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Reactive Oxygen Species (ROS) Scavenging in Hot Air Preconditioning Mediated Alleviation of Chilling Injury in Banana Fruits

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Abstract

Banana fruits (*Musa sp*., AAA group, Cavendish subgroup cv. 'Williams') were exposed to two hot air treatment regimes namely 50℃ for 10 minutes (HAT A) and 40℃ for 60 minutes (HAT B). The fruits were then stored at chilling temperatures (8℃) for up to 21 days to evaluate the efficacy the treatments on chilling injury (CI) alleviation and activity of reactive oxygen species (ROS) following the treatments. The hot air treatments initially disrupted normal cellular functions as evidenced by higher percentage of initial ion leakage. However upon transfer to cold storage, the trend was reversed and ion leakage was higher in the untreated controls compared to the treated bananas. Symptoms of CI appeared earlier (5^{th} day) and progressed faster in the untreated controls compared to the treated bananas. The slow progression of CI in treated bananas was accompanied by increased reactive oxygen species (ROS) scavenging capacity. This was evidenced by higher activity of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD) and ascorbate peroxidase (APX), in the treated bananas. Similarly, the components of the ascorbate-glutathione cycle were positively affected by the hot air treatments. The content of reduced glutathione (GSH) and reduced ascorbic acid (AsA) were slightly higher, contributing to the higher antioxidant potential in the treated bananas; this further enhanced the ROS scavenging capacity. Moreover the activity of glutathione reductase (GR) which is essential in recycling glutathione was slightly higher in the treated bananas. These results indicate that the increased antioxidants' content and antioxidant enzymes' activity triggered by hot air treatments positively enhanced the bananas' tolerance to chilling temperature.

Keywords: *Musa spp,* ROS, SOD, POD, catalase, glutathione, chilling injury

1. Introduction

Low temperature storage is the most effective way to slow down deteriorative metabolic and pathological processes in harvested commodities. However, chilling injury poses a major limitation to cold storage in most tropical and subtropical fruits. Banana is very sensitive to chilling stress and even temperatures as high as 13℃ can cause chilling injury (Chaiprasart, 2002; Pongrasert et al., 2006). Banana fruits' sensitivity to chilling injury deters the possibility of prolonging the postharvest shelf life through low temperature storage. In previous reports, there are various strategies which have been used or have been proposed to alleviate chilling injury in several fruits and vegetables. Examples of these strategies include enhancing polyamine activity (Shen et al., 2000; Martinez-Tellez et al., 2002), application of jasmonic and salicylic acid derivatives (Chaiprasart, 2002; Pongrasert et al., 2006). Temperature management is a non-chemical alternative that has been shown to alleviate chilling injury in some commodities. High temperature pre-conditioning has been used in postharvest handling of fruits for insect disinfestation, decay control, ripening delay and modification of fruit responses to other stresses (Lurie, 1998; Paull & Chen, 2000). Exposure to high temperature is known to induce thermotolerance and in harvested commodities it has been shown to induce tolerance to chilling stress in some tropical and subtropical fruits. Exposure of plant tissues to a moderate stress not only induces the resistance to this kind of severe stress, but can also improve tolerance to other stresses (Wang et al., 2003). This cross-protection

following heat shock has been reported to result in tolerance to chilling stress in various fruits including grape fruits (Zhang et al., 2005), avocado (Woolf & Lay-Yee, 1997) and tomato (Lurie & Klein, 1991). High temperature preconditioning is also thought to induce reactive oxygen species (ROS), followed by the production of ROS scavengers such as superoxide dismutases (SOD), peroxidases (POD) and catalases (Holmberg & Bulow, 1998; Mittler et al., 2004). These ROS scavengers are in turn believed to offer cell protection from oxidative stress resulting from destructive conditions such as chilling injury (Soto et al., 2005; Yahia et al., 2007). Commodities' response to heat treatments vary depending on the temperature and time of exposure, species (cultivar) as well as the preharvest factors. Although heat treatments have been used successfully to control postharvest pests and fungal infections in banana fruits, there are no reports on the use of such treatments with the objective of alleviating chilling injury. Therefore the objective of this study was to determine the effectiveness of hot air treatments in alleviating chilling injury in banana fruits and to elucidate the role of antioxidants and antioxidant enzymes in this process.

2. Materials and Methods

2.1 Plant Materials and Treatments

Banana fruits (*Musa sp*., AAA group, Cavendish subgroup cv. 'Williams') imported from Ecuador were used. Prior to the actual experiment, various temperature and time combinations were tried in a preliminary study, to determine the best combination that imparted some level of cold tolerance without causing severe heat injury. After this preliminary phase, two treatment regimes were selected for further investigations; hot air treatment at 50℃ for 10 minutes (HAT A) and hot air treatment at 40℃ for 1 hour (HAT B). The fruits were arranged in single layer on a flat plastic tray and placed in a preheated hot air chamber for the prescribed time. After the heat treatment, the fruits were allowed to cool to room conditions for one hour then packaged into separate paper bags and stored at 8℃ for 21 days. Two controls were used in this experiment; the first control included untreated fruits stored at 8℃; in the second control, untreated fruits were stored at ambient room conditions throughout the 21 days period. A random sample was taken from each treatment before cold storage, and then 2, 5, 8, 14 and 21 days after cold storage. These randomly selected samples were separated into two batches. One batch was used to measure various parameters immediately after removal from cold storage. The second batch was kept at room conditions for 5 days to allow full expression of chilling injury and then evaluated for the same parameters. In each case, determination of chilling injury index and ion leakage was done on fresh fruit samples. Thereafter samples of the peel tissues were frozen in liquid nitrogen and stored at -80℃ for later determination of antioxidants and antioxidant enzymatic activities.

2.2 Evaluation of Chilling Injury (CI)

Ten fruits were chosen randomly from each treatment at each sampling time and assessed for CI. The extend of CI was determined from visual observation of the bananas immediately after cold storage and after 5 days storage at ambient room conditions. Scores were made on a scale of 0 to 5 as follows; 0=no injury, 1=very mild injury, 2=mild injury, 3=moderate injury, 4=severe injury and 5=very severe injury.

2.3 Evaluation of Membrane Permeability

Membrane permeability was determined based on electrolyte (ion) leakage. Ten discs of similar dimensions (2mm thick) were excised from the banana peel using a 15 mm diameter cork borer. The discs were rinsed in distilled water, placed into a conical flask with 50ml of distilled water and then incubated on a shaking water bath for 3 hours, at a frequency of 50 stokes per min and temperature 25℃. After the 3-hour incubation period, the initial electrical conductivity (EC) of the suspending solution was measured using a conductivity meter (COA Conductivity meter, Model CM-30ET, TOA Electronics Ltd, Japan). Thereafter the discs were returned to their respective solutions and autoclaved for 15 minutes to determine 100% leakage resulting from complete cell death. Electrolyte leakage was calculated as follows: % ion leakage=(initial EC/final EC) x 100.

