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Full Length Research Paper

Improved preservation effects of litchi fruit by combining chitosan coating with ascorbic acid treatment during postharvest storage

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Pericarp browning and aril decay of litchi fruits shorten post-harvest storage and thus reduce market value. Effects of chitosan coating and ascorbic acid (AsA) on litchi fruits storage were investigated in this paper. Based on the fact that AsA increases anti-oxidation capacity and chitosan inhibits dehydration and microbial attack, the novel strategies of combining chitosan with AsA treatment were developed to improve litchi storability. By treating harvest fruits with 40 mmol/l AsA and 1.0% (w/v) chitosan solution, parameters of browning index and relative leakage rate and activities of polyphenol oxidase (PPO) and peroxidase (POD) in pericarp were markedly lowered in treated fruits. Moreover, increased activities of super oxide dismutase (SOD) and catalase (CAT) and contents of AsA and glutathione were observed in pulp of treated fruit, thus leading to lowered contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). Correspondingly, total soluble solids, soluble sugar and titratable acidity were significantly increased and thus lowered decay rate was achieved. It was suggested that chitosan and AsA play active roles in inhibiting pericarp browning, dehydration and microbial attack and maintaining membrane integrity, thus improved litchi storability being achieved.

Key words: Aril decay, ascorbic acid, chitosan coating, litchi fruit, pericarp browning.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) belongs to the subtropical fruit with high commercial value for its white tasty flesh and attractive red color of anthocyanins at full maturity (Holcroft and Mitcham, 1996). Tragically, harvest litchi fruit easily loses its commercial value resulting from pericarp browning and aril decay (Ray, 1998). Pericarp browning was considered to be a major problem affecting litchi fruits market value. During the past decades, much work has been conducted on analyzing roles of pigments, hormones and other related factors responsible for pericarp browning (Zhang and Quantick, 1997), selecting suitable temperature and chemicals to inhibit pericarp browning and control aril decay during post-harvest storage (Paull and Chen, 1987; Jiang and Fu, 1998). Moreover, the effects of some substances, such as anthocyanidin, extracted from litchi pericarp on browning were also investigated (Yang et al., 2006; Duan et al., 2007).

Generally, pericarp browning of harvest litchi fruit was believed to be a rapid degradation of anthocyanidin by polyphenol oxidase (PPO) and peroxidase (POD) (Chen and Wang, 1989; Lee and Wicker, 1991). In addition, dehydration was another key factor leading to pericarp browning (Scott et al., 1982; Underhill and Simons, 1993). Pericarp dehydration may result in 40% decrease in water content after 48h storage at 25℃ with relative

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Abbreviations: **PPO**, Polyphenol oxidase; **POD**, peroxidase; **AsA**, ascorbic acid; **RH**, relative humidity; **SOD**, super oxide dismutase; **CAT**, catalase; **NBT**, nitro blue tetrazolium chloride; **MDA**, malondialdehyde; **TCA**, thiobarbituric acid; **BHT**, butylated hydrosytoluen.

humidity of 60% (Underhill and Critchley, 1994). Such a drastic change undoubtedly causes cell damage and plasmolysis. As a result, tissue wounding induces a high respiration rate which triggers faster tissue deterioration and increased contact between PPO and POD from damaged chloroplast, leucoplast, anthocyanins and phenols from vacuole, thus accelerating enzymatic action of phenols oxidation (Jiang et al., 1997). Concisely, pericarp browning of post-harvest litchi mainly resulted from degradation of anthocyanidin by PPO and POD actions and decrease in antioxidants levels besides rapid water loss and microbial attack. As a result, active oxygen species originated from pericarp browning will inevitably do harm to cell plasma membranes of litchi flesh, thus leading to aril decay. Correspondingly, research concentrated on delaying pericarp browning or aril decay of harvest litchi fruits has been conducted in the recent years. Firstly, sulfur dioxide fumigation was used to delay loss of skin color of litchi fruits (Tongdee, 1994; Underhill et al., 1992). Tragically, sulfur residues left in fruits were accompanied simultaneously. As a result, alternative chemicals for color control without toxic effects in harvested litchi fruits were greatly needed (Jiang et al., 2003). Correspondingly, alternative means for browning control were widely sought to improve harvested litchi storability during the past few years.

