Heat Shocks Applied either before or after Wounding Reduce Browning of Lettuce Leaf Tissue

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ABSTRACT. Wounds and injuries incurred during preparation of fresh-cut lettuce (*Lactuca sativa* L.) stimulate phenolic metabolism, which leads to tissue browning. Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5.) is the first committed enzyme in the phenylpropanoid pathway and regulates its overall activity. Maximum activity of wound-induced PAL occurred sooner as the storage temperature increased from 0 to 25 °C, but the maximums were lower. A heat shock at 50 °C for 90 seconds protected fresh-cut lettuce tissue against browning, helped retain greenness, and decreased subsequent production of phenolics when applied either after or before wounding. Browning was reduced when the heat shock was applied up to 36 hours after wounding, while the maximum effect occurs around 6 hours before cutting. Like the heat-shock treatment, the protein synthesis inhibitor cycloheximide reduced wound-induced PAL activity, but it did not prevent tissue browning. When cycloheximide was applied in combination with heat-shock treatments, browning did not occur. Heat shocks may control tissue browning by more than just interfering with protein synthesis.

Preparation of fresh-cut fruit and vegetables necessarily involves physically injuring the tissue. This unavoidable trauma causes both an immediate and a subsequent physical response that alters the physiological activities and subsequent quality of the product (Brecht, 1995; Saltveit, 1997; Watada et al., 1990). Enzymatic and nonenzymatic reactions lead to browning of many foods (Iyengar and McEvily, 1992). Browning is beneficial when it contributes to the desirable color and flavor of such products as raisins, prunes, coffee, tea, and cocoa (Mayer and Harel, 1979; Vamos-Vigyazo, 1981). However, for other products, browning usually impairs their quality because of associated changes in color, flavor, aroma and texture (Hall, 1989; Huxsoll et al., 1989; Shewfelt, 1986).

In lettuce (*Lactuca sativa*) leaf tissue, the intensity of browning has been correlated with both an increase in phenylpropanoid metabolism and a decrease in final visual quality (Couture et al., 1993). Browning involves a complex set of interrelated metabolic reactions (Sapers, 1993). Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5.) is the first committed enzyme in the phenylpropanoid pathway and regulates its overall activity (Dixon and Paiva, 1995). PAL activity is increased by a number of biotic and abiotic stresses. The most important factors that determine the rate of enzymatic browning of fruit and vegetables are the concentration of phenolic compounds in the tissue, activity of polyphenol oxidase (PPO, EC 1.10.3.1), tissue pH, temperature, and oxygen availability (Martinez and Whitaker, 1995; Rhodes and Woollorton, 1978). All of the PPOs discovered have the ability to convert o-dihydroxyphenols to o-benzoquinones using O₂ as the second substrate (catecholase activity) (Fujita et al., 1991). The presence of a wide range of o-dihydroxyphenols in plant tissue constitutes a great deal of potential for browning (Strack, 1997). Quinones condense and react nonenzymatically with other phenolic compounds, amino acids, and other constituents to produce pigments of indeterminate structure.

Browning can be effectively inhibited by sulfites, but the hazard of allergic-like reactions in sulfite-sensitive asthmatics currently restricts their use (U.S. Food and Drug Administration, 1986, 1990). The search for alternative browning inhibitors has stimulated considerable research. Ascorbic acid-based browning inhibitors are

usually not as effective as sulfites because of the greater stability and better penetration of the latter (Sapers and Ziolkowski, 1987). A novel approach for the control of PPO in vivo is the use of antisense RNA techniques (Bird and Ray, 1991), which has resulted in about 70% of the transformed plants showing lower PPO activity when compared to controls. Other researchers have detected the existence of a natural PAL inactivating system in potato (*Solanum tuberosum* L.) (Zucker, 1968), sunflower (*Helianthus annuus* L.) (Creasy, 1976; Gupta and Creasy, 1991), and lettuce (Ritenour and Saltveit, 1996). Methods to rapidly induce this PAL inactivating system should reduce subsequent browning.

