International Food Research Journal 25(5): 2195-2203 (October 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of hot air treatment on cell wall-degrading enzymes, pulp softening and ripening in bananas

[1]Chopsri, A., [2]Sekozawa, Y. and [2*]Sugaya, S.

[1]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, 305-8572, Japan
[2]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan

Article history

Received: 6 March 2017
Received in revised form: 4 May 2017
Accepted: 11 May 2017

Keywords

Cavendish banana
Heat treatments
Pectate lyase
Polygalacturonase
Pectin methyl esterase

Abstract

Heat treatments can prolong the shelf-life of some fruits and sometimes provide effective alternatives to chemical insecticides. However, effects of sufficient hot air treatment to kill insects (50°C for 10 min) on cell wall-degrading enzyme activities in banana and associated processes, which could potentially be either positive or negative, have not been previously described. Thus, this study addressed these effects by examining selected enzyme activities, pulp softening and phenomena associated with ripening (changes in peel color, weight, firmness, respiration rates and ethylene production) in hot air-treated and control bananas. The results show that ethylene production, respiration rates, color changes of peel and pulp softening in bananas were all inhibited by hot air treatment. It also inhibited the increases in pectin methyl esterase, polygalacturonase and pectate lyase activities that typically occur in the pulp during ripening. Of these three enzymes, polygalacturonase was found to be most strongly correlated with pulp softening. The results suggest that hot air treatment retards ripening processes in bananas including changes in color and pulp softening by inhibiting respiration and ethylene production.

Introduction

Bananas are among the most important agricultural commodities in the world, with annual production (in more than 50 countries)amounting to 106 million tons in 2013 (FAOSTAT, 2016). The bananas with AAA triploid phenotype is the most distributed worldwide trade (Paull and Odilo, 2011) and a great majority in the share of banana global market are Cavendish subgroup (47%) (FAO, 2003). As part of efforts to prevent exotic pests entering Japan and causing economic damage to agricultural products (Plant protection station, 2016), imported bananas must be quarantined in accordance with the Plant Quarantine Law of 1914.

Postharvest quarantine treatments involving high temperatures targeting a wide range of insect pests have been developed for various commodities (Neven, 2000). These include disinfestation and disinfection treatments of diverse crops via exposure to hot air, hot water or hot water-saturated air (Lurie, 1998). Effects of hot water treatments as possible measures to control various postharvest disorders and diseases of bananas, and infestation by surface insects, have been examined in several studies (Acedo *et al*., 2001; Wall, 2004;Promyou *et al.*, 2008; Amnuaysin *et al*., 2012; Mirshekari *et al.,* 2015). Other studies have shown that hot air treatment can alleviate chilling injury in bananas (Ambuko *et al.*, 2012), and both hot air treatment (Nakamura *et al.*, 2008) and hyper vapor heat treatment (Nakamura *et al*., 2011) at 50°C for 10 min to 20 min can control mealybugs on bananas. Furthermore, treatment by hyper vapor heat treatment reportedly has no detrimental effect on fruit quality, but seems to delay softening of banana pulp (Nakamura *et al.*, 2012).

Bananas are climacteric fruits, a typical characteristic of ripeness during post-harvest storage is a rise in respiration rate and ethylene production. As the ethylene is produced, the fruit undergo respiration where organic materials break down into end products and lead fruits into senescence (Hailu *et al*., 2012). Subsequently, changes in biochemistry and physiology proceed from the onset of the fruit ripening: e.g. the accumulation of soluble solid, peel color development, loss of textural intensity and starch content (Palmer and McGlasson, 1969, Hailu *et al*., 2012).

Banana pulp softens rapidly in a climacteric ripening phase, particularlywhen ethylene gas is applied (Han *et al.*, 2016). A key process in tissue

*Corresponding author.
Email: *sugaya.sumiko.fw@u.tsukuba.ac.jp*
Tel: +81-298-53-4790

softening of various fruits appears to be degradation of pectin (Prasana *et al.*, 2007), a major component of primary cell walls and the most soluble wall polysaccharides, composed of (1-4) α-D-galacturonic acids (Rose, 2003). Several major cell wall-degrading enzymes are involved in the softening. One is pectin methyl esterase (PME), which hydrolyses the methyl esters in pectin. This enables subsequent hydrolysis of the (1-4) α-D-galacturonan backbones by polygalacturonase (PG) (Bennett, 2003; Rose, 2003). Another enzyme that plays an important role in fruit softening is pectate lyase (PL) (Marin *et al*., 2002), which also catalyzes cleavage of (1-4) α-D-galacturonan backbones, but by a β–elimination (Fischer and Bennett, 1991). Loss of the integrity and structure of pectin via activities of these enzymes increases its solubility, and decreases in water-soluble pectin contents are strongly associated with softening during ripening (Gwanpua *et al.*, 2014).

Heating can reportedly disrupt cell wall-degrading enzymes and ethylene production in fruits, but their responses depend on various factors, including the threshold temperature of their tissues for heat stress, which is typically ca. 45°C (Paull and Chen, 2000). The species involved may also be important. For example, Klein *et al.* (1990) found that incubating apples at 38°C for 4 days resulted in firmer pulp, but hot water treatment at 46°C resulted in softer mango pulp according to Jacobi *et al.* (1995). Moreover, the sensitivity of enzymes to heat treatment may depend on the ripeness of the tissues (Lazan *et al*., 1989).

