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Hot Water Treatment of Postharvest Mei Fruit to Delay Ripening

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Abstract. **Mei (*Prunus mume* 'Daqinghe') fruit were immersed in 20 °C (control), 47 °C (HWT_{47}), 50 °C (HWT_{50}), or 53°C (HWT_{53}) water for 3 min after harvest, then stored at 20 °C. Firmness, peel color, chlorophyll, chlorophyllase activity, soluble solids content (SSC), titratable acidity (TA), respiration, ethylene production, and pectinmethylesterase (PME) and polygalacturonase (PG) activity were monitored to determine the effects of hot water treatment in delaying fruit ripening. Control fruit displayed a typical climacteric pattern of respiration and ethylene production. Peak CO_2 production and ethylene production were observed 6 days after harvest. Fruit softening was accompanied by decreases in hue angle, chlorophyll content, SSC, and TA and increases in chlorophyllase and PME and PG activity. Hot water treatment delayed the onset of the climacteric peaks of CO_2 and ethylene production. The delays were associated with delays in fruit softening, consistent with lags in the rise of PME and PG activity; delays in yellowing and chlorophyll breakdown, consistent with lags in the rise of chlorophyllase activity; and delays in loss of SSC and TA. The shelf life of fruit increased by 6 days, or 60%, with HWT_{47}, and by 8 days, or 80%, with HWT_{50} or HWT_{53}.**

Mei (*Prunus mume* Sieb.et Zucc.) originating from China, is one of the important fruit crops in China. The fruit softens quickly at ambient temperatures after harvest. Therefore, there is a need for technologies to slow the physicochemical changes that occur during postharvest storage, for the development of a sound mei industry in China.

There has been increasing interest in the use of heat treatments to control insect pests, prevent fungal rots, increase resistance to chilling injury, delay fruit ripening and extend postharvest shelf life of fruit and vegetables (Lurie, 1998). To understand whether heat treatment may be commercially practical for mei fruit, it is important to determine how it affects the ripening process so that it can be used in a predictable and reliable manner by the industry. However, as to my knowledge, application of heat treatment has not been elucidated in mei fruit in detail. The present study was performed to characterize the physiological and biochemical responses of mei fruit to hot water treatment and to evaluate its ability as a postharvest tool for regulating the ripening of mei fruit. The effects of hot water treatment on firmness, peel color, chlorophyll, chlorophyllase activity, soluble solids content (SSC), titratable acidity (TA), respiration, ethylene production and activity of enzymes associated with cell wall hydrolysis of mei fruit during ripening were examined.

Materials and Methods

Fruit material. Mature mei (*Prunus mume* 'Daqinghe') fruit [firmness = 55 ± 5 (N), h° = 125 ± 5, SSC = 8.2 ± 0.3 (%,) TA = 2.7 ± 0.2 (%)] were harvested from a commercial orchard in Xiaoshan, Zhejiang Province of China. Then fruit were packed into fibreboard cartons, transferred to the laboratory on the same day, and sorted to uniform size and freedom from blemishes. The fruit were divided into four groups of 420 fruit, comprising three replications of 140. The fruit were immersed in 20 °C (control), 47 °C (HWT_{47}), 50 °C (HWT_{50}) or 53 °C (HWT_{53}) hot water for 3 min respectively. Then fruit were stored at 20 °C with a relative humidity of 90% for ripening. Fruit firmness, peel color, respiration and ethylene production were assessed every two days. Fruit flesh samples (about 100 g each) were frozen in liquid nitrogen and stored at –70 °C until used for the measurement of SSC, TA, PME and PG, and similar treatment on fruit peel samples (about 15 g each) for the measurement of chlorophyll and chlorophyllase activity.

Texture measurements. Texture measurements were conducted using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd, U.K.) incorporating a 5-mm-diameter probe. Firmness was measured on the side along the equatorial region of the fruit. At least six fruit were measured for each treatment.

