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Influence of cultivar and harvest method on postharvest storage quality of pepper (*Capsicum annuum* L.) fruit

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Abstract

The principal physiological factors that negatively impact pepper fruit during shipment and storage and subsequent marketing are water loss and chilling injury. The current study evaluated the effect of harvest methodology on postharvest water loss from sweet bell pepper fruit and the potential relationship between water loss and chilling injury in cold-stored fruit. The influence of cultivar, epicuticular wax, and AOX gene expression on water loss and chilling injury were examined. Our results demonstrated that the degree of water loss in pepper fruit is subject to effects of genotype and pre- and postharvest environments as evidenced by year to year variation in fruit storage attributes. A comparison of pepper fruit harvest methods, wherein peduncles were either torn or cut, showed that harvest method had little effect on percent water loss. Observations on fruit water loss in relation to fruit size suggested that fruit cuticles are the primary barrier to water loss. A clear relationship between epicuticular wax content and fruit water loss was not evident. Cultivars varied in their susceptibility to chilling injury and fruit water loss was positively correlated with the severity of chilling injury. No correlation was found between endogenous AOX transcript levels and cultivar-specific susceptibility to chilling injury. The results illustrate the difficulty of identifying indices correlated with water loss that could be used to develop or identify cultivars with improved storability. We did, however, find that there are significant differences in storage attributes of pepper cultivars and that routine screening for water loss and chilling injury are advantageous for selection of cultivars most suitable for cold-storage.
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Keywords: *Capsicum annuum*; Water loss; Chilling injury; Harvest method; Postharvest storage

1. Introduction

Postharvest quality of fresh pepper (*Capsicum annuum* L.) fruit is influenced by physiological and pathological factors. The principal physiological factors that negatively impact pepper fruit during shipment and storage and subsequent marketing are water loss (Lownds et al., 1993; Maalekuu et al., 2002; Watada et al., 1987) and chilling injury (Hardenburg et al., 1986; Paull, 1990).

Postharvest water loss of fruits and vegetables results in fruit softening, and reduced glossiness and shelf life. The cuticle is considered the primary barrier against uncontrolled water loss from plant tissues (Kerstiens, 1996). The cuticle is composed of cutin and nonpolar solvent soluble cuticular waxes. There is general consensus that cuticle thickness has little relationship with cuticular transpiration (Riederer and Schreiber, 2001). There are conflicting reports on the relative importance of wax content in regulating water loss (Lownds et al., 1993; Maalekuu et al., 2004; Riederer and Schreiber, 2001). Utilizing mutant tomato lines with altered wax composition, Vogg et al. (2004) determined that fruit epicuticular wax composition had a significant effect on cuticular transpiration. In addition to diffusion through the cuticle, water loss

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in fruit tissue may also occur through the stem scar (Cameron and Yang, 1982; Diaz-Perez, 1998).

Damage caused by chilling injury in peppers is typified by dot-pitting followed by sheet-pitting. These morphological lesions may lead to alternaria-induced rot on pods and calyxes, seed darkening, and fruit shrinkage due to moisture loss (Hardenburg et al., 1986). This chilling induced fruit damage markedly reduces the quality and shelf life of pepper fruit (Paull, 1990). Reactive oxygen species (ROS) often contribute to chilling injury in sensitive plant tissues and resistance to chilling injury has been correlated with an increase of enzymatic activities in antioxidant systems that reduce the generation of ROS or scavenge those that are generated (Moller, 2001; Walker and McKersie, 1993). Increased alternative oxidase (AOX) gene expression and AOX protein level are associated with a reduction in the formation of ROS and have been demonstrated to increase significantly when pepper fruit were stored at low temperature (Fung et al., 2004; Purvis, 2002).

The current study evaluates the effect of harvest methodology on postharvest water loss from sweet bell pepper fruit and the potential relationship between water loss and chilling injury in stored fruit. The influence of cultivar, epicuticular wax abundance, and AOX gene expression on water loss and chilling injury were examined.

2. Materials and methods

2.1. Plant material

Seven-week-old greenhouse grown plants of the commercially available sweet bell pepper cultivars 'Bell Captain', 'Midway', 'Northstar', and 'Sentry' were transplanted to field plots at the Beltsville Agricultural Research Center and at a local farm in Beltsville, MD, into Keyport fine loam soil (clayey, mixed, mesic Aquic Hapludult). Field grown plants were spaced at 0.45 m intervals in single rows on polyethylene covered raised beds positioned on 1.5 m centers with trickle irrigation. Pest control and fertilization regimes followed standard horticultural practices for pepper production in Maryland (University of Maryland, 2000).