2.4 Extraction of Antioxidants and Antioxidant Enzymes

Ten grams of frozen peel tissue was homogenized in 40ml of ice-cold 50mM sodium phosphate buffer (pH 7.0) containing 3mM EDTA and 0.2g polyvinylpolypyrrolidine (PVPP). The homogenate was then filtered through 3 layers of miracloth and then centrifuged at 10,000 g (4℃) for 20 minutes. The supernatant was used for the analyses of SOD, POD, CAT and APX. For the determination of glutathione, 2 grams of the frozen tissue was homogenized in 20ml ice-cold sodium phosphate buffer (pH 7.5) containing 0.1 M HCl, 1 mM EDTA, 0.5% TritonX-100 and 1.25% PVPP. The homogenate was filtered through 3 layers of miracloth then centrifuged at 15,000 g and temperature 4℃ for 30 minutes. The supernatant was used for the determination of total glutathione and oxidized glutathione (GSSG). To determine the activity of glutathione reductase (GR), 2 grams

of frozen peel tissue was homogenized in ice-cold potassium phosphate buffer (pH 7.0) containing 1mM EDTA, 2% PVPP and 0.05% TritonX-100. The homogenate was then filtered through 3 layers of miracloth before being centrifuged at 10,000 g and temperature 4℃ for 10 minutes. The supernatant was used for the assay of GR activity. For the determination of ascorbic acid, 5 grams of tissue sample was homogenized in 20ml of ice-cold 5% metaphosphoric acid. The homogenate was then filtered through whatman filter paper and the supernatant used for the analysis.

2.5 Antioxidant Enzyme Assays

2.5.1 Superoxide Dismutase (SOD) Activity

SOD activity was determined according to the Xanthine oxidase/Nitroblue tetrazolium (XO/NBT) system as described by Ukeda et al. (1997), with some modification. The assay mixture was composed of 0.1 ml of the enzyme extract, 2.3 ml of sodium phosphate buffer, 0.1 ml of 1mM NBT, 0.1 ml of 4mM xanthine and 0.1 ml of 0.15% BSA. Water was used instead of the enzyme extract in the blank sample. The reaction was initiated by addition of 0.1 ml of xanthine oxidase and the reaction mixture incubated on a water bath at 30℃ for 20 minutes. The reaction was stopped by addition of 0.2 ml of 8mM $CuCl_2$. The differences in the intensity of the blue color were measured as the absorbance at 560 nm and temperature 25℃. Activity of SOD in the enzyme extract was expressed in terms of its ability to inhibit superoxide radical production from the xanthine-XO activity.

2.5.2 Catalase (CAT) Activity

CAT activity was determined based on the ability to decompose H_2O_2 at assay conditions as described by Aebi (1984), with some modification. The reaction mixture was composed of 2.2 ml of 50mM sodium phosphate buffer and 0.2 ml of the enzyme extract. The reaction was initiated by addition of 0.1 ml of 100 mM H_2O_2. One unit of CAT was defined as the amount of enzyme that resulted in a 0.01 decrease in absorbance at 240nm per minute at temperature 25℃.

2.5.3 Guaiacol Peroxidase (POD) Activity

POD activity was determined as described by Jiang et al. (1984) with some modification. The assay mixture was composed of 7.9 ml of the sodium phosphate buffer, 1 ml of 20mM guaiacol and 0.1 ml of the enzyme extract. The reaction was initiated by adding H_2O_2 followed by 10 minutes incubation at 25℃. One unit of POD was defined as the amount of enzyme that caused a 0.001 increase in absorbance at 470nm at temperature 25℃.

2.5.4 Ascorbate Peroxidase (APX) Activity

APX activity was assayed according to Nakano and Asada (1981) with some modification. APX activity was based on the decrease in absorbance at 290nm as ascorbate was oxidized by H_2O_2 .The assay mixture was composed of 0.3 ml of the enzyme extract, 0.2 ml of 0.5 mM sodium ascorbate and 1.9 ml of sodium phosphate buffer. The reaction was initiated by adding 0.1 ml of H_2O_2. One unit of APX was defined as the amount of enzyme that resulted in a 0.01 decrease in absorbance at 290nm per minute at 25℃.

2.6 Determination of Glutathione Content and Glutathione Reductase (GR) Activity

2.6.1 Glutathione Content

Reduced (GSH) and oxidized glutathione (GSSG) levels were determined as described by Griffith (1980). For total glutathione, 0.2 ml of the enzyme extract was neutralized with 0.4 ml of sodium phosphate buffer (0.5 M, pH 7.5). Thereafter, 1.0 ml of 100 mM potassium phosphate buffer (pH 7.5 containing 1mM EDTA), 0.2 ml of 5 mM DTNB and 0.1 ml of GR (35 units/ml) were added. The reaction was started by addition of 0.1 ml of 1.5 mM NADPH. In this assay, GSH was oxidized by DNTB, 5,5'-dithiobis(2-nitrobenzoic acid) with stoichiometric formation of TNB (5-thio-2-nitrobenzoic acid). While GSSG was reduced by GR in the presence of NADPH. The rate of formation of TNB which was monitored as change in absorbance at 412 nm in this assay is proportional to the sum of GSH and GSSG present. To determine amount of GSSG only, 0.4 ml of the enzyme extract was neutralized by adding 18µl of triethanolamine, and then pretreated with 6µl of 2-vinvyl pyridine for 60 minutes at room temperature to mask GSH by derivatization and allow the determination of GSSG alone. Thereafter, the same procedure as that used for the determination of total glutathione was used. GSH content was determined from the difference between total glutathione and GSSG.

2.6.2 Glutathione Reductase (GR) Activity

GR activity was assayed only in banana fruits from HAT A treatment regime. The GR activity was determined as described by Halliwell and Foyer (1978) with some modification. The assay mixture was composed o 0.5 l of 2 mM oxidized glutathione (GSSG), 1.05 ml of potassium buffer and 0.2 ml of the enzyme extract. The reaction

was initiated by adding 0.05 ml of mM NAPDH. GR activity was determined by measuring the rate of NADPH oxidation, as the decrease in absorbance at 340 nm.

2.7 Determination of Ascorbic Acid Content

Five grams of tissue sample was homogenized in 20ml of ice-cold 5% metaphosphoric acid. The homogenate was then filtered through whatman filter paper and the supernatant used for ascorbic acid analysis. Total ascorbic and dehydroascorbic acid was analyzed following the 2, 4-Dinitrophenylhydrazine (DNP) method as described by Roe et al. (1948) with some modifications. For total ascorbic acid, 0.2ml of 0.2% indolephenol was added to 0.4ml of the enzyme extract, incubated for 3 minutes before adding 0.4ml of 2% thiourea. Thereafter 0.2ml of 2% DNP was added before the samples were incubated for 3 hours on a water bath which was set at 37℃. After this incubation period, 1ml of 85% H_2SO_4 was added and the sample left at room temperature for 30 minutes, then the absorbance at 540nm (26℃) was checked. The same procedure was used in the determination of dehydroascorbic acid, but in this case, instead of adding 0.2ml of 0.2% indolephenol to enzyme extract, 0.2ml of 5% metaphosphoric acid was added. A standard curve was prepared from L-ascorbic acid and used to determine the actual levels of ascorbic acid in the enzyme extracts.

