Physical and Physiological Changes in Low-Temperature-Stored Pitahaya Fruit (*Hylocereus undatus*)♦

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ABSTRACT

Pitahaya fruit (*Hylocereus undatus*) is highly perishable, and so commercialization is limited. Refrigeration prolongs shelf life of fruits and vegetables. Nevertheless, some produce may be injured by low temperatures during storage. Studies on chilling injury in pitahaya fruit are scarce and this damage is mostly judged subjectively. This study was conducted to contribute to the knowledge and understanding of chilling injury in pitahaya, to eventually identify indicators that would be useful and consistent in describing degree of injury more objectively for future studies. Pitahaya fruits were stored at 4 ±2ºC and 8 ±2ºC for 5, 10, 16, or 21 d. At the end of each low-temperature storage period the fruits were removed from cold storage and kept at 26 ±2ºC and RH 72 ±2% for 0, 3, or 6 d. An additional group of fruits (used as reference) was kept under environmental conditions (6 d at 26±2ºC). At the end of each treatment period, external color, respiration rate, production of ethanol and acetaldehyde in pulp, and observable damage were assessed. Fruits not stored at low temperatures (reference) showed good color development (hue angle diminished from 45.09° to 17.5°) and good external appearance. However, external damage was exhibited in low-temperature-stored fruits at both low temperatures, although more intensely and sooner in fruits stored at 4ºC than in those stored at 8°C. For both temperatures, after 5, 10, or 16 d of cold storage, a moderate reduction of hue angle was registered (around 18.6° to 26.7°) when removed from low-temperature storage, indicating moderate development of color. In fruits stored for 21 d at 4ºC and ripening at 26±2°C, hue angle remained high (37.6º), indicating that the long period of cold storage inhibited natural reduction of hue angle, and so the development of the desirable external pinkish-red color did not occur. A 28% increase in respiration rate was also recorded, while production of ethanol and acetaldehyde increased 37 and 35 times. Also, external and internal appearance of the fruit deteriorated. In general, observable damage increased in fruits stored for longer periods, particularly at the lower temperature. It is suggested that external color (hue angle) and respiration rate are objective, useful, and consistent variables for measuring chilling injury in pitahaya fruits.

Key words: Color, respiration, ethanol, acetaldehyde, chilling injury, pitahaya.

INTRODUCTION

Pitahaya with red-peel and red or white pulp, belonging to the genus *Hylocereus*, is cultivated in Nicaragua, Guatemala, Vietnam, Taiwan, Thailand, Cambodia, Philippines, Mexico, Israel, and El Salvador. Other common names of this fruit are dragonfruit in Asia and eden in Israel (Nerd *et al*., 2002). *Hylocereus undatus* is native to tropical America and grows throughout Central America and southern Mexico. This plant is a very drought-resistant tropical cactus adapted to environments with average

♦ Received 29 December 2007; Accepted 6 September 2008

temperatures of 21 to 29°C and precipitations of 600 to 1300 mm, with alternating dry and humid seasons. It produces best at altitudes between 200 to 600 meters, but one can find it from 0 to 1500 meters above sea level. Given their production of aerial roots, plants can creep onto supporting structures such as live or dead scaffolds, trees, poles, stone walls, and rocks. The pitahaya plant is best adapted to loamy, sandy or rocky soils with good drainage (Barbeau, 1990). There are different recommended doses of fertilization. In Israel, 20N-20P-20K (Weiss *et al*., 1994) and 23N-7P-23K (Mizrahi and Nerd, 1999) are employed. The fruit is a large globose berry with white pulp covered by bract; its peel develops a characteristic rose color when ripe (Centurión *et al*., 1999). The fruit is important for its genetic variability, its adaptability to adverse environmental conditions, its many uses, its possibilities for industrialization, its productivity, and its high profitability and demand (Rodríguez, 2003). However, it is highly perishable, and therefore its commercialization as fresh fruit is limited (Corrales-García, 2003).

Low-temperature storage is the commercial technology that is thought to be the most adequate for prolonging shelf life of fruits and vegetables in postharvest. However, some fruits, especially tropical and subtropical fruits, are injured by temperatures lower than their critical temperatures (Couey, 1982; Morris, 1982, Kader, 2002).

Typical symptoms of chilling injury are spotting or internal and external browning, depressions, areas with aqueous tissue, abnormal ripening, development of unpleasant odors and flavors, and marked incidence of external rotting. These symptoms become more acute when the fruit is transferred to market temperatures (Kader 2002).