Peel color assessment. The color was measured on opposite sides along the equator of each six fruit, using a chromameter (CR-200; Minolta Camera Co., Japan). The parameters of 'a' and 'b' were measured and the final results was expressed as hue angle ($h°$) according to McGuire (1992).

Chlorophyll extraction and measurement. Chlorophyll was determined according to Knee (1972). The frozen peel (about 5 g) was ground and extracted with 50 mL of cold acetone containing 1% w/v calcium carbonate to prevent degradation. The residue was washed with cold acetone until the residue became colorless. The extracts were combined, adjusted to 100 mL with acetone, and centrifuged at 14,000 g for 10 min. Chlorophyll was calculated by the absorbance of the supernatant at 645 and 663 nm.

Chlorophyllase extraction and activity assay. The frozen peel (about 5 g) was ground with 25 mL of 10 mmol·L^{-1} phosphate buffer (pH 7.0) containing 50 mmol·L^{-1} KCl and 0.24% w/v Triton X-100. The crude enzyme was stirred for 1 h at 0 °C and the mixture was filtered through Whatman No. 1. Afterwards, the filtrate was centrifuged at 14,000 *g* for 15 min at 4 °C. The supernatant was used as the crude enzyme extract.

Chlorophyllase activity was determined by a modification of the method of Amir-Shapira et al. (1987). The reaction mixture contained 0.5 mL enzyme solution, 0.1 mL 1.44% w/v Triton X-100, 0.2 mL chlorophyll acetone solution (chlorophyll a 100 μg·mL^{-1}) and 0.5 mL 0.1 mol·L^{-1} phosphate buffer (pH 7.5). The mixture was incubated in a water bath at 25 °C for 40 min, and the enzyme reaction was stopped by the addition of 4 mL of acetone. The remaining (nondegraded) chlorophyll were extracted with 4 mL of hexane and assayed by reading the absorbance at 663 nm. One unit of enzyme activity was defined as the change in absorbance per minute per initial fresh weight.

SSC and TA measurement. Frozen flesh was ground and juiced. SSC was determined by a digital refractometer (Atago, Tokyo, Japan). TA was determined by titration with 0.1 mol·L^{-1} NaOH up to pH 8.1, using 5 mL of diluted juice in 25 mL distilled H_2O. The results were expressed as g of malic acid per 100 g fresh weight.

Respiration and ethylene evolution. Fruit respiration (20 fruit for each treatment) was measured as CO_2 production using an open gas exchange system (1.5 L) linked to an infrared gas analyzer with flow rate of 1 L·min^{-1} (GXH-3051; Institute of Junfang Scientific Instruments of Beijing, China).

For ethylene determination, fruit from each treatment were enclosed in 1-L airtight jars for 1 h at 20 °C, then a 1-mL gas sample was collected by syringe and injected into a gas chromatograph (SP 6800-A, Lunan Chemical Engineering Instrument Ltd, Shandong Province, China) equipped with an flame-ionization detector and an activated alumina column.

Cell wall hydrolysis enzymes extraction and activity assay. For enzyme extraction, 30 g of frozen fruit flesh was ground in two volumes of cold 100 mmol·L^{-1} sodium acetate buffer (pH 6.0) containing 1% w/v polyvinyl pyrrolidone. The homogenate was centrifuged at 14,000 *g* for 20 min. The supernatant was discarded. The residue was suspended in two volumes of 1 mol·L^{-1} sodium acetate buffer, pH 6.0, containing 6% w/v NaCl. The pH of the suspension was adjusted to 8.2 with 2 mol·L^{-1} NaOH. The sample was kept overnight at 4 °C with continuous stirring and then centrifuged. The supernatant was filtered twice using Whatman No. 1 filter paper. The filtrate was dialysed against distilled water for 48 h with four changes. All operations were carried out in an ice bath. This dialysed sample constituted the enzyme extract.

