Effect of Different Treatments on the Evolution of Polyamines during Refrigerated Storage of Eggplants

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The applicability of a thermal treatment was compared with modified-atmosphere (MA) storage in relation to chilling injury (CI) and polyamines evolution in eggplants. Fruits underwent physiological disorders at 3 °C, evidenced by the appearance of surface injuries at the third day of storage, and, after moving the fruits to 20 °C, by increased respiratory activity and more intense ethylene production. Storage of fruits in sealed low-density polyethylene bags and a previous treatment with heated air (1 h at 35 °C) were both effective in retarding chilling injury, though the former was better. Two free polyamines were found in cv. Black Nite: putrescine, in greater proportion, and spermidine. Putrescine increased in control (untreated) fruits stored at 3 °C in parallel with the external appearance of chilling injury, whereas this increase was either not exhibited or retarded in treated or MA stored fruits. Spermidine did not change in control fruits at 3 °C, remaining almost constant over the whole storage period, whereas in heat- and MAP-treated fruits spermidine levels exhibited a decrease.

Keywords: *Polyamines; eggplant fruit; chilling injury; heat treatment; modified storage*

INTRODUCTION

Refrigerated storage is an effective method to preserve the quality of fruits and vegetables. However, some products of tropical origin, such as eggplants, are sensitive to chilling injury (CI) when stored at temperatures under their critical value. Below 12 °C, eggplants suffer physiological disorders, manifested mainly by appearance of surface injuries, such as pitting, and seed darkening.

Biochemical and physiological changes in plant tissues, such as increases in polyamine levels, are caused by environmental factors such as nutritional stress, oxygen deficiency, salinity, low pH, magnesium and potassium deficiency, and exposure to low temperatures (*1*).

Several stress-inducing conditions were related to putrescine. Flores and Galston (*2*) found putrescine build-up in cereal leaf tissues exposed to osmotic shock; Wang and Steffens (*3*) encountered putrescine in apple tree leaves under hydric stress; and McDonald and Kushad (*4*) observed it in cold-damaged lemons, peppers, and grapefruit. In this regard, Slocum et al. (*5*) have suggested that putrescine accumulation could cause the damage induced by abiotic stress.

One of the goals in studying CI suffered by some vegetable produce is to find effective methods for eliminating or retarding it (*6*). Nakamura et al. (*7*) reported that eggplant conditioning at two temperatures, 15 °C for 1 or 2 days, and 10 °C for 1 day, before storage at 6.5 °C retarded the appearance of CI symptoms.

Fallik et al. (*8*) indicated that refrigerated storage of eggplants can be prolonged by placing the product in sealed bags, thereby reducing or retarding CI. This conditioning technique also allowed CI to be retarded in other fruits such as tomatoes (*9*), melons (*10*), and red (*11*) and green peppers (*12*).

According to Woolf et al. (*13*), heat treatments are receiving more attention as a way to reduce CI in some fruits. Heated-air or hot-water treatments were reported to reduce CI in mangoes (*14*), tomatoes (*15*−*17*), oranges (*18*−*20*), persimmons (*21*−*23*), and zucchini (*6*).

The literature offers no antecedents of studies dealing with polyamines in eggplant fruits, nor on their evolution during cold storage. No previous work on the application of heat treatments aimed at reducing CI was found either.

This work studies the applicability of a thermal treatment, compared with storage in modified atmosphere (MAP), on the retardation of CI and polyamine evolution in eggplants.

MATERIALS AND METHODS

Plant Material. Eggplant fruits (*Solanum melongena)* cv. Black Nite were used in this work. Fruits were provided by a farm at Colonia María Luisa, Departamento Banda, Province of Santiago del Estero, Argentina. Eggplants were harvested manually, early in the mornings, at commercial ripening stage (500 cm^3 volume). Fruits were chosen according to shape, size, and absence of defects, then washed with water and drained.

Treatments and Storage Conditions. Four different treatments were applied to retard or diminish chilling injury in eggplants.

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Table 1. Chilling Injury Scale as Determined by External and Internal Visual Symptoms in Eggplants Stored at 3 °C

n_i	extent of damage	symptoms
1	none	not visible
2	incipient	green calix with few surface injuries; incipient pitting
3	moderate	medium-sized surface injuries (brown-colored scalded zones with surface depressions); slight flesh darkening; damaged fruits
4	severe	dark-brown, large surface injuries, extended over not more than a third of fruit surface; moderate flesh darkening; damaged fruits, not suitable for trade
5	very severe	large necrotic surfaces; flesh and seed darkening; aqueous appearance of eggplants; severe fruit damage

Hot Water Treatment. 30 °C for 1h; 35 °C for 1 h; 40 °C for 1, 2, and 3 h; 45 °C for 30 min, 1 h, and 2 h.