Since lettuce tissue browning requires the wound-induced synthesis of certain proteins, chemical inhibitors of protein synthesis could be effective at reducing browning by interfering with the synthesis of enzymes involved in phenolic metabolism. However, their toxic nature automatically precludes their use on foods. Other less restrictive methods also have the ability to reduce protein synthesis. Brief abiotic shocks (e.g., heat shocks) are known to interfere with 'normal' protein synthesis by preferentially inducing the synthesis of a unique set of stress proteins (Saltveit, 2001). To date, postharvest heat treatments have been applied for extended periods as quarantine treatments to eliminate insect larvae or as alternatives to postharvest chemical dips and sprays (Klein and Lurie, 1992). Blanching at >50 °C inactivates PPO, but it may produce undesirable changes in color, flavor, and texture in some crops (Klein and Lurie, 1991; Ma et al., 1992). A less stringent, brief heat-shock treatment delays the browning of lettuce midrib tissue without causing any undesirable changes in appearance or texture (Loaiza-Velarde et al., 1997).

The object of this research was to evaluate the effect of applying a brief heat shock to lettuce tissue before and after it was wounded by cutting. We also compared the effect of chemical protein-synthesis inhibitors to the effects of heat-shock treatments on the quality of fresh-cut lettuce.

Materials and Methods

PLANT MATERIAL. Freshly harvested iceberg lettuce was obtained from a local wholesale market, transported to the Mann Laboratory, University of California, Davis, and kept at ≈0.5 °C until used. Outer and damaged leaves were discarded and only the

next approximately eight sound leaves were carefully separated from the head for use in experiments. Leaves were excised starting 3 cm away from their base and either used whole or cut into 2 × 2-cm pieces according to the needs of each experiment.

TREATMENTS. Cut lettuce midrib tissue segments were randomly selected and stored at 0, 5, 10, 15, 20, or 25 °C. The tissue was kept under a flow of humidified air (≈90% relative humidity) at a rate sufficient to prevent the accumulation of CO₂ above 0.15%. Replicate samples were withdrawn at different times and assayed for PAL activity or frozen at –80 °C until analyzed.

Heat shocks were applied 0, 12, 24, 36, or 48 h after wounding by immersing the leaf pieces in a model 9001 Fisher Scientific (San Francisco, Calif.) hot-water bath at 50 °C for 90 s. Following the heat shocks, the tissue was placed in 10 °C deionized water for 30 s, and then centrifuged for 15 s in a home salad spinner to remove excess water. The tissue was then put into 200-mL jars and held at 10 °C in airtight metal boxes through which a flow of ethylene-free, humidified air was maintained at a sufficient rate to keep the CO₂ level below 0.15%. Replicated samples were assayed for PAL activity, phenolic content, and L* and a* values at various times after the heat-shock treatment, or frozen at –80 °C until analyzed.

Lettuce tissue was heat shocked before wounding by immersing excised whole leaves in water at 50 °C for 90 s as in the previous experiment. After 0, 2, 6, 12, 24, or 36 h following heat shock, 2 × 2-cm pieces of midrib tissue were excised starting 6 cm away from the original site of excision. The wounded tissue was placed into 200-mL jars and held at 10 °C as previously described.

Lettuce leaf pieces were exposed to 0.0 or 8 mM aqueous solutions of the protein synthesis inhibitor cycloheximide (CHX; Sigma Chemical Co., St. Louis, Mo.) by completely submerging 40 g of the 2 × 2-cm segments in 250 mL of the 10 °C solutions for 30 min with shaking. The treatments were applied 0, 2, 6, 12, 24, or 36 h after wounding. After immersion, the tissue was centrifuged in a salad spinner for 1 min to eliminate excess solution and held at 10 °C, as previously described, until analyzed.

