ELSEVIER

Postharvest Biology and Technology 21 (2000) 61–69

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock

Mikal E. Saltveit *

Mann Laboratory, Department of Vegetable Crops, University of California, One Shields Ave., Davis, CA 95616-8631, USA

Received 4 May 2000; accepted 21 August 2000

Abstract

Wounding is one of many abiotic stresses that produce signals that propagate from injured into adjacent non-injured tissues and induce the de novo synthesis of specific wound-induced proteins. Some of these induced proteins are enzymes of phenolic metabolism, such as phenylalanine ammonia-lyase (PAL), whose increased activity leads to the accumulation of phenolic compounds (e.g., chlorogenic acid, dicaffeoyl tartaric acid and isochlorogenic acid) and tissue browning. Wounding of iceberg lettuce leaves increases PAL activity six- to 12-fold over 24 h at 10°C and leads to a three-fold increase in the total phenolic content within 3 days. There may be a hierarchical order to the plant's response to different abiotic stresses. Plant tissue simultaneously exposed to a heat shock and wounding responds to the heat shock in preference to wounding by producing heat shock proteins instead of PAL. A 90 s, 45°C heat shock prevents an increase in PAL activity if administered either 4 h before or 2 h after wounding. This diversion of wound-induced protein synthesis by heat shock might be used to prevent browning in other crops that normally have low phenolic content; e.g., celery and lettuce. The persistence of the ability of a heat shock to preferentially induce the synthesis of heat shock proteins (hsps) in place of wound-induced enzymes of phenylpropanoid metabolism offers a new way to control browning in lightly processed fruits and vegetables. The design of processing lines using a heat shock to extend the shelf-life of fresh-cut lettuce will need to be modified from existing designs to take full advantage of the effect of the heat treatment.

Keywords: Fresh-cut; Lettuce; Phenols; Phenylpropanoid metabolism; Quality; Wounding

1. Introduction

The mechanical and physical stresses used to segment whole commodities during the preparation of fresh-cut produce create a wound signal that migrates into adjacent, uninjured tissue and induces a number of physiological responses (Saltveit, 1997). Fresh-cut produce comprises fruits and vegetables that have been cut into small, easily prepared and/or consumed pieces. One of the most detrimental changes induced by wounding is the induction of phenylpropanoid metabolism that results in the accumulation of phenolic compounds and subsequent tissue browning. Browning of fresh fruits and vegetables

* Tel.: +1-530-7521815; fax: +1-530-7524554.
E-mail address: mesaltveit@ucdavis.edu. (M.E. Saltveit).

0925-5214/00/$ - see front matter
PII: S0925-5214(00)00165-4

reduces quality (Shewfelt, 1987) and is often the factor limiting shelf-life and marketability of fresh-cut lettuce (Bolin and Huxsoll, 1991; Couture et al., 1993; Lopez-Galvez et al., 1996a).

Wounding (e.g., cutting, cracking or breaking) of lettuce produces a signal that migrates through the tissue and induces the synthesis of enzymes in the metabolic pathway responsible for increased production of phenolic compounds and browning (Ke and Saltveit, 1989; Lopez-Galvez et al., 1996b; Peiser et al., 1998). Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) is the first committed enzyme of the primary phenolic pathway, and its activity controls the rate through the pathway. Caffeic acid is produced from phenylalanine via cinnamate and *p*-coumarate and conjugates with quinic acid to form chlorogenic and isochlorogenic acid, and with tartaric acid to form caffeoltartaric and dicaffeoltartaric acids (Tomás-Barberán et al., 1997). These four phenolic compounds accumulate in fresh-cut lettuce, and are associated with subsequent tissue browning.

There are many points in the sequence from wounding to browning (Fig. 1) where this process could be interrupted. For example, the synthesis or propagation of the wound signal could be stopped, recognition and response to the signal could be lessened, and transcription and translation of the gene for PAL could be diminished. PAL activity could also be lessened by sequentially inducing the synthesis of the PAL-inactivating factor (PAL-IF). Increase in the PAL-IF lags about 12 h behind the induction of PAL and this lag allows sufficient PAL activity to accumulate to make enough phenolic compounds to cause browning (Ritenour and Saltveit, 1996). Fusion of the PAL-IF gene to the same wound promoter that induces PAL could be used to eliminate this lag and could possibly prevent the transitory rise in PAL activity. Membrane integrity could be strengthened with the application of calcium salts, activity of polyphenol oxidase (PPO) and/or peroxidase (POD) could be reduced with chemical inhibitors, and finally oxygen could be excluded. Some of the current treatments used to control tissue browning in fresh cut horticultural commodities are based on these ideas and include cold storage, storage under reduced oxygen and elevated carbon dioxide atmospheres, and the application of chemical anti-oxidants.