The aim of the study presented here was to elucidate both effects of hot air treatment on banana fruit quality and the mechanisms involved by examining its effects on peel color changes, pulp softness, respiration and ethylene production rates, and cell wall-degrading enzyme activities in bananas.

Materials and Methods

Materials

Green mature bananas (*Musa acuminate* 'Dwarf Cavendish' subgroup) were purchased from a local market in Ibaraki prefecture, Japan. They were transferred to the Pomology laboratory, University of Tsukuba, and kept in a temperature-controlled incubator set at 13.5°C. Fresh materials were prepared by separating each banana cluster into individual fingers, which were divided into two groups, one assigned to a heat treatment (described below) and the other to a control treatment.

Hot air treatment

Sets of five fingers with a T-type thermocouple, connected to a GL400 midi logger (Graphtec Corp., Japan), attached to the peel surface were arranged on an aluminum tray and placed in a central position in a CI-310 incubator(ADVANTEC, Japan). When the peel temperature reached 50°C, the fruits were held at that temperature for 10 min(Figure 1)which adapted from Nakamura *et al.* (2008), then cooled in water at room-temperature (20°C), air-dried and allowed to ripen during storage at 20°C in an incubator. Control bananas were treated identically except that they were incubated at room temperature when the heated bananas were incubated at 50°C.

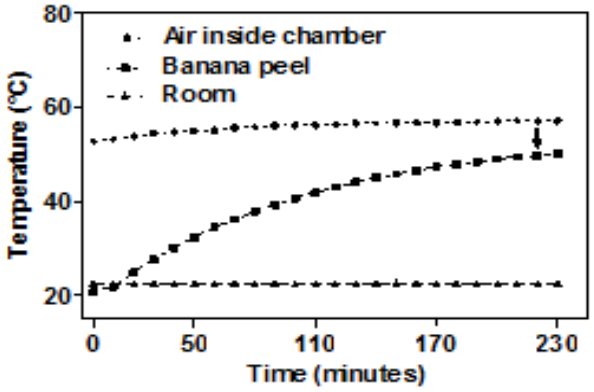

Figure 1. Time course of temperature changes during the hot air treatment. The surface temperature of maturegreen bananasreached 50°C at the time indicated by the arrow, and were then held at that temperature for 10 min. Data are means ± S.E. of three replications.

After 1, 3, 9 and 15 days of storage three hot air-treated bananas and threecontrols were subjected to firmness measurements then the flesh pulp was cut into small pieces, frozen with liquid nitrogen and immediately kept at -80°C until further analysis, as described below. Three replications of hot air-treated bananas were also subjected to firmness measurement, cutting and freezing after 33 and 36 days of storage. Sampling for non-destructive measurements, also described below, was done daily.

Peel color

A portable colorimeter (Model CR-400/410, Konica Minolta Inc., Japan) was used to measure color of bananas peel and the values was chosen as Hue angle (H°). The machine calibrated so that three different values were obtained from each side of the banana. An average Hue angle of each banana then calculated.

Pulp firmness

A FRT-50N food rheology tester (Imada Co.Ltd., Japan) equipped with a 2 mm diameter cylindrical probe connected to a 100 mm probe shaft was used to

measure the firmness of the bananas' pulp. To prepare samples, each finger was divided into three equal parts using a sharp knife and one side was peeled. A cut part was placed on the tester's platform, the bar penetrated 10 mm into the pulp at 5 mm sec^{-1} and the maximum penetration force was recorded.

Weight loss

The weight of each finger was recorded daily using a digital scale, and the resulting data were used to calculate the percentage weight loss at each day since the start of storage.

Respiration and ethylene production of fruits

To collect gasses released from the bananas they were incubated in a 1-liter closed plastic container at 20°C for 1 hour. Then, 1 ml of the head space gas was taken and injected into a GC - 18A gas chromatograph equipped with a total conductivity detector and a Porapak Q (Mesh 60/80) column (Shimadzu Co., Japan) to determine the CO_2 concentration. In addition, a 1 ml sample was used to measure the ethylene concentration in the headspace, using a GC - 8A gas chromatograph equipped with a flame thermionic detector and a WG - 100 column (also supplied by Shimadzu Co.).

Enzyme preparation

Extracts for measuring enzyme activities were prepared following Pathak and Sanwal (1998), with a slight modification. Samples (ca. 5 g) of pulp tissue were homogenized in buffer containing 0.02 M Na-Pi buffer, 0.02 M EDTA, 1% Triton X-100, 0.02 M cysteine-HCl, and 1 mM PMSF (pH 7), then proteins were precipitated with $(NH_4)_2SO_4$, dialyzed to remove excess salt and centrifuged as described by the cited authors. The supernatants were then used for the analyses described in the following sections.

