HOT AIR TREATMENT IMPROVED THE CHILLING RESISTANCE OF LOQUAT FRUIT UNDER COLD STORAGE

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ABSRACT

Hot air treatment at 38C for 36 h significantly improved the chilling resistance of loquat during 4C storage for 28 days, appeared as higher extractable juice, lower firmness, internal browning and decay development. This treatment reduced hydrogen peroxide level by improving superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase activities, then maintained higher unsaturated/saturated fatty acid ratio and lower permeability of membrane at the end of storage. It also reduced phenylalanine ammonia lyase, peroxidase and polyphenol oxidase activities, and total phenolic content to control internal browning and lignin synthesis; the enhanced antioxidant enzyme activities also contribute to control them. Meanwhile, heat-treated fruits showed significantly higher levels of water- and chelator-soluble pectins and lower Na_2CO_3-soluble pectin than controls at the later of storage. It was suggested that the improved chilling resistance by heat treatment maybe mainly due to the enhanced scavenging capacity of oxygen species and solubilization of cell wall polysaccharides.

PRACTICAL APPLICATIONS

Loquat fruit is very perishable because of microbial decay and mechanical damage after harvest. Low-temperature storage is an effective method to control pathogenic decay and preserve quality. However, loquat fruit are chilling-sensitive, and chilling injury limits the storage period and shelf-life. This work investigated the effects of hot air treatment on the chilling resistance of loquat fruit. It was found that heat treatment at 38C for 36 h can enhance the chilling resistance, reduce the chilling symptoms and maintain higher quality after storage at 4C for 28 days. The information obtained from this study suggested that this heat treatment may be a suitable method for the commercial application on loquat fruits.

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl.) fruit is famous for its soft, juicy taste and nutritional value, highly favored by consumer. It is harvested at hot and rainy season, and has a short postharvest life because of fungi disease, mechanical damage and senescence. Low-temperature storage is a commonly used method to delay senescence and decay development. However, loquat fruit are chilling-sensitive; the major symptoms of chilling injury (CI) express as flesh woodiness, adhesion of peel to the flesh, leathery and juiceless pulp, and internal browning (IB) (Zheng *et al.* 2000). In order to alleviate or delay CI, different postharvest treatments have been applied to loquat fruits, such as polyamine (Zheng *et al.* 2000), modified atmosphere packaging (Ding *et al.* 2002), controlled atmospheres (Ding *et al.* 2006), 1-methylcyopropene (Cai *et al.* 2006a), low-temperature conditioning (Cai *et al.* 2006b) and methyl jasmonate (Cao *et al.* 2009).

As a safe physical treatment, heat treatment can increase the tolerance to subsequent chilling and delay ripening. In addition, this treatment also reduces pathogen levels and disease development in several fruits (Zhang *et al.* 2005; Luo 2006; Mirdehghan *et al.* 2007; Ghasemnezhad *et al.* 2008; Jemric *et al.* 2011). For loquat fruit, our previous study found that hot air treatment at 38C for 36 h not

only effectively alleviated CI during the cold storage (Liu *et al.* 2009) but also control anthracnose rot caused by *Colletotrichum acutatum*, which was the main disease on postharvest loquat fruits (Liu *et al.* 2010). Other report found that heat treatment at 38C for 5 h was also maybe useful to reduce CI symptoms of loquat fruit (Rui *et al.* 2010).

The rationale for the use of heat treatment is that exposure to a high temperature triggers physiological responses that allow the tissue to cope in a better way with subsequent stress conditions (Lara *et al.* 2009). Some of the beneficial effects of heat treatment in reducing CI have been mainly reported to be contingent on heat-shock proteins (Hsp), oxidative stress and membrane integrality (Lurie 1998). Heat treatment could induce expression of Hsp70 and small Hsp (17–18 kDa) to improve chilling tolerance (Zhang *et al.* 2005; Lara *et al.* 2009). It increased the activities of antioxidant enzymes, superoxide dismutase (SOD), peroxidase (POD) or catalase (CAT) to prevent the accumulation of active oxygen species (AOS) (Zhang *et al.* 2005; Ghasemnezhad *et al.* 2008), then reduced membrane lipid oxidation and malondialdehyde content (Zhang *et al.* 2005), and maintained higher unsaturated/saturated fatty acid ratio and membrane integrity (Mirdehghan *et al.* 2007; Rui *et al.* 2010) in fruits under chilling stress, including loquat.

The abnormal modifications of cell wall material, pectin and lignin, were also detailed and reported in chilling susceptible fruit and vegetables under CI (Meng *et al.* 2009; Cao *et al.* 2010; Rugkong *et al.* 2010; Zhu *et al.* 2010). Heat treatments retarded polyuronide release but promoted degradation of solubilized polyuronides to alleviate CI in sweet persimmon (Woolf *et al.* 1997). For loquat fruits, it was well known that the increasing of firmness positively related with the increasing of lignin content during the cold stress (Zheng *et al.* 2000; Cai *et al.* 2006a; Cao *et al.* 2010). In addition, the solubilization of cell wall polysaccharides might also relate with firmness changes and CI happening (Cao *et al.* 2010). Heat treatment reduced the accumulation of lignin (Liu *et al.* 2009).

