Original Research Article

POSTHARVEST CONSERVATION OF 'THOMSON' PITAYA AS A FUNCTION OF STORAGE TEMPERATURE

ABSTRACT

Aims: The aims of this work was to evaluate the physicochemical and biochemical attributes and the postharvest conservation period of 'Thomson' pitaya under different storage conditions.
Study design: The experimental design used was completely randomized with three treatments and four replications of four fruits/repetition.
Place and Duration of Study: The experiment was carried out with 'Thomson' pitayas harvested in an organic orchard located in the municipality of São Miguel do Oeste, Santa Catarina, Brazil, during the 2018/2019 harvest.
Methodology: The treatments evaluated were 23°C, 9°C and 4°C. The storage of the fruits was carried out in chambers with Biochemical Oxygen Demand (BOD). The fruits were evaluated at different dates (7, 14, 21, 28 and 35 days) during cold storage.
Results: At 7 days of storage, the fruits stored at 23 °C showed higher values of mass loss, lipid peroxidation, pulp browning and activity of peroxidase (POX) and ascorbate peroxidase (APX) enzymes, as well as reductions in soluble solids contents (SS), titratable acidity (AT) and pulp firmness. At 14 and 21 days, storage at 9°C showed reductions in lipid peroxidation and pulp browning, as well as higher vitamin C contents and lighter and more intense skin color. Also, at 21 days, the fruits stored at 9°C showed higher values of SS, AT and pulp firmness, and reductions in mass loss and in the activity of POX and APX enzymes.
Conclusion: It is concluded that the storage of 'Thomson' pitayas at 23 °C provides a short post-harvest period and early senescence of the fruits, while the temperature of 9 ºC increases the shelf life of the fruits and proves to be quite efficient in the conservation of the attributes of quality. The storage at 4°C is not recommended due to high internal browning, oxidative stress and loss of quality attributes.

Keywords: Hylocereus undatus (Haworth) Britton & Rose, storage time, chilled atmosphere, post-harvest conservation.

1. INTRODUCTION

The pitaya [*Hylocereus undatus* (Haw.) Britton e Rose (Cactaceae)] also known as dragon fruit [1] is a fruit native to Mexico, Central and South America [2]. It is widely cultivated in tropical and subtropical regions, including Thailand, Philippines, Vietnam, Malaysia, South China [3], Mexico, Central America [2] and Brazil [4]. The *H. undatus* produces non-climacteric fruits [5], with red skin and white pulp, spherical or semi-globose, measuring from 10 to 20 cm in diameter and weighing from 350 to 450 g [6]. The fruits of *H. undatus* have been commercialized and consumed in different regions of the world not only for their organoleptic characteristics and nutraceutical properties [7], but also for the high commercial value added to the fruits. Furthermore, *H. undatus* contributes to agricultural development in dry regions due to its greater tolerance to water stress and poor soils [2].

Although it is an important source of income for small producers, post-harvest losses caused by mechanical damage, cold injuries, rotting and water loss (withering) have been the main factors that reduce the quality and shelf life of fruits [8, 9, 6]. In this context, the storage of fruits at low temperatures can be an important alternative to reduce not only the post-harvest losses of pitayas, but also as a strategy for maintaining the quality and prolonging the shelf life of the fruits after harvest.

However, storage of pitaya at low temperatures can induce chilling and/or browning of pitaya pulp [1]. The chilling injuries are characterized by skin lesions, translucency and darkening of the pulp

[10]. The pulp browning is a physiological disorder that induces dark spots internally, reduces fruit firmness and loss of flavor [11]. Previous studies have shown that *Cereus undatus* Haworth fruits should be stored at 5 ºC [12, 13], while other authors concluded that fruits of *H. undatus* and of *Hylocereus polyrhizus* (Weber) Britton & Rose should be stored at 10 ºC to reduce quality losses and avoid cold injuries [14].

Despite being an important tool for the conservation and maintenance of fruit quality, storage at low temperatures can also cause stresses that favor the formation of reactive oxygen species (ROS), which induce the formation of membrane damage and tissue death. and, consequently, a reduction in the quality and shelf life of the fruits [15]. To prevent the accumulation of ROS at the toxic level, the fruits can increase the concentration of antioxidant compounds and enzymes of antioxidative stress such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX) [16]. Thus, the increase in enzymatic activity can contribute to maintaining the quality and prolonging the period of conservation and commercialization of the fruits.

The post-harvest studies of pitaya have mostly focused on the evaluation of the physicochemical attributes of the fruit. However, there is little information regarding changes in quality attributes, post-harvest conservation and enzyme activity of the antioxidative stress of pitayas during the post-harvest period. The objective of this work was to evaluate the physicochemical and biochemical attributes and the postharvest conservation period of 'Thomson' pitaya under different storage conditions.

