

Effect of saturated air heat treatments on weight loss reduction and epicuticular changes in six varieties of cactus pear fruit (*Opuntia* spp.)

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Abstract

Cactus pear (*Opuntia* spp.) is a not climacteric fruit, nonetheless it is highly perishable. Fruit is easily damaged showing lesions, rotting, and high transpiration rates. These events occur in the peel, and the impact can depend on, among other factors, the morpho–anatomical characteristics of its structure. In this study weight loss, respiration rate, cuticle thickness, and epicuticular micro–structure were evaluated in six varieties of cactus pear in response to different vapor heat treatments (35, 38 and 42 ± 1°C for 12 and 24 h) after storage for 15 d at 20°C and 75% RH and 30 d at 10°C and 95% RH. Vapor heat treatment reduced fruit weight loss, in general, an effect related to treatment. For non refrigerated fruits, the treatments that caused the highest weight loss reduction, compared to the control were 38°C for 12 h in Alfajayucan variety (61% reduction), and 35°C or 38°C for 12 or 24 h in Sangre de Toro variety (up to 51% reduction). For cold stored fruits, the treatments in which weight loss was lowest were 38°C for 12 or 24 h with the variety Alfajayucan (1.4 or 1.53% weight loss, respectively); 38°C for 12 h with the varieties Cristalina and Rojo Pelón (0.92 and 2.27% weight loss, respectively). All these treatments caused rearrangement of the epicuticular wax layers, minimizing or eliminating cracking. This rearrangement can partially explain the positive effect in weight loss reduction. However, vapor heat treatment in general, caused little effect on respiration rates and cuticle thickness of fruit.

Key words: postharvest, micro–structure, epicuticular wax, transpiration.

Introduction

Cactus pear deterioration is caused by removal of spines (glochids), inappropriate handling, rotting, and high transpiration rates. Resistance to these factors is different among varieties making some less perishable than others (Corrales and Flores–Valdez, 2003). However, the degree of tolerance to these factors is associated with morpho–anatomical characteristics of the peel (Corrales *et al.*, 1997). Particularly, the structure and chemical composition of its waxy layers and the particular response of each Mexican variety to vapor heat are unknown.

In Italy, Schirra *et al.* (1996, 1997, 1999) and D'hallewin *et al.* (1999) have studied the application of vapor heat treatments on an Italian cactus pear cultivar. Schirra *et al.* (1997) found that early ripening cactus pear fruit Gialla variety exposed to 38°C for 24 h had a positive effect and improved efficiency of the peel as a protective barrier in the Gialla variety, significantly reducing weight loss during storage for 21 d at 6°C plus 7 d at 20°C.

Mexico has a high diversity of cactus pear varieties (Gallegos *et al.*, 2006; Corrales *et al.*, 2006), which under simple observation exhibit different morpho-anatomical characteristics, and likely their responses to heat treatments are different. However, there is no published information on the effect of vapor heat treatments on any of the Mexican varieties. This study was conducted to evaluate the response, in terms of weight loss, respiration rate, cuticle thickness, and re-accommodation of epicuticular waxy layers, of six varieties of cactus pear fruit to postharvest application of different conditions of vapor heat.

Materials and methods

The study was conducted through three experiments. In the first, physiological (weight loss and respiration rate) responses to vapor heat application were observed during and after storage for 15 d at room temperature (approximately 20–22°C and 70–75% RH). In the second experiment, fruit weight loss was evaluated after 30 d in cold storage at 10°C and 95% RH. Finally, in the third experiment, vapor heat treatment was applied, and changes in cuticle thickness and epicuticular wax microstructure were determined directly after treatment without storage.