3. Results

3.1 Effect of Hot Air Treatments on Chilling Injury-Chilling Injury Index

The extend of chilling injury increased with extension in the storage duration and was aggravated upon transfer to ambient room conditions. In the untreated bananas CI symptoms appeared from the 5th day of cold storage and became more severe as the storage time was prolonged (Figure 1A). This was evidenced by the higher CI index in the untreated bananas compared to the treated bananas. Severity of chilling increased upon transfer to ambient room conditions (Figure 1B). Between the two hot air treatments, short term exposure to higher temperature (10 minutes at 50℃-HAT A) tended to have better effect compared to the long term duration of exposure to relatively lower temperature (1 hour at 40℃-HAT B).

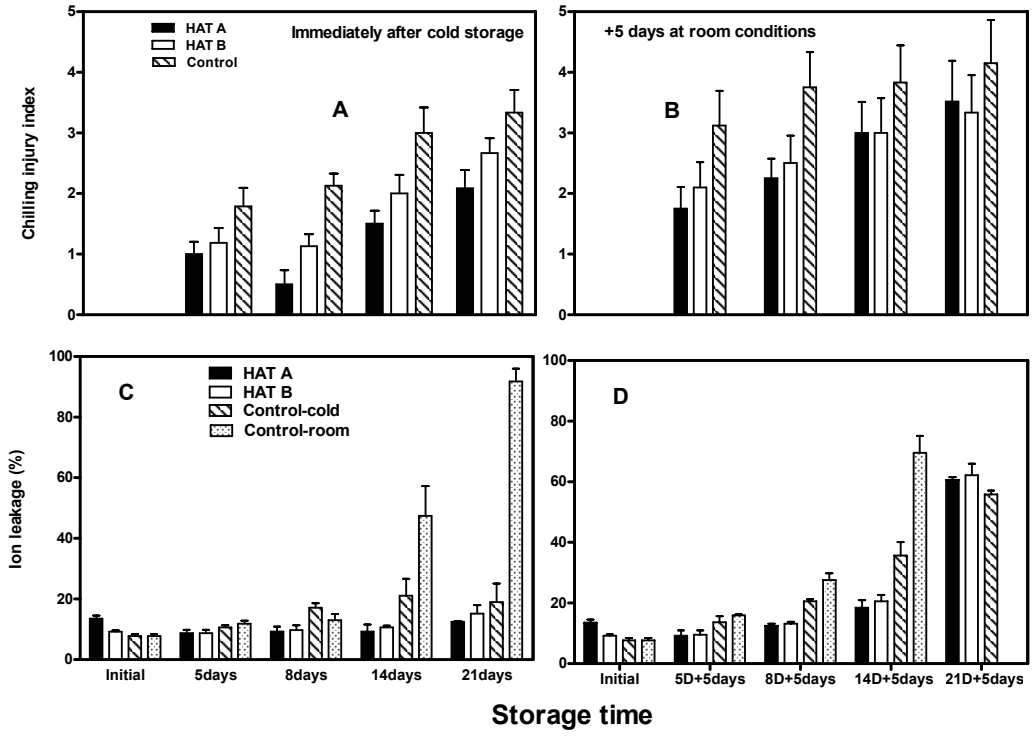

Figure 1. Effect of hot air treatments at 50℃ for 10 minutes (HAT A) and 40℃ for 60 minutes (HAT B) on chilling injury index and ion leakage from peel tissue of banana fruits; immediately after storage at 8℃ (A and C) and upon transfer from cold storage to ambient room conditions for 5 days (B and D). Vertical bars denote SE (±), n=15

3.2 Effect of Hot Air Treatments on Ion Leakage

Immediately after the hot air treatments and prior to low temperature storage, treated bananas had significantly higher levels of ion leakage relative to the untreated fruits; over 70% and 20% in HAT A and HAT B respectively (Figure 1C). However upon transfer to cold temperature storage, ion leakage in both treatments decreased and remained lower than that of the untreated bananas throughout the cold storage period. Transfer from cold storage to ambient room conditions aggravated the extend of ion leakage in both the treated and the untreated bananas (1D). Untreated bananas which were stored at ambient room conditions throughout generally had higher levels of ion leakage and by the end of the storage, there was nearly 100% ion leakage.

3.3 Effect of Hot Air Treatments on Antioxidant Enzyme Activities

3.3.1 Superoxide Dismutase (SOD)

Immediately after hot air treatments, there was a 20% increased in SOD activity in treated bananas, relative to the untreated ones (Figure 2A). SOD activity in the untreated and HAT B treated bananas reduced slightly after 5 days of cold storage. Thereafter, the activity in the two batches increased reaching the highest activity at 8 days, and then gradually decreased until the end of the storage period. SOD activity in HAT A increased gradually to a peak level at 8 days and then decreased gradually. The hot air-treated bananas generally achieved and maintained higher SOD activity than the untreated fruits throughout the storage period.

3.3.2 Guaiacol Peroxidase (POD) Activity

During cold storage, POD activity increased gradually but no significant differences between the treated and untreated bananas was evident (Figure 2C). Upon transfer to ambient room conditions, POD activity in all the bananas increased significantly with the treated bananas showing higher POD activity (Figure 2D). HAT A treated bananas had peak POD activity after 8 days of cold storage followed by 5 days at ambient room conditions, thereafter the activity decreased gradually. HAT B treated bananas had the peak POD activity after 2 weeks of cold storage followed by 5 days of storage at ambient room conditions, thereafter there was a gradual decreased in the activity.

3.3.3 Ascorbate Peroxidase (APX) Activity

Immediately after hot air treated there was a 15% and 22% increase in APX activity in HAT A and HAT B treated bananas respectively, relative to the untreated bananas (Figure 2E). APX activity increased further upon transfer to cold storage in all the bananas but the hot air treated bananas exhibited higher APX activity (Figure 2F). The peak level of APX activity in all the bananas occurred after 8 days of cold storage and thereafter declined gradually.

3.3.4 Catalase (CAT) Activity

Immediately after hot air treatment, HAT A treated bananas had slightly higher CAT activity (15%) compared to the untreated bananas (Figure 2G). On the contrary, in HAT B treated bananas there was a slight decrease (10%) in CAT activity. When the fruits were transferred to cold storage, there was a sharp increase in CAT activity in both the treated and untreated bananas. The peak level of CAT activity was achieved after 8 days of cold storage in all the bananas. HAT A bananas achieved and maintained higher CAT activity compared to the HAT B and the untreated bananas. However at the end of the cold storage period, CAT activity in the untreated bananas was higher than that of the treated bananas.