PGactivity was assayed following Pathak and Sanwal (1998) with a slight modification. To start the reaction, 0.3 ml of the supernatant described above was mixed with 0.3 ml of 0.2 M HOAc buffer (pH 4.5), 0.3 ml of 1% polygalacturonic acid (Sigma-Andrich) and 0.1 ml distilled water. The resulting preparation was thoroughly vortex-mixed then incubated at 37°C for 60 min. Then, 0.5 ml of DNS solution (Miller, 1958), containing 1% of 3, 5-dinitrosalicylic acid, 1% NaOH and 0.05% Na_2SO_4 was added. The mixture was boiled for 10 min, then 1 ml of 40% potassium sodium tartrate was immediately added to stabilize color (formed from the reaction between DNS and reducing sugars) prior to cooling at room temperature.

Control tubes were not incubated after receiving the supernatant. The absorbance of the mixtures at 540 nm was measured using a V-550 spectrophotometer (JASCO Corporation, Tokyo, Japan). A standard curve was obtained using D-galacturonic acid, and one unit of PG was defined as the amount required to release 1 nmol D-galacturonic acid in 1 min at 37°C.

Pectate lyase(PL) assay

PLactivity was assayed using the 2-thiobarbituric acid (TBA) method of Waravdekar and Saslaw (1959) as described by Payasi and Sanwal (2004). A 5% (w/v) solution of polygalacturonic acid in 0.05 M Tris-HCl buffer (pH 8.5) was prepared as the enzyme substrate. 0.20 ml of this solution was mixed with 0.45 ml of Tris-buffer (pH 8.5), 0.15 ml of 0.01 M $CaCl_2$, 0.20 ml of the supernatant described above and appropriate amount of distilled water.

The mixture was then incubated at 37°C for 7 hours. To terminate the reaction, 75 µl of 9% $ZnSO_4.7H_2O$ and 75 µl of 0.5 M NaOH were added. The white jelly-like pellets formed were removed by centrifugation at 1,500 xg for 10 min. A 0.2 ml portion of the supernatant was then mixed with 0.25 ml of 0.025 N periodic acid (prepared in 0.125 N H_2SO_4). The solution was subsequently incubated at room temperature for 40 min, the optimal oxidation period for sugars with trans groups (Waravdekar and Saslaw, 1959). Next, 0.5 ml of 2% (w/v) $NaAsO_2$ in 0.5 N HCl was gradually added and the mixture was allowed to stand for 2 min. The solution (pH 2.0) was finally mixed with 2 ml of 3% (w/v) TBA then boiled for 10 min. After cooling the absorbance at 548 nm of the solutions (and non-incubated counterparts as controls)was measured, and one unit of PL was defined as the amount required for a 0.01 increase in absorbance units relative to controls under these conditions.

Pectin methyl esterase (PME) assay

To assay PME activity, initially 0.5% (w/v) pectin substrate, 0.015% bromothymol blue in 3 mM KH_2PO_4-KH_2PO_4 buffer, and 3 mM KH_2PO_4-KH_2PO_4 buffer solutions, all adjusted to pH 7, were prepared. 2 ml of the pectin and 0.15 ml of the bromothymol blue solutions were mixed and incubated at 60°C for 2 min, then cooled to room temperature. Subsequently, 0.1 ml of the supernatant described above was added, the mixture was immediately stirred and 5 min later the absorbance at 620 nm was measured. One min after addition of the supernatant incubation tubes were used as controls and one unit of PME was defined as the amount required to liberate 1 µmol D-galacturonic acid in 1 min under these conditions.

Protein determination

An RC DC™ Protein kit (Bio-Rad Laboratories, Inc., Tokyo, Japan) was used to determine amounts of proteins in the samples, following Lowry *et al.* (1951). Calibration curves were prepared using serum BSA.

Results and Discussion

Hue angle

Yellow coloration rapidly developed in the control bananas' peel during storage as the Hue angle of their peel sharply decreased from 113.74°to 89.28°between day 6 and day 9, then further declined to 85.11° by day 15 (Figure 2A). The Hue angle of the peel of heat-treated bananas changed more slowly and remained higher than that of controls from day 9 to day 20.Considering the ripening stage of banana fruit, storage of control group was ended on 15 days while heat treated group was delayed until day 36.

It is known that storage at temperatures above ca. 24°C retard loss of greenness and changes in Hue angle of banana peel (Kajuna *et al.*, 1998). This is due to inhibition of chlorophyll degradation (Seymour *et al*., 1987; Thomas and Janave, 1992; Drury *et al*., 1999), which may involve inhibition of Mg-dechelatase activity (Yang *et al*., 2009). Our results show that hot air treatment can inhibit chlorophyll degradation, resulting in maintenance of green banana peel for up to 20 days.