PME activity was determined according to the technique described by Nagel and Patterson (1967). The substrate used was a solution of

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1% w/v pectin (Sigma). The pH of the pectin solution was adjusted to 7.0 with 0.02 mol·L^{-1} NaOH. The reaction mixture contained 10 mL of the crude enzyme, 5 mL of 0.2 mol·L^{-1} sodium oxalate and 25 mL of substrate. The reaction mixture was incubated at 30 °C and continuously stirred by bubbling CO_2-free air through it. During the course of the reaction, the pH of the reaction mixture was maintained at 7.0 with 0.02 mol·L^{-1} NaOH. The amount of 0.02 mol·L^{-1} NaOH added in 30 min was recorded. Enzyme activity was expressed as milliequivalents of ester hydrolysed per minute per initial fresh weight.

PG activity was assayed by measuring an increase in reducing sugars with 2-cyanoacetamide described by Gross (1982). Reaction mixtures contained 10 mL of 1% w/v polygalacturonic acid (Sigma), which was washed with 80% v/v ethanol before use to remove oligosaccharides, and 10 mL of crude enzyme. Following incubation for 20 min at 37 °C, the reaction were terminated by adding 5 mL of cold 100 mmol·L^{-1} borate buffer (pH 9.0). Then, 1 mL of 2% w/v 2-cyanoacetamide was added, the sample mixed, and immersed in a boiling water bath for 10 min. After cooling on ice, the absorbance at 276 nm was determined. A calibration curve was obtained using D-galacturonic acid as a standard. PG activity was expressed as µmol of galacturonosyl reducing groups liberated per minute per initial fresh weight.

Statistical analysis. The experiments were conducted in a completely randomized design. Means were compared by the least significant difference ($P = 0.05$) within analysis of variance. Regression analysis use MS Excel 2000.

Results and Discussion

Effect of hot water treatment on firmness and peel color of mei fruit. Control fruit softened rapidly during ripening at 20 °C (Fig. 1A), firmness declining about 8-fold within 10 d ($P < 0.05$). Fruit softening was greatly inhibited by hot water treatment, with treated fruit firmer than control fruit on days 4, 6, 8, and 10 postharvest ($P < 0.05$). There was no significant difference in firmness between the HWT_{50} and HWT_{53} fruit ($P > 0.05$). Hot water treated fruit took 6 or 8 d more to reach the same value of firmness as the control.

The peel of mei fruit before storage had a moderate green color (hue angle = 126.4°, where pure yellow = 90° and pure green = 180°). The hue angle of control fruit decreased sharply during ripening at 20 °C (Fig. 1B; $P < 0.05$), with most of the decline occurring during the first 6 d. Hot water treatment delayed changes in peel color of mei fruit. The hue angle of hot water treated fruit was higher than control fruit on days 6, 8, and 10 postharvest ($P < 0.05$).

Effect of hot water treatment on chlorophyll content and chlorophyllase activity of mei fruit

The chlorophyll content of control fruit peel decreased quickly when ripening at 20 °C (Fig. 2A). After 10 d of storage the chlorophyll content of peel was 10.7% that at harvest. A positive correlation was observed between the chlorophyll content and hue angle of mei fruit ($r = 0.99$), consistent with chlorophyll degradation as an important factor in color chang. Hot water treatment delayed changes in peel color of mei fruit. Statistical analysis showed that the hue angle of hot water treated fruit was higher than control fruit on day 6, 8, and 10 ($P < 0.05$). At 16 or 18 d postharvest the chlorophyll content of hot water treated fruit was similar to that of the control fruit. There was no effects detected among the HWT_{47}, HWT_{50} and HWT_{53} fruit ($P > 0.05$).

Chlorophyllase is considered the first enzyme in the pathway of chlorophyll degradation (Matile et al., 1997). Chlorophyllase activity of control fruit increased sharply during ripening at 20 °C (Fig. 2B). Maximum chlorophyllase activity of control fruit was observed on day 8, being about twice that at harvest ($P < 0.05$). A negative correlation was observed between the chlorophyllase activity and chlorophyll content of mei fruit ($r = –0.98$), consistent with involvement of chlorophyllase in the degradation of chlorophyll during the ripening of mei fruit. Chlorophyllase activity was inhibited by hot water treatment. Maximum chlorophyllase activity was recorded on days 12, 14, or 16 postharvest for the HWT_{47}, HWT_{53} and HWT_{50} fruit, respectively. Chlorophyllase activity of hot water treated fruit was lower than that of control fruit on days 4, 6, 8, and 10 postharvest ($P < 0.05$).