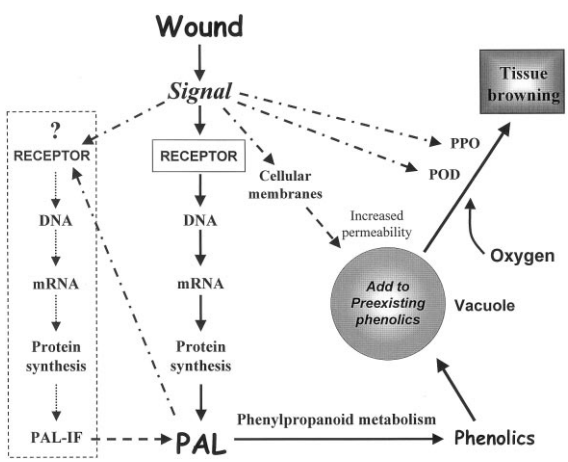

Fig. 1. The interrelationships between wounding lettuce leaf tissue and the subsequent induced changes in phenolic metabolism that leads to tissue browning. Many control points for postharvest modification of the wound-induced browning processes are evident in this diagram.

2. Wound-induced synthesis of PAL

Wounding iceberg lettuce leaves by cutting them into 2×2 cm pieces induces the synthesis of PAL and increases its activity six- to 12-fold within 24 h (Fig. 2; and Ke and Saltveit, 1989). By 48 h after wounding the content of phenolic compounds in a tissue extract and its absorbance at 320 nm had also increased. A 90 s heat shock at 45°C effectively prevented these wound-induced changes. Exposure to hormonal levels of ethylene also leads to an increase in PAL activity, but the rate of increase following wounding is much faster than from exposure to ethylene (Ke and Saltveit, 1989). Work with inhibitors of ethylene action (e.g., 1-MCP) also showed that ethylene was not involved in the wound response in lettuce (data not shown). This suggests that wounding does not act through the induction of ethylene synthesis, which is low and transitory, but through some other signal to increase PAL activity.

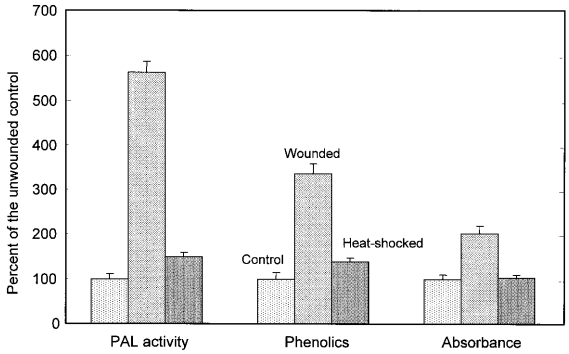

Fig. 2. The effect of wounding and heat shocking (45°C for 90 s) on the development of phenylalanine ammonia-lyase (PAL) activity (μmol cinnamic acid produced/g/h) after 24 h, and the concentration of total phenolic compounds (μg/g), and absorbance at 320 nm (a measure of browning) in iceberg lettuce tissue after 28 h. The line on top of each bar represents the standard deviation of that mean. (Redrawn from Saltveit, 1998).

Four major phenolic compounds are induced by either wounding or exposure to hormonal levels of ethylene (10 μl l^{-1} ethylene in air) in whole heads and excised midrib sections of iceberg, butter leaf and romaine lettuce (Tomás-Barberán et al., 1997). After 3 days, 5-caffeoylquinic acid (chlorogenic acid), 3,5-dicaffeoylquinic acid (isochlorogenic acid), caffeoyltartaric acid, and dicaffeoyltartaric acid were the predominant phenolic compounds. Of these four compounds, chlorogenic acid accumulated to the highest level in all three lettuce types; however, wounding did not induce the synthesis of caffeoyltartaric acid in iceberg lettuce. Similar kinetics for the induction and relative phenolic compositions were detected in the three lettuce types studied, although concentrations among the lettuce types differed by almost two-fold. These data suggest that the mechanism by which wounding and ethylene induces phenylpropanoid synthesis may be common for the different lettuce types, but that there are differences in the fine control of the rate of synthesis and the accumulation of phenolic compounds. These differences, though small, may be responsible for the observed differences in browning potential among various lettuce types and cultivars (Couture et al., 1993; Lopez-Galvez et al., 1996a,b; Tomás-Barberán et al., 1997).

