficial properties and the fruit's attractiveness at the consumer end due to its unique taste, appealing appearance, and classification in the exotic produce category.

Since pitaya fruit is non-climacteric, it should be harvested at full maturity, when quality is at least minimally acceptable. Pitaya are usually harvested by hand and picked when the pericarp reaches red stage (commercial maturity) and packed into 4.5-kg cartons for shipping. Postharvest development and final quality of the fruit depend on the stage of maturation at harvest. Premature fruit is susceptible to cell wall rupture and physiological disorders caused by cellular disorganization; however, overripe fruit is likely to show senescence symptoms, causing quality losses and reduced shelf life (Chitarra and Chitarra, 2005).

Postharvest coatings have beneficial effects on fruit quality such as improving shelf life, suppressing ripening, retarding water loss, enhancing visual quality and providing resistance to fruit decay (Amarante et al., 2001; Arowora et al., 2013). Coatings such as polyethylene, chitosan and carnauba are semi-permeable and if properly formulated, can perform similar to modified atmosphere packaging. Ali et al. (2014) reported that pitaya treated with chitosan coating maintained fruit quality for up to 28 days without any off-flavors. Propolis coating also helped slow the ripening process while increasing the biosynthesis of nutritional components in pitaya (Zahid et al., 2013). Vegetable oil and carnauba wax are lipid-based coatings shown to have beneficial effects on fresh produce such as tomato (Dávila-Aviña et al., 2014). These coatings are stable at ambient temperature, emulsify and do not interfere with the quality of fresh fruits and vegetables.

Low temperature storage has many benefits in prolonging shelf life of fresh produce after harvest by reducing the respiration rate, water loss and the development of decay. However, the optimum storage temperature for pitaya is dependent on the genetic background, growing conditions and harvest maturity (Nerd et al., 1999). Storing pitaya fruit at lower than their optimum temperatures may cause a physiological disorder known as chilling injury. Chilling injury symptoms in pitaya were described as peel translucency, scale darkening, fruit softening, shriveling and loss of flavor (Nerd et al., 1999). Severity of visual chilling injury symptoms also increases as storage duration increases. Thus, different minimum postharvest temperatures have been recommended for pitaya fruit, ranging from 10 °C to as low as 5 °C, depending upon ripeness stage and storage duration. While *H. undatus* in California and Vietnam stored at 5 °C for 20

days in good quality (Freitas and Mitcham, 2013; To et al., 2002; Hoa et al., 2006), *H. undatus* grown in Florida is recommended to be stored between 7 to 10 °C to maintain quality (Crane and Balerdi, 2005). Pitaya (*H. undatus* and *H. polyrhizus*) cultivated in Israel are also recommended to be stored at 10 °C to avoid chilling injury (Nerd et al., 1999). Red-fleshed pitaya has higher antioxidant activity compared to white-fleshed pitaya (Mahattanatawee et al., 2006) due to betalain pigment that expresses the red color. However, the stability of betalains are affected by temperature, pH, oxygen, light, and moisture (Saguy et al., 1978; Delgado-Vargas et al., 2000; Wong & Siow, 2015; Montes-Lora et al. 2016) with temperature being the main factor affecting betalains stability (Anni et al., 2015). Several studies have been conducted to determine the stability of betalains in high temperature especially for food colorant purposes (Anni et al., 2015; Woo, et al. 2011). However, very few studies have measured the stability of betalains during low temperature storage. A study on betalains pigments from the cactus species (*Myrtillocactus geometrizans*) reported that storage at 4 °C could prevent the degradation of betalains and antioxidant capacity (Reynoso et al. 1997).

In this study, the goal was to optimize the procedures to prolong the postharvest quality of red-fleshed pitaya (*Hylocereus costaricensis*) grown in South Florida, USA. The hypotheses were that the ½-red and ¾-red maturity stages would have longer shelf life than full-red (commercial maturity) without compromising the quality presented by full-red maturity stage. It was further proposed that use of postharvest coatings would reduce fruit weight loss and maintain quality as well as sensory attributes during storage for distribution and marketing. Another hypothesis was that low temperature storage will prolong the postharvest quality of red pitaya without chilling injury and affect the quality.

The postharvest behavior in terms of respiration and ethylene production of fruit harvested at different maturity stages was compared. Furthermore, the impact of postharvest coatings on pitaya flavor was studied using consumer taste panels. The use of low temperature storage to reduce decay and extend the storage life was also studied. The low temperature storage was tested for their effects on total betalains and other quality attributes.

CHAPTER 2 LITERATURE REVIEW

Origin and Distribution

Pitaya or dragon fruit (*Hylocereus* spp) is a member of the Cactaceae family. Most of the genus of *Hylocereus* originate from Latin America and some from West Indies (Britton and Rose, 1963). Nowadays, they are grown in tropical and subtropical areas all over the world, mainly in China, Vietnam, Malaysia, Indonesia and Israel, with the white-fleshed *H. undatus* being the most cultivated species (Le Bellec et al., 2006).

In Vietnam, historical evidence indicates that pitaya was introduced by French about 100 years ago and it was grown for the king and wealthy families (Gunasena et al., 2006). In North America, pitaya was mostly unknown until the mid-1990s (Le Bellec and Vailant, 2011). A study on variability of *Hylocereus* genotypes in Mexico reported that the robustness of this species represents better adaptability in different environments (Grimaldo-Juarez et al., 2007). For example, pitaya is grown in very hot climates above 38 to 40 °C to prevent excess water in the soil that contributes to abscission of flowers and immature fruit (Le Bellec et al., 2006). As pitaya belongs to the Cactaceae family, the crassulacean acid metabolism (CAM) pathways allow it to adapt to arid conditions (Micheal, 2011). Pitaya has a great potential as a water efficient crop and it can tolerate hot climates of up to 40 °C (Le Bellec et al., 2006). Pitaya also can survive prolonged drought up to 6 weeks without any negative effect on growth (Nobel, 2006). Plants employing CAM can keep most stomata in the shoot closed during the day. Per Mizrahi et al. (2007), the vine cacti pitaya, the *Cereus peruvianus* pitaya and the cactus-pear *Opuntia ficus-indica* which only consumes 10% of the water used by C3 crops such as pear, peach, citrus and avocado. Nevertheless, some

species prefer rainy areas (340 to 3500 mm year) and high altitudes of 2750 m above sea level in Mexico (Mizrahi et al., 1997). The rainy season offers cooler temperatures and low vapor pressure during the night that provides optimum condition for photosynthesis in pitaya (Andrade et al., 2006).

In the United States, pitaya is produced in tropical and subtropical areas, notably South Florida, California and Hawaii and the acreage is increasing rapidly. Florida is the main producer in the United States with the acreage being between 200 to 250 ha, followed by California (160 to 200 ha) and Hawaii (40 to 80 ha) (De Soto, J.F. undated). Although there are many species, only five are cultivated to produce fruit. These are red pericarp with white-fleshed (*H. undatus*), red pericarp with red-fleshed *H. costaricensis*, *H. polyrhizus* and *H. ocamponis*) and yellow pericarp with white flesh (*H. megalanthus*) (Le Bellec and Vaillant, 2011). Several cultivars of *H. costaricensis* are widely cultivated in North, Central and South America; “Lisa”, “Orejona”, “Rosa” and “San Ignacio” are the most common genotypes commercialized mainly in Costa Rica (Vaillant et al., 2005). For *H. costaricensis*, fruit diameter ranges from 10 to 15 cm, the weight from 250 to 600 g and has many small black seeds.

Botanical Characteristics

Cactus Plant

Pitaya, a vine cactus, and belongs to the genus *Hylocereus* (Berger) Britton & Rose from Cactaceae family. As described by Le Bellec and Vaillant (2011), it is characterized as a climbing plant with aerial roots with stems that bear a large berry (Britton and Rose, 1963). The fruit is derived from a single, compound pistil and belongs to the bacca group in berry, in which the entire peel is soft and the flesh contains many small seeds. The pericarp consists of three layers, the outer exocarp, middle fleshy

mesocarp and inner endocarp. This endocarp may not be distinct from the mesocarp in berry type fruits. The bracts of pericarp also called scales are overlaid leaves.

It is a perennial with triangular or seldom 4- or 5- sided stem. Each stem has many branch segments and each segment has many spines. The stem's length is dependent on growing environment and the aerial root enables them to climb over rocks, trees or cling to walls. Some species such as *H. polyrhizus* have waxy epicarpal layer but other species such as *H. undatus* does not have wax layer, thus less drought tolerant (Mizrahi and Nerd, 1999).

H. costaricensis (Web.) Britton & Rose, which is the most robust of this genus, was selected for this present study. This species is characterized by vigorous vines, waxy, white stems and margined flowers. Its fruit is oblong and with various size of scales also known as bracts. It has a red-purple flesh with numerous black tiny seeds (Le Bellec and Vaillant, 2011). Pitaya is widely consumed fresh and the peel is removed prior to eating. It is refreshing especially if chilled and used in sorbet and fruit salads. The flowers can be eaten as vegetables. The betalain pigments from the fruit can be commercialized as colorants for the food and beverage industry (Wybraniec and Mizrahi, 2002) due to the pigments' stability during processing and storage (Herback et al., 2007).

Flower Morphology

Hylocereus species have large flowers (15 to 25 cm, creamy white in color) that bloom at night (Le Bellec and Vaillant, 2011). At the onset of flowering, up to five spherical buttons will arise from the stem margins and some of them may emerge into flower buds in about 13 days (Gunasena et al., 2006). The flower induction depends on

the day length; in Taiwan flowering is induced by extending photoperiod to increase day length (Jiang et al., 2012).

In the United States, pitaya flowers from May to October and the flowering episodes are seven to eight cycles over the whole period per year (Weiss et al., 1994). Whether fertilized or not, flowers bloom only once at night and then close in the next morning. Petals become yellowish and soft the following day and then slowly dry dropping off 4 to 6 days later. If the flower is fertilized, its lower part remains greenish and the volume increases enormously, indicating that the fruit has set. In the flower, both ovary (flesh) and the receptacle that surrounds the ovary (peel) develop into fruit (Gunasena et al., 2006). Each flowering phase is short; stage 1, forming of floral buds and flowering ranging from 15 to 20 days, and stage 2, flowering to harvest maturity around 30 days.

Propagation and Production

Pitaya can be propagated from seeds obtained from selected fruit of mother plants. However, seed propagation resulted in variation in fruit and stem characteristics due to cross pollination and needed longer time from planting to fruit production (Crane and Balerdi, 2009). These same authors recommend pitaya to be propagated by cuttings (15 to 60 cm length), in which the fruiting stage is achieved promptly, under 1 year after planting, contrasted with plants grown from seed that need 3 years to reach fruiting stage (Zee et al., 2004). This vegetative propagation can be done throughout the year; however, it is preferable to collect the cuttings after fruiting season of the mother plants. The cutting is potted in a suitable media for rooting and root formation significantly benefits from the application of rooting hormone (Seran and Thresh, 2015). The cuttings should be watered regularly but not excessively, and once well

established, they can be transferred to the field. Another method for pitaya propagation is grafting, however it is not very common as cuttings are more convenient. The grafting method is beneficial when purposely need to use selected rootstocks and scions.

Compared to other fruit trees, pitaya requires relatively low maintenance and the duration between planting and harvesting is short, between 12 to 18 months (Gunasena et al., 2006). In Mexico and Central America, pitaya is planted in family orchards using a traditional method in which the pitaya plant is supported on a tree or on land terrace system (Ortiz-Hernandez and Carillo-Salazar, 2012). On the other hand, Thailand, Malaysia, Israel and the United States use advanced technology resulting in high yields; up to 40 tons per ha of fruit are harvested in Israel (Mizrahi and Nerd, 1999). Pitaya prefers half-shaded environments, however most of them can tolerate solar radiation exposure and thus can grow in open areas (Nerd et al., 2002; Andrade et al., 2006). A study in Israel showed that the most favorable condition for pitaya *H. polyrhizus* is 30% shade (Raveh et al., 1996).

Pitaya requires mineral fertilization, particularly when annual yields are high between 20 to 45 tons per hectare (Mizrahi, 2014). However, as a semi-epiphytic plant, pitaya can climb and may become quite large and spreading, and therefore a strong trellis needs to be established to support the plant. It consists of vertical post and a support system on top of the post. The distance between planting lines requires 2 to 3 m and averages three cuttings per post (N'guyen, 1996). Pruning is very important when using vertical post, to influence the plant to climb over the entire support. Major pruning is done during the first year of planting and continues every subsequent year for maintenance. Based on a study by Evans et al. (2013), the estimated cost to set up a

pitaya orchard is about \$37,840 per hectare, where the first year encountered the highest costs. The estimated costs are based on the costs for orchard preparation, planting work, trellis system construction and agricultural practices.

Economic Importance

The market trend is continuously shifting and consumers are more aware of the impact of food and its ingredients on their health. Demand for products with antioxidant and beneficial properties has increased and contributes to the increase of production and consumption of pitaya. Besides the health-conscious trend, the high returns based on an increasing population and the fruit's attractiveness at the consumer end due to its unique taste, appealing appearance and exotic nature - all factors for pitaya to become more popular. As the market is expanding, the profits also increase. With a total receipt assessed at \$64,1250 per ha (21,546 kg·ha⁻¹ of marketable yield), net profits are estimated at \$38,808 per ha and this denotes a very satisfactory return, compared with other tropical fruit crops, for example mango and avocado, with an average return of \$3,750 per ha (Evans et al., 2013). The latest report by USDA (2017), as of March 2017, showed the market price of pitaya for a 4.5-kg box as high as \$55 per carton or an average price of \$12.22 per kg. The life of pitaya trees is estimated to be 20 years; thus, it provides a steady income to the producer (Crane and Balerdi 2009; Gunasena et al., 2006).

The increasing worldwide production of *Hylocereus* under various environments demands more study concerning plant breeding and propagation, fruit production, disease and pest control and postharvest handling (Ortiz-Hernández and Carrillo-Salazar, 2012).

Fruit Quality Components

Physico-chemical Properties

The edible portion of pitaya is the mesocarp which consists of fruit tissue containing juice and tiny seeds. Depending on varieties and cultivars, the juice has about 12% dry matter consisting of reducing sugars, mainly glucose and fructose, that range between 30-103 g·L⁻¹ and 19-29 g·L⁻¹, respectively (Esquivel et al., 2007). Sucrose accounts for less than 3% of the total sugar content (Nomura et al., 2005). Various authors have not detected the presence of sucrose when using HPLC methods for the determination of sugars (Vaillant et al., 2005; Stintzing et al., 2003), but when using enzymatic methods, Wu and Chen (1997) found that sucrose accounts for 2.8 to 7.5% of total sugars. Soluble solids content increases gradually when the fruit mature and is observed from the external section to the center of the fruit (Nomura et al., 2005). Soluble solids content is about 140 g per 100 g fresh weight (Nomura et al., 2005) or 7.1 to 10.7 °Brix (Le Bellec and Vaillant, 2011).

Pitaya had low total titratable acidity, only 2.4 to 3.4 g·L⁻¹ depending on varieties and cultivars (Le Bellec et al., 2006), and the predominant acid is malic acid (Nomura et al., 2005). The total titratable acidity in pitaya was quite low and similar between species and cultivar. However, *H. costaricensis* cultivar Rosa is often most preferred by consumers because it is more acidic with a better sugar-to-acid ratio than the other cultivar (Vaillant et al., 2005). Even so, they concluded that pitaya regardless of variety, generally showed a low sugar/acid ratio, presenting a low sensorial quality, which can be improved by adding an acid juice such as lemon juice.

The protein content varies around 1.25 g·L⁻¹ depending on methods used (Le Bellec and Vaillant, 2011). The predominant amino acid in pitaya happens to be proline

with very high content up to $1.6 \text{ g}\cdot\text{L}^{-1}$ (Stintzing et al., 2001). Mineral content is high with potassium the most predominant mineral followed by sodium and magnesium (Stintzing et al., 2003). A high total dietary fiber was reported about 3.2 g per 100 g fresh weight (Mahattanatawee et al., 2006) with pectin about 0.27% might be due to mucilage content. Betalains are the main pigments present in red-fleshed pitaya while non-colored compounds are present in the white-fleshed pitaya.

Health Benefits

The fruit has gained popularity due to the attractive appearance and the potential health benefits. Nowadays, the consumption of products with high antioxidant activity has increased tremendously. Pitaya is a source of lycopene, beta-carotene and vitamin E and the seed is rich in essential fatty acids (Ariffin et al., 2009; Charoensiri et al., 2009). In recent studies oligosaccharides extracted from pitaya showed prebiotic properties (Wichienchot et al., 2010) and consumption of red pitaya may prevent cardiovascular disease (Mohd Adzim Khalili et al., 2009). A study on red-fleshed *H. polyrhizus* and white-fleshed *H. undatus* showed both have antimicrobial activity against bacteria, with red-fleshed pitaya having greater effect (Nurmahani et al., 2012). The pitaya had antioxidant properties and medicinal potential especially in the flesh (Tenore et al. 2012). A study conducted at the University of Florida measured the antioxidant activity of two types of pitaya grown locally and showed that red-fleshed pitaya has higher antioxidant activity (ORAC: 7.6 ± 0.1 , DPPH 134.1 ± 30.1) compared to white-fleshed pitaya (ORAC: 3.0 ± 0.2 , DPPH: 34.7 ± 7.3) due to betalains pigment that express the red color (Mahattanatawee et al., 2006). The total antioxidant capacities from the cultivar 'Lisa' of pitaya was $8.8 \mu\text{mol Trolox}\cdot\text{g}^{-1}$ of fresh tissue without seeds as assessed by the ORAC method (Vailant et al., 2005). The ORAC value of pitaya is

double that of apple and white grape juice and it is the same amount as beetroot and strawberry measured by the same method (Ou et al., 2002 and Prior et al., 2003).