Plant material

Fruits were obtained from a collection of cactus pear varieties at the experimental cactus pear orchard “Facundo Barrientos Pérez” of the ‘Universidad Autónoma Chapingo’ (Mexico). Six varieties were selected according to postharvest weight loss, including two groups (Corrales *et al.* 2006). Group one included varieties of high weight losses through transpiration, such as Alfajayucan, Amarillo Milpa Alta, and Sangre de Toro. The second group included varieties characterized by low postharvest weight losses, such as, Rojo Pelón, Cristalina, and Rojo 3589. Fruits were manually harvested with knife and gloves, and with a minimum part of the cladode attached to the fruit, taking as much care as possible to avoid causing lesions. Marketing criteria were used for selection in terms of ripening stage (fruits with flat floral cavity) and size (typical for each variety: Alfajayucan, 130 g; Amarillo Milpa Alta, 240 g; Sangre de Toro, 150 g; Rojo Pelón, 150 g; Cristalina, 220 g; and Rojo 3589, 175 g). Removal of the spines was done manually to simulate normal marketing conditions.

Sixty-three fruits of each variety were harvested and divided at random into three groups of 21 fruits, one group for each experiment. The fruits of each group were again divided into seven subgroups of three fruits allotted at random to each of seven treatments.

Treatments

Vapor heat was applied in six treatments derived from the combination of three temperatures (35, 38, and 42±1°C), and two exposure times (12 and 24 h). These temperatures were arbitrarily selected, around the temperature recommended by Schirra *et al.* (1997) for the Gialla variety. Control fruits (seventh treatment) were not treated. In this way, for each of the six varieties studied, seven treatments were applied.

Vapor heat application

A Burrows Equipment™ germinator, model 1000A, with six trays and temperature regulator (20 to 45°C range) was adapted as a vapor (saturated air) generating device. Fruits were introduced into the germinator by groups when it reached a stable the desired temperature and relative humidity. Each group of fruits occupied six trays, one for each variety, and 18 fruits per variety were placed in the trays. Of these, 9 fruits were removed after 12 h of exposure and the remaining were removed after 24 h.

Variables

Weight loss

Each fruit was weighed with an Adam Equipment™, model ADP 2100L, top-loading balance, which has a precision of ±10 mg. Fruits stored for 15 d at room temperature were weighed every 3 d and those stored for 30 d at 10°C were weighed every 5 d. Results were converted into percentages with the equation: %WL=[(Wi-Wf)/Wi] (100), where %WL=percentage weight loss, Wi=initial fruit weight in g, Wf=final fruit weight in g at the indicated period.

Cuticle thickness

Cuticle thickness was determined in cross sections of peel fragments (0.5 X 0.5 cm). The fragments were fixed in a formaldehyde-glacial acetic acid-ethyl alcohol solution (Ruzin, 1999) for 24 h. Later, samples were dehydrated in an automatic changer, Leica TP1020, and embedded in paraffin. Cross-sections, 15 µm thick, were then made with a rotatory microtome, stained with safranin and fast green, and mounted in synthetic resin (Ruzin, 1999). The cuticle thickness was measured using an Olympus microscope adapted with an image-analyzer (Image ProPlus version 3.1, Media Cybernetics 1997); 25 measurements per sample were taken.

Respiration rate

This variable was determined in treated fruits during storage for 15 d at room temperature only. Every 3 d during the storage period fruit respiration rates were measured. Respiration rates were based on the colorimetric method of Claypool and Keefer (1943) modified by Pratt and Mendoza (1979). Transmittance was determined in a spectrophotometer Spectronic 20D at 615 nm; these data were transformed into CO₂ concentration percentages using an equation derived from a previously determined CO₂ standard curve:

$$\text{Log \%CO}_2 = -4.01037 + [0.141937(T)] + [-0.00173(T^2)] + [8.24^{-6}(T^3)]$$

Where T=Fruit transmittance reading

To calculate respiration rate, the value of CO₂ concentration was substituted in the following formula:

$$\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1} = \text{net \%CO}_2 \cdot 10 \text{ (air flow rate / fruit weight),}$$

where air flow is 7.4 Lh⁻¹, and fruit weight is expressed in kg.

Epicuticular wax microstructure

Visual analysis of epicuticular waxy layers was conducted on without spines cactus pear, before (control) and after each vapor heat treatment. Three portions of peel (2 x 2 cm) were cut from each cactus pear and immediately frozen in liquid nitrogen for lyophilization. The lyophilized samples were fractioned into smaller portions approximately 3 x 3 mm and fixed to aluminum specimen holders with double-sided tape and coated with gold in a Hitachi-S-2460N sputter coater prior to observation under a JEOL-JSM-5310LV electron scanning microscope at the "Instituto de Biología de the Universidad Nacional Autónoma de México". Analysis was based on visual examination of microphotographs of the epicuticular surface at two magnifications (125 x and 600 x). Terminology for epicuticular waxes follows Barthlott *et al.* (1998).