Based on our previous study, postharvest hot air treatment at 38C for 36 h has been confirmed as an effective method to enhance chilling tolerance, control disease and maintain quality on "Jiefangzhong" loquat fruits (Liu *et al.* 2009, 2010). In order to reveal the possible mechanism of heat treatment in the reduction of CI, the objective of this work was to investigate the effects of this treatment (38C hot air for 36 h) on the physiological responses related with chilling tolerance, including whether the heat treatment modification in the antioxidant enzymes, cell membrane composition, IB and cell wall modification in "Jiefangzhong" loquat fruits during chilling stress.

MATERIALS AND METHODS

Fruit Material and Treatments

Fresh "Jiefangzhong" loquat (*Eriobotrya japonica* Lindl.) fruits were hand-harvested from a commercial orchard in Fujian, China. The fruits were selected for uniform size and color, and absence of visual defects. Then, it was distributed at random into two groups. In the control group (Control), fruits were untreated and directly placed into storage at 4C for 28 days. The second group (HT), fruits were treated at 38C for 36 h and then stored at 4C to 28 days. Several quality parameters and selected enzymes were measured during or at the end of the storage: fruit firmness, extractable juice, IB index, soluble solids contents (SSCs), titratable acidity (TA), decay development, hydrogen peroxides (H_2O_2) content, the activity of SOD, ascorbate peroxidase (APX), glutathione reductase (GR), CAT, phenylalanine ammonia lyase (PAL), POD and polyphenol oxidase (PPO), membrane permeability, fatty acid composition, and lignin content. There were three replicates of about 6 kg of fruits each per treatment, and the experiment was conducted twice. Average values of each sample and standard deviation from six replicates were calculated.

Determinations of Fruit Firmness, Extractable Juice and IB Index

Fruit firmness were made using a TA-XT2i texture analyzer (Stable Micro System Ltd., Haslemere, Surrey, U.K.) equipped with a probe 5 mm in diameter, a penetration depth of 5 mm and a rate of penetration of 1 mm/s. Measurements were made on two sides of each fruit after removal of a small piece of peel (Cai *et al.* 2006b).

Extractable juice was estimated via the weight loss from placental tissue plugs in response to low-speed centrifugation (Cao *et al.* 2009). Four plugs (7 mm wide and 10 mm thick) were placed over sterile cotton in a 50-mL centrifuge tube and centrifuged at $3{,}000 \times g$ for 15 min at room temperature. Results were expressed as weight loss of the tissue plugs after centrifugation.

IB index was measured according to Cai *et al.* (2006a) as 0: none; 1: browning area <5%; 2: browning area 5–25%; 3: browning area 25–50%; and 4: browning area >50%. The results were expressed as an IB index calculated using the following formula: IB index = (Σ[IB level] × [number of fruit at the IB level])/(total number of fruit in the treatment group).

Determinations of SSC, TA and Decay Index

SSC was determined by measuring the refractive index of expressed juice with a hand refractometer (WYT-4; Quan-

zhou Optical Instrument Co., Ltd., Quanzhou, China), and the results were expressed as °Brix values. TA was measured by titrating 50 mL of the filtered expressed juice to pH 8.1 with 0.1 M NaOH and calculating the result as malic acid percentage.

Decay was scored according to a four-grade scale, where 0: none; 1: decay area <25%; 2: decay area 25–50%; and 3: decay area >50%. Decay index (%) = (Σ[decay level] × [number of fruit at the decay level] × 100)/(total number of fruit in the treatment group) (Liu *et al.* 2010).

Determinations of H_2O_2 Content and Membrane Permeability

For H_2O_2 determination, 2 g of fresh tissue was homogenized with 5 mL of chilled 100% acetone and then centrifuged at 10,000 × *g* for 20 min at 4C. The supernatant was collected immediately for H_2O_2 analysis according to the method of Patterson *et al.* (1984). H_2O_2 content was expressed as μmol/g fresh weight (FW).

Membrane permeability was analyzed according to a modification method (Cai *et al.* 2006a). Flesh fruit disks (10 mm diameter × 2 mm height) from fruit were incubated in 20-mL deionized water. The solution was then heated in a boiling water bath for 30 min. The conductivity of the solution was measured using a conductivity meter (DDS-11A, Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China) at the start of incubation (EC_0) and at 3 h afterwards (EC_T). Results of membrane permeability were expressed as relative electric conductivity (%) = (EC_0/EC_T) × 100%.