2. MATERIAL AND METHODS

The experiment was carried out with 'Thomson' pitayas harvested in an organic orchard located in the municipality of São Miguel do Oeste, Santa Catarina, Brazil, during the 2018/2019 harvest. After harvesting and sorting, the fruits were randomly selected and separated into replicates and treatments.

The treatments evaluated were: 23°C, 9°C and 4°C. These temperatures were chosen based on results obtained in other studies with refrigerated storage of tropical fruits. The storage of the fruits was carried out in chambers with *Biochemical Oxygen Demand* (BOD). The temperatures in the fruit pulp were monitored with mercury thermometers. The relative humidity of the air was maintained at 80 ± 3% and monitored using a thermo-hygrometer (INCOTERM®, Porto Alegre RS, Brazil) installed inside the BOD chambers.

Immediately after harvest, the fruits were evaluated for the initial mass of repetitions, skin color, pulp darkening (PD), respiratory rate, soluble solids (SS), titratable acidity (TA) and pulp firmness to verify the physicochemical characteristics before the storage period. At each of the dates after storage (7, 14, 21, 28 and 35 days), the same attributes as at the time of harvest were evaluated, in addition to lipid peroxidation and activities of peroxidase (POX) and ascorbate peroxidase (APX) enzymes.

The fresh weight (*FW*) of the fruits was determined at 0, 7, 14, 21, 28 and 35 days of storage using a digital analytical balance (accuracy of 0.001 g). Fruit mass loss was expressed as a percentage (%) [17].

Respiratory rates were quantified by the method proposed by Stanger et al. [18] placing 3 fruits of each sample in an airtight container with a volume of 7000 mL^{-1} and obtained by the difference in the concentration of CO_2 inside the container, immediately after closing and after 90 minutes.

Fruit firmness was measured with a penetrometer (GÜSS Manufacturing, South Africa) equipped with an 8 mm probe. For this, the epidermis on the two opposite sides of each fruit was removed and the firmness recorded on each side [1].

The SS content (ºBrix) was determined with a precision digital refractometer (°Brix ± 0.2%), with automatic temperature measurement and compensation to 20 °C (PR 201α, Atago®, Japan) [17].

The TA (g^{-1} citric acid/100 g^{-1} of pulp) was determined using 10 g^{-1} of pulp, diluted in 40 mL^{-1} of distilled water and titrated with sodium hydroxide (0,1 M) up to pH 8,2 [19].

The vitamin C (mg ascorbic acid/100 g^{-1} of pulp) was determined using 4 mL^{-1} of pitaya extract (1 g^{-1} of homogenized pulp + 100 mL^{-1} of oxalic acid 0,5%), diluted in 50 mL^{-1} of distilled water and titrated with Tillmans solution [20].

The lipid peroxidation was determined following the procedure described by Heath & Packer [21]. The activities of the enzymes peroxidase (POX) and ascorbate peroxidase (APX) were evaluated, according to the protocols described by Sekita [22]. The enzymatic activity was expressed on a protein basis, and the protein content was determined according to Bradford [23]. In both

analyses, the reading of the samples was performed in a K37-UV/VIS spectrophotometer (KASVI® Produtos e Equipamentos para Laboratórios, Brazil).

The color of the epidermis and the browning of the pulp (BP) were analyzed in terms of *L, C* and *hue angle* (h°) attributes using a Delta Vista 450 G colorimeter (Delta Color Industria e Comércio de Equipamentos Eletrônicos Ltda., Brazil). The *L* values (*lightness*) indicate the brightness of the epidermis or pulp of the fruits. *L* values close to 0 and 100 indicate values with low and high brightness intensity in the fruits, respectively. The *C* (*crome*) indicates chromaticity, where values close to 0 and 90 indicate darker and lighter shades of color, respectively. The *h*° defines the basic coloring, where 0°=red, 90°=yellow, 180°=green, 270°=blue, respectively [24]. For skin coloring, two readings were performed on opposite sides in the equatorial region of the fruits. For BP, the fruits were transversally cut in the equatorial region, and the readings were carried out in the seedless regions of the pulp. The DP was determined based on the product ratio of the attributes *lightness* and *crome* with the *hue* angle [DP = (L×C)/*h*°] measured in the pulp of the fruits.