Statistical analysis

For the tree experiments and for each variety, variance analysis and the Tukey comparison of means ($\alpha \leq 0.05$) were performed with the Statistical Analysis System (SAS, version 8). A completely random experimental design was used, considering 7 treatments with 3 replicates each.

Results

Weight loss

Table 1(A) shows the effect of vapor heat treatments on fruit weight loss of each of the varieties studied after 15 d of storage at room temperature. The treatments where weight loss was highly reduced, were as follows: 38°C for 12 h in Alfajayucan (61% reduction), 35°C for 12 h and 24 h in Amarillo Milpa Alta (up to 38% and 43%, respectively), 38°C for 24 h in Cristalina and Rojo 3589 (44 and 43% reduction, respectively), 38°C for 12 and 24 h in Rojo Pelón (up to 42% reduction), and 35 or 38°C for 12 and 24 h in Sangre de Toro (up to 51% reduction). The treatments that, instead of decreasing weight loss, resulted in greater weight loss, relative to the control, were 42°C for 12 and 24 h in Amarillo Milpa Alta, Rojo 3589, and Sangre de Toro (48, 44, and 52% higher, respectively); and 42°C for 24 h in Cristalina (47% higher). However, only in the Alfajayucan variety did the treatments 42°C for 12 and 24 h had the opposite effect; that is weight loss was reduced up to 21%, relative to the control. In the Rojo Pelón variety, the treatments had little effect.

In general, except Rojo Pelón variety, it was evident (statistic analysis was not needed) that refrigeration caused an important weight loss reduction, this reduction was lower in the group one varieties (characterized by high postharvest weight losses) than in the second group (characterized by low postharvest weight losses). The reduction in weight loss caused by cold storage for control fruits was as follows for each variety: Alfajayucan (78%), Amarillo Milpa Alta (57%), Cristalina (53%), Rojo 3589 (46%), Sangre de Toro (64%) (Table 1A, B). Similarly, cold storage highly reduced weight loss of the treated fruits.

After cold storage for 30 d at 10°C, the reduction of weight loss, of the best treatment, was as follows for each variety: Alfajayucan exposed at 38°C for 12 h (40% reduction), Rojo Pelón Rojo Pelón exposed at 38°C for 12 h (up to 41% reduction), Cristalina exposed at 38°C for 12 h (up to 58% reduction). None of the treatments reduced weight loss (relative to the control) in the varieties Amarillo Milpa Alta, Rojo 3589 and Sangre de Toro. The treatments at 42°C for 12 and 24 h were those with which weight loss was the highest in refrigerated fruits of the varieties Alfajayucan, Amarillo Milpa Alta, Rojo 3589, and Sangre de Toro (up to 60, 127, 147 and 200% greater than the control, respectively). At 38°C for 24 h Amarillo Milpa Alta also exhibited greater weight loss. All treatments increased weight loss in Rojo 3589 variety.

Table 1. Weight loss (%) in six varieties of cactus pear fruits treated with different combinations of temperature and exposure time to vapor heat after 15 days of storage at 20–22°C and 70–75% RH (A) or 30 days at 10°C and 95% RH (B).