Determinations of Membrane Fatty Acid Composition

Total lipids were extracted according to Cao *et al.* (2009). Fatty acids were separated and quantified by gas chromatography (GS)-mass spectrometry (MS) (QP2010 Plus, Shimadzu, Kyoto, Japan). GC condition were as follows: SPB-50 silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness); injection temperature, 250C; and carrier (nitrogen) flow rate, 0.81 mL/min. Oven temperature was initially 150C for 3.5 min, raised to 200C at a rate of 20C/min and held for 5 min, then to 280C at a rate of 5C/min, and finally held at that temperature for 20 min. The MS conditions were as follows: solvent delay, 3.5 min; transfer line temperature, 250C; ion source temperature, 200C; electron ionization mode at 70 eV; acquisition mode, selected ion monitoring, 50–650 m/z. Twenty microliters of each sample dissolved in CH_2Cl_2 was injected.

The unsaturated/saturated fatty acid ratio was calculated by the formula: (18:1 + 18:2 + 18:3)/(12:0 + 14:0 + 16:0 + 18:0), where 12:0 is lauric acid, 14:0 is myristic acid, 16:0 is palmitic acid, 18:0 is stearic acid, 18:1 is oleic acid, 18:2 is linoleic acid and 18:3 is linolenic acid.

Measurements of Phenolic Compounds and Lignin Content

For the determination of phenolic compounds content, 2 g of fresh tissue was homogenized with 5 mL of 80% ethanol and centrifuged at 10,000 × *g* at 4C for 20 min. The supernatant was immediately collected for analysis of phenolic content by a method of Pan *et al.* (2004). The content was calculated by using gallic acid as the standard, and results were expressed as mg/kg FW.

Lignin content was gravimetrically determined according to the modified method (Femenia *et al.* 1998). Fresh sample (2 g) was dispersed in 10 mL of 98% H_2SO_4 at room temperature for 12 h, diluted to 200 mL deionized water and heated in a boiling water bath for 6 h. Insoluble material was recovered by vacuum filtration with core sand funnel and vacuum pump (SHZ-D, Shanghai Zhixing Scientific Instrument Co., Ltd., Shanghai, China), and then washed thoroughly with hot water (90C) until acid-free before drying at 105C overnight. The residue was recorded as the lignin content expressed in %.

Extraction and Analysis for Cell Wall Polysaccharides

The alcohol-insoluble solids (AISs) were prepared by using a modified method of Shalom *et al.* (1996). The tissues (100 g) were homogenized in 200 mL of cold 70% alcohol for 2 min. The residual was filtered, homogenized twice with 200 mL of 70% alcohol and once with absolute ethanol, and left to dry overnight at room temperature. One gram of the AIS were serially extracted for 1 h in water, for 6 h in 0.02 M *trans*-1,2-diaminocyclohexane–*N,N,N′,N′*-tetraacetic acid (CDTA) and pH 6.5, and for 2 h in 0.05 mol/L Na_2CO_3. The uronic acid content in each fraction was measured by the method of Bitter and Muir (1962).

Enzyme Assays

All enzyme extraction procedures were conducted at 4C. For SOD, 1 g of fresh sample was ground with 5 mL of 50-mM sodium phosphate buffer (pH 7.8). Fresh sample (1 g) was ground with 5 mL of 100-mM sodium phosphate buffer (pH 7.0) for CAT or 100-mM sodium phosphate buffer (pH 7.0), containing 0.1-mM ethylenediaminetetraacetic acid, 1-mM sodium ascorbate, and 1% polyvinylpyrrolidone for APX and GR. For measurement of POD and PPO, 2 g of the fresh sample was ground with 5 mL of 100-mM sodium phosphate buffer (pH 6.4). PAL was extracted with 100-mM sodium borate buffer at pH 8.7.

The extracts were then homogenized and centrifuged at 10,000 × *g* for 20 min at 4C. The supernatant was used for the enzyme assay.

SOD activity was determined using the method of Dhindsa *et al.* (1981). One unit of SOD was defined as the amount of enzyme that caused a 50% decrease in SOD-inhibitable nitroblue tetrazolium reduction. CAT activity was determined by adding 0.2 mL of the enzyme preparation to 3 mL of sodium phosphate buffer containing 0.2-mL H_2O_2 as a substrate (Wang *et al.* 2004). APX was carried out as described by Cao *et al.* (2010), and one unit of APX activity was defined as the amount of enzyme that oxidizes 1 μmol ascorbate/min at 25C. GR were conducted using the assay kits purchased from Nanjing Jiancheng Insititute of Bioengineering (Nanjing, Jiangsu, China) according to the instructions of the manufacturer. POD activity was determined by the method of Kochba *et al.* (1977). Enzyme activity was defined as the increase in absorbance, where one unit was defined as the change in 0.01 absorbance units per minute at 460 nm. PPO activity was determined using the method of Tian *et al.* (2002). Enzyme activity was defined as the increase of absorbance, where one unit was defined as the change of 0.1 absorbance unit per minute at 400 nm. PAL activity was analyzed using the method of Assis *et al.* (2001). One unit was defined as the change in 0.01 absorbance units per hour at 290 nm. The specific activity of all the enzymes was expressed as units per gram FW.