The experimental design used was completely randomized with three treatments and four replications of four fruits/repetition. The data obtained in the different variables were initially submitted to Bartlett [25] and Shapiro-Wilk [26] analysis to verify the homogeneity of variances and normality of residuals, respectively. The evaluation means performed at 7 days after storage were compared by the Tukey test (5% probability), while the averages of the analyzes performed at 14 and 21 days after storage were compared by Student's t test (5% probability). All analyzes were performed using *R* statistical software, version 3.6.1 [27].

3. RESULTS AND DISCUSSION

At harvest time, the fruits had the following attributes: respiratory rate of 0,67 mmol CO_2 kg^{-1} h^{-1}, pulp firmness of 10,9 N, TA of 1,6 g^{-1} citric acid/100 g^{-1} of pulp, SS of 12,6 °Brix, vitamin C 31,61 mg ascorbic acid/100 g^{-1} of pulp, coloring of the epidermis of 37,4; 47,7 and 25,3, for *L*, *C* e *h*°, respectively and BP of 0,64.

The storage temperature influenced the conservation period and the loss of fresh weight of 'Thomson' pitaya. Fruits stored at 23 °C showed a reduced shelf life, while those stored at 9 °C and 4 °C extended their storage period to 35 and 21 days after harvest, respectively (Figure 1). Fresh weight loss was also influenced by storage temperatures. Fruits stored at 23 °C exhibited greater loss of fresh weight at 7 days after storage compared to fruits stored at 9 °C and 4 °C (Figure 1). However, at 21 days after storage, fruits stored at 4°C exhibited greater mass loss when compared to fruits stored at 9°C (Figure 1).

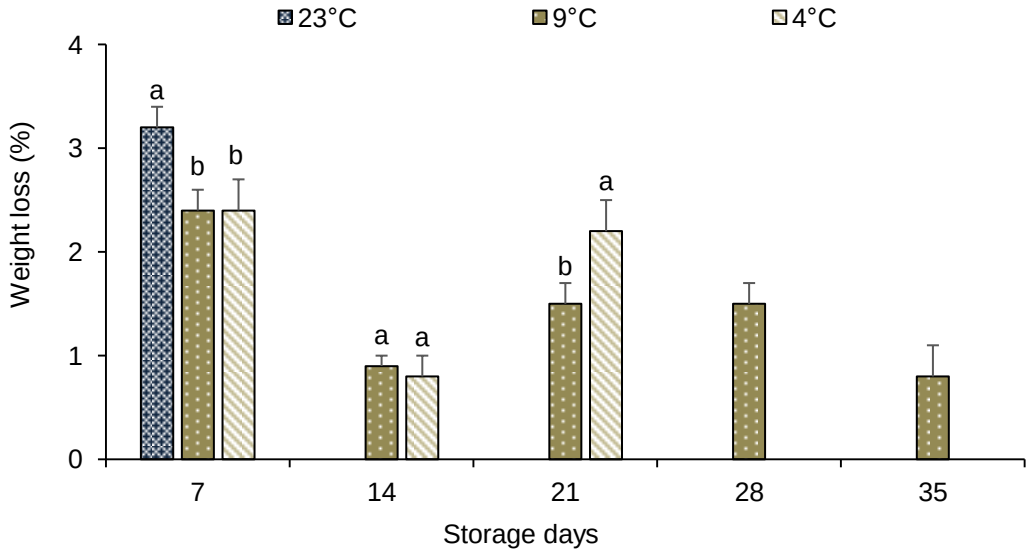

Fig. 1. Percentage of fresh weight loss of 'Thomson' pitayas stored for 7, 14, 21, 28 and 35 days at a temperature of 23 ± 1 °C, 9 ± 1 ° C or 4 ± 0.4 °C and relative humidity of 80 ± 3%.

The loss of fresh weight occurs due to metabolic reactions such as respiration and transpiration. In pitaya, the thin thickness of the epidermis increases the susceptibility to water loss, and consequently affects the quality of the fruit, since its commercialization is carried out by weight and appearance [28]. The greater mass loss exhibited by fruits stored at 23 °C at 7 days of storage is

justified by the fact that in fruits and vegetables, higher temperatures increase the respiratory rate, transpiration and consequently the loss of fresh weight [29,30]. In other non-climacteric fruits, such as tangerine, refrigeration at 10 °C also contributed to lower mass loss when compared to temperatures of 24 and 5 °C [31].

However, although the refrigerated atmosphere stands out among post-harvest preservation methods, for some tropical fruits cold storage at very low temperatures causes refrigeration injuries, which result in enzyme denaturation, increased respiratory activity and acceleration of the metabolism of the fruits [10, 32], which explains the greater loss of mass of the fruits conditioned at 4°C at 21 days of storage. In mango, avocado and peach, associations were also observed between greater mass loss and the occurrence of cold injuries [33, 34].