Total phenolic content is as high as blueberry, white grape, apple, plum and apple (Vinson et al., 2001). Surprisingly, pitaya is poor in total ascorbic acid content between 12 to 17 mg per 100 g fresh weight compared to other cactus species that have higher ascorbic acid on par with that of citrus (To et al., 2002). Mahattanatawee et al. (2006) reported that pitaya contains 84% moisture, 1076 $\mu\text{g GA}\cdot\text{g}^{-1}$ of total soluble phenolic specifically hydroxycinnamates and 56mg per 100g puree of total ascorbic acid.

Betalain Pigments

Betalains are water soluble pigments consisting of a basic structure of betalamic acid that comprise red-violet betacyanin and yellow betaxanthin which is synthesized from hydroxylation of tyrosine (Sunnadeniya et al., 2016). Betacyanin is reddish to violet pigment that is commonly found in fruits and flowers. This pigment is known to have antioxidant and radical scavenging activities that could help protect against oxidative stress in the body (Kanner et al., 2001 and Wu et al., 2006). Betalains exclusively substitute for anthocyanins in the taxa where they occur and play important roles in attracting pollinators and seed dispersers (Sunnadeniya et al., 2016). Betalains which have high antioxidant activity are reported to have potential as commercial food coloring (Cai et al., 2003, Vailant et al., 2005). Different from beetroot or other cactus species, red-fleshed pitaya is a pure source of betacyanin, devoid of betaxanthin (Stintzing et al., 2002). At least ten betacyanin compounds have been found in pitaya, predominantly betanin and phyllocactin, with all these pigments exhibiting the similar red color with an

absorbance peak at about 536 nm (Stintzing et al., 2002; Wybraniec and Mizrahi, 2002; Rebecca et al., 2012).

The stability of betalains are affected by temperature, pH, oxygen, light, and moisture (Saguy et al., 1978; Delgado-Vargas et al., 2000; Wong & Siow, 2015; Montes-Lora et al., 2016); temperature is the main factor affecting betalains stability (Anni et al., 2015). The betacyanin is degraded to the colorless cyclo-dopa 5-O- β -glucoside and bright yellow betalamic acid (Herbach et al., 2004). However, if adverse conditions are only temporary, this degradation was shown to be partially reversible (Degenhardt and Winterhalter, 2001). Several studies had been conducted to determine the stability of betalains in high temperature especially for food colorant purposes (Anni et al., 2015; Woo et al., 2011). However, very few studies measure the stability of betalains in low temperature storage. A study on betalains pigments from the cactus species (*Myrtillocactus geometrizans*) reported that storage at 4 °C could prevent the degradation of betalains and antioxidant capacity (Reynoso et al., 1997).

Problems in Postharvest Handling

Postharvest deterioration of pitaya is mainly caused by mechanical injury, decay development, water loss, chilling injury, anthracnose disease and scale drying (Freitas and Mitcham, 2013; Nerd et al., 1999; Chandran, 2010). Mechanical injury led to increased water loss resulting in the development of sunken areas. By harvesting fruit at the optimum maturity and by careful postharvest handling, this injury can be avoided (To et al., 2002). Since pitaya fruit has non-climacteric ripening pattern, it should be harvested at full maturity when quality is at maximum acceptability. Postharvest development and final fruit quality depend on the stage of maturation at harvest. Premature fruit are susceptible to cell wall rupture and physiological disorders caused

by cellular disorganization; however, overripe fruit rapidly senesce, causing quality losses and reduced shelf life (Chitarra and Chitarra, 2005).

Pitaya is prone to decay and has a short shelf life due to high rates of respiration, water loss, rapid ripening, and shriveling. The shelf life of this fruit is only eight days at ambient temperature and is evident from change of chemical composition as well as softening of the peel tissue (Arevalo-Galarza and Ortiuz-Hernandez, 2004). Low temperature storage has plenty of benefits in prolonging shelf life of fresh produce after harvest by reducing respiration and water loss as well as controlling decay. However, some tropical and subtropical fruits are susceptible to chilling injury when exposed to temperatures lower than their optimum temperatures (Kader, 2002). Pitaya preserves high water content due to the peel thickness, scales and high amount of mucilage content in the pulp. Pitayas undergo accelerated softening and decrease of sugar and acidity when stored above 20 °C (Punitha et al., 2010). According to Nerd et al. (1999), moisture loss reached 4.2% after storage at 20 °C for one week, while that for those stored at 6 °C lost generally lesser, around 2.2%. The firmness of pitaya also was considerably reduced from 6.3 N to 3.8 N after 14 d of storage at 20 °C (To et al., 2002). These losses may affect the fresh weight, flavor and appearance of the fruit as well as the market price.

In order to reduce sugar loss and rapid softening, therefore, pitaya is generally stored at temperatures below 14 °C. However, when the fruit are stored at lower temperatures, for example below 5 °C, chilling injury can occur with symptoms like peel translucency, scales darkening, fruit softening, shriveling, increased respiration rate, increase in the production of ethanol and acetaldehyde in the flesh and loss of flavor

(Nerd et al., 1999; Corrales-García and Canche-Canche, 2008; Freitas and Mitcham, 2013). These symptoms depend on the genetic background, growing conditions and harvest maturity (Nerd et al., 1999). *H. undatus* in California was stored at 5 °C for 20 days with only minor chilling injury (Freitas and Mitcham, 2013). With same species, in Vietnam, it was stored at 5 °C for 17 days in good quality (To et al., 2002). On the other hand, pitaya (*H. undatus* and *H. polyrhizus*) cultivated in Israel may be stored at 14 °C for 14 days to avoid chilling injury and decrease quality loss (Nerd et al., 1999). The authors stated that the titratable acidity of the pitaya was considerably reduced during storage, dropping 50 % upon transfer to 20 °C after 2 weeks at 6 °C.

Anthrachnose infection in *Hylocereus* is caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. which can be present at the farm and during storage (Phoulivong et al. 2010). This disease has been identified in *Hylocereus* sp. fruits in Malaysia (Masyahit et al., 2009). The symptoms of infection are described by the appearance of yellowish or brown to black lesions with different sizes merging to form large irregular areas (Narayanasamy, 2005). The discolored area expands, typically in a tear-stain pattern, developing from the basal toward the distal end of the fruit (Narayanasamy, 2005). Generally, the lesions may be limited to the skin but may enter the flesh in the case of severe infections (Narayanasamy, 2005). The host barrier becomes breached, the fruit losing structural integrity. Currently, synthetic fungicides such as carbendazim, benomyl, difenoconazole and propineb are applied in the field to control pitaya diseases. However, the effects of fungicides to health, environment and the buildup of fungal pathogen resistance had been rising public awareness thus there

is interest in the development of bio-fungicides as substitutes for the synthetic fungicides (Ali et al., 2010).

Postharvest Physiology and Handling

Harvesting Method and Maturity

Pitaya with thorns are more difficult to harvest than thornless types. Leather gloves and long sleeved shirts are recommended for harvesting. Pitayas are commonly harvested by hand using hand clippers to detach fruits from the plants. Any stub with thorns at the stem attachment is removed by cutting the fruit stem. The current commercial practice is to harvest only red colored, mature fruit.

Earlier postharvest research on pitaya concentrated on harvesting the fruits. Studies with another climbing cactus, yellow pitaya (*Selenicereus megalanthus*) showed that the fruit development depends on seasonal temperatures and the fruit harvested at full color maturity had the best flavor (Nerd and Mizrahi, 1998). This characteristic is reported to be same with red pitaya (*Hylocereus undatus*), that the harvesting occurred when the fruit turn red color, between 28 and 30 days after anthesis (DAA) (Nerd et al., 1999). *H. undatus* grown in Brazil achieved physiological maturity between 30 to 32 DAA (Ortiz, and Takahashi, 2015). Merten (2003) indicated that in California, USA, ripening of pitaya occurs between 40 and 45 days after flowering.

Peel color is considered an indicator of maturity. It is an important parameter because it indicates if the fruit is best for market and ready to consume. Consumers also prefer to buy fruit that is vivid and shiny (Hirsch et al., 2012). The peel color of pitaya altered from green to red as DAA progressed from 25 to 35 DAA, while the flesh turned from reddish creamy white mixture to red-violet in fruit harvested at 25 and 30 DAA (Le Bellec et al., 2006). The peel and flesh color changes corresponded with

increase of protein content, total and types of betacyanin being separated during ripening (Nerd et al. 1999). Depending on the variety, pitaya fruit can be harvested between 25 to 30 DAA when the peel turns fully red (Nerd et al., 1999; To et al., 2002). Fruit at this maturity can last for 1 weeks at 20 °C and 2 weeks at 14 °C (Nerd et al., 1999). Per Nerd et al. (1999), when the fruit matures, the respiration rate is about 95 to 144 mg CO₂·kg·h⁻¹ at 20 °C. Delay in harvesting of pitaya fruits at 5 to 8 days after fully red will slightly shorten their storage life, even though the sensory properties are improved and result in sweeter taste (Le Bellec., et al. 2006).

Other than pericarp color, the soluble solids content and acidity are also considered an indicator of maturity (Nerd et al., 1999). According to Wu and Chen (1997), white-fleshed pitaya has higher soluble solids content than red-fleshed pitaya and the distribution of soluble solids in the flesh is not homogeneous, the core part contains more sugars than the peripheral part. Merten (2003) indicated that the soluble solids content of *H. undatus* at the time of harvest fluctuated between 13 and 16 °Brix and for *H. costaricensis* is between 7 and 11 °Brix (Vaillant et al., 2005).

Grades, Sizes, and Shipping Container

Fruit should be separated by size after the elimination of undersized, damaged, spotted and malformed fruits. There are no U.S. or international standards to grade the pitaya fruit. Generally, they are graded by size and color. The weight of red Nicaraguan pitayas varies from 200 to 550 g. Size grades suggested for Vietnam are extra-large (over 500 g), large (380 to 500 g), regular (300 to 380 g), medium (260 to 300 g), and small (under 260 g) (To et al., 2000). Fruit exported from Israel to Europe are graded by number of fruit per 4.5-kg cardboard box: 6, 8, 10, 12, 14, and 16 (Paull, 2016). According to Crane and Balerdi (2009), container of size 4.5-kg (dimensions: 40 cm x

30 cm x 9 cm) are preferred to ship to the market. These boxes should have adequate ventilation holes for good air circulation. Fruit should be packed with similar color in the boxes. Cushioning the bottom of boxes with one layer of fruit per box is recommended.

Modified Atmosphere Packaging (MAP)

Modified atmosphere packaging (MAP) and the use perforated plastic bags have also been used to extend storage life at low temperature (To et al., 1999; Freitas and Mitcham, 2013). MAP is a technique of sealing produce in a sealed package to modify the oxygen and carbon dioxide levels within the package atmosphere. A study in Vietnam showed that pitaya has a good response in MAP where fruit harvested 28 days after flowering could be stored in a MAP polyethylene bag with an oxygen transmission rate of $4 \text{ L}\cdot\text{m}^2\cdot\text{hour}^{-1}$ at $10 \text{ }^\circ\text{C}$ for 35 days (To et al., 2002). Without a bag, the same authors reported that pitaya could only be stored for 14 days at the same above temperature. They also reported that, with advanced maturity (harvest at 40 days after flowering), the shelf life was reduced to 21 days even using MAP bag storage.

Low Temperature Storage

Low temperature storage has plenty of benefits in prolonging shelf life of fresh produce after harvest by reducing respiration and water loss as well as controlling decay. Crane and Balerdi (2005) recommended *H. undatus* in Florida to be stored between 7 to $10 \text{ }^\circ\text{C}$ to maintain their quality. Storage at $10 \text{ }^\circ\text{C}$ may change many quality parameters of pitaya such as losses in sugar and organic acids but these changes did not affect the sensory quality of the fruit as no significant difference reported by panelists in overall liking, sweetness, flavor, texture or tartness (Obenland et al., 2016). Nerd et al. (1999) also reported no decline in flavor for either *H. undatus* or *H. polyrhizus* after 3 weeks at $6 \text{ }^\circ\text{C}$, compared to pitayas that were stored at higher

temperatures. Although sensory evaluation did not reveal any differences, color data showed that low temperature storage resulted in slight darkening of the flesh (Obenland et al., 2016). In terms of aroma, these same authors identified 34 aroma volatiles in the pitaya juice and aldehydes were the most abundant, consisting of 90% of the total volatile amount. Various hydrocarbons, alcohols, a ketone, an ester and a furan making up the rest of the identified volatiles (Obenland et al., 2016). The authors also observed that from 34 aroma volatiles, 19 were different depending on variety or storage treatment. Storage increased the total aldehyde concentration with fruit stored at 10 °C having a greater concentration than that stored at 5 °C (Obenland et al., 2016).

1-Methylcyclopropene (1-MCP) Treatment

To further extend the storage life of pitaya, use of the ethylene-action inhibitor 1-methylcyclopropene (1-MCP) has been explored. Cock et al. (2013) found that pitaya exposed to 1-MCP at 200 or 400 mg/L maintained its firmness and had less weight loss. Deaquiz et al. (2014) observed that yellow pitaya exposed to 1-MCP at 600 mg·L⁻¹ had low respiration rates, maintained the firmness, and had decreased weight loss, less peel yellowing and carotenoid accumulation after harvest. Li et al. (2016) found that red pitaya cv. 'Bilu' exposed to 1-MCP at 1 mL·L⁻¹ had delayed senescence as observed after 16 days at 10 °C followed by 5 days at 20 °C (Li et al., 2016).

Edible Coatings

Food-grade coatings such as polyethylene, chitosan and carnauba are semi-permeable and can perform similar to MAP (Espino-Díaz et al., 2010). By modifying the internal fruit atmosphere including carbon dioxide and oxygen concentrations, under optimum storage environment conditions, postharvest coatings have been shown to reduce water vapor loss, suppress ripening, enhance visual quality, reduce fruit decay

and improve the storage life of many fruits and vegetables (Amarante et al., 2001; Arowora et al., 2013; Del-Valle et al., 2005; Hagenmaier, 2005). The use of natural ingredients is in high demand due to the low impact on the environment and human health, as well as eliminating the use of chemical fungicide.

An effective coating application has the potential to reduce postharvest deterioration and extend the shelf life. Ali et al. (2013) reported that chitosan coating applied to pitaya maintained quality for up to 28 days at 10 °C without any off-flavors. Propolis (natural glue produced by honey bees) coating also slowed down the ripening process and increased the biosynthesis of nutritional components in pitaya (Zahid et al., 2013). Vegetable oil and carnauba wax are lipid-based coatings that have been reported to have beneficial effects on fresh produce, for example tomatoes (Dávila-Aviña et al., 2012). They are stable at ambient temperatures, emulsify and do not interfere with the quality of fresh fruit and vegetables. Vegetable oils (soybean, corn, peanut, linseed, and cottonseed) are widely used as coatings to delay ripening and maintain fruit quality (Ju et al., 2000). Carnauba wax comes from the carnauba palm, native to northeastern Brazil (Puttalingamma, 2014). Machado et al. (2012) reported that carnauba-based waxes preserve the freshness of Ortanique' tangor by reducing mass loss, peel dehydration and retaining green color.

Minimally Processed Pitaya

Besides marketing pitaya as a whole fruit, the fruit are also suitable for minimal processing. Minimally processed fruits have a high demand because consumer like products that are ready to eat, convenient with reduced preparation time. Minimally processed fruits are safe to be stored between 4 to 8 °C (Wiley, 1994). However, minimally processed pitaya rapidly loses its quality, developing a brown surface

appearance due to oxidation in wounded tissue (Baldwin et al., 1995). Additionally, minimally processed pitaya has a short shelf life because it is highly perishable. Low molecular weight chitosan coating delayed browning and reduced water loss as well as decay symptoms of minimally processed pitaya (*H. undatus*) without influencing the flavor, thus maintaining the quality during handling, distribution and retail sale (Chien et al., 2007).

Research Objectives

Despite the numerous postharvest studies conducted with pitaya, there is insufficient information related to prolonging the postharvest quality and storage life of the fruit due to different genetic background, growing conditions and harvest maturity. This led to the interest of this study for red-fleshed pitaya grown in southern Florida. The objectives were to:

- 1) determine the effect of harvest maturity on respiration and ethylene production and fruit quality during storage,
- 2) compare the effectiveness of coatings in reducing fruit weight loss and maintaining quality and sensory attributes during simulated commercial storage,
- 3) determine the lowest safe storage temperature that does not induce chilling injury by measuring the effect on betacyanin concentration as well as other fruit quality parameters during extended storage.



