Advance Journal of Food Science and Technology 10(10): 726-734, 2016
DOI: 10.19026/ajfst.10.2253
ISSN: 2042-4868; e-ISSN: 2042-4876
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Submitted: May 1, 2015 Accepted: May 10, 2015 Published: April 05, 2016

Research Article

Effects of Micro-vacuum Storage on Post-harvest Physiological and Physicochemical Characteristics of Laiyang Pear (*Pyrus bretschneideri* Reld)

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Abstract: The aim of this study was to compare the physiological and physicochemical characteristics of Laiyang pears after 40 days of storage at 3±1°C under both MV and CA storage systems. During the storage period, physiological and physicochemical characteristics of the fruits were evaluated at an interval of 8 days. The results showed that respiratory intensity, ethylene production, relative conductivity and malondialdehyde were decreased in fruits stored under MV storage systems. It could also inhibit the reduction of fruit weight loss, firmness, total soluble solids and titratable acidity. Compared to CA storage systems, MV storage systems have better ability to prolong the shelf life and to maintain the fruit quality of Laiyang pears.

Keywords: Laiyang pear, micro-vacuum equipment, physiological characteristics, physicochemical characteristics

INTRODUCTION

Laiyang pear (*Pyrus bretschneideri* Reld), a popular pear cultivar, is grown in the Laiyang City, Shandong Province of China. This fruit is mainly consumed fresh, since it is juicy, crisp and sweet. It has high nutritional value and helps in curing phlegm and relieving cough (Liu *et al*., 2013). Its skin is yellow-green with rough, rusty-brown spots. The quality of pear is defined by its physical properties such as texture, size, color and odor of the fruit, as well as chemical parameters such as sugar, organic acids, minerals and vitamins. This fruit has a short post-harvest storage time of not more than a month in air at room temperature (Li *et al*., 2013). During this period the fruit presents increased respiration, metabolic rate and several biochemical reactions. The commercial value of the fruit also decreases drastically in this period (Ju *et al*., 2000). Hence, effective control of physiological processes to prolong the post-harvest storage time of the Laiyang pear is an important area of research.

Modified Atmosphere Packaging (MAP) and Controlled Atmosphere (CA) storage are the techniques used to delay ripening and thereby to reduce physiological disorders in "Bartlett" pears during storage and transportation at low temperatures (Wang and Sugar, 2013). Fresh pears are susceptible to physiological damage due to increased rate of post-harvest respiration. Modified atmosphere packaging alters the atmosphere surrounding fruits that is created by respiration and reduces water loss to preserve the quality of pear (Mir and Beaudry, 2004). However, exposure of fruits to a level above their CO_2 tolerance limit can increase physiological damage; exposure below their O_2 tolerance limit may create an anaerobic condition and accumulation of acetaldehyde leading to change in flavor. In spite of the benefits, film gas permeability must match the requirement of fruits and the storage temperature to prevent creating undesirable conditions (e.g., anaerobic condition, CO_2 damage, condensation within the package) (Lange, 2000). It has been shown that by combining CA with low temperature storage, it is possible to delay the subsequent ripening of the fruit and prolong its storage (Sommer *et al*., 1979). Although the senescence and decay of Laiyang pear can be controlled by means of CA storage, gas composition cannot be changed momentarily. This leads to diminished food quality such as loss of ascorbic acid (Veltman *et al*., 2000) and aroma of the fruit (Lara *et al*., 2003). Furthermore, requirement of large storage environment and the difficulties in the maintenance of uniform temperature also contribute to the reduced effect of CA storage.