Since browning of lettuce is the result of an active inductive process, requiring the de novo synthesis of PAL and the accumulation of phenolic compounds, rather than a passive oxidation of pre-existing phenolic compounds, browning could be effectively prevented by interfering with the wound-inductive process. The two pivotal steps in this process appear to be creation of the wound signal and the subsequent synthesis of PAL (Fig. 1).

The initial physiological steps following wounding of plant tissue and the formation of a wound signal are incompletely understood (Peña-Cortes and Willmitzer, 1995). Products of lipid metabolism and oxidation, e.g., jasmonic acid, are currently thought to be major constituents of the plethora of possible wound signals in plants. Other candidates for the wound signal include chemical compounds such as ethylene, systemin, ABA, salicylic acid, elicitors; and physical changes such as electric and hydraulic waves. Although many of these candidates are very potent wound signals in specific plants and tissues, they are inactive in others. The possibility that the wound signal in lettuce is related to one of these commonly accepted signaling molecules has been examined. Neither ABA, ethylene, methyl jasmonate, or salicylic acid appear to be the wound signal in lettuce (Ke and Saltveit, 1988, 1989; Campos-Vargas and Saltveit, 2000; data not shown). Whatever the signal may be, it is becoming increasingly obvious that a better understanding of the induction, movement and perception of the wound signal is necessary to reduce its impact on the quality of the processed product.

Wound-induced synthesis of PAL could be reduced with general inhibitors of protein synthesis such as cycloheximide (CHX), or by diverting the synthetic capacity of the cell to the production of other proteins. The first approach is theoretically interesting, but not practical since no protein synthesis inhibitor has been approved for use on foods. An 8 μM aqueous solution of CHX did reduce wound-induced PAL activity in mid-rib segments of lettuce, but it had to be applied within a few hours of wounding for maximal effect (Loaiza-Velarde and Saltveit, 2000).

The capacity of a cell to synthesize proteins, although plastic, is finite and any process that preferentially occupied the synthetic capacity of the cell would by necessity prevent the synthesis of other proteins. This approach could be used to lessen the wound response if the wound-induced cell could be induced to preferentially synthesize some other proteins. Fortunately, there does appear to be a hierarchical order in the response of plant cells to certain abiotic stresses with the response to some stresses superceding and being preferentially expressed in relation to other stresses. This idea is diagrammatically represented in Fig. 3. In non-stressed tissue, the total protein synthetic capacity of the cell is occupied by the production of proteins from existing mRNAs (i.e., proteins A, B, C, and D from mRNAs A, B, C, and D) (Fig. 3A). Wounding produces a signal that causes the transcription of PAL mRNA and its translation into PAL (Fig. 3B). Wound-induction of PAL synthesis occupies a small portion of the total synthetic capacity of the cell, whereas the induction of the synthesis of heat shock proteins (hsps) occupies most of the synthetic capacity (Fig. 3C). In heat-shocked tissue, the existing mRNAs are sequestered in heat-shock granules (HSG). When confronted with both a wound and heat shock, the cell appears to preferentially synthesize hsps, and this could lead to a loss in capacity to synthesize PAL (Fig. 3D). In this scenario, the wound signal dissipates before the tissue recovers from the heat shock signal and the mRNAs are released from the HSG. Therefore, by the time normal protein synthesis resumes there is no wound signal left to induce the synthesis of PAL (Fig. 3D). Experimental data presented below support this scenario.