Figure 2-1. Pitaya (*H. costaricensis*) planting in trellis system in private farm in Homestead, Florida.



Figure 2-2. Pitaya (*H. costaricensis*) planting estimated about 2 m height in vertical post with support system



Figure 2-3. Pitaya (*H. costaricensis*). Note: 1=floral button, 2=floral buds, 3=flower at anthesis, 4= pitaya fruit on stem



Figure 2-4. Red-fleshed pitaya fruit (*Hylocereus costaricensis*) cv. Lisa.

CHAPTER 3 HARVEST MATURITY AFFECTS FRUIT QUALITY AND POSTHARVEST BEHAVIOR OF RED-FLESHED PITAYA FRUIT

Introduction

Pitaya is a non-climacteric fruit, originating from Latin America, and belongs to the botanical family Cactaceae and genus *Hylocereus*. It is a cactus plant with vigorous vines. The fruit has a red peel and white or red flesh and many small black seeds. In south Florida, red pitaya is grown commercially and demand is increasing for niche markets as well for domestic consumption. With a total receipt assessed at \$64,1250 per ha (21,546 kg·ha⁻¹ of marketable yield), net profits are estimated at \$38,808 per ha and this denotes a very satisfactory return (Evans et al., 2016). In March 2017, the market price of Florida pitaya packed in a 4.5-kg box ranged from \$32 to \$42 per carton and can occasionally be as high as \$55 per box. (USDA, 2017).

The fruit has gained popularity due to its attractive appearance and potential health benefits. Pitaya is a source of lycopene, beta-carotene and vitamin E and the seeds are rich with essential fatty acids (Ariffin et al., 2008; Charoensiri et al., 2009). In recent studies oligosaccharides extracted from pitaya showed probiotic properties (Wichienchot et al., 2010) and consumption of red pitaya may prevent cardiovascular disease (Mohd Adzim Khalili et al., 2009). A study conducted at the University of Florida measured the antioxidant activity of two types of pitaya grown locally and showed that red-pulp pitaya had higher antioxidant activity (ORAC: 7.6 ± 0.1, DPPH 134.1 ± 30.1) compared to white-pulp pitaya due to betalains pigment (ORAC: 3.0 ± 0.2, DPPH: 34.7 ± 7.3) (Mahattanatawee et al., 2006).

Postharvest development and final fruit quality depend on the stage of maturation at harvest. Since pitaya fruit is non-climacteric, it should be harvested at full, ripe

maturity stage when quality is at least minimally acceptable because ripening processes do not continue after harvest. Immature fruit are susceptible to cell wall rupture and physiological disorders caused by cellular disorganization; however, overripe fruit are prone to show senescence symptoms, causing quality losses and reduced shelf life (Chitarra and Chitarra, 2005). Peel color is considered an indicator of pitaya maturity. It is an important parameter because it indicates whether the fruit is ideal for market and ready to consume. Consumers also prefer to buy fruit that is firm and has a shiny peel (Hirsch et al., 2012). Additionally, the number of days after anthesis (DAA) until the fruit is ripe, soluble solids content and sugar:acid ratio are often used to determine the timing of harvest. Studies in Vietnam and Brazil found that *H. undatus* fruit are harvested when they turn red, between the 28th and 30th DAA and 30th and 32nd DAA, respectively (To et al., 2002; Ortiz and Takahashi, 2015). However, the time it takes for fruit to develop depends on seasonal environments. Nerd et al. (1999) reported that, during fruit growth, these fruit (*H. undatus*) reach the maximum respiration rate. When the fruit matures, the respiration rate is about 95 to 144 mg·kg⁻¹·h⁻¹ at 20 °C (52 to 80 mL·kg⁻¹·h⁻¹). However, Corrales-García & Canche-Canche (2008) reported lower respiration rate (26 to 32 mL·kg⁻¹·h⁻¹) at 26 °C for the same species. Jamaluddin et al. (2011) studied red-fleshed pitaya (*H. polyrhizus*) and reported very low respiration rate during development at 26 °C between 0.14 to 0.03 mL·kg⁻¹·h⁻¹.

The objectives of this study were to prolong the postharvest quality of red fleshed pitaya (*Hylocereus costaricensis*) grown in South Florida, USA, by 1) measuring the effects of different maturity stages at harvest on fruit quality during simulated

commercial storage, and 2) comparing the respiration rate and ethylene production of fruit harvested at different maturity stages.

Materials and Methods

Plant Materials

The pitaya (*Hylocereus costaricensis*) cv Lisa fruit were harvested on July 29, 2016 at three different maturities, ½-red (T1), ¾-red (T2) and full-red (commercial maturity) (T3) from Red Pitaya Growers in Homestead, Florida and transported to the Postharvest Laboratory at the University of Florida in Gainesville. Fruit were held at 15 °C overnight and the next day were sorted for quality, uniformity and freedom from decay. Fruit were cleaned by wiping each fruit with soft dry wipes to remove dirt particles and visible insects. Fruit from each maturity group were randomized into four lots of four fruit each. Fruit were placed on single-layer trays and stored at 20 °C with 85% relative humidity (RH) for 11 days.

Quality Analyses

Four fruit from each maturity stage were subjectively evaluated every 2 to 3 days for peel color, yellow lesions, disease area (symptoms other than yellow lesions), and overall appearance ratings (Table 3-1); they were objectively measured for weight loss, firmness and external and internal color. Visual peel color was rated as 0% (green unripe), 25% (green with slightly red), 50% (half-green and half-red), 75% (red with slightly green) and 100% (full-red). Severity of diseases and yellow lesions area were rated as 0% (no trace), <15% (slightly affected), 16-25% (moderately affected), 25-50% (badly affected) and >50% (severely affected). For overall acceptability ratings, fruit were rated as 5 (excellent), 4 (good), 3 (acceptable), 2 (poor) and 1 (very poor). Fruit with more than 50% symptoms and rated as poor or very poor were considered

unacceptable for sale and consumption. Fruit weight loss (% fresh weight basis) was calculated as the difference between the initial fruit weight at harvest and fruit weight after 3, 6, 9 and 11 d of storage. Mesocarp firmness was measured by using a computer-controlled texture analyzer (model TA.HD*Plus*, Texture Technologies Corp, NY) equipped with a 50-N load cell (n=8). Two lengthwise slices were made on opposite sides of the fruit to a depth of 5 mm at the equator, and the outer portions were discarded. Mesocarp firmness was measured on each sliced side at the fruit equator using an 11-mm diameter convex probe with the crosshead speed of 2 mm/sec and expressed in Newtons (N) at the bioyield point. External color was determined using a colorimeter (Konica Minolta Sensing Model CR-400; Tokyo, Japan) with D₆₅ illuminant. In this system, L* refers to lightness, on a scale of 0=black to 100=white, hue* angle is the actual color in degrees, where 0°=red, 90°=yellow, 180°=green and 270°=blue, and chroma* value indicates color saturation (McGuire, 1992).

After each evaluation, fruit were peeled and the flesh was frozen (-20 °C) for later analysis of soluble solids content (SSC), total titratable acidity (TA), and pH. Upon thawing for about 1 h, each sample (15 g) was added to distilled water (1:1 w/v), homogenized, then centrifuged (Thermo Scientific Lynx 4000 Sorvall) for 20 min at 19,319 x g at 4 °C. The juice was then filtered with 4 layers of cheese cloth and the filtrate collected. SSC was quantified by placing a drop of juice on a digital refractometer (model r2i300, Reichert Technologies, Depew, N.Y.). TTA and pH were determined with an automatic titrator (model 905 Titrando; Metrohm Ion Analysis, Switzerland) for which 3 mL of juice diluted with 15 mL of water was titrated with 0.1 M NaOH to pH 8.2 and reported as percent malic acid.

Respiration and ethylene measurements were also taken periodically throughout storage at 20 °C. A sound fruit (n=3) from each treatment was placed in each jar (1.7-L each) and sealed for 2 h. A headspace sample (3 mL) was taken from each jar and injected into a gas chromatography (Varian CP-3800, CA) and CO₂ and ethylene concentrations were determined. Following sampling, lids were removed from each jar; samples were determined every 2 days for 8 days.

Data Analysis

Each treatment was comprised of five replications with four fruit per replicate and was evaluated every 3 d. The analysis of variance (ANOVA) for a completely randomized design was performed for each variable within each storage period (SAS 9.4, Cary, North Carolina). Means separation for each variable was performed using Duncan's Multiple Range test ($p \leq 0.05$).

Results and Discussions

Appearance and Acceptability Score

Florida pitaya harvested at ½-red stage (T1) ripened to 85% red at day 6 whereas ¾-red stage (T2) fruit ripened to 85% red at day 3 (Figure 3-1). While other fruit ripened evenly, T1 fruit exhibited non-uniform ripening; 50% of this group didn't begin to change color until day 6. By day 9 of storage, T1 and T2 fruit ripened to full-red. At day 6, T1 and T2 fruit had no disease, but T3 fruit had 20 to 40% severity of yellow lesions (Figure 3-2). By day 9, the T3 fruit were discarded with up to 60% of the fruit showing fungal decay and developing severe yellow lesions. Yellow lesions in this study was similar to the symptom called body rot according to Woolf et al. (2006) for pitaya harvested in Vietnam. Fruit with yellow lesions and fungal decay were sent to the Plant

Diagnostic Center, University of Florida and both lesion types were diagnosed as anthracnose fruit rot from *Colletotrichum gloeosporioides* based on visual inspection of the cultures but not DNA. This fungus was present in the field; thus, it is very important to control the pathogen at the field level because the symptoms only appeared during storage.

For overall acceptability, fruit harvested at T1 and T2 maturity stages showed excellent (rating 5) quality at day 6 and good (rating 4) quality up to 9 days of storage. At day 10, the fruit were rated as acceptable (rating 3) and by day 11, they became unacceptable as the quality deteriorated even faster at the end of storage. In the present study, a treatment was considered unacceptable if more than 25% of all parameters were affected, whereas in the study with fruit grown in Vietnam (Woolf et al., 2006), fruit with more than 15% decay were considered unacceptable.

Weight Loss

Percent weight loss was recorded at 3 d, 6 d, 9 d and 11 d; fruit harvested at T3 had highest weight loss (Figure 3-3). At day 9, weight loss for T3 was 8.24% compared with fruit harvested at T1 and T2 at 6.39 % and 5.89 %, respectively. While T3 fruit storage was ended at day 9, T1 and T2 fruit were observed until day 11 when their weight loss was similar (7.4%). Fruit harvested at T3 had the highest weight loss due to their late maturity during harvest. The fruit became more perishable as the harvest maturity advanced, being more susceptible to mechanical injury and higher water loss during storage. Physical changes in *H. undatus* were assessed by Ortiz and Takahashi (2015) who concluded that as fruit mature, the ratio of pericarp mass to whole fruit mass decreased from 69% pericarp at 21 DAA to 41% at 31 DAA. This reduction makes the

pericarp of the fruit thinner and more susceptible to postharvest injury (Centurion Yah et al., 2008).

Color and Firmness

There were significant differences in external and internal color parameters related to the harvest maturities. Fruit harvested at T1 maturity stage had the highest L* (37.15) and highest h* (49.96°) and lowest C* (29.39) compared with T2 and T3 (Table 3-2). A similar pattern was observed in the three harvested ripeness stages for internal (flesh) color. This finding is in conjunction with study on *H. polyrhizus* (Phebe et al., 2009), where the L* and h* values declined as maturation progressed, reflecting the development of redded color in both peel and flesh as pigment synthesis occurred during maturation. These authors also reported that the increase of C* indicate the development of more intense color in pitaya peel and pulp. Fruit harvested at T1 maturity stage experienced significant changes in peel and pulp color during ripening (Figure 3-4). Decreasing values of peel and pulp h*, which means redder color, represents fruit ripening. Betacyanins had an important role in the red coloration of the pericarp in *Hylocereus* species fruit, for which high availability of sugar and light were reported as being among the factors that promote their synthesis (Castellar et al., 2003). The amount of soluble sugars increases as the maturation process advances, leading to increased betacyanin production and lower hue angle values.

Fruit firmness decreased equally between treatments during storage except at day 3, when the T1 fruit were firmer due to half of them still being immature (Table 3-3). Fruit harvested at T1 had significantly firmer texture (8.58 N) compared to the other maturities. This finding was expected since the T1 fruit were immature at harvest and the softening process did not begin until day 6. This finding was similar to the trend

reported by Nerd et al. (1999), that fruit firmness decreased as maturity advanced. These authors reported that as the fruit matured, the moisture content in the peel decreased, hence reducing the fruit firmness.

Soluble Solids Content, Titratable Acidity and Sugar/Acid Ratio

SSC was significantly lower in T1 (5.80%) compared to T2 and T3 (9.36% and 8.67%, respectively) (Table 3-3). However, there were no significant differences among treatments for TTA throughout the storage period (mean = 0.27%). Fruit harvested at T2 maturity stage had significantly higher sugar:acid ratio (52.43) compared to other treatments, which indicates sweeter flavor. Osuna Enciso et al. (2011) also measured soluble solids content of pitaya fruit (*H. undatus*) harvested at different stages based on a subjective scale of red color of the peel: 25-50%, 50-75% and 75-100%. In that study, similar values were found to those of this study in which the fruit harvested at full or $\frac{3}{4}$ red color had higher SSC values than the fruit harvested at the least mature stage.

Respiration Rate and Ethylene Production

T1 fruit had higher respiration rate (17 to 25 mL·kg⁻¹·h⁻¹) compared to those harvested at the other maturity stages (Figure 3-5), indicating that the former had higher metabolism during fruit development. The respiration rate declined and ranged between 12 and 17 mL·kg⁻¹·h⁻¹ as the fruit matured. The respiration rate of T3 fruit was lower (11 to 15 mL·kg⁻¹·h⁻¹) compared to previous data reported by Nerd et al. (1999) and Corrales-García and Canche-Canche (2008) for white-fleshed pitaya at 26 °C (*H. undatus*) (52 to 80 and 26 to 32 mL·kg⁻¹·h⁻¹, respectively). However, for red-fleshed pitaya (*H. polyrhizus*), Jamaluddin et al. (2011) reported lower respiration rate at 26 °C, between 0.14 to 0.03 mL·kg⁻¹·h⁻¹. The authors reported the decreased in stomatal density in red-fleshed pitaya as ripening progressed presumably that inhibition of

respiration might be due to modification of the fruit internal atmosphere. The pattern was similar for ethylene production, for which T1 fruit produced more than twice ($1.66 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) the amount of ethylene (Figure 3-4) compared to the other T2 and T3 fruit (0.69 and $0.42 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, respectively). The ethylene production decreased slowly during the storage period, thus confirming that pitaya exhibits non-climacteric respiratory activity. Similar results were reported for pitaya harvested at several stages of maturation, as no ethylene peak or increased production was observed (Wu, C. 2010) and for red-fleshed pitaya grown in Malaysia (Jamaluddin et al., 2011).

Conclusions

Pitaya harvested at the full-red stage (current practice) had the shortest shelf life, only 8 days at $20\text{ }^{\circ}\text{C}$ due to development of severe yellow lesions on the peel. Although pitaya can be harvested at the $\frac{1}{2}$ -red stage, it is not advisable since those fruit displayed non-uniform ripening and had low soluble solids content. Pitaya harvested at the $\frac{3}{4}$ -red maturity stage ripened normally and had acceptable appearance until day 10 with significantly higher sugar:acid ratio. Therefore, they had better quality and longer shelf life compared to full-red stage.

Table 3-1. Visual peel color, disease and yellow lesions area rating and overall acceptability ratings based on each fruit (n=4)

Visual Peel Color (%)	Disease and Yellow Lesion Area Rating (%)	Overall Acceptability Ratings
100 = Full-red	0 = No trace	5 = Excellent
75 = Red with slight green	<15 = Slightly affected	4 = Good
50 = Half-green and half-red	16-25 = Moderately affected	3 = Acceptable
25 = Green with slight red	25-50 = Badly affected	2 = Poor
0 = Green unripe	>50 = Severely affected	1 = Very poor

Table 3-2. Peel and flesh color of pitaya (n=4) harvested at three maturity stages after 11 days of storage at 20 °C.

Main Effect	Peel			Flesh		
	L*	C*	h*	L*	C*	H*
Harvest Maturity						
½ red (T1)	37.15a ^z	29.39b	49.96a	35.67a	26.30b	52.15a
¾ red (T2)	35.66b	30.57b	19.94b	20.54b	28.23ab	12.82b
Full-red (T3)	36.32ab	38.49a	14.54b	20.84b	31.16a	13.73b
Storage Period						
Day 0	38.33a	24.78d	53.44a	38.54a	23.07b	56.36a
Day 3	37.38a	30.56c	33.00b	33.04a	30.53a	38.51ab
Day 6	35.33b	33.56bc	20.03c	20.91b	30.36a	10.94b
Day 9	35.37b	36.95ab	17.26c	20.25b	28.36a	12.36b
Day 11	35.06b	38.09a	18.22c	21.16b	30.14a	12.62b
Interactions						
Harvest Maturity x Storage Period	**y	**	**	**	**	**

^zMeans in each column with the same letter are not significantly different.

