Polyamines induced by hot water treatments reduce chilling injury and decay in pepper fruit

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Abstract

Treatment of peppers with hot water (53°C) for 4 min was found to be effective in alleviating chilling injury and reducing decay after 14 and 28 days of storage at 8°C. Treatment at 45°C for 15 min was less effective in maintaining pepper quality during storage. Packaging with low density polyethylene film significantly reduced weight loss and chilling injury during low temperature storage. Lower O2 and higher CO2 levels were found in internal and in-package atmospheres of heated fruit than controls. Ethylene was not detected in the in-package atmosphere of treated fruit, but was present in the control. Polyamine levels increased immediately after hot water treatments. Putrescine levels increased during storage at 8°C particularly in heat-treated fruit and in packaged fruit. A significant increase in putrescine was noted in packaged fruit treated at 53°C for 4 min after 14 days of storage. Spermine levels decreased in control fruit during storage. However, heat treatment in combination with film packaging maintained higher levels of spermine in peppers during storage than controls. These results indicated that hot water treatment in conjunction with film packaging may delay chilling injury and decay of bell peppers through a mechanism that involved elevation of polyamine levels. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Polyamine; Hot water dips; Film packaging; Chilling injury; Decay; Pepper

1. Introduction

Bell peppers are susceptible to chilling injury (CI) during storage at temperatures of 7°C and below (Hardenburg et al., 1986). Development of CI symptoms is a primary postharvest problem during storage of peppers and many other horticultural crops (Wang, 1993). The main symptoms of cold-induced damage in pepper fruits are surface pitting and calyx discoloration. In addition to the visible symptoms, several biochemical and physiological processes are altered as a direct effect of low temperature on cellular constituents.
(Lurie, 1998). This phenomena limits the storage life and leads to significant degradation of produce quality. Therefore, different methods have been developed to alleviate CI symptoms. These include postharvest treatments with fungicides, plant growth regulators and other chemicals, waxing, plastic wrapping, controlled atmosphere, intermittent warming and temperature conditioning (Wang, 1990, 1994; Lurie, 1998). The mechanisms involved in the beneficial effects of these treatments and the physiological basis associated with CI are, however, still unclear.

Changes in polyamine (PA) biosynthesis in plant tissues have been correlated with various kinds of stress (Faust and Wang, 1993). Some evidence suggests a relationship between PA and CI with substantial variations among species. However, the role of PA in CI is unknown. It has been observed that conditioning treatments effective in reducing chilling symptoms have increased PA (McDonald, 1989; Rodov et al., 1995; González-Aguilar et al., 1997). Prestorage hot water dipping (HWD) of fruit has been investigated as a way of enhancing fruit resistance to CI (Lurie, 1998). Film wrapping has also been proven to effectively reduce CI symptoms in pepper fruits (Ben-Yehoshua et al., 1983; Forney and Lipton, 1990; González and Tiznado, 1993). However, the mechanism involved in the reduction of CI by these methods is still unclear. Furthermore, little information is available in literature about the effect of HWD’s in conjunction with film wrapping in reducing CI symptoms and changing the PA levels of pepper fruits.

The present work was initiated to evaluate the effectiveness of hot water in combination with film packaging as a postharvest treatment to control CI and decay of bell peppers and whether this effectiveness was related to changes in PA content.

2. Materials and methods

2.1. Plant material

Freshly harvested green bell peppers (Capsicum annuum L.) were obtained from packing houses in Guaymas, Mexico. Fruit were delivered to the laboratory immediately after harvest and uniform sized peppers, free of blemishes or defects, were selected for this study.