Statistical Analysis

The data were analyzed by one-way analysis of variance and Pearson's correlation. SAS Software (Version 8.2; SAS Institute, Cary, NC) was used to conduct all statistical analyses. Comparison of means was performed by Duncan's multiple range tests. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of Heat Treatment on the Quality of Loquat during the Cold Storage

Compared with harvest, the firmness, IB and decay index in both groups were significantly ($P < 0.05$) increased, and the extractable juice, SSC and TA were significantly ($P < 0.05$) decreased at the end of cold storage (Table 1). Heat treatment significantly ($P < 0.05$) inhibited the increasing of firmness and IB index, and the decreasing of extractable juice. Meanwhile, this treatment also significantly ($P < 0.05$) reduced the TA content and decay index but no significant effect on SSC ($P > 0.05$).

Effects of Heat Treatment on the SOD, APX, GR and CAT Activities, and the H_2O_2 Content

The effects of heating on the antioxidant enzymes' activity and H_2O_2 content were summarized in Fig. 1. SOD activity in control fruit decreased with storage time, and heat-treated fruit maintained remarkably ($P < 0.05$) higher SOD activity through the storage (Fig. 1A). APX activity in control group increased and reached peak values at 7 days and then decreased dramatically (Fig. 1B). A significantly ($P < 0.05$) higher level of that was exhibited in the treated group. GR activity decreased slightly during the first 14 days of storage and then increased during the following storage (Fig. 1C). Heat-treated fruit showed significantly ($P < 0.05$) higher GR activity than that of control. CAT activity increased gradually during the first 21 days of storage and then slightly decreased (Fig. 1D). Heat-treated fruit also showed higher CAT activity during the whole cold storage.

H_2O_2 contents in control and heat-treated fruits generally increased with storage time (Fig. 1E). The content of H_2O_2 in the heat-treated was significantly lower ($P < 0.05$) than that of control. Large difference between them could be observed after 14 days storage.

Effects of Heat Treatment on the Membranes Permeability and Fatty Acid Composition

Membranes permeability in both groups generally increased during the cold storage for 28 days (Fig. 1F). Heat-treated fruit showed lower membrane permeability than the value of the control fruit though the storage, with significant ($P < 0.05$) difference from day 21 of storage.

Lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were identified in

TABLE 1. EFFECTS OF HEAT TREATMENT ON QUALITY PARAMETERS OF LOQUAT FRUITS

Treatment	Firmness (N)	Extractable juice (%)	IB index	SSC (%)	TA (%)	Decay index (%)
0 day	2.55 ± 0.14^c	78.29 ± 3.10^a	0^c	8.14 ± 0.45^a	0.49 ± 0.01^a	0^c
28-day control	4.20 ± 0.05^a	52.19 ± 2.28^c	0.41 ± 0.02^a	6.95 ± 0.53^b	0.35 ± 0.02^b	35.10 ± 0.44^a
Heat treatment	3.63 ± 0.04^b	63.65 ± 0.71^b	0.26 ± 0.01^b	7.10 ± 0.41^b	0.25 ± 0.01^c	12.93 ± 0.88^b

Note: Means followed by different letters (a, b and c) indicate significantly different scores, according to Duncan's multiple range tests at $P = 0.05$ level.

IB, internal browning; SSC, soluble solids content; TA, titratable acidity.