To reduce use of potentially hazardous chemicals, litchi fruits were treated with hot water and hydrochloric acid instead of fumigation to delay pericarp browning (Lichter et al., 2000). Additionally, anthocyanins extracted from litchi pericarp showed positive effect on preventing litchi pericarp browning (Zhang et al., 2004). Moreover, coating techniques were also carried out to improve litchi fruits storability (Ghosh et al., 1998). For example, chitosan, a cationic polysaccharide was used to inhibit pericarp browning of litchi fruits (Zhang and Quantick, 1997). In the recent years, ascorbic acid (AsA) has been reported to effectively control enzymatic browning of fruits and vegetables (Santerre et al., 1988; Sapers et al., 1989). Unfortunately, there were few reports on application of AsA to inhibiting pericarp browning of litchi fruits. Moreover, knowledge concerned with the effects of AsA and chitosan coating on pericarp browning and aril decay of litchi fruits is scanty. Accordingly, the present study was aimed to investigate the effects of combining AsA with chitosan on pericarp browning and aril decay of litchi fruits. Emphasis was laid on developing the novel strategies of improving litchi storability during its post-harvest storage.

MATERIALS AND METHODS

Plant materials and treatments

Litchi fruits (*Litchi chinensis* Sonn. cv. Feizixiao) at about 80% matured stage were harvested from an orchard in Zhanjiang city, Guangdong Province, China and were immediately transported to the laboratory for experiment. Fruits with uniform size and appearance were selected and treated with fungicide solution (Bavistin 0.05%) for 10 min. Afterwards, the treated fruits were divided into four groups, placed in four clean plastic boxes each with 150 fruits and were dipped in water (as control), 1%(w/v) chitosan solution, 40 mmol/l AsA solution and mixed solutions containing 40 mmol/l AsA + 1%(w/v) chitosan for 15 min, respectively. After air drying, each box was wrapped in a polyethylene bag and kept in a room at 5℃ with 90% relative humidity (RH). Five fruits from each box were randomly sampled every 6 days to determine physic-biochemical changes during storage periods.

Browning index assessment in pericarp

Appearance was assessed by measuring the extent of total browned area on each fruit pericarp using the following scale (Jiang and Chen, 1995): (1) no browning (excellent quality); (2) slight browning; (3) <1/4 browning; (4) 1/4 - 1/2 browning and (5) >1/2 browning (poor quality). The browning index was calculated as R (browning scale × % of corresponding fruit within each class). Fruit lots higher than 3.0 were considered unacceptable for marketing.

Measurement of relative leakage rate in pericarp

Membrane permeability, expressed by relative leakage rate, was determined according to the method of Zheng and Tian (2006). Discs were removed with a cork borer (10 mm in diameter) from the equatorial region of nine fruit pericarp. Thirty discs were rinsed twice and then incubated in 25 ml of distilled water at 23℃ and shaken for 30 min. Electrolyte leakage was determined with a conductivity meter (model DDS-11A, Shanghai Scientific Instruments, China) and again after boiling another batch of discs for 15 min and cooling to 25℃ (total electrolytes). Relative leakage rate was expressed as percent of total electrolytes.

Assay of super oxide dismutase (SOD) and catalase (CAT) in pulp

Extraction of SOD and CTA in pulp was based on the description of Mo et al. (2008). One fruit was randomly selected from six samples and fruit pulp (1.0 g) was used and ground in a mortar and pestle in 5 ml of 50 mmol/l cool phosphate buffer (pH 7.8). The homogenate was centrifuged at 13 000 × *g* for 15 min at 4℃ and the supernatant was used for assays of antioxidant enzyme activities of SOD and CAT.

SOD activity was determined according to the method of Giannoplitis and Ries (1977). 3 ml reaction solution contained 13 µmol/l methionine, 63 µmol/l ρ-nitro blue tetrazoliumchloride (NBT), 1.3 µmol/ l riboflavin, 50 mmol/l phosphate buffer (pH 7.8) and 50 µl enzymes extract. The reaction solution was incubated for 10 minutes under fluorescent light with 80 µmol /m^2 /s. Absorbance at 560 nm was determined by spectrophotometer (Beckman DU-7). One unit of SOD activity was defined as the amount of enzyme required for inhibiting photochemical reduction of NBT by 50%. CAT activity was determined spectrophotometerically according to the method of Change and Maehly (1955). 3 ml reaction solution contained 15 mmol/ l H_2O_2, 50 mmol/ l phosphate buffer (pH 7.0) and 100 µl of enzyme extract. The reaction was initiated by adding enzyme extract and determined with absorbance at 240 nm by Beckman DU-7.

Measurement of POD and PPO activities in pericarp

Extraction of POD and PPO in pericarp was accorded to the method of Jiang et al. (2005). Litchi peel (5.0 g) was homogenized

in 30 ml of 0.02 mol/l phosphate buffer (pH 6.8) containing 0.6 g polyvinylpyrrolidone (insoluble). The homogenate was centrifuged at 19000× *g* (Beckman J20-2) for 20 min at 4℃ and the supernatant was collected as crude enzyme extract for assaying POD and PPO activities.