The storage temperature of 9°C delayed the loss of firmness of the fruits, when compared with the other temperatures (Figure 2).

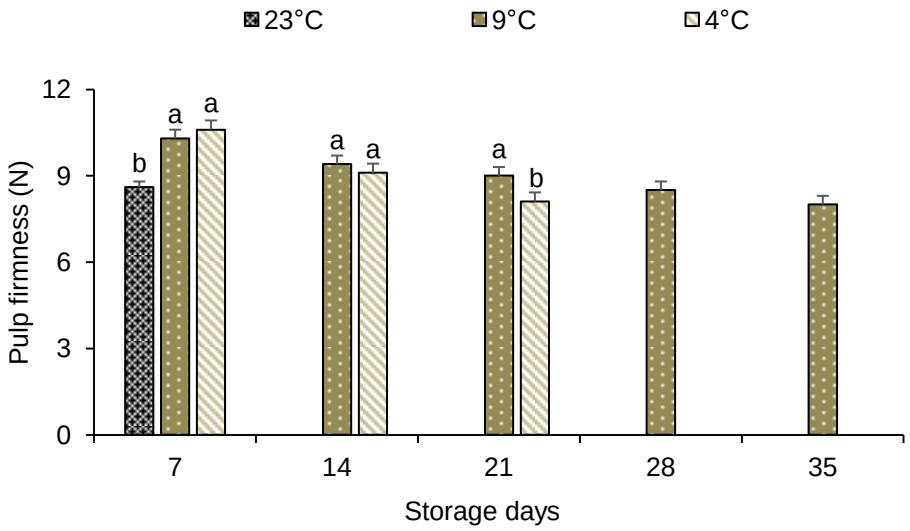

Fig. 2. Pulp firmness (N) of 'Thomson' pitayas stored for 7, 14, 21, 28 and 35 days at a temperature of 23 ± 1 °C, 9 ± 1 ° C or 4 ± 0.4 °C and humidity relative air of 80 ± 3%.

The firmness of the fruit is one of the aspects most valued by consumers when purchasing pitayas fruit. This attribute begins to change when the fruit reaches its physiological maturity due to the action of cell wall enzymes (pectinmethylesterase and polygalacturonase) that act in the breakdown of pectic substances [35]. Storage at low temperatures decreases fruit metabolism, cell wall enzyme activity, and consequently reduces softening [1,36], which explains the greater pulp firmness presented by the fruits stored at 9 and 4 °C at 7 days. However, the occurrence of chilling injuries increases the loss of firmness and reduces the storage potential of tropical fruits [37]. NERD et al. [14] report that high storage temperatures resulted in lower firmness of the fruits of *H. undatus*, however, the same authors also reported the occurrence of loss of firmness in the fruits, when stored at low temperatures due to the occurrence of cold lesions. Other authors also report a decrease in Kiwi firmness after the occurrence of cold injuries [36, 38].

At 7 days of storage, the temperature of 23°C promoted the respiration rate of the fruits, while the temperatures of 9ºC and 4ºC significantly reduced respiration (Figure 3). In the other storage periods, the temperature of 4ºC presented greater respiration, when compared to the fruits stored at 9ºC (Figure 3).

Respiration is essential for fruits to maintain their metabolic activity during storage, however, adequate reduction in respiratory rate is beneficial to prolong the storage period of vegetables [39]. Possibly, after 7 days of storage, the temperature of 23°C increased the respiratory rate of the fruits, because high temperatures potentiate metabolic activities such as respiration and transpiration, leading to early senescence of the fruits [30]. On the other hand, fruits stored at 4 ºC showed higher respiratory rate at 14 and 21 days due to injuries caused by cold, which intensified fruit metabolism. WANG [32] explains that the increase in respiratory rate is one of the physiological changes caused by low storage temperatures. Freitas & Mitcham [1] also reported an increase in respiratory rates during the occurrence of chilling injuries in pitaya. The lower respiration during the storage period justifies the longer storage period and the better quality of the fruits stored at 9°C [39].