PHENOLIC COMPOUND DETERMINATION. Phenolic compounds were extracted as previously described Ke and Saltveit (1988); 10 g of tissue was homogenized with 20 mL of methanol using an Ultra-Turrax tissue homogenizer (Takmar, Cincinnati, Ohio) at moderate speed for 30 s. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000 *g*_a for 15 min. The supernatant was used directly to measure the browning potential and soluble o-quinones of lettuce tissue. The absorbance of an aliquot of the supernatant was read at 320 nm to determine browning potential, and at 437 nm to determine the relative concentration of soluble o-quinones with an UV-VIS recording spectrophotometer (UV-160A; Shimadzu Scientific Instrument, Columbia, Md.).

EVALUATION OF a* AND L* VALUES. The a* and L* values were determined from the remnants of the filtration for the phenolic extraction. They were placed in a transparent, multiwell tissue culture plate. Upon drying, a* and L* values were measured

with a chromameter (CR-200; Minolta Camera Co., Palo Alto, Calif.) after calibrating with a white plate (L* = 97.63; a* = –0.53; and b* = 2.38).

ENZYME ASSAY. PAL activity was assayed as previously described (Ke and Saltveit, 1986).

STATISTICAL ANALYSIS. Results reported in this paper are means of duplicate samples ± SD. All experiments were performed more than once. Even though slight variations existed from experiment to experiment, identical trends were detected.

Results and Discussion

Maximal wound-induced PAL activity of $0.10 \pm 0.06 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ was reached in ≈6 h at 20 or 25 °C (Fig. 1). At 5 °C, maximal wound-induced PAL activity of $0.18 \pm 0.02 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ occurred at 24 h, while maximums at 0.17 ± 0.03 and $0.16 \pm 0.01 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ occurred after 12 h at 10 °C and after 6 h at 15 °C, respectively. PAL activity continued to increase at 0 °C for the 64-h duration of the experiment. Although maximal PAL activity occurred faster at the higher temperatures, the peaks were smaller than at the lower temperatures and there was a decline to initial levels of activity by 18 h at 20 or 25 °C, 30 h at 15 °C, and 64 h at 10 °C. The decline occurred more slowly at 5 °C, and not at all at 0 °C.

Wound-induced PAL activity increased in a roughly linear fashion for the first 24 h after wounding at 5 °C, and then declined slightly for the next 12 h (Fig. 2). This decline after 24 h is coincident with the previously reported time required for induction of the PAL inactivating factor (Ritenour and Saltveit, 1996). A heat shock (i.e., 50 °C for 90 s) was applied 1, 8, or 24 h after wounding. The heat shock at 1 h had no immediate effect on PAL activity, but prevented any further increase in activity, which remained constant at $0.06 \pm 0.02 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ for the duration of the experiment. In contrast, heat shocks applied 8 or 24 h after

Fig. 1. Effect of storage temperature on fresh-cut lettuce, evaluated as differences in PAL activity (μmol cinnamic acid $\text{g}^{-1}\cdot\text{h}^{-1}$). PAL activity was periodically assayed during 64 h of storage. Symbols represent means ($n = 6$) ± SD, SD bars are present only when larger than the symbols.

Fig. 2. Effect of heat-shock treatment (50 °C, 90 s) applied after wounding on subsequent development of PAL activity (mmol cinnamic acid g⁻¹·h⁻¹) at 5 °C. Symbols represent means (n = 6) ± SD.

wounding resulted in immediate decreases in PAL activity from 0.15 ± 0.02 to 0.09 ± 0.02 $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ and from 0.33 ± 0.02 to 0.18 ± 0.03 $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, respectively. The subsequent fairly stable rates persisted for the duration of the experiment. The magnitude of the decrease in PAL activity following the heat shock at 8 or 24 h was about 40% from the control rate induced by wounding. There was no drop in PAL activity following a heat shock at 36 h, but the rate of activity remained stable after the heat shock (data not presented).