Dry Heated-Air Treatment. 20 °C for 24 and 48 h; 30 °C for 24 h; 35 °C for 30 min and 1 h; 40 °C for 30 min and 1 h; and 45° for 30 min and 1 h.

Rich Carbon Dioxide Atmosphere. 20% CO_2, 16% O_2 (completed with nitrogen) for 24 h.

Modified Atmosphere (MAP). Fruits were individually packed in low-density polyethylene (LDPE) sealed bags.

After treatments, 1 to 3 fruits were placed individually in perforated LPDE bags (192 perforations/m^2, 13 mm diameter each). In a first experiment, all fruits were stored at 3 °C for 9 days, after which the best treatments were selected. Then, fruits treated with selected treatments, conditioning as indicated, were stored at 3 °C for 18 days. After 3, 6, 9, 12, and 18 storage days, five fruits of each treatment were analyzed immediately after being removed from the cold storage, whereas five other fruits were transferred to a 20 °C room and kept for 48 h before analysis. Eggplants placed in perforated bags and stored at 3 °C were employed as controls. Fruits placed in perforated bags and kept at 20 °C were also used as controls.

Quality Evolution. Fruits were evaluated upon the characteristic symptoms of cold-induced physiological disturbance, using a numerical scale from 1 to 5, as indicated in Table 1. The chilling injury index (CI) was calculated as

$$\mathrm{CI} = \frac{\sum(n_i \times i)}{N}$$

where, for each treatment and storage time, n_i was the number of fruits receiving the mark "*i*" (from 1 to 5) and *N* was the total number of fruits. Values above 2.5 are representative of fruits with considerable damage, not suitable for trade.

Determination of Respiratory Activity and Ethylene Production. Carbon dioxide and ethylene productions were determined as described elsewhere (*24*).

Extraction and Determination of Polyamines. Five fruits were selected, and in each the samples were prepared by cutting a 1-cm thick slice from the widest part of each fruit. Each slice was divided radially into four portions, taking two opposite portions for analysis. Tissue (20 g) was taken from the eggplant portions, finely homogenized for 2 min with a solution of 0.5% perchloric acid in a 1:1 w/v ratio, kept for 1 h under refrigeration with periodic stirring, and centrifuged at 5000*g* for 8 min. The supernatant, containing free polyamines, was placed in plastic jars and kept in a freezer at −20 °C until used.

Free polyamines were benzoylated as described by Kushad et al. (*25*) with some modifications. Samples (500 μL) were taken for polyamine analysis and mixed with 20 μL of a 1 mM solution of 1,6 hexane diamine as internal standard, 500 μL of 2 M NaOH, and 10 μL of benzoyl chloride. The mixture was stirred in a vortex for 15 s and allowed to rest for 20 min at room temperature. Subsequent to this, 2 mL of saturated sodium chloride were added, and the system was stirred while 2 mL of diethyl ether was added. The system was left at rest for 30 min at −20 °C. The diethyl ether phase was extracted and evaporated under nitrogen. The residue was resuspended in 500 μL of methanol/water (55:45).

The polyamines present were analyzed with a Waters HPLC equipped with a UVdetector (254 nm) and a reversed-phase column (Beckman, Ultrasphere ODS 5 μm, 4.6 mm × 25 cm) at 30 °C. A mixture of 55:45 methanol/water was used as elution solvent, with a flow rate of 1 mL/min. The injection volume used was 20 μL. Polyamines were identified using 1 mM standard solutions of putrescine, spermidine, spermine, cadaverine, and agmatine and were quantified using the internal standard method.

Experimental Design. A factorial design was employed, defining selected treatments (3−4 levels) and storage times (5−9 levels) as factors. Determinations (at each factor combination) were realized in triplicate on a pool of five fruits. Six replications were carried out. Data were processed by analysis of variance (ANOVA), and the means were compared by the LSD test using a significance level of 0.05 (SYSTAT, Inc.).

RESULTS AND DISCUSSION

To select the best treatment to retard or diminish chilling injury in eggplants, different treatments were applied: hot-water treatment, dry heated-air treatment, rich carbon dioxide atmospheres applied for 24 h (20% CO_2, 16% O_2, completed with nitrogen), and passively modified atmosphere.