Low temperatures provoke physiological and biochemical responses in fruits and vegetables such as changes in membrane structure, alterations in rate of respiration and ethylene synthesis (Wang, 1982). In cherimoya, low temperatures cause increases in reducing sugars (Alique and Zamorano, 2000). In refrigerated avocado, the production of ethanol and acetaldehyde was associated with chilling injury (Corrales and Tlapa, 1999). This was also shown in frozen apples later exposed to high temperatures (Song *et al*., 1996).

It has been proposed that ethanol and acetaldehyde produced by fruit may be possible indicators of chilling injury (Schirra, 1993; Forney and Jordan, 1996; Corrales, 1997), and that these metabolites are associated with secondary responses such as browning and discoloring (Cantwell *et al*., 2002). However, the effect of low-temperature storage on pitahaya fruits has been subject to few studies (Nerd *et al*., 1999; Centurion *et al*., 2000).

The objective of this study was to determine physical and physiological responses to different temperatures and periods of low-temperature storage to eventually identify indicators that would be useful and consistent in describing the degree of chilling injury more objectively for future studies on pitahaya.

MATERIALS AND METHODS

Origin of fruit

White-pulped, red pitahaya fruit (*Hylocereus undatus*), 350 ±50 g, on which 70% of the outer surface was red, were harvested in the community of Yaxcopoil, municipality of Umán, Yucatan, Mexico, located 22 km southwest of Merida. The fruits were carefully harvested, packed in plastic crates, and taken to the laboratories of the Instituto Tecnológico de Mérida, where the experiment was set up.

Conditioning of the fruit and description of treatments

The fruits and their bracts (scales) were cleaned with a cotton cloth impregnated with a solution of 3% sodium hypochlorite and grouped randomly into nine lots of 12 fruits each. One of these lots (used as reference) was stored for 6 d at 26 ±2ºC and relative humidity (RH) of 72 ±2%. The remaining lots were stored in two refrigerated chambers: one at 4 ±2ºC and the other at 8 ±2ºC, both with 92 ±2% (RH). The fruits were low-temperature stored for 5, 10, 16, or 21 d. At the end of each low-temperature storage period they were removed from cold storage and kept at 26 ±2ºC and RH 72 ±2% for 0, 3, or 6 d. From each lot, three fruits were used to determine external color and respiration; the remaining nine were used to assess firmness and ethanol and acetaldehyde production in the pulp (destructive tests) after 0, 3, and 6 d. The combination of temperatures (2), low-temperature storage periods (4), and post-cold-storage periods (3) defined the 24 treatments, each of which was replicated three times; one fruit was one experimental unit.

Determination of response variables

Color

External fruit color was determined using a Minolta Colorimeter Model CR-200. The dimensions L*, a*, and b* were obtained, and the hue angle was calculated (hue = arc tan b*/a*) and expressed in degrees (º). Saturation index, or purity of color (chroma), was also calculated [$C = (a^{*2} + b^{*2})^{1/2}$] and expressed numerically.

Firmness

Firmness was determined with a manual penetrometer, Effegi™ Model FT 011, (R. Lusa™). The fruit was cut in halves along the equatorial line, and firmness was observed at three equidistant points of the pulp. Firmness of each fruit was expressed in newtons (N) as the average of the three measurements.

Production of ethanol and acetaldehyde in the pulp

Ethanol and acetaldehyde in the pulp were quantified by gas chromatography using the headspace technique (Davis and Chase, 1969). The pulp was extracted with a punch: a portion of the peel was lifted and a sample of approximately 5 g was extracted and deposited in 25 mL glass vials. These were frozen in liquid nitrogen and stored in a freezer at −20 ºC for the duration of the experiment (approximately 30 d). For the determinations, the samples were thawed in a double boiler at 60ºC for 10 min to facilitate the release of the volatile components. A 3 mL sample of gas from the vial headspace was extracted with a syringe and injected into the gas chromatograph.