Hypobaric storage is an effective storage measure which can rapidly remove the respiration heat, reduce the oxygen concentration and the accumulation of detrimental volatile metabolic products such as ethylene and α-farnesene. This storage system can significantly overcome the disadvantages of refrigeration and CA storage (Dilley, 2003, Li *et al*., 2006). Nevertheless, based on the design principle, the construction of the storeroom should bear greater atmospheric pressure than CA storage. The possible solution is to increase the rigidity of storeroom wall material or equip with upholders inside the facility. This results in heavy

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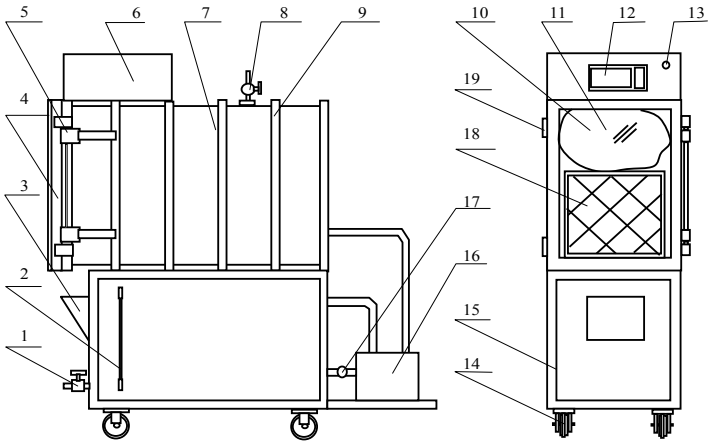

Fig. 1: Structure schematic diagram of Micro-vacuum storage experimental equipment; 1: blow-down valve, 2: water level gauge, 3: filling pipe end, 4: air-tight door, 5: hinge, 6: control box, 7: storage chamber, 8: inlet or outlet air valve, 9: stiffener, 10: air bag, 11: glass, 12: PLC display screen, 13: indicator lamp, 14: return pulley, 15: water tank, 16: vacuum pump, 17: electromagnetic valve, 18: turnover box, 19: fastening handle

containers and increased cost. In addition, it has been reported that maintenance of low vacuum in hypobaric storage facility results in high moisture loss (Li *et al*., 2006). These difficulties make the hypobaric storage technology less acceptable despite its advantages over CA storage. In an effort to overcome the limitations of hypobaric storage, a "three-stage hypobaric storage" technology was established (Li and Zhang, 2006). But this technique was suitable only for products with high surface area such as soft-textured leafy vegetable. The applicability of this system on the storage of Laiyang pear (Li *et al*., 2008) is not well defined.

This study used equipment, Micro-Vacuum (MV) storage experimental equipment (Fig. 1), designed and manufactured by the Food Science and Engineering College of Qingdao Agriculture University. This device is a modified hypobaric storage equipment based on the invention patent CN1530290A (Wang, 2004). The most characteristic part of this experimental equipment is the soft air bag inside the storage chamber which is connected to the surrounding atmosphere. The air bag expands automatically when the internal air is excluded from the storage chamber. The expanding air bag formed by the decline of air pressure inside the storage chamber occupies the little chamber space. It buffers the change of internal air pressure and creates a "micro-vacuum" inside the chamber. In this MV condition, the air constituents in the chamber, including the oxygen and harmful gases, are decreased dramatically. The buffer action caused by the soft air bag lowers the vacuum of storage chamber, due to which MV storage equipment bears lesser atmospheric pressure. This reduces the material and manufacturing costs. In addition, the expanding air bag in the MV storage chamber inhibits the water loss of the products and plays an important role in improved preservation for a longer time.

The objective of this study was to investigate the effect of MV storage and CA storage on physiological and physicochemical characteristics of Laiyang pear in post-harvest period.

MATERIALS AND METHODS

Plant materials: Laiyang pears were harvested from plantations in Laiyang and transported to the laboratory of Qingdao Agricultural University, within 3 h of harvest. They were then sorted and pears of uniform size, free of scars and insects were selected and pre-cooled at 3°C for 12 h. The pears were packaged using a plastic bag (low-density polyethylene LDPE).