Results obtained for visual symptoms of CI in fruits stored 9 days at 3 °C plus 48 h at 20 °C with different treatments are shown in Figure 1. CI values demonstrated that hot water and rich CO_2 treatments failed to retain the quality characteristics of the fruits (Figure 1). In the case of dry air treatments, the treatment applied at 35 °C for 1 h was the only one that had success in retarding CI symptoms (Figure 1). Similar results were obtained for MAP storage. Thus, these last two treatments were selected for long storage effect evaluation.

Figure 2 presents the evolution of the CI index as a function of storage time, under the selected conditions of the test. During storage at 3 °C, the control fruits conditioned in perforated bags showed incipient visible symptoms of chilling injury at the third day (CI = 2.3, Table 1). Physiological disorders manifested externally by the appearance of pitting in the smooth eggplant surface. This damage soon became more evident during refrigerated storage, with flesh browning and seed darkening after 6 days, the time at which the CI index surpassed 2.5, the limit previously established as a limit for trade.

Fruits treated in air at 35 °C for 1 h before refrigerated storage began to show external damage plus flesh and seed darkening at day 9. This damage was incipient (CI = 1.7), and they were still fit for trade. Such fruits surpassed the limiting CI index value of 2.5 at day 12.

The MAP stored fruits presented an index of 1.3 at 9 days and of 2.2 at 12 days. Up to that time, the external damage appeared as a slight surface alteration caused by pitting. Although the fruits stored in these conditions were those that best conserved the original external characteristics, they began to show incipient damage of flesh darkening from day 9. As shown in Figure 2, these fruits did not reach the limiting value of 2.5, even after 12 days at 3 °C.

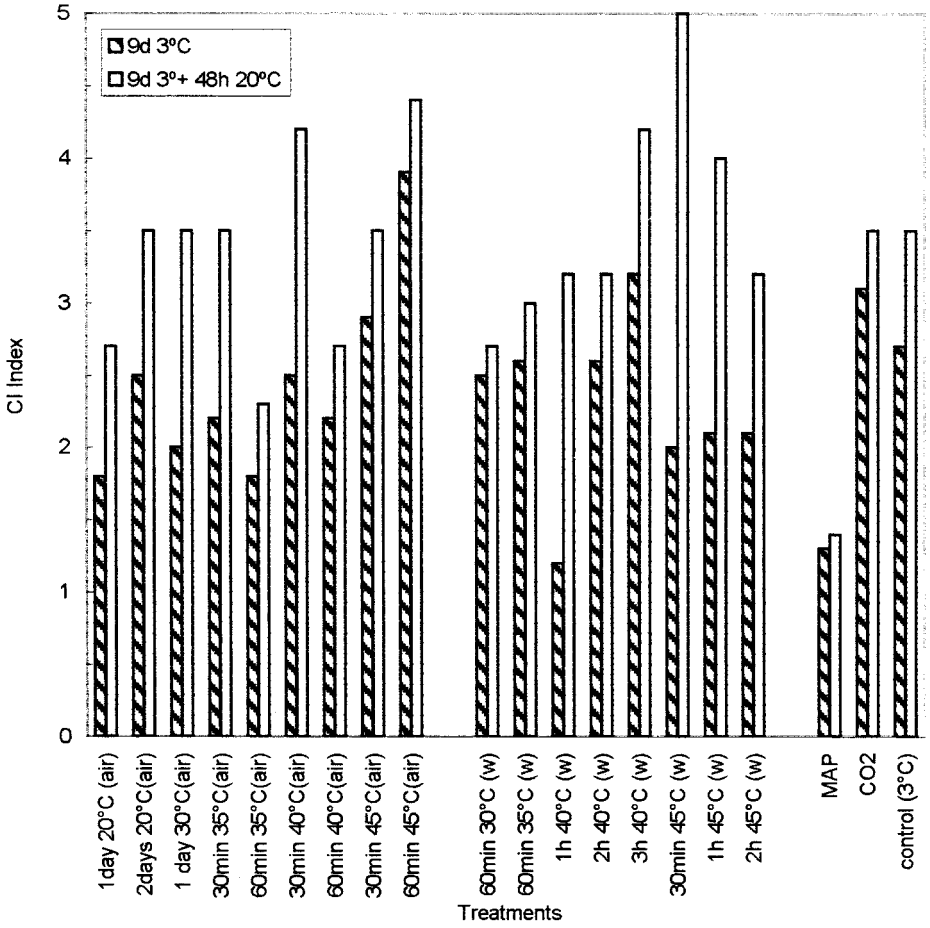

Figure 1. CI index of eggplant fruits after 9 days of storage at 3 °C (striped bar) and after 9 days at 3 °C plus 48 h at 20 °C (white bar), with indicated treatments.