Treatment	Alfajayucan	Amarillo Milpa Alta	Cristalina	Rojo 3589	Rojo Pelón	Sangre de Toro
(A)						
Control	10.91a	10.25b	4.76c	6.96c	4.06a	8.69c
35°Cx12h	7.74c	6.53c	4.04d	4.63d	2.77c	5.38d
35°Cx24h	7.55c	6.32c	3.65d	7.31c	3.31b	4.26d
38°Cx12h	4.19e	9.32b	4.03d	7.71c	2.32c	4.82d
38°Cx24h	5.31d	9.31b	2.63e	3.91d	2.48c	4.23d
42°Cx12h	8.52bc	13.82a	5.47b	8.83b	3.83a	11.48b
42°Cx24h	9.48b	15.22a	7.02a	10.07a	4.17a	13.22a
(B)						
Control	2.33b	3.96cd	2.20b	3.73d	3.86a	3.05cd
35°Cx12h	2.22b	3.41d	1.95b	6.06c	2.82bc	2.97cd
35°Cx24h	2.10b	3.23d	1.43c	6.80bc	3.24ab	2.53d
38°Cx12h	1.40c	3.48d	0.92d	5.90c	2.27c	3.56c
38°Cx24h	1.53c	5.06c	1.07cd	6.01c	2.79bc	2.42d
42°Cx12h	3.31a	7.33b	2.26b	7.51b	3.64a	6.06b
42°Cx24h	3.75a	9.02a	3.25a	9.22a	3.72a	9.20a

For each storage period and variety, means with the same letter are statistically not different (Tukey; $\alpha=0.05$).

Respiration rate

During 15 d of storage at room temperature, respiration rates of the varieties Rojo 3589, Rojo Pelón and Alfajayucan (22.5, 22.0 and 31.1 mL CO₂ kg⁻¹h⁻¹, respectively) were not affected by the treatments. Respiration rate of Amarillo Milpa Alta variety was higher with the treatment of 38°C for 12 h than with the treatments of 35°C for 12 h, 38°C for 24 h or 42°C for 24 h. Respiration rate of Cristalina was higher with the treatments 42 °C for 12 and 24 h than with 35 °C for 24 h. For Sangre de Toro the only treatment that increased the mean respiration rate, relative to the control, was the treatment 42 °C for 24 h (Table 2).

Cuticle thickness

Table 3 shows that cuticle thickness of the varieties Alfajayucan, Amarillo Milpa Alta, and Cristalina increased 30 to 47%, relative to the control, with the treatments 35°C for 12 and 24 h and 38°C for 12 h. However, in the variety Rojo 3589, all of the treatments reduced cuticle thickness, up to 44.7%, in the variety Rojo Pelón, the cuticle thickness was reduced by treatments 42°C for 12 and 24 h (21.35 and 16%) and increased by the treatment 38°C for 24 h (13.43%), while in Sangre de Toro cuticle thickness was not affected by any of the treatments.

Epicuticular structure

Treatments had a differential effect on the rearrangement of epicuticular wax layers among varieties (Figure 1 and 2). Comparing the SEM photographs of the control, most varieties showed differences in layer appearance, fissured or smooth, as well as in the size of wax crusts, while SEM

photographs of the treatments showed a rearrangement of those epicuticular wax layers, particularly in Rojo 3589, Rojo Pelón, and Sangre de Toro, varieties in which the rearrangement of the large fissured layers caused the least weight loss (Figure 2).

Table 2. Effect of different vapor heat treatment on respiration rate ($\text{mLCO}_2 \text{kg}^{-1}\text{h}^{-1}$) of six cactus pear fruit varieties during 15 d of storage at 20–22°C and 70–75% RH.

Treatment	Alfajayucan	Amarillo Milpa Alta	Cristalina	Rojo 3589	Rojo Pelón	Sangre de Toro
Control	31.15	27.60ab	24.26ab	22.48	21.97	31.29b
35°C X 12h	30.24	26.71b	24.18ab	21.19	21.40	31.80ab
35°C X 24h	30.30	28.74ab	22.39b	21.43	21.81	31.49b
38°C X 12h	31.03	31.11a	23.18ab	19.83	21.14	31.94ab
38°C X 24h	31.08	27.07b	25.00ab	21.12	21.22	31.17b
42°C X 12h	32.92	28.19ab	26.16a	22.18	22.81	32.50ab
42°C X 24h	31.07	25.76b	26.78a	22.40	22.82	35.14a

For each variety, means with the same letter are statistically not different (Tukey; $\alpha=0.05$). Means without letters are statistically not different.

Table 3. Cuticle thickness (μm) of six varieties of cactus pear fruit after vapor heat treatment.