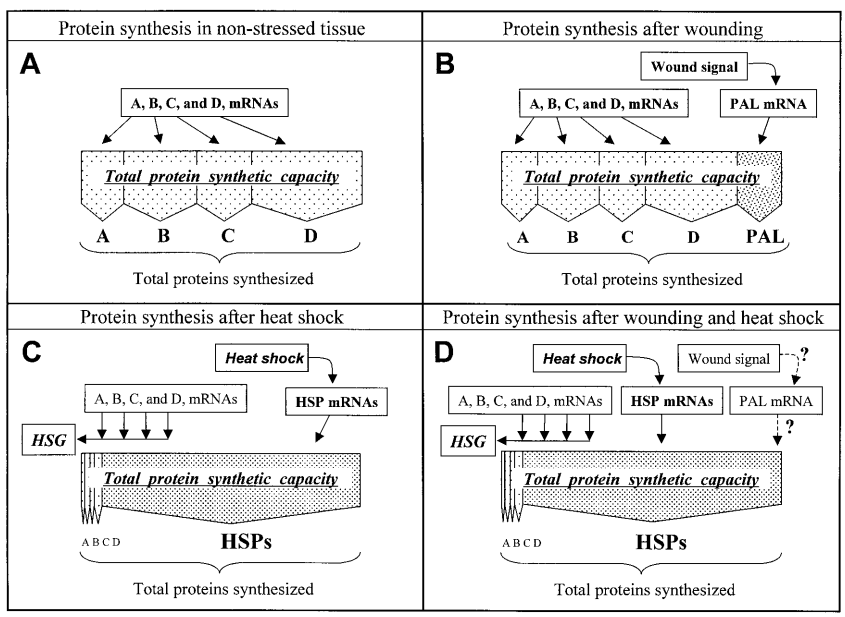

Fig. 3. Diagram showing the scenario in which the synthesis of wound-induced PAL is prevented by induction of the synthesis of heat shock proteins (hsps). The total protein synthetic capacity of the cell is occupied by the synthesized proteins A, B, C, and D from mRNAs A, B, C, and D (A). The synthesis of wound-induced PAL from PAL mRNA occupies a small fraction of the total synthetic capacity of the cell and slightly reduces the synthesis of the other proteins (B). In contrast, the synthesis of hsps occupies most of the total synthetic capacity of the cell (C), and pre-existing mRNAs are sequestered in heat-shock granules (HSG) with an almost complete cessation of their translation into proteins. When simultaneously wounded and heat shocked, cells preferentially synthesise hsps and no synthetic capacity is left for the synthesis of wound-induced PAL (D). The question marks between the wound signal and PAL mRNA and between PAL mRNA and protein synthesis indicate an incomplete understanding of the interaction between these events and the heat shock response.

3. Heat shocks and protein synthesis

Exposure of plant tissue to temperatures about 10°C above the normal growing temperature induces the synthesis of a unique set of proteins called heat shock proteins (hsps). This response is ubiquitous to microorganisms, plants and animals, and protects the induced tissue from subsequent high temperature stress (Vierling, 1991). It has long been known that the induction and synthesis of hsps is accompanied by a general inhibition of normal protein synthesis (Zhang et al., 1984; Ougham and Stoddart, 1986; Brodl, 1989; Fourre and Lhoest, 1989; Somers et al., 1989).

The alteration in translation during heat stress is controlled differently in plants than in other eukaryotic organisms. In plants the repressed mRNAs are dissociated from polysomes and are transiently stored in the cytoplasm (Nover et al., 1983, 1989; Apuya and Zimmerman, 1992). This translation reprogramming occurs immediately upon heat-stress, with protein synthesis shifting almost exclusively to the production of hsps (Lindquist, 1981, 1986; Nover, 1991; Sierra and Zapata, 1994; Brostrom and Brostrom, 1998). Experiments with transcriptional and translational inhibitors showed that normal mRNAs are not degraded during heat shock, but are sequestered in some fashion so that they may be re-used when the heat shock response has dissipated (Barnett et al., 1980; Lindquist, 1981). For example, treatment with cycloheximide and with chloramphenicol demonstrated that hsp synthesis in spinach leaves was restricted to cytosolic ribosomes (Somers et al., 1989).

The exact mechanism by which the repressed mRNAs are sequestered and stored in heat shocked tissue is still unresolved (Stuger et al., 1999). Messages may be stored in RNA and protease-containing particles called proteasomes (Scherrer and Bey, 1994; Schmid et al., 1995), or in large RNA-containing particles called HSG (Nover et al., 1983, 1989). The HSG are rich in small hsps and appear after the heat stress has induced their synthesis and accumulation. Small hsps may therefore not only function as molecular chaperones (Jakob et al., 1993), but also as components of these protective HSG. In vitro translation of polysomes from stressed cells mainly yielded hsps, while in vitro translation of a cytoplasmic fraction rich in small hsps and mRNAs produced proteins similar to those encoded by polysomes from nonstressed cells (Nover et al., 1989). Upon recovery from the heat shock, the sequestered mRNAs are released and again function in protein synthesis.

Treatment temperature appears to be critical in suppressing normal protein synthesis while inducing the synthesis of hsps. For example, tobacco cell suspension cultures heat shocked at 38°C produced hsps without inhibition of the synthesis of normal proteins while at a heat shock of 42°C normal proteins were made at a reduced rate or not at all (Kanabus et al., 1984). Normal protein synthesis was also reported to continue with the synthesis of hsps in 3-day-old maize seedlings exposed to 40°C (Cooper and Ho-T, 1983). Other responses to heat shock include the rapid delamination and fragmentation of the endoplasmic reticulum lamellae on which the mRNA was translated in both barley aleurone layers and carrot root discs (Brodl, 1989).

