# Improving preservation effects of Taiwan jujube fruits by using chitosan coating combined with ascorbic acid during postharvest period

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**Abstract:** In order to demonstrate the fact that ascorbic acid (AsA) increased anti-oxidation and chitosan prevented dehydration and microbial attack, this paper systematically investigate effects of chitosan coating, AsA, and their combinational treatment on preservation of harvested Taiwan jujube fruits during storage. In treatment of 30.0 mmol/L AsA, 8 g/L chitosan, 30.0 mmol/L AsA combined with 8 g/L chitosan, and dipped in deionized water (as control), respectively, all samples were stored in darkness at (25±1)℃ and 90%-95% relative humidity. Relative physiological parameters were measured and cells structures were observed by scanning electron microscope in pericarp and pulp tissue. Results showed that, compared to the control, AsA or chitosan alone improved storability of harvested fruits and their combinational treatment showed a better effect. Fruits water loss and relative leakage rate of pericarp were markedly reduced ($P$<0.05), and an increase in pericarp chlorophyll degradation and activities of pectin methylesterases (PME) and polygalacturonases (PG) in pulp was inhibited, and degradation rate of protopectin to soluble pectin was markedly reduced ($P$<0.05). Thus, high degree of fruits firmness and integral cell structure was maintained. A decrease in activities of superoxide dismutase (SOD) and catalase (CAT) was alleviated, membrane oxidation rate was reduced, and thus contents of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) were markedly decreased ($P$<0.05). An increase in contents of soluble sugars, total soluble solids, titratable acidity, glutathione and AsA was achieved. It was indicated that chitosan coupled with AsA treatment play active roles in enhancing anti-oxidation, decreasing oxidation injury, fruits water loss, and chlorophyll degradation, alleviating pericarp browning, delaying fruits softening, maintaining cell integrity, and reducing decay rate, thus realizing improved preservation effects of harvested Taiwan jujube during storage.

**Keywords:** storage; quality control; fruits; Taiwan jujube; ascorbic acid; chitosan; oxidation stress; antioxidant enzymes
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梁国斌，王 海，张耀红，莫亿伟. 壳聚糖和抗坏血酸复合处理提高台湾青枣采后保鲜效果[J]. 农业工程学报，2017，33(17)：304－312. doi：10.11975/j.issn.1002-6819.2017.17.040 http://www.tcsae.org

## 0 Introduction

Taiwan jujube (*Ziziphus mauritiana* Lam.), belongs to a member of *Rhamnaceae* family which includes approximately 86 plant species. As a fruit tree, it mainly grows in tropical and subtropical regions including Southern China and Burma[1]. Different parts of Taiwan jujube are used traditionally for treatment of asthma, allergies, depression and other disease[2]. For example, its fruit contains sufficient amounts of potassium, calcium, sodium, magnesium, phosphorous, manganese, iron, vitamin C and minerals[3]. Compared to other species, freshly harvested Taiwan jujube fruits easily became senescent and browning, thus causing a shorted shelf-life[4]. As its pericarp was very thin, water loss and softening were considered to be major factors affecting storability and marketability of Taiwan jujube fruits[5]. Cold storage can be used to slow down these processes and thus reduce decay rate, but chilling injury occur and cause browning in pericarp simultaneously[6].

Under normal condition, water evaporation occurs on pericarp surface of harvested fruits. Also, strong respiration causes water to lose quickly. As a result, turgor pressure inside cell decrease and then fruits will become softening[7-8]. In addition, softening of harvested fruits can be attributed to cell wall modification caused by several degrading enzymes. For example, fruit softening accelerated with an increase in activities of polygalacturonases(PG), pectin methylesterases

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(PME), and galactosidase(GAL)[9-11]. Meanwhile, a decrease in activities of these enzymes could keep firmness and extend shelf life of fresh fruits[12-13]. As is known, with conversion of insoluble pection into soluble form, adhesion degree between cells decreases and fruits softening will occur. So, it is necessary to control these enzymes activities for enhancing preservation effects of Taiwan jujube fruits during storage.

Recently, biomaterials were developed and used to extend storage life and improve quality of fresh, frozen and formulated food products[14]. Chitosan, as versatile biopolymer, was widely used in fresh-keeping field[15]. Chitosan coating has been applied to prolonging shelf life by reducing water loss of harvested fruits during storage[16-18].

Owing to rapid degradation of chlorophyll in pericarp, Freshly harvested Taiwan jujube fruits easily became browning during storage. Publications showed that pericarp browning in color was associated with defensive system including antioxidant enzymes of superoxide dismutase (SOD), catalase (CAT), peroxides (POD) and ascorbic acid oxidase[19-20]. It was believed that an efficient antioxidant system could delay senescence process and thus control enzymatic browning of fruits[21]. For instance, it was reported that AsA could effectively control enzymatic browning of apple slices and litchi[22-24].

In recent years, many techniques or methods were adopted to improve storability of Taiwan jujube fruits during postharvest period. It was showed that 1-MCP treatment could delay senescence of harvested *Ziziphus mauritiana* fruit by retaining higher cellular energy charge[25]. Also, *Agave americana* leaf extracts could delay fruit senescence and had a good fresh-keeping effect on Taiwan green jujube[26]. In addition, Beeswax coating[27], calcium, chitosan and hot water treatments[28] were used to improve storage of Indian jujube fruits. Evidently, these methods had positive effects on storage of jujube fruits. However, some more effective and environmentally friendly methods need to be further developed.