Treatment	Alfajayucan	Amarillo Milpa Alta	Cristalina	Rojo 3589	Rojo Pelón	Sangre de Toro
Control	6.29cd	5.23c	7.62b	10.37a	7.54bc	6.97ab
35°Cx12h	6.68bc	6.54b	9.93a	7.56b	7.75ab	6.27ab
35°Cx24h	7.40b	8.34a	7.27bc	6.65c	6.56cd	8.08a
38°Cx12h	9.30a	7.78a	6.41cd	6.24bc	7.92ab	6.33ab
38°Cx24h	5.40c	5.79bc	7.60b	7.67b	8.71a	7.39ab
42°Cx12h	6.29cd	5.37c	6.74bcd	6.47c	5.93d	6.31ab
42°Cx24h	5.83cd	6.06bc	6.01d	5.73c	6.33d	5.72b

For each variety, means with the same letter are statistically not different (Tukey; $\alpha=0.05$).

Discussion

Weight loss depends on water loss mainly and it is important because it affects the visual appearance and texture of the fruits and causes a reduction in saleable weight. Generally, products must lose about 5 % of their fresh weight before visual appearance is affected (Kader, 2002). However, for cactus fruits, a weight loss of 8 % was necessary to affect their visual appearance (Rodríguez-Félix *et al.*, 1992). In this study, application of vapor heat reduced weight loss in all six varieties of cactus pear fruits stored at room temperature. Each variety had a reduction in weight loss that was different from the response to its best treatment, varying from 38 % in Amarillo Milpa Alta to 61 % in Alfajayucan. The best treatments for the varieties Alfajayucan, Rojo 3589 and Sangre de Toro induced weight loss that was lower than that considered by Rodríguez-Félix *et al.* (1992) as commercially tolerable threshold (5%). With Cristalina and Rojo Pelón the reduction in weight loss was not as notable because these varieties normally lose less weight, and their control fruits did not surpass the commercially tolerable threshold, a result that agrees with Corrales *et al.*

(2006). These results suggest that the amount and chemical nature of the epicuticular wax is probably different in each variety since the highest reduction in weight loss did not occur with the same treatment, this suggest that partial fusion and rearrangement occurred under different temperature conditions and exposure time in each variety. These results suggest the need to determine the amount and chemical nature of the epicuticular wax in future studies.