3.4 Effect of Hot Air Treatments on Glutathione Content and Glutathione Reductase (GR) Activity

There was a slight increase in the total glutathione and reduced glutathione (GSH) content during cold storage until the 8^{th} day of cold storage, thereafter a gradual decrease until the end of storage (Figure 3A). A similar trend was observed upon transfer from cold storage to ambient room conditions (Figure 3B). The treated bananas maintained slightly higher levels of total glutathione and GSH compared to the untreated bananas both during cold storage and after transfer to ambient room conditions. Levels of oxidized glutathione (GSSG) increased slightly during cold storage, with the untreated bananas having higher levels of GSSG compared to the treated bananas (3C). This trend resulted in changes in the GSH/GSSG ratio which followed a similar trend as GSH (Figure 3E). Treated bananas had a significantly higher GSH/GSSG ratio compared to the untreated bananas immediately after cold storage and even upon transfer to ambient room conditions (3F).

Hot air treatments resulted in increased GR activity in the fruits prior to cold storage. Transfer to cold storage led to a steady rise in GR activity in the treated bananas, reaching the peak 2 weeks after cold storage and thereafter reduced gradually (Figure 4). HAT A treated bananas maintained higher GR activity compared to HAT B

bananas. In the control, GR activity also increased gradually during cold storage, reached peak activity 8 days after cold storage and then decreased gradually until the end of storage.

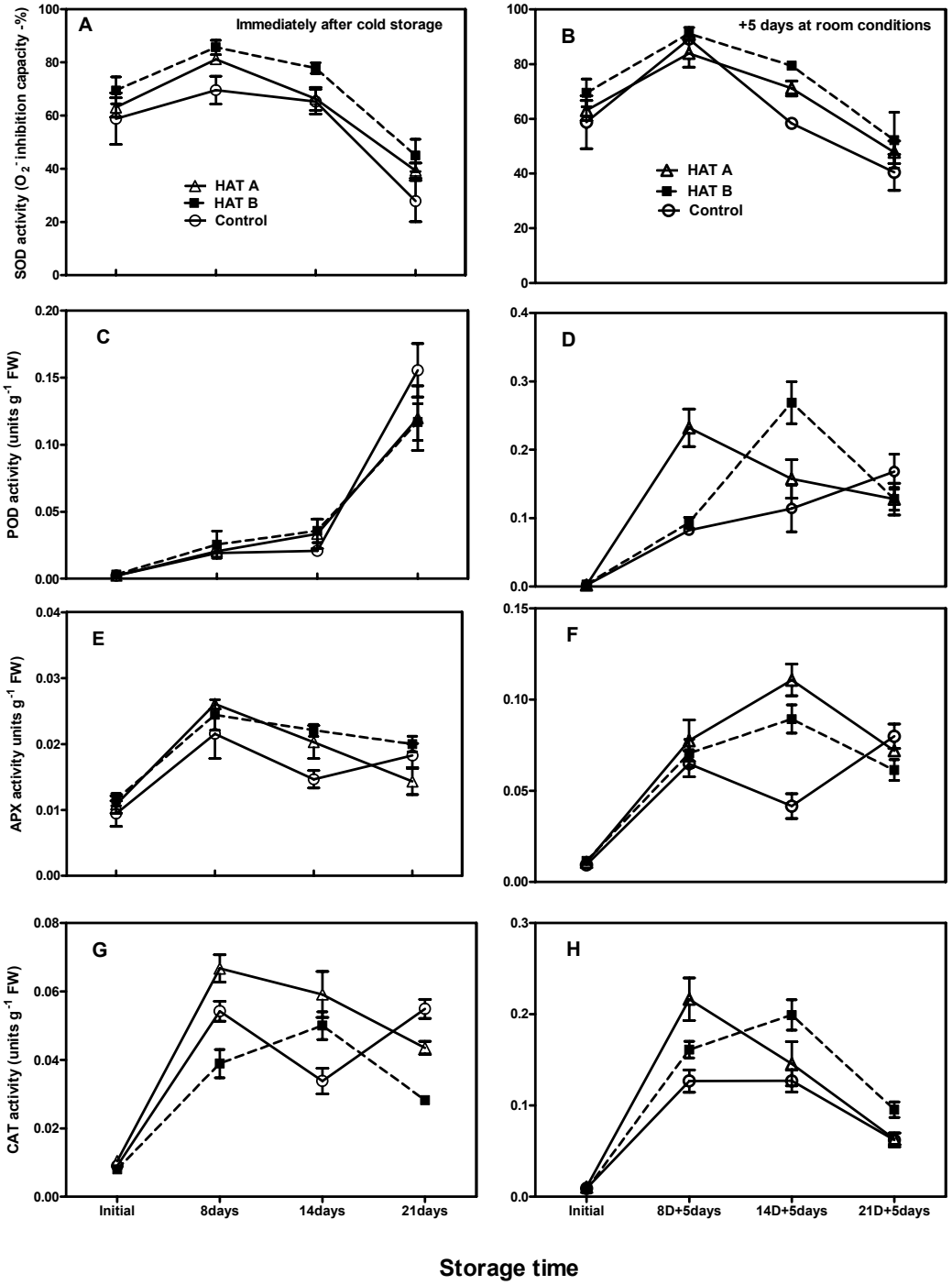

Figure 2. Effect of hot air treatments at 50℃ for 10 minutes (HAT A) and 40℃ for 60 minutes (HAT B) on antioxidant enzyme activities; SOD (A), POD (C), APX (E) and CAT (G) in the peel tissue of banana fruits immediately after storage at 8℃ and upon transfer from cold storage to ambient room conditions for 5 days (B, D, F and H). Vertical bars denote SE (±), n=10