The gas chromatograph used was a Varian Star 3400 CX with a Poraplot Q capillary column (27.5 m/0.32 mm). Flow in the column was 36.14 mL min^{-1}. The gases used and their flow in the detector were: He, as carrier gas (4.77 mL min^{-1}), H_2 (34.5 mL min^{-1}) and air (331 mL min^{-1}), with a pressure of 5 psi in the column head. The conditions were 80ºC in the column, 220ºC in the injector, 240ºC in the flame ionization detector (FID) and 210ºC in the thermal conductivity detector (TCD). Run time was 4.2 min and determined retention time in the FID for ethanol and acetaldehyde was 3.52 and 2.74 min, respectively. Ethanol and acetaldehyde standards of known concentration were injected to construct a standard curve. The results are expressed in mg 100 g^{-1}.

Respiration rate
Respiration was determined by gas chromatography with the equipment described above. Each fruit was placed in a hermetically sealed recipient (2.15 L) for 2 h. A sample of gas (2 mL) was extracted and injected in the chromatograph to be analyzed with a TCD. The chambers were opened and each fruit was weighed.

Chromatographic conditions were those used to determine ethanol and acetaldehyde. Run time was 3.5 min and CO_2 retention time was 3.12 min. The concentrations of the samples were calculated using a standard curve and the results were expressed in mL CO_2 $kg^{-1}h^{-1}$.

Visual assessment of damage
Damage was assessed with a 5-point hedonic scale (0, 1, 2, 3, and 4), which corresponded to the following percentages of damaged areas of the peel and pulp (shriveling, brown spots, depressions, rot, among others): 0, 1-20, 21-40, 41-60, and 61-100.

Statistical analysis
The statistical analysis was performed using SAS (1993). The variables color, firmness, ethanol, and acetaldehyde were analyzed with a completely random design in a factorial arrangement of 2×4×3: temperatures (4 and 8ºC), low-temperature storage periods (5, 10, 16, and 21) and post-cold-storage periods (0, 3, and 6 d). For the variable respiration, the same levels of low-temperature storage period were considered, but in the post-refrigeration period there were seven levels (1, 2, 3, 4, 5, 6, and 7 d) with a factorial arrangement of 2×4×7. Means were compared with the Tukey test ($p \leq 0.05$). Nonrefrigerated fruit data were used only as a reference and were not included in the statistical analysis.

RESULTS AND DISCUSSION

Color

Hue angle
Hue angle revealed that the period in which fruits were stored at low temperatures was critical for the development of color after removal from cold storage. Nonrefrigerated fruits had the best external color development, indicated by a notable reduction in hue angle (Table 1). According to the factorial analysis of the cold-storage fruits, the smallest average hue angle (20.23º) was observed in those stored for the shortest period (5 d) and ripened for 6 d at 26 ±2°C, and the largest average hue angle (29.88º) was observed in fruits stored for the longest period (21 d) and ripened for 6 d. For the factor temperature it was found that in fruits stored at 8ºC the average hue angle was significantly smaller (22.16º) than the average for those stored at 4ºC (26.89º). The analysis of the interaction between the factors temperature and low-temperature storage period (Figure 1) indicated that the ability to develop the pinkish red color after removal from low-temperature storage decreased slightly in fruits that were stored for longer periods (up to 16 d). In fruits stored for 21 d, however, this ability decreased significantly at 4°C, whereas at 8°C color development was not greatly affected. This manifestation of chilling injury occurred only when the long period of cold storage was combined with the lower storage temperature.

Table 1. Physical, biochemical and physiological characteristics of non-refrigerated pitahaya fruits during 0, 3, and 6 d of storage at 26 ±2ºC and 72 ±2% RH.
(For each variable, means with different letters are statistically different (p 0.05) for n = 24 and for respiration rate n = 56.)

Variable	0 d	3 d	6 d
Hue (°)	45.09 a	20.4 b	17.53 bc
Chroma	25.54 b	33.25 ab	36.35 a
Firmness (N)	5.16 a	4.9 a	5.1 a
Ethanol (mg/100g)	0 a	2.99 a	2.84 a
Acetaldehyde (mg/100g)	0.15 a	0.13 a	0.14 a
Respiration rate (mL CO_2 $kg^{-1}h^{-1}$)	26.52 b	31.26 a	31.97 a