2.2. Hot water treatments

Peppers were grouped into lots of 50 and subjected to the following six treatments: (1) control (C); (2) dipped in water at 53°C for 4 min (D1); (3) dipped at 45°C for 15 min (D2); (4) D1 plus film packaging; (5) D2 plus film packaging; (6) control plus film packaging (control). A 20 × 30 cm rectangle of low density polyethylene (Industrias Plasticas del Noroeste, Hermosillo, Mexico) was used to wrap two peppers. In previous work (González and Tiznado, 1993), this film proved effective in maintaining the quality of peppers. Thickness, O₂, CO₂ and water permeability rates of the film were 0.065 mm, 19.093 × 10⁻⁶ nmol m⁻² s⁻¹ Pa⁻¹, 7.43 × 10⁻⁶ nmol m⁻² s⁻¹ Pa⁻¹, and 0.02559 nmol m⁻² s⁻¹ Pa⁻¹, respectively (González and Tiznado, 1993). The baths contained chlorinated water at 200 ppm. Water temperature was maintained within ±1°C. Preliminary tests were carried out to determine the best temperatures and durations of hot water dips to use for the experiments. After dip treatments, fruit were dried in still, 20°C air and then stored either directly at 8°C or packaged in groups of two peppers in low density polyethylene before 8°C storage for 28 days. Each group of 50 peppers selected for continuous cold storage was placed in a clean plastic box. The six groups of peppers treated and packaged were placed in the same cold room chamber and maintained at 8 ± 1°C with 80–85% relative humidity (RH). A silicone septum in each package was used for withdrawing headspace gas for analysis. Headspace concentrations of O₂, CO₂ and ethylene where measured weekly by withdrawing 1 ml of gas from each of 15 samples using a hypodermic syringe. After 14 and 28 days of storage, 30-fruit replicates from each treatment were sampled to evaluate changes in weight loss and decay. Thereafter, 1 ml of internal atmosphere of five fruit was withdrawn from the internal cavity to analyze the O₂, CO₂ and ethylene
concentrations. Chilling injury symptoms were evaluated after the fruit (15 fruit/treatment) had been held for 2 days in air at 20°C and 75% RH. For polyamine analysis three spatially distributed replicates (2 g) were sampled from fresh tissue of each of the 15 fruit and stored at −20°C until analysis. Polyamine analysis was performed on freshly harvested fruit immediately after hot water treatments and after 14 and 28 days at 8°C.

2.3. Quality measurements

Peppers were weighed before and after storage to calculate the percentage of fresh weight loss. Extent of decay was assessed based on the area of decay and the surface area covered with microorganisms growing on it. Decay was rated on a scale of 1–5 where: 1 = none, 2 = trace, 3 = slight, 4 = moderate, 5 = severe. The score of CI was based on the fraction of total surface areas affected by sheet pitting: 0 = no injury, 1 = slight, 2 = moderate, 3 = severe, depending on the extent of damage. A CI index was determined by summing the number of fruit in each category by the score of each category and then dividing this sum by the total number of fruit evaluated (González-Aguilar et al., 1997).

2.4. Polyamine analysis

Free polyamines were analyzed according to the method of Carbonell and Navarro (1989). A total of 2 g of frozen tissue was homogenized in a chilled mortar with 3 ml of 0.2 N HClO₄ and 1 ml of 0.6 mM 1,6-hexanodiamine as an internal standard. The homogenate was then centrifuged at 12,000 × g for 20 min at 4°C. Free polyamines left in the supernatant were dansylated as previously described (González-Aguilar et al., 1997) and then extracted with 500 μl toluene. The organic phase was dried under a stream of N₂ at 70°C and the residue was resuspended in 200 μl of acetonitrile (HPLC grade) and filtered through a HV-4 filter (Millipore, pore size 0.45μm) for HPLC analysis. Polyamines were eluted through a 200 × 4.6 mm reverse-phase C18 column packed with 5 μm Hypersil ODS resin. Elution was performed at a flow rate of 1.5 ml min⁻¹ with a gradient of 60 to 90% acetonitrile for 25 min at 35°C. Dansylated polyamines in the extracts were detected by fluorescence at an excitation wavelength of 365 nm and an emission wavelength of 447 nm and quantified by peak area comparison using 1,6-hexanediamine as the internal standard and standard curves for putrescine (Put), spermidine (Spd) and spermine (Spm).

2.5. Measurements of O₂, CO₂ and ethylene

Oxygen content in the package was measured using a portable O₂ analyzer (Mocon LC 700F). Headspace CO₂ content was analyzed using an infra red CO₂ analyzer (Horiba PIR 700). The accumulation of C₂H₄ in the package was analyzed using a gas chromatograph (HATCH CARLE series 400) with a FID detector.

2.6. Statistical design

Experimental data are the mean ± SE of three replicates of the determinations for each sample. A variance analysis using the Tukey test at the 5% level was performed to determine if the polyamine content induced by different times of conditioning were significantly different (P < 0.05).

3. Results and discussion

3.1. O₂ and CO₂ levels

The CO₂ production of bell pepper measured over 28 days varied from 0.434 to 1.086 mmol kg⁻¹ h⁻¹, following a typical non-climacteric respiratory pattern (data not shown). Initially, HWD’s increased the respiration rate, however, after 1 week a similar pattern was observed in treated and control fruits. Fruit treated at 45°C for 15 min presented the highest respiration rate throughout the storage period. This increase could be due to the higher temperature and longer period in comparison with control and 4 min at 53°C treatments.