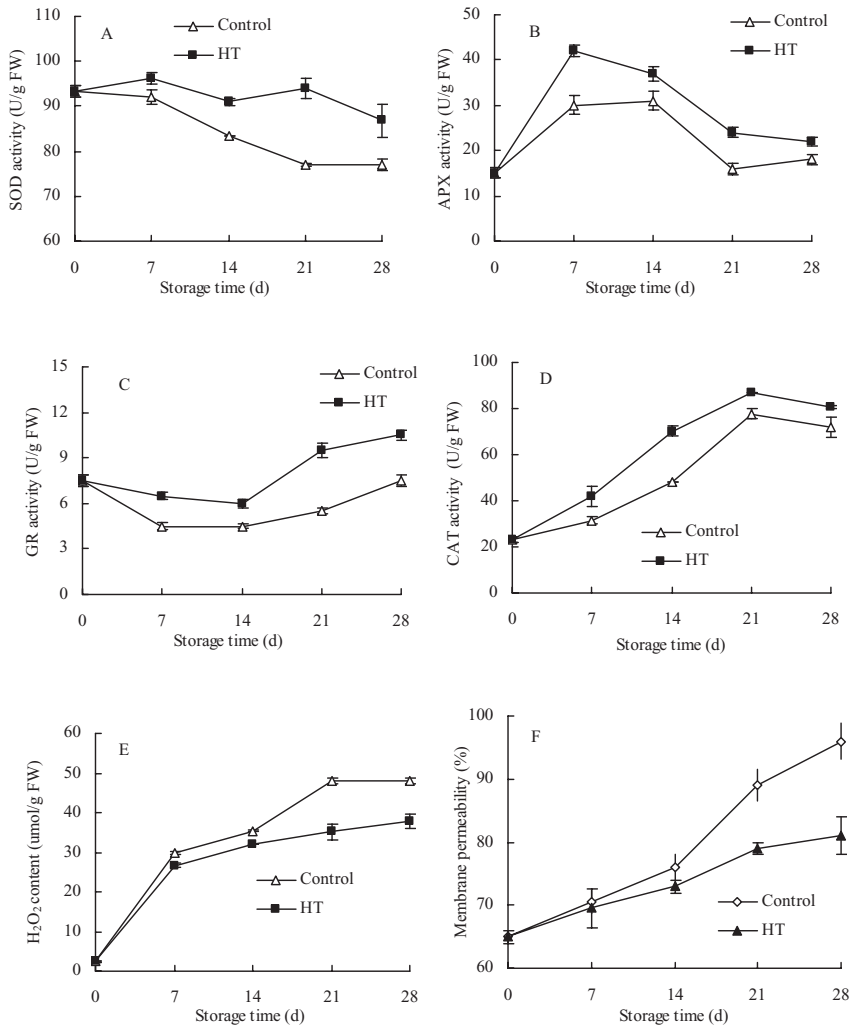

FIG. 1. EFFECTS OF HEAT TREATMENT ON THE ACTIVITY OF SUPEROXIDE DISMUTASE (SOD) (A), PEROXIDASE (B) AND CAT (C), HYDROGEN PEROXIDE (H_2O_2) LEVEL (D), AND MEMBRANES PERMEABILITY (E) OF LOQUAT FRUITS DURING STORAGE AT 4C Values are the means ± standard error of triplicate assays. Vertical bars represent the standard errors of the means. APX, ascorbate peroxidase; GR, glutathione reductase.

membrane fatty acids of loquat fruit after harvest (Table 2). Among them, the first four are saturated fatty acids (12:0, 14:0, 16:0, 18:0), and the latter three are unsaturated fatty acids (18:1, 18:2, 18:3). The content of the first two were lower than 1%, and the last five were the major fatty acids in loquat fruit.

Compared with control group, heat-treated groups showed significantly ($P < 0.05$) lower content of three saturated fatty acids (lauric acid, palmitic acid and stearic acid) and higher content of two polyunsaturated fatty acids (oleic acid and linoleic acid) than those of control at the end of cold storage. At the same time, heat treatment has no significant ($P > 0.05$) effect on myristic acid and linolenic acid content. Therefore, heat-treated fruit had significant ($P < 0.05$) higher unsaturated/saturated fatty acid ratio than control fruit at the end of storage.

TABLE 2. EFFECTS OF HEAT TREATMENT ON THE MEMBRANE FATTY ACID COMPOSITION AND UNSATURATED/SATURATED FATTY ACID RATIO OF LOQUAT FRUITS

Treatment	Lauric acid C12:0 (%)	Myristic acid C14:0 (%)	Palmitic acid C16:0 (%)	Stearic acid C18:0 (%)	Oleic acid C18:1 (%)	Linoleic acid C18:2 (%)	Linolenic acid C18:3 (%)	Uns./sat. fatty acid ratio
0 day	0.74 ± 0.10^{c}	0.89 ± 0.08^{a}	20.98 ± 1.50^{b}	14.34 ± 0.75^{a}	25.71 ± 1.34^{c}	29.44 ± 1.73^{a}	7.90 ± 0.32a	1.70 ± 0.11^{a}
28-day control	1.36 ± 0.10^{a}	0.88 ± 0.07^{a}	25.28 ± 1.26^{a}	15.18 ± 1.34^{a}	29.13 ± 1.33^{b}	22.14 ± 1.55^{c}	5.18 ± 0.56^{b}	1.32 ± 0.15^{c}
Heat treatment	0.82 ± 0.03^{b}	0.81 ± 0.04^{a}	21.37 ± 1.71^{b}	11.66 ± 0.82^{b}	32.87 ± 1.64^{a}	26.43 ± 2.11^{b}	5.48 ± 0.44^{b}	1.86 ± 0.12^{a}

Note: Means followed by different letters (a, b and c) indicate significantly different scores, according to Duncan's multiple range tests at $P = 0.05$ level.

Effects of Heat Treatment on the PAL, POD and PPO Activities, and Phenolic and Lignin Content

For the control fruit, PAL activity increased with storage time (Fig. 2A). Heat treatment significantly ($P < 0.05$) decreased that during the storage. POD activity in both groups decreased during the first 7 days of storage and then increased during the remainder of storage (Fig. 2B). Heat treatment significantly ($P < 0.05$) reduced POD activity from day 14 of storage. For PPO activity, heat-treated fruit showed significant ($P < 0.05$) lower activity than that of the control during whole storage (Fig. 2C).

Total phenolic contents of control group declined first and then increased; the lowest value was found at 14th day (Fig. 2D). Heat-treated fruit showed significant ($P < 0.05$) lower level than control fruit after 7 days of storage.

