

Heat shock reduces browning of fresh-cut celery petioles

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Abstract

Preparation of 5 mm segments of celery petioles induced an increase in the activity of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), and subsequent tissue browning potential (absorbance of a 70% methanol tissue extract at 320 nm g^{-1} FW). The level of constitutive and wound-induced PAL activity was higher in vascular than in cortex tissue, and lowest in epidermal tissue. Heat shocking excised petiole segments (e.g. 50 °C for 90 s) significantly reduced the rise in wound-induced PAL and browning potential. Pithiness of the petiole segments did not alter the effectiveness of the heat shock treatments, nor was pithiness enhanced by the treatments. As storage life was extended, however, decay and the protrusion of vascular bundles from the cut ends of the segments became important factors in limiting shelf-life.

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1. Introduction

Wounding during the preparation of fresh-cut fruits and vegetables induces the synthesis of enzymes of phenylpropanoid metabolism, the synthesis and accumulation of phenolic compounds, and subsequent tissue browning (Ke and Saltveit, 1989; Brecht, 1995; Lopez-Galvez et al., 1997; Saltveit, 1997). In iceberg (crisphead) lettuce leaf tissue, phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), the first committed enzyme in the phenylpropanoid pathway, increased 6-fold in activity during the first 24 h at 10 °C after

wounding (Tomás-Barberán et al., 1997). The phenolic compounds chlorogenic and isochlorogenic acid, and dicaffeoyl tartaric acid increased thereafter and accumulated to levels 3-, 6- and 4-fold higher, respectively, than in unwounded tissue after 72 h. Upon oxidation and/or polymerization, these phenolic compounds cause tissue browning that reduces quality and shortens shelf-life (Bolin and Huxsoll, 1991). During this same 72 h period after wounding, browning increased 2-fold in fresh-cut lettuce (Lopez-Galvez et al., 1997).

Vascular browning at the ends of fresh-cut celery petioles is one of three major changes that reduce its quality. The other two changes are flaring of the cut ends and development of pithiness (i.e. the formation of aerenchyma in the pith) (Saltveit and Mangrich, 1996). Excision and

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mechanical perturbation induces pithiness in celery petioles, and the induction appears to involve the plant hormones ABA and ethylene (Pressman et al., 1984; Saltveit and Mangrich, 1996). Mechanical wounding (i.e. cutting) of harvested celery also appears to exacerbate aerenchyma formation (i.e. pithiness) during storage. Pithiness can be minimized by selecting dense petiole segments, by excluding ethylene from the storage atmosphere, and by maintaining high relative humidity and the recommended storage temperature of 0 °C. The third deleterious change, flaring of the cut ends, has not received much attention, but it appears to be the result of cellular expansion and rupturing of the parenchymatous cortex and pith tissues.

In tissue with initial low levels of preformed phenolic compounds (e.g. celery, lettuce) browning results from the induced synthesis and subsequent accumulation of phenolic compounds. A heat-shock treatment that reduces browning in fresh cut lettuce (e.g. 90 s at 45 °C) may work by redirecting protein synthesis away from the production of wound-induced enzymes of phenolic metabolism, and toward the production of innocuous heat shock proteins (hsps) (Saltveit, 2000). Administering the heat shock up to 24 h before, or 8 h after wounding significantly reduced the wound-induced increase in PAL activity, the accumulation of phenolic compounds, and the subsequent browning of excised lettuce leaf tissue (Saltveit and Loaiza-Velarde, 2000; Loaiza-Velarde and Saltveit, 2001).

Experiments reported in this paper examined the effect of heat shock treatments on wound-induced PAL activity and browning potential in fresh-cut celery petiole segments. Effective heat shock treatments were identified, and their effects on quality characterized.