Quality deterioration of harvested Taiwan jujube fruit is closely related to water loss, softening and browning in appearance[29]. Accordingly, effects of chitosan coating, AsA, and their combinational treatments on storability of harvested Taiwan jujube fruits were investigated and we aimed to develop an effective method for improving fruits preservation effects during storage.

## 1 Materials and methods

### 1.1 Plant materials and treatments

Fruits (*Ziziphus mauritiana* Lam. Gao lang No.1) were harvested (about 85% matured stage) from an orchard of Zhanjiang city, Guangdong Province and transported to laboratory for experiments. Fruits with uniform size and appearance were selected and treated with fungicide solution (Bavistin 0.05%) for 10 min to kill bacteria on fruits surface.

Based on our previous publication[17], 20.0, 30.0 and 40.0 mmol/L AsA and 6, 8 and 10 g/L chitosan were adopted to investigate independent effect of AsA and chitosan on storability of Taiwan Jujube fruits. To make it brevity and clarity, experimental results were not shown and an optimal concentration of AsA (30.0 mmol/L) and chitosan (8 g/L) was chosen. Chitosan coating and 98% glacial acetic acid (as cosolvent of chitosan) were used to prepare film-forming dispersions according to the method[19].

After treated with Bavistin, fruits were divided into four groups by placing them into four clean plastic boxes (300 fruits each box) and dipped into deionized water (as control), 30.0 mmol/L AsA solution, 8 g/L chitosan solution, and mixed solutions containing 30.0 mmol/L AsA + 8 g/L chitosan for 5 min, respectively. After air drying in cool room, each box was wrapped in a polyethylene bag and kept in artificial box at (25±1)℃ with 90%-95% relative humidity.

### 1.2 Measurement of fruits water loss, firmness, decay rate, chlorophyll content and relative leakage rate in pericarp

Water loss is defined as decrease in weight of harvested fruits between the initial and end of storage during postharvest period. Calculation formula for water loss can be expressed as

$$\text{Water loss} = \frac{FW_{initial} - FW_{end}}{FW_{initial}} \times 100\%$$

Where $FW_{initial}$ and $FW_{end}$ represent fruits weight at the initial and end storage (g), respectively. Each time thirty fruits were weighed to measure water loss on 0, 2, 4, 6, 8, 10 and 12 days, respectively.

Fruit firmness was measured according to the method[25]. At each time, ten fruits from each treatment were randomly chosen to measure fruit firmness on 0, 2, 4, 6, 8, 10, and 12 days, respectively. A texture analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY) fitted with a 3 mm diameter flat probe was used. It was compressed 1 cm in middle part of each fruit at a constant speed and maximum force developed during the text was recorded.

Fruit decay rate is defined as the ratio of decay fruits at end storage to total number of fruits at the initial. Ten fruits from each treatment (300 fruits) were randomly chosen and decay rate was calculated.

Chlorophyll content in pericarp was usually used to reflect fruits browning during postharvest storage[25]. Chlorophyll content was analyzed according to the method[30]. Chlorophylls were extracted from pericarp using a mixed solvent of 80% acetone. Concentrations of chlorophyll a and b were calculated from absorbance measured at 652 nm with spectrophotometer (Beckman Co, California, USA ).

Membrane permeability, expressed by relative leakage rate, was determined according to the method [21]. Each time ten fruits from treatment were randomly selected. Discs were removed with a cork borer (10 mm in diameter) from equatorial region of ten fruits pericarp. Thirty discs were rinsed twice and then incubated in 25.0 mL of distilled water at 25 ℃ and shook for 30 min. Electrolyte leakage was determined with a DDS-11A conductivity meter (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 percentage of total electrolytes.

### 1.3 Assay of activities of PME and PG in pulp

Tissue (2.0 g) from 3 fruits pulp was homogenized in 10 mL of 0.2 mol/L acetic acid buffer (pH value 6.0) and then centrifuged at 11 000×*g* for 20 min. The supernatant was used for PME and PG assay. Pectinmethylesterase (PME) activity was measured based on the method[31] with minor modifications. Reaction mixture was prepared in a 3.0 mL glass cuvette. It contained 1.0 mL pectin solution (0.01% aqueous solution adjusted to pH value 7.5 using 0.1 mol/L NaOH), 0.2 mL NaCl (0.15 mol/L), 0.1 mL bromothymol blue solution (0.01%), 0.2 mL water and 0.1 mL homogenate. After adding prepared enzyme, cuvette was shaken gently. Absorbance was measured at 620 nm and repeated 3 min later. The different values in absorbance between 0 and 3 min represent PME activity. One unit was defined as the amount of PME required for liberating 1 $\mu$mol of methylester per min.