^y** = Statistically different at $p \leq 0.01$. Separated using Duncan's Multiple Range Test.

Table 3-3. Compositional data for pitaya (n=4) harvested at different maturity stages at the end of 11 days of storage at 20 °C.

Main Effect	Soluble Solids Content (%)	Total Titratable Acidity (Malic acid, %)	SSC/TTA	Flesh Firmness (N)
Harvest Maturity				
½ red (T1)	5.80b ^z	0.22a	35.12b	8.58a
¾ red (T2)	9.36a	0.23a	52.43a	5.98b
Full-red (T3)	8.67a	0.22a	45.52ab	6.60ab
Storage Period				
Day 0	8.22a	0.31a	26.81c	6.88b
Day 3	7.48a	0.25ab	31.74c	11.87a
Day 6	8.41a	0.22ab	42.45bc	5.45b
Day 9	7.35a	0.17bc	51.40b	5.42b
Day 11	8.04a	0.11c	76.82a	5.18b
Interaction				
Harvest Maturity x Storage Period	ns ^y	ns	ns	**

^zMeans in each column with the same letter are not significantly different.

^y** = Statistically different at $p \leq 0.01$, ns = not statistically different. Separated using Duncan's Multiple Range test.

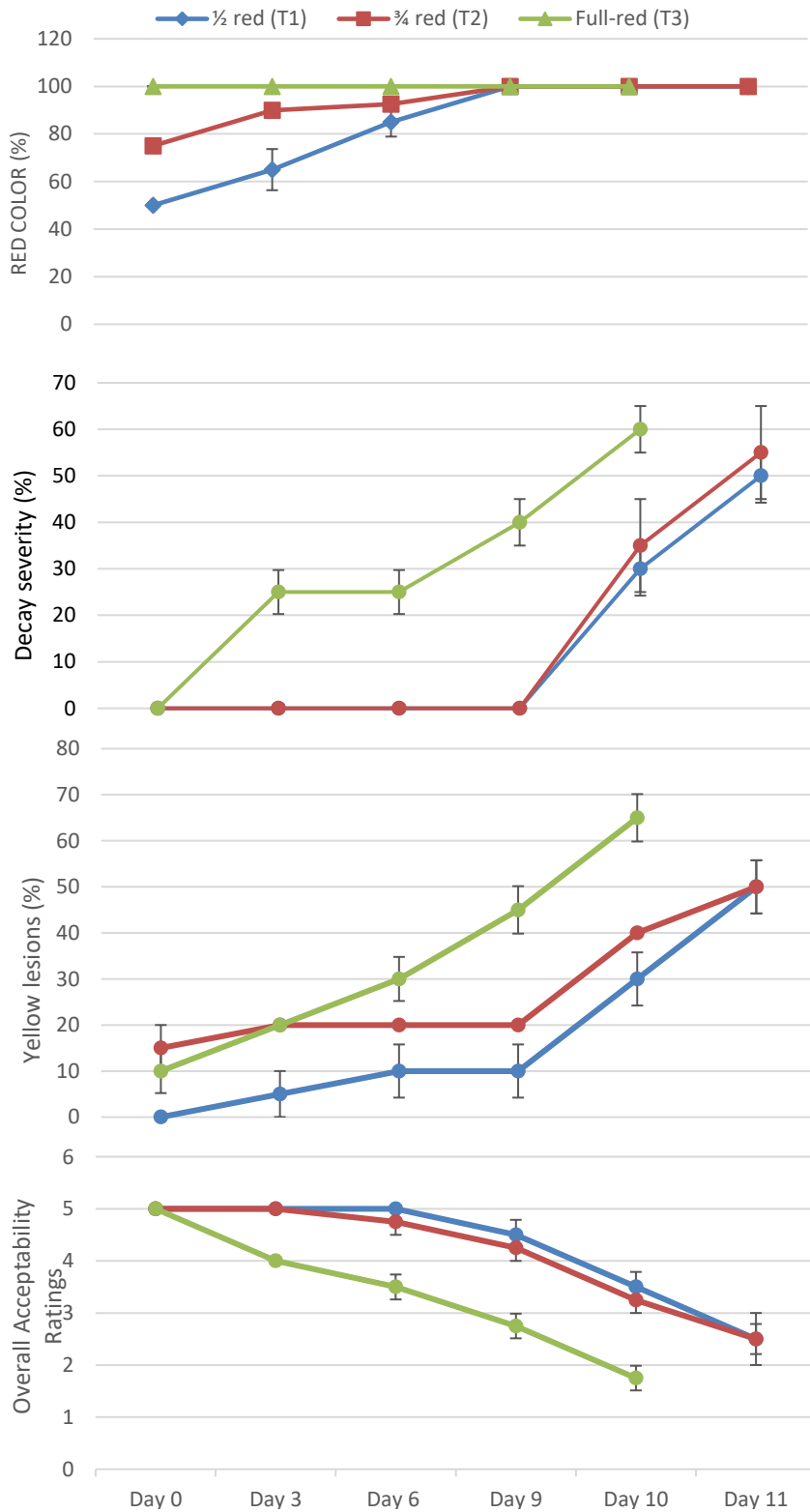


Figure 3-1. Red color and disease/yellow lesion severity and overall acceptability of pitaya harvested at three color stages during storage at 20 °C. The values are means (n=4) and vertical bars are standard errors.



Figure 3-2. Yellow lesion severity (fungal decay) diagnosed as anthracnose fruit rot from *Colletotrichum gloeosporioides*.

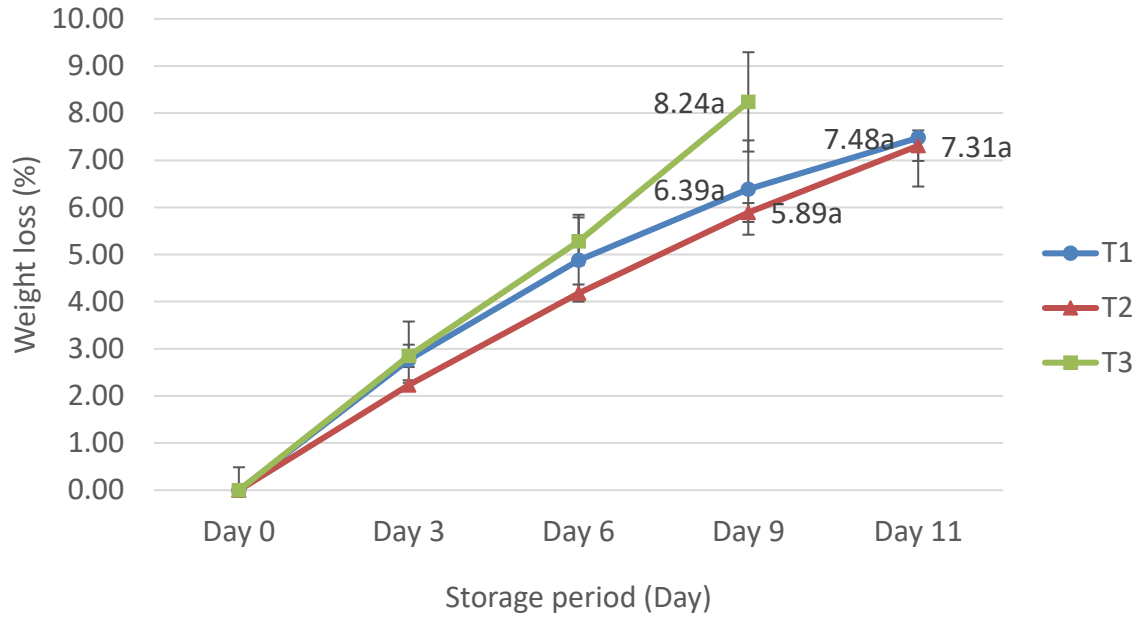


Figure 3-3. Weight loss of pitaya harvested at 1/2- (T1), 3/4- (T2) and full- (T3) color stages and stored at 20 °C with 65% RH. The values are means (n=4) and vertical bars are standard errors.

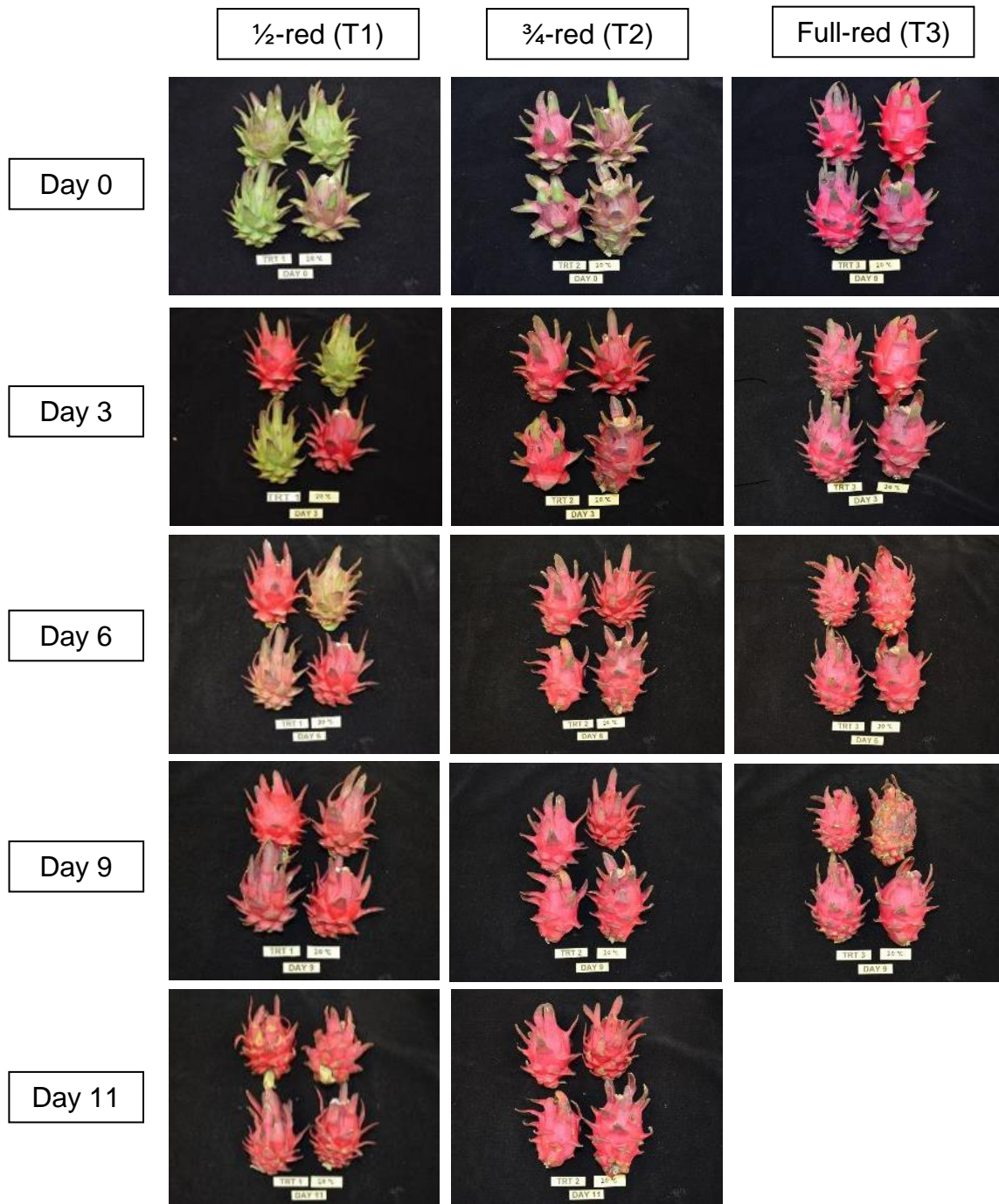


Figure 3-4. Color changes of pitaya harvested at ½ (T1), ¾ (T2) and full-red (T3) maturity stages during storage at 20°C.

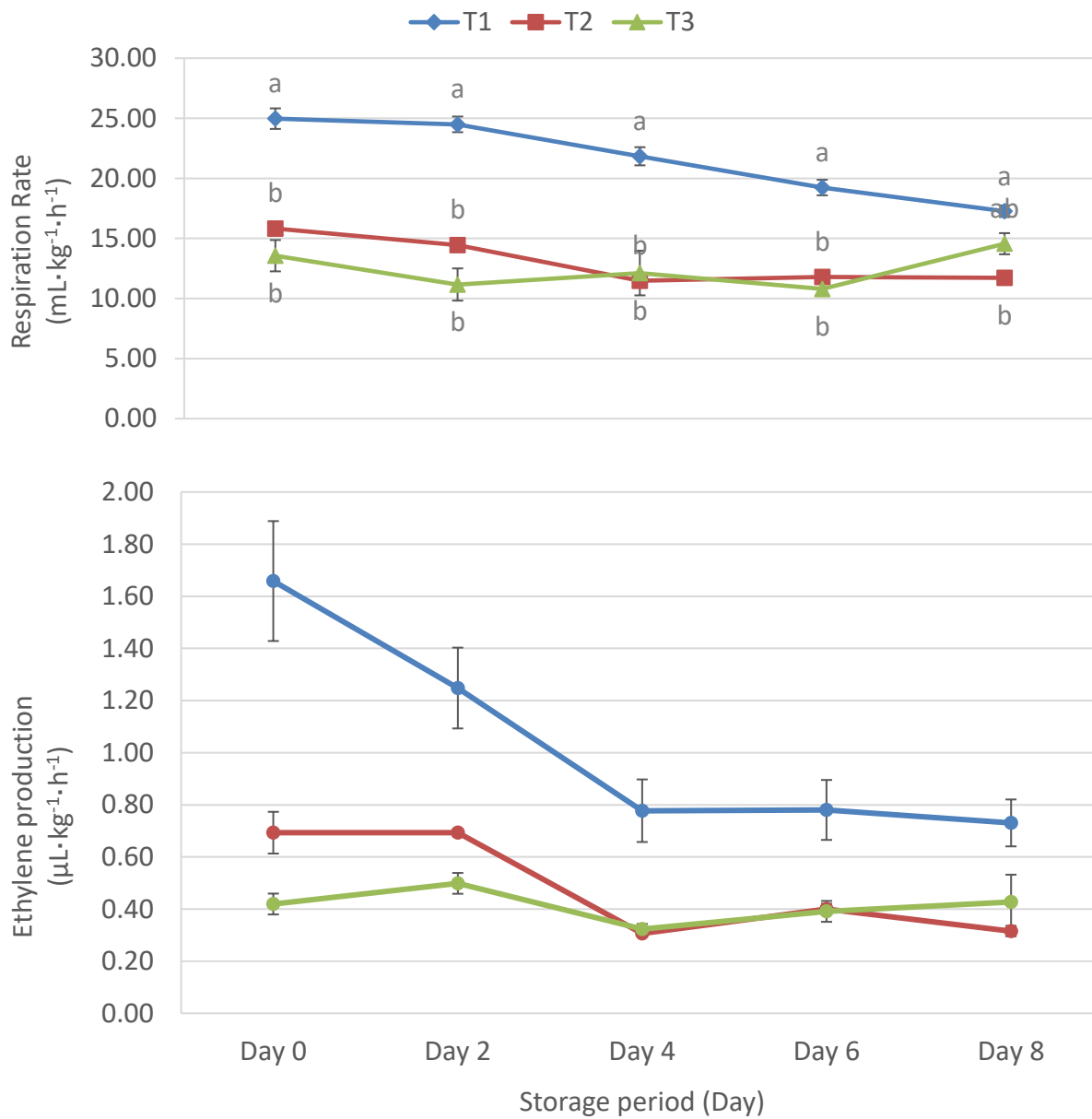


Figure 3-5. Respiration rate and ethylene production of pitaya harvested at different maturity stages during storage at 20 °C. The values are means (n=3) and vertical bars are standard errors.

CHAPTER 4 POTENTIAL OF POSTHARVEST COATINGS TO MAINTAIN FRESHNESS OF RED- FLESHED PITAYA

Introduction

Pitaya originates from Central America and belongs to the genus *Hylocereus* of the botanical family Cactaceae. It is a climbing plant with vigorous vines and the scarlet fruit have white or red flesh with numerous small black seeds. Fruit diameter ranges from 10 to 15 cm and weight between 250 to 600 g. Since pitaya is a non-climacteric fruit, it should be harvested near full maturity when the quality is acceptable. However, without proper handling, the quality of pitaya picked at optimum maturity decreases rapidly during storage.

Pitayas preserve high water content due to the thickness of the exocarp, the presence of scales and the high mucilage content in the mesocarp. According to Nerd et al. (1999), water loss was 4.2% after 1 week of storage at 20 °C; at 6 °C, water loss was generally 6% after 3 weeks of storage. Water loss affects the fresh weight, firmness and appearance of the fruit as well as the market price. In March 2017, the market price of Florida's pitaya in 4.5-kg (10-lb.) package was \$55 per box (USDA, 2017).