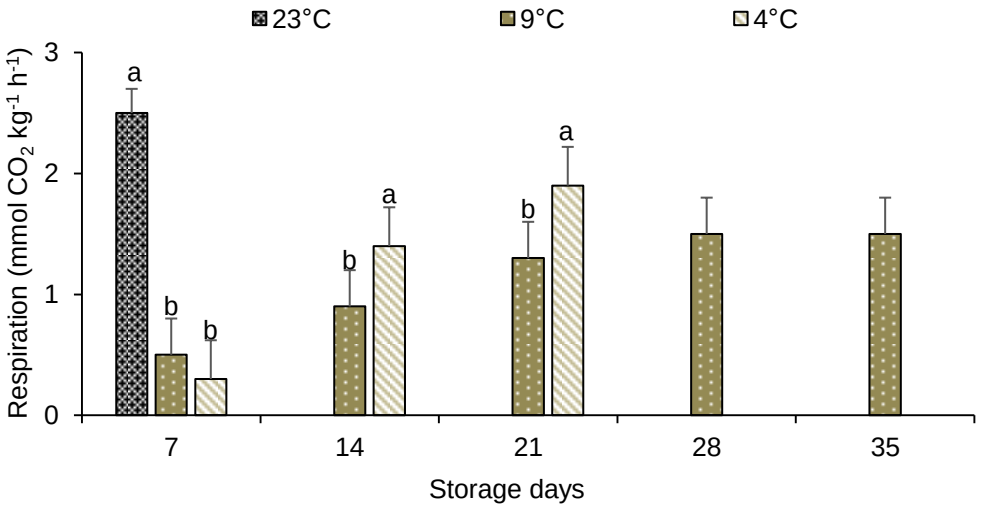

Fig. 3. Respiratory rate (mmol CO_2 kg^{-1} h^{-1}) of 'Thomson' pitaya stored for 7, 14, 21, 28 and 35 days at 23 ± 1 °C, 9 ± 1° C or 4 ± 0,4 °C and relative humidity of 80 ± 3%.

The fruits stored at 23°C had lower SS and TA values at seven days of storage, when compared to the other treatments (Table 1). At 21 days of storage, fruits stored at 4 °C showed lower values of SS and TA, when compared to those stored at 9 °C (Table 1).

The higher values of SS and TA presented by the fruits conditioned at 9 ºC are explained by the capacity of the adequate storage temperature to decrease the metabolism, the synthesis of sugars and the use of organic acids [40, 35]. On the other hand, the low temperature of 4 ºC promoted greater thermal stress and increased respiratory rates in the post-storage period, reducing the levels of SS and TA, since sugars and organic acids are used as substrates in respiratory and metabolic processes for cell maintenance [41, 42].

When comparing different storage temperatures VELHO et al. [43], also observed a greater decrease in SS and TA contents of *Acca sellowiana* stored at 23 ºC, compared to fruits stored at 4 ºC.

Table 1. Content of soluble solids (SS) and titratable acidity (TA) of 'Thomson' pitayas stored by 7, 14, 21, 28 and 35 days under temperature of 23 ±1 °C, 9 ± 1 °C or 4 ± 0,4 °C and relative humidity of 80 ± 3%.

Temperature	Fruit storage time (days)				
	7	14	21	28	35
	Soluble solids content (°Brix)*				
23°C	9,2 b	-	-	-	-
9°C	11,7 a	13,6 a	10,2 a	8,9	8,0
4°C	11,8 a	14,9 a	8,4 b	-	-
CV (%)	9,4	7,1	7,5	-	-
	Titratable acidity (% citric acid/100g^{-1} of pulp)*				
23°C	0,9 b	-	-	-	-
9°C	1,6 a	1,5 a	1,3 a	1,2	0,9
4°C	1,6 a	1,6 a	0,7 b	-	-
CV (%)	5,7	6,3	8,2	-	-

**Means followed by the same letter, in the columns, do not differ statistically by the Tukey test or t (5% probability). CV (%): Coefficient of variation.*

The pitaya stored at 9 °C had higher levels of vitamin C in all the analysis dates, when compared to the other treatments (Table 2).

The vitamin C is an antioxidant and, in association with other components, protects plants against oxidative damage [34]. The decrease in vitamin C levels during storage is due to the use of this antioxidant in tissue protection, especially in delaying cell oxidation and inhibiting free radicals and reactive oxygen species formed under stress conditions [44, 45], like low temperature. Thus, the higher levels of vitamin C and lower values of MDA (Figure 4) presented by the treatment stored at 9°C indicate that this treatment was more effective in protecting tissues against lipid peroxidation by non-enzymatic pathways.

Table 2. Vitamin C content (mg ascorbic acid /100 g^{-1} of pulp) in 'Thomson' pitayas stored for 7, 14, 21, 28 and 35 days at a temperature of 23 ± 1 °C, 9 ± 1 °C or 4 ± 0,4 °C and relative humidity of 80 ± 3%.