Polygalacturonase (PG) activity was assayed according to the method[32]. Reaction mixture contained 0.2 mL sodium acetate (200 mmol/L, pH value 4.5), 0.1 mL NaCl (200 mmol/L), 0.3 mL polygalacturonic acid (PGA, 1% aqueous solution adjusted to pH value 4.5), 0.1 mL enzyme extract and appropriate amount deionized $H_2O$ in a total volume of 1.0 mL. Reaction was initiated by adding PGA substrate. The mixture was incubated at 37 ℃ for 1 h followed by adding DNS. The reaction was terminated by heating reaction mixture in a boiling water bath for 5 min. For control, substrate was added after heat treatment. Formation of reducing group was estimated against d-galacturonic acid as standard after measuring absorbance at 540 nm. One unit of enzyme was defined as amount of enzyme required to liberate 1 nmol of galacturonic acid per min.

### 1.4 Cell wall extraction and analysis

Cell wall polysaccharides were obtained as alcohol insoluble residue according to the method[33]. Fruit pulp (20.0 g) from 3 fruits was homogenized with 80.0 mL ethanol and boiled for 30 min. Homogenate was filtered and residue was washed three times with ethanol and then solvent was evaporated at 20 ℃. The dried residue obtained was used to extract different cell wall fractions. Polyuronides were isolated according to the description[34] with minor modifications. 100 mg aliquot of dried residue was homogenized in 100 mL water and stirred overnight at 20 ℃. Then, homogenate was filtered and solid was washed three times with 10.0 mL water. The filtrates were labeled as water-soluble pectins. The residue was then resuspended in 100 mL of 0.5 mol/L $H_2SO_4$, and stirred for 1 h at 100 ℃ for protopectin decomposition. The polyuronides concentration of all fractions was measured by carbazole method using galacturonic acid (GA) as standard.

### 1.5 Assay of superoxide dismutase (SOD) and catalase (CAT) in pulp

Extraction of antioxidant enzymes of SOD and CAT in pulp was based on the description[35]. Fruit pulp (2.0 g) from 3 fruits was collected and ground in a mortar and pestle in 5.0 mL of 50 mmol/L cool phosphate buffer (pH value 7.8). The homogenate was centrifuged at 13 000×*g* for 15 min at 4 ℃ and the supernatant was used for SOD and CAT assays.

SOD activity was determined according to the method[30]. 3.0 mL reaction solution contained 13.0 $\mu$mol/L methionine, 63.0 $\mu$mol/L $\rho$-nitro blue tetrazolium chloride (NBT), 1.3 $\mu$mol/L riboflavin, 50.0 mmol/L phosphate buffer (pH value 7.8), and 50.0 $\mu$L enzyme extract. The reaction solution was incubated for 10 min under fluorescent light with 80.0 $\mu$mol/($m^2$·s). Absorbance at 560 nm was determined by a DU-7 spectrophotometer (Beckman, California, USA). 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[30]. 3.0 mL reaction solutions contained 15.0 mmol/L $H_2O_2$, 50 mmol/L phosphate buffer (pH value 7.0) and 100.0 $\mu$L of enzyme extract. Reaction was initiated by adding enzyme extract and determined with absorbance at 240 nm by DU-7 spectrophotometer.

### 1.6 Measurements of $HB_{2B}OB_{2B}$ and MDA levels in pulp

$H_2O_2$ level was determined according to the method [36]. Pulp (2.0 g) from 3 fruits was homogenized in 5.0 mL chilled acetone and centrifuged at 6 000×*g* for 15 min at 4 ℃. Supernatant (1.0 mL) was mixed with 0.1 mL 20% (v/v) titanium tetrachloride ($TiCl_4$) and centrifuged at 6 000×*g* for 15 min. Precipitate was collected and 3.0 mL 1 mol/L $H_2SO_4$ was added to mixture and centrifuged at 6 000×*g* for 15 min. The absorbance of solution was measured at 415 nm against a blank which was carried through the same procedure. $H_2O_2$ content was determined using a standard curve.

A modified thiobarbituric acid (TCA) reactive substance assay was used as an alternative assessment of lipid oxidation according to the method[30]. Pulp (2.0 g) from 3 fruits was ground in 80:20 ethanol: methanol (V/V) containing 1 g/L butylated hydrosytoluen (BHT). After centrifugation at 13 000×*g* for 15 min, supernatant was recovered (200 mL) and added to 1.0 mL solution of 0.2 kg/L TCA and 0.1 g/L BHT containing 6.5 g/L thiobarbituric acid for 25 min at 95 ℃. After centrifugation, sample absorbance was measured at 532 nm. Blank measurements were performed using reagent solution without TCA. Nonspecific turbidity was subtracted from 532 nm signals by using measurements at 600 and 440 nm. The results were expressed as MDA equivalent.

### 1.7 Measurements of TTS, SS, TA, GSH and AsA in pulp

Pulp (5.0 g) from 3 fruits was ground in a mortar and pestle in 25.0 mL of 50.0 mmol/L phosphate buffer (pH value 7.8). Homogenate was centrifuged at 13 000×*g* for 15 min and supernatant was used for these parameters analysis. Ascorbic acid (AsA) was estimated by titrating sample extract with 2, 6 dichlorophenol indophenol dye[30].

Glutathione (GSH) was measured according to the method[37]. The percentage of total soluble solids (TSS) was determined by using a J1-3a hand refractometer (Guangdong Scientific Instruments, China). Titratable acidity (TA) was determined by titration with 0.1mol/L NaOH solution[38]. Soluble sugar (SS) was measured by phenol-sulfuric acid method[39].