Market size green fruit of each cultivar were harvested via cutting or tearing fruit from plants for water loss studies.

2.2. Wax content

In 2004, six 1 cm diameter pericarp discs (ca. 5–6 g fresh wt./fruit) were excised from around the equator of each fruit from a random sample of 10 individual fruit of each cultivar on the day of harvest, frozen in liquid nitrogen, lyophilized, and stored at −80 °C prior to total wax extraction. Epidermal tissue of lyophilized discs was peeled from pericarp discs with a sharp razor, scraped lightly to remove any residual pericarp tissue, and shaken in 5 ml hexane at room temperature for 30 min. The hexane extract was collected and filtered through a Whatman GF/C glass microfiber filter. Remaining tissue residue was washed with additional hexane, filtered, and the extracts were combined. Hexane extracts were dried under nitrogen and the dry weights of the insoluble wax residue recorded.

2.3. Storage conditions

Fruit were harvested, rinsed with water, air dried, placed in single layers in large plastic bins, covered loosely with plastic wrap, and stored in walk in chambers held at either 12.5 °C (45–55% relative humidity) or 0 °C (80–85% relative humidity). Thirty fruit from each cultivar were weighed at the start of the each experiment and at days 4, 7, 11, and 14 in storage. Fruit were weighed within the storage chambers. The 12.5 °C storage conditions were chosen to eliminate possible chilling injury and closely mimic commercial storage temperature. The 0 °C storage temperature was chosen to insure that every fruit had some area of chilling injury (Fung et al., 2004). The percent weight loss of individual fruit was used for statistical analysis using SAS software (SAS Institute, Cary, NC).

2.4. Evaluation of chilling injury

Chilling injury symptoms were evaluated during and after cold storage (Fung et al., 2004). Chilling injury appeared as surface-pitting followed by a combination of both surface-pitting and sheet-pitting starting after 2–3 days at 0 °C. The severity of the symptoms was assessed visually and the percentage of fruit surface covered by pitting was scored for each fruit every 3–4 days. The final score was made after 14 days at 0 °C followed by 2 days at 20 °C.

2.5. RNA gel blot analysis

Fruit were processed immediately after harvest by chilling on ice, excising the pericarp tissue and freezing in liquid nitrogen. Tissue samples were ground using a mortar and pestle and stored at −80 °C. RNA was extracted using the method described by Verwoerd et al. (1989) except that a second chloroform extraction was performed.

Total RNA (10 μg/lane) was separated in a formaldehyde/MOPS agarose gel, transferred to a Hybond-N^+ nylon membrane (Amersham, Arlington Heights, IL), fixed by incubating for 2 h at 80 °C, hybridized to radiolabeled DNA probes overnight in a hybridization incubator using a buffer described by Church and Gilbert (1984), washed to a final stringency of 0.1 × SSC with 0.2% SDS at 65 °C, and autoradiographed. An RNA ladder standard was used to estimate the length of the mRNAs. Probes were synthesized using a Random Primed DNA Labeling Kit (Roche Molecular Biochemicals) with ^{32}P-dATP (3000 Ci/mmol) as the label and DNA fragments derived from PCR-amplification of the AOX1 gene from pepper (Fung et al., 2004). Blots were exposed to X-OMAT (Kodak) film using an intensifying screen for 2 days at −80 °C. As a loading control, RNA blots were stripped

and re-probed at a reduced hybridization and washing stringency using a soybean 26S rDNA fragment (Turano et al., 1997). Films were scanned in a Bio-Rad Flour-S Multilmager (Bio-Rad Laboratories, Hercules, CA) and the data quantified using Quantity One software, normalized against the loading controls.

3. Results and discussion

Percent water loss for the respective cultivars varied across years with relatively higher levels of water loss observed in 2004 than in 2002 (Fig. 1). Northstar exhibited consistently high levels of water loss in both 2002 and 2004 in comparison with other cultivars examined. Conversely, Bell Captain consistently exhibited the least percent water loss in both years. The relative percent water loss rankings of the cultivars Midway and Sentry varied as a function of year. In consecutive harvests over a 4-month period in a single growing season, Maalekuu et al. (2004) observed a significant harvest date × cultivar interaction for fruit water loss after prolonged storage.