POD activity was measured by the procedure of MacAdam et al. (1992). Assay mixture consisted of 0.05 mol/l sodium phosphate buffer (pH 7.0), 0.012 mol/l H_2O_2, 0.007 mol/l guaiacol and 0.1 ml enzyme solution in a final volume of 3.0 ml. The increase in absorbance at 470 nm was recorded for 3 min using a Beckman DU-7 spectrophotometer. PPO activity was assayed with 4-methyl-catechol as a substrate according to the method of Zauberman et al. (1991). Assay of PPO activity was performed using 1.0 ml of 0.1 mol/l phosphate buffer (pH 6.8), 0.5 ml of 0.1 mol/l 4-methylcatechol and 0.5 ml enzyme solution. The increase in absorbance at 410 nm at 25℃ was automatically recorded for 3 min (Beckman DU-7). One unit of enzyme activity was defined as the amount which caused a change of 0.01 in absorbance per minute.

Measurements of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content in pulp

Extraction of H_2O_2 was done as described by Rao et al. (2000) with some modifications. Pulp (0.5 g) was ground with a pre-chilled mortar and pestle in liquid nitrogen and extracted with 0.2 mmol/l perchloric acid (11 ml) by ultrasonication (Vibra-Cell Ultrasonic Processor; Sonics & Materials Inc., Danbury, CT, USA). After centrifugation at 5000 × g for 5 min at4℃, the pellet was re-extracted three times with same solvent. The supernatant was pooled and a sample of 0.5 ml was applied to 2 ml columns of AG 1-X8 Resin 200-400 mesh chloride form, 0.834 cm (Bio-Rad Laboratories, Inc., Hercules, CA, USA) previously calibrated with $HClO_4$ and eluted with double-distilled water (3 ml). Acidic purified extract was neutralized to pH 7 with 0.2mol/l NH_4OH.

Quantification of H_2O_2 was based on the method of Tana et al. (2009). An Amplex Red Hydrogen Peroxide Assay kit (Molecular Probes, Invitrogen Detection Technologies, Leiden, Netherlands) was used. Briefly, 50 µl of extract was mixed with 50 µl of solution containing 1 U/ ml horseradish peroxidase in 50 mmol/l sodium phosphate buffer, pH 7.4 and was incubated for 15 min at 25℃. Fluorescence was measured with a fluorescence microplate reader (Fluostar Optima, BMG Labtechnologies, Germany) equipped for excitation at 520 nm and emission at 590 nm. Concentration of H_2O_2 was calculated using a standard curve obtained with known concentrations of pure H_2O_2 (Molecular Probes) diluted in 0.2 mol/l $HClO_4$.

A modified thiobarbituric acid (TCA) reactive substance assay was used as an alternative assessment of lipid oxidation according to the method of Gutteridge and Wilkins (1982). Fruit pulp (1.0 g) was ground in 80: 20 ethanol: methanol (V/V) containing 0.01% (W/V) butylated hydrosytoluen (BHT). After centrifugation at 13000 × *g* for 15min at 4℃, the supernatant was recovered (200 ml) and added to 1 ml of a solution of 20% (W/V) TCA and 0.01% (W/V) BHT containing 0.65% (W/V) thiobarbituric acid for 25 min at 95℃. After centrifugation, sample absorbance was measured at 532 nm. Blank measurements were performed using reagent solution without thiobarbituric acid. Nonspecific turbidity was subtracted from the 532 nm signal by using the measurements at 600 and 440 nm. The results were expressed as MDA equivalent.

Measurements of total soluble solids, soluble sugar, titratable acidity, ascorbic acid and glutathione in pulp

Fruit pulp (5.0 g) was ground in a mortar and pestle in 25 ml of 50 mmol/l cool phosphate buffer (pH 7.8). The homogenate was centrifuged at 13000 × *g* for 15 min at 4℃. The supernatant was collected to analyze total soluble solids, soluble sugar, titratable acidity, ascorbic acid and glutathione.

The percentage of total soluble solids was determined by using a hand refractometer (J1-3a, Guangdong Scientific Instruments). Titratable acidity was determined by titration with 0.1 mol/l NaOH solution (Jiang et al., 2005). Soluble sugar (total carbohydrate) was measured by phenol-sulfuric acid method described by Tatsuya et al. (2005). AsA was estimated by titrating sample extract with 2, 6 dichlorophenol indophenol dye (Chen et al., 1986). Glutathione was measured according to the method described by Ruiz and Blumwald (2002). Water loss was calculated according to the methods of Zhang and Quantick (1997). Decay rate was represented by percentage of completely brown and soften fruit.

Statistical analysis

All experiments were performed with six replications and the average value was used. The data were analyzed by analysis of variance and means were compared by Duncan's multiple range test.