Whether the mRNAs are sequestered with hsps in HSG or with proteases in proteasomes, the result is the same: the synthesis of normal proteins is repressed and the synthetic capacity of the cell is occupied with the synthesis of hsps. The diversion of protein synthesis, and not the synthesis and accumulation of specific hsps by themselves, has been proposed as the basis for some of the physiological effects of heat stress (Saltveit, 1997). This capacity for heat stress to divert protein synthesis and the hierarchical response of plants to specific stresses could provide a whole new method to control the response of plants to stresses. The plant's response to one stress, such as wounding, could be attenuated by the simultaneous imposition of another stress such as heat stress.

4. Effects of heat shock on wound-induced PAL

Wound-induced browning can be significantly reduced in iceberg lettuce by a short thermal

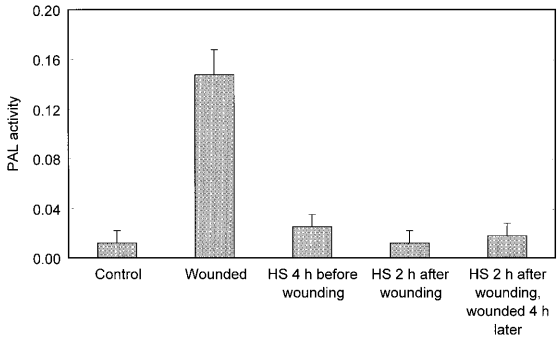

Fig. 4. The effect of wounding and the timing of the application of a heat shock (45°C for 90 s) to iceberg lettuce tissue on the development of phenylalanine ammonia-lyase (PAL) activity (μmol cinnamic acid produced/g/h) after 24 h. The heat shocks (HS) were applied 4 h before wounding, and 2 h after wounding. The tissue heat shocked 2 h after wounding was cut in half after an additional 4 h. The line on top of each bar represents the standard deviation of that mean. (Redrawn from Saltveit, 1998).

stress (Loaiza-Velarde et al., 1997). A heat shock of 45°C for 90 s effectively prevents the synthesis of PAL by wounded lettuce leaf tissue and its subsequent browning (Fig. 4). Inhibition of PAL synthesis appears to result from a redirecting of protein synthesis away from wound-induced proteins (e.g., PAL) to the synthesis of hsps. The heat shock does not act through interfering with the wound signal since it is effective when administered either 4 h before or 4 h after wounding. The heat shock effect was so persistent that fresh-cut iceberg lettuce did not show any browning even after being held for 15 days in air at 5°C.

However, the caveat must be emphasized that browning is prevented by the heat stress response only in tissue with initially low levels of phenolic compounds and low levels of phenylpropanoid enzyme activity. The slight heat stress that prevents the rise in PAL activity and browning in lettuce, unlike the much higher temperature treatments used in thermal denaturation of proteins and blanching, does not eliminate the activity of enzymes involved in tissue browning (e.g., PAL, PPO or POD) that are already present in the tissue (Loaiza-Velarde et al., 1997). Therefore, it does not work in lettuce or other tissues with constitutively or induced high levels of phenolic compounds or high levels of phenylpropanoid enzyme activity. Short heat-stress treatments have no effect on the browning of artichokes (which constitutively have high levels of phenolic compounds and phenylpropanoid enzyme activity), or in lettuce stressed in the field by disease or drought (which have high levels of phenolic compounds and phenylpropanoid enzyme activity) (data not shown).

The phenolic content of lettuce leaves varies with the type of lettuce (e.g., iceberg, butter leaf, Romaine), the type of stress (e.g., wounding and exposure to ethylene) and the temperature (Tomás-Barberán et al., 1997). As would be expected from an examination of the phenolic content of the different lettuces, iceberg is the least likely to brown while butter leaf is the most likely. The same pattern holds for wounded lettuce. The heat shock treatment that was previously described (Loaiza-Velarde et al., 1997), and is so effective in preventing PAL induction and browning in iceberg lettuce, was not as effective in delaying the browning of fresh-cut Romaine or butter leaf lettuces (data not shown). Whereas browning of fresh-cut iceberg lettuce could be delayed for 15 days at 5°C, browning of fresh-cut Romaine and butter leaf lettuces was only delayed 3 and 1.5 days, respectively. Alternative (e.g., higher temperatures, longer treatment time) and combined (e.g., antioxidants, low oxygen CA) treatments may be necessary to control browning in lettuce types that have higher levels of phenolic compounds and activities of enzymes of phenolic metabolism due to inherent differences or induced by prior stresses.