### 1.8 Microscopic preparation and observation of pericarp and pulp

Pericarp (3 mm×3 mm×2 mm) and pulp tissue (3 mm×3 mm×3 mm) near equatorial region of fruit was taken by scalpel on 12th day. Specimens were prepared and processed according to the method[40]. Samples slices were fixed in 2.0% glutaraldehyde (v/v) in 50 mmol/L phosphate buffer (pH value 7.2). After fixing, samples were washed three times with 50 mmol/L phosphate buffer (pH value 7.2) and post fixed in 10 g/L $OsO_4$ solution (Spectrum, USA) for 3 h. Afterwards, samples were dehydrated in a series of ethanol solutions (20%, 40%, 60%, 80%, 90%, 95%, 100%) and dried at a critical point of liquid $CO_2$ in a desiccator. The specimens were mounted onto aluminum stubs using conductive silver glue and sputter coated with gold. Scanning electron microscope (JSM-6360LV, Japan) was used.

### 1.9 Statistical analysis

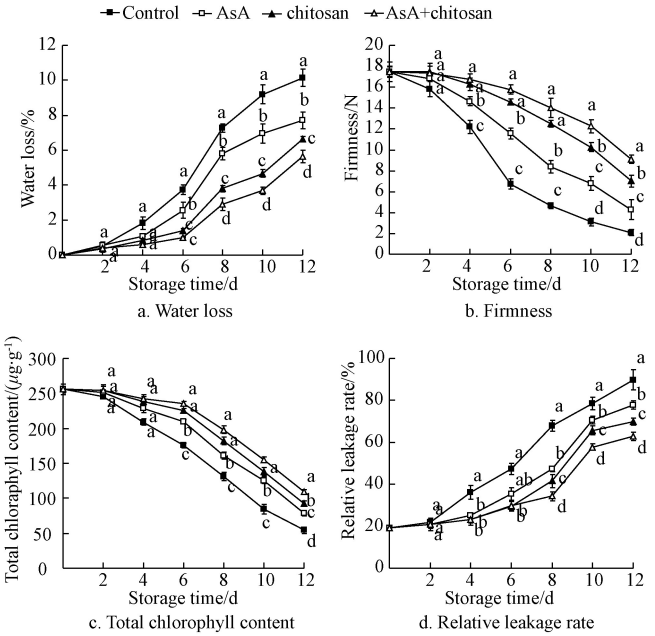
All experiments were in triplicates and statistical analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way ANOVA, and means were compared by Duncan's multiple range test. The level of significance of $P<0.05$ was accepted. The error estimates given in text and error bars in figures were standard deviation (SD) of means.

## 2 Results and Analysis

### 2.1 Effects of AsA and chitosan on water loss, fruit firmness, chlorophyll content and relative leakage rate in pericarp

Water loss (Fig.1a) in all samples continuously increased during storage. Compared to maximal value of 10.14% in control on 12th day, water loss of AsA, chitosan coating, and AsA combined with chitosan were 7.71%, 6.63% and 5.63% ($P<0.05$), respectively. Meanwhile, shown in Figure 1B, fruit firmness of the control on 12th day was 2.08 N, and corresponding values for AsA, chitosan coating and their combinational treatment were 4.27, 7.01 and 9.11 N ($P<0.05$), respectively. For all treatments, chlorophyll content in pericarp (Fig.1c) decreased gradually during storage. Compared to AsA, chitosan coating showed a more remarkable effect on inhibiting chlorophyll degradation and AsA coupled with chitosan treatment had the most significant effect ($P<0.05$). Relative leakage rates (Fig.1d) in all samples increased gradually during storage. The order of increase degree is AsA combined with chitosan< chitosan coating<AsA and a distinct difference among three treatments was observed ($P<0.05$).

In short, in contrast to the control, treatments showed remarkable effects on decreasing fruits water loss, chlorophyll content and relative leakage rate in pericarp and maintaining fruits firmness. Interestingly, a distinct difference among three treatments was observed and AsA coupled with chitosan had the most significant effect.




Note: Different letters indicate significant difference at $P<0.05$, the same below.

Fig.1 Effects of AsA and chitosan coating on water loss, firmness, chlorophyll content and relative leakage rate in pericarp

### 2.2 Effects of AsA and chitosan on PME and PG activities and water soluble pectin and protopectin contents in pulp

PME activity (Fig.2a) in control increased sharply, reached plateau on the 6th day and decreased rapidly afterwards. In contrast, PME activity in AsA treated fruit reached a peak on the 8th day, 3.52-fold higher than that at the beginning. Chitosan coating, and AsA combined with chitosan treatment markedly retarded increase of PME activity, reaching maximal values on the 10th day. PG activity (Fig.2b) increased at initial storage and decreased afterwards. For instance, PG activity (control) reached maximal value on the 6th day, 8.79-fold higher than that at the initial. Compared to the control, a distinct difference was observed among three treatments and AsA combined with chitosan had the most significant effect on inhibiting PG increase ($P<0.05$). It was shown in Fig.2c that water soluble pectin (WSP) content in control from 1.02% increased to 5.28% on the 12th day. In treatment of AsA, chitosan coating, and AsA coupled with chitosan, WSP was decreased by 12.10%, 24.24% and 38.22% ($P<0.05$), respectively, in contrast to the control. Protopectin content (Fig.2d) in treated fruits was higher than that in control during storage. For instance, it was 1.06 % in control on the 12th day, and corresponding values were 1.57%, 2.26% and 2.79% ($P<0.05$) for AsA, chitosan coating, and AsA coupled with chitosan treatment, respectively.