RESULTS

Effects of AsA and chitosan on browning index and relative leakage rate in pericarp

As shown in Figure 1, browning index in all fruits increased continuously with treatment time. AsA and chitosan treatment showed a similar pattern in browning index as the control, but with lowered value in the treated fruit. For example, the browning index in the control reached 5.3 after 30 days storage. In contrast, by treating with 1% chitosan, 40 mmol/l AsA and 1% chitosan coupled with 40 mmol/l AsA, respectively, browning indices were 4.6, 4.1 and 3.3 on day 30, and decreased by 13.2, 22.6 and 37.7% when compared with the control. Fascinatingly, mixed treatments of AsA and chitosan showed a most significant effect in inhibiting browning.

As it is known, injury degree of treated litchi fruits can be expressed as relative leakage rate (Jiang et al., 2001). As indicated in Figure 1, the relative leakage rates in all fruits increased during storage periods, indicating that litchi pericarp became more vulnerable to leakage. However, compared to untreated (control) fruits, relative leakage rates were markedly lowered in treated fruits. Moreover, AsA combined with chitosan treatments had a most significant effect.

Effect of AsA and chitosan on PPO and POD activities in pericarp

The bright red color of litchi fruit has been attributed to the anthocyanins in the pericarp. It was known that PPO and POD accelerate enzymatic browning, thus forming brown-color by-products. To further understand roles of AsA and chitosan in controlling pericarp browning, PPO and POD activities in litchi pericarp were analyzed. As shown in Figure 2, both enzymes activities in all litchi

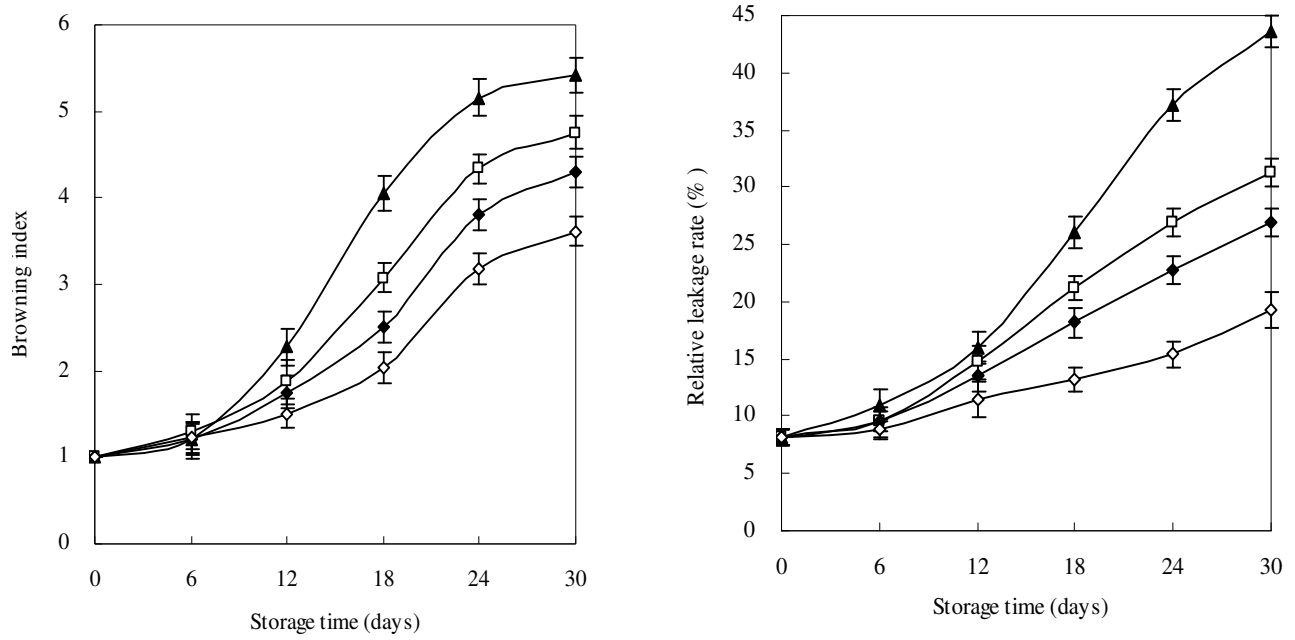

Figure 1. Effects of AsA and chitosan on browning index and relative leakage rate. ▲, Control; □, 1% chitosan; □, 40 mmol/l; □, 1% chitosan and 40 mmol/l.