By the time the heat shock response has dissipated and normal protein synthesis resumes, the wound signal appears to have also dissipated and is no longer capable of inducing the wound response. Wounding induces elevated levels of PAL in lettuce tissue that has recovered normal protein synthesis after a heat shock. Therefore, if the initial wound signal was still present upon recovery from the heat shock, PAL synthesis should have been observed. The heat shock does

not appear to destroy the wound signal, nor the capacity of the tissue to make a wound signal. Heat shocking the cut edge of a lettuce leaf segment prevents those cells from producing PAL, but it does not prevent adjacent cells a few millimeters away that were not heat shocked from responding to the wound signal diffusing from the cut edge and increasing PAL activity.

5. Effects of heat shock on chilling injury

One of the earliest proposals for the basis of chilling injury was altered metabolism and the accumulation of phytotoxic compounds (Molisch, 1896; Pentzer and Heinze, 1954; Saltveit, 2000). Chilling alters the rate and products of many metabolic reactions and induces the synthesis of specific enzymes and isoenzymes (Saltveit, 2000). Certain treatments, such as intermittent warming, appear to reduce the development of chilling injury because they facilitate the metabolism of these accumulated toxic compounds and allow repair of damaged cellular components. Altered metabolism, as the result of chilling, was the only theory discussed at length in an early review on chilling (Pentzer and Heinze, 1954), and it was a major component of the membrane phase transition hypothesis presented in 1973 by Lyons (Lyons, 1973).

A short heat shock (Lafuente et al., 1991; Collins et al., 1993; Jennings and Saltveit, 1994) or prolonged heat treatment (Whitaker, 1994; Sabehat et al., 1995) confers chilling tolerance in a number of plant tissues. The protective effect of a short heat stress could be through the interruption of these altered normal metabolic processes or through inhibiting the synthesis of enzymes related to deleterious pathways, rather than through the synthesis of protective levels of some specific hsps. Although the appearance of hsps is correlated with the acquisition of tolerance to both high (Vierling, 1991) and chilling (Lafuente et al., 1991; Collins et al., 1995; Sabehat et al., 1995) temperatures, the ability of a heat shock to prevent the synthesis of proteins induced by the second stress may be as important as its ability to induce the synthesis of hsps.

6. Possible disadvantages of heat shock treatments

Elevated temperatures stimulate respiration and the accelerated respiration at the heat shock temperature could reduce shelf-life. However, the short period of exposure (less than 2 min) and the rapid cooling to 0°C after the heat treatment should minimize the effect in lettuce. This is not the case when whole fruit are held at 38°C for around 3 days to increase their subsequent chilling tolerance (Sabehat et al., 1995). In that case, the significant increase in respiration during the prolonged treatment at the higher inductive temperature would have to be offset by a significantly reduced rate of respiration and increased shelf-life at previously chilling temperatures.

7. Commercial implication

The preparation of fresh-cut lettuce is currently done as follows. Freshly harvested lettuce is cooled and then manually trimmed of unwanted outer leaves. The cored and trimmed heads are mechanically chopped into salad pieces that are washed and then dried to remove excess moisture. Drying can be done with forced air, but most processing lines use a batch centrifugation step. The lettuce pieces are flumed into large, perforated stainless-steel baskets that are placed into centrifuges and spun at high rates of speed to remove excess water. The high centrifugal force not only removes water, but also cracks and crushes the tissue. An examination of packaged fresh-cut lettuce will reveal many pieces that contain cracked and crushed tissue. Damaged tissue segments in these packages are usually the ones that show extensive browning and decay. The de-watered lettuce is then packed in plastic film bags that are purged with an atmosphere low in oxygen and high in carbon dioxide before being sealed. The bags are made of plastics with specific permeability properties so that the combination of tissue respiration and gas diffusion maintains the desired atmosphere within the package. All these steps should be done as close to 0°C as possible to maintain quality and optimize shelf-life.

The ability of a heat shock to control browning when administered either before or after the preparation of fresh-cut lettuce has some added benefits besides controlling browning. After the heat treatment, the lettuce is warm and wet. Water at 45°C is half as viscous as water at 0°C and will therefore drain away faster and to a greater extent than water at 0°C. Lower centrifugal forces could be used to de-water the lettuce and thereby reduce tissue damage. However, the de-watered lettuce would still have to be cooled to 0°C. Both de-watering and cooling could be achieved by vacuum cooling. The energy contained in the heated lettuce could be removed by evaporating the water remaining on the lettuce after draining. Preliminary experiments have shown that vacuum cooling can both de-water and cool fresh-cut lettuce from 45 to 0°C (data not shown). Vacuum cooling is not possible with 0°C lettuce, since the heat needed to vaporize the water and provide the cooling is not there.