About 40% of the PAL synthesized in response to wounding appears to be sensitive to the heat shock, as shown by the immediate reduction in activity following the heat shock, while the other 60% appears resistant, as shown by the stable rate after the heat shock (Fig. 2). Different isozymes of PAL could be synthesized that have different thermal stabilities, or the synthesized PAL could

Fig. 3. Effect of heat-shock treatment (50 °C, 90 s) applied 0, 12, 24, 36, or 48 h after wounding. Affected quality was evaluated as changes in (A) L* value, (B) a* value, (C) browning potential (absorbance at 320 nm), and (D) accumulation of soluble o-quinones (absorbance at 437 nm) 3 d after receiving the treatment. Symbols represent means (n = 6) ± SD, SD bars are present only when larger than the symbols.

Fig. 4. Effect of a heat-shock treatment (50 °C, 90 s) applied before wounding on the subsequent development of PAL activity (mmol cinnamic acid g⁻¹·h⁻¹) at 5 °C. Symbols represent means (n = 6) ± SD.

be sequestered in different cellular compartments whose internal composition alters the thermal sensitivity of the enzyme. A number of PAL isozymes are synthesized by plant tissues, and many of them have slightly different enzyme kinetics and cellular distribution (Appert et al., 1991; Neumann et al., 1991; Sarma et al., 1998).

There was a slight increase in subsequent tissue browning reactions and loss of quality when the heat shock was delayed for up to 36 h following wounding (Fig. 3A–D). A much greater change occurred when the heat shock was delayed 48 h. Tissue browning (i.e., lower L* value) (Fig. 3A), loss of green color (i.e., higher a* value) (Fig. 3B), and accumulation of soluble o-quinones (Fig. 3D) showed only slight changes when the heat shock was

Fig. 5. Effect of heat-shock treatment (50 °C, 90 s) applied 2, 6, 12, 24, or 36 h before wounding. Affected quality was evaluated as changes in (A) L* value, (B) a* value, (C) browning potential (absorbance at 320 nm), and (D) accumulation of soluble o-quinones (absorbance at 437 nm) 3 d after receiving the treatment. Symbols represent means (n = 6) ± SD, SD bars are present only when larger than the symbols.

Fig. 6. Effect of cycloheximide treatment (8-mM solution for 30 min) on PAL activity in fresh-cut lettuce. PAL activity ($\mu\text{mol cinnamic acid g}^{-1}\text{h}^{-1}$) was evaluated 24 h after the treatment was received. Symbols represent means ($n = 6$) $\pm$ SD.

delayed 0, 12, 24, or 36 h after cutting. A much greater change occurred in the browning potential of the tissue (Fig. 3C) under similar conditions. All four measures showed a rapid change between the 36 h and 48 h treatment. Significantly, these rapid detrimental changes in all four measures of quality seemed to lag behind the maximum increase in PAL activity (Fig. 1) by about 24 h at 5 °C. Keeping the delay between wounding and heat shock to within 36 h maintained the greatest benefit from the heat shock.

When the heat shock was applied to whole leaves of lettuce before wounding, the wound-induced increase in PAL activity was suppressed for at least 36 h at 5 °C (Fig.

Fig. 7. Evaluation of the effect of cycloheximide treatment (8-mM solution for 30 min) applied at 0, 2, 6, 12, 24, or 36 h after wounding. Affected quality was assessed as changes in (A) L* value, (B) a* value, (C) browning potential (absorbance at 320 nm), and (D) accumulation of soluble o-quinones (absorbance at 437 nm) 3 d after receiving the treatment. Symbols represent means ($n = 6$) $\pm$ SD, SD bars are present only when larger than the symbols.

Fig. 8. Comparison of the effects of heat shock (HS) (50 °C, 90 s), cycloheximide (CHX) (8-mM solution for 30 min), and the combination of both treatments on PAL activity in wounded lettuce tissue. PAL activity ($\text{mmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ cinnamic acid) was assayed 24 hours after the treatment. The line on top of each bar represents SD ($n = 6$).