Temperature	Fruit storage time (days)				
	7	14	21	28	35
	Vitamin C content (mg ascorbic acid /100 g^{-1} of pulp)*				
23°C	22,8 b	-	-	-	-
9°C	41,1 a	41,1 a	37,6 a	31,4	28,4
4°C	28,4 b	26,9 b	24,7 b	-	-
CV (%)	11,7	10,2	11,4	-	-

**Means followed by the same letter, in the columns, do not differ statistically by the Tukey test or t (5% probability). CV (%): Coefficient of variation.*

At 7 days of storage, pitaya kept at 4 °C and 9 °C exhibited lower lipid peroxidation than fruits stored at 23 °C (Figure 4). At 14 and 21 days after storage, lower lipid peroxidation was observed in fruits stored at 9 °C (Figure 4).

The lipid peroxidation is an indicator of cell membrane damage caused by chilling injury to fruits. When subjected to low or high temperatures for long periods of storage, fruits can undergo oxidative stress [46], due to the accumulation of reactive oxygen species (ROS). This is because there is a super-reduction in the electron transport chain, one of the steps of respiration, in which electrons react with molecular oxygen and form superoxides (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH-). These compounds cause severe damage to cell membranes and result in tissue death and, consequently, fruit deterioration [15].

Thus, it can be inferred that the highest lipid peroxidation observed in fruits stored at temperatures of 23 and 4°C occurred due to the accumulation of reactive oxygen species that damaged cell membranes and accelerated the senescence process [47,48]. Other studies have also related higher lipid peroxidation with the occurrence of cold injuries at low storage temperatures [48,49].

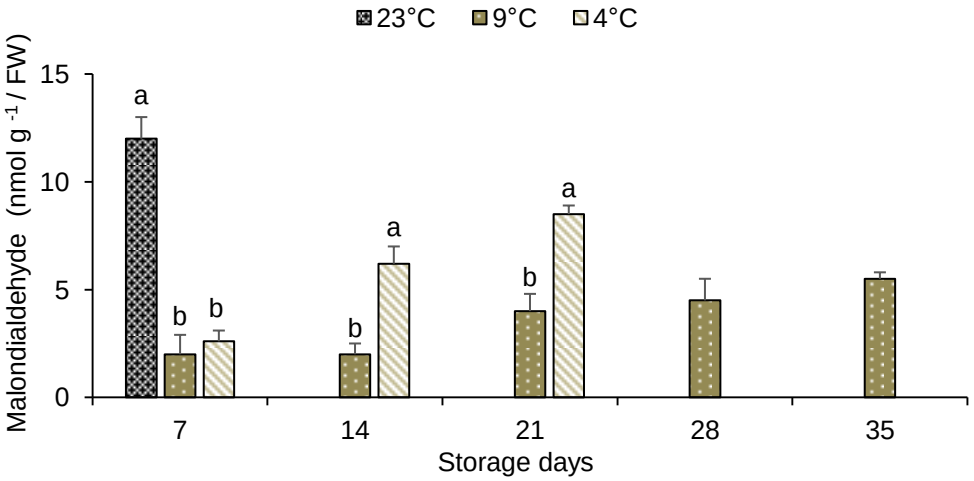

Fig. 4. Lipid peroxidation (MDA nmol g^{-1} / FW) in 'Thomson' pitayas stored for 7, 14, 21, 28 and 35 days at a temperature of 23 ± 1 °C, 9 ± 1 °C and 4 ± 0.4 °C and a relative humidity of 80 ± 3%.

At 7 days of storage, fruits stored at 23 °C exhibited higher activities of POX and APX enzymes in relation to the other treatments (Figure 5). However, at 21 days of storage, pitayas

conditioned at 4 °C had higher POX and APX activity compared to fruits submitted to a temperature of 9 °C (Figure 5).