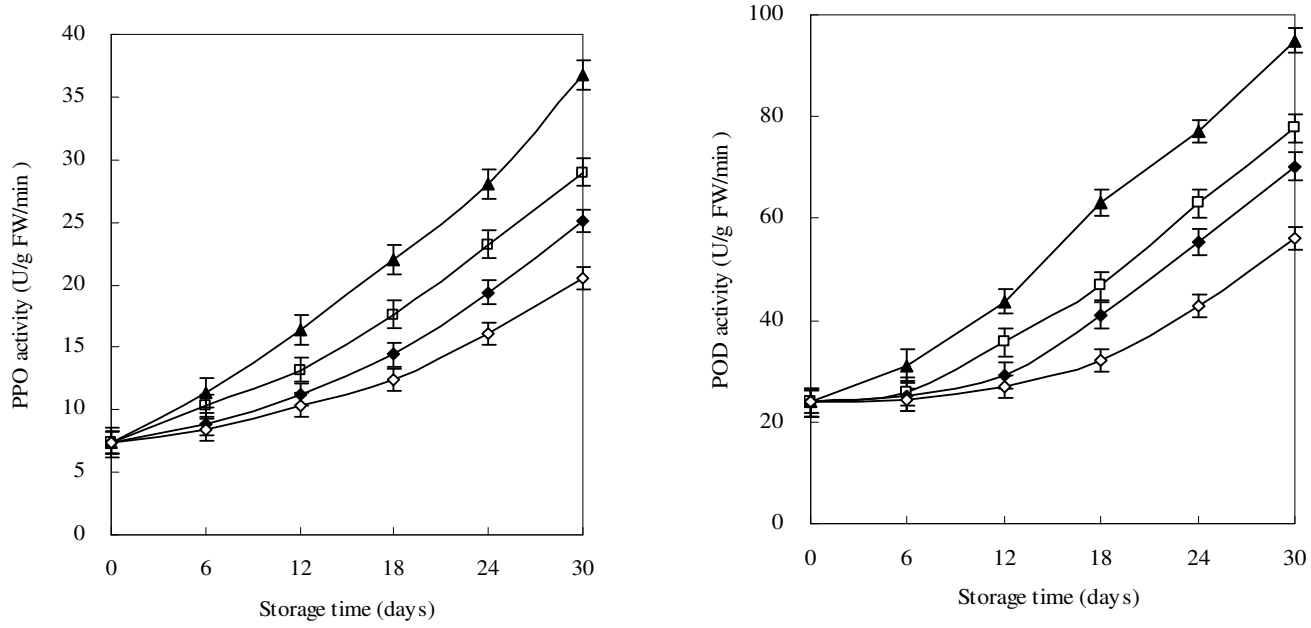

Figure 2. Effects of AsA and chitosan on POD and PPO activities. ▲, Control; □, 1% chitosan; □, 40 mmol/l; □, 1% chitosan and 40 mmol/l.

fruits were enhanced continuously along extending of treatment time. However, treated litchi fruits showed relatively lowered PPO and POD activities as compared to the control during the whole storage time. For instance, without treatment, both PPO and POD activities reached 36 and 95 U/g Fw/min, respectively, at the end of storage. In comparison, by treating with 40 mmol/l AsA coupled with 1% chitosan, both enzymes activities of PPO and POD were 20 and 56 U/g Fw/min on day 30, which were 44 and 41% lower than the control, suggesting that PPO and POD activities were markedly inhibited by AsA and chitosan treatments.