Recently, coating technology has been developed to improve the storage life of fruits and vegetables by using edible ingredients. The use of natural ingredients is in high demand due to the low impact on the environment and human health, as well as eliminating the use of fungicides. According to Amarante et al. (2001) and Arowora et al. (2013), postharvest coatings can have beneficial effects on fruit quality such as improving shelf life, suppressing ripening, retarding water loss, enhancing visual quality and providing resistance to fruit decay. Coatings such as chitosan and carnauba are semi-permeable and if properly formulated, can behave similarly to modified

atmosphere packaging (MAP). By modifying the internal atmosphere of carbon dioxide and oxygen concentrations to optimum storage environment conditions, postharvest coatings have been shown to reduce water vapor loss and improve the storage life of fruits and vegetables (Del-Valle et al., 2005; Hagenmaier, 2005).

An effective coating application has the potential to reduce the perishability of pitaya fruit and extend the shelf life. Ali et al. (2014) reported that pitaya treated with chitosan coating maintained fruit quality for up to 28 d at 10 °C without any off-flavors. Propolis (natural “glue” produced by honey bees) coating also helped slow the ripening process while increasing the biosynthesis of nutritional components in pitaya (Zahid et al., 2013). Vegetable oil and carnauba wax are lipid-based coatings that have been reported to have beneficial effects on fresh produce such as Chinese pear and tomato (Ju et al., 2000; Dávila-Aviña et al., 2014). These coatings are stable at ambient temperature, emulsify and do not interfere with the quality of fresh fruit and vegetables. Vegetable oils (soybean, corn, peanut, linseed, and cottonseed) are widely used as coatings to delay ripening and maintain fruit quality (Ju et al., 2000). Carnauba wax comes from the carnauba palm, grown in northeastern Brazil (Puttalingamma, 2014). Machado et al. (2012) reported that carnauba-based waxes preserve the freshness of citrus fruit by reducing mass loss, peel dehydration and retaining green color. However, each lipid-based coating has different behavior against moisture transfer due to its hydrophobic substance (Morillon et al., 2002).

The objective of this study was to study the postharvest quality of red-fleshed pitaya (*Hylocereus costaricensis*) as affected by the application of commercial

coatings, a vegetable oil-based coating or a carnauba-based coating, during simulated commercial storage.

Materials and Methods

Plant Material

Pitaya fruit ('Lisa') at commercial maturity (full-red stage) were acquired from Red Pitaya Growers in Homestead, FL in two subsequent years (Nov 20, 2015 and Oct 27, 2016). The fruit were harvested at commercial maturity stage and transported in coolers on the day of harvest to the Postharvest Horticulture Laboratory at the University of Florida in Gainesville. Following overnight storage at 15 °C, the fruit were sorted for uniformity of size and freedom from decay, and cleaned with soft, dry wipes to remove dirt particles and visible insects. Fruit (n=60) were randomized into three treatment groups: VOC (SF2981, vegetable oil-based), CC (EF6100, carnauba-based) (JBT Corporation, Lakeland, Florida, USA) and control (dipped in water). Each treatment included 40 replications of one fruit per replicate. Fruit were dipped for 30 s, air-dried using a fan at room temperature for about 20 min, then placed on single-layer trays and stored at 7 °C and 85% relative humidity for 20 days.

Quality Analysis

Fruit (n=4) from each treatment were removed from storage every 5 days for evaluation of shriveling and incidence of yellow lesions (an anthracnose infection), and overall appearance ratings (Table 4-1), and measurement of weight loss, external color and firmness. Severity of shriveling and yellow lesions area were rated as 0% (no trace), <15% (slightly affected), 16-25% (moderately affected), 25-50% (badly affected) and >50% (severely affected). For overall acceptability ratings, fruit were rated as 5 (excellent), 4 (good), 3 (acceptable), 2 (poor) and 1 (very poor) (Table 4-1). Fruit with

more than 50% symptoms and rated as poor or very poor were considered unacceptable for sale and consumption. Fruit weight loss (% fresh weight basis) was calculated as the difference between fruit weight at harvest and fruit weight after 5, 10, 15 and 20 d of storage. External color was determined using a colorimeter (Konica Minolta Sensing Model CR-400; Tokyo, Japan) with D_{65} illuminant, obtaining L^* , a^* and b^* and these values were converted into hue angle (h^*) and chroma (C^*) value as described by McGuire (1992). Mesocarp firmness for pitaya was identified by using a computer-controlled texture analyzer (model TA.HD*Plus*, Texture Technologies Corp, NY) equipped with a 50-N load cell. Firmness was measured after fruit were conditioned to ambient temperature for about 1 hour. Two lengthwise slices were made on opposite sides of the fruit to a depth of 5 mm at the equator, and the outer portions were discarded. Mesocarp firmness was measured on each sliced side at the fruit equator using an 11-mm diameter convex probe with the crosshead speed of 2mm·sec⁻¹. Firmness was expressed in Newtons (N) at the the bioyield point (n=8).

After each evaluation, fruit were peeled and the fruit flesh were frozen (-20 °C) for later analysis of soluble solids content (SSC), total titratable acidity (TTA), and pH. Each sample was blended then centrifuged (Thermo Scientific Lynx 4000 Sorvall) for 20 min at 19,319 x g at 4 °C. The juice was filtered with 4 layers of cheese cloth and the filtrate was collected. Soluble solids content (SSC) was quantified by placing a drop of juice on a digital refractometer (model r2i300, Reichert Technologies, Depew, N.Y.); total titratable acidity (TTA) was determined with an automatic titrator (model 905 Titrando; Metrohm Ion Analysis, Switzerland). For total titratable acidity, 3 mL of juice

diluted with 15 mL of water was titrated with 0.1 M NaOH to pH 8.2 and reported as percent malic acid.

Sensory Evaluation

Consumer testing was conducted in the Sensory Laboratory, Food Science & Human Nutrition Department, University of Florida. The objective of this test was to determine fruit acceptance and any differences between fruit coated with VOC, CC or control. Panelist rated each sample for like or dislike of external and internal appearance, overall acceptability, flavor and texture using 9-point hedonic scales (1=dislike extremely and 9=like extremely) (Table 4-2) and 5-point Just About Right scales (1=much too soft/dry and 5=much too firm/juicy) for firmness and juiciness (Table 4-3). Additionally, ranking was also used to determine fruit preference. A questionnaire consisting of those six sensory characteristics was prepared and responses were analyzed using Compusense Software.

Fruit (n=20) from each treatment were cut into wedge shape and served on individual plates. Each plate consisted of two wedges for each treatment. Fruit were prepared immediately prior to serving to the panelists at room temperature. Unsalted cracker and water were given as a palate cleanser between samples. The coding used three-digit random numbers (VOC = 429, CC = 165 and control = 738). The randomization was set by Compusense Software and the order of presentation for each panelist was included in the master sheet to avoid error. A sticker gun was used to apply labels on the plates.

Panelists (n=71) were recruited through emails targeted to students and staff at the University of Florida. Each panelist was assigned an identification number. For the test, each panelist was asked to taste the fruit according to instructions displayed on

their individual monitor and to enter their answers directly into the program. Each panelist received a coupon for participating in this testing. No debriefing was conducted in this testing.

Statistical Analysis

The same experiment was repeated twice, once in each of two subsequent years. An analysis of variance (ANOVA) test was performed on each variable using a completely randomized design. The analyses were accomplished using the SAS statistical package (SAS Institute, 2002-2012). The mean values of four replicate samples were compared using Duncan Multiple Range test ($p \leq 0.05$). Sensory analysis was conducted once; 2-way ANOVA was performed using a randomized complete block design (RCBD). Tukey's HSD was used to compare the mean values of samples.

Results and Discussions

Appearance and Acceptability Score

The appearance of the fruits is very important in relation to consumer acceptability. Fruit that look fresh and are free from decay will attract consumers as well as command a higher market price. From this study, CC significantly ($p \leq 0.05$) prevented shriveling (0%) compared with uncoated pitaya (16.3%) after 15 days' storage (Table 4-4). At day 20, 50% of uncoated pitaya were shriveled (Figure 4-2) while the pitaya coated with CC or VOC exhibited only 20% and 25%, respectively. CC also reduced the severity of yellow lesions (2%) compared to uncoated pitaya that developed 10% of yellow lesions as early as day 15, however no significant difference among treatments. Fruit with yellow lesions were diagnosed as anthracnose fruit rot from *Colletotrichum gloeosporioides* based on visual inspection of the cultures but not DNA. This result was similar with chitosan coating, which reduced the onset of

anthracnose in pitaya stored at 10 °C (Ali et al., 2014). Pitaya coated with CC was more acceptable at day 15, however, fruit from all treatments became unacceptable at the end of the 20-day storage at 7 °C.

Weight Loss

Data analysis revealed that there was no effect of coating on weight loss during 20 d of storage (mean=5.5%; Table 4-5). This finding was different from the report by Machado et al. (2012) in which carnauba-based coating better reduced the mass loss of citrus fruit (16%) compared with the control (20%) when stored at 22 °C, 60% RH for 15 days. The 5.5 % weight loss of pitaya measured here after 20 days of storage was significantly high and according to Wills et al. (2007), just 5% moisture loss can generate shrivel symptoms that affect the quality of fresh produce. There was an interaction between weight loss and year, because weight loss during year 1 was slightly higher than year 2 (3.24% and 2.34%, respectively). However, there was no three-way interaction among year, storage period and coating treatment for percentage of weight loss.

Color and Firmness

There were no significant differences among coating treatments for external color (mean=35.7; Table 4-5). Overall means for C* and h* were 33.06 and 23.08, respectively. No significant difference in color among coating treatments showed that these coatings did not interfere with the color characteristics of pitaya after 20 days of storage. Study on mango reported that CC reduced the L*, increased C* and had higher h* compared to uncoated fruit, which might indicate a delay in color development, thus delaying fruit ripening (Dang et al., 2008). The C* increased significantly during pitaya storage (from 31.12 at day 0 to 36.45 at day 20), which indicated that the fruit

developed more intense red color. Result showed the same for h^* that increased during storage from 17.12 at day 0 to 23.08 at day 20, suggesting that the color shifted slightly, which might be due to senescence.

Mesocarp firmness was significantly affected by coating treatments (Table 4-5). Coated fruit maintained firmness equally for 20 d at 7 °C with VOC and CC (7.94 N and 7.98 N, respectively). This finding was similar to the reports by Ju et al. (2000) on Chinese pear coated with vegetable oil and Ergun et al. (2005) on mamey sapote coated with carnauba wax, in which these coatings retained firmness during storage at 0 °C for 6 months and 9 days at 20 °C, respectively. Other coating applications such as chitosan also have shown potential in retaining pitaya fruit firmness during 28 days' storage at 10 °C (Ali et al., 2013). The coating may have formed a semipermeable film on the fruit surface, resulting in development of an internal modified atmosphere that improved retention of fruit firmness. Fruit firmness can be maintained with high levels of CO₂ and low levels of O₂ that limit the activity of cell wall degrading enzymes such as polygalacturonase and pectin methylesterase (Salunkhe et al., 1991). However, there was a significant increase in firmness of uncoated pitaya from 15 to 20 d storage (11.15 N) (Figure 4-2). This might indicate that the fruit did not soften, but rather the mesocarp tissue became more dense, probably due to water loss. The mesocarp firmness increased in parallel to the increased shriveling of pericarp. Initially, mesocarp firmness was 7.24 N at the beginning of the storage, but then increased after being stored at 7 °C for 20 days (9.02 N) as a consequence of continued water loss. While firmness is normally expected to decline during storage (Zahid et al., 2013), this opposite firmness outcome was similar to the findings on carnauba-coated citrus in which the puncture

force increased with extension of the storage period, associated with loss of moisture from the peel (Machado et al., 2012). An increase of firmness due to moisture loss during storage was also observed in a study of blueberry fruit (Paniagua et al., 2013).

Soluble Solids Content, Total Titratable Acidity and pH

Results for soluble solids content (SSC), total titratable acidity (TTA), pH and sugar:acid ratio (SSC:TTA) showed no significant differences due to coating (means: SSC = 11.41 %, TTA = 0.25 %, SSC:TTA = 46.27) (Table 4-6). Another study on mamey sapote fruit coated with carnauba wax also showed no effect on SSC or pH (Ergun et al., 2005). There was a gradual decrease in TTA values during storage, similar to a study by Nerd et al., (1999) in which it was reported that the TTA declined during storage at 6 °C for 21 days. By day 20, the pitaya TTA decreased to 0.25%; therefore, the SSC:TA ratio doubled from the initial value of 22.03 to 46.26. This is typical of fruit crops, an indication that the acids (substrates of respiration) were metabolized to a greater extent than sugars during storage because of normal respiration. Valero and Serrano, (2010) explained that TTA decreased due to metabolism of organic acids to simpler molecules such as CO₂ and H₂O during respiration.

Sensory Evaluation

In this study, the sensory evaluation was conducted using 71 panelists of which 57.8% were female. The panelist demographics were Caucasian (54.9%), Asian (23.9%), Hispanic (11.3%), African American (4.2%) and Other (5.6%). Median age was 25 years. There were no statistical differences among treatments for any of the sensory attributes evaluated (Table 4-7). These results indicate that the presence of the coating did not change the sensory attributes. Results of sensory evaluations of pears

and apples coated with candelilla wax coating (a lipid-based coating) also showed no significant differences between coated and uncoated fruit (Cruz et al., 2015; Ochoa et al., 2011). From this study, external and internal appearance was rated an average of 6.68 and 6.95, respectively, while the overall acceptability was rated 6.5. In relation to the 1-9 rating scale used, this can be described as panelists liked the fruit moderately. Even though the fruit firmness data showed significant differences among treatments, sensory data showed no significant differences between treatments for both texture (mean = 6.65) and firmness (mean = 2.94) of the fruit. Panelists disliked the firmness moderately because the seeds were too large and crunchy. Panelists also found no difference in flavor (mean = 6.29), juiciness (mean = 2.94) or preference between the treatments (Table 4-8). The comments provided by the panelists mentioned that pitaya, coated or not coated, tasted similar, with mild, bland, or earthy flavor. Some thought the texture was similar to a pomegranate or kiwi fruit.

Conclusions

Pitaya coated with CC or VOC had significantly less shriveling and retained greater firmness than uncoated pitaya over the 20-d storage period at 7 °C. There were no significant differences in color or chemical composition between the treatments indicating that these coatings did not interfere with the quality of pitaya during storage. The result of sensory evaluation showed that the presence of the coating did not change the sensory attributes. Although the coatings did not prolong storage life, freshness was maintained for 15 days' storage.

Table 4-1. Percentage of shriveling, yellow lesions area rating and overall acceptability ratings based on each fruit (n=4)

Shriveling and Yellow Lesion Area Rating (%)	Overall acceptability ratings
0 = No trace	5= Excellent
<15 = Slightly affected	4= Good
16-25 = Moderately affected	3= Acceptable
25-50 = Badly affected	2= Poor
>50 = Severely affected	1=Very poor

Table 4-2. Sensory evaluation using 9-point hedonic scale for external and internal appearance, overall acceptability, flavor and texture.

Value	Descriptor
1	Dislike extremely
2	Dislike very much
3	Dislike moderately
4	Dislike slightly
5	Neither like nor dislike
6	Like slightly
7	Like moderately
8	Like very much
9	Like extremely

Table 4-3. Sensory evaluation using 5-point Just About Right scale for firmness and juiciness.

Value	Descriptor
1	Much Too Soft
2	Slightly Too Soft
3	Just About Right
4	Slightly Too Firm
5	Much Too Firm

Table 4-4. Shriveling (% surface area), yellow lesions (% surface area) and overall acceptability ratings (scale 1-5) for pitaya during 20 days' storage at 7°C

	Treatment	Day 0	Day 5	Day 10	Day 15	Day 20
Shriveling (%)	VOC	0.0a ^z	0.0a	3.8a	5.0ab	25.0b
	CC	0.0a	0.0a	0.0a	0.0b	20.0b
	Control	0.0a	0.0a	7.5a	16.3a	50.0a
Yellow lesions (%)	VOC	0.0a	0.0a	0.0a	5.3a	17.5a
	CC	0.0a	0.0a	0.0a	2.8a	15.0a
	Control	0.0a	0.0a	0.0a	10.0a	25.0a
Overall acceptability	VOC	5.0a	5.0a	5.0a	3.8a	2.8a
	CC	5.0a	5.0a	5.0a	4.0a	2.8a
	Control	5.0a	5.0a	5.0a	3.8a	2.5a

^zMeans in each column with the same letter are not significantly different. Data represents the mean (n=4). Separated using Duncan's Multiple Range test.

Table 4-5. Pitaya weight loss, external color and flesh firmness after 20 days' storage at 7 °C.