Weight loss

All bananas dramatically lost weight during storage at 20°C (Figure 2B), and the weight losses of hot air-treated and control bananas were similar until day 15, after which storage of controls ended and treated bananas continued to lose weight until the end of the experiment at day 36. Banana fruits inevitably lose weight during storage (Ahmad *et al*., 2001), due to losses of respiratory gases and water vapor, and hot water treatment reportedly accelerates the losses both temperature- and duration-dependently (Amin and Hossain, 2013). This conflict with our finding that weight losses of hot air-treated and control bananas were very similar. A possible explanation for the discrepancy in results of the two thermal treatments is that the hot water treatment applied by Amin and Hossain caused re-arrangements of epicuticular layers that accelerated weight losses. For example, López-Castañeda *et al.* (2010) found that heat treatment caused fissure-reducing rearrangement of the epicuticular wax layer of apples that reduced water losses. However, further research on the structure of peel and the structural

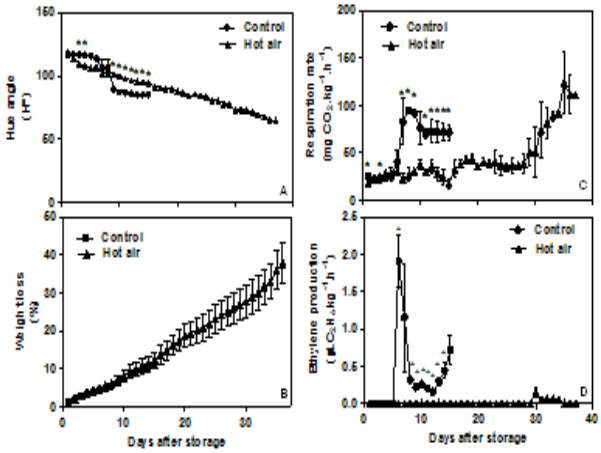

Figure 2.Changes in hue angle (A) of the peel, weight loss (B), respiration rate (C) and ethylene production (D) of bananas subjected to the hot air treatment (50°C, 10 min) or control treatment (room temperature, 10 min) then stored at 20°C. Data are means ± S.E. of three replications. Significant between-treatment differences ($P \leq 0.05$) are indicated by asterisks.

effects of heat treatment is needed to elucidate the mechanisms involved.

Respiration and ethylene production of fruits

Respiratory CO_2 and ethylene production rates of control bananas strongly rose after storage for 5 days, and peaked at days 6 and 8, respectively (Figure 2C, 2D). In contrast, no ethylene production by the hot air-treated bananas was detected until day 29 after treatment and their respiration rate remained continuously low until day 28 (Figure 2C, 2D). A small peak in ethylene production (0.2 µl/ kg/ h) was observed at day 30, and the respiration rate gradually rose after day 28 in the hot air-treated bananas.

A sharp increase in ethylene followed by a climacteric peak of respiration are general characteristics of banana fruit ripening (Liu *et al*., 1999; Pathak *et al*., 2003), which demonstrate that initiation of fruit ripening leads to major physiological and chemical changes (Millerd, 1953). Our results show that exposure to hot air at 50°C for 10 min. can strongly retard the increases in respiration and ethylene production in stored bananas, and thus the metabolic changes associated with ripening. In addition, the effects on ethylene production were strongest, in accordance with findings by Antunes and Sfakiotakis (2000) in a study of heat treatment effects in kiwifruits. Discussion of heat effects on ripening of fruits and vegetables has also focused heavily on ethylene (Luries, 1998) as it promotes climacteric ripening (Saltveit, 1999), and it is strongly involved in heat stress responses (Larkindale *et al*., 2005). Thus, the ripening-retarding effects of high

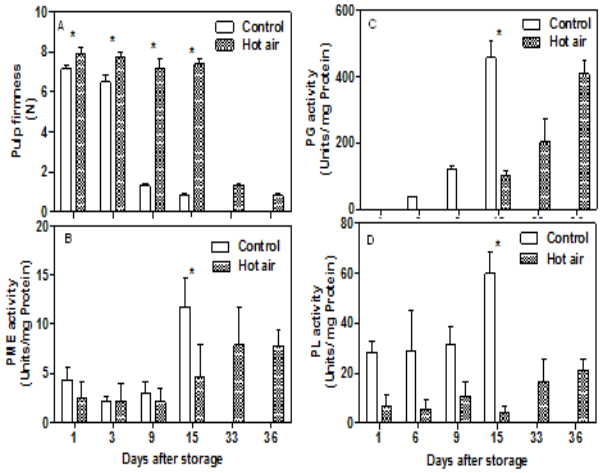

Figure 3. Changes in pulp firmness (A), and activities of PME (B), PG (C) and PL (D) enzymes in the pulp of bananas subjected to the hot air treatment (50°C, 10 min) or control treatment (room temperature, 10 min) then stored at 20°C. Data are means ± S.E. of three replications. Significant between-treatment differences ($P \leq 0.05$) are indicated by asterisks.

temperature we observed may be due to inhibition of ethylene synthesis enzymes (Morgan and Drew, 1997), e.g.1-aminocyclopropane-1-carboxylic acid synthase and 1-aminocyclopropane-carboxylic acid oxidase (Ketsa *et al*., 1999).

Heat stress responses are mediated by heat shock proteins induced by heat stress transcriptional factors in plants (Kotak *et al*., 2007). Temperatures above 45°C, around the short-term thermal damage threshold may perturb induction of heat shock-mRNAs in fruit, and hence general repair responses to less severe heat shocks, which include (inter alia) increases in respiration and suppression of ethylene production rate (Paull and Chen, 2000), in accordance with our findings.