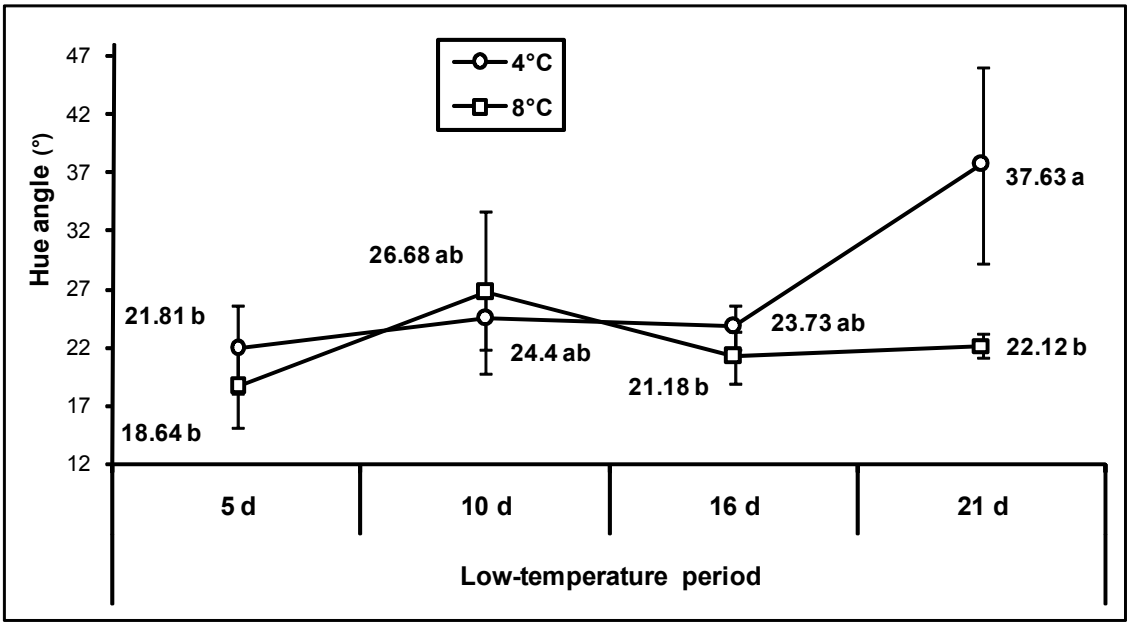

Figure 1. The effect of interaction between factors temperature*storage time (low-temperature storage period) on peel color change (hue angle) during cold storage of pitahaya fruit.
(Means with different letters are statistically different (p≤0.05). Vertical bars indicated standard error for n = 9.)

Chroma

Low-temperature storage impeded development of chroma. In nonrefrigerated fruits, chroma increased notably (Table 1), indicating that the red pigment increased. The low-temperature stored fruits did not exhibit significant differences between the tested temperatures. The factors low-temperature storage time and post-cold storage time, however, did have a significant effect; greater average chroma (38.98) was observed in fruits stored for 5 d (shortest low-temperature storage period), but in fruits stored for a longer period, chroma remained virtually unchanged during post-cold storage, within the interval of 30.4 to 31.6. In post-cold storage, the highest chroma was recorded on d 3 (C = 35.93), but this decreased significantly

(C = 32.26) on d 6. The analysis of the interaction between temperature and post-cold storage period (Figure 2A) indicated that at the end of cold storage (d 0) chroma was lower in fruits that had been stored at the lower temperature, but after 6 d at 26 ±2°C there were no differences. There was practically no interaction between the factors low-temperature storage period and post-cold storage period except for fruits low-temperature stored for 5 d (Figure 2B). Apparently, the fruits stored at the lowest temperature for 10 d or more lost the capacity to increase chroma in post-cold storage, an incapacity that could also be interpreted as a manifestation of chilling injury.