2. Materials and methods

2.1. Plant material

Horticulturally mature celery (*Apium graveolens* L.) stalks were obtained from local wholesalers and stored at 2.5 °C until used. Individual petioles

were removed from the stalk and segments were excised from each petiole with a razor blade. Only stalks and petioles free of external defects were used. After excision, the segments were washed for 30 s in a 1:20 dilution of commercial bleach (5.25% sodium hypochlorite), blotted dry and randomly assigned to groups for treatment. After treatment, the segments were held in humidified, ethylene-free air at 10 °C. Some segments were dissected into epidermal, cortex, and vascular tissue to measure the level of PAL in these various tissues. In another series of experiments, entire petioles were either kept turgid by being enclosed with moist paper towels in plastic bags, or left on the lab bench at 20 °C for 6 h to accelerate water loss and produce flaccid petioles. The flaccid and turgid petioles were individually enclosed in plastic bags and held at 10 °C for 48 h. Segments were then excised from the middle of the petioles, and used to study the effect of water loss on browning potential.

2.2. Assay for PAL activity and browning potential

PAL was assayed 24 h after excision as previously described (Ke and Saltveit, 1988) and is presented as μmol of cinnamic acid produced in 1 h by 1 g FW of tissue. Tissue browning potential was assayed 48 h after excision as absorbance at 320 nm of a 70% methanol extract as previously described (Loaiza-Velarde et al., 1997). Briefly, diced fresh tissue was crushed in twice its weight of HPLC grade 100% methanol and held at -20 °C overnight. The petiole tissue was about 90% water, so the resulting extract was 70% methanol. The cloudy supernatant was centrifuged at $3000 \times g$ for 5 min and the clear supernatant decanted. The absorbance of an aliquot of the supernatant was measured with a spectrophotometer at 320 nm using a 70% methanol in water solution as the blank.

2.3. Density assay

The density of individual petioles segments was calculated from measurements of their weight and volume. The volume of each segment was calcu-

lated from its buoyant density as described by Saltveit and Mangrich (1996).

2.4. Heat shock treatment

Randomly selected groups of excised petiole segments were immersed for 60 s in water at 20–70 °C in 10 °C increments. After the heat treatments, the segments were put in 10 °C water for 3 min. Segments were then held at 10 °C in a flow of ethylene-free, humidified-air that was maintained at a sufficient rate to keep the CO₂ level below 0.15%. After 24 or 48 h, replicated samples were taken and assayed for PAL activity or browning potential, respectively.

Based on the ability of the previous heat treatments to reduce PAL activity and browning potential, three times temperature heat treatments were selected for more detailed study. Petiole segments were subjected to 45 °C for 30–480 s, 50 °C for 15–150 s, and 55 °C for 10–80 s. After 24 or 48 h, replicated samples were taken and assayed for PAL activity or browning potential, respectively.

2.5. Statistical analysis

The results reported in this paper are the mean values of triplicate samples accompanied by their standard deviations (S.D.). All experiments were performed more than once. Even though slight variation existed in the levels of wound-induced PAL and browning potential from experiment to experiment, similar trends were detected.

3. Results and discussion

3.1. Effect of wounding on PAL activity and browning potential

Wounding celery petioles by excision of 5 mm segments rapidly induced a 12-fold increase in PAL activity from an initial value of 0.01 to around 0.12 $\mu\text{mol g}^{-1} \text{h}^{-1}$ by 36 h (Fig. 1). Enzyme activity declined with almost the same rapidity that it increased, reaching levels of activity that were similar to initial levels by 96 h, and

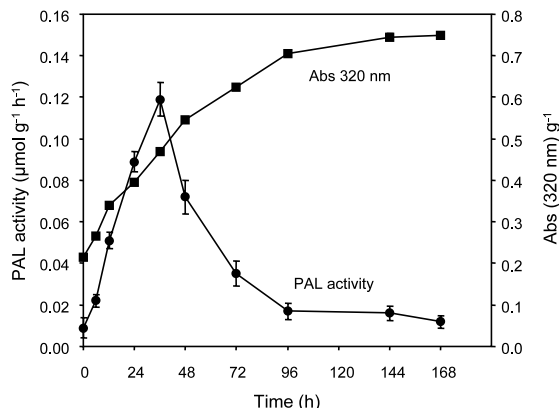


Fig. 1. Effect of wounding (i.e. excision of 5 mm long segments of celery petioles) on the subsequent activity of PAL after 24 h, and browning potential (absorbance of a 70% methanol extract at 320 nm g^{-1} FW) after 48 h at 10 °C. PAL activity is expressed as the formation of cinnamic acid ($\mu\text{mol h}^{-1} \text{g}^{-1}$ FW). The vertical line at each data point is the S.D. about the mean.