Another advantage of the heat shock treatment is that since phenolic compounds are not synthesized and since the wounded lettuce has naturally low levels of preformed phenolic compounds, the use of expensive barrier bags to maintain modified atmospheres is not required. Exclusion of oxygen and elevation of carbon dioxide in the atmosphere are not required to prevent the browning of phenolic compounds. An inexpensive polyethylene bag could be used to reduce moisture loss and maintain the cleanliness of the tissue.

Consumers do not want their fresh fruits and vegetables to contain additives or chemical residues (Bruhn, 1995). No chemicals are used in the heat shock treatment, and the hsps that are produced by the lettuce are natural compounds found in many fresh fruits and vegetables. The ease with which a heat shock can be administered to lettuce and the lack of an offensive chemical residue makes this technique an attractive method to control browning in fresh-cut lettuce, and perhaps in other fresh fruits and vegetables as well.

References

Apuya, N.R., Zimmerman, J.L., 1992. Heat shock gene expression is controlled primarily at the translational level in carrot cells and somatic embryos. Plant Cell 4, 657–665.

Barnett, T., Altschuler, M., Mcdaniel, C.N., Mascarenhas, J.P., 1980. Heat shock induced proteins in plant cells. Dev. Gen. 1, 331–340.

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.

Brodl, M.R., 1989. Regulation of the synthesis of normal cellular proteins during heat shock. Physiol. Plant 75, 439–443.

Brostrom, C.O., Brostrom, M.A., 1998. Regulation of translational initiation during cellular responses to stress. Prog. Nucleic Acid. Res. Mol. Biol. 58, 79–125.

Bruhn, C., 1995. Consumer perception of fresh-cut produce. Perishables Handling Newsletter: UC Davis 81, 18–19.

Campos-Vargas, R., Saltveit, M.E.; 2000. Exposure to methyl jasmonate does not induce phenolic metabolism in harvested lettuce, Physiol. Plant, (in press).

Collins, G.G., Nie, X.L., Saltveit, M.E., 1993. Heat shock increases chilling tolerance of mung bean hypocotyl tissue. Physiol. Plant 89, 117–124.

Collins, G.G., Nie, X.L., Saltveit, M.E., 1995. Heat shock proteins and chilling injury of mung bean hypocotyls. J. Expt. Bot. 46, 795–802.

Cooper, P., Ho-T, H.D., 1983. Heat shock proteins in maize. Plant. Physiol. 71, 215–222.

Couture, R., Cantwell, M.I., Ke, D., Saltveit, M.E., 1993. Physiological attributes and storage life of minimally processed lettuce. HortScience 28, 723–725.

Fourre, J.L., Lhoest, J., 1989. Protein synthesis and modification by heat in rice cell culture. Plant Sci. 61, 69–74.

Jakob, U., Gaestel, M., Engel, K., Buchner, J., 1993. Small heat shock proteins are molecular chaperones. J. Biol. Chem. 268, 1517–1520.

Jennings, P., Saltveit, M.E., 1994. Temperature and chemical shocks induce chilling tolerance of germinating *Cucumis sativus* (cv. Poinsett 76) seeds. Physiol. Plant 91, 703–707.

Kanabus, J., Pikaard, C.S., Cherry, J.H., 1984. Heat shock proteins in tobacco cell suspension during growth cycle. Plant Physiol. 75, 639–644.

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.

Lafuente, M.T., Belver, A., Guye, M.G., Saltveit, M.E., 1991. Effect of temperature conditioning on chilling injury of cucumber cotyledons. Plant Physiol. 95, 443–449.

Lindquist, S., 1981. Regulation of protein synthesis during heat shock. Nature 294, 311–314.

Lindquist, S., 1986. The heat stock response. Annu. Rev. Biochem. 55, 1151–1191.

Loaiza-Velarde, J.G., Tomás-Barberá, 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. Hortic. Sci. 122, 873–877.

Loaiza-Velarde, J.G.; Saltveit, M.E.; 2000. Heat shocks applied either before or after wounding reduced browning of lettuce leaf tissue, J. Am. Soc. Hortic. Sci., (in press).