A comparison of pepper fruit harvest methods, wherein peduncles were either torn or cut, demonstrated little difference in percent water loss for the two methods (Fig. 2). In fruit harvested by cutting in 2002, those of Bell Captain exhibited

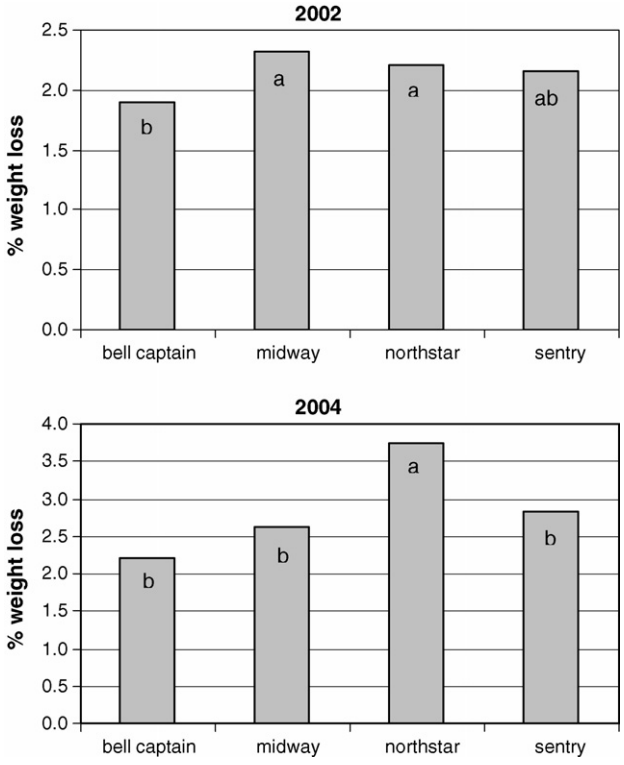

Fig. 1. Weight (water) loss data from four pepper fruit cultivars harvested in the 2002 and 2004 seasons. Fruit were harvested manually by tearing the stems. Percent weight loss was the mean of 30 fruit after 14 days storage at 12 °C. Means with the same letter are not significantly different; $P < 0.05$, Tukey–Kramer comparison.

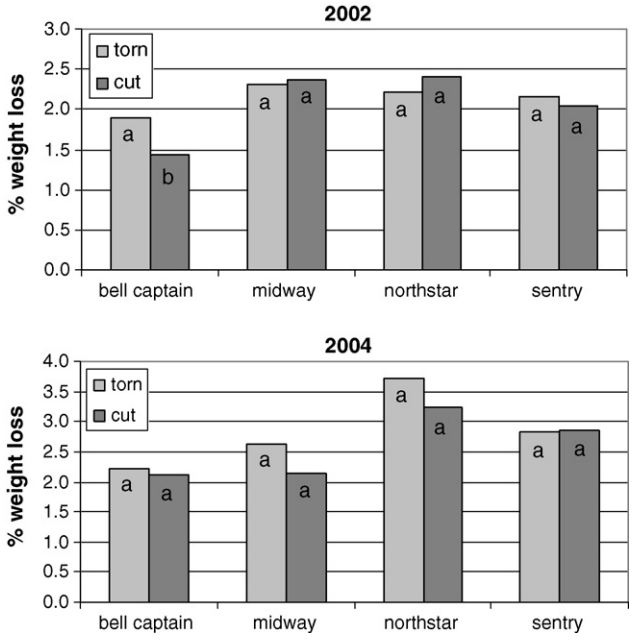

Fig. 2. Weight loss comparison of pepper fruit harvested by tearing vs. cutting the stems. Fruit from four cultivars were harvested during the 2002 and 2004 seasons. Percent weight loss was the mean of 30 fruit after 14 days storage at 12 °C. Statistical analysis was performed comparing the two harvest methods for each cultivar. Means with the same letter are not significantly different; $P < 0.05$, Tukey–Kramer comparison.

significantly less water loss relative to those of other cultivars. This difference, however, was not observed in 2004. Lownds et al. (1993) noted preliminary data suggesting that sealing the cut stem end of pepper fruit had no effect on the rate of water loss during storage. In eggplant, the calyx was the main route for fruit water loss, accounting for at least 60% of fruit transpiration (Diaz-Perez, 1998). In contrast with eggplant, it appears that harvest methodology of pepper fruit and postharvest treatments which could potentially reduce water loss from the calyx have little effect on improving postharvest fruit quality.