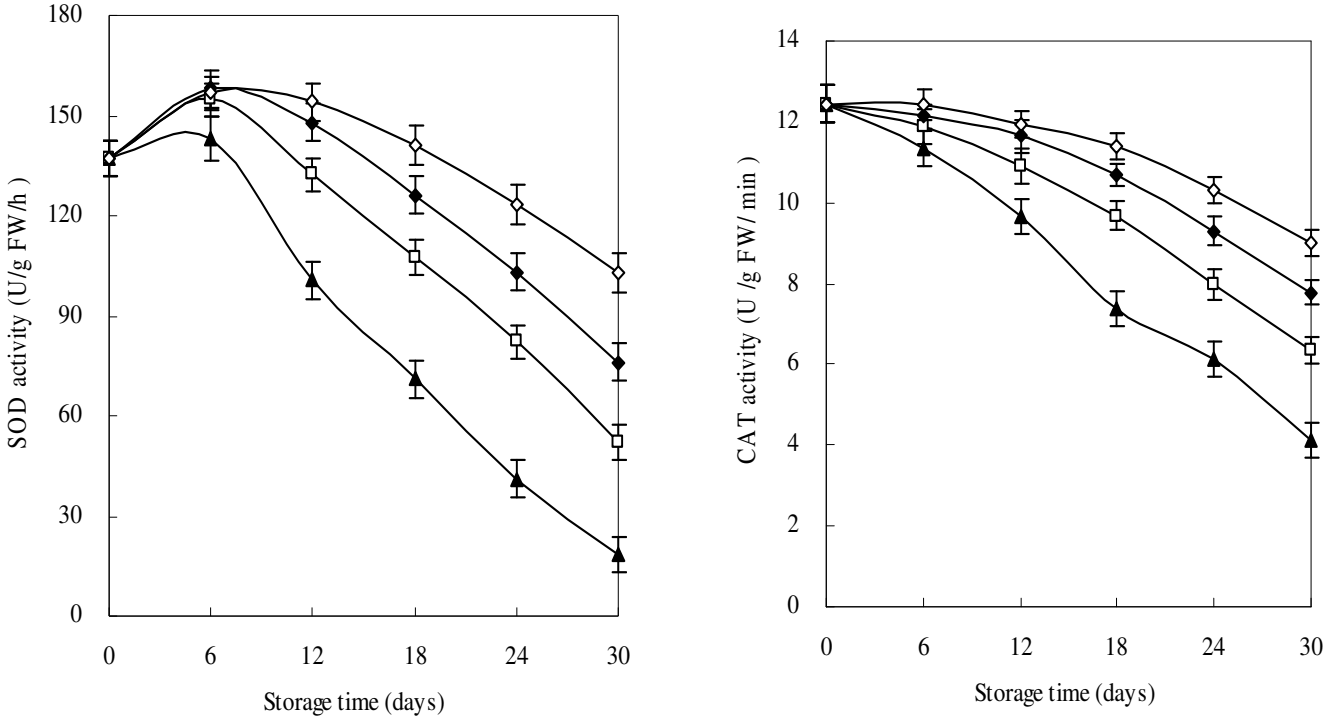

Figure 3. Effects of AsA and chitosan on SOD and CAT activities. ▲, Control; □, 1% chitosan; □, 40 mmol/l; □, 1% chitosan and 40 mmol/l.

Effect of AsA and chitosan on antioxidant enzymes activities of SOD and CAT in pulp

Effects of AsA and chitosan on the activities of anti-oxidant enzymes of SOD and CAT during storage were investigated and the results are shown in Figure 3. SOD activities in all fruits increased at the beginning of storage time and markedly decreased after 6 days storage and at each storage time, the levels of SOD activities in treated fruit were similar to those in the control. However, the treated litchi fruits showed a relative higher value of SOD than the control. For example, without treatment, SOD activity increased from initial value of 137 to 143U/g Fw/h on day 6 and then decreased rapidly to a lowered value of 19 on day 30. In comparison, in the fruits treated with 1% chitosan, 40 mmol/l AsA and 1% chitosan coupled with 40 mmol/l AsA, SOD activities were elevated with peak value of 154, 158 and 166 on day 6 and then decreased to 52, 76 and 102 U/g Fw/h respectively, after 30 days storage.

The patterns of CAT activities in all fruits were similar during storage, but its activities were significantly higher in the treated fruit than the control throughout storage. For example, CAT activity decreased from initial value of 12.5 to 4.1 U/g Fw/min on day 30 in the control. In comparison, in treatment of 1% chitosan, 40mmol/l AsA and 1% chitosan coupled with 40mmol/l AsA, CAT activities were 6.4, 7.8 and 9.1 U/g Fw/min after 30 days storage, which were 56, 90 and 122% higher than the control.

Effects of AsA and chitosan on $HB_{2B}OB_{2B}$ and MDA contents in pulp

It is well known that H_2O_2, as the typical oxidant, can result in lipid oxidation and thus membrane destruction (Chih et al., 2003). Accordingly, the effects of AsA and chitosan on H_2O_2 and MDA contents in pulp during storage were also investigated.

As indicated in Figure 4, H_2O_2 in all litchi fruits increased continuously during storage process. Fascinatingly, treated fruits showed a lowered H_2O_2 content as compared to the control, suggesting that AsA and chitosan showed positive roles in getting rid of H_2O_2. Furthermore, the patterns of MDA contents in all fruits were similar during storage, but the MDA contents were significantly lowered in the treated fruit than that in the control.

As it is known, lipid oxidation produces MDA, accumulation of which can reflect degree of membrane integrity. Our results indicated that H_2O_2 content was significantly reduced by AsA and chitosan treatments and in turn MDA content was markedly lowered, suggesting that AsA and chitosan play active roles in maintaining membrane integrity.