Experimental apparatus: The MV storage experimental equipment consisted of a vacuum pump, compressor, moistener, soft air bag, storage chamber (100×75×65 cm) and a control box (Fig. 1). This equipment was placed in a refrigeration house (3±1°C, 85-95% relative humidity [RH]).

Treatments: Laiyang pear fruits were divided into two sets of 30 kg each. One set of pears was placed in MV experimental equipment storage chamber and stored for 40 days at 70 kPa (3±1°C; 85-95% RH). The other set of pears was stored in CA store room (10555×100 cm), under controlled conditions (2% O_2 plus 2% CO_2). This was monitored by an atmosphere analyzer (FC-701, Italy) kept at the same temperature and RH. Freshly harvested pears were used as the 0 day sample. Eight pears were taken randomly at an interval of 8 days to determine the characteristics. Each treatment was repeated thrice.

Determination of physiological characteristics:

Ethylene production and respiration rate: For each treatment, eight fruits were sealed in 5L air-tight jars at room temperature (25±3°C). After 3 h, a 50 mL gas sample was collected from each jar using a syringe.

Ethylene concentration was determined using gas chromatography (Agilent, 6890N, USA) equipped with a Flame Ionization Detector (FID) and an HP-5 column. Respiration rate was measured by the same gas chromatograph fitted with a thermal conductivity detector. Ethylene production rate was expressed in nmol/kg/FWh and the respiration rate in $mLCO_2$/kg/FWh.

Relative conductivity and malondialdehyde content: The cell-membrane permeability was measured according to the methods of Liu *et al*. (2013). From each treatment, 10 g of flesh was collected from 8 fruits and placed into 50 mL of distilled water. The sample was then incubated for 3 h. The initial electric conductivity was measured with a conductivity meter (DDS-11C, Shanghai, China). Disks were boiled for 30 min, cooled in room temperature and then the total conductivity was measured. Membrane permeability was expressed as relative electric conductivity percentage of the initial electric conductivity.

Malondialdehyde (MDA) content was assayed using the Thiobarbituric Acid (TBA) reaction (Li *et al*., 2013). Approximately 1 g of flesh from the fruits was homogenized with 10 mL of 5% Trichloroacetic Acid (TCA) and then centrifuged at 10000g for 10 min at 4°C. The supernatant (2 mL) was incubated with 2 mL of 6.7 g/L TBA for 30 min at 95°C and the mixture was then cooled rapidly in an ice bath. It was centrifuged again at 4000 g for 10 min. The absorbance of the supernatant was recorded at 532, 450 and 600 nm using a UV-2000 spectrophotometer (Unico Instrument Co., Shanghai, China) and the results were expressed as μmol/g using the formula: MDA content (μmol/g/FW) = $6.45\times(OD_{532}-OD_{600})-0.56 \times OD_{450}$. All measurements were repeated at least three-times.

Determination of physicochemical characteristics:
Firmness and weight loss rate: Firmness (g) was determined at the center of the pear fruits using a TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK) fitted with a 5 mm-diameter probe. The penetration rate was 1 mm/s, with a final penetration depth of 10 mm.

To assess weight (water) loss, 10 fruits were weighed before and after the storage period in triplicates. The results were expressed as the percentage loss of initial weight.

Total soluble solids and titratable acid: About 20 g of edible tissue of pear fruit was homogenized in a mortar. Soluble solids were obtained by filtering through a cellulose nitrate membrane filter (1.0 μm pore size) and Total Soluble Solids (TSS) were determined on a WAY-Refractometer (ShangHai Exact Science Instrument Limited Company, China) as reported earlier (Cavalcanti *et al*., 2010).

Ten grams of edible part of pear tissue was mixed with 0.5 g sea sand and homogenized in a mortar. After adding 100 mL distilled water, the homogenate was centrifuged at 6000 g for 15 min. Titratable acid (TA) content in the supernatant was determined by titration with 0.1 N NaOH (Cavalcanti *et al*., 2010).