Pulp firmness

The firmness of the control bananas' pulp dramatically declined during storage (Figure 3A), dropping from 7.15 to 1.32 Newton,as measured by the rheology tester,by day 9 and 0.87 Newton by day 15. In contrast, the pulp of hot air-treated bananas lost very little firmness during the first 15 days of storage although it fell strongly between 15 and 33 days. Our other findings show that the hot air treatment strongly retarded other ripening-related changes. Other authors have shown that heat treatments can retard softening of bananas (Ummarat *et al*., 2011) and various other fruits (Vicente *et al*., 2005; Luo *et al*., 2008;Dotto *et al*., 2011; Ornelas-Paz and Yahia, 2014).

Shalom *et al.* (1993) suggested that increases in fruits' firmness resulting from the loss of neutral sugar side chains during heating may lead to closer packing of pectin strands and thus hinder enzymatic cleavage during and after storage. Paull and Chen (2000) noted that heating often disrupts cell wall-degrading enzymes and ethylene production, which may explain the delayed softening in banana pulp induced by the hot air treatment and (inter alia) observations that heat treatment can impair ethylene production and softening in papaya (An and Paull, 1990). In our experiment the small peak of ethylene detected at day 30 (Figure 2D) may have initiated ripening and the loss of pulp firmness in hot-air treated bananas we recorded at day 33.

Pectin methyl esterase (PME) activity

PME activity did not significantly change in either hot air-treated or control bananas up to day 9 (Figure 3B). Between days 9 and 15 it increased in both sets, but significantly more strongly in controls. It also further increased after day 15 in the hot air-treated bananas. These findings are consistent with previous observations that PME activity in banana pulp increases during ripening (Hultin and Levine, 1965).

The findings also clearly show that the hot air treatment inhibited increases in PME activity. Suppression of PME activity by heat has been previously observed in Chinese bayberry (Luo *et al*., 2009) and banana (Mirshekari *et al.,* 2015). Intriguingly, cold shock treatment can also reduce PME activity in banana fruits (Zhang *et al*., 2010). However, according to Brummell and Harpster (2001), transgenic suppression of PME activity does not affect the firmness of fruit during ripening in the absence of thermal shocks.

Polygalacturonase (PG) activity

PG plays an important role in banana softening, and increases in its activity coincide with reductions in pulp firmness during ripening (Pathak and Sanwal, 1998). We detected no PG activity at day 1 of storage in either hot air-treated or control bananas (Figure 3C). However, while PG activity progressively increased thereafter (and peaked at day 15) in the pulp of control bananas, no PG activity was detected in hot air-treated bananas until day 15 although it increased strongly thereafter until the end of the experiment at day 36. In both cases, as expected, the PG activity closely correlated with loss of pulp firmness. The results clearly suggest that ripening-associated increases in PG activity were strongly suppressed by the hot air treatment, confirming conclusions by Chan *et al.* (1981) that heat treatments depress PG activity, thereby delaying fruit softening.

Heat-induced reductions in PG activity have also been previously observed in mango (Ketsa *et al.*, 1988) and banana (Mirshekari *et al*., 2015).

These observations are consistent with several previous reports and conclusions regarding ripening and activities of PG and other pectin-metabolizing enzymes. Notably, Gwanpua *et al.* (2014) concluded that losses of neutral sugars, increases in pectin solubility and reductions in molar masses of water-soluble pectin are all associated with softening during apple ripening. In addition, Luo *et al.* (2009) found that suppression of PG and PME activity by heat resulted in delayed polymerization of pectic substances that can be dissolved by alkaline solutions containing chelators, and reduced increases in water–soluble pectic substances. As PG activity is largely responsible for pectin depolymerization and increases in its solubility (Brummell and Harper, 2001), as well as major changes in pectin structure (Fischer and Bennett, 1991) during ripening, the inhibitory effect of heat on PG activity may have contributed to the retarded softening in our hot air-treated bananas. However, a study with transgenic plants indicated that suppression of PG activity only slightly reduces fruit softening (Brummell and Harper, 2001). Furthermore, although we detected substantial PG activity at day 15 the pulp was still firm in our heat-treated bananas, presumably because the PG had not had sufficient time to significantly influence firmness and/or activities of other enzymes that may be involved in softening were not perturbed by the hot air treatment.

Pectate lyase (PL) activity

PL activity of control bananas continuously increased during their 15 days of storage (Figure 3D), as expected because it generally increases in pulp during ripening (Payasi and Sanwal, 2003; Lohani *et al.*, 2004). In contrast, PL activity in hot air-treated bananas slowly increased and remained at low levels for 36 days. Thus, PL activity was strongly affected by the hot air treatment. Similarly, hot water treatment can reduce PL activity in banana peel (Amnuaysin *et al.*, 2012) and pulp (Mirshekari *et al.*, 2015).