Main Effect	Weight Loss (%)	L*	Hue* Angle	Chroma*	Mesocarp Firmness (N)
Coating					
VOC	5.45a ^z	35.83a	23.23a	32.83a	7.94b
CC	5.25a	36.68a	26.78a	31.88a	7.98b
Control	5.80a	34.60a	19.22a	34.48a	11.15a
Significance	ns ^y	ns	ns	ns	*
Storage Period					
Day 0	0.00e	35.52a	17.12b	31.12c	7.24b
Day 5	1.44d	33.06c	16.28b	33.96abc	8.71a
Day 10	2.85c	33.25b	15.53b	35.55ab	8.60ab
Day 15	4.22b	34.88a	15.60b	36.45a	8.14ab
Day 20	5.50a	35.70a	23.08a	33.06bc	9.02a
Significance	**	**	**	*	*
Year					
1	3.24a	34.85a	16.59a	33.01b	na ^x
2	2.34b	34.14b	18.59a	35.04a	na
Significance	**	*	ns	*	na
Interactions					
Year*Storage	**	ns	**	ns	na
Year*Coating	ns	ns	*	ns	na
Storage*Coating	ns	ns	ns	ns	*
Year*Storage*Coating	ns	ns	ns	ns	na

^zMeans in each column with the same letter are not significantly different.
^yns=not statistically different, *, **=statistically different at p≤0.05, p≤0.01, respectively.

^xna=not available. Separated using Duncan's Multiple Range test.

Table 4-6. Compositional data for pitaya after 20 days' storage at 7 °C.

Treatment	Soluble solids content (SSC) (%)	Total titratable acidity (TTA) (malic acid, %)	SSC:TTA
Coating			
VOC	11.63a ^z	0.25a	46.62a
CC	11.43a	0.24a	47.17a
Control	11.18a	0.25a	45.01a
Significance	ns ^y	ns	ns
Storage Period			
Day 0	11.71ab	0.59a	22.03d
Day 5	11.32b	0.41b	29.17c
Day 10	11.87ab	0.37b	33.77b
Day 15	11.99a	0.26c	47.37a
Day 20	11.41ab	0.25c	46.26a
Significance	ns	**	**
Year			
1	11.70a	0.34b	37.96a
2	11.62a	0.42a	33.31b
Significance	ns	**	**
Interactions			
Year*Storage	**	*	**
Year*Coating	*	*	**
Storage*Coating	ns	ns	*
Year*Storage*Coating	ns	ns	ns

^zMeans in each column with the same letter are not significantly different.

^yns=not statistically different, *, **=statistically different at p≤0.05, p≤0.01, respectively. Separated using Duncan's Multiple Range test.

Table 4-7. Sensory panel results for appearance, overall acceptability, texture and flavor (9-point Hedonic Scale); firmness and juiciness (5-point Just About Right Scale) of pitaya after 14 days' storage at 7 °C

Main Effect	External Appearance	Internal Appearance	Overall acceptability	Texture	Flavor	Firmness	Juiciness
Coating							
VOC	6.75a	7.06a	6.65a	6.77a	6.41a	2.93a	3.01a
CC	6.51a	6.96a	6.39a	6.62a	6.14a	2.92a	2.89a
Control	6.77a	6.83b	6.46a	6.56a	6.32a	2.97a	2.92a
Significance	ns	ns	ns	ns	ns	ns	ns
Panelist							
Significance	*	*	*	*	*	*	*

^zMeans in each column with the same letter are not significantly different.

^yns=not statistically different, *=statistically different at $p \leq 0.05$. Separated using Tukey's HSD test.

Table 4-8. Preference ranking of sensory panelists for pitaya after 14 days' storage at 7 °C.

Calculated Friedman statistic	Degrees of freedom	p-value
0.45	2	0.798
Ranking	Treatments	Ranking
1	CC	146a ^z
2	VOC	142a
3	Control	138a

^zMeans in each column with the same letter are not significantly different. Separated using Tukey's HSD test.



Figure 4-1. Shriveling in uncoated pitaya after 20 days' storage at 7 °C.

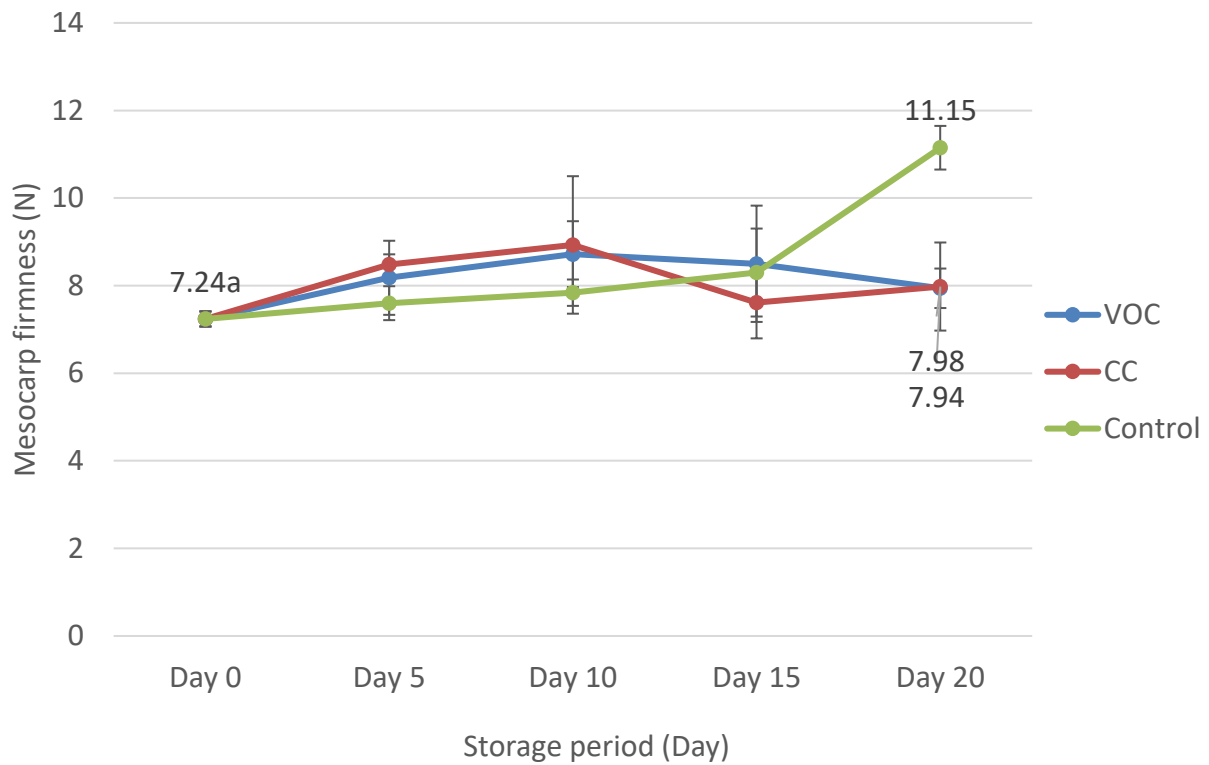


Figure 4-2. Mesocarp firmness of pitaya during storage at 7 °C. The values are means (n=4) and vertical bars are standard errors.

CHAPTER 5 STORAGE TEMPERATURE AFFECTS PITAYA FRUIT QUALITY AND BETALAINS CONTENT

Introduction

Low temperature storage has benefits in prolonging shelf life of fresh produce after harvest by reducing respiration and water loss as well as controlling decay. However, some fruits, especially tropical and subtropical fruits, are susceptible to chilling injury when exposed to temperatures lower than their optimum temperatures (Kader, 2002). Pitaya preserve high water content during handling and storage due to the peel thickness, scales and high amount of mucilage content in the pulp. Pitaya undergo accelerated softening and decreased sugar and acidity when stored above 20 °C (Punitha et al., 2010). According to Nerd et al. (1999), red-fleshed pitaya (*H. polyrhizus*) lost about 4.9 % fresh weight after 7 days of storage at 20 °C, while those stored at 6° C lost generally lesser around 2.2 %. Water loss may affect the fresh weight, firmness and appearance of the fruit which, in turn, can lower the typically high market price. In March 2017, the market price of Florida's pitaya in 4.5 kg (10 lb.) package was \$55 per box (USDA, 2017).

Other than water loss, it has been reported that pitaya stored below 5 °C developed chilling injury. Chilling injury symptoms were described as peel translucency, scale wilting and darkening, browning of the pulp, fruit softening, shriveling and loss of flavor (Nerd et al., 1999; Freitas and Mitcham, 2013). These symptoms depend on the genetic background, growing conditions and harvest maturity (Nerd et al., 1999). Crane and Balerdi (2005) recommended *H. undatus* grown in Florida be stored at 7 to 10 °C to maintain quality. However, *H. undatus* grown in California and stored at 5 °C for 20 d had only minor chilling injury (Freitas and Mitcham, 2013). This same species grown in

Vietnam and stored at 5 °C for four weeks had good quality (Hoa et al., 2006). On the other hand, pitaya (*H. undatus* and *H. polyrhizus*) cultivated in Israel are recommended to be stored at 10 °C to avoid chilling injury and quality loss (Nerd et al., 1999). Storage at 10 °C may negatively affect many quality parameters of pitaya, but sensory panelists found no significant difference in overall liking, sweetness, flavor, texture or tartness (Obenland et al., 2016). Nerd et al. (1999) also reported no decline in flavor for either *H. undatus* or *H. polyrhizus* after 14 days at 6 °C, compared to pitaya stored at 14 °C and 20 °C. Although low temperature storage did not affect sensory evaluation, internal pulp color was affected where there was significant increment in hue angle (Obenland et al., 2016). These same authors identified 34 aroma volatiles in the pitaya juice; aldehydes were the most abundant, comprising 90% of the total volatile amount. These authors also observed increase in aldehydes and loss in alcohols due to low temperature storage both at 5 °C and 10 °C.

Red-fleshed pitaya has higher antioxidant activity compared to white-fleshed types (Mahattanatawee et al., 2006) due to betalains that express the red color. Betalains exhibit antioxidant with radical scavenging activities that could help protect against oxidative stress in the body (Kanner et al., 2001; Wu et al., 2006). Betalains are water soluble pigments whose basic structure consists of a moiety of betalamic acid comprised of red-violet betacyanin and yellow betaxanthin that are synthesized from hydroxylation of tyrosine (Sunnadeniya et al., 2016). Different from beet root or from other cactus fruits, red-fleshed pitaya is a pure source of betacyanin, totally lacking of betaxanthin (Stintzing et al, 2002). At least 10 betacyanin compounds have been identified in pitaya, predominantly betanin and phyllocactin; all exhibit the similar deep

red color with an absorbance peak at 536 nm (Stintzing et al., 2002; Wybraniec and Mizrahi, 2002; Rebecca et al., 2012).

The stability of betalains are affected by temperature, pH, oxygen, lightness, and moisture (Saguy et al., 1978; Delgado-Vargas et al., 2000; Wong & Siow, 2015; Montes-Lora et al., 2016), and of these, temperature has the greatest influence on betalains stability (Anni et al., 2015). The betacyanins are degraded to the colorless cyclo-dopa 5-O- β -glucoside and bright yellow betalamic acid (Herbach et al. 2004). However, if adverse conditions are only temporary, this degradation was found to be partially reversible (Degenhardt and Winterhalter, 2001). Numerous studies have been conducted to determine the stability of betalains at high temperatures, especially for food colorant purposes (Anni et al., 2015; Woo et al., 2011). However, very few studies have measured the stability of betalains during low temperature storage. A study on red beet pigments from garambullo tree (*Myrtillocactus geometrizans*) reported that storage at 4 °C could prevent the degradation of betalains and antioxidant capacity (Reynoso et al., 1997). Due to the health benefits contributed by the betalains (Wu et al., 2006; Tenore et al., 2012), it became an interest to determine the effects of low temperature storage on betalains in pitaya.

The objective of this study was to prolong the postharvest quality of red-fleshed pitaya (*Hylocereus costaricensis*) grown in South Florida, USA, by determining the lowest safe temperature that does not induce chilling injury and maintains the betacyanins concentration during 25 days' storage.

Materials and Methods

Plant Material

Pitaya (*Hylocereus costaricensis*) cv Lisa commercially grown in Homestead, Florida, were harvested twice (Nov 20, 2015 and Oct 27, 2016) at commercial maturity (full-red stage) and transported in coolers the same day to the Postharvest Lab at the University of Florida in Gainesville. The next day, fruit were sorted for uniformity of size and freedom from decay. Fruit were cleaned by wiping each fruit with soft, dry wipes to remove dirt particles and visible insects.

Storage and Sampling

Fruit were randomized into three groups for storage on single-layer trays at 7 °C, 4 °C or 1 °C. Each treatment included 30 replications with one fruit per replicate. Fruit (n=4) from each treatment were analyzed initially and every 5 days during 25 d storage. At each analysis, fruit were transferred to 20 °C for 24 h to allow for development of any chilling injury symptoms.

Quality Analysis

At each evaluation, individual fruit were subjectively rated for chilling injury symptoms, scale dryness, yellow lesions area and overall acceptability ratings (Table 5-1). Severity of chilling injury, scale dryness and yellow lesions area were rated as 0% (no trace), <15% (slightly affected), 16-25% (moderately affected), 25-50% (badly affected) and >50% (severely affected) (Table 5-1). For overall acceptability ratings, fruit were rated as 5 (excellent), 4 (good), 3 (acceptable), 2 (poor) and 1 (very poor) (Table 5-1). Fruit with more than 50% symptoms and rated as poor or very poor were considered unacceptable for sale and consumption. Fruit weight loss (% fresh weight basis) was calculated at each evaluation and after transfer to 20 °C for 24 h. External

color was determined using a colorimeter (Konica Minolta Sensing Model CR-400; Tokyo, Japan) with D₆₅ illuminant. In this system, L* refers to lightness, on a scale of 0=black to 100=white, hue* angle is the actual color in degrees, where 0°=red, 90°=yellow, 180°=green and 270°=blue, and chroma* value indicates color saturation (McGuire, 1992).

Mesocarp firmness for pitaya was identified by using a computer-controlled texture analyzer (model TA.HD*Plus*, Texture Technologies Corp, NY) equipped with a 50-N load cell. Firmness was measured after fruit were conditioned to ambient temperature for about 1 hour. Two lengthwise slices were made on opposite sides of the fruit to a depth of 5 mm at the equator, and the outer portions were discarded. Mesocarp firmness was measured on each sliced side at the fruit equator using an 11-mm diameter convex probe with the crosshead speed of 2mm·sec⁻¹. Firmness was expressed in Newtons (N) at the the bioyield point (n=8).

After each evaluation, whole fruit were peeled and the edible portion was frozen (-20 °C) for later analysis. Upon thawing for about 1 h, each sample was blended then centrifuged (Thermo Scientific Lynx 4000 Sorvall) for 20 min at 19,319 x g at 4 °C. The juice was filtered with 4 layers of cheese cloth and the filtrate collected. Soluble solids content (SSC) was quantified by placing a drop of juice on a digital refractometer (model r2i300, Reichert Technologies, Depew, N.Y.); total titratable acidity (TTA) and pH were determined with an automatic titrator (model 905 Titrando; Metrohm Ion Analysis, Switzerland). For TTA, 3 mL of juice diluted with 15 mL of water was titrated with 0.1 M NaOH to pH 8.2 and reported as percent malic acid.

Determination of Betalains Concentration

The betalains absorption spectrum was determined for pitaya and betalains standard spectrophotometrically by scanning $1 \mu\text{L}\cdot\text{mL}^{-1}$ solution between 200 nm to 700 nm in a quartz cuvette set in a UV–Vis spectrophotometer (model DU730; Beckman Coulter, California); this was later used as the wavelength for betalains measurement. In this spectrophotometric analysis, a calibration curve method was used to determine the concentration of a sample by comparing the samples to a set of standard samples of known concentration. A series of different concentrations of betalain standard, betanin (CAS number: 7659-95-2, TCI, Japan) was prepared from a stock solution of $100 \mu\text{g}\cdot\text{mL}^{-1}$. To prepare the stock solution, 2.5 mg of betalains standard was weighed, transferred to a 25-mL volumetric flask, to which distilled water was filled up to the mark and shaken until the betalains powder was dissolved. The stock solution was then diluted into six different concentrations of working standard (20, 10, 5, 2.5, 1.25 and $0.625 \mu\text{g}\cdot\text{mL}^{-1}$). Each standard was pipetted in triplicate into a microplate and distilled water served as the blank. Absorbance was measured at 535 nm using a spectrophotometer (BioTek PowerWave XS2, BioTek Instrument Inc., Highland Park, USA). The standard curve was drawn by plotting the concentration on the x-axis and absorbance on the y-axis.

To determine the concentration of betalains in pitaya, fruit mesocarp (flesh) ($n=4$) from each treatment were blended, clarified by centrifugation and filtered as above. Each sample was diluted 1:20 with distilled water to get a concentration within the standard curve range, pipetted onto the microplate and read with the spectrophotometer as above. The equation from the standard curve was used to convert the absorbance of

betalains sample from pitaya concentration, which was then converted back to the concentration in the extracted juice.