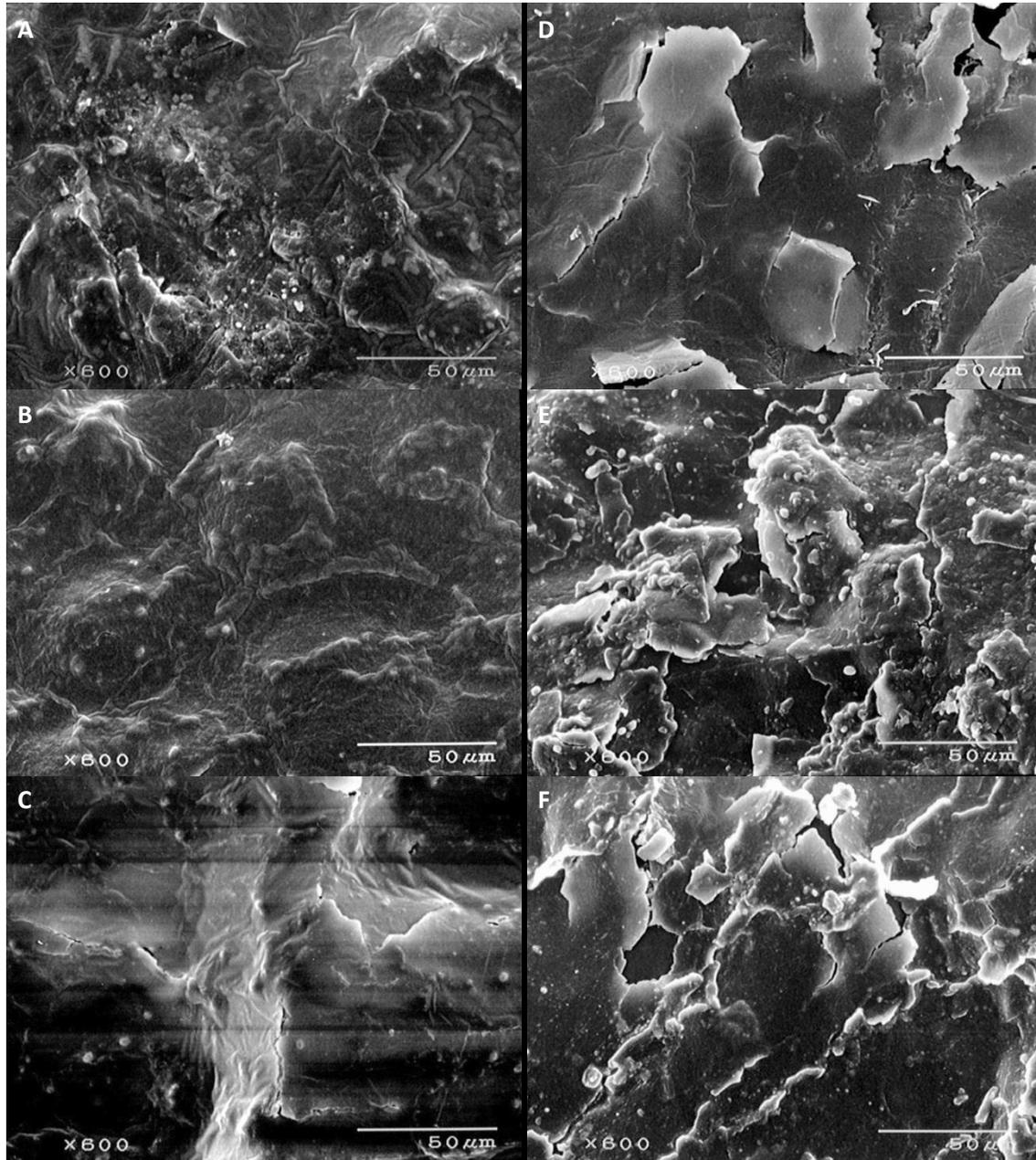


Figure 1. Effect of the best vapor heat treatment on weight loss reduction on the epicuticular waxes of non-refrigerated cactus pear fruit of the varieties: A) Alfajayucan (38°C for 12 h); B) Amarillo Milpa Alta (35°C for 24 h); and C) Cristalina at 38°C for 24 h. On the right of each microphotograph the corresponding control (D, E, F) is shown.