Effect of hot water treatment on SSC and TA of mei fruit. SSC of control fruit decreased slowly from 8.34% to 7.26% from harvest to day 10 (Fig. 3A; $P < 0.05$). Hot water treatment delayed SSC decrease. SSC of hot water treated

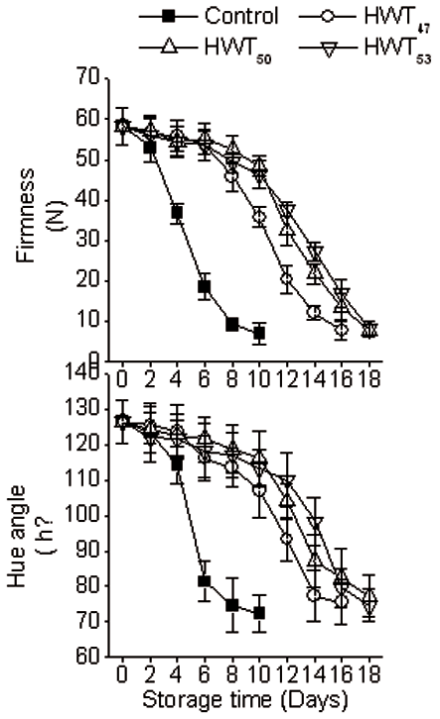

Fig. 1. Firmness and peel color of mei fruit during ripening at 20 °C after postharvest water treatment for 3 min at 20 °C (control), 47 °C (HWT_{47}), 50 °C HWT_{50} or 53 °C (HWT_{53}). Each data point is the average of six independent fruit. Vertical bars represent standard deviation of the mean.

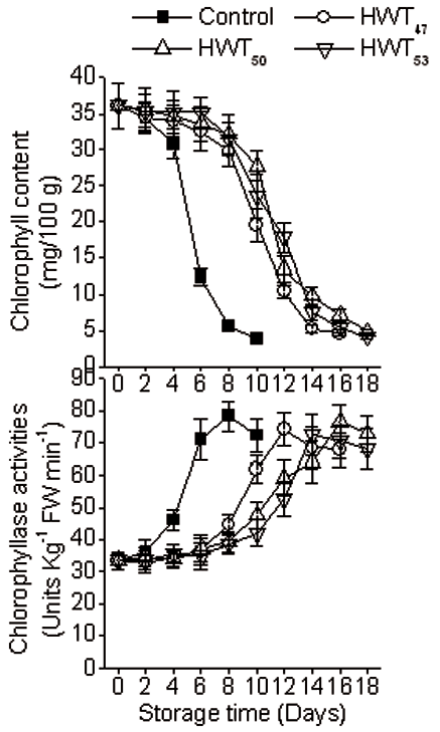

Fig. 2. Chlorophyll content and chlorophyllase activity of mei fruit during ripening at 20 °C after postharvest water treatment for 3 min at 20 °C (control), 47 °C (HWT_{47}), 50 °C HWT_{50} or 53 °C (HWT_{53}). Each data point is the average of three independent samples. Vertical bars represent standard deviation of the mean.