Statistical Analysis

The same experiment was conducted twice, once in each of two subsequent years. The experimental design was a completely randomized design. The effects of storage temperature and storage duration were examined independently as well as their interactions. For this purpose, a one-way ANOVA test was used to evaluate the effects of the treatments ($p \leq 0.05$) on each measurement day. Duncan's Multiple Range test was used to separate means. SAS statistical software Version 9.4 was used to perform the statistical analyses.

Results and Discussions

Appearance and Acceptability Score

Previous studies on pitaya have reported the cold storage benefits by reducing respiration, decay and water loss. Pitaya fruit are very susceptible to decay. The decay symptoms were observed as yellow lesions that developed on the peel of pitaya during storage (Figure 5-1). The yellow lesions were observed after 10 d at all storage temperatures. At 20 days, the incidence and severity of yellow lesions was higher. There was a significant difference ($p \leq 0.05$) among treatments as pitaya stored at 1 °C had the least yellow lesions (3.8%) compared to 4 °C and 7 °C (6.35% and 5.0%, respectively). This became obvious at the end of 25 days' storage where pitaya stored at 1 °C only had 13.8% yellow lesions while fruit from the other treatments developed more than 35% (Table 5-2). This suggests that storage at 1 °C could suppress the development of decay during commercial handling. Freitas and Mitcham (2013) also found that storage at lower temperature (5 °C), was better than either 7 °C or 10 °C for

maintaining pitaya visual appearance. The yellow lesions in this study were diagnosed as anthracnose fruit rot from *Colletotrichum gloeosporioides* by Plant Diagnostic Center, University of Florida. This fungus typically inoculates the fruit in the field; thus, it is very important to control at the field level because the symptoms only appeared during storage.

Even the cold storage benefits in reducing decay, it can induce chilling injury (CI) symptoms if pitaya fruit had exposed to the below optimum temperature for longer period. In this study, no CI symptoms as described by Nerd et al. (1999) were observed in pitaya during storage at 1 °C, 4 °C or 7 °C. Only scale drying (Figure 5-2) was related to low temperature storage (10-20 %) after 25 days of storage and the dryness affected the overall appearance of pitaya fruit in all treatments (Table 5-2). Nerd et al. (1999) reported chilling injury symptoms in *H. undatus* and *H. polyrhizus* pitaya fruit (harvested at first color stage (green fruit with a verge of red) or 2–3 days after appearance of first color stage) as peel browning and translucency, scales turning dark, tissue softening, shriveling, and poor flavor, while Corrales-García and Canche-Canche (2008) and Freitas and Mitcham (2013) noted excessive softening and flesh translucency of *H. undatus* pitaya harvested at 70%-red stage and 45-days after flowering, respectively.

Pitaya stored at 7 °C had fungal infection at the stem end as early as day 15. This infection was similar to stem end rot that develops in many fruits during storage and is usually observed at the cut end of fruit (Figure 5-3). Storage at 4 °C and 1 °C might retard the development of stem end rot by drying the tissue at the cut area. For overall acceptability, based on factors like scale dryness, anthracnose (yellow lesions) and stem end rot infection, pitaya stored at 7 °C and 4 °C were rated as acceptable

(rating 3) until day 15 while pitaya stored at 1 °C was rated as acceptable until day 20. Consumer acceptability is influenced by the appearance of the product. Fruit that looks fresh and is free from decay will be more attractive to consumers and sell at higher prices.

Weight Loss

There was no treatment effect on weight loss, which increased during storage to 5.2% at 25 d (Table 5-3). Following transfer to 20 °C for 24 h, fruit lost an additional 0.5% weight. In a study on red-fleshed pitaya (*H. polyrhizus*), it was reported that the water loss was as high as 5.4% for fruit stored at 6 °C at the end of 21 d of storage (Nerd et al., 1999).

Color and Firmness

The initial color measurements were the following: L* 35.26, C* 31.12 and h* 17.66. Low temperature storage had only minor effects on peel color. There were no significant differences due to storage temperature for external color, and the overall means for L*, C* and h* were 35.25, 31.46 and 22.06, respectively, after 25 d (Table 5-3). No color degradation was observed in any of the treatments during 25 days' storage as indicated by the L* and C*. The main effect of storage time showed C* and h* increased significantly during storage (C* from 30.65 to 36.55 at day 20, and h* from 18.00 to 22.06 at day 25), which indicates that fruit had a more intense red color and the actual red color shifted slightly. The increase of C* and h* showed that the color change might be have been due to water loss, concentrating the red color.

Fruit firmness was not significantly affected by storage temperature (mean = 8.15 N) (Table 5-4). This result was dissimilar to a study reported by Corrales-García and Canche-Canche (2008), in which they found fruit stored at 4 °C was softer than those at

8 °C and that the fruit firmness was more stable at higher temperature. The result of the present study also showed no reduction in fruit firmness during 25 d of storage (mean = 7.93 N). No softening during storage disagrees with a report by Marangony et al. (1996), in which it was explained that exposure to near-threshold temperatures for chilling injury for longer storage period produced irreversible effects such as the rupture of cell compartments, destruction of membranes and electrolyte leakage, all of which lead to softening of the tissue.

Soluble Solids Content, Total Titratable Acidity, Sugar:Acid Ratio and pH

Results for SSC, TTA, sugar:acid ratio (SSC:TA) and pH showed no significant differences due to storage temperature (means: SSC = 10.94%, TA = 0.56%, SSC:TA = 20.16, pH = 4.2) (Table 5-4). The SSC and TTA were higher at day 0 (12.78 % and 0.73%, respectively) and decreased to (11.24% and 0.56%, respectively) at day 5 and thereafter remained constant during storage. However, the SSC:TA ratio remained constant during storage (21.35). Reduction in sugars and acids during storage were attributed to decreases in fructose, glucose and malic acids in pitaya after 2 weeks' storage at 10 °C (Obenland et al., 2016). This is typical of fruit, an indication that the acids were metabolized during storage because of normal respiration. Valero and Serrano (2010) mentioned that TTA decreased due to metabolism of organic acids. The initial pH of pitaya was 4.0 and it increased slightly to 4.3 at day 5 and thereafter remained constant during storage. pH in the range of pH 3.0 to 7.0 maintains red pigment stability in pitaya (Stintzing and Carle, 2004).

Determination of Betalains Concentration

The measurement of UV-Vis spectra of the standard and sample solution showed that the peak wavelength was at 535 nm as shown in Figure 5-4 and Figure 5-

5. This result was in the range of betalains peak wavelength as reported by Woo et al. (2011) who reported the betalains peak at 537 nm and Ami et al. (2012) who reported the betalains peak at 540 nm. The absorbance peak at 535 nm was explained by optical active chiral carbon on the C-2 and C-15 position (Wybraniec and Mizrahi, 2002). In contrast to the commercial betalains source, which is from beetroot (*Beta vulgaris* L.) (Bilyk, 1979), no absorbance peak was observed between 470-480 nm in red pitaya (Figure 5-5). This indicated the lack of betaxanthin present in the red pitaya (Woo et al., 2011). Thus, our results are consistent with the hypothesis that the compound responsible for the red color of pitaya is betacyanin and not betaxanthin.

A calibration curve by Beer's Law was obtained by plotting the concentration against the absorbance. The correlation (R^2) value and regression equation from the standard curve of betalains standard (Figure 5-6) was used to calculate betacyanin concentration in pitaya. Betacyanin concentration showed no difference among storage treatments with an average of $148 \mu\text{g}\cdot\text{mL}^{-1}$ [$0.148 \text{ mg}\cdot\text{g}^{-1}$ fwt] (Table 5-7). This result was slightly lower than the betacyanin concentration reported for *Hylocereus* sp. pitaya fruit by Vailant et al. (2005) with betacyanin content ranging between 0.32 to $0.41 \text{ mg}\cdot\text{g}^{-1}$ of fresh pulp without seeds.

Low temperature storage did not affect the betacyanin concentration during storage duration except the betacyanin concentration in pitaya stored at 7°C where it increased significantly from $83.3 \mu\text{g}\cdot\text{mL}^{-1}$ at day 0 to $224.8 \mu\text{g}\cdot\text{mL}^{-1}$ at day 20. This finding also is in line with the C^* measured as highest at day 20 might be due to water loss and that the red color became concentrated. Reshmi et al. (2012) worked on *Basella alba* fruit, and reported that there was an increase in betacyanin absorbance in

samples stored at 0 °C, 10 °C and 20 °C. Obenland et al. (2016) also reported that betacyanin content of pitaya fruit in their study did not differ due to low temperature storage either at 5 °C or 10 °C.

Conclusions

No chilling injury symptoms were observed in pitaya during storage at 1 °C, 4 °C or 7 °C during 25 days' storage. Storage at 1 °C could suppress the development of the anthracnose (yellow lesions) decay problem and fungal infection at the stem end. There were no significant differences observed among the three storage treatments for weight loss, color, firmness or chemical composition of pitaya. Low temperature storage did not alter the betacyanin content during 25 days' storage.

Table 5-1. Percentage of chilling injury symptoms, scale dryness and yellow lesions area rating and overall acceptability ratings based on each fruit (n=4)

Chilling Injury Symptoms, Scale Dryness and Yellow Lesion Area Rating (%)	Overall Acceptability Ratings
0 = No trace	5 = Excellent
<15 = Slightly affected	4 = Good
16-25 = Moderately affected	3 = Acceptable
25-50 = Badly affected	2 = Poor
>50 = Severely affected	1 = Very poor

Table 5-2. Percentage of chilling injury symptoms, scale dryness, yellow lesions (% surface area) and overall acceptability ratings (scale 1-5) for pitaya during 25 days' storage at 1 °C, 4 °C and 7 °C.

Days	Treatment	Chilling injury symptoms (%)	Scale dryness (%)	Yellow lesions (%)	Overall acceptability ratings
Day 0	7°C	0.0a ^z	0.0a	0.0a	5.0a
	4°C	0.0a	0.0a	0.0a	5.0a
	1°C	0.0a	0.0a	0.0a	5.0a
Day 5	7°C	0.0a	0.0a	0.0a	5.0a
	4°C	0.0a	0.0a	0.0a	5.0a
	1°C	0.0a	0.0a	0.0a	5.0a
Day 10	7°C	0.0a	0.0a	1.3a	4.8a
	4°C	0.0a	0.0a	2.5a	4.5a
	1°C	0.0a	0.0a	2.5a	4.5a
Day 15	7°C	0.0a	0.0a	5.0a	3.0a
	4°C	0.0a	0.0a	6.3a	3.0a
	1°C	0.0a	0.0a	3.8a	3.5a
Day 20	7°C	0.0a	11.3a	18.8a	2.5b
	4°C	0.0a	12.5a	17.5a	2.5b
	1°C	0.0a	5.0a	3.8b	3.3a
Day 25	7°C	0.0a	18.8a	37.5a	1.5b
	4°C	0.0a	20.0a	35.0a	1.8b
	1°C	0.0a	10.0a	13.8b	2.8a

^zMeans in each column with the same letter are not significantly different. The values are means of four independent determinations, separated using Duncan's Multiple Range test.

Table 5-3. Weight loss and external color for pitaya at the end of 25 days' storage at 1 °C, 4 °C and 7 °C.

Treatment	Weight loss (%)	Weight loss +24h (%)	L*	Chroma*	Hue* angle (°)
Temperature					
7°C	4.9a ^z	0.72a	35.32a	36.29a	19.19a
4°C	5.8a	0.46a	34.78a	27.99a	24.66a
1°C	4.9a	0.45a	35.64a	30.09a	22.33a
Significance	ns ^y	ns	ns	ns	ns
Storage Period					
Day 0	0.00e	0.00b	35.40a	30.65b	18.00b
Day 5	1.10d	0.44a	33.21bc	31.41b	22.72a
Day 10	2.74c	na	33.09c	33.00ab	17.05b
Day 15	3.55b	0.65a	33.54bc	35.67a	15.93b
Day 20	4.90a	na	34.05b	36.55a	16.98b
Day 25	5.19a	0.54a	35.25a	31.46b	22.06a
Significance	**	**	**	*	*
Interaction					
Temperature x Storage Period	ns	ns	ns	ns	*

^zMeans in each column with the same letter are not significantly different.

^yns=not statistically different, *, **=statistically different at p≤0.05, p≤0.01.

Separated using Duncan's Multiple Range test.

Table 5-4. Firmness and compositional data for pitaya at the end of 25 days at 1 °C, 4 °C and 7 °C.

Treatment	Flesh firmness (N)	Soluble solids content (%)	Total titratable acidity (malic acid, %)	SSC:TA	pH
Temperature					
7°C	8.17a ^z	10.73a	0.47a	21.88a	4.3a
4°C	8.27a	10.65a	0.63a	18.66a	4.2a
1°C	8.02a	11.45a	0.58a	19.95a	4.2a
Significance	ns ^y	ns	ns	ns	ns
Storage Period					
Day 0	7.24a	12.78a	0.73a	18.64a	4.0b
Day 5	8.51a	11.24b	0.56b	22.49a	4.3a
Day 10	7.55a	10.96b	0.49b	23.40a	4.4a
Day 15	8.35a	10.56b	0.53b	23.17a	4.3a
Day 20	7.75a	10.65b	0.58b	20.73a	4.3a
Day 25	8.15a	10.94b	0.57b	20.02a	4.3a
Significance	ns	**	*	ns	*
Interaction					
Temperature x Storage Period	*	ns	ns	ns	ns

^zMeans in each column with the same letter are not significantly different.

^yns=not statistically different, *, **=statistically different at p≤0.05, p≤0.01, respectively. Separated using Duncan's Multiple Range test.



Figure 5-1. Yellow lesions in pitaya (*H. costaricensis*) diagnosed as anthracnose fruit rot from *Colletotrichum gloeosporioides*.



Figure 5-2. No scale dryness at day 15 (1), and scale dryness after 25 day (2) of storage in pitaya (*H. costaricensis*) at 1 °C, 4 °C or 7 °C.



Figure 5-3. Symptom consistent with fungal infection at the stem end after 20 days of storage in pitaya (*H. costaricensis*) at 7 °C.

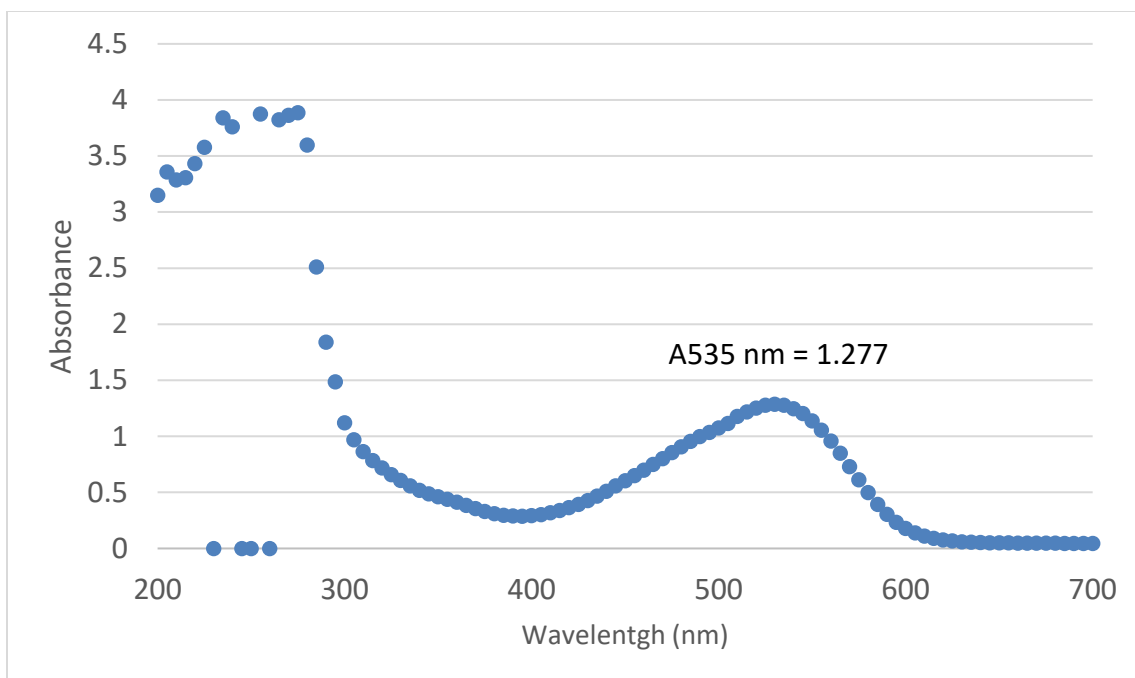


Figure 5-4. Absorbance of betalains UV-Vis spectrum of the standard solution ($10 \mu\text{g}\cdot\text{mL}^{-1}$) extracted with water.

