

# Effect of three-stage hypobaric storage on membrane lipid peroxidation and activities of defense enzyme in green asparagus

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## Abstract

The effect of three-stage hypobaric storage on free radical content, membrane lipid peroxidation, activities of cell defense enzyme and surface color ( $L^*$  value,  $a^*$  value and  $b^*$  value) in green asparagus was studied as compared with the atmosphere cold storage. The three-stage hypobaric condition significantly increased the activities of superoxide dismutase (SOD) and catalase (CAT), decreased the accumulation of malondialdehyde (MDA), superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ), inhibited the increase of relative leakage rate, delayed the degeneration of surface color, and controlled the increase of peroxidase (POD) activity. It was concluded that three-stage hypobaric storage could obviously inhibit asparagus senescence and that the storage life of 50 days could be achieved as compared with the storage life of only 25 days in atmosphere cold condition.

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**Keywords:** Green asparagus; Hypobaric storage; Lipid peroxidation; Free radical; Defense enzyme

## 1. Introduction

Senescence of postharvest fruits and vegetables is a complicated physiological process. Several studies have been carried out on mechanism of organism senescence with various hypotheses. Since free radical injury theory of senescence was proposed in 1956 by Denham Harman, many investigations have been conducted (Huang, Huang, & Song, 1995). Many researchers have investigated the activity of correlative defense enzyme (Droillard, Bureau, & Paulin, 1989; Masia, 1998; Rabinowitch & Sklan, 1981), membrane lipid peroxidation (Lin, Li, & Zhang, 1988; Paliyath & Droillard, 1992; Paulin & Droillard, 1989) and active oxygen radical metabolism (Guan & Shu, 1996; Huang et al., 1995) in fruits and vegetables. There are protective enzyme systems such as

superoxide dismutase (SOD) and catalase (CAT), which can eliminate active oxygen radical in organism. However, the activity of SOD and CAT usually decreases with the extension of postharvest time, which leads to the accumulation of hydrogen peroxide ( $H_2O_2$ ) in organism (Baker, 1976; Droillard, Paulin, & Massot, 1987; Rajinder, 1981). Brennan and Frenkle (1977) pointed out that  $H_2O_2$  might participate in regulating senescence of pear and might be a part for starting senescence mechanism. In general, the free radical production and elimination are always in a dynamic equilibrium state and the free radical level is too low to injure organism cell. But this balance can be broken by the increase of active oxygen radical during senescence process or in adverse conditions. Cell membrane systems can be injured when the free radical accumulates enough (Hu, Zhang, & Ji, 2004; Lin, Li, & Lin, 1988; Liu, Yao, & Feng, 2004). Active oxygen radical can set off a chain reaction of membrane lipid peroxidation. As a result, it can lead to the accumulation of lipid peroxidation product MDA. This may injure the membrane permeability and damage the membrane integrity. Therefore, membrane lipid

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peroxidation can accelerate senescence of membrane systems (Droillard et al., 1987; Lin, Li, & Zhang, 1988).

As one of the most important fresh vegetables, green asparagus (*Asparagus officinalis* L.) is becoming more and more popular due to its special flavor and taste in China in recent years. However, it is a highly perishable vegetable, which deteriorates rapidly after harvest, and only has a shelf life of 3–5 days under normal postharvest handling at ambient temperatures (Baxter & Waters, 1991; Lipton, 1990). Undesirable changes can be reduced by rapid cooling after harvest, keeping at low temperatures (2–5 °C) and use of controlled or modified atmosphere storage (Lipton, 1990; Villanueva, Tenorio, & Sagardoy, 2005), but Lipton (1990) and Siomos, Sfakiotakis, and Dogras (2000) also reported that controlled atmospheres provided little added benefit when it was used to retard deterioration of *Asparagus* during cold storage. In our previous research, hypobaric storage, combination of hypobaric and atmosphere cold storage, and three-stage hypobaric storage were found to be all beneficial to green asparagus storage (Li & Zhang, 2005; Li & Zhang, 2006; Li, Zhang, & Yu, 2006). The three-stage hypobaric storage technology of green asparagus was proposed to solve the contradiction between vacuum pressure and weight loss rate during the hypobaric storage. The effect of keeping green asparagus fresh was quantitatively evaluated from the view of mathematics which makes use of fuzzy mathematics theory. The result showed that the effect of three-stage hypobaric storage was obviously better than the normal hypobaric storage (Li & Zhang, 2007).

The objective of this paper was to investigate the relationship between activity of defense enzyme, free radical content and membrane lipid peroxidation in green asparagus under hypobaric storage thus to explore the senescence mechanism of postharvest asparagus.

## 2. Materials and methods

### 2.1. Raw materials

The green asparagus variety UC800 (Nantong Hua-Lin Agriculture Company, China) was 220 ± 20 mm in length and 10 ± 1 mm in diameter, with an average weight of roughly 500 g per bundle. Raw asparagus were harvested in the morning and sent to the laboratory immediately.

### 2.2. Treatment methods

Each five bundles of asparagus spears were put in a plastic basket (450 mm × 350 × 250 mm) with cover and every plastic basket was a treatment. After weighed, the raw asparagus samples were treated in parallel with the atmosphere cold storage and three-stage hypobaric storage. The atmosphere cold storage was used as a control, in which the samples were placed directly into a refrigerator (Model ZB-1.5M<sup>3</sup>, Qi-Hong Refrigerators Company Ltd., Wuxi, China). The storage temperature and RH were controlled at 3 ± 1 °C and 85–95%, respectively. The three-stage hypobaric storage was used as a real treatment, in which the optimal treatment conditions

determined by (Li & Zhang, 2005) were followed. The samples were placed into a Model JZ2 hypobaric chamber (Fig. 1) (Qi-Hong Refrigerators Company Ltd., Wuxi, China) at the cold storage temperature of 3 ± 1 °C. The different vacuum pressures and RH were adopted in different phases: 15 ± 5 kPa and RH 80–85% in the initial 3 days; 25 ± 5 kPa and RH 85–90% in the following 7 days, and 45 ± 10 kPa and RH 90–95% till the end of the experiment. Samples were taken about 100 g randomized compete for measurement every 5 days. The experiment was carried out until 50% asparagus were unable to eat, and three trials were conducted with similar storage conditions.