These findings are consistent with reports that PL plays a significant role in wall disassembly during ripening, and is strongly up-regulated in ripening banana pulp (Medina-Suarez *et al.,* 1997). In addition, Baldwin and Pressey (1989) found that both PG and PL solubilized uronic acids from washed cell wall fragments, but the PLs were most effective. Moreover, PL inhibition can significantly increase strawberry fruit firmness (Jiménez-Bermúdez *et al*., 2002). Thus, in our study the low activity of PL in heat-treated bananas relative to controls (detected at days 9 and 15) may have contributed to their weak losses of pulp firmness.

Conclusion

Our hot air treatment (50°C for 10 min) in the tested bananas, clearlyhad inhibitory effects on their ethylene production and respiration, and delayed ripening processes including changes in peel color and pulp softening. Softening is an important indicator of banana ripening that is caused by cell wall-degrading enzymes. Accordingly, increases in PG, PME and PL activities in banana pulp during storage were all reduced and retarded (relative to control levels) by the hot air treatment. Hence, in addition to other benefits of heat treatments they can retard softening of bananas. Our results may facilitate efforts both to understand effects of heat treatments on banana quality and improve postharvest treatments.

References

Acedo Jr., A. L., Benetiz, M. M. and Millan, L. A. 2001. Heat treatment effects on ripening and disease infection of Philippine bananas and mangoes. Acta Horticulture 553: 417-420.

Ahmad, S., Thompson, A. K., Hafiz, I. A. and Asi, A. A. 2001. Effect of temperature on the ripening behavior and quality of banana fruit.International Journal of Agriculture and Biology 3: 224–227.

Ambuko, J.L., Sekozawa, Y., Sugaya, S., Zanol, G. and Gemma, H. 2012. Effect of postharvest hot air treatments on ripening and soluble sugars in banana fruits *Musa* spp. 'Williams'. Acta Horticulture 934: 719-725

Amin, M. N. and Hossain, M. M. 2013. Reduction of postharvest loss and prolong the shelf-life of banana through hot water treatment. Journal of Chemical Engineering 27: 42–47.

Amnuaysin, N., Jones, M. L. and Seraypheap, K. 2012. Changes in activities and gene expression of enzymes associated with cell wall modification in peels of hot water treated bananas. Scientia Horticulturae 142: 98–104.

An, J. and Paull, R. E. 1990. Storage temperature and ethylene influence on ripening of papaya fruit. Journal of the American Society for Horticultural Science 115: 949–953.

Antunes, M. D. C. and Sfakiotakis, E. M. 2000.Effect of high temperature stress on ethylene biosynthesis, respiration and ripening of "Hayward" kiwifruit. Postharvest Biology and Technology 20: 251–259.

Baldwin, E. A. and Pressey, R. 1989.Pectic enzymes in pectolyase. Plant Physiology 90: 191–196.

Brummell, D. A. and Harpster, M. H. 2001.Cell wall metabolism in fruit softening and quality and its

manipulation in transgenic plants. Plant Molecular Biology 47: 311–340.

Chan, H. T., JR, Tam, S. Y. and Seo, S. T. 1981. Papaya polygalacturonase and its role in thermally injured ripening fruit. Journal of Food Science 46: 190–197.

Dixon, J. and Hewett, E. W. 1998. Temperature affects postharvest color change of apples. Journal of the American Society for Horticultural Science 123: 305–310.

Drury, R., Hoertensteiner, S., Donnison, I., Bird, C. R. and Seymour, G. B. 1999. Chlorophyll catabolism and gene expression in the peel of ripening banana fruits. PhysiologiaPlantarum 107: 32–38.

FAO. 2003. The world banana economy 1985-2002 (FAO commodity studies). Rome.

Fischer, R. L. and Bennett, A. B. 1991.Role of cell wall hydrolases in fruit ripening. Annual Review of Plant Physiology and Plant Molecular Biology 42: 675–703.

Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT).July, 2016. Food and Agricultural commodities production / Countries by commodity. Retrieved on July 1, 2016 from FAOSTAT website:*http://faostat3.fao.org/browse/rankings/countries_by_commodity/E*

Garcia, J. M., Aguilera, C. and Albi, M. A. 1995.Postharvest heat treatment on Spanish strawberry (Fragaria X ananassa cv. Tudla). Journal of Agriculture and Food Chemistry 43: 1489–1492.

Gwanpua, S. G., Buggenhout, V. S., Verlinden, B. E., Christiaens, S., Shpigelman, A., Vicent, V., Kermani, Z. J., Nicolai, B. M., Hendrickx, M. and Geeraerd, A. 2014. Pectin modifications and the role of pectin-degrading enzymes during postharvest softening of Jonagold apples. Food Chemistry 158: 283–291.

Hailu,M., Workneh T. S. and Belew, D.2013.Review on postharvest technology of banana fruit. African Journal of Biotechnology 12: 635-647.

Han, Y.-C., Kuang, J.-F., Chen, J.-Y., Liu, X.-C., Xiao, Y.-Y., Fu, C.-C., Wang, J.-N., Wu, K.-Q.and Lu, W.-J. 2016. Banana transcription factor MaERF11 recruits histone deacetylase MaHDA1 and represses the expression of MaACO1 and expansins during fruit ripening. Plant Physiology 171: 1070–1084.