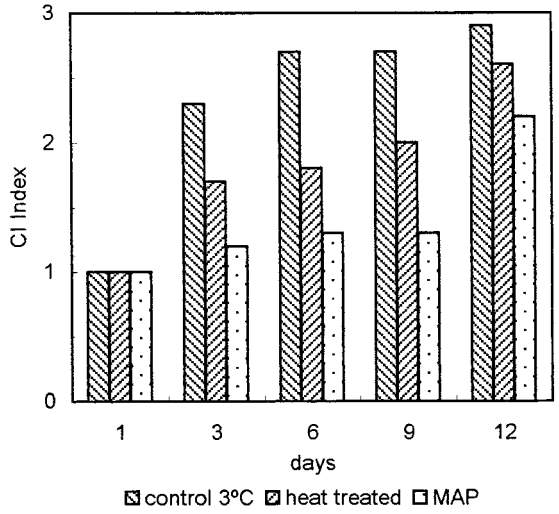

Figure 2. Evolution of CI index of eggplant fruits stored at 3 °C with different treatments: control 3 °C , left bar; heat-treated, middle bar; MAP, right bar. $LSD_{0.05} = 0.32$.

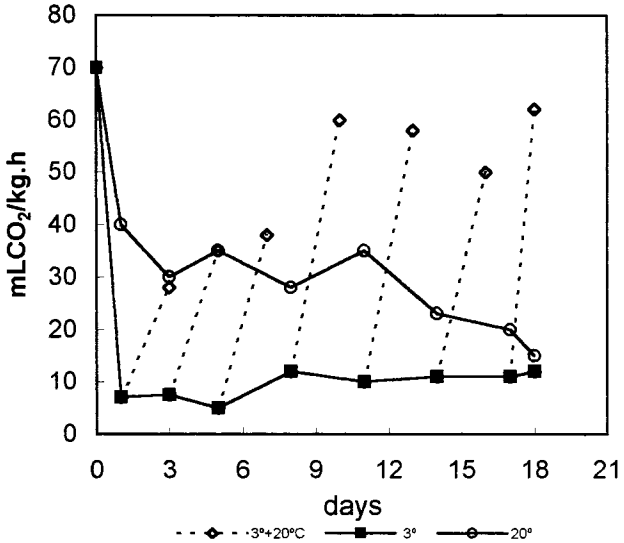

Figure 3. Changes in CO_2 production (mL/kg·h) during refrigerated storage of eggplant fruits at 3 °C (■), and after transfer at 20 °C for 48 h (◇), and in fruits kept at 20 °C (control) (○). $LSD_{0.05} = 10.8$.

The fruits kept at 20 °C were not suitable for trade after 6 days because of the marked damage signs they presented due to senescence (data not shown).

In fruits stored at 20 °C, carbon dioxide production decreased from 70 to 30 mL/kg·h over the first 3 days to remain around that value up to day 11, from which it decreased slowly to about 15 mL/kg·h, as indicated in Figure 3.

Figure 3 also shows that, at 3 °C, the respiration rate decreased to 8 mL/kg·h during the first storage day, keeping that value almost constant ($P > 0.05$) up to the end of the treatment.

Concerning the samples stored at 3 °C and moved to 20 °C, the respiratory intensity increased markedly for fruits transferred at or after 5 storage days, and the values reached were higher than in fruits stored at 20 °C during the same period (Figure 3). In this regard, the evidence indicated that the more damaged the fruit