maintaining these low levels until 168 h. Browning potential roughly paralleled the integrated area under the PAL activity curve; rising rapidly from 0 to 36 h as PAL activity increased, rising less rapidly from 36 to 96 h as PAL activity declined to initial levels, and then rising very slowly after 96 h as PAL activity stabilized at initial levels.

The correlation between wound-induced PAL activity after 24 h and browning potential after 48 h was highly significant (Fig. 2). PAL activity after 24 h is calculated as $0.177 \times$ (the absorbance after 48 h) $- 0.0494$, with an R^2 of 0.97. This correlation is similar to that reported for wounded Romaine lettuce (Campos-Vargas and Saltveit, 2002).

Dehydration stimulated an increase in browning potential. In contrast to turgid petioles that had a non-wounded browning potential of 0.23 ± 0.10 abs 320 nm g^{-1} FW, petioles that had been held for 48 h after becoming limp had browning potential levels of 0.59 ± 0.18 abs 320 nm g^{-1} FW. This almost 2.6-fold increase in browning potential was accompanied by pronounced vascular browning that adversely affected segment quality. Since phenylpropanoid metabolism (e.g. PAL activity and browning potential) is related to many defense responses of the plant, both biotic and abiotic stresses need to be minimized during

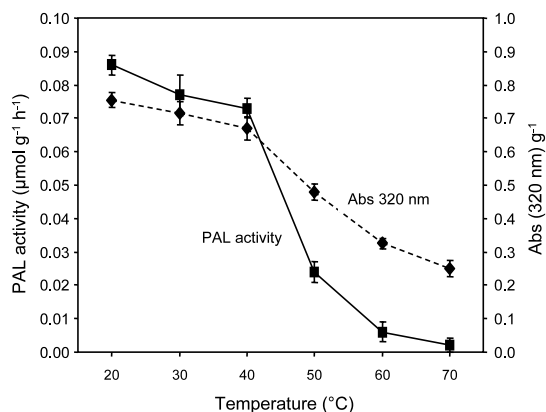


Fig. 2. Effect of a 60 s heat treatment at 20–70 °C on the activity of wound-induced PAL after 24 h, and browning potential (absorbance of a 70% methanol extract at 320 nm g⁻¹ FW) after 48 h in excised 5 mm segments held at 10 °C. PAL activity is expressed as the formation of cinnamic acid (μmol h⁻¹ g⁻¹ FW). The vertical line at each data point is the S.D. about the mean.

the growth and postharvest handling of celery to maximize the shelf-life of fresh cut celery.

3.2. Effect of heat shock on PAL activity and browning potential

A 60 s heat treatment at 50–70 °C significantly affected both PAL activity and browning potential (Fig. 2). Temperatures of 20–40 °C only slightly decreased wound-induced PAL activity measured 24 h after wounding. PAL activity declined more rapidly at higher temperature, falling from 0.075 μmol g⁻¹ h⁻¹ at 40 °C to 0.02, 0.01, and 0.005 μmol g⁻¹ h⁻¹ at 50, 60, and 70 °C, respectively. Browning potential followed an almost identical trajectory, declining slightly from 20 to 40 °C and then falling precipitously from 40 to 70 °C.