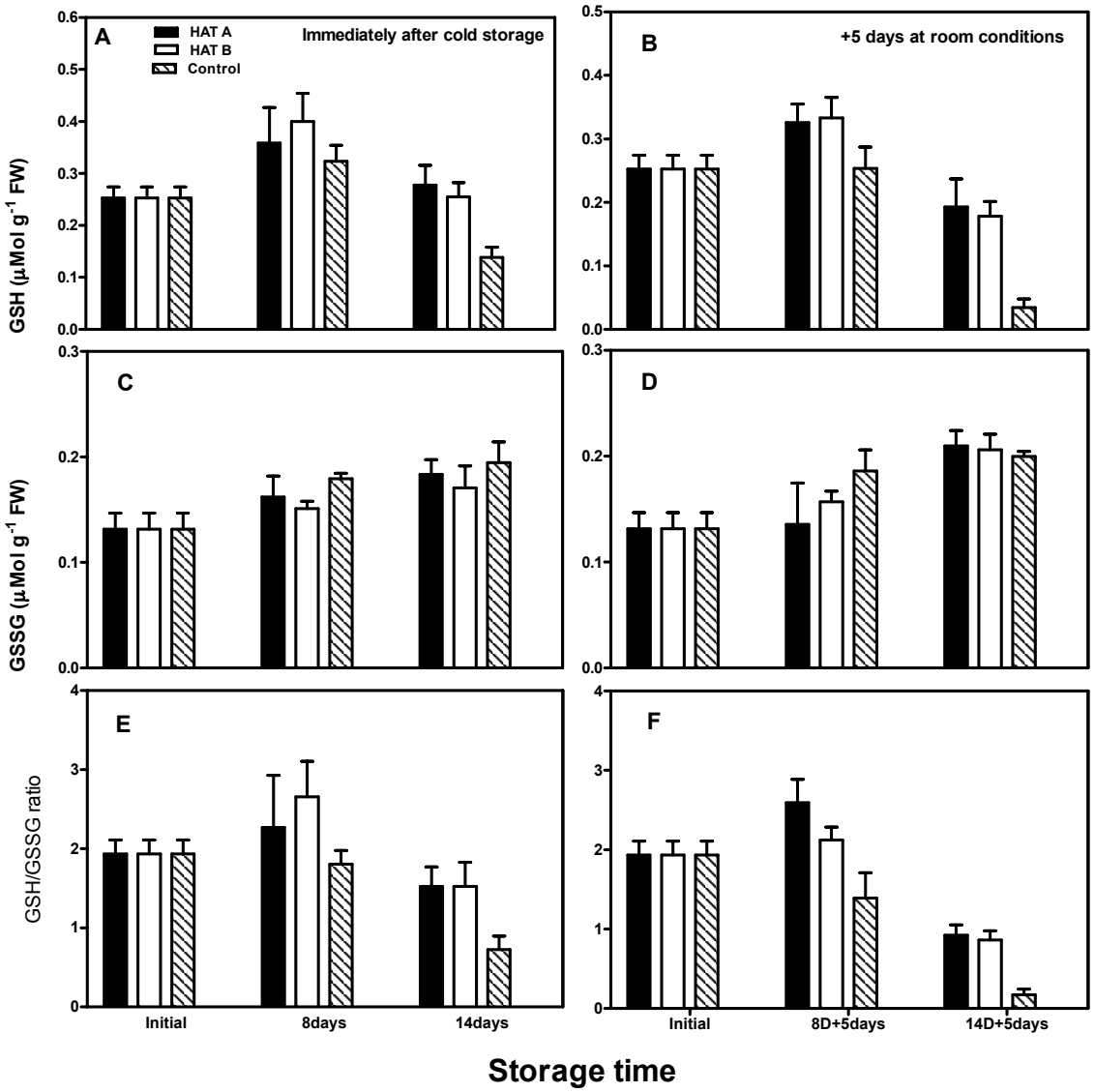

Figure 3. Effect of hot air treatments at 50℃ for 10 minutes (HAT A) and 40℃ for 60 minutes (HAT B) on the content of reduced glutathione-GSH (A), oxidized glutathione-GSSG (C) and the GSH/GSSG ratio (E) in the peel tissue of banana fruits immediately after cold storage at 8℃ and upon transfer to ambient room conditions for 5 days (B, D and F). Vertical bars denote SE (±), n=10

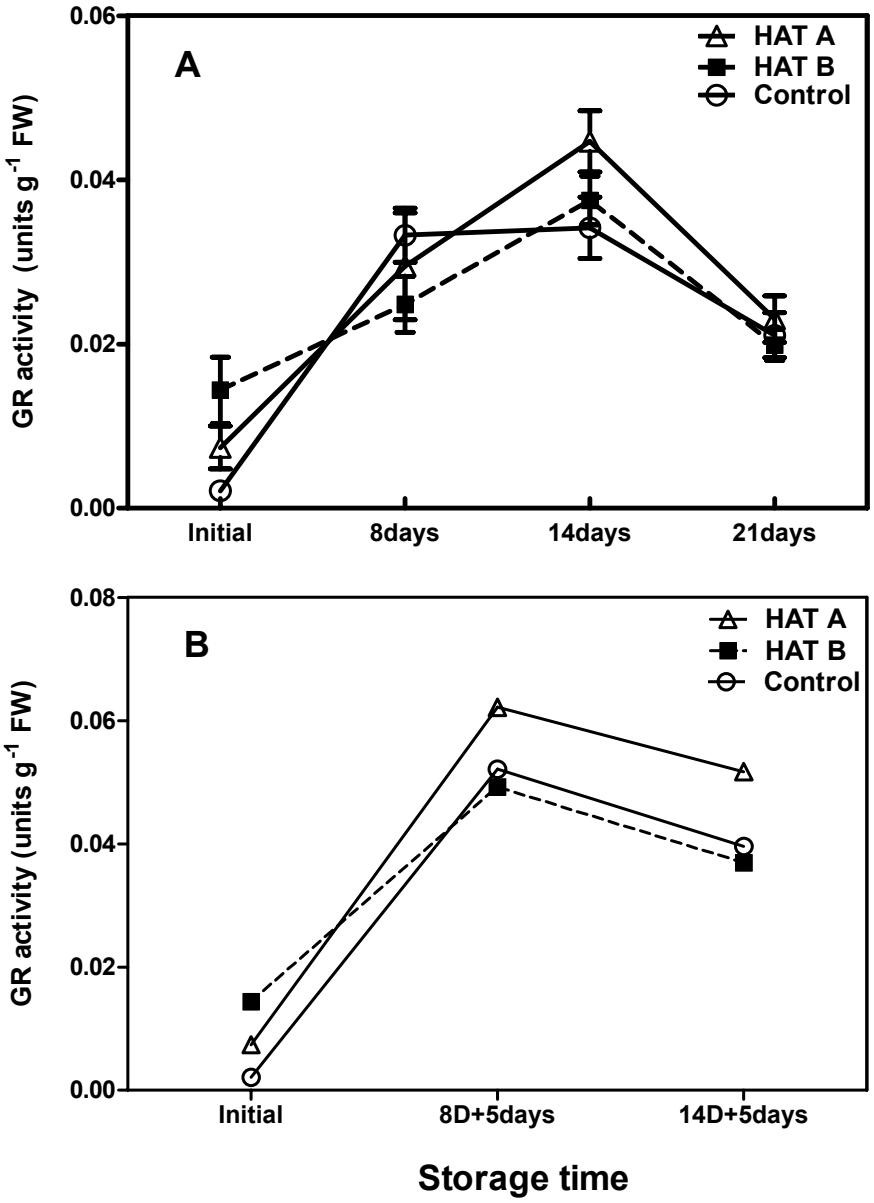

Figure 4. Effect of hot air treatments at 50℃ for 10 minutes (HAT A) and 40℃ for 60 minutes (HAT B) on glutathione reductase (GR) activity in the peel tissue of banana fruits immediately after storage at 8℃ (A) and upon transfer from cold storage to ambient room conditions for 5 days (B). Vertical bars denote SE (±), n=10

3.5 Effect of Hot Air Treatment on Ascorbic Acid Content

The levels of reduced ascorbic acid (AsA) declined gradually during storage for both the HAT A treated bananas and the control (Figure 5A). On the other hand, oxidized ascorbic acid (DHAsA) increased slightly at the end of storage in both the HAT A treated bananas and the control (Figure 5C). Following these changes, the ratio of AsA/ DHAsA gradually declined during storage, the changes followed the same pattern as AsA (Figure 5 E). HAT A treated bananas maintained a slightly higher AsA/ DHAsA ratio, which increased slightly after 8 days of cold storage and thereafter reduced gradually until the end of the storage period. Changes in AsA, DHAsA and AsA/DHAsA ratio followed a similar trend when the bananas were transferred from cold storage to ambient room conditions for days 5 days (Figure 5B, D and F).