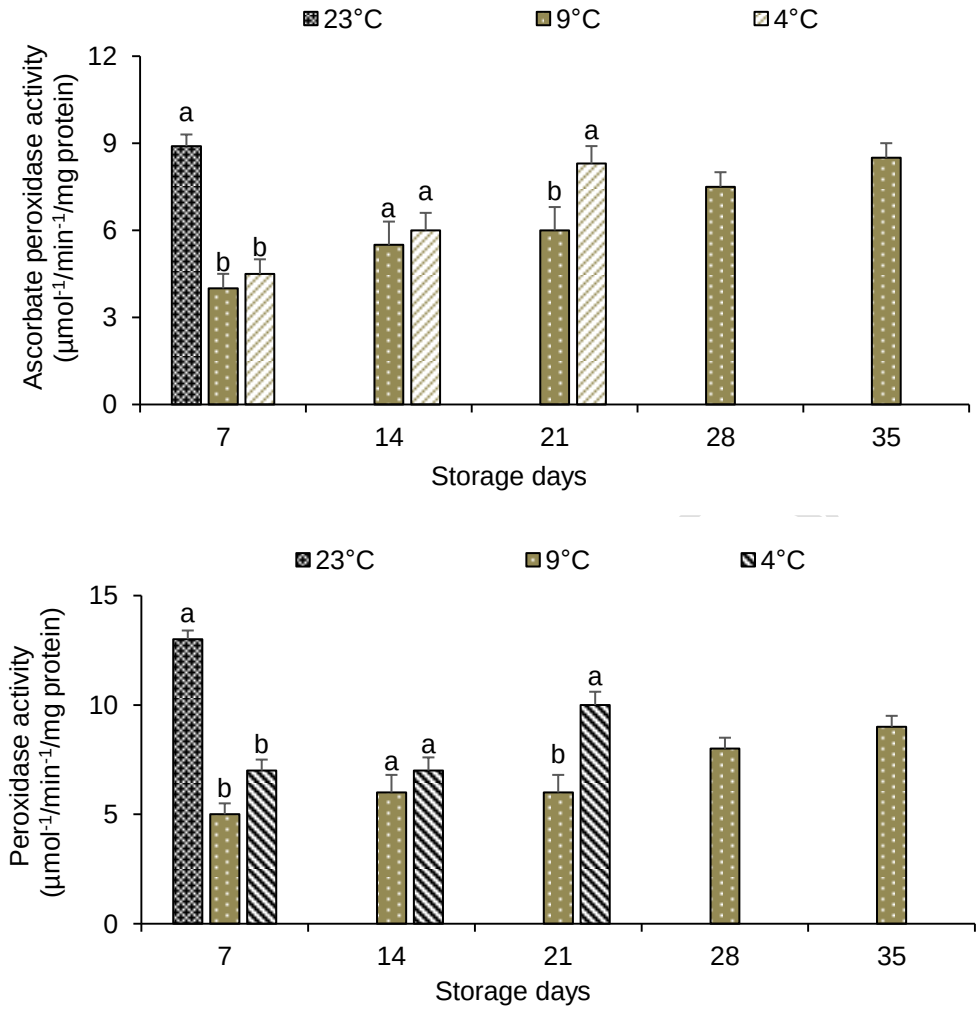

Fig. 5. Activity (µmol^{-1}/min^{-1}/mg protein) of ascorbate peroxidase and peroxidase enzymes in 'Thomson' pitayas stored for 7, 14, 21, 28 and 35 days at 23 ± 1 °C, 9 ± 1 °C or 4 ± 0.4 °C and relative humidity of 80 ± 3%.

When fruits are exposed to stresses (such as refrigeration), the balance between free radicals and antioxidant compounds is disturbed, resulting in oxidative stress in plant cells. [16,50]. POX and APX are detoxifying enzymes used as a strategy to combat reactive oxygen species (O_2− and H_2O_2) and protect the cells/tissues [39]. These enzymes act as antioxidants in plants in different subcellular compartments, sequestering the free radicals formed and decomposing them [16,50]. Thus, higher activities of these enzymes at 7 and 21 days of storage, for temperatures of 23 °C and 4 °C, respectively, indicate greater oxidative stress, which culminated in increased respiratory rate, acceleration of degradative processes and accumulation of free radicals. free, especially reactive oxygen species [50], causing loss in fruit quality and senescence. On the other hand, fruits stored at a temperature of 9 °C for 21 days had lower activity of APX and POX enzymes, which may be an indication that these fruits had adequate conservation and quality.

Regarding the coloring attributes of the epidermis *L*, *C* and *h*° there was no significant difference between temperatures at 7 days of storage (Table 3). However, at 14 and 21 days, fruits kept at 4 °C had higher values of *C* and *h*º, compared to fruits stored at 9 °C (Table 3).

The highest values of *C* and *h*° presented by the fruits stored at 4°C indicate a darker and less intense red color. The transformation in the color of the fruit epidermis is related to the degradation of chlorophyll, which favors visual perception or the formation of carotenoids, increasing the appearance of yellowish and/or orange colors [51]. The degradation and alteration of anthocyanins and

anthoxanthins are accelerated and promoted by incorrect storage conditions and high respiratory rates [35], which explains the greater browning and the accelerated loss of red color in the fruits stored at 4°C.

Table 3. Luminosity (*L*), chroma (*C*) and hue angle (*h°*) of the epidermis of 'Thomson' pitayas stored for 7, 14, 21, 28 and 35 days at a temperature of 23 ± 1 °C, 9 ± 1 °C or 4 ± 0.4 °C and relative humidity of 80 ± 3%.