Significant differences in total wax content were evident among cultivars sampled in 2004. Bell Captain contained the highest and Sentry the lowest amount of wax (Fig. 3). Wax levels in Midway and North Star were intermediate in comparison with the other cultivars examined. Although a trend correlating fruit wax content with water loss was apparent for some cultivars, sample variation negated drawing definitive conclusions. For example, Bell Captain had significantly greater wax content than Sentry, but a significant difference in water loss between these cultivars was not evident. Likewise, Northstar exhibited significantly greater water loss than Midway, but wax content did not differ significantly between these cultivars.

Lownds et al. (1993) found that water-loss rate and epicuticular wax content of three New Mexican-type pepper fruit were negatively correlated, suggesting that wax content was important in regulating water loss in those cultivars. Their study also noted that stomata were absent from pepper

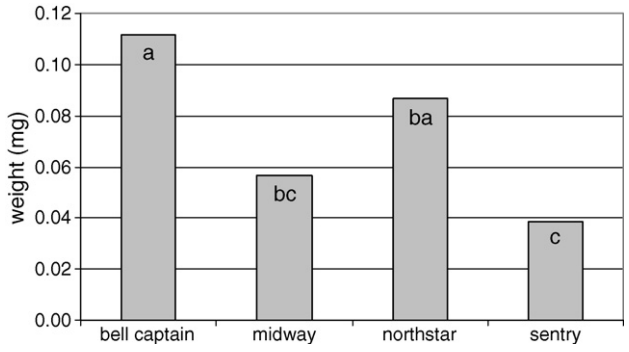

Fig. 3. Weight of cuticular wax in hexane extracts of pericarp epidermal tissue from fruit of the four bell pepper cultivars. Data are the mean values obtained by sampling 10 fruit from each cultivar harvested in the 2004 season. Means with the same letter are not significantly different; $P < 0.05$, Tukey–Kramer comparison.

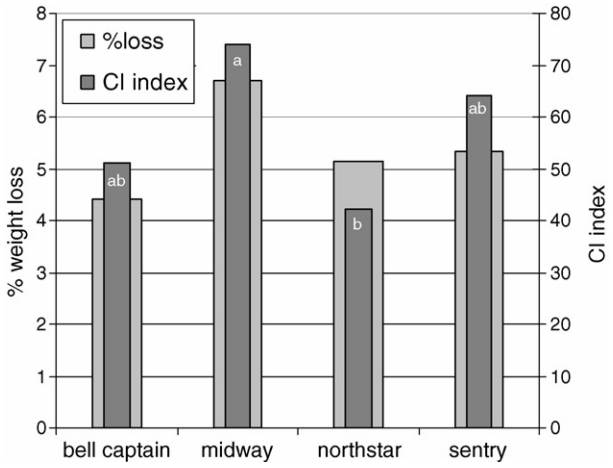

Fig. 4. Percent weight loss and chilling injury index (CI) of fruit harvested during the 2004 growing season. Percent weight loss was the mean of 30 fruit after 14 days storage at 0 °C. The CI was determined for the same fruit after two additional days at 20 °C. Statistical analysis was performed comparing the CI among the four cultivars. Means with the same letter are not significantly different; $P < 0.05$, Tukey–Kramer comparison.

fruit surfaces and epicuticular wax was amorphous for the cultivars examined, thus discounting those factors for differences in water loss rates observed among the cultivars. Similarly, Maalekuu et al. (2004) reported a negative correlation between pepper fruit water loss and skin wax content. The absence of a relationship between wax content and water loss in our samples may be due to environmental influences. Varying reports on the role of wax content in regulating water loss in different plant species have been described and attributed to environmental factors and cultivar × environment interactions (Baker, 1974; Hunt and Baker, 1982; Riederer and Schreiber, 2001).