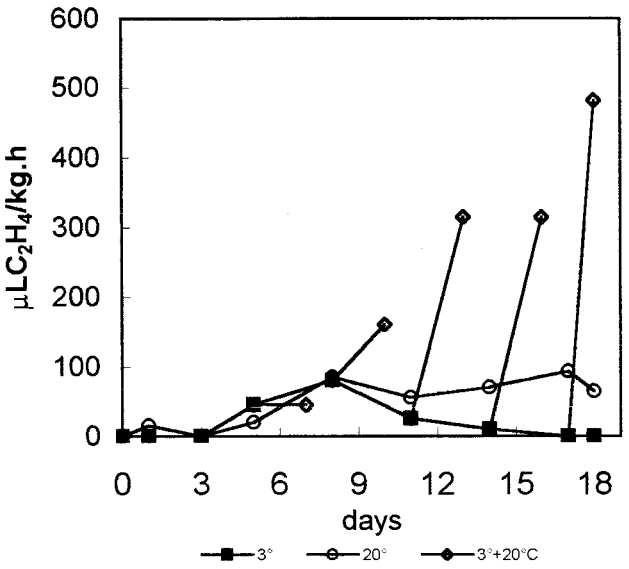

Figure 4. Variation in ethylene production during storage of eggplant fruits at 3 °C (■), and after transfer at 20 °C for 48 h (◊), and in fruits kept at 20 °C (control) (○). $LSD_{0.05} = 37.7$.

was, the stronger the increase in respiratory rate. These results agree with those of Saltveit and Morris (*26*) and Wang (*6*) who have reported that in fruits and vegetables stored at temperatures low enough to produce chilling injury, the respiratory rate increased rapidly after transferring the fruits to room temperature, and that this increase was related to the extent of chilling injury. Sigrist (*27*) found the same behavior in eggplants.

Figure 4 shows the evolution of ethylene with time at 3 and 20 °C. For both temperatures, ethylene production increased gradually from undetectable values to about 85 μL/kg·h which was reached after 8 storage days. From then on, this value was almost constant at 20 °C ($P < 0.05$) up to the end of storage, whereas at 3 °C ethylene production decreased slowly to reach again the initial very low values, after 14 days. These low values were then kept until the end of the storage period. The ethylene production decrease observed in fruits stored at 3 °C could be related to CI damage extent.

Concerning the transfer of refrigerated fruits to 20 °C, ethylene production rapidly increased in fruits previously kept at 3 °C for 8 days ($P < 0.05$), reaching values from 100 to 300 times as high as those measured at the cold storage outlet. According to Figure 4, these values were also much higher ($P > 0.05$) than those exhibited by fruits stored at 20 °C for the same period. These values agree with those found by Sigrist (*27*) in eggplants, because, as in the case of respiratory rate, longer storage periods at temperatures low enough to produce chilling injury led to higher ethylene production after moving the fruits to temperate conditions.

Polyamines. Two free polyamines were found in eggplants: putrescine and spermidine. Other polyamines such as spermine, cadaverine, and agmatine were not detected.

Putrescine was found in greater proportion than spermidine. Freshly harvested fruits had a putrescine concentration of about 100 nmol/g fresh tissue and a spermidine concentration of about 75 nmol/g.

Putrescine concentration remained nearly constant ($P > 0.05$) over the entire storage time at 20 °C, as shown in Figure 5, where concentration results were related to the initial value. In contrast, at 3 °C putrescine concentration quickly increased from storage day 3 to reach, after 18 days, values that were 4 to 6 times as high as the initial value. McDonald and Kushad (*4*), Young and Galston (*28*), Guye et al. (*29*), and Slocum and Galston (*30*) have reported sharp increases of free polyamine concentration in plant tissues subjected to different stresses such as acidic treatment, osmotic shock, and hydric stress. This increase of putrescine observed from day 3 of storage coincides with the onset of external appearance of chilling injury, as indicated in Figure 2. This is in agreement with Galston (*31*) who has found, for lemons, a linear relationship between the extent of chilling injury and putrescine levels.