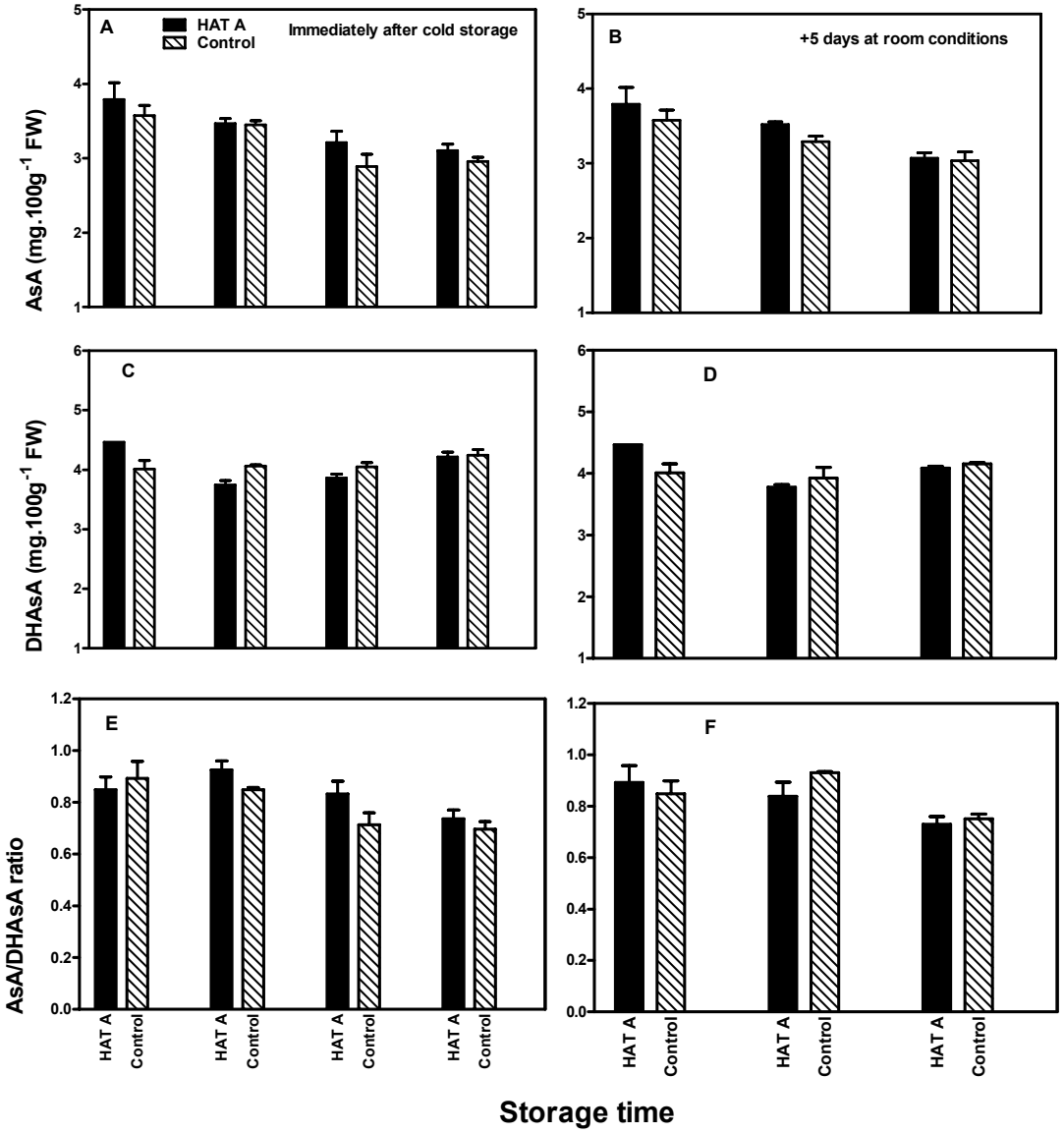

Figure 5. Effect of hot air treatments at 50℃ for 10 minutes (HAT A) on the content of reduced ascorbic acid-AsA (A), oxidized ascorbic acid-DHAsA (C) and the AsA/DHAsA ratio (E) in the peel tissue of banana fruits immediately after storage at 8℃ and upon transfer from cold storage to ambient room conditions for 5 days (B, D and F). Vertical bars denote SE (±), n=10

4. Discussion and Conclusion

In banana, prestorage high temperature (hot air and hot water) treatments are traditionally used for disinfestations from insect pests and to control of fungal infections. The use of such treatments has become particularly important because of the growing need to decrease the postharvest use of chemicals to control fungi and insects. In the present study, other benefits of high temperature pretreatments were explored. Hot air treatments significantly enhanced bananas' tolerance to chilling injury as evidenced by the lower chilling injury index compared to the untreated controls. The peel color of the untreated bananas started darken by the 5th day of cold storage signifying early progression of chilling injury in the untreated bananas. This peel darkening is associated with oxidation of phenolic substrates by polyphenol oxidase. This is thought to be the major cause of the browning or darkening discoloration of many fruits and vegetables (Vaughn et al., 1988; Walker & Ferrar, 1998). Pongprasert et al. (2006) reported increased activity of polyphenol oxidase that correlated with increased

CI symptoms in cold-stored bananas. Chilling injury is thought to result in increased membrane permeability leading to an increase in leakage of cellular constituents (Murata, 1990; Sharom et al., 1994). The rate of ion leakage from excised tissue into an isotonic aqueous solution is a useful measure of the severity of chilling-induced increase in membrane permeability (King & Ludford, 1983). In the present study, initial exposure to hot air treatment resulted in a significant increase in ion leakage, relative to the untreated control. This could have resulted from initial heat injury, leading to a disruption of normal cellular functions in the treated tissues. However upon transfer to low temperature, ion leakage in the treated bananas reduced to levels lower than that of the untreated control. This reduction in ion leakage correlated with lower CI symptoms in treated bananas. These findings are in agreement with previous reports in other commodities exposed to high temperature preconditioning. A 50% higher electrolyte leakage was found from apple fruit discs isolated after being held at 38°C for 2 days (Lurie & Klein, 1990). When the discs were transferred to 20°C, leakage declined within 2 days, to the levels of the untreated controls. Heat treatments have been reported to reduce CI-induced ion leakage in tomato (Saltveit, 2001; 2005), grape berries (Zhang et al., 2005). It is hypothesized that heat-shock treatments confer chilling tolerance by inducing the production of proteins that interact with and stabilize proteins and/or membranes (Saltveit, 2001).