The level of lignin increased steadily in the whole storage period (Fig. 2E). The lignin content in the control fruit was 31.14% higher than the level at harvest. Heat treatment significantly ($P < 0.05$) inhibited the accumulation of lignin, and after 28 days of storage, the content was only 19.68% higher than the level at harvest.

Effects of Heat Treatment on the Water-, CDTA- and Na_2CO_3-Soluble Pectins in Loquat Fruit

Figure 3 showed that the levels of water- and CDTA-soluble pectin decreased, while the Na_2CO_3-soluble pectin level increased during the cold storage. Heat-treated fruit showed higher water-soluble pectin and lower Na_2CO_3-soluble pectin level than that of the control, but the significant ($P < 0.05$) difference could be found after 7 days of storage.

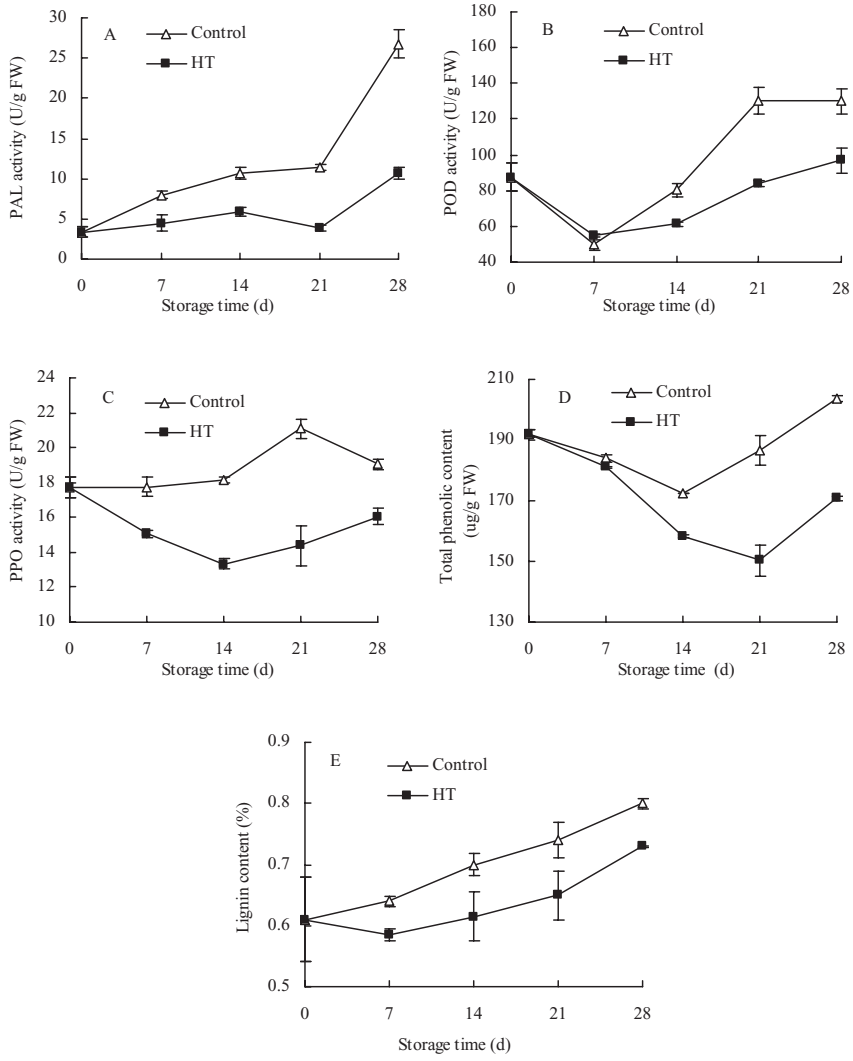

FIG. 2. EFFECTS OF HEAT TREATMENT ON THE PHENYLALANINE AMMONIA LYASE (PAL) (A), PEROXIDASE (POD) (B) AND POLYPHENOL OXIDASE (PPO) (C) ACTIVITIES, AND TOTAL PHENOLIC (D) AND LIGNIN (E) CONTENT OF LOQUAT FRUIT DURING STORAGE AT 4C
Values are the means ± standard error of triplicate assays. Vertical bars represent the standard errors of the means. FW, fresh weight.