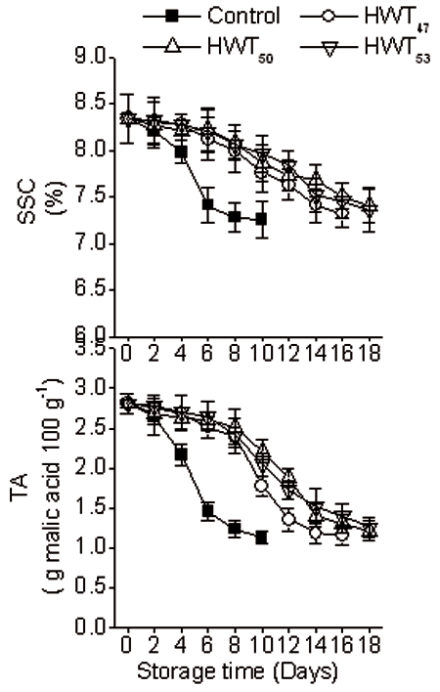

Fig. 3. SSC and TA of mei fruit during ripening at 20 °C after postharvest water treatment for 3 min at 20 °C (control), 47 °C (HWT_{47}), 50 °C HWT_{50} or 53 °C (HWT_{53}). Each data point is the average of three independent samples. Vertical bars represent standard deviation of the mean.

fruit was higher than control fruit on days 6, 8 and 10 postharvest ($P < 0.05$). There was no significant difference in SSC among HWT_{47}, HWT_{50} and HWT_{53} fruit ($P > 0.05$).

TA decreased quickly during ripening of mei fruit at 20 °C (Fig. 3B). TA of control fruit decreased to 1.14 (g/100 g) on day 10, showing about 41% compared with day 0 ($P < 0.05$). Hot water treatment greatly delayed the loss of TA in the first 8 d, and then TA began to decrease sharply. TA of hot water treated fruit was higher than that of control fruit on days 4, 6, 8, and 10 ($P < 0.05$).

Effect of hot water treatment on respiration and ethylene production of mei fruit. Mei fruit is climacteric, with the characteristic peak in CO_2 production occurring several days after harvest (Fig. 4A). The climacteric peak of control fruit was observed on day 6, being about 2-fold compared with day 0 ($P < 0.05$), then the rate of CO_2 production began to decrease sharply. The CO_2 production rate of hot water treatment fruit was greatly inhibited in the first 6 d, the CO_2 production peaks of hot water treatment fruit were recorded on days 12 or 14 respectively. The differences in the maximum CO_2 production rate between control and hot water treated fruit were significant ($P < 0.05$). Inhibition of respiration by hot water treatment has been reported for tomato (McDonald et al., 1999) and sweet pepper (Fallik et al., 1999).

Ethylene production of control fruit increased rapidly and reached the maximum values on day 6 (Fig. 4B), being about 31-fold compared with day 0 ($P < 0.05$). Thereafter, the ethylene production decreased quickly. A good positive correlation was observed between the respiration and ethylene production of mei fruit ($r = 0.88$). The ethylene production peaks of hot water treated fruit were recorded on days 12 or 14, representing a 6 or 8 days lag later compared with that of control fruit. The delayed the onset of ethylene climacteric by heat treatment has also been reported for mango fruit (Ketsa et al., 1999). There were no significant differences in the maximum ethylene production between control fruit and hot water treatment fruit (Fig. 4B; $P > 0.05$).

Effect of hot water treatment on PME and PG activity of mei fruit. In general, softening of many ripening fruit is associated with solubilization of pectic substances in the cell wall (Brummell and Labavitch, 1997; Huber and O'Donoghue, 1993). The change of solubility of pectic substances involves the action of cell wall hydrolysis enzymes such as PME and PG (Fischer and Bennett, 1991). These enzymes may have profound effects on the cohesiveness of the wall during ripening (Carrington et al., 1993).

The PME activity of control fruit increased sharply, peak PME activity was recorded on day 6, being about 3-fold compared with on day 0 (Fig. 5A; $P < 0.05$). Thereafter, PME activity decreased slowly. A negative correlation was observed between the PME activity and firmness of mei fruit ($r = -0.74$). PME activity of hot water treatment fruit was almost completely suppressed in the first 6 d, and then increased sharply and reached maximum PME activity on days 10 or 12, showing about 259%, 251%, or 271% compare to day 0 ($P < 0.05$), respectively. However, this increase of PME activity was much less than in control fruit ($P < 0.05$). This suppression in PME activity by heat treatment has also been reported for mango (Ketsa et al., 1998). This suppression in PME activity may be associated with mRNA synthesis and stability, or protein synthesis and degradation (Paull, and Chen 2000).