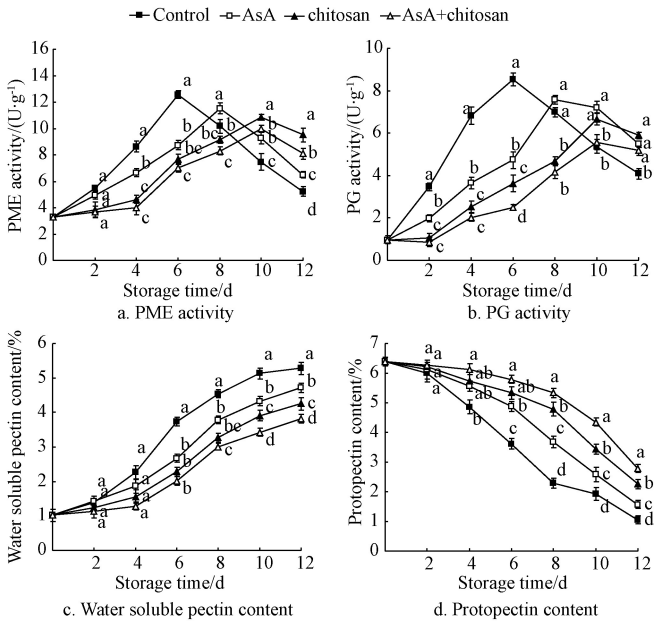



Fig.2 Effects of AsA and chitosan coating on activities of PME and PG and contents of water soluble pectin and protopectin in pulp

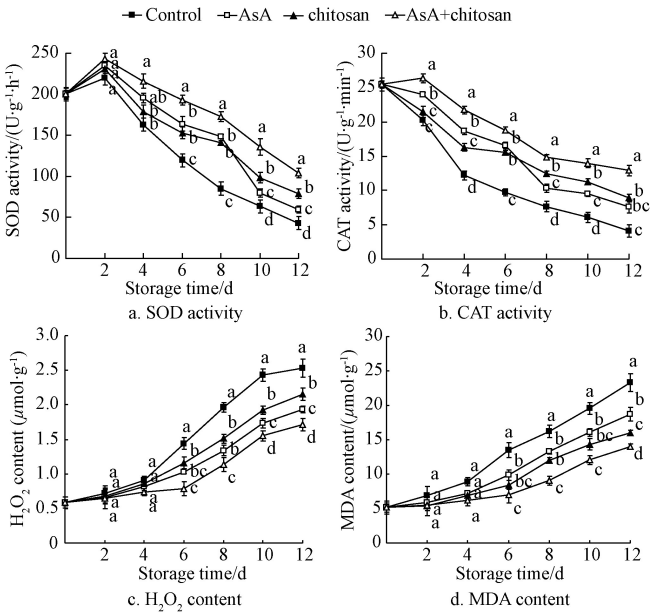



Fig.3 Effects of AsA and chitosan on SOD and CAT activities, and $H_2O_2$ and MDA contents in pulp

### 2.3 Effects of AsA and chitosan on SOD and CAT activities, and $H_2O_2$ and MDA contents in pulp

SOD activity (Fig.3a) in all samples increased at the initial two days and decreased afterwards. However, treated fruits showed a relatively higher value of SOD than control during whole storage. For instance, it was 42.85 U/(g·h) in control on the 12th day. In comparison, treated with AsA, chitosan coating, and AsA coupled with chitosan, corresponding values were 59.67, 78.98 and 103.94 U/(g·h) ($P$<0.05), respectively. The decrease degree of CAT activities (Fig.3b) in control was higher than those in treated fruits. For instance, CAT activity in control decreased from 25.45 U/(g·min)at the beginning to 4.08 U/(g·min) on the 12th day. Treated with AsA, chitosan coating, and AsA coupled with chitosan, corresponding values were 87.99%, 117.89% and 216.42% ($P$<0.05) higher than the control on the 12th day, respectively.

It was known that $H_2O_2$, as typical oxidant, can result in lipid oxidation and membrane destruction[41]. Malondialdehyde (MDA) generated from lipid oxidation can be used to reflect membrane integrity[17]. $H_2O_2$ level (Fig.3c) in all samples increased gradually during storage. Fascinatingly, treated fruits showed a relatively lowered $H_2O_2$ compared to the control. For instance, compared to 2.53 $\mu$mol/g in control, treated with AsA, chitosan coating, and AsA combined with chitosan, $H_2O_2$ were 1.93, 2.15 and 1.71 $\mu$mol/g ($P$<0.05) on the 12th day, respectively. MDA contents (Fig.3d) in treated fruits were markedly lower than in control during storage. For example, MDA content in control was 23.34 $\mu$mol/g on the 12th day. Treated with AsA, chitosan coating, and AsA coupled with chitosan, corresponding values were 24.74%, 32.40% and 39.97% ($P$<0.05) lower than the control on the 12th day, respectively.