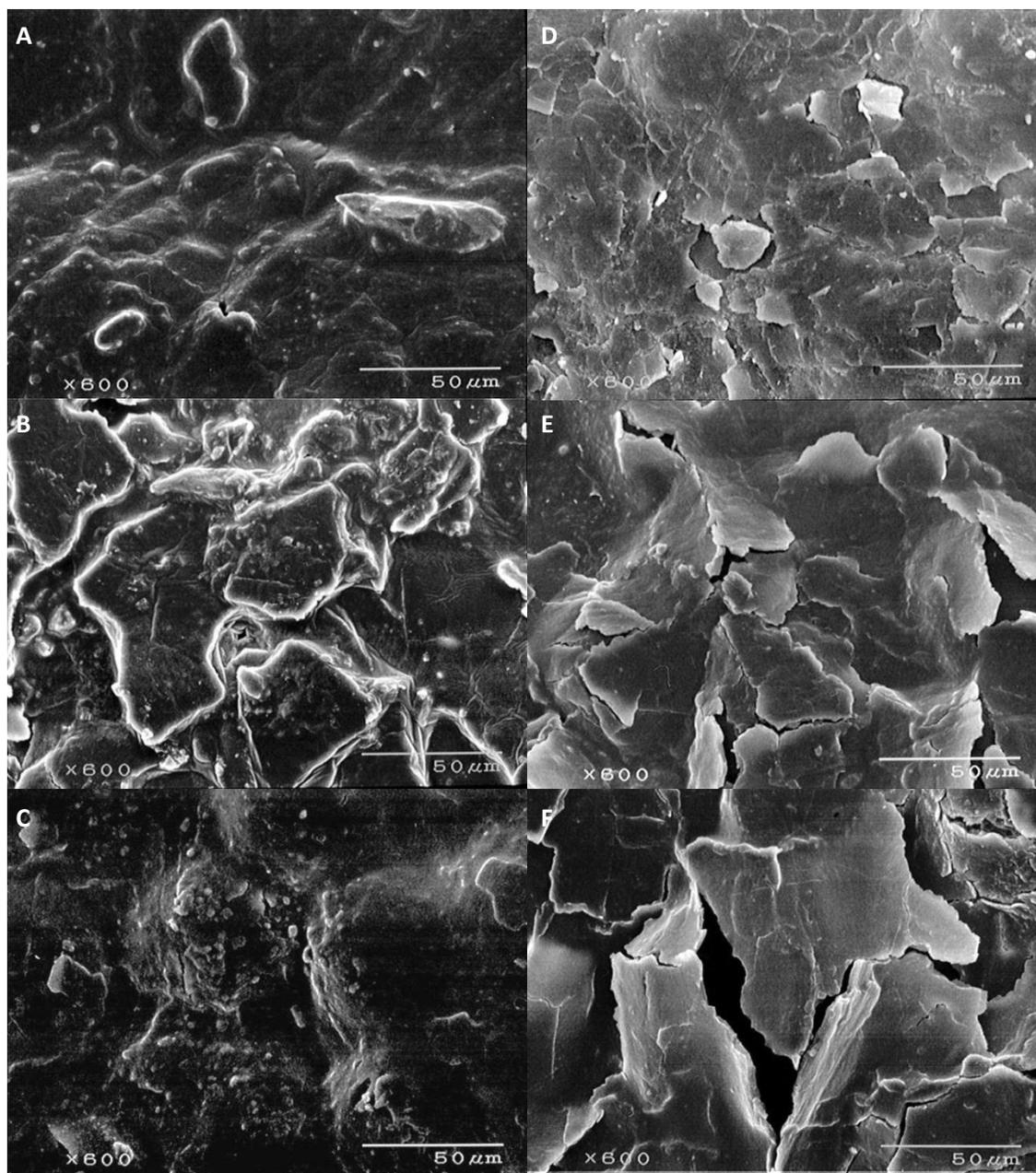


Figure 2. Effect of the best vapor heat treatment on weight loss reduction on the epicuticular waxes of non-refrigerated cactus pear fruit of the varieties: (A) Rojo 3589 (38°C for 24 h), (B) Rojo Pelón (38°C for 12 h), and (C) Sangre de Toro (38°C for 24 h). On the right of each microphotograph the corresponding control (D, E, F) is shown.

The treatments that most reduced weight loss of non-refrigerated fruits are not necessarily the best treatments for refrigerated fruits. Only in the varieties Alfajayucan and Rojo Pelón did the vapor heat treatment at 38°C for 12 h coincide. It is notable that the untreated fruits (controls) had higher reductions in weight loss only under refrigeration. However, this effect is highly variable among varieties. For example, Alfajayucan, Amarillo Milpa Alta and Sangre de Toro were outstanding for

the smallest weight loss. This variability in response to refrigeration among varieties was also observed by Corrales *et al.* (1997). Under refrigeration, reductions in weight loss associated to the best treatments were not as large as those observed in unrefrigerated fruits.

The varieties that best responded to vapor heat in terms of reducing weight loss were Alfajayucan, Amarillo Milpa Alta, and Sangre de Toro. Therefore, the greatest commercial potential for this treatment corresponds to these varieties. Although, there may be other varieties with the same or higher potential, therefore it will be necessary to study their behavior in future studies.

The varieties Cristalina and Rojo Pelón were outstanding for their small weight loss under both storage conditions. This was previously observed in Cristalina by Corrales *et al.* (1997). For this reason, for these varieties vapor heat may be applicable only for its possible effect in reducing chilling injury, as suggested by Schirra *et al.* (1996, 1997, 1999), rather than in reducing weight loss.

Results on respiration agree with Cantwell (1991) and Corrales *et al.* (1997) who observed low metabolic activity in cactus pear fruit. In general, vapor heat treatments did not increase respiration rate, this suggest that metabolic activity in the fruit of the cactus pear varieties studied was not significantly affected by vapor heat treatments proved.