Statistical analysis: The experimental design was completely randomized with three replications. Statistical evaluation of the different storage conditions were performed using analysis of variance (ANOVA). The treatment means were separated using the Least Significant Difference (LSD) method. Mean values were considered significantly different at $p<0.05$.

RESULTS AND DISCUSSION

Effects of MV and CA storage on physiological characteristics of fruit:
Changes in respiration intensity and ethylene production: Ripening and senescence of the pear fruits is accompanied by physiological and biochemical changes, including an increase in respiration and ethylene production (McMurchie *et al*., 1972). Under room temperature, the respiration intensity of Laiyang pears reaches a climacteric peak after 15-20 days. The quality and nutrition of the fruit decreases dramatically, following this respiration peak (Li *et al*., 2013). However, low temperature condition reduces the rate of respiration and prolongs the respiratory peak. In this study, rate of respiration slowed down under both storage conditions (Fig. 2A). The respiratory peak appeared after 32 days in fruits stored under CA storage condition but could not detect the same till the end of experiment in fruits stored under MV storage condition. At the end of the experiment, it was noted that the respiratory intensity of the Laiyang pears under MV storage was lower than CA storage ($p<0.05$). The respiratory intensity under MV storage was found to be 79.4% of that under CA storage. The increase in ethylene production associated with the respiration climacteric was observed in all treatments. Ethylene production of pear fruit reached the maximum after 32 days of storage. Compared to CA storage, ethylene production was significantly lower ($p<0.05$) in MV storage condition (Fig. 2B). The maximum ethylene production under MV storage was 83.7% of that under CA storage. These results indicate that MV storage could reduce respiration rate and ethylene production, leading to delay in the process of physiological damage and metabolism of Laiyang pear fruit.

Changes in relative conductivity and MDA content: The changes in relative conductivity and MDA content under two different storage conditions are presented in