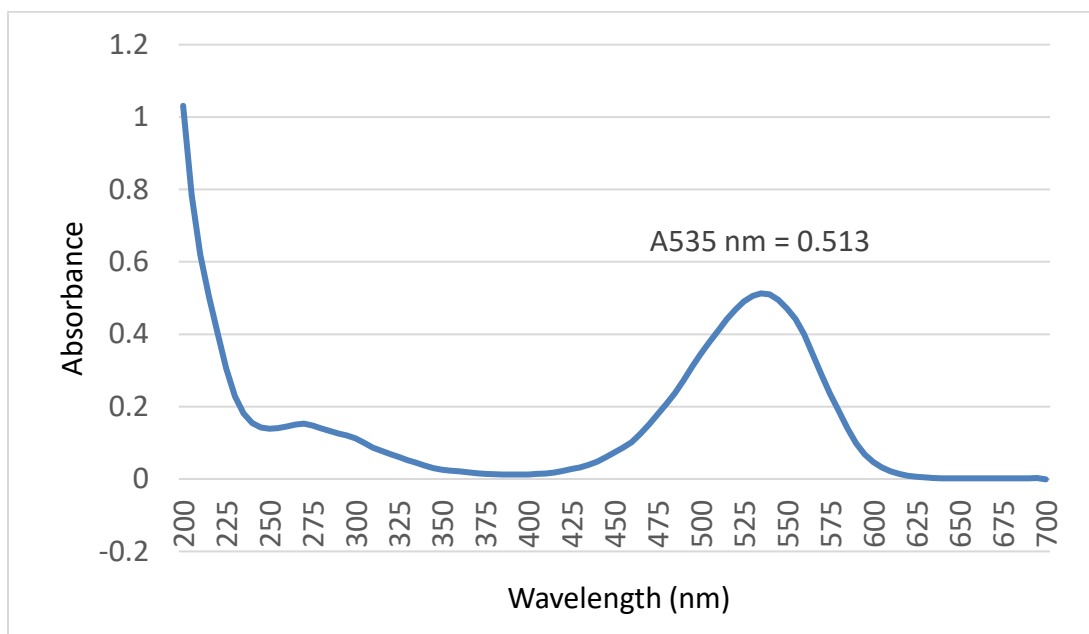


Figure 5-5. Absorbance of betacyanin UV-Vis spectrum of the sample solution ($1 \mu\text{L}\cdot\text{mL}^{-1}$) of pitaya (*H. costaricensis*) extracted with water.

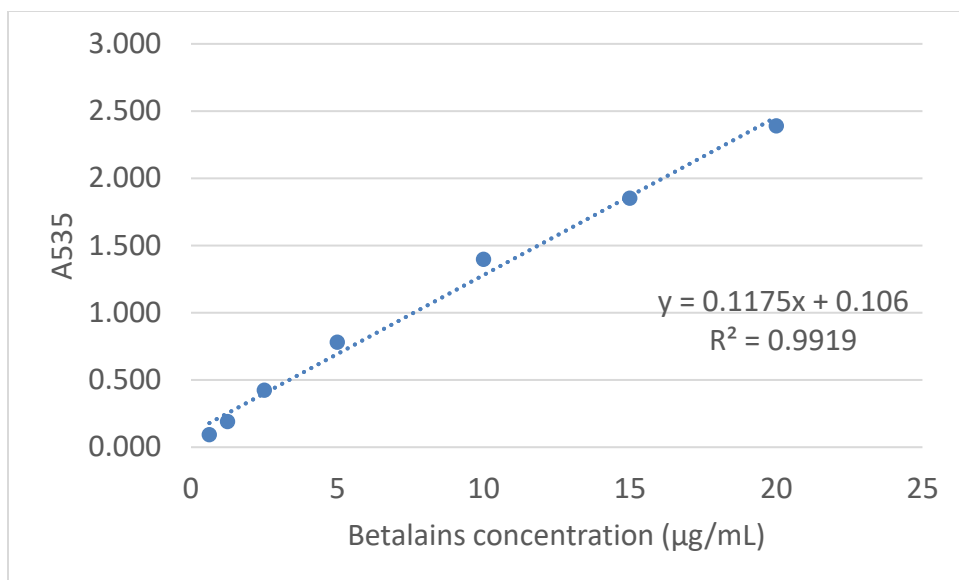


Figure 5-6. Standard curve of betalains standard.

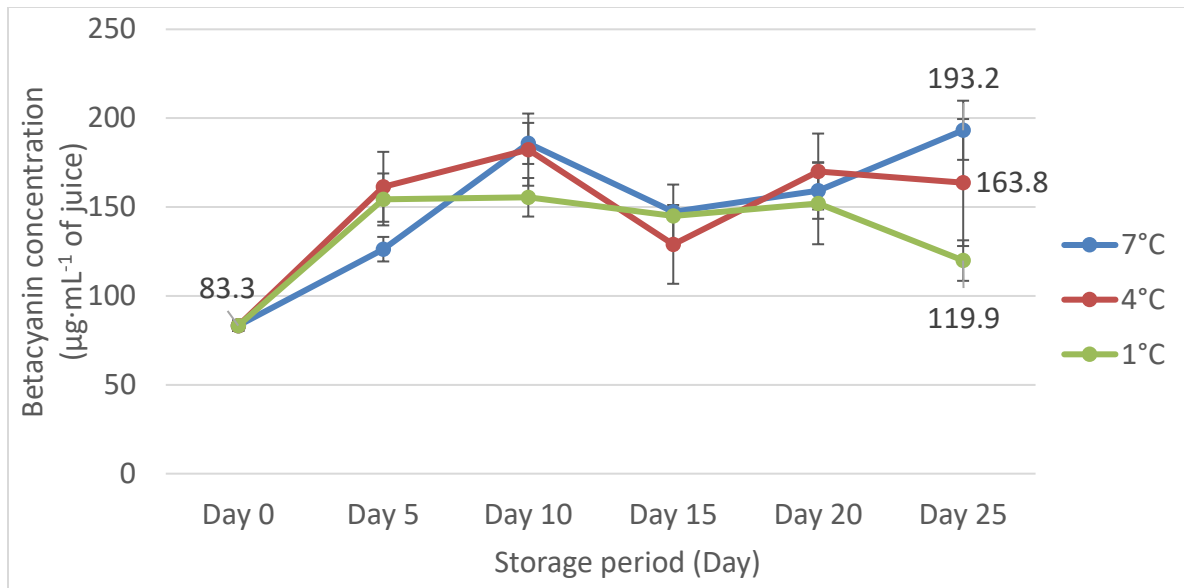


Figure 5-7. Betacyanin concentration in pitaya during 25 days' storage at 1 °C, 4 °C or 7 °C. The values are means (n=4) and vertical bars are standard errors.

CHAPTER 6 CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Current commercial practices for pitaya involving harvest at full-red stage results in short shelf life: only 8 d at ambient temperature. Full-red pitaya develop the highest weight loss compared with fruit harvested at $\frac{1}{2}$ -red or $\frac{3}{4}$ -red maturity stages and become unacceptable in appearance due to development of severe anthracnose disease on the peel after a week of storage. The fruit became more perishable as the harvest maturity advanced, being more susceptible to injury and moisture loss during storage. Pitaya harvested at $\frac{1}{2}$ -red stage exhibited non-uniform ripening among fruit and had low soluble solids content. These fruit had higher respiration rate (17 to 25 mL·kg⁻¹·h⁻¹) compared with those harvested at the other maturity stages, indicating the high metabolism during fruit development. Pitaya harvested at $\frac{3}{4}$ -red maturity stage ripened normally with extended shelf life until 10 days' storage at 20 °C. With high sugar:acid ratio, therefore, they had better sweet taste and longer shelf life compared to other maturities. Thus, the optimum harvest maturity, based on the above quality parameters as well as the ability to extend the shelf life, should be at the $\frac{3}{4}$ -red maturity stage.

An effective coating application shows potential to reduce the perishability of pitaya fruit and extend shelf life. Pitaya harvested at the full-red stage and coated with CC or VOC had significantly reduced shriveling and retained firmness over the 20-d storage period at 7 °C and 85% RH. However, these coatings did not reduce weight loss during storage. There were no significant differences in color or chemical composition (SSC and TTA) compared to uncoated pitaya, proving that these coatings did not interfere with the quality of pitaya during storage. The result of sensory

evaluation showed no significant difference among coating treatments, indicating that the presence of coatings did not change the sensory attributes. With the ability to reduce shriveling, CC and VOC coating have the potential to maintain the freshness of full-red pitaya up to 15 days' storage at 7 °C.

Low temperature storage at 1 °C, 4 °C or 7 °C with 85% RH did not induce chilling injury symptoms in full-red stage pitaya grown in south Florida. Only scale drying (10 to 20%) was observed related to low temperature storage after 25 days of storage and this dryness affected the overall appearance of pitaya in all storage temperatures. Pitaya stored at 1 °C had the least anthracnose disease (13.8% severity) compared to 4 °C and 7 °C, which developed more than 35% by the end of 25 days' storage. This suggests that storage at 1 °C could suppress the development of decay during commercial handling. Pitaya stored at 7 °C had fungal infection at the stem end as early as day 15. No significant differences in color, firmness and chemical composition among storage conditions indicate that this low temperature storage did not affect the quality of pitaya during storage. The stability of betalains in this red-fleshed pitaya were not affected by low temperature storage. Thus, based on this finding, 1 °C is considered to be the optimum storage temperature for pitaya harvested at commercial maturity, full-red ripeness stage.

In conclusion, red-fleshed pitaya *Hylocereus costaricensis* grown in south Florida should be harvested at the ¾-red maturity stage to extend shelf life until 10 days of storage at 20 °C. With application of carnauba wax coating, pitaya had less shriveling and retained firmness, thus the storage life may be extended until 15 days of storage at 7 °C with 85% RH. If the storage temperature is lowered to 1 °C, it will maintain the

quality of pitaya by preventing fungal infection at the stem end as well as controlling anthracnose disease on the peel of pitaya, resulting in extended storage life until 20 days of storage.

Commercially, pitaya has plenty of selling points. The fruit is attractive with many good properties for the health benefits. Red-fleshed pitaya is additionally rich in betalains, which is of increased interest to industry for antioxidant products and as a natural food colorant. Pitaya fruit are very perishable and thus need a lot of effort to keep them fresh. The increasing demand worldwide has encouraged production under various environments, requiring more study concerning plant breeding and propagation, fruit production, disease and pest control and postharvest handling. For postharvest handling, heat treatment has shown potential to maintain quality and control postharvest disease in many fruits and vegetables. Combination treatments, for example, heat treatment with application of a natural compound as sanitizer or coating's incorporating active compounds on pitaya may be studied to see the synergistic actions of various recommended treatments that have been studied previously. Integrated pest management in the field may be strengthened to control diseases that become the main postharvest problem in the pitaya industry.

APPENDIX PLANT SPECIMEN DIAGNOSTIC REPORT

3/25/2017

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PLANT SPECIMEN DIAGNOSTIC REPORT Specimen # 2016-1555

SUBMITTED BY Adrian Berry UF 2550 Hull Road Room Gainesville, FL 32614 adberr@ufl.edu		PLANT	METHOD SUBMITTED
		dragon fruit(<i>Hylocereus</i> sp.)	Walk-In
		VARIETY	CLASS
		Pitaya	Fruit/Nut
		INTERNAL LAB NO.	LAB FEE
			\$40.00
		REPLY FROM LAB	
		November 22, 2016	
PHONE	COUNTY	PLANT MATERIAL	RECEIVED BY LAB
352-392-4802	MIAMI-DADE, FL	fruit	November 15, 2016
CONDITION UPON ARRIVAL		DIAGNOSTICIAN(S)	
Good, Sample and Information		Dr. Sladana Bec Dr. John Bonkowski	
GENERAL OBSERVATIONS		DIAGNOSTIC TECHNIQUE(S)	
		<input type="checkbox"/> Baiting - \$65 <input type="checkbox"/> Inoculation <input type="checkbox"/> Referral to another lab <input type="checkbox"/> Biochemical Analysis <input checked="" type="checkbox"/> Microscopy <input type="checkbox"/> Sequencing - \$35 <input checked="" type="checkbox"/> Culturing <input type="checkbox"/> Molecular prep <input type="checkbox"/> Serological - \$20 <input type="checkbox"/> HR Test <input type="checkbox"/> Nested PCR - \$75 <input checked="" type="checkbox"/> Visual Observation <input type="checkbox"/> Immunostrip - \$5 <input type="checkbox"/> PCR - \$35 <input checked="" type="checkbox"/> Incubation <input type="checkbox"/> qPCR - \$75	
GROWER INFORMATION		REFERRAL INFORMATION	

Diagnosis/Recommendations

Diagnosis: Anthracnose Fruit Rot
(*Colletotrichum gloeosporioides*)

Category: Fungi

Comments: Tropical Fruit Rot
Glomerella acutata (anamorph: *Colletotrichum acutatum*) affects avocado, breadfruit, carambola, citrus, fig, guava, kiwifruit, lychee, mango and papaya. It causes anthracnose primarily on fruit, but is usually less important than *G. cingulata*. *Glomerella cingulata* (anamorph: *Colletotrichum gloeosporioides*) has the widest host range of any of the pathogens that are covered in this book. It causes significant problems on avocado, birba, breadfruit, carambola, cherimoya, citrus, custard apple,

<https://outlook.office.com/owa/?realm=ufl.edu>

1/3

durian, fig, guava, ilama, jackfruit, lychee, mango, mangosteen, papaya, passion fruit, soursop, sugar apple and rambutan. It is also responsible for a serious fruit set disease on citrus, postbloom fruit drop. Colonies are effuse, white becoming pale orange then greenish grey or black, often with a pink or reddish purple reverse. *C. acutatum* differs from *C. gloeosporioides* in its orange to pink colony coloration during the first few weeks of growth and its fusiform conidia. Although *C. gloeosporioides* is the most important as a fruit pathogen, it also causes branch and leaf diseases. It is also a common endophyte and saprophyte. On PDA, colonies are whitish to dark grey with thick to sparse lawns of aerial mycelium. Conidia are hyaline, one-celled, and either cylindrical with obtuse ends or ellipsoidal with a rounded apex and a narrow, truncate base. They form on light brown conidiophores in irregular acervuli and, upon maturity, appear orange and slimy en masse. Acervuli develop in lesions on leaves, branches and fruit, and conidia in acervuli remain viable for long periods, even under adverse climatic conditions. Setae that form in acervuli are brown and two- to five-celled. The fungus is heterothallic and, although the teleomorph can be induced readily in vitro, it is observed rarely in the field. Perithecia are subspherical, dark brown to black, 90–220 µm in diameter and contain hyaline, unitunicate asci. Ascospores are unicellular, curved, hyaline. Conidia are the most important type of inoculum. They are produced on virtually all host tissues and are usually dispersed by rainsplash. Moderate temperatures (25–30°C) and free moisture are needed for optimum production, germination and infection. New leaf flushes usually are most susceptible. Although fruits can be infected at any stage of development, infections

that occur before ripening usually progress no further than the formation of appressoria. Once ripening commences, infection pegs that forcefully penetrate the host are formed.

Fungicide treatments are focused on reducing damage to fruit and inflorescences. Overhead irrigation is not recommended because it spreads the inoculum throughout the canopy. Cultural practices include removal and destroying of the infected leaves and fruits with spore producing fruiting bodies that serve as sources of secondary inoculum.
(20160404)

Thank you for using the diagnostic services of the University of Florida Gainesville Plant Diagnostic Center. Questions or comments about this service should be directed to Dr. Carrie Lapaire Harmon at cdc@ifas.ufl.edu. Find us on Facebook

at www.facebook.com/PlantDiagnosticCenter and <http://plantpath.ifas.ufl.edu/Clinic/index.shtml>.

Management recommendations are based on UF-IFAS research when possible. Mention or omission of product names does not imply endorsement or exclusion; product names are included as examples only. Always read, understand, and follow all label instructions including safety precautions, required personal protective equipment (PPE), range of target organisms, rates of application, risks of phytotoxicity, and compatibility with other chemicals before application. The applicator assumes all liability for following the label and any application activities or outcomes. Use of the UF PDC diagnostic service implies an understanding of this responsibility.

Captions for attached images

Image 19377[1].jpg: G16-1555

Image 19377[2].jpg: G16-1555

Image 19377[3].jpg: G16-1555

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BIOGRAPHICAL SKETCH

Nur Azlin Razali is from Kuala Lumpur, Malaysia. She received a Bachelor of Science degree (with Honors) in Biochemistry from the University of Malaya, Malaysia in 2006. The title of her undergraduate thesis was Large Scale Production of Roselle Anthocyanin Pigment. After her graduation, she worked for one year at Malaysian Nuclear Agency, Malaysia, as Research Officer, conducted research on biochemistry aspect of crops that were treated with irradiation to increase the production and enhance the quality of the industrial crop in Malaysia.

She has been working as Research Scientist in Postharvest Program at Malaysian Agricultural Research and Development Institute (MARDI), Malaysia since 2007 until present. Her job requires her to plan and execute research activities in developing new technology and methodology for quality control on fresh produce (fruits, vegetables and flowers) as well as whole process of postharvest handling. After securing a MSc Scholarship Award from MARDI, she joined Prof Dr. Steven Sargent's Lab to pursue a Master of Science degree in Horticultural Sciences. Prof Dr. Jeffrey Brecht, Prof Dr. Bala Rathinasabapathi and Prof Dr. Charles Sims (Food Science) are members of her graduate advisory committee. Upon completion of her master's degree, she will continue her work in MARDI in postharvest physiology and technology.