No pattern of change in cuticle thickness was observed in response to vapor heat application. The reduction in cuticle thickness on some varieties, relative to the control fruits, was probably due to the effect of the heat treatment. D'Hallewin *et al.* (1999) observed that, when vapor heat is applied, the epicuticular wax plates on cactus pear fruits flatten. On the other hand, the increase in cuticle thickness of the varieties Alfajayucan, Amarillo Milpa Alta, and Cristalina would not be explained by wax deposition by the treatment, but rather it is likely that, under these conditions (temperature and exposure time) during the vapor heat treatment, water vapor temporarily filled the spaces between the epicuticular wax plates and increased the volume. As can be seen, the results referring to cuticle thickness are contrasting, but this characteristic does not totally determine cactus pear shelf life. According to Knoche *et al.* (2004) and Rogiers *et al.* (2004) the resistance provided by the cuticle against mechanical damage, gas diffusion, changes in water permeability, and microorganisms penetration does not depend specifically on thickness, but on changes in its structure and the proportions in which its components are found.

The notably fissured layers and crusts of epicuticular wax seen in the microphotographs of control fruits are considered normal because they are characteristic of succulent fruits (Barthlott, 1998). In this respect, Wiedermann and Neinhuis (1998) pointed out that epicuticular waxes accumulate during fruit growth as flat plates that later become hard and brittle. During ripening they break and form laminar masses. Cracking (fissures) decrease the effectiveness of the structure as a protective barrier. Moreover, the epicuticular wax layers were smooth in the fruits of those treatments that resulted in less weight loss, while the epicuticular wax layers of control fruits had large fissures (Figure 2). This suggests that vapor heat probably caused a partial phase change in the wax, which melted and filled in the fissures and cavities present before treatment, thus reordering and smoothing out the layers. This partially explains the lower weight loss caused by the treatment. The same effect of smoothing the wax layers was observed by Schirra *et al.* (1997, 1999) and D'Hallewin *et al.* (1999) in Gialla variety cactus pear fruits. In addition, wax content has been associated with regulation of water lost in vapor form, and differences among species have been demonstrated, for example, Knoche *et al.* (2004) correlated the fissures in the cuticle with moisture loss and physiological disorders in cherries. The number of cracks formed during ripening has also been related to susceptibility of grapes to *Botrytis cinerea* (Commenil *et al.*, 1997). In mangoes

similar relationships between fissures and pathogen susceptibility have been found (Petit *et al.*, 2007).

In the varieties studied the highest temperature treatments (42°C) for 12 and 24 h also caused wax layers to become smooth (figures not shown). However, as discussed above, these treatments were not the best conditions to reduce weight loss and prolong shelf life. This is likely due to the high temperature, which melted the wax to such an extent that it ran off and was lost and caused major injuries to fruit tissues.

Varieties respond different in terms of weight loss, and the best vapor heat treatment condition should be determined for each variety to reduce weight loss and prolong shelf life. There are also likely differences among varieties in terms of the best treatment for reducing susceptibility to chilling injury, which should be studied in future works.

Conclusions

The results of this study show that vapor heat treatments reduced weight loss, but the degree of reduction depends on the variety. For fruits stored during 15 d at room temperature, the treatments in which weight loss was lowest were 35°C for 12 h with the varieties Amarillo Milpa Alta, Rojo 3589, Rojo Pelón and Sangre de Toro; 35°C for 24 h with the varieties Amarillo Milpa Alta and Sangre de Toro; 38°C for 12 h with Alfajayucan, Rojo Pelón, and Sangre de Toro; 38°C for 24 h with Cristalina, Rojo 3589, Rojo Pelón and Sangre de Toro. For stored fruits during 30 d at 10 °C, the treatments causing lower weight loss were: 38°C for 12 h or 24 h with the variety Alfajayucan; 38°C for 12 h with the varieties Cristalina and Rojo Pelón. In general, treatments with vapor heat had little effect on respiration rate and cuticle thickness of fruits. Microphotographs of non-treated (controls) fruits of the six cactus pear varieties revealed epicuticular wax with smooth and fissured layers. Vapor heat caused rearrangement of these layers, minimizing or eliminating fissures. This rearrangement can partially explain the positive effect on weight loss reduction.

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