The headspace CO₂ concentration never exceeded 7% and that of O₂ never dropped below 14.5% throughout the 28 days of the experiment.
The concentration of O$_2$ declined in the three treatments, reaching 18.2, 18.0 and 15.3\% after 7 days at 8°C in control, fruit treated at 45°C for 15 min and fruit treated at 53°C for 3 min, respectively. Thereafter, O$_2$ concentration was stable in control fruit and fruit treated at 53°C for 3 min, whereas, O$_2$ levels in packages of fruit treated at 45°C for 15 min, continued to decrease slightly during the storage period. The maximum accumulation of CO$_2$ in the packages was observed after 1 week of storage. The CO$_2$ content increased to 6\% in packages of fruits treated at 45°C for 15 min and 4 and 2\% in fruits treated at 53°C for 3 min and control, respectively. Thereafter, steady state in the packages was reached after 1 week at 8°C. The relative reduction in O$_2$ levels was accompanied with increases of CO$_2$, following a similar pattern in the three treatments. No ethylene was detected in the packages of fruits treated with hot water (data not shown). This could be due to the higher CO$_2$ levels found in the packages, because CO$_2$ is an antagonist of ethylene action and impedes its autocatalytic synthesis. However, ethylene was detected (<0.3 ppm) in the headspace surrounding control packaged fruits, where the lowest levels of CO$_2$ and highest of O$_2$ were found.

Fruit treated at 45°C for 15 min presented higher internal CO$_2$ and lower O$_2$ levels than fruit of other treatments (Table 1). The use of plastic packaging enhanced the accumulation of CO$_2$ and reduction of O$_2$. The highest levels of CO$_2$ were observed in fruit treated at 45°C for 15 min and packaged, followed by fruit treated at 53°C for 3 min and control. These levels could be related to the low ethylene content found in the package headspace.

### 3.2. Water loss, chilling injury and decay

Fruit water loss was consistently lower in untreated fruit than those treated with hot water (Fig. 2A). Kerbel et al. (1987) found that HWD increased fresh weight loss in some fruits. Schirra and D’Hallewin, (1997) also found that HWD ranging 56–58°C that inhibited CI in the same magnitude affect weight loss of ‘Fortune’ mandarin. However, González-Aguilar et al. (1997) found in the same cultivar that HWD (ranging 44–53°C) did not affect weight loss during cold storage. It has been observed in some citrus fruit,
that HWD alone reduced weight loss of stored fruit, probably by improving the membrane function of the cells or the cuticular properties at the fruit surface (Rodov et al., 1995). The increase in water loss may be associated with cellular breakdown, loss of membrane integrity and by the apparent removal of epicuticular waxes, known to play an important role in water exchange through the rind (Schirra and D’Hallewin, 1997). Film packaging did significantly delay water loss of treated fruit. Storage conditions or treatments that reduce fruit water loss have been shown to alleviate CI (Wang, 1993). Nevertheless, it has been suggested that in addition to water loss, other factors may contribute to CI symptom development (Hardenburg et al., 1986). In the present study, the symptoms of CI appeared in the form of skin depressions irregularly distributed over the fruit surface and calyx discoloration. After 14 days of storage, the percentage of fruit with moderate to severe peel pitting was very low in treated and packaged fruit, except for 45°C for 15 min (Fig. 2B). After 28 days of storage the incidence of CI increased considerably in control and fruit treated at 45°C for 15 min. In contrast, fruit treated at 53°C for 3 min delayed the appearance of CI. Reduction in sensitivity to CI by hot water treatments has been observed in avocado (Woolf et al., 1996), cucumbers (McCollum and McDonald, 1993), mandarins (González-Aguilar et al., 1997), oranges (Wild and Hood, 1989) and other citrus fruits (Rodov et al., 1995). The physiological basis for this response has not been elucidated. The beneficial effect of hot water has been related to the partial removal and/or inhibition of pathogen spores (Couey, 1989). The reduction of CI symptoms in peppers by film packaging could be due to the increase in humidity, the reduction in O₂ and accumulation of CO₂ within the package. Meir et al. (1995) found that storing bell peppers at 3°C in polyethylene bags extended their quality and shelf life. Also, low O₂ (2–5%) and high CO₂ (up to 20%) can alleviate CI symptoms in bell peppers and zucchini squash (Wang, 1977; Wang and Ji, 1989; Luo and Mikkelsen, 1996). It has been observed in many sensitive crops that modification of the atmosphere surrounding the commodity and the high humidity created in-package, suppressed CI (Forney and Lipton, 1990). In the present study, HWD’s in conjunction with plastic film were found effective in reducing CI and decay of peppers. Treatment at 53°C for 3 min considerably reduced the cold-induced damage.
Fruit treated with hot water plus film packaging had a better appearance, expressed as fraction of decayed surface, than non-packed fruit (Fig. 2C). In general, HWD reduced the decay symptoms while they were more severe on control fruits after 28 days at 8°C. Fruit treated at 53°C for 4 min and packaged presented the least deterioration at the end of storage (Fig. 2C).