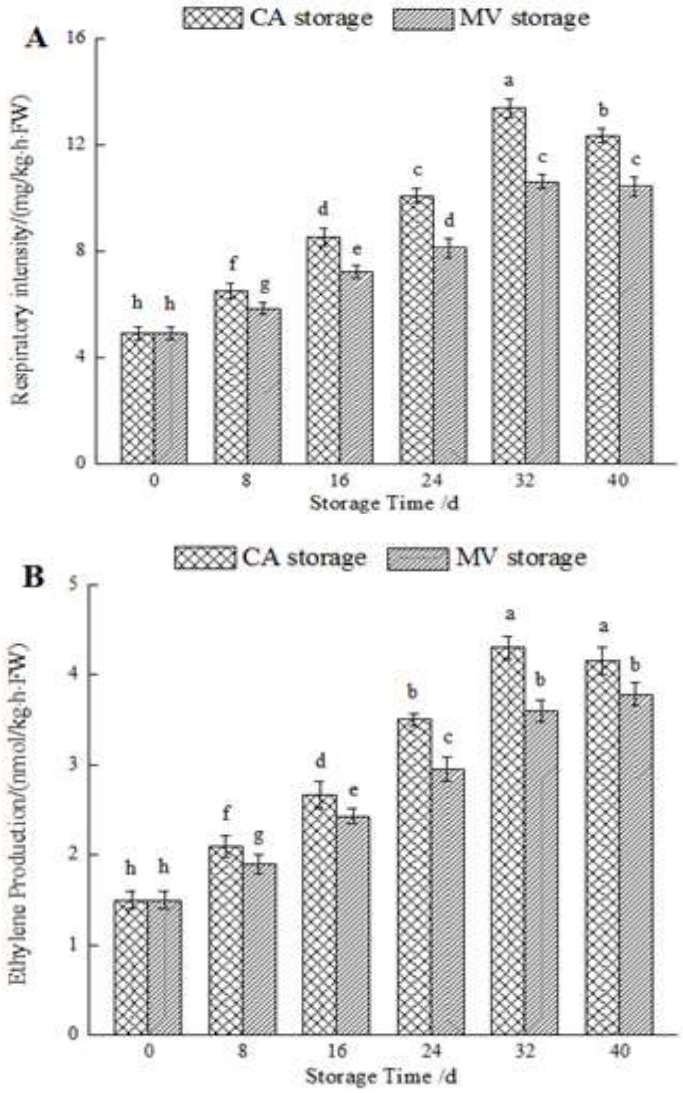

Fig. 2: Changes of respiratory intensity (A) and ethylene production (B) in MV and CA storage Laiyang pear fruits during 40d storage at 3±1°C. Each value is the average of three replicates ± standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

Fig. 3. There was a continuous increase in relative conductivity during the storage period, showing a gradual loss of cell membrane integrity (Fig. 3A). However, MV storage was found to maintain the cell membrane integrity better than CA treatment ($p<0.05$) (Fig. 3B). At the end of storage, the relative conductivity of fruits under MV storage treatment was 66.5% of that under CA storage. MDA is one of the intermediate products of lipid peroxidation. The content of MDA is often used as an indicator of lipid peroxidation resulting from oxidative stress (Dhindsa *et al*., 1981). Lower MDA content indicates less lipid peroxidation. As shown in Fig. 3B, MDA content under two storage conditions increased with prolonged storage time. It was observed that MDA content of CA storage treatment was higher than that of MV storage treatment ($p<0.05$). At the end of storage, MDA content of MV storage treatment was 80.2% of that under CA storage.

The breakdown of cell wall component and membrane disruption is important physiological phenomena of fruit senescence, resulting in increased membrane permeability (Paliyath and Droillard, 1992). Membrane permeability is expressed as increasing leakage of ions (Marangoni *et al*., 1996). Peroxidation of fatty acids with resulting free radical formation has been described as one of the major deteriorative processes of membranes. This process is accompanied by increased contents of MDA (Ye *et al*., 2000), higher production of Reactive Oxygen Species (ROS; Thompson *et al*., 1987) and gradual loss in the ability of scavenging enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD) to neutralize the free radicals (Pastori and Del Rio, 1997). Our