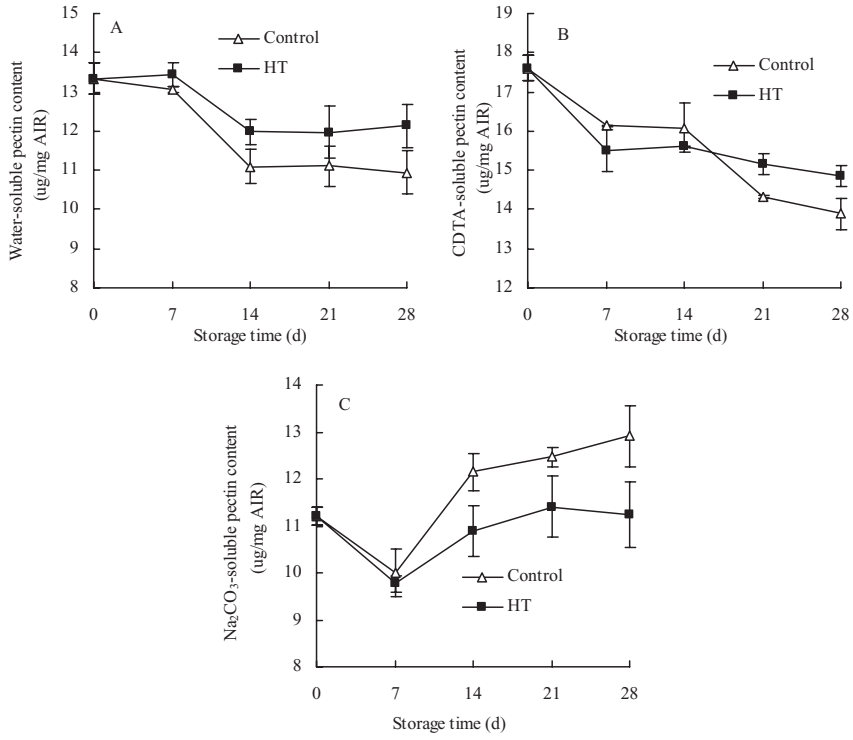

FIG. 3. EFFECTS OF HEAT TREATMENT ON THE WATER- (A), *trans*-1,2-DIAMINOCYCLOHEXANE–*N,N,N′,N′*-TETRAACETIC ACID (CDTA)- (B) AND NA_2CO_3-SOLUBLE (C) PECTIN LEVEL OF LOQUAT FRUIT DURING STORAGE AT 4C Values are the means ± standard error of triplicate assays. Vertical bars represent the standard errors of the means.

For CDTA-soluble pectin, heat-treated fruit showed lower level than control during the first 14 days of storage; however, significant ($P < 0.05$) higher level than control was obtained during the remaining storage period.

DISCUSSION

The major CI symptoms in loquat fruit are stuck peel, firm and juiceless texture, and IB. Therefore, fruit firmness, IB and extractable juice were used to evaluate the development of CI of loquat fruit during the chilling stress (Cao *et al.* 2006a,b, 2009, 2010). In our study, hot air treatment (38C for 36 h) could delay the increase in fruit firmness, IB index, and maintained higher levels of extractable juice (Table 1), thereby alleviating CI and maintaining fruit quality. Rui *et al.* (2010) found that hot air treatment at 38C for 5 h can delay the occurrence of CI symptoms on loquat fruits; however, our previous study revealed that heat-treated at 38C for 36 h was more efficient to resist CI than for 12 or 24 h (Liu *et al.* 2009). Lara *et al.* (2009) also used hot air treatment at 39C for 3 days to prevent CI of peach fruits. Whitaker *et al.* (1997) suggest that heat-treated at 38C less than 3–4 days has an adverse effect on poststorage quality. Besides, for controlling fungi disease by hot air, the processing times are running from 12 to 96 h, and the temperatures are ranging from 38 to 46C (Lurie 1998). The treatment at 38C for 36 h can effectively reduce the natural decay development (Table 1) but also control artificial inoculated disease (Liu *et al.* 2010). Therefore, it was suggested that hot air treatment at 38C for 36 h can play the both roles to improve chilling resistant and control fungi disease of loquat fruit.

It is widely accepted that the development of CI symptoms can be the consequence of oxidative stress in the tissues occurring when AOS such as H_2O_2 and superoxide anion radical ($O_2^{-}\cdot$) are in excess of the scavenging capacity of fresh tissue. AOS can induce peroxidation and breakdown of unsaturated fatty acids in membrane lipids (Hodges *et al.* 2004). The metabolism of AOS is controlled by an array of interrelated antioxidant enzymes. $O_2^{-}\cdot$ is efficiently converted to H_2O_2 by the action of SOD (Mittler 2002). Ascorbate–glutathione cycle performs effectively to scavenge H_2O_2 in the plant cell (Nishikawa *et al.* 2003). In this cycle, APX catalyzes the reduction of H_2O_2 to water with the simultaneous oxidation of ascorbic acid. Oxidized glutathione is reduced by GR, which contributes to the maintenance of the glutathione pool in the reduced state (Nishikawa *et al.* 2003; Cai *et al.* 2011). Besides, H_2O_2 is also destroyed by CAT (Mittler 2002). Postharvest treatments that induce chilling tolerance and alleviate CI can enhance these antioxidant enzyme activity, then reduce peroxidation of membrane lipids, and maintain higher unsaturated/saturated fatty acid ratio and cell membrane integrity in fresh loquat fruit (Cai *et al.* 2006a,b; Cao *et al.* 2009; Rui *et al.* 2010). In the present work, heat treatment significantly reduced H_2O_2 level by increasing SOD, APX, GR and CAT

activity (Fig. 1A–E), then maintained higher unsaturated/saturated fatty acid ratio and lower membrane permeability by significantly improving the oleic and linoleic acid content, and decreasing lauric, myristic, palmitic and stearic acid level at the end of storage (Table 2). These results suggest that heat treatment improving chilling resistance in loquat fruit was correlated with enhancing antioxidant enzyme activities in scavenging the AOS and reducing peroxidation and breakdown of membrane fatty acids.