Oxidative damage resulting from increased ROS production is considered to be an early response of sensitive tissues to chilling temperatures (Hariyadi & Parkin, 1991). Accumulation of ROS under stress results in an imbalance between ROS production and the cell's scavenging capacity (Mittler, 2002). An enhanced antioxidant defense from oxidative stress is thought to be one of the mechanisms involved in the cold tolerance conferred to chilling sensitive tissues by high temperature preconditioning. In the present study, hot air treatments resulted in increased activities of antioxidant enzymes (SOD, POD, APX and CAT) and enhanced levels of non-enzymatic antioxidants (glutathione and ascorbic acid). A balance between the formation and detoxification of ROS is critical to cell survival during cold storage (Zhang et al., 1995). SOD activity was significantly higher in treated bananas compared to the untreated control. These findings concur with previous reports in strawberry (Vicente et al., 2006) and cucumber (Kang et al., 2002). SOD is found in all aerobic organisms and most subcellular compartments that generate ROS (Scandalios, 1993). It is considered to be a key player within the antioxidant defense system because it regulates the cellular concentration of the superoxide radical, which is dismutated to H_2O_2. Therefore, increased SOD activity as seen in the present study would probably imply increased protection from the reactive superoxide ion whose production is increased during cold storage (Pongrasert, 2006). The resultant H_2O_2 is less reactive compared to other ROS but it may be harmful because of its relative stability hence ability to spread within and among the cells by diffusion. H_2O_2 can also give rise to more ROS which greatly increases its cytotoxicity (Feierabend, 2005). Survival of the plant cell therefore depends on the efficient scavenging of H_2O_2. Catalase enzyme is the primary H_2O_2 scavenger in glyoxysomes, peroxisomes and mitochondria (Prasad et al., 1994). In the present study, increased activity of catalase was observed during and after cold storage; the treated bananas maintained relatively higher catalase activity which coincided with lower CI symptoms. The role of catalase in oxidative stress has been documented in other commodities including mandarin (Yahia et al., 2007), cucumber (Kang & Saltveit, 2001) and zucchini squash (Wang, 1995). H_2O_2 generated in the chloroplasts is removed from the cell by the various forms of peroxidases through the ascorbate-glutathione cycle. Ascorbate peroxidase (APX) is found in almost every component of the plant cell and participates in the removal of H_2O_2 as part of the ascorbate-glutathione pathway. In the present study, APX activity in the treated bananas was slightly higher compared to the untreated bananas, thereby enhancing the ROS scavenging capacity in the former. APX function is dependent upon the availability of a pool of ascorbate and glutathione. Both ascorbate and glutathione constitute the ascorbate-glutathione cycle together with APX, MDHAR, DHAR and GR. This redox cycle has been reported to be present in chloroplasts, mitochondria, peroxisomes and cytosol and its activation results in scavenging H_2O_2 produced in the cell (Noctor & Foyer, 1998; Asada, 1999). In the present study, the treated bananas had slightly higher levels of reduced ascorbic acid (AsA) and a resultant AsA: DHAsA ratio. As antioxidant, ascorbic acid plays a significant role in the ROS scavenging system through direct detoxification of ROS or indirectly by providing the reducing power to APX which plays a role in the detoxification of H_2O_2. The results indicate a synergistic role between APX and AsA, with the latter probably acting as the reductant, thereby ensuring sustained APX activity. Just like ascorbic acid, glutathione which has antioxidant capacity can quench ROS directly or indirectly as a reductant. In the present study, treated bananas had slightly higher levels of reduced glutathione (GSH) and lower levels of oxidized glutathione (GSSG). This resulted in a higher GSH/GSSG ratio hence greater redox capacity in tissues of the treated bananas. Apart from its direct effect in detoxification of ROS, glutathione serves also as a cofactor for glutathione-dependent peroxidase (Chaudiere & Ferrari-Iliou, 1999). It has been shown that depletion of the glutathione pool may lead to oxidative stress (De Vos et al., 1992). Indirectly, glutathione contributes to the ROS

scavenging capacity by recycling ascorbic acid, which is oxidized in the H_2O_2 scavenging process. Reduced glutathione reacts with the oxidized form of ascorbic acid (dehydroascorbate) to produce ascorbate and oxidized glutathione (GSSG). For effective scavenging, glutathione should be maintained in its physiologically active form, GSH, a role played by glutathione reductase (GR). GR catalyses the NADPH-dependent reduction of GSSG to GSH, thereby maintaining high GSH levels and a high GSH/GSSG ratio (Gamble & Burke, 1984). In the present study, GR activity was slightly higher in the treated bananas. This probably contributed to the overall effect by maintaining higher levels of GSH in the treated banana tissues. In conclusion, this study revealed a synergistic role played amongst various antioxidant enzymes, ascorbic acid and glutathione in detoxification of ROS generated due to stress resulting from cold storage. The study also showed that hot air treatments positively affected the antioxidant system thereby resulting in the ability of the treated bananas to tolerate chilling stress better than the untreated bananas.

References

Aebi, H. (1984). Catalase in vitro. *Methods Enzymol, 105*, 121-126. http://dx.doi.org/10.1016/S0076-6879(84)05016-3

Asada, K. (1999). The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annu. Rev. Plant Physiol. *Plant Mol. Biol., 50*, 601-639. http://dx.doi.org/10.1146/annurev.arplant.50.1.601

Chaiprasart, P. (2002). *Physiological and biochemical changes during occurrence of chilling injury in banana fruits and applicable techniques to reduce its damage*. Ph.D. Thesis. University of Tsukuba, Division of Agricultural Forestry.

Chaudiere, J., & Ferrari-Iliou, R. (1999). Intracellular antioxidants: from chemical to biochemical mechanism. *Food and Chem. Toxic., 37*, 949-962. http://dx.doi.org/10.1016/S0278-6915(99)00090-3

De Vos, C. H., Vonk, M. J., Voojis, R., & Schat, H. (1992). Glutathione depletion due to copper-induced phytochelatin syntheis causes oxidative stress in *Silene cucubalus. Plant Physiol, 98*, 853-858. http://dx.doi.org/10.1104/pp.98.3.853

Gamble, P. E., & Burke, J. J. (1984). Effect of water stress on the chloroplast antioxidant system: 1. Alterations in glutathione reductase activity. *Plant Physiol, 76*, 615-621. http://dx.doi.org/10.1104/pp.76.3.615

Griffith, O. W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry, 106*, 207-212. http://dx.doi.org/10.1016/0003-2697(80)90139-6

Halliwel, B., & Foyer, C. H. (1978). Properties and physiological function of a glutathione reductase by affinity chromatography. *Planta, 139*, 9-17. http://dx.doi.org/10.1007/BF00390803

Hariyadi, P., & Parkin, K. L. (1991). Chilling-induced oxidative stress in cucumber fruits. *Postharvest Biol. Technol, 1*, 33-45. http://dx.doi.org/10.1016/0925-5214(91)90017-6

Holmberg, N., & Bulow, L. (1998). Improving stress tolerance in plants by gene transfer. *Trends Plant Sci., 3*, 61-66.