PG activity of control fruit increased markedly and reach to maximum activity on day 8 during ripening at 20 °C (fig. 5B), being about 4-fold compare with day 0 ($P < 0.05$). The increase in PG activity and loss in flesh firmness were correlated ($r = -0.98$). Hot water treatment greatly retarded PG activity increase in the first 8 d. Maximum PG activity was recorded on days 12, 14, or 16 respectively. The application of hot water treatment only slightly suppressed the magnitude of the PG activity, and there were no significant differences in the maximum PG activity between control fruit and hot water treatments fruit ($P > 0.05$). It should be noted that the PG peaks lag of PME in both control and hot water treated mei fruit. It has been widely recognized that PME removes the methyl groups from the C_6 position of the galacturonic acid polymers, which then enables PG to depolymerise the de-esterified polygalacturonide chain (Koch and Nevins 1989). Since PG acts preferentially on the demethylated substrate, the action of PME may be a prerequisite for optimal PG activity. The activity of PME in mei fruit preceded the activity of PG, indicating the coordinated action of both enzymes.

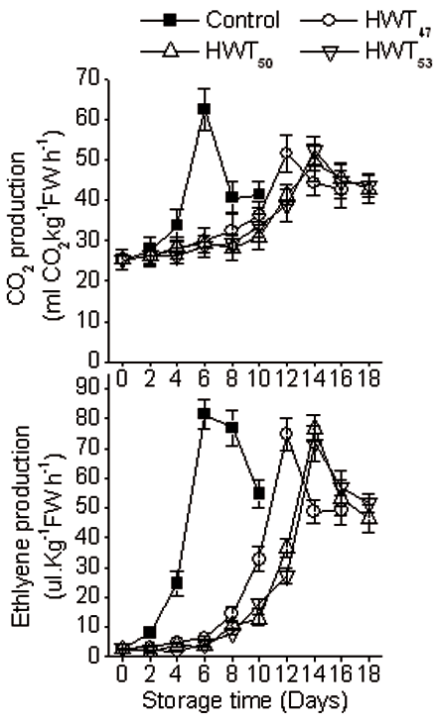

Fig. 4. Respiration and ethylene production of mei fruit during ripening at 20 °C after postharvest water treatment for 3 min at 20 °C (control), 47 °C (HWT_{47}), 50 °C HWT_{50} or 53 °C (HWT_{53}). Each data point is the average of three independent samples. Vertical bars represent standard deviation of the mean.

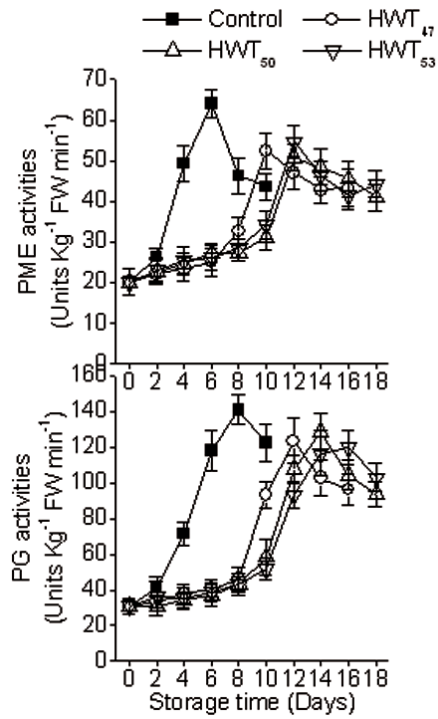

Fig. 5. PME and PG activity of mei fruit during ripening at 20 °C after postharvest water treatment for 3 min at 20 °C (control), 47 °C (HWT_{47}), 50 °C HWT_{50} or 53 °C HWT_{53}. Each data point is the average of three independent samples. Vertical bars represent standard deviation of the mean.