Loquat fruit with an IB index <0.4 can be commercially acceptable (Cai *et al.* 2006b). It was observed that the IB index reached 0.41 after 28 days of storage, and heat treatment significantly reduced that (Table 1). Tissue browning is due to oxidative reactions of phenolic compounds by oxidases (PPO and POD) and the reaction products. PAL is a key enzyme closely related with the synthesis of phenolic compounds in tissue (Ding *et al.* 2006). Different treatments inhibited the increasing of PAL, PPO and/or POD activity (Ding *et al.* 2006; Cao *et al.* 2010), or decrease the content of total phenol (Cai *et al.* 2006b) to control brown in loquat. Our research found that heat treatment reduced the IB index by reducing PPO, POD and PAL activity, and total phenolic content (Fig. 2A–D) in loquat fruits.

For loquat fruit, lignin content increased during the cold storage because of the coordinated action of many related enzymes including PAL, POD and PPO (Cai *et al.* 2006b; Cao *et al.* 2010). PAL is a key enzyme that catalyzes the conversion of phenylalanine to *trans*-cinnamic acid in the phenylpropanoid pathway, which is the first step in the biosynthesis of lignin in plants. POD catalyzes the polymerization of monolignol to form lignin. PPO can use as substrates those phenolic compounds that are precursors of lignin synthesis (Cao *et al.* 2010). In this study, heat treatment reduced lignin content (Fig. 2E) by reducing the activity of PAL, POD and PPO (Fig. 2A–C) during the whole or later storage period.

In addition, H_2O_2 also act as a substrate of the lignifications process, and the inhibited H_2O_2 production will strongly reduce lignin (Chin *et al.* 1999; Liu *et al.* 2010). There was a very significant ($P < 0.01$) positive correlation between H_2O_2 and lignin level ($r = 0.76$) in loquat fruit. Heat treatment delayed the increasing of H_2O_2 level (Fig. 1E), and this might contribute to control lignin accumulation. At the same time, H_2O_2 caused peroxidation and breakdown of membrane fatty acids (Hodges *et al.* 2004); a very positive correlation ($r = 0.88$, $P < 0.01$) was observed between H_2O_2 contents and membranes permeability in loquat. Tissue browning results from loss of compartmentalization within the cells when exposed to physical and/or physiological stresses (Ding *et al.* 2002), and maintaining membrane integrity was beneficial for controlling flesh browning (Meng *et al.* 2009; Rui *et al.* 2010). The correlation between IB and membranes permeability was very positive ($r = 0.91$, $P < 0.01$). Thus, heat treatment favors the maintenance of membrane stability (Fig. 1F), and this might contribute to control browning. It was suggested that the enhanced activities of antioxidant enzymes may play important role to control these CI symptoms of loquat fruit.

During fruit softening, depolymerization of cell wall polysaccharides was accompanied by the increases in the levels of water- and chelator-soluble pectins, while the levels of Na_2CO_3-soluble pectin decrease (Chin *et al.* 1999). It was observed that the development of irreversible CI symptoms was accompanied by decreased levels of water- and CDTA-soluble pectins and increased contents of Na_2CO_3-soluble pectin in the control fruit (Fig. 3), while heat treatment maintained significantly ($P < 0.05$) higher contents of the first two pectins and lower contents of the latter substance at the later, just similar to the effect of methyl jasmonate and 1-methylcyclopropene on the CI of loquat (Cao *et al.* 2010).

CONCLUSIONS

The present study confirmed the beneficial effects of hot air treatment (38C for 36 h) on improving chilling tolerance in loquat fruit during cold storage. Our result found that heat treatment prevents the H_2O_2 accumulation by improvement in the activities of antioxidant enzymes (SOD, APX, GR and CAT), which reduces the peroxidation and breakdown of unsaturated fatty acids in membrane lipids and therefore retained higher membrane integrity and higher unsaturated/saturated fatty acid ratio. The reduction of PAL, POD and PPO activities combined with lower membrane permeability or H_2O_2 level may contribute to the controlling IB or lignin synthesis, respectively. In addition, heat treatment can also affect the solubilization of cell wall polysaccharides, showed as higher levels of water- and chelator-soluble pectins and lower Na_2CO_3-soluble pectin than controls, which may also explain the changes of firmness in loquat during cold storage. It was suggested that the enhanced antioxidant enzymes and cell wall polysaccharides solubilization by heat treatment may be the crucial reason related with the reduction of CI symptoms in loquat fruit.

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