4); longer times before wounding were not tested. Wounding increased PAL activity about 3-fold from 0.03 ± 0.004 to $0.10 \pm 0.005 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ in 24 h, while tissue previously given the heat shock up to 36 h before wounding maintained PAL activity near the level found in the nonwounded control ($0.02 \pm 0.005 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$).

Not all the measures of lettuce quality were equally affected by the heat shock (Fig. 5). An increase in browning (i.e., the decline in L^*) started at -6 h and continued to -36 h (Fig. 5A). Loss of greenness (i.e., larger a^* values) started at -2 h and continued to -36 h (Fig. 5B). Both browning potential (Fig. 5C) and soluble o-quinones

Fig. 9. Effects of heat shock (50 °C, 90 s), cycloheximide (8 mM, 30 min), and the combination of both treatments on the quality of fresh-cut lettuce. Affected quality was evaluated as changes in (A) L^* value, (B) a^* value, (C) browning potential (absorbance at 320 nm), and (D) accumulation of soluble o-quinones (absorbance at 437 nm) 3 d after receiving the treatment. The line on top of each bar represents SD ($n = 6$).

(Fig. 5D) started to increase at -6 h and reached constant levels at -24 h. Although the heat shock delayed the increase in wound-induced PAL activity when applied up to 36 h before wounding (Fig. 4), the other aspects of the wound response were not inhibited as much and recovered earlier than PAL synthesis (Fig. 5A-D). Application of the heat shock before wounding allowed some of the wound-induced responses to recover and responded similarly to the wounded control tissue that was not heat shocked.

If the heat-shock treatment prevents the increase in wound-induced PAL activity and browning by interfering with protein synthesis, then an inhibitor of protein synthesis such as CHX should show a similar effect. Preliminary experiments with various concentrations of CHX showed that 8.0 mM was effective at reducing protein synthesis (data not presented) and the wound-induced synthesis of PAL (Fig. 6). The effectiveness of the CHX treatment depended on the time of application. The greatest control occurred when treatment was within 2 h of wounding. PAL activity in tissue treated with CHX 2 h after wounding doubled from 0.02 ± 0.003 to $0.04 \pm 0.006 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ within 24 h of wounding, while PAL increased 6-fold to $0.12 \pm 0.006 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ in nontreated, wounded tissue. Extending the time between wounding and treatment with CHX to 6, 12 and 24 h resulted in a progressive decrease in its effectiveness, such that there was no statistical difference between the wounded treated and nontreated tissue when treatment was delayed 24 h.

Even though CHX reduced the increase in wound-induced PAL activity when applied close to the time of wounding (Fig. 6), it also caused tissue browning (Fig. 7A). CHX may be inducing some aspects of phenylpropanoid metabolism since there was a significant increase in browning. No matter when the CHX treatment was applied, all the treated tissue lost green color (a^*), turned brown (L^*), accumulated phenolic compounds, and progressively converted them into soluble o-quinones to a greater extent than the wounded tissue not treated with CHX (Fig. 7A-D). Every CHX treatment increased the level of soluble o-quinones from 3- to 7-fold over the wounded control, while they increased browning potential about 35% over the wounded control.

Wounding caused a 7-fold increase in PAL activity which was reduced by over 50% to a 2.7-fold increase by immediately treating the wounded lettuce with CHX (Fig. 8). The heat shock applied at the time of wounding reduced the rise in wound-induced PAL activity to only 27% of that in wounded tissue (from 0.131 ± 0.006 to $0.024 \pm 0.003 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). When applied together, the heat shock and CHX treatments completely prevented any increase in PAL activity following wounding. The heat-shock treatment not only reduced the wound-induced increase in PAL activity, but it also helped maintain the four measured attributes of lettuce quality near their control, nonwounded levels (Fig. 9A-D). In contrast, CHX reduced the rise in wound-induced PAL activity, but it also reduced all four parameters of lettuce quality. These two treatments had opposite effects with regard to the four measurements of quality. When they were combined, the heat shock effect predominated over the CHX effect, and became the prevalent treatment with all its benefits. Once again, the major difference among the treatments was the large increase in soluble o-quinones in the tissue treated with CHX.