Based on the ability of the heat treatments to reduce browning potential, 5 mm tissue segments were subjected to 45 °C for 30–480 s, 50 °C for 15–150 s, and 55 °C for 10–80 s (Fig. 3). The browning potential of the non-heat-shocked control tissue increased 3.2-fold from an initial value of 0.22 ± 0.02 abs 320 nm g⁻¹ FW immediately after wounding to 0.70 ± 0.05 abs 320 nm g⁻¹ FW after 48 h at 10 °C. The heat shock treatments had significant effects on subsequent browning poten-

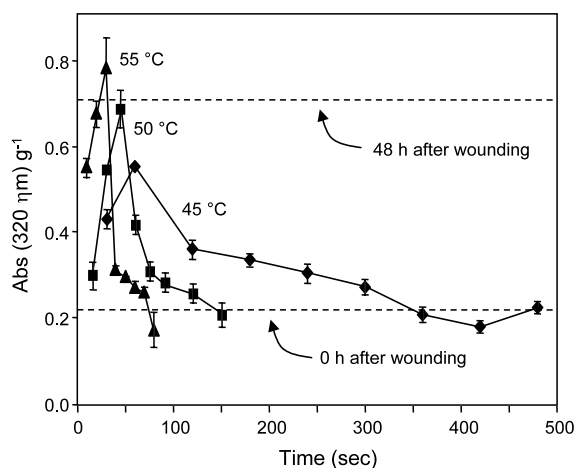


Fig. 3. Effect of various heat shock treatments on browning potential (absorbance of a 70% methanol extract at 320 nm g⁻¹ FW) after 48 h at 10 °C. The 5 mm long segments of celery petioles were stored at 10 °C after excision and treatment. Wounded, non-heat-shocked tissue was assayed initially (0 h) and after 48 h. The vertical line at each data point is the S.D. about the mean.

tial. The maximum browning potential increased as the heat shock temperature increased from 45 to 50 to 55 °C, and the duration of exposure to elicit this maximum response declined from 60 to 45 to 30 s, respectively. The duration of heat shock necessary to reduce the wound-induced level of browning potential to the initial control level was 360 s at 45 °C, 150 s at 50 °C, and 75 s at 55 °C.

The effectiveness of a 90 s 50 °C heat shock treatment in reducing wound-induced PAL activity was maximized if administered immediately after wounding, and rapidly declined in effectiveness as the time between administering the heat-shock and wounding increased beyond 2 h (Fig. 4). Applying the heat shock treatment 10 h before or 10 h after wounding did not reduce the 3.7-fold increase in wound-induced PAL activity to a level below that of the wounded, non-heat shocked control; while applying it within a few minutes of wounding reduced the wound-induced increase in PAL activity 75%, to a level only 60% greater than the non-wounded, non-heat-shocked control. This response was quite unlike lettuce in which a heat-shock could be administered at least 24 h before wounding or 8 h after wounding and still have a

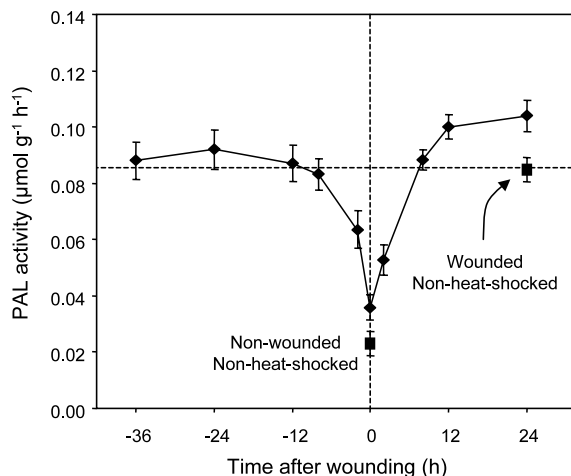


Fig. 4. Effect of a heat shock (90 s at 50 °C) administered 36, 24, 12, or 2 h before or 0, 2, 8, 12, or 24 h after wounding on PAL activity after 24 h. The 5 mm long segments of celery petioles were stored at 10 °C after excision and treatment. PAL activity is expressed as the formation of cinnamic acid ($\mu\text{mol h}^{-1} \text{g}^{-1} \text{FW}$). The vertical line at each data point is the S.D. about the mean.

significant effect on reducing wound-induced PAL activity (Loaiza-Velarde and Saltveit, 2001).