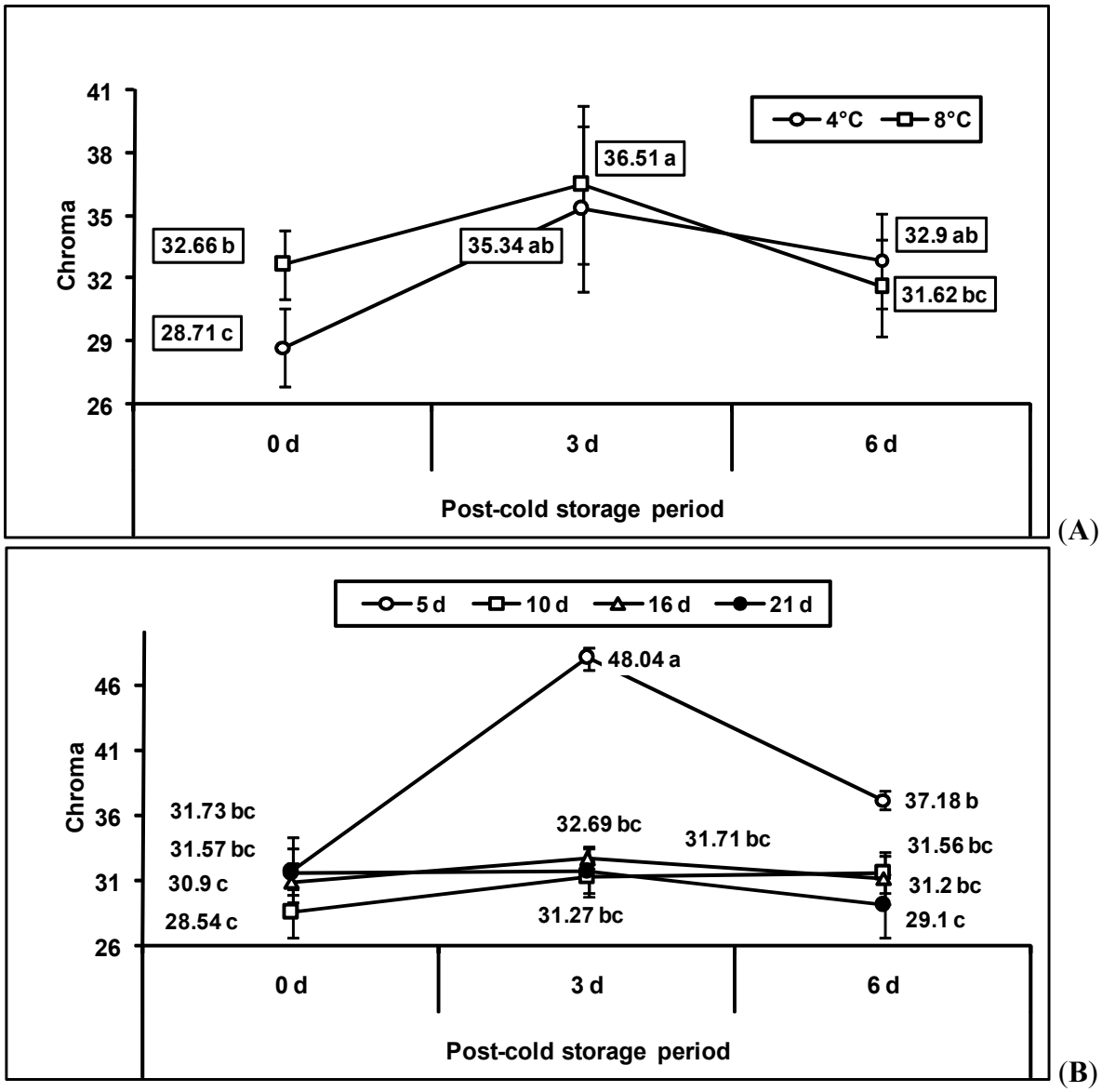

Figure 2. The effect of interaction (**A**) between temperature and post storage time (post-cold storage period) and (**B**) between factors storage time (low-temperature storage period) and post storage time (post-cold storage period) on peel color change (chroma) during cold storage of pitahaya fruit.
(Means with different letters are statistically different ($p \leq 0.05$).
Vertical bars indicate standard error for (**A**) n = 12 and (**B**) n = 6.)

Firmness

Firmness of nonrefrigerated fruits remained virtually unchanged (Table 1); for this variable an effect of temperature and post-cold storage period was found. Thus, fruits stored at 4ºC had a general average firmness significantly lower (4.8 N) than those stored at 8ºC (5.6 N). At the end of cold storage, the fruits had a general average firmness of 5.4 N, which later decreased significantly (4.9 N) on the sixth day of post-cold storage.

The interaction between the factors temperature and low-temperature storage period (Figure 3) indicated that at 8ºC, firmness stabilized, while at 4ºC lower firmness was recorded. This trend became more evident with the longer period of cold storage, especially in fruits stored for 16 d. For fruits in general, this behavior is explained by disorders caused by exposure to near-threshold temperatures for prolonged periods, producing irreversible effects such as the destruction of membranes, rupture of cell compartments, and electrolyte leakage (Marangoni *et al*., 1996). These effects have direct repercussions on fruit firmness. Bauchot *et al*. (1999) reported excessive softening of kiwi fruits stored below threshold temperatures.