Hazbavi, I., Khoshtaghaza, M. H., Mostaan, A. and Banakar, A. 2015.Effect of postharvest hot-water and heat treatment on quality of date palm (cv. Stamaran). Journal of the Saudi Society of Agricultural Sciences 14: 153–159.

Hultin, H. O. and Levine, A. S. 1965.Pectin methyl esterase in the ripening banana. Journal of Food Science 30: 917–921.

Jacobi, K., Giles, J., MacRae, E. and Wegrzyn, T. 1995. Conditioning "Kensington" mango with hot air alleviates hot water disinfestation injuries. Horticultural Science 30: 562–565.

Jiménez-Bermúdez, S., Redondo-Nevado, J., Muñoz-Blanco, J., Caballero, J. L., López-Aranda, J. M., Valpuesta, V., Pliego-Alfaro, F., Quesada, M. A. and Mercado, J. A. 2002.Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiology 128: 751–759.

Kajuna, S., Bilanski, W. K. and Mittal, G. S. 1998. Color changes in bananas and plantains during storage. Journal of Food and Processing and Preservation 22: 27–40.

Ketsa, S., Chidtragool, S., Klein, J. D. and Lurie, S. 1998. Effect of heat treatment on changes in softening, pectic substances and activities of polygalacturonase, pectinesterase and β-galactosidase of ripening mango. Journal of Plant Physiology 153: 457–461.

Ketsa, S., Chidtragool, S., Klein, J. D. and Lurie, S. 1999. Ethylene synthesis in mango fruit following heat treatment. Postharvest Biology and Technology 15: 65–72.

Klein, J. D., Lurie, S. and Ben-arie, R. 1990. Quality and cell wall components of "Anna" and "Granny Smith" apples treated with heat, calcium, and ethylene. Journal of the American Society for Horticultural Science 115: 954–958.

Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E. and Scharf, K. 2007. Complexity of the heat stress response in plants. Current Opinion in Plant Biology 10: 310–316.

Larkindale, J., Hall, J. D., Knight, M. R. and Vierling, E. 2005. Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiology 138: 882–897.

Lazan, H., Ali, Z. M., Liang, K. S. and Yee, K. L. 1989. Polygalacturonase activity and variation in ripening of papaya fruit with tissue depth and heat treatment. PhysiologiaPlantarum 77: 93–98.

Liu, X., Shiomi, S., Nakatsuka, A., Kubo, Y., Nakamura, R. and Inaba, A. 1999. Characterization of ethylene biosynthesis associated with ripening in banana fruit. Plant Physiology 121: 1257–1266.

Lohani, S., Trivedi, P. K. and Nath, P. 2004. Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA and IAA. Postharvest Biology and Technology 31: 119–126.

López-Castañeda, J., Corrales-García, J., Terrazas–Salgado, T. and Colinas–León, T. 2010.Effect of saturated air heat treatments on weight loss reduction and epicuticular changes in six varieties of cactus pear fruit (*Opuntia* spp.). Journal of the Professional Association for Cactus Development 12: 37–47.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951.Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193: 265–275.

Luo, Z., Xu, T., Xie, J., Zhang, L. and Xi, Y., 2009.Effect of hot air treatment on quality and ripening of Chinese bayberry fruit. Journal of the Science of Food and Agriculture 89: 443–448.

Lurie, S., 1998.Postharvest heat treatments. Postharvest Biology and Technology 14: 257–269.

Marin-Rodriguez, M. C., Orchard, J. and Seymour, G. 2002.Pectate lyases, cell wall degradation and fruit softening. Journal of Experimental Botany 53: 2115–