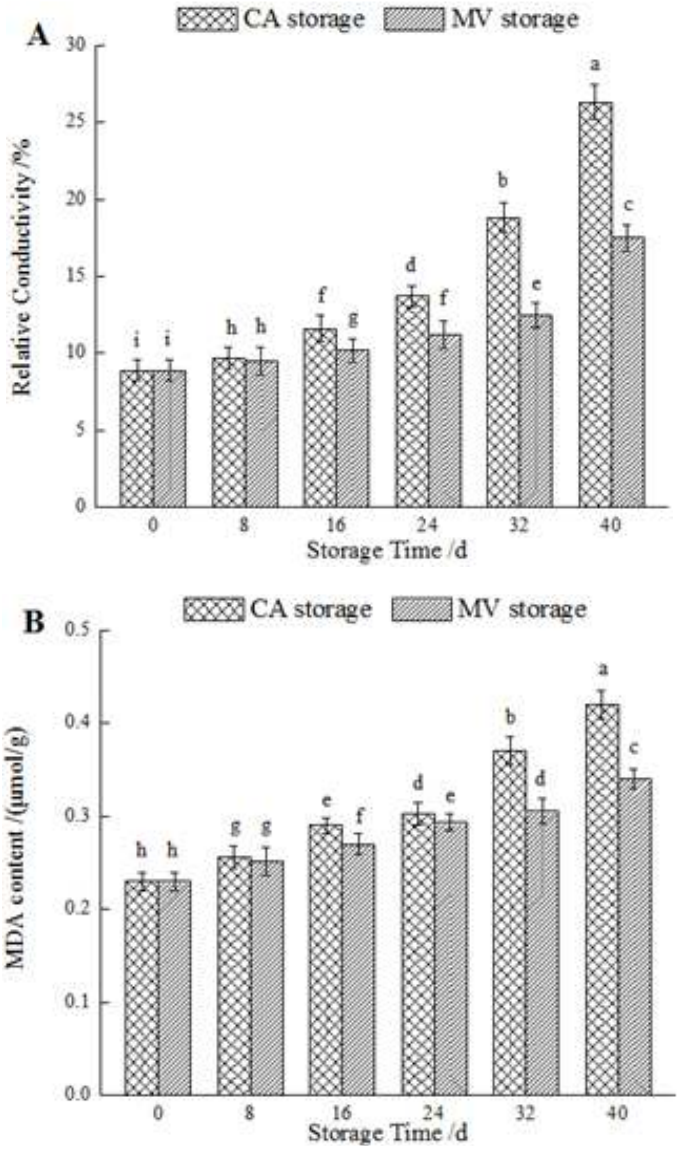

Fig. 3: Changes of relative conductivity (A) and malondialdehyde (MDA) (B) in MV and CA storage Laiyang pear fruits during 40d storage at 3±1°C. Each value is the average of three replicates ± standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

results suggest that MV storage condition could inhibit cell membrane deterioration and accumulation of the MDA. The effect of MV storage on the production of ROS and activity of the certain antioxidant enzymes needs to be studied further.

Effects of MV and CA storage on fruit physicochemical characteristics:

Changes in physical properties: weight loss and firmness: The changes in weight loss and firmness of the fruits were studied as indicators of physical properties (Fig. 4). The weight of the fruits was found to decrease slowly until 16 days and rapidly then after (Fig. 4A). The rate of weight loss under MV storage condition was lower than the CA storage condition throughout the storage period ($p<0.05$). Finally, the weight loss rate under MV storage condition was 59.1% of that under CA storage condition. The firmness of the fruits showed a declining trend during storage time under all conditions (Fig. 4B). At the end of storage, the firmness of fruits under MV storage treatment was 66.5% of that under CA storage. Compared to CA storage condition, the decline of fruit firmness under MV storage condition was significantly lower ($p<0.05$).

The weight loss of fruits is due to the process of transpiration, which is generally determined by the gradient of water vapor pressure between the fruit and the surrounding air. The transpiration is reduced by both the epidermal cell layer and the cuticle. The surface/volume ratio of the fruit and epidermis and cuticle structures differ among fruits (Pasquariello *et al*., 2013). The weight loss including the water loss in