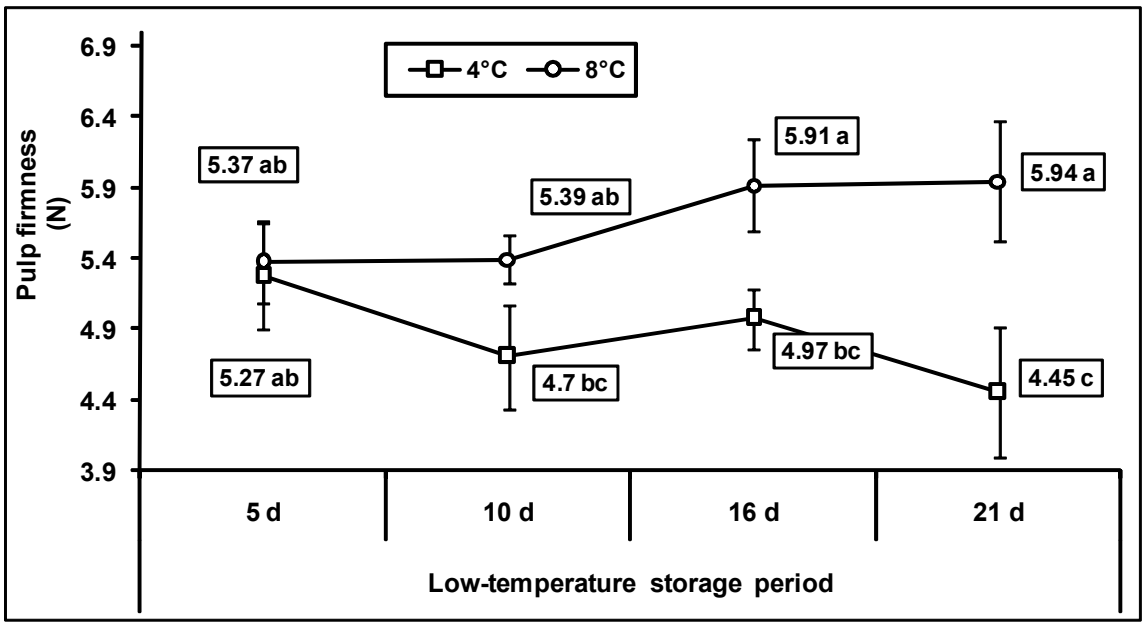

Figure 3. The effect of interaction between temperature and storage time (low-temperature storage period) on pulp firmness during cold storage of pitahaya fruit.
(Means with different letters are statistically different ($p \leq 0.05$).
Vertical bars indicated standard error for n = 9.)

The interaction between temperature and post-cold storage period (Figure 4) indicated an analogous situation, that is, at lower temperatures pulp firmness is also lower. This trend became more evident as the post-refrigeration period increased, particularly as of day 3.