Effects of AsA and chitosan on AsA and glutathione contents in pulp

As two typical antioxidants, AsA and glutathione contents

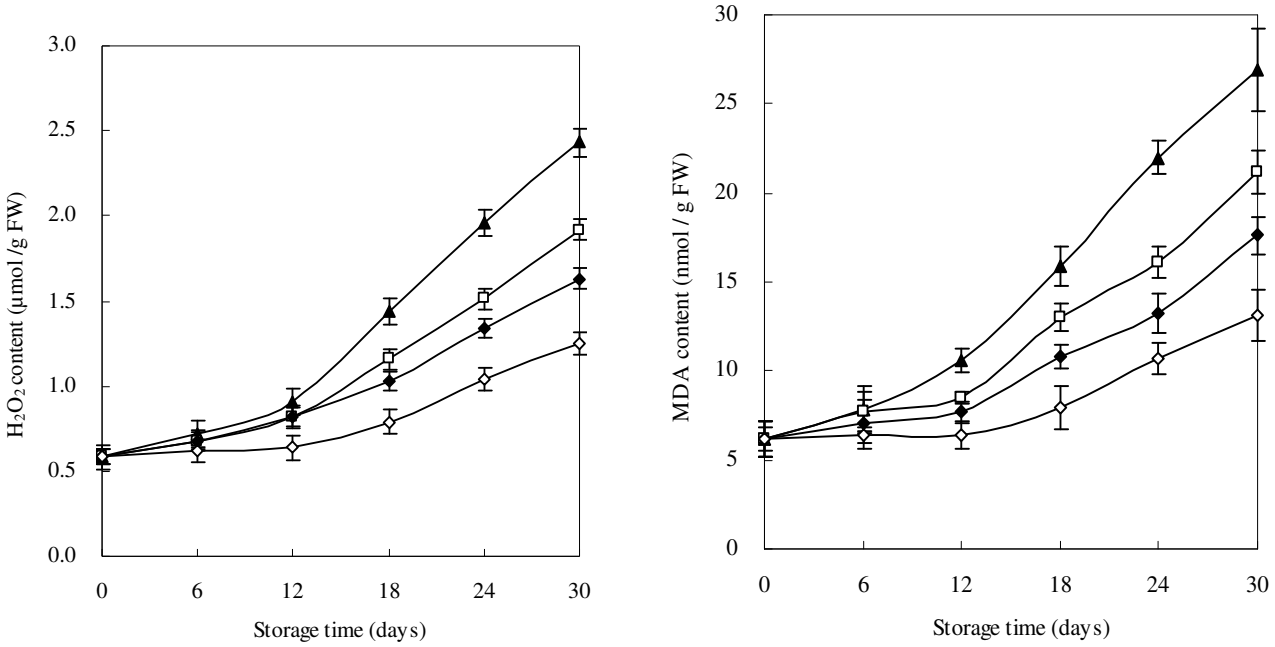

Figure 4. Effects of AsA and chitosan on H_2O_2 and MDH contents. ▲, Control; □, 1% chitosan; □, 40 mmol/l; □, 1% chitosan and 40 mmol/l.

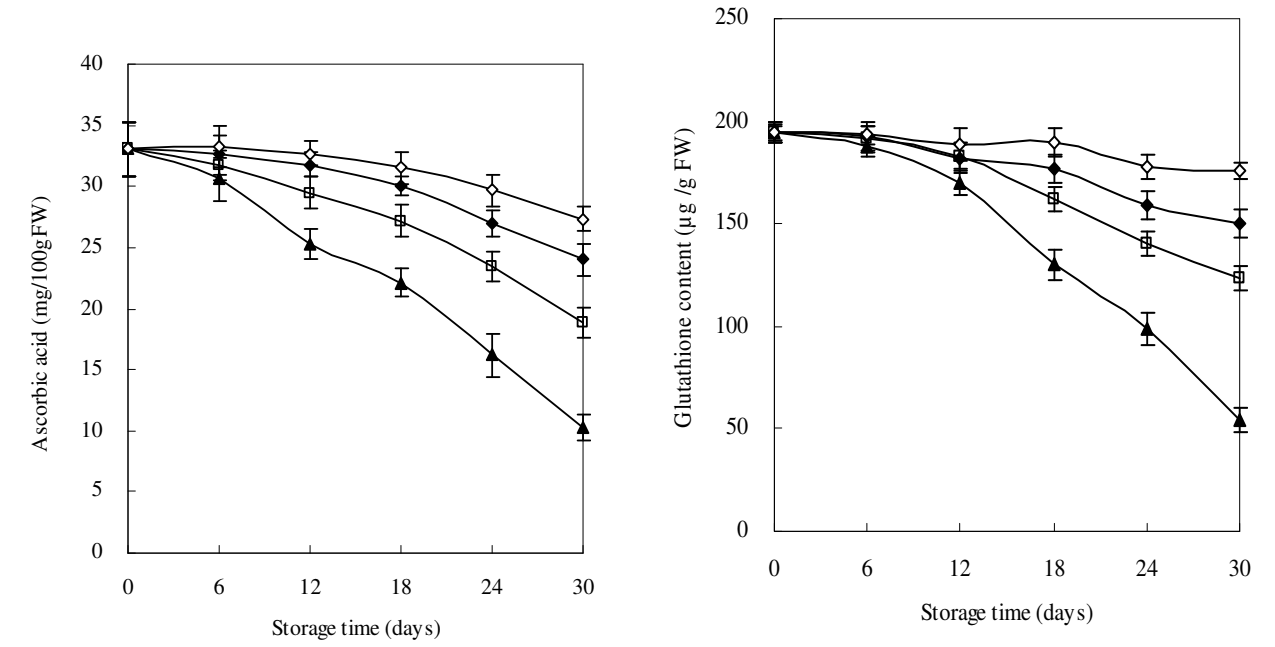

Figure 5. Effects of AsA and chitosan on AsA and glutathione contents. ▲, Control; □, 1% chitosan; □, 40 mmol/l; □, 1% chitosan and 40 mmol/l.