Temperature	Luminosity (L)*	Crome (C)*	Hue angle (*h°*)*
	7 days of storage		
23 ºC	43,8 a	46,2 a	76,1 a
9 ºC	44,9 a	46,3 a	98,1 a
4 ºC	43,7 a	49,5 a	95,6 a
CV (%)	11,6	8,9	12,5
	14 days of storage		
9 ºC	40,5 a	44,5 b	62,3 b
4 ºC	46,1 a	49,5 a	89,2 a
CV (%)	10,5	12,2	8,1
	21 days of storage		
9 ºC	42,8 a	42,5 a	65,1 b
4 ºC	41,6 a	46,8 b	90,4 a
CV (%)	7,2	11,4	9,2
	28 days of storage		
9 ºC	43,0	42,2	65,8
	35 days of storage		
9 ºC	42,1	40,4	69,19

**Means followed by the same letter, in the columns, do not differ statistically by the Tukey test or t (5% probability). CV (%): Coefficient of variation.*

At 7 days of storage, higher values of pulp browning were observed in fruits stored at 23 °C and 4 °C (Figure 6). Between the 14th and 21st day of storage, fruits kept at 4 °C showed greater pulp darkening in relation to those exposed to a temperature of 9 °C. Fruits stored at 9 °C showed lower pulp darkening when compared to fruits kept at 4 °C (Figure 6).

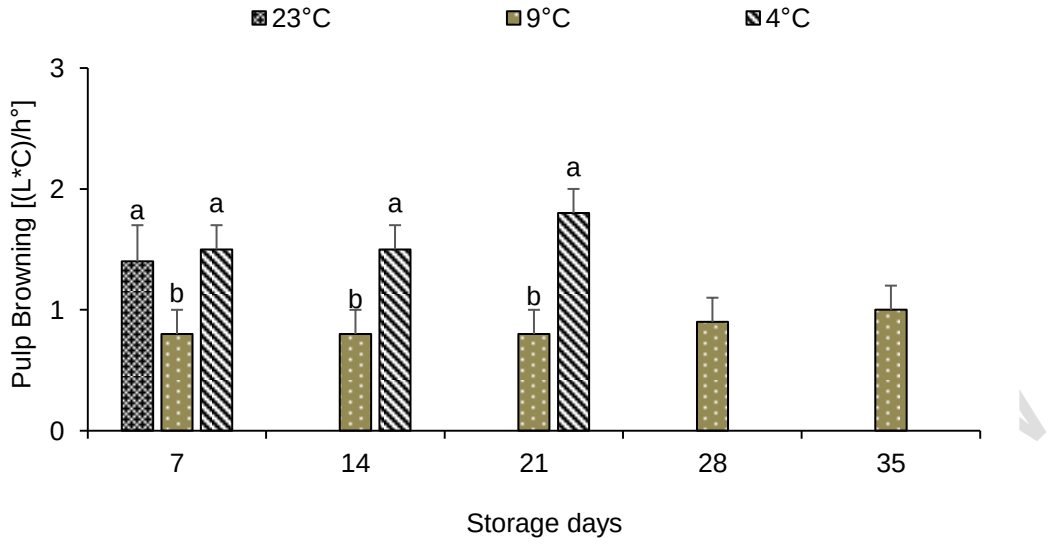

Fig. 6. Pulp browning [(L×C)/h°] of 'Thomson' pitayas stored for 7, 14, 21, 28 and 35 days at 23 ± 1 °C, 9 ± 1 °C or 4 ± 0.4°C and relative humidity of 80 ± 3%.

The pulp browning is one of the main symptoms of cold injuries due to cold storage [52]. Thus, the greater pulp browning observed in dragon fruit stored at 4°C occurred due to the exposure of the fruits for long periods at low temperatures, which results in the solidification of protoplasmic lipids, in the alteration of the metabolism, in the reduction of essential components and in the accumulation of toxic compounds. These changes cause deformations in cell permeability and physical change in membranes [37,46,32]. The phase transition of membranes causes visual changes and the appearance of lesions, due to the passage of this structure from a crystalline, liquid, flexible and normal structure to a solid gel structure [37].

4. CONCLUSION

The storage of 'Thomson' pitaya at 23 °C provides a short post-harvest period (7 days), rapid deterioration and early fruit senescence.

The temperature of 9 ºC provides a longer shelf life of 'Thomson' pitaya fruits, as well as being quite efficient in the conservation of quality attributes. The storage capacity of 'Thomson' pitaya fruits at 9 °C is approximately 28 days, after this period, there is a reduction in the quality of the fruits and the beginning of the senescence process.

The storage of 'Thomson' pitaya at 4°C is not recommended due to high internal browning, oxidative stress and loss of quality attributes.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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