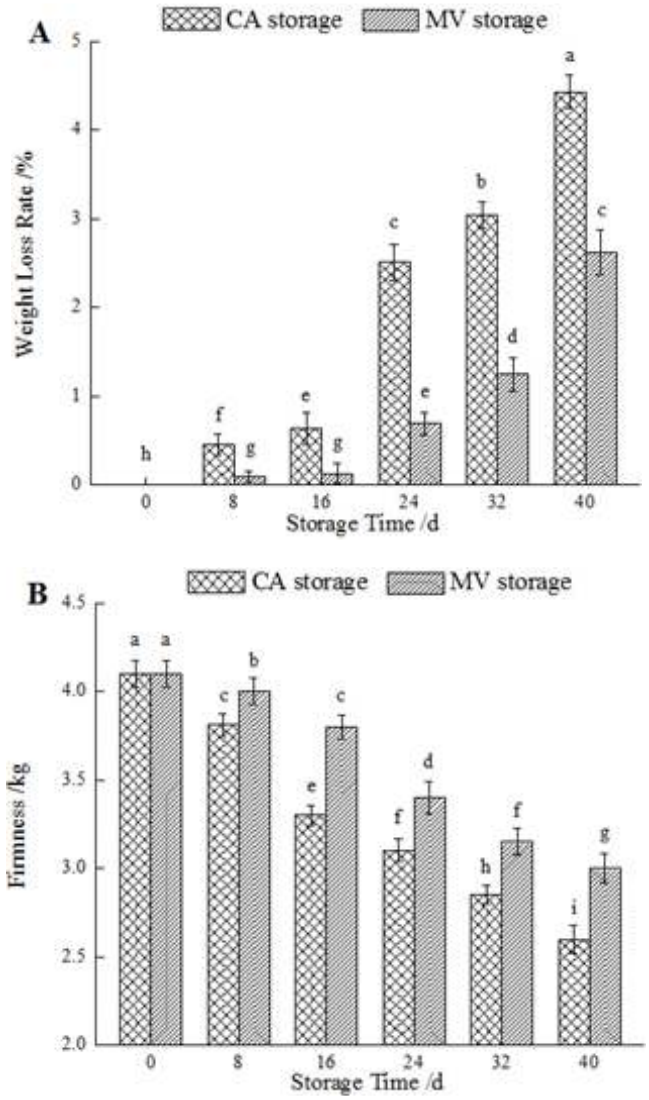

Fig. 4: Changes of weight loss (A) and firmness (B) in MV and CA storage Laiyang pear fruits during 40d storage at 3±1°C. Each value is the average of three replicates ± standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

Laiyang pears were found to be nondestructive indicators before the visible symptoms of senescence. It has been observed that high weight loss levels also affect the visual appearance of fruit, such as fruit wilting, yellowing and softening.

Firmness and crispness are critical quality factors required for fresh and minimally processed fruits. Fruit flesh firmness is most closely associated with the cell wall structure and composition, particularly, with the cell wall changes that occur during ripening. The cell wall provides rigidity and strength and it is against the resistance of the wall that the osmotic pressure of the protoplast exerts force and provides turgor pressure (Glenn and Poovaiah, 1990). Firmness is largely determined by the physical anatomy of the tissue (particularly cell size and shape and cell wall thickness and strength) and the cell-to-cell adhesion status, together with the turgor status. During ripening, these factors change, leading to creation of larger air spaces and reduced intercellular contact (Hallett *et al*., 1992). Fruit tissues with larger cells and more intercellular spaces are generally considered to be weaker than tissues with smaller cells and fewer intercellular spaces (Harker *et al*., 1997). This finding may explain why post-harvest pear fruits tend to soften more rapidly in storage period.

Changes in chemical properties: total soluble solids and titratable acid: The results of TSS and TA under two different storage conditions of the fruits are presented in Fig. 5. TSS content (Fig. 5A) increased with the storage intervals up to 16 days of storage and declined thereafter with subsequent storage. The TSS content of the fruits under MV storage treatment was