Kang, H. M., & Saltveit, M. E. (2001). Enzymatic antioxidant defense systems in chilled and head shocked cucumber seedlings radicles. *Physiol. Plant, 113*, 548-556. http://dx.doi.org/10.1016/S1360-1385(97)01163-1

Kanga, H. M., Park, K.W., & Salveit, M. E. (2002). Elevated growing temperatures during the day improve the postharvest chilling tolerance of greenhouse-grown cucumber (*Cucumis sativs*) fruit, *Postharvest Biol. Technol, 24*, 49-57. http://dx.doi.org/10.1034/j.1399-3054.2001.1130414.x

King, M. M., & Ludford, P. M. (1983). Chilling injury and electrolyte leakage in fruit of different tomato cultivars. *J. Am. Soc. Hortic. Sci., 108*, 74-77. http://dx.doi.org/10.1016/S0925-5214(01)00129-6

Lurie, S. (1998). Postharves heat treatments. *Postharvest Biol. Technol., 14*, 257-269. http://dx.doi.org/10.1016/S0925-5214(98)00045-3

Lurie, S., & Klein J. D. (1991). Acquisition of low temperature tolerance in tomatoes by exposure to high temperature stress. *J. Am. Soc. Hort. Sci., 116*, 1007-1012.

Lurie, S., & Klein, J. D. (1990). Heat treatment on ripeness apples differential effects on physiology and biochemistry. *Physiol. Plant, 78*, 181-186. http://dx.doi.org/10.1111/j.1399-3054.1990.tb02078.x

Martinez-Tellez, M. A., Ramos-Clamont, M. G., Gardea, A. A., & Vargas-Arispuro, I. (2002). Effect of infiltrated polyamines on polygalaturonase activity and chilling injury reponses in zucchini squash (*Cucurbita pepo L.*). *Biochem. Biophys. Res. Commun., 295*, 98-101. http://dx.doi.org/10.1016/S0006-291X(02)00631-9

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci., 7*, 405-410. http://dx.doi.org/10.1016/S1360-1385(02)02312-9

Mittler, R. Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends Plant Sci., 9*, 490-498. http://dx.doi.org/10.1016/j.tplants.2004.08.009

Murata, T. (1990). Relation of chilling stress to membrance permeatility. In C. Y. Wang (Ed.). *Chilling Injury of Horticultural Crops* (pp. 201-209), CRC Press, Boca Raton, FL.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidases in spinach chloroplasts. *Plant Cell Physiol, 22*, 867-880

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control, Annu. Rev. Plant Physiol. *Plant Mol. Biol., 49*, 249-279. http://dx.doi.org/10.1146/annurev.arplant.49.1.249

Paull, R. E., & Chen, N. J. (2000). Heat treatment and fruit ripening. *Postharvest Biol. Technol, 21*, 21-37. http://dx.doi.org/10.1016/S0925-5214(00)00162-9

Pongprasert, N., Kanlaynarat, S., Sekozawa, Y., Sugaya, S., & Gemma, H. (2006). Postharvest n-propyl dihydrojasmonate and abscisic acid application on reducing chilling injury of banana fruit (*Musa sp*. Cv. Grande Naine). *Acta Hort., 712*, 741-746.

Prasad, T. K., Anderson, M. D., Martin, B. A., & Stewart, C. R. (1994). Evidence for chilling induced oxidative stress in maize seedlings and regulatory role of hydrogen peroxide. *Plant cell, 6*, 65-74.

Roe, J. H., Mills, M. B., Oesterling, M. J., & Damron, C. M. (1948). The determination of diketo-L gulonic acid, dehydro-L-ascorbic acid and L-ascorbic acid in the same tissue extract by the 2,4-dinitrophenylhydrazine method. *J. Biol. Chem., 174*, 201-208.

Saltveit, M. E. (2001). Chilling injury is reduced in cucumber and rice seedling and in tomato pericarp discs by heat shocks applied after chilling. *Postharvest Biol. Technol., 21*, 169-177. http://dx.doi.org/10.1016/S0925-5214(00)00132-0

Saltveit, M. E. (2005). Influence of heat shocks on the kinetics of chilling-induced ion leakage from tomato pericarp discs. *Postharvest Biol. Technol., 36*, 87-92. http://dx.doi.org/10.1016/j.postharvbio.2004.10.007

Scandalios, J. G. (1993). Oxygen stress and superoxide dismutase. *Plant physiol., 101*, 7-12.

Sharom, M., Willemot, C., & Thompson, J. E. (1994). Chilling injury induces lipid phase changes in membrance of tomato fruit. *Plant Physiol., 105*, 305-308.

Shen, W., Nada, K., & Tachibana, S. (2000). Involvement of polyamines in the chilling tolerance of cucumber cultivars. *Plant physiol., 124*, 431-439. http://dx.doi.org/10.1104/pp.124.1.431

Soto, G., Yahia, E. M. Brecht, J. K., & Gardea, A. (2005). Effects of postharvest hot air treatments on the quality and antioxidant levels in tomato furit. Lebsnsm. *Wiss. U-Technol., 38*, 657-663.

Ukeda H, Maeda, S, Ishii T, & Sawamura, M. (1997). Spectrophotometric assay for superoxide dismutase based on tetrazolium salt 3′-1-(phenylamino)-carbonyl-3, 4-tetrazolium]-bis(4-methoxy-6-nitro)ben zenesulfonicacid hydrate reduction by xanthine-xanthine oxidase. *Anal Biochem, 251*, 206-209. http://dx.doi.org/10.1006/abio.1997.2273

Vaughn, K. C., Lax, A. R., & Duke S. O. (1988). Polyphenol oxidase with no established function. *Plant physiol., 72*, 659-665. http://dx.doi.org/10.1111/j.1399-3054.1988.tb09180.x

Walker, J. R. L., & Ferrar, P. H. (1998). Diphenol oxidase, enzyme catalysed browning and plant disease resistance. Biotech. *Gen. Eng. Rev. 15*, 457-498.

Wang, C. Y. (1995). Effect of temperature preconditioning on catalase, peroxidase, and superoxide dismutase in chilled zucchini squash. *Postharvest Biol. Technol., 5*, 67-76. http://dx.doi.org/10.1016/0925-5214(94)00020-S

Wang, D, & Luthe, D. S. (2003). Heat sensitivity in bentgraaa variant. Failure to accumulate a chloroplast heat shock protein isoform implicated in heta tolerance. *Plant Physiol., 133*, 319-327. http://dx.doi.org/10.1104/pp.102.018309

Woolf, A. B., & Lay-Yee, M. (1997). Pretreatments at 38℃ of 'Hass' avocado confer thermotolernace to 50℃ hot-water treatments. *HortScience, 32*, 705-708.

Yahia, E. M., Soto, G. Z. Brecht, J. K., & Gardea, A. (2007). Postharvest hot air treatment effects on the antioxidant system in stored mature-green tomatoes. *Postharvest Biol. Technol., 44*, 107-115. http://dx.doi.org/10.1016/j.postharvbio.2006.11.017

Zhang, J., Huang, W., Pan, Q., & Liu, Y. (2005). Improvement of chilling tolerance and accumulation of heat shock proteins in grape berries (*Vitis vinifera* cv. Jingxiu) by heat pretreatment. *Postharvest Biol. Technol., 38*, 80-90. http://dx.doi.org/10.1016/j.postharvbio.2005.05.008