3.3. Responses of different petiole tissues to wounding and heat-shock

There were significant differences in PAL activity among the tissues in the celery petiole (Fig. 5). The level of constitutive and wound-induced PAL activity was higher in vascular than in cortex tissue, and lowest in epidermal tissue. The higher level of PAL in vascular tissue would likely result in a higher production and accumulation of phenolic compounds, and probably accounts for the selective browning of the vascular strands in wounded petiole segments. The vascular tissue in lettuce is not as prominent as in celery petioles and is not a main site of tissue browning (Lopez-Galvez et al., 1997).

A 50 °C heat shock for 90 s did not significantly affect the level of PAL activity immediately after excision and treatment, but it did depress the wound-induced rise in PAL activity in all tissues (Fig. 5). After 24 h at 10 °C, the level of PAL activity in the epidermal, cortex and vascular

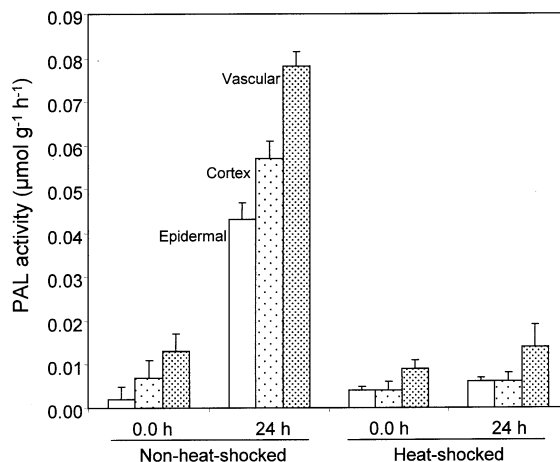


Fig. 5. Activity of PAL in various tissues of wounded celery petiole segments. After excision, the 5 mm segments were heat-shocked at 0 or 90 s at 50 °C. After being held at 10 °C for 0 or 24 h, the segments were dissected into epidermal, cortex, and vascular tissue. PAL activity is expressed as the formation of cinnamic acid ($\mu\text{mol h}^{-1} \text{g}^{-1} \text{FW}$). The vertical line atop each bar is the S.D. about the mean.

tissue of heat-shocked segments was only 13, 10 and 18%, respectively, of that in wounded, non-heat-shocked tissue. The higher temperatures needed to get significant suppression of PAL activity and tissue browning in celery versus lettuce (Loaiza-Velarde et al., 1997) may result from the more localized concentration of activity in the vascular tissue of celery petioles.

3.4. Effect of heat shock on pithiness of petiole segments

Both non-heat-shocked and heat-shocked segments lost density slowly during the first 2 weeks of storage at 10 °C; declining from 1.00 ± 0.005 to $0.98 \pm 0.02 \text{ g ml}^{-1}$. The heat-shocked segments continued to lose density at the same uniform rate for another week, while the decline in the density of the non-heat-shocked segments increased between weeks 2 and 3 to such an extent that they became significantly less dense than the heat-shocked segments (0.95 ± 0.02 vs. $0.92 \pm 0.02 \text{ g ml}^{-1}$) by 3 weeks. The linear regression for the heat shocked segments was: $\text{density} = 1.00 - (0.016 \times \text{weeks})$ with an r^2 of 0.92. The 90 s heat shock at 50 °C did not increase development of

pithiness, and actually retarded its development during 3 weeks of storage at 10 °C.