In tomato (Ehret and Ho, 1986), pepper (Cohen and Hicks, 1985; Lownds et al., 1993), and eggplant (Diaz-Perez, 1998), an inverse relationship between fruit transpiration rate and fruit size has been observed for fruit stored at 20 °C. This relationship was also apparent for a number of the pepper varieties evaluated here under 12.5 °C storage conditions (average fruit weight for 2002/2004: 119 ± 21 g/115 ± 31 g, Bell Captain; 93 ± 15 g/88 ± 23 g, Midway; 108 ± 15 g/75 ± 20 g, Northstar, and 123 ± 21 g/85 ± 47 g, Sentry). Fruit weight of Bell Captain was greater than Midway and Northstar in 2002 and also exhibited significantly less water loss than Midway or Northstar. This relationship was not apparent, however, for Sentry. Fruit weight of Bell Captain was greater than that of all other cultivars in 2004 and Bell Captain fruit exhibited the least mean water loss, although water loss was not significantly different from that in Midway or Sentry fruit. Conversely, fruit of Northstar were smallest in 2004 and displayed the greatest water loss. Thus, a trend towards decreasing water loss with increased fruit size was evident here, but it was subject to a harvest × cultivar interaction. The cooler commercial storage temperatures utilized in our study also likely reduced the magnitude of the inverse relationship between fruit size and water loss relative to those reported at higher storage temperatures. Temperature is considered one of the predominant physical factors influencing cuticle permeability (Riederer and Schreiber, 2001). In a survey of 12 plant species, cuticle permeability to water was shown to increase by a factor of 2 in the temperature range from 15 to 35 °C (Riederer and Schreiber, 2001).

The cultivar Northstar exhibited the lowest chilling injury index whereas Midway displayed the greatest susceptibility to chilling injury (Fig. 4). Chilling injury of pepper fruit was examined together with percent water loss in order to assess the potential relationship between these parameters. The analysis demonstrated a significant correlation between chilling injury and percent water loss ($r = 0.51$; $P < 0.0001$). An increase in damage due to chilling injury generally corresponded with increased fruit water loss (Fig. 4). In comparison with percent water loss measured under commercial storage conditions for bell pepper (Fig. 1), the colder temperatures used to elicit chilling injury resulted in relatively higher percent water loss (Fig. 4). There was no correlation between fruit wax content and chilling injury ($r = -0.20$; $P < 0.21$).

Having observed significant differences in chilling injury among the cultivars, we examined AOX gene expression in response to chilling injury. Fung et al. (2004) demonstrated that AOX transcripts increased in fruit of the pepper cultivar Sentry in response to cold storage. Their study further demonstrated that treatment with methyl salicylate and methyl jasmonate vapors prior to cold storage increased the resistance against chilling injury, and that this resistance was correlated with elevated levels of AOX mRNA immediately after treatment. In the current study, we examined whether cultivars with lower chilling injury had naturally higher levels of AOX mRNA. All of the cultivars had nearly the same level of AOX mRNA at harvest (day 0) and the AOX mRNA levels increased in all cultivars by day 3 in cold storage (Fig. 5). However, the percent increase in AOX mRNA levels by day 3 showed little relationship with differences observed in the chilling injury index for these cultivars. In the absence of exogenously applied AOX elicitors, it appears that levels of

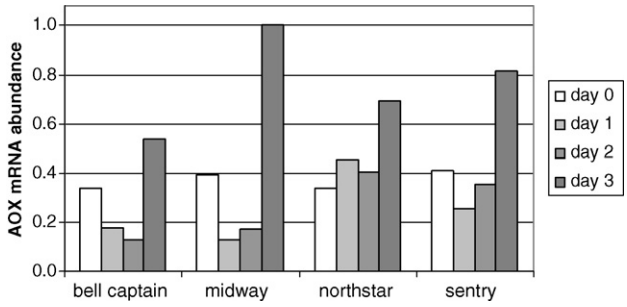

Fig. 5. Graphic representation of RNA gel blot analysis of alternative oxidase (AOX) mRNA levels in pepper fruit pericarp. RNA was prepared from fruit at harvest (day 0), and after 1, 2, and 3 days storage at 0 °C. Abundance units were arbitrarily set at 1.0 for the sample with the highest value.

AOX gene expression are insufficient to protect pepper fruit against chilling injury during the early stages of cold storage for these cultivars.

Numerous factors influence postharvest pepper fruit quality. Our results demonstrate that the extent of water loss in pepper fruit is subject to genotype effects and pre- and postharvest environments. Water loss from fruit stem ends explained little of the variation observed among cultivars for fruit water loss. Observations on fruit water loss in relation to fruit size suggested that fruit cuticles are the primary barrier to water loss. A clear relationship between epicuticular wax abundance and fruit water loss was not evident in our study. Cultivars varied in their susceptibility to chilling injury, and fruit water loss was positively correlated with the degree of chilling injury. Levels of endogenous AOX transcript were not found to be correlated with levels of chilling injury and resultant water loss.

Our results illustrate the difficulty of identifying indices correlated with water loss that could be used to develop or identify cultivars with improved storability. There are significant differences in storage attributes of pepper cultivars and routine screening for water loss and chilling injury would be advantageous for selection of cultivars most suitable for storage.

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