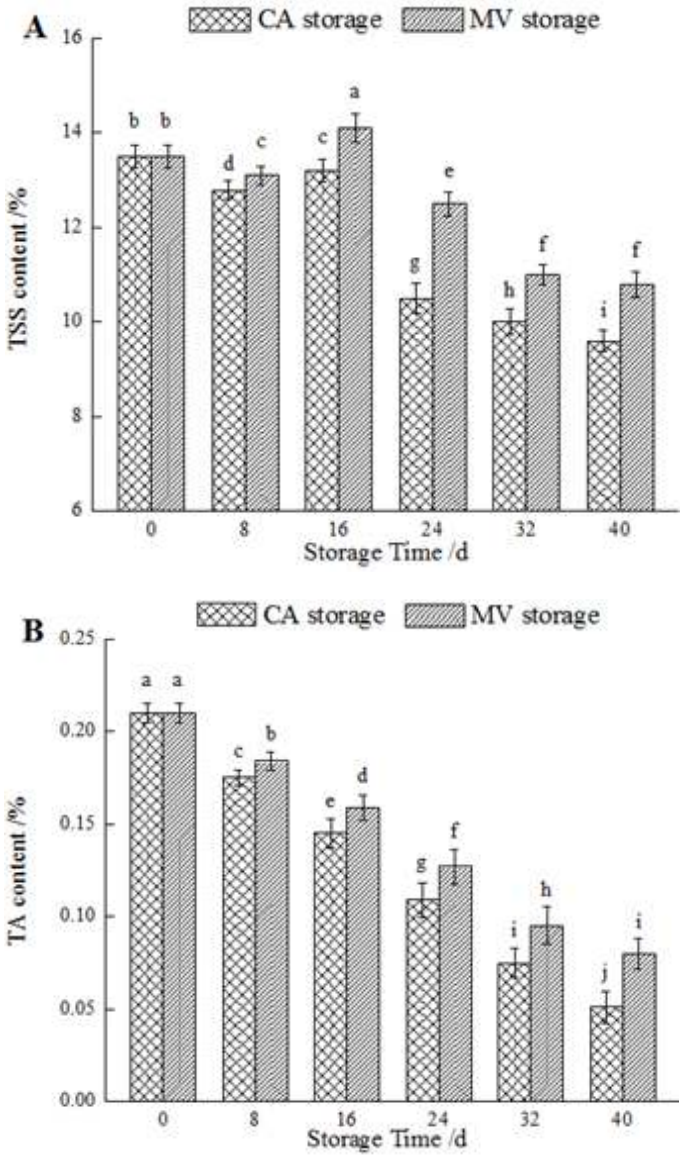

Fig. 5: Changes of total soluble solids (TSS) (A) and titratable acid (TA) (B) in MV and CA storage Laiyang pear fruits during 40d storage at 3±1°C. Each value is the average of three replicates±standard deviation. Values with different letters are significantly different according to Duncan's multiple range test at $p<0.05$

higher than those under the CA storage treatment throughout the storage period ($p<0.05$). The TSS content was 10.8% after 40 days MV storage while it decreased to 9.6% in CA treatment. The TA content of the fruits showed a negative correlation with storage time. The TA content gradually decreased with storage time. At the end of storage, the TA content of the fruits under MV storage was higher (TA: 0.082%) than that under CA storage (TA: 0.051%) ($p<0.05$).

Fruits generally contain different carbohydrate and acid compositions, which may influence the consumers' perception of the fruit quality. The balance between TSS and acidity decides the overall acceptability of fruits. During storage many biochemical changes take place which disturbs the TSS/TA ratio, ultimately rendering the fruit unacceptable for consumption (Kirandeep *et al*., 2013). The changes in TA and TSS contents could be associated with the metabolic activity and respiratory rates of the fruits (Chen *et al*., 2013). The TSS increased up to 16 days of storage and declined thereafter which can be attributed to the fact that on complete hydrolysis of carbohydrate, no further increase in TSS could occur and consequently a decline in TSS is predictable as they are the primary substrates for respiration (Wills *et al*., 1980, 1983). The decrease in TA content could be attributed to the use of organic acids as respiratory substrate during storage and to the conversion of acids into sugars (Echeverria and Valich, 1989). This study showed that the MV storage could inhibit the decreasing TSS and TA content of the

Laiyang pear fruits. The possible reason might be related with the ability of MV storage toward decreasing the respiration intensity of the pear fruits.

CONCLUSION

This study showed that by decreasing the respiratory activity, ethylene production and by reducing the rate of weight loss and fruit firmness, MV storage can delay fruit ripening and senescence, when compared with CA storage. During the MV storage period, the TSS and TA content were at higher level than that during the CA storage. MV storage was more effective in inhibiting the increase in relative conductivity and MDA accumulation. The ability of MV storage in delaying the senescence of pear fruit could be associated with the inhibition of ROS accumulation and maintenance of the activity of certain antioxidant enzymes. However, the mechanisms of inhibiting fruit senescence need further study.

ACKNOWLEDGMENT

The authors would like to thank the Shandong Province Modern Agricultural Industry Technology System (SDAIT-11-011-09) and Shandong Province Natural Science Foundation (ZR2011CL009) of China for supporting this research.

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