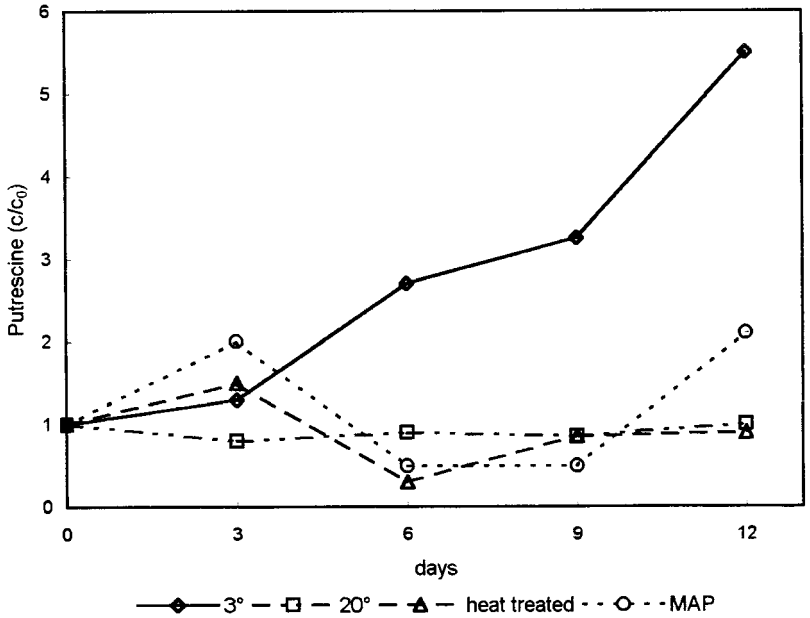

Figure 5. Evolution of putrescine during storage of eggplant fruits at 3 °C (◊), with different treatments (heat (△); MAP (○)), and fruits stored at 20 °C (□). $LSD_{0.05} = 1.0$.

Table 2. Spermidine Concentration (nmol/g of fresh tissue) in Eggplants at Different Times during Storage

time (days)	stored at 20 °C	stored at 3 °C	treated in heated air (1 h, 35 °C)	MAP (3 °C)
0	75.0	75.0	75.0	75.0
3	74.3	61.5	41.5	90.0
6	63.0	44.3	34.5	25.0
9	72.7	44.3	20.0	25.0
12	82.0	70.0	32.0	65.0

In MAP stored fruits, the putrescine content remained approximately constant until day 9, to increase again rapidly up to 200 nmol/g at day 12 of storage (Figure 5).

Concerning the fruits previously treated at 35 °C (heat treated), their putrescine concentration began to decrease from a value of 135 nmol/g measured at day 3 of storage, to about 30 nmol/g at day 6; this last value remained then mostly constant until the end of storage. In both the heat-treated fruits and those stored in MAP, putrescine concentration stayed below that of fruits stored at 3 °C, with the difference being significant after 6 days ($P < 0.05$). These results are in agreement with those found by Serrano et al. (*32*) for CO_2-pretreated zucchini squash, where the chilling injury was reduced, showing a lower increase in putrescine levels.

Table 2 presents the evolution of spermidine concentration as a function of storage time, and it can be observed that levels stayed substantially constant at both 20 and 3 °C, with no significant differences ($P < 0.05$) between the two temperatures. Our results agree with a report by McDonald and Kushad (*4*) who did not find any significant relationship between spermine or spermidine levels and chilling injury in lemons. In unripe peaches stored at 1 and 5 °C, Valero et al. (*33*) found a decrease of spermidine followed by an increase of its level, though the contents measured at the end of storage were still lower than the initial values. Serrano et al. (*32*), working on zucchini squash, also reported a decrease of spermidine and increase of putrescine with chilling injury.

For the fruits treated in air at 35 °C, Table 2 also shows a continuous decrease in spermidine concentration from the first day of refrigerated storage, to reach a value of about 20−32 nmol/g at the end of the testing period. Spermidine concentration was always lower than that in the fruits stored at 3 °C, but it became significantly different ($P < 0.05$) from it only after 12 days.

Fruits stored in sealed bags exhibited a rapid decrease of spermidine concentration from day 3, reaching about 25 nmol/g at day 6, remaining approximately constant from then until storage day 9. Subsequently, its concentration increased until reaching values like that observed at the beginning of the storage. No significant differences ($P > 0.05$) were found between fruits stored in sealed bags and control fruits stored at 3 °C, nor with those treated in heated air.

According to our results, the chilling injury symptoms presented by eggplants, after 12 storage days, ranged from incipient to moderate in fruits stored at 3 °C in sealed bags (MAP). Within this period, putrescine levels did not increase up to day 9, whereas spermidine concentration decreased to lowest level at day 9 and then increased to reach, after 12 days, values like that of freshly harvested fruits.

Guye et al. (*29*) have observed that plants respond to diverse stresses by inducing putrescine synthesis. McDonald and Kushad (*4*) reported that the low-temperature stress could affect putrescine levels in fruits sensitive to chilling injury. Slocum et al. (*5*) also suggested that putrescine build-up could be a cause of the stress-induced damage, in this case low-temperature stress. This agrees with our results, as putrescine levels manifested rapidly in fruits stored at 3 °C in parallel to the increase of chilling injury. In treated fruits, putrescine levels did not increase, or did so very slightly, according to whether the fruits were treated in air at 35 °C or stored in MAP, respectively. Chilling injury was significantly lower in these fruits: apparently CI was retarded during storage. Thus, the increase in putrescine levels found in chilling injured fruits could be a response of fruit tissues to this stress as has been proposed in pepper and zucchini (*12*, *32*).

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