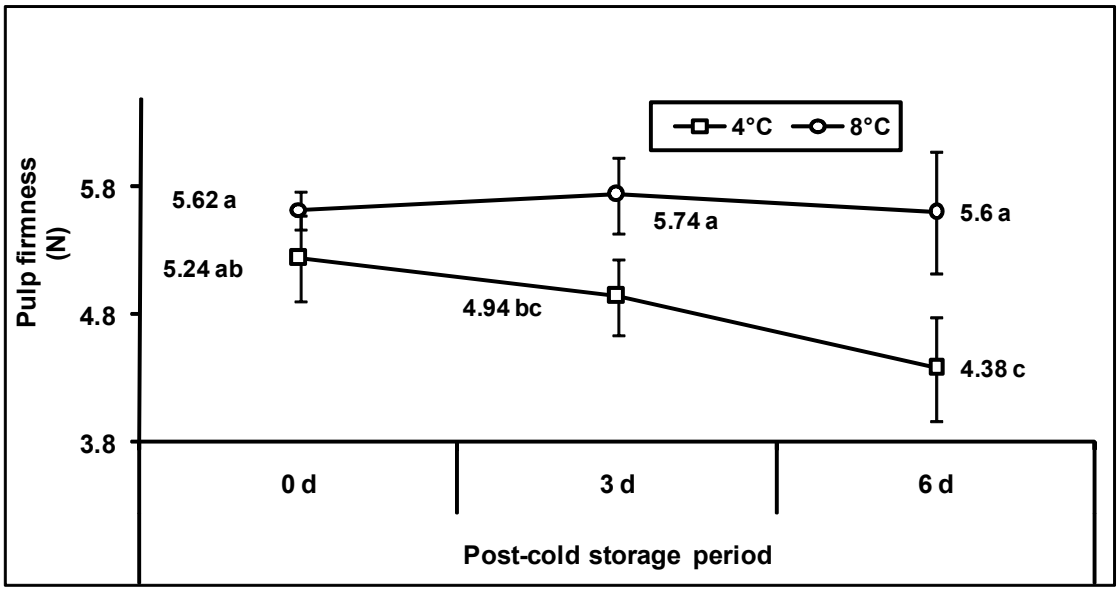

Figure 4. The effect of interaction between temperature and post storage time (post-cold storage period) after cold storage of pitahaya fruit.
(Means with different letters are statistically different ($p \leq 0.05$).
Vertical bars indicated standard error for n = 12.)

Ethanol and acetaldehyde production

Nonrefrigerated control fruits had minimal quantities of ethanol and acetaldehyde, which did not surpass 3.0 and 0.15 mg 100 g^{-1}, respectively (Table 1). The production of these metabolites, however, increased notably in low-temperature stored fruits. As the periods of cold storage and post-cold storage increased (particularly fruits low-temperature stored for 10 d or longer), the production of both metabolites also increased (Table 2). However, there were no significant differences between the temperatures tested. Chromatograph readings indicated high production of these metabolites, but even at the highest contents recorded they were not perceived by the authors in the flavor or aroma of the fruit when they cut and took samples of the fruit for analysis. If the production of these metabolites is due to stress (Kimmerer and Koslowski, 1982; Schirra, 1993; Forney and Jordan, 1996), besides those of anaerobiosis and other factors, then the results obtained suggest that the longer the period of low-temperature storage, the greater the stress and production of these metabolites. The results also coincide with those of Corrales and Tlapa (1999), who found an increase in the production of these metabolites as a manifestation of chilling injury in avocado. For ethanol and acetaldehyde production no interactions among the factors studied were found.

Table 2. Effect of low-temperature storage period and post-cold storage period on the production of ethanol and acetaldehyde in pulp of low-temperature stored pitahaya fruit at 4 ±2ºC and 8 ±2ºC, with 92 ±2% RH.
(Vertically and within each factor, the means followed by different letters are statistically different ($p \leq 0.05$). For low temperature storage period n = 18; for post-cold storage period n = 24.)

Factor	Level	Ethanol (mg/100g)	Acetaldehyde (mg/100g)
Low-temperature storage period	5 d	23.2 b	1.87 b
	10 d	99.8 a	4.06 ab
	16 d	138.99 a	4.50 a
	21 d	111.04 a	5.23 a
Post-cold storage period	0 d	12.31 c	1.68 b
	3 d	81.45 b	3.18 b
	6 d	186.02 a	6.84 a

Respiration

Nonrefrigerated reference fruit respiration rate was between 26 and 32 mL $CO_2 kg^{-1} h^{-1}$ (Table1), and according to the factorial analysis, a significantly higher respiration rate was recorded at 4°C than at 8ºC (37.9 and 33.27 mL $CO_2 kg^{-1} h^{-1}$, respectively). With longer low-temperature storage periods, respiration increased significantly, almost linearly, from 31.6 (5 d low-temperature stored fruits) up to 39.6 mL $CO_2 kg^{-1} h^{-1}$ (21 d low temperature stored fruits). The interaction between temperature and period in low-temperature storage indicated that respiration increased during both 4°C and 8°C storage, but rose more quickly in 4°C storage. In fruits stored for 10 d, respiration rate at 4ºC was significantly higher than at 8ºC, but in fruits stored for 16 d, respiration at 8°C equaled that of the fruit stored at 4°C (Figure 5).