3.3. Polyamine levels

High PA levels have been correlated with increased chilling resistance in many horticultural crops (Faust and Wang, 1993). It has been hypothesized that PA’s protect the integrity of membranes and in turn alleviate CI. In order to determine if PA’s were involved in changes in chilling sensitivity among the treatments, these compounds were analyzed after the treatment and during storage.

HWD’s, in general, increased significantly the Put content in treated and control fruit throughout the storage period. A 4- and 2-fold increase in Put was observed in fruit treated at 53°C for 4 min and 45°C for 15 min, respectively (Fig. 3). However, the accumulation of Put in hot water treated fruit was higher than that observed in control fruit. A significant increase in Put content (2.045 μmol g⁻¹) was observed in fruit treated at 53°C and wrapped in polyethylene, after 14 days. Accumulation of Put in tissues seems to be a general response of plants to chilling temperatures (Faust and Wang, 1993). Film packaging in this study increased the accumulation of Put. After 28 days at 8°C, the largest accumulation of Put was observed in hot water treated fruit plus film packaging, followed by non-packed and hot water treated fruit. Put content increased during the first 14 days and then remained stable in control fruit. Wang and Qi (1997) found that sealed cucumbers maintained higher levels of PA’s than fruit sealed in perforated bags or non-wrapped samples. These higher levels could be attributed to the elevated CO₂ concentration inside the packages. However, Serrano et al. (1997) found that film packaging that reduces CI symptoms in peppers did not affect the Put levels. Rodov et al. (1995) demonstrated that a significantly higher level of Put was found in hot-dip treated grapefruit (53°C, 3 min) as compared with the untreated fruit stored 4 weeks at the same temperature (2°C). McDonald (1989) found that conditioning reduced CI injury symptoms in lemons and increased Put levels.

HWD’s also increased Spd content but to a lesser extent than Put (Fig. 4). Fruit treated at 53°C for 3 min showed the highest mean values, whereas the lowest Spd induction occurred in fruit treated at 45°C for 15 min. Spd levels continuously increased with storage period in control and hot water (45°C, 15 min) treated fruit. After 28 days, Spd content was higher in non-packaged fruits than in hot water and packaged fruit (Fig. 4). This is contrary to other results in sealed cucumbers where higher levels of Spd than in control fruit were maintained during cold storage.

Fig. 3. Effect of hot water dips and film packaging on the putrescine levels of bell pepper fruit, after 14 and 28 days at 8°C. T₀ = immediately after hot water treatment. Each bar is the mean of three replicates with standard error (±) indicated.
Fig. 4. Effect of hot water dips and film packaging on the spermidine levels of bell pepper fruit, after 14 and 28 days at 8°C. \( T_0 \) immediately after hot water treatment. Each bar is the mean of three replicates with standard error (±) indicated.

Fig. 5. Effect of hot water dips and film packaging on the spermine levels of bell pepper fruit, after 14 and 28 days at 8°C. \( T_0 \) immediately after hot water treatment. Each bar is the mean of three replicates with standard error (±) indicated.

(Wang and Qi, 1997). In general, the levels of PA changed widely during cold storage and no consistent differences were found among treatments. In a previous study, it was observed that Spd content did not correlate with the effectiveness of HWD or conditioning treatments (González-Aguilar et al., 1997).

A 2-fold increase in Spm content was observed in fruit treated for 3 min at 53°C (Fig. 5). However, treatment of 45°C for 15 min barely modified the Spm content. After cold storage, Spm levels continuously decreased in non-packaged fruit. The concentrations of this PA during storage were comparable among these treatments and were always lower than those of freshly harvested fruit. Film packaging suppressed the reduction in Spm content. This PA had a similar behavior as Put in response to film packaging and hot water treatments. The major increase in Spm was noticed after 14 days in fruit treated with hot water and film packaging. Fruit treated at 53°C for 3 min and packaged showed the highest levels in Spm, followed by fruit treated at 45°C for 15 min or non-treated and packaged (Fig. 5). In general, HWD’s alone did not modify the Spm content throughout the cold storage period. However, HWD’s plus film packaging increased this PA levels.

Taken together, results obtained in this study indicate that the induction of higher levels of PA’s by HWD’s and film packaging are related to the reduction in CI and decay of pepper fruit. The reduction in CI symptoms by PA’s might be due to their capacity to preserve membrane integrity, both by lowering the membrane phase-transition temperature fluidity and by retarding lipid peroxidation, resulting in increased cell viability (Drolet et al., 1986, Kramer and Wang, 1989).
It may be concluded that treatment of 53°C for 4 min in conjunction with film packaging can be effectively used to reduce CI and decay of bell peppers stored at 8°C and that PA's appear to be directly related to the hot water-induced tolerance to chilling.

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