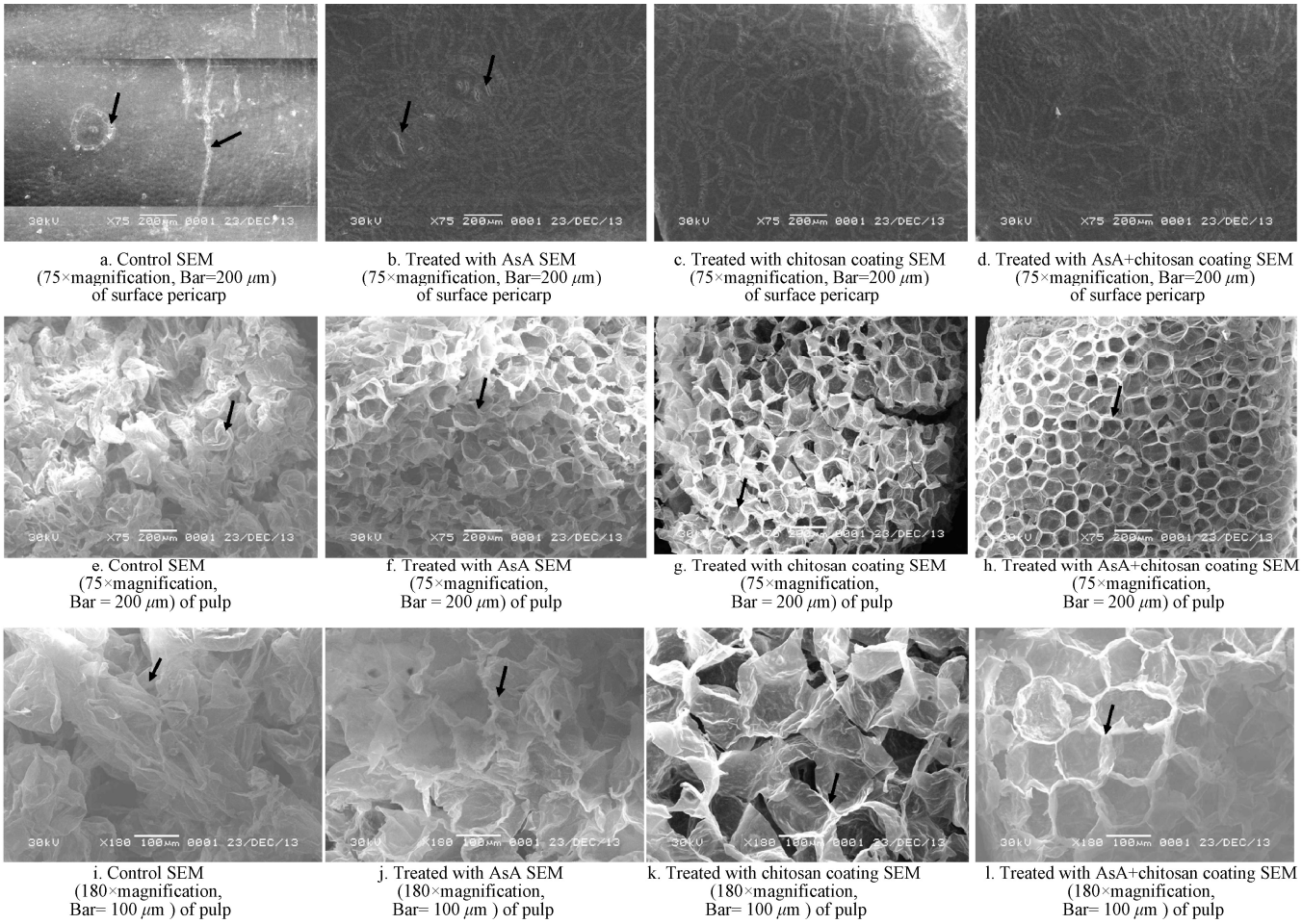
### 2.4 Effects of AsA and chitosan on integrity of cell structure in pericarp and pulp

Scanning electron microscopic (SEM) picture was carried out to investigate the effects of AsA, chitosan coating, and their combination treatments on cell structure integrity in pericapr and pulp tissues. Evidently, many big splits were observed (Fig.4a) on pericarp surface of the control. In contrast, only a few small splits existed as treated with AsA (Fig.4b). In treatment of chitosan coating, and AsA coupled with chitosan, no split appeared on pericarp surface (Fig.4c, d), which indicates that integral pericarp is being maintained. In addition, (shown in Fig.4e, i), pulp cells integrity in control was completely damaged, and cell wall became much looser on the 12th day. In comparison, pulp cells in treated fruits showed normal morphology with clear cellular framework (Fig.4 f, g, h, j, k, l) on the 12th day. Moreover, among three treatments, AsA combined with chitosan coating had the most significant effect on maintaining cell structure integrity of pericarp and pulp.