in litchi fruits during storage were measured and results are shown in Figure 5. In all fruits, AsA and glutathione were found to decrease continuously during storage periods. Although treated fruits showed a similar pattern as the control, relatively higher contents of AsA and glutathione were observed in treated fruits. For example, by treating with 1% chitosan, 40 mmol/l AsA and 40 mmol/l AsA coupled with1% chitosan, AsA content was 82, 123 and 152% higher than the control after 30 days storage. Correspondingly, glutathione content was

Table 1. Effects of AsA and chitosan on total soluble solid, soluble sugar, titratalbe acidity, weight loss and decay rate.

Treatment	Total soluble solids (%)	Soluble sugar (mg/g)	Titratable acidity (%)	Weight loss (%)	Decay rate (%)
Control	14.45±2.34^{d}	120.09±5.78^{d}	0.37±0.09^{d}	8.98±0.46^{a}	70.56 ± 5.78^{a}
Chitosan	16.41±2.13^{c}	150.23±3.28^{bc}	0.56±0.13^{bc}	6.23±1.34b^{c}	20.47±0.66^{b}
AsA	16.85±1.34^{ab}	158.27±1.28^{ab}	0.59±0.15^{b}	6.95±0.89^{b}	23.45±0.86^{c}
AsA+ Chitosan	17.95±2.34	166.59±7.86^{a}	0.63±0.17^{a}	5.98±0.49^{d}	10.58±2.56^{d}

*Values are mean ± standard deviation; means with the same letters in the each column are no significant difference at P ≤ 0.05.

increased by 127, 270 and 224% in treated fruits as compared to the control.

Effects of ASA and chitosan on total soluble solid, soluble sugar, titratalbe acidity, weight loss and decay rate

Total soluble solids and titratable acidity are important factors in flavor and nutritive quality of litchi fruit (Jiang and Fu, 1998).The parameters of total soluble solids, soluble sugar, titratable acidity, weight loss and decay rate of litchi flesh after 30 days storage were determined and the results are shown in Table 1.

It is clear that total soluble solids, soluble sugars and titratable acidity in the treated fruit were higher than those in the control. In particular, the fruits in treatment of chitosan combined with AsA had highest contents of total soluble solid, soluble sugar and titrable acidity while with lowest water loss and decay rate.

DISCCUSSION

The major limitation in litchi marketing is the rapid loss of the red color after harvest (Jiang et al., 2003). Post-harvest browning of litchi fruit was generally thought to be a rapid degradation of anthocyanins caused by PPO, producing brown by-products (Jiang and Li, 2000). In addition, rapid propagation and growth of pathogen microorganisms resulting from tissue deterioration and dehydration lead to aril decay, further limiting litchi market value (Dong et al., 2004). Accordingly, in the present study, we mainly focused on improving post-harvest litchi fruits storability by preventing pericarp browning and aril decay. In a concrete way, pericarp browning was delayed by enhancing anti-oxidation capacity through AsA treatment. Meanwhile, water loss and microbial attack were avoided by chitosan coating. As a result, pericarp browning and aril decay were significantly inhibited and thus improved litchi preservation effect was achieved. As it is expected, by developing novel strategies of combining AsA with chitosan coating, firstly, pericarp browning and its injury degree were significantly reduced. For example, a lowered browning index and relative leakage rate were observed in the treated fruits, suggesting that degradation of anthocyanins slowed down and in turn the injury degree was decreased accordingly.

Moreover, decreased PPO and POD activities in pericarp of treated fruits further demonstrated the inhibitory effects of AsA and chitosan on pericarp browning. Meanwhile, increased SOD and CAT activities and AsA and glutathione contents in pulp of treated fruits indicated the occurrence of improved anti-oxidation capacity, thus delaying sene-scence process. It is known that MDA accumulation can indirectly reflect the degree of membrane integrity. In addition, H_2O_2, as a typical oxidant, can result in lipid oxidation and thus membrane destruction. Our results indicated that H_2O_2 content was largely decreased by AsA and chitosan and in turn MDA content was significantly lowered, suggesting that AsA and chitosan showed positive roles in maintaining membrane integrity. As a result, total soluble solids, soluble sugars and titratable acidity in treated fruits were significantly increased, thus resulting in lowered fruits decay rate. The results safely suggested that AsA and chitosan showed positive effects on inhibiting pericarp browning and aril decay by increasing anti-oxidation capacity while avoiding dehydration and microbial attack simultaneously.

In conclusion, quality maintenance and shelf life extension of litchi fruits by AsA coupled with chitosan treatments in the present study suggests that application of the novel strategies could be feasible for litchi fruits storage on commercial scale.

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