Treatment with CHX produced some unexpected results. Even while CHX reduced the wound-induced increase in PAL activity in lettuce tissue, it stimulated the accumulation of soluble o-quinones and tissue browning. The heat-shock treatment used

in this study reduced PAL activity and protected wounded lettuce tissue against browning. As a physical treatment, heat shocks proved to be more effective in preventing browning than chemical treatments with an inhibitor of protein synthesis (e.g., CHX), which caused browning. The window of time for the beneficial application of the heat-shock treatments to prevent wound-induced increases in PAL activity, accumulation of soluble o-quinones, and tissue browning was 24 h before and 12 h after wounding. Thus, heat shocks may control tissue browning in cut lettuce by more than just interfering with protein synthesis.

Literature cited

- Appert, C., E. Logemann, K. Hahlbrock, J. Schmid, and N. Amrhein. 1991. Structural and catalytic properties of the four phenylalanine ammonia-lyase isoenzymes from parsley (*Petroselinum crispum* Nym.). *European J. Biochem.* 225:491-499.
- Bird, C.R. and J.A. Ray. 1991. Manipulation of plant gene expression by antisense RNA. *Biotechnol. Genet. Eng. Rev.* 9:207-227.
- Brecht, J.K. 1995. Physiology of lightly processed fruits and vegetables. *HortScience* 30:18-22.
- Couture, R., M.I. Cantwell, D. Ke, and M.E. Saltveit. 1993. Physiological attributes and storage life of minimally processed lettuce. *HortScience* 28:723-725.
- Creasy, L.L. 1976. Phenylalanine ammonia-lyase-inactivating system in sunflower leaves. *Phytochemistry* 15:673-675.
- Dixon, R.A. and N.L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* 7:1085-1097.
- Fujita, S., T. Tono, and H. Kawahara. 1991. Purification and properties of polyphenol oxidase in head lettuce (*Lactuca sativa*). *J. Sci. Food Agr.* 55:643-651.
- Gupta, S.C. and L.L. Creasy. 1991. The effects of sunflower L-phenylalanine ammonia-lyase inactivating factor on *Rhodotula glutinis* L-phenylalanine ammonia-lyase. *Phytochem.* 30:85-88.
- Hall, G.C. 1989. Refrigerated, frozen and dehydrofrozen apples, p. 239-256. In: D.D. Downing (ed.). *Processed apple products*. Van Nostrand Reinhold, New York.
- Huxoll, C.C., H.R. Bolin, and A.D. King, Jr. 1989. Physicochemical changes and treatments for lightly processed fruits and vegetables, p. 203-215. In: J.J. Jen (ed.). *Quality factors of fruits and vegetables. Chemistry and technology*, ACS Symp. Ser. 405.
- Iyengar, R. and A.J. McEvily. 1992. Anti-browning agents: Alternatives to the use of sulfites in foods. *Trends Food Sci. Technol.* 3:60-64.
- Ke, D. and M.E. Saltveit. 1986. Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. *HortScience* 21:1169-1171.
- Ke, D. and M.E. Saltveit. 1988. Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. *Plant Physiol.* 88:1136-1140.
- Klein, J.D. and S. Lurie. 1991. Postharvest heat treatment and fruit quality. *Postharvest News Info.* 2:15-19.
- Klein, J.D. and S. Lurie. 1992. Heat treatments for improved postharvest quality of horticultural crops. *HortTechnology* 2:316-319.
- Loaiza-Velarde, J.G., F.A. Tomás-Barberá, and M.E. Saltveit. 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. *J. Amer. Soc. Hort. Sci.* 122:873-877.
- Ma, S., J.L. Silva, J.O. Hearnberger, and J.O. Garner, Jr. 1992. Prevention of enzymatic darkening in frozen sweet potatoes [*Ipomoea batatas* (L.) Lam.] by water blanching: Relationship among darkening, phenols, and polyphenol oxidase activity. *J. Agr. Food Chem.* 40:864-867.
- Martinez, M.V. and J.R. Whitaker. 1995. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* 6:195-200.
- Mayer, A.M. and E. Harrel. 1979. Polyphenol oxidases in plants. *Phytochem.* 18:193-215.
- Neumann, G., A. El-Aschker, and B. Schwemmler. 1991. L-Phenylalanine ammonia-lyase and chalcone synthase in seedlings of *Oenothera*:

- Plasmatype dependent regulation and tissue specific distribution. *J. Plant Physiol.* 138:263–269.
- Rhodes, J.M. and L.S.C. Wooldorton. 1978. The biosynthesis of phenolic compounds in wounded plant storage tissues, p. 243–285. In: Gunter Kahl (ed.). *Biochemistry of wounded plant tissues*, Walter de Gruyter & Co., Berlin/New York.
- Ritenour, M.A. and M.E. Saltveit. 1996. Identification of a phenylalanine ammonia-lyase inactivating factor in harvested iceberg lettuce (*Lactuca sativa* L). *Physiol. Plant.* 97:327–331.
- Saltveit, M.E. 1997. Physical and physiological changes in minimally processed fruits and vegetables, p. 205–220. In: F.A. Tomás-Barberán (ed.). *Phytochemistry of fruits and vegetables*. Oxford Univ. Press, Oxford, United Kingdom.
- Saltveit, M.E. 2001. Heat shock prevents tissue browning by redirecting stress induced protein synthesis. *Postharvest Biol. Technol.* (in press).
- Sapers, G.M. 1993. Browning of foods: Control by sulfites, antioxidants, and other means. *Food Technol.* 68:75–84.
- Sapers, G.M. and M.A. Ziolkowski. 1987. Comparison of erythorbic and ascorbic acids as inhibitors of enzymatic browning in apple. *J. Food Sci.* 52:1732–1747.
- Sarma, A.D., S. Yellamraju, S. Rameshwar, Y. Sreelakshmi, and R. Sharma. 1998. Differential expression and properties of phenylalanine ammonia-lyase isoforms in tomato leaves. *Phytochemistry* 49:2233–2243.
- Shewfelt, R.L. 1986. Flavor and color of fruits as affected by processing, p. 481–529. In: J.G. Woodroof and B.S. Luh (eds.). *Commercial fruit processing*. 2nd ed. AVI Publ. Co., Westport, Conn.
- Strack, D. 1997. Phenolic metabolism, p. 387–416. In: P.M. Dey and J.B. Harborne (eds.). *Plant biochemistry*. Academic Press, London.
- U.S. Food and Drug Administration. 1986. Sulfiting agents: Revocation of GRAS status for use on fruits and vegetables intended to be served or sold raw to consumers. U.S. Food Drug Admin. Fed. Reg. 51:25021–25026.
- U.S. Food and Drug Administration. 1990. Sulfiting agents: Revocation of GRAS status for use on “fresh” potatoes served or sold unpackaged or unlabeled to consumers. U.S. Food Drug Admin. Fed. Reg. 55:9826–9833.
- Vamos-Vigyazo, L. 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. *CRC Crit. Rev. Food Sci. Human Nutr.* 15:49–127.
- Watada, A.E., K. Abe, and N. Yamuchi. 1990. Physiological activities of partially processed fruits and vegetables. *Food Tech.* 44:116, 118, 120–122.
- Zucker, M. 1968. Sequential induction of phenylalanine ammonia-lyase and a lyase-inactivating system in potato tuber disks. *Plant Physiol.* 43:365–374.