The heat shock treatment was equally effective in reducing the increase in wound-induced PAL in non-pithy and pithy petiole segments (Fig. 6). Segments were sorted into those exhibiting no pithiness (density $0.99 \pm 0.01 \text{ g ml}^{-1}$) and those exhibiting significant levels of pithiness ($0.96 \pm 0.02 \text{ g ml}^{-1}$) (Saltveit and Mangrich, 1996). Without the 90 s 50 °C heat shock treatment, the browning potential of the excised 5 mm segments increased almost 3-fold from 0.27 ± 0.04 to $0.76 \pm 0.05 \text{ abs } 320 \text{ nm g}^{-1} \text{ FW}$ during storage for 48 h at 10 °C. The heat shock treatments reduced the increase to only 40% of the control level (to $0.37 \pm 0.07 \text{ abs } 320 \text{ nm g}^{-1} \text{ FW}$).

3.5. Effect of wounding and heat-shock on shelf-life of petiole segments

The browning potential of non-heat-shocked (20 °C for 90 s) excised 5 mm petiole segments increased rapidly over 2 weeks of storage at 0 °C from 0.22 ± 0.03 to $0.63 \pm 0.03 \text{ abs } 320 \text{ nm g}^{-1} \text{ FW}$, and then remained constant ($0.64 \pm 0.02 \text{ abs } 320 \text{ nm g}^{-1} \text{ FW}$) for the remainder of the 5 week

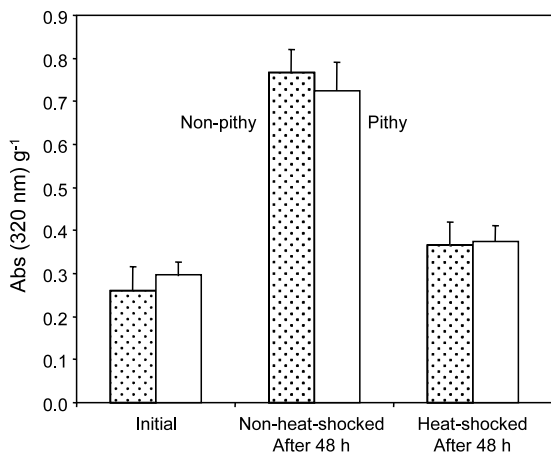


Fig. 6. Effect of wounding and heat shock on the browning potential of pithy and non-pithy 5 mm segments. Segments were selected that had no (density $0.99 \pm 0.01 \text{ g ml}^{-1}$) or significant levels of pithiness ($0.96 \pm 0.02 \text{ g ml}^{-1}$) (Saltveit and Mangrich, 1996). Browning potential (absorbance of a 70% methanol extract at 320 nm $\text{g}^{-1} \text{ FW}$) was measured after 48 h at 10 °C. The vertical line atop each bar is the S.D. for that mean.

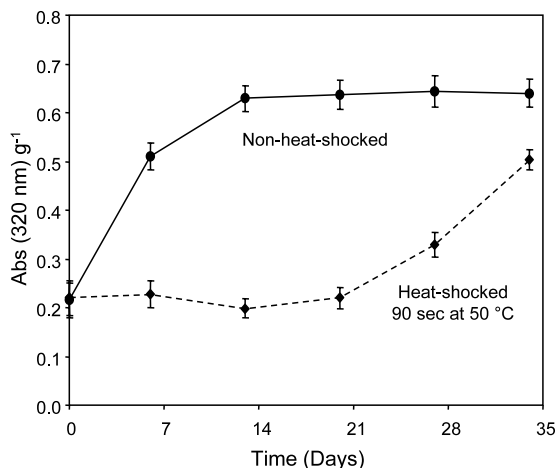


Fig. 7. Effect of wounding and heat shock on the browning potential (absorbance of a 70% methanol extract at $320 \text{ nm g}^{-1} \text{ FW}$) of 5 mm petiole segments. Segments were immersed in 20 or 50 °C water for 90 s before being held in humidified, ethylene-free air for up to 5 weeks of storage at 0 °C. The vertical line at each data point is the S.D. for that mean.

experiment (Fig. 7). Application of a 90 s 50 °C heat shock within 10 min of excision delayed the rise in browning potential for 3 weeks. Browning potential started to rise after 4 weeks of storage, and continued with a linear increase until the end of the experiment. The heat shock treatment was very effective in reducing tissue browning in cut petiole segments for up to 4 weeks of storage at 0 °C.