### 2.5 Effects of AsA and chotosan on ascorbic acid (AsA), glutathione (GSH), total soluble solid(TSS), titratalbe acidity(TA), total soluble sugar(SS) and decay rate

Total soluble solids (TSS), titratable acidity (TA) and other indexes were considered as important factors in flavor and nutritive quality of many fruits[42]. So, the main physiological parameters concerned fruits quality were determined after 12 days storage and shown in Table 1. Compared to the control, relatively higher contents of AsA and GSH were observed in treated fruit. For example, treated with 30 mmol/L AsA, 8 g/L chitosan, and 30 mmol/L AsA coupled with 8 g/L chitosan, AsA content was 18.80%, 30.08% and 54.89% ($P$<0.05) higher than the control after 12 days storage, respectively. Correspondingly, GSH content was increased by 138.93%,

153.02% and 198.73% ($P$<0.05) in treated fruit, respectively. In addition, TSS, SS and TA in treated fruit were higher than those in control. Fascinatingly, decay rate in control was remarkably higher than that in treated fruits. Among three treatments, AsA combined with chitosan had the lowest decay rate.



a. Control SEM (75×magnification, Bar=200 $\mu$m) of surface pericarp
b. Treated with AsA SEM (75×magnification, Bar=200 $\mu$m) of surface pericarp
c. Treated with chitosan coating SEM (75×magnification, Bar=200 $\mu$m) of surface pericarp
d. Treated with AsA+chitosan coating SEM (75×magnification, Bar=200 $\mu$m) of surface pericarp
e. Control SEM (75×magnification, Bar = 200 $\mu$m) of pulp
f. Treated with AsA SEM (75×magnification, Bar = 200 $\mu$m) of pulp
g. Treated with chitosan coating SEM (75×magnification, Bar = 200 $\mu$m) of pulp
h. Treated with AsA+chitosan coating SEM (75×magnification, Bar = 200 $\mu$m) of pulp
i. Control SEM (180×magnification, Bar= 100 $\mu$m ) of pulp
j. Treated with AsA SEM (180×magnification, Bar= 100 $\mu$m ) of pulp
k. Treated with chitosan coating SEM (180×magnification, Bar= 100 $\mu$m ) of pulp
l. Treated with AsA+chitosan coating SEM (180×magnification, Bar= 100 $\mu$m ) of pulp

Note: Arrows in picture indicate integrity degree of cell wall in pericarp and pulp tissues.

Fig.4　SEM picture of cell structure integrity of pericarp and pulp after 12 days storage

**Table 1　Effects of AsA and chotosan on ascorbic acid, glutathione, total soluble solids, titratable acidity, total soluble sugar and decay rate**

| Treatment | Ascorbic acid/ ($mg \cdot g^{-1}$) | GSH content/ ($\mu g \cdot g^{-1}$) | Total soluble solids/% | Titratable acidity/% | Total soluble sugar/ ($mg \cdot g^{-1}$) | Decay rate/% |
|---|---|---|---|---|---|---|
| Control | 1.33±0.04d | 32.95±3.01d | 8.82±0.28c | 0.18±0.02d | 51.01±2.41d | 89.18±5.65a |
| AsA | 1.58±0.02c | 78.73±1.28c | 14.67±1.15b | 0.29±0.05bc | 73.44±1.28c | 56.35±4.11b |
| Chitosan | 1.73±0.02b | 83.12±2.15b | 15.42±0.15b | 0.24±0.03c | 80.29±1.01b | 21.15±3.27c |
| AsA+ Chitosan | 2.06±0.03a | 98.43±4.24a | 16.47±0.15a | 0.35±0.02a | 86.23±2.12a | 13.37±2.75d |

Note: Values are mean of three replicates per treatment± standard deviation ($n$=3), the same letters in each column mean no significant difference at $P$<0.05.

## 3　Discussion

Water content in harvested fruits represents status of metabolic activity, which makes most fruits highly perishable commodities. This perishability presents the greatest problem for marketability of fresh fruits. Rapid water loss, softening and pericarp browning are major limitation for harvested Taiwan jujube fruits during storage[43]. Browning in pericarp was thought to be a rapid degradation of chlorophyll resulted from oxidation injury [44]. Also, rapid propagation and growth of pathogen microorganisms caused fruit tissue deterioration and thus reduced fruit market value[45].

Besides directly inhibits microbial activity, chitosan coating can form semi-permeable film on surface of fresh fruit to alleviate water loss and slow down respiration[46]. In addition, reactive oxygen species (such as $O_2^{\bullet-}$), generated during fruits storage, can cause serious damage to cell membrane[47]. As a result, plants have developed a defensive system to get ride of $O_2^{\bullet-}$ by enzymes-induced reaction including SOD and CAT or antioxidants such as AsA and glutathione[35].

Consequently, in this paper, we mainly focused on improving storability of harvested Taiwan jujube fruits by using chitosan coating combined with AsA during postharvest period. In a concrete way, three treatments of AsA, chitosan, and their combinational treatment were adopted. Compared to AsA, chitosan coating showed a more

remarkable effect on inhibiting water loss, chlorophyll degradation and membrane oxidation in pericarp. Moreover, chitosan coating had a more significant effect on inhibiting PME and PG activities. As a result, a relatively lower water soluble pectin and higher protopectin contents in pulp was observed. Meanwhile, compared to chitosan coating, AsA markedly prevented decreasing of SOD and CAT activities and a relatively lower $H_2O_2$ was observed. Also, chitosan coating had significant effect on maintaining cell structure integrity of pericarp and pulp. Fascinatingly, AsA coupled with chitosan had the most significant effect on these physiological parameters. Evidently, combinational treatment showed a better effect on storability of harvested fruits in contrast to AsA or chitosan treatment alone.