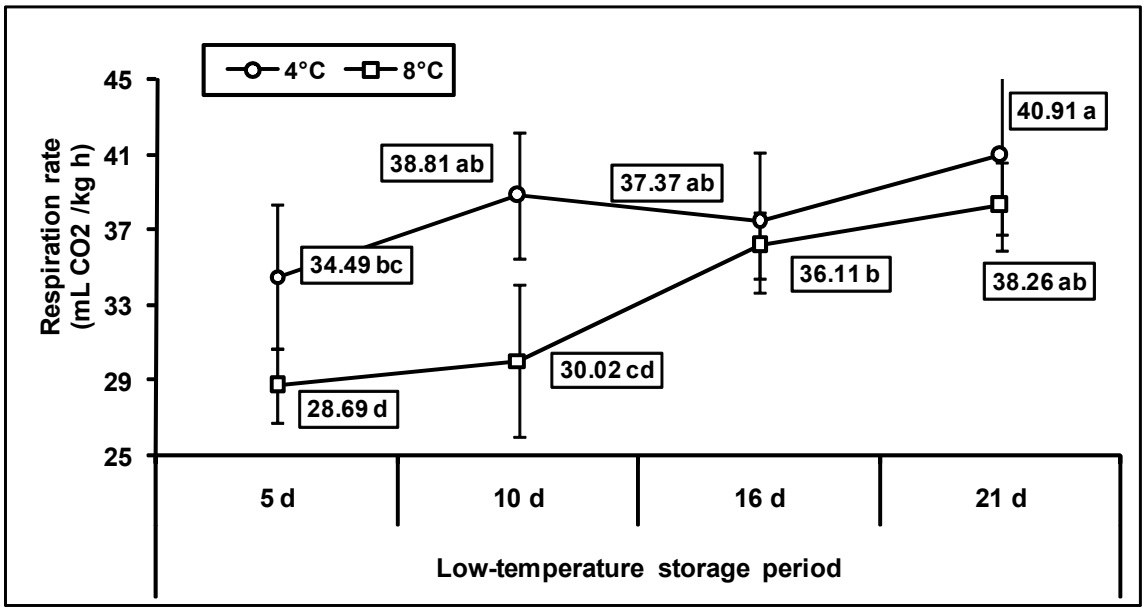

Figure 5. The effect of interaction between temperature and storage time (low-temperature storage period) on respiration rate during cold storage of pitahaya fruit.
(Means with different letters are statistically different ($p \leq 0.05$).
Vertical bars indicated standard error for n = 21.)

Lower temperature and longer exposure time cause greater stress; consequently, there was more respiration. Therefore, the metabolic oxidation process accelerated in response to stress caused by low temperatures. The increase in respiration has been considered an indicator of sensitivity to cold (Wang, 1982); this coincides also with results for refrigerated kiwi fruits (Lee *et al*., 2001).

Visual assessment

External damage was exhibited in fruits stored at both low temperatures, although more intensely and sooner in fruits stored at 4ºC. This damage is characterized mainly by the appearance of depressions and brown spots on the surface of the peel of fruits stored for 16 d or longer, and became more evident when the post-cold storage period increased. The appearance of depressions has been observed in citrus fruits, cucumber, papaya, and beans (Wang, 1994); browning has been reported as a manifestation of cold damage in products such as jicama (Cantwell *et al*., 2002). In pulp, the damage was manifested as an alteration of its characteristic appearance (white and succulent), becoming vitrescent. At 4ºC vitrescence was homogeneous and juicy; at 8ºC it was more heterogeneous and reticulate. This alteration in the pulp was observed first in the pitahaya stored for 16 d at 4ºC, but at 8ºC it was observed only in those stored for 21 d.

CONCLUSIONS

Pitahaya sensitivity to low temperatures was manifested in undesirable appearance of the fruit due to slight browning, loss of firmness, and increase in the production of ethanol and acetaldehyde in the pulp, as well as to the scarce development of pinkish-red coloring in the peel and increased respiration rate of the fruit. Chilling injury appeared more intensely and sooner in fruits stored for longer periods at 4ºC.

The magnitude of ethanol and acetaldehyde production depended on the level of stress caused by prolonged exposure to low temperatures. Chromatograph readings indicated high production of these metabolites, but even at the highest contents recorded they were not perceived in the flavor or aroma of the fruit.

Finally, the results suggest that external color (hue angle and chroma), pulp firmness, and respiration rate are useful, objective, and consistent variables for measuring chilling injury in pitahaya fruits.

ACKNOWLEDGEMENTS

The authors sincerely thank Dra. Diane Miyoshi for the translation of this article and Dr. Enrique Sauri and the Instituto Tecnológico de Mérida for facilitating their installations for the realization of this study.

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