The shelf-life of the segments was not terminated by tissue browning, but by drying of the cut ends of the segments and decay. Decay became a serious problem after 3 weeks of storage and by 5 weeks over half of the segment in both treatments had developed extensive decay (watery breakdown of the tissue). More stringent sanitation measures or other treatments (Buta and Moline, 1998) may be necessary to control decay during the long-term storage of cut petiole segments.

Water loss from the cut ends of the segments from both treatments caused the first mm or so of parenchymatous cortex tissue to collapse within 2 weeks of storage. The vascular strands did not collapse and, therefore, protruded from the cut ends of the segments. Storage of the excised segments with wet paper towels in plastic bags

did not prevent this disorder. Application of an edible coating to the cut ends to retard moisture loss (Baldwin et al., 1995) may be necessary to prevent the development of this disorder during the long-term storage of cut petiole segments.

We have shown that a heat-shock treatment can diminish wound-induced physiological changes that led to reduced quality (i.e. tissue browning) and shortened shelf-life. As storage life was extended, however, other changes (i.e. vascular protrusion from the cut ends and decay) became the principal delineators of shelf-life.

References

- Baldwin, E.A., Nisperos-Carriedo, M.O., Baker, R.A., 1995. Edible coatings for lightly processed fruits and vegetables. *HortScience* 30, 35–38.
- Bolin, H.R., Huxsoll, C.C., 1991. Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *J. Food Sci.* 56, 60–67.
- Brecht, J.K., 1995. Physiology of lightly processed fruits and vegetables. *HortScience* 30, 18–22.
- Buta, J.G., Moline, H.E., 1998. Methyl jasmonate extends shelf life and reduces microbial contamination of fresh-cut celery and peppers. *J. Agric. Food Chem.* 46, 1253–1256.
- Campos-Vargas, R., Saltveit, M.E., 2002. Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiol. Plant* 114, 73–84.
- Ke, D., Saltveit, M.E., 1988. Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. *Plant Physiol.* 88, 1136–1140.
- Ke, D., Saltveit, M.E., 1989. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiol. Plant* 76, 412–418.
- Loaiza-Velarde, J.G., Saltveit, M.E., 2001. Heat shocks applied either before or after wounding reduce browning of lettuce leaf tissue. *J. Am. Soc. Hort. Sci.* 126, 227–234.
- Loaiza-Velarde, J.G., Tomás-Barberán, F.A., Saltveit, M.E., 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in Iceberg lettuce. *J. Am. Soc. Hort. Sci.* 122, 873–877.
- Lopez-Galvez, G., Saltveit, M.E., Cantwell, M., 1997. Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuce. *Postharvest Biol. Technol.* 9, 223–233.
- Pressman, E., Huberman, H., Aloni, B., Jaffe, M.J., 1984. Pithiness in plants: I. The effect of mechanical perturbation and the involvement of ethylene in petiole pithiness in celery. *Plant Cell Physiol.* 25, 891–897.
- Saltveit, M.E., 1997. Physical and physiological changes in minimally processed fruits and vegetables. In: Tomás-Barberán, F.A. (Ed.), *Phytochemistry of Fruit and Vegetables*. Oxford University Press, pp. 205–220.
- Saltveit, M.E., 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biol. Technol.* 21, 61–69.
- Saltveit, M.E., Mangrich, M.E., 1996. Use of density measurements to study the effect of excision, storage, abscisic acid and ethylene on the development of pithiness in celery (*Apium graveolens* L.) petioles. *J. Am. Soc. Hort. Sci.* 121, 137–141.
- Saltveit, M.E., Loaiza-Velarde, J.G., 2000. Method of preventing wound-induced browning in produce. Patent 6,113,958 issued September 5, 2000.
- Tomás-Barberán, F.A., Loaiza-Velarde, J., Bonfanti, A., Saltveit, M.E., 1997. Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. *J. Am. Soc. Hort. Sci.* 122, 399–404.