Lopez-Galvez, G., Saltveit, M.E., Cantwell, M.I., 1996a. The visual quality of minimally processed lettuce stored in air or controlled atmospheres with emphasis on romaine and iceberg types. Postharvest Biol. Technol. 8, 179–190.

Lopez-Galvez, G., Saltveit, M.E., Cantwell, M.I., 1996b. 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.

Lyons, J.M., 1973. Chilling injury in plants. Annu. Rev. Plant. Physiol. 24, 445–466.

Molisch, H., 1896. Das Erfriern von Pflanzen bei Temperaturen uber dem Eispunkt. Sitzungsber. d. Kaiserlichen Akademie der Wissenschaften zu Wien. Bd. 105, Abth. I.

Nover, L., 1991. Translational control. In: Nover, L. (Ed.), Heat Shock Response. CRC Press, Boca Raton, FL, pp. 299–324.

Nover, L., Scharf, K.D., Neumann, D., 1983. Formation of cytoplasmic heat shock granules in tomato cell cultures and leaves. Mol. Cell. Biol. 3, 1648–1655.

Nover, L., Scharf, K.D., Neumann, D., 1989. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol. Cell. Biol. 9, 1298–1308.

Ougham, H.J., Stoddart, J.L., 1986. Synthesis of heat shock protein and acquisition of thermotolerance in high-temperature tolerant and high-temperature susceptible lines of Sorghum. Plant. Sci. 44, 163–167.

Peiser, G., López-Gálvez, G., Cantwell, M.I., Saltveit, M.E., 1998. Phenylalnine ammonia-lyase inhibitors control browning of cut lettuce. Postharvest Biol. Technol. 14, 171–177.

Peña-Cortes, H., Willmitzer, L., 1995. The role of hormones in gene activation in response to wounding. In: Davies, P.J. (Ed.), Plant Hormones. Kluwer Academic Press, pp. 395–414.

Pentzer, W.T., Heinze, P.H., 1954. Postharvest physiology of fruits and vegetables. Annu. Rev. Plant. Physiol. 5, 205–224.

Ritenour, M.A., Saltveit, M.E., 1996. Identification of a phenylalanine ammonia-lyase inactivating factor in harvested iceberg lettuce (*Lactuca sativa* L.). Physiol. Plant. 97, 327–331.

Sabehat, A., Weiss, D., Lurie, S., 1995. Persistence of heat shock proteins in heated tomato fruit and the resistance to chilling injury of the fruit. Acta. Hortic. 398, 11–21.

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., 1998. Heat-shock and Fresh Cut Lettuce. Perishables Handling Q. 95, 5–6.

Saltveit, M.E., 2000. Discovery of Chilling Injury. In: Kung, S.D., Yang, S.F. (Eds.), Discoveries in Plant Biology, vol. 3. World Scientific Publishing Co, Singapore, pp. 423–448.

Scherrer, K., Bey, F., 1994. The prosomes (multicatalytic proteinases; proteasomes) and their relationship to the untranslated messenger ribonucleoproteins, the cytoskeleton, and cell differentiation. Prog. Nucleic Acid Res. Mol. Biol. 49, 1–64.

Schmid, H.P., Pouch, M.N., Petit, F., Dadet, M.H., Badaoui, S., Boissonnet, G., Buri, J., Norris, V., Briand, Y., 1995. Relationships between proteasomes and RNA. Mol. Biol. Rep. 21, 43–47.

Shewfelt, R.L., 1987. Quality of minimally processed fruits and vegetables. J. Food. Qual. 10, 143–156.

Sierra, J.M., Zapata, J.M., 1994. Translational regulation of the heat shock response. Mol. Biol. Rep. 19, 211–220.

Somers, D.J., Cummins, W.R., Filion, W.G., 1989. Characterization of the heat shock response in spinach (*Spinacia oleracea* L.). Biochem. Cell. Biol. 67, 113–120.

Stuger, R., Ranostaj, S., Materna, T., Forreiter, C., 1999. Messenger RNA-binding properties of nonpolysomal ribonucleoproteins from heat-stressed tomato cells. Plant. Physiol. 120, 23–31.

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. Hortic. Sci. 122, 399–404.

Vierling, E., 1991. The role of heat shock proteins in plants. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 42, 579–620.

Whitaker, B.D., 1994. A reassessment of heat treatment as a means of reducing chilling injury in tomato fruit. Postharvest. Biol. Technol. 4, 75–83.

Zhang, H.Q., Croes, A.F., Linskens, H.F., 1984. Qualitative changes in protein synthesis in germinating pollen of Lilium longiflorum after a heat shock. Plant. Cell. Environ. 79, 689–691.