Conclusions

This study has shown that ripening of 'daqinghe' mei fruit is coupled with climacteric ethylene production and respiration on day 6. Hot water treatment has the potential to control the ripening of 'daqinghe' mei fruit. Hot water treatments delayed the onset of the climacteric peaks of CO_2 and ethylene production by about 6 or 8 d. Consistent with the reductions in fruit softening, hot water treatment delayed increases in the activity of PME and PG.. Hot water treatment retarded chlorophyllase activity, and resulted in less peel color change and less chlorophyll breakdown in mei fruit. Hot water treated fruit also had less loss of SSC and TA compared with control fruit. The efficacy of hot water treatment varied with temperature, with the shelf life of mei fruit increased by 6 d, or 60%, with HWT_{47}, and by 8 d, or 80%, with HWT_{50} or HWT_{53}. We recommend a hot water treatment at 50 or 53 °C for 3 min for 'daqinghe' mei fruit. The magnitude of the extension to postharvest life needs to be determined for other mei cultivars and other growing districts.

Literature Cited

Amir-Shapira, D., E.E. Goldschmidt, and A. Altman. 1987. Chlorophyll catabolism in senescing plant tissues: In vivo breakdown intermediates suggest different degradative pathways for citrus fruit and parsley leaves. Proc. Natl. Acad. Sci. USA 84:1901–1905.

Brummell, D.A. and J.M. Labavitch. 1997. Effect of

antisense suppression of endopolygalacturonase activity on polyuronide molecular weight in ripening tomato fruit and in fruit homogenates. Plant Physiol. 115:717–725.
Carrington, C.M., S.L.C. Greve, and J.M. Labavitch. 1993. Cell wall metabolism in ripening fruit. Plant Physiol. 103:429–433.
Fallik, E., S. Grinberg, S. Alkalai, O. Yekutieli, A. Wiseblum, and R. Regev. 1999. A unique rapid hot water treatment to improve storage quality of sweet pepper. Posthavest Biol. Technol. 15:25–32.
Fischer, R.L. and A.B. Bennett. 1991. Role of cell wall hydrolases in fruit ripening. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:675–703.
Gross, K.C. 1982. A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. HortScience 17:933–934.
Huber, D.J. and E.M. O'Donoghue. 1993. Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) fruit exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol. 102:473–480.
Ketsa, S., S. Chidtragool, J.D. Klein, and S. Lurie. 1999. Ethylene synthesis in mango fruit following heat treatment. Posthavest Biol. Technol. 15:65–72.
Ketsa, S., S. Chidtragool, J.D. Klein, and S. Lurie. 1998. Effect of heat treatment on changes in softening, pectic substances and activities of polygalacturonase, pectinesterase of ripening mango. J. Plant Physiol. 153:457–461.
Knee, M. 1972. Anthocyanin, carotenoid, and chlorophyll changes in the peel of Cox's Orange Pippin apples during ripening on and off the tree. J. Expt. Bot. 23:184–196.
Koch, J.L. and D.J. Nevins. 1989. Tomato fruit cell wall. I. Use of purified tomato polygalacturonase and pectin methylesterase to identify developmental changes in pectins. Plant Physiol. 91 816–22.
Lurie, S. 1998. Postharvest heat treatments. *Posthavest Biol. Technol.* 14:257–269.
Matile, P., M. Schellenberg, and F. Vicentini. 1997. Locatization of chlorophyllase in the chloroplast envelope. Planta 201:96–99.
McDonald, R.E., T.G. McCollum, and E.A. Baldwin. 1999. Temperature of water heat treatments influences tomato fruit quality following low-temperature storage. Posthavest Biol. Technol. 16:147–155.
McGuire, R.G. 1992. Reporting of objective color measurements. HortScience 27:1254–1255.
Nagel, C.W. and M.E. Patterson. 1967. Pectic enzymes and developments of the pear (*Pyrus communis*). J. Food Sci. 32:292–297.
Paull, R.E. and N.J. Chen. 2000. Heat treatment and fruit ripening. Posthavest Biol. Technol. 21:21–37.