2119.
McGuire, R. G. 1992. Reporting of objective colour measurements. Horticultural Science 27: 1254–1255.
Medina-Suárez, R., Manning, K., Fletcher, J., Aked, J., Bird, C. R. and Seymour, G. B. 1997.Gene expression in the pulp of ripening bananas. Plant Physiology 115: 453–461.
Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31: 426–428.
Millerd, A., Bonner, J. and Biale, J. B. 1953.The climacteric rise in fruit respiration as controlled by phosphorylative coupling. Plant Physiology 28: 521–531.
Mirshekari, A., Ding, P. and Ghazali, H. M. 2015. Enzymatic activity and microstructural changes of hot water treated banana during ripening. Journal of Agricultural Science and Technology 17: 949–962.
Mitcham, E. J. and McDonald, R. E. 1993.Respiration rate, internal atmosphere, and ethanol and acetaldehyde accumulation in heat-treated mango fruit. Postharvest Biology and Technology 3: 77–86.
Morgan, P. W. and Drew, M. C. 1997.Ethylene and plant responses to stress.PhysiologiaPlantarum 100: 620–630.
Nakamura, K., Chopsri, A., Uchiyama, T., Sotome, I. and Gemma, H. 2012. Effect of vapor heat treatment with superheated stream for fumigation on postharvest quality of imported banana fruit. Research for Tropical Agriculture (Extra issue in Japanese) 5: 61-62.
Nakamura, K., Miyazaki, M. and Gemma, H. 2008. Development of non-chemical fumigation method with controlled atmosphere and high-pressure hot-water washing treatment for imported banana fruit. Research for Tropical Agriculture (Extra issue in Japanese) 1: 105-106.
Nakamura, K., Uchiyama, T., Sotome, I., and Gemma, H. 2011. Development of non-chemical fumigation method with vapor heat treatment by using superheated steam for imported banana fruit. Research for Tropical Agriculture (Extra issue in Japanese) 4: 75-76.
Neven, L. G. 2000. Physiological responses of insects to heat. Postharvest Biology and Technology 21: 103–111.
Ornelas-Paz, J. de J. and Yahia, E. M. 2014.Effect of the moisture content of forced hot air on the postharvest quality and bioactive compounds of mango fruit (*Mangifera indica* L. cv. Manila). Journal of the Science of Food and Agriculture 94: 1078–1083.
Palmer, J. K. and McGlasson, W. B. 1969.Respiration and ripening of banana fruit slices. Australian journal of biological sciences22: 87-99.
Pathak, N. and Sanwal, G. G. 1998.Multiple forms of polygalacturonase from banana fruits.Phytochemistry 48: 249–255.
Pathak, N., Asif, M. H., Dhawan, P., Srivastava, M. K. and Nath, P. 2003. Expression and activities of ethylene biosynthesis enzymes during ripening of banana fruits and effect of 1-MCP treatment. Plant Growth Regulation 40: 11–19.
Paull, R. E. and Chen, N. J., 2000.Heat treatment and fruit ripening. Postharvest Biology and Technology 21: 21–37.
Paull, R. E. and Odilo D. 2011.Tropical fruits.2nd ed. Crop production science in horticulture series; no. 20. London: MPG Books Group.
Payasi, A. and Sanwal, G. G., 2003.Pectate lyase activity during ripening of banana fruit.Phytochemistry 63: 243–248.
Payasi, A., Misra, P. C. and Sanwal, G. G. 2004.Effect of phytohormones on pectate lyase activity in ripening Musa acuminata. Plant Physiology and Biochemistry 42: 861–865.
Plant Protection Station. July, 2016. Functions of the Plant Protection station. Retrieved on July 1, 2016 from website:*http://www.pps.go.jp/english/jobs/index.html*
Promyou, S., Ketsa, S. and van Doorn, W. G. 2008. Hot water treatments delay cold-induced banana peel blackening. Postharvest Biology and Technology 48: 132–138.
Rose, J. K. C. 2003. The plant cell wall.In Rose, J. K. C., Catalá, C., Gonzalez-Carranza, Z. H. and Roberts, J. A. (Eds).Cell wall disassembly, p. 264-275.Bodmin, Cornwall: MPG Book Ltd.
Roy, S., Conway, W. S., Watada, A. E. and Sams, C. E. 1994. Heat treatment affects epicuticular wax structure and postharvest calcium uptake in "Golden Delicious" apples. Horticultural Science 29: 1056–1058.
Saltveit, M. E. 1999. Effect of ethylene on quality of fresh fruits and vegetables. Postharvest Biology and Technology 15: 276–292.
Seymour, G. B., Thompson, A. K. and John, P. 1987. Inhibition of degreening in the peel of bananas ripened at tropical temperatures. I. Effect of high temperature on changes in the pulp and peel during ripening. Annals of Applied Biology 110: 145–151.
Shalom, N. B., Hanzon, J., Klein, J. D. and Lurie, S. 1993. A postharvest heat treatment inhibits cell wall degradation in apples during storage. Phytochemistry 34: 955–958.
Thomas, P. and Janave, M. T. 1992.Effect of temperature on chlorophyllase activity, chlorophyll degradation and carotenoids of Cavendish bananas during ripening. International Journal of Food Science and Technology 27: 57–63.
Ummarat, N., Matsumoto, T. K. and Wall, M. M., Seraypheap, K. 2011. Changes in antioxidants and fruit quality in hot water-treated "Hom Thong" banana fruit during storage. Scientia Horticulturae 130: 801–807.
Vicente, A. R., Costa, M. L., Martínez, G. A., Chaves, A. R. and Civello, P. M. 2005.Effect of heat treatments on cell wall degradation and softening in strawberry fruit. Postharvest Biology and Technology 38: 213–222.
Wall, M.M., 2004.Ripening behavior and quality of "Brazilian" bananas following hot water immersion to disinfest surface insects. Horticultural Science 39: 1349–1353.
Waravdekar, V. S.andSaslaw, L. D. 1959. A sensitive colorimetric method for the estimation of 2-deoxy

sugars with the use of the malonaldehydethiobarbituric acid reaction. Journal of Biological Chemistry 234: 1945–1950.

Yang, X., Zhang, Z., Joyce, D., Huang, X., Xu, L. and Pang, X. 2009.Characterization of chlorophyll degradation in banana and plantain during ripening at high temperature. Food Chemistry 114: 383–390.

Zhang, H., Yang, S., Joyce, D. C., Jiang, Y., Qu, H. and Duan, X. 2010. Physiology and quality response of harvested banana fruit to cold shock. Postharvest Biology and Technology 55: 154–159.