It was known that freshly harvested fruits showed a strong respiration, which symbolizes rapid water loss. As chitosan coating can directly prevent water loss resulted from respiration as well as natural evaporation on pericarp surface, water loss was significantly reduced in treated fruits. Thus, turgor pressure inside cell was slightly affected[8]. Also, inhibition on increase in PME and PG activities markedly prevented protopectin degradation, thus leading to relatively lower soluble pectin in treated fruits. As water loss was remarkably reduced and higher protopectin was observed, relatively higher fruits firmness was obtained, indicating that softening was markedly delayed in treated fruits.

It was believed that increased activities of enzymes such as SOD and CAT or antioxidants level can decrease oxidative stress[48-49]. We found that activities of SOD and CAT and GSH content in treated fruits were relatively higher than those in control. Thus, chlorophyll degradation in pericarp was markedly alleviated and fruits browning were effectively controlled. Also, a relatively lowered relative leakage rate in pericarp was observed, suggesting that membrane permeability was reduced. In addition, as typical oxidant, $H_2O_2$ could result in lipid oxidation and membrane destruction[17]. As is known, lipid oxidation produces MDA, accumulation of which can reflect degree of membrane integrity[38]. Our results indicated that increase degree of $H_2O_2$ and MDA were significantly reduced in treated fruits, suggesting that integral membrane is maintained, which was further proved by SEM picture in pericarp and pulp of treated fruits. So, we reasonably concluded that destruction of cell walls is minimized and integral cell structure is maintained.

In short, water loss was significantly reduced and pericarp browning was markedly delayed. Thus, higher degree of firmness and integral cell structure in treated fruits was obtained. As a result, an increase in contents of total soluble solids, soluble sugars, glutathione, ascorbic acid and titratable acidity and a decrease in decay rate were achieved. It was fascinating to observe that chitosan had a more remarkable effect on preserving harvested fruits than that of AsA. Chitosan mainly prevented dehydration and microbial attack and AsA function as typical antioxidant to increase anti-oxidation. So, we can conclude that chitosan play more important roles in enhancing storability than AsA. More interestingly, their combinational treatment showed the most significant effect.

## 4 Conclusions

In the present study, the effects of chitosan, AsA, and their combinational treatment on storability of harvested Taiwan jujube fruits were systematically investigated. Chitosan showed remarkable effect ($P$<0.05) than AsA on inhibiting water loss, chlorophyll degradation, membrane oxidation, and increase of PME and PG activities. In contrast, AsA had a better effect ($P$<0.05). than chitosan coating on preventing decrease of SOD and CAT activities. More fascinatingly, AsA coupled with chitosan showed a combinational effect on storability of harvested fruits. Water loss was reduced, fruits firmness and integral cell structure were maintained, and pericarp browning was alleviated. As a result, fruits softening were effectively delayed and decay rate was minimized. It was suggested that the strategies of chitosan combined with AsA on improving preservation effects of harvested Taiwan jujube fruits during storage is being feasible.

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# 壳聚糖和抗坏血酸复合处理提高台湾青枣采后保鲜效果

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**摘　要：**为了延长台湾青枣（*Ziziphus mauritiana* Lam.）的采后保鲜时间，基于抗坏血酸（ascorbic acid, AsA）可提高果实抗氧化能力，壳聚糖涂膜处理能防止果实失水和微生物侵染，该文探讨抗坏血酸、壳聚糖涂膜及抗坏血酸和壳聚糖复合处理对台湾青枣（*Ziziphus mauritiana* Lam.）采后保鲜效果的影响。果实采收后当天，分别用 30.0 mmol/L 抗坏血酸浸泡处理、8 g/L 壳聚糖涂膜以及 30.0 mmol/L 抗坏血酸浸泡后并用 8 g/L 壳聚糖涂膜复合处理，以双蒸水浸泡处理为对照，处理结束后，置于（25±1）℃和 90%～95%相对湿度下贮藏，定期测定果实相关生理参数，扫描电镜观察果皮果实组织结构。结果表明，与对照相比，抗坏血酸或壳聚糖单独处理虽然对台湾青枣保鲜有一定效果，但复合处理后保鲜效果更好。表现为显著减少果实失水率和相对电导率（$P$<0.05），抑制果皮叶绿素降解及果实果胶酶和多聚半乳糖醛酸酶活性增加，降低原果胶分解成可溶性果胶的速率（$P$<0.05），使果实硬度和细胞完整性得以维持；延缓果实超氧化物歧化酶（superoxide dismutase, SOD）和过氧化氢酶（catalase, CAT）活性下降，降低膜脂氧化速率，果实过氧化氢和膜脂过氧化产物丙二醛含量显著下降（$P$<0.05）；维持果实较高的可溶性固形物（total soluble solids, SS）、可溶性总糖（total soluble sugar, TSS）、可滴定酸（titratable acidity, TA）、谷胱甘肽（glutathione, GSH）及抗坏血酸含量。研究结果说明，抗坏血酸和壳聚糖复合处理可提高台湾青枣贮藏过程中的抗氧化能力，降低氧化伤害；降低果实失水率，减缓果皮叶绿素降解速率，延缓细胞降解和软化速率，维持果实细胞完整性，降低果实的腐烂率，从而达到延长采后果实保鲜的效果。

**关键词：**贮藏；品质控制；水果；台湾青枣；抗坏血酸；壳聚糖；氧化胁迫；抗氧化酶