### 2.3. Measurement methods

#### 2.3.1. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content

Ten asparagus spear were diced and 2 g samples were ground in 10 ml acetone with a mortar and pestle at 4 °C. The homogenate was centrifuged at 10000 × g for 15 min and the supernatant used to determine hydrogen peroxide contents. The assay based on the oxidation of ferrous (Fe<sup>2+</sup>) to ferric ions (Fe<sup>3+</sup>) by hydrogen peroxide under acidic conditions. The ferric ions bind with the indicator dye xylenol orange to form a stable coloured complex, which can be measured at 560 nm (Lin, Li, & Lin 1988). Hydrogen peroxide content was expressed as μmol g<sup>-1</sup> fresh weight.

#### 2.3.2. Superoxide anion (O<sub>2</sub><sup>-</sup>) content

Asparagus samples were homogenized at 4 °C in extraction buffer [50 mM phosphate buffer, pH 7.8 containing 0.1 mM EDTA, 0.3% (w/v) TritonX-100 and 4% (W/V) Polyvinylpyrrolidone (PVPP)]. The homogenate was centrifuged at 10000 × g for 15 min at 4 °C and the supernatant used to determine superoxide anion content by hydroxyl amide oxidation method (Zhu, 1990). Superoxide anion content was expressed as nmol min<sup>-1</sup> g<sup>-1</sup> of protein fresh weight and soluble protein was determined by the method of Bradford (Bradford, 1976), using bovine serum albumin (BSA) as a standard.

#### 2.3.3. Superoxide dismutase (SOD) activity

SOD was extracted by the method of Zhu (Zhu, 1990). Samples were homogenized at 4 °C in extraction buffer [50 mM phosphate buffer, pH 7.8 containing 0.1 mM EDTA, 0.3% (w/v) TritonX-100 and 4% (W/V) Polyvinylpyrrolidone (PVPP)]. The homogenate was centrifuged at 10000 × g for 15 min at 4 °C and the supernatant used as the crude enzyme extract.

SOD activity was assayed by measuring its ability to inhibit the nitroblue tetrazolium (NBT) photochemical reduction using the method of Ginnopolitis and Ries (1977) with some modifications. The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA, and 50 μl enzyme extract. Then placed 30 cm below a light bank consisting of two 15 W fluorescent lamps for 20 min. The absorbance by the reaction mixture was read at 560 nm. One unit of SOD was considered to be the amount of enzyme that inhibited NBT reduction by 50%.

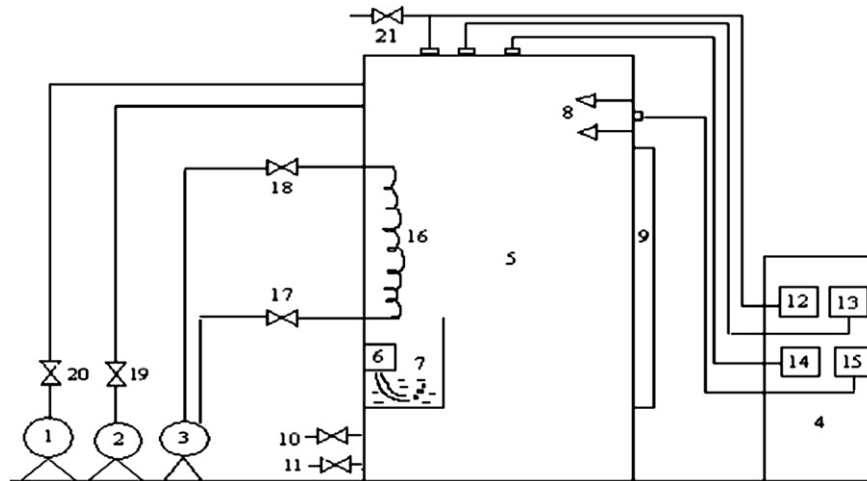


Fig. 1. Schematic diagram of Model JZ2 hypobaric chamber. Note: 1. Vacuum pump, 2. Air pump, 3. Air compressor, 4. Automatic control panel, 5. Storage chamber, 6. Ozone disinfection apparatus, 7. Cold water trough, 8. Spray nozzle, 9. Airtight door, 10. Vent valve, 11. Drain valve, 12. Vacuum pressure indicator and controller, 13. Ozone disinfection controller, 14. Temperature indicator and controller, 15. Relative humidity indicator and controller, 16. Evaporator, 17. Control valve, 18. Stop valve, 19. Moistener pressure control valve, 20. Vacuum control valve, 21. Bleeding valve.

#### 2.3.4. Catalase (CAT) activity

CAT was extracted in buffer [0.2 M phosphate buffer, pH 7.8 containing 1% (W/V) PVPP]. The homogenate was centrifuged at  $10000 \times g$  for 15 min at  $4^\circ\text{C}$  and the supernatant used as to determine CAT activity by measuring the rate of disappearance of hydrogen peroxide using the method of Aebi (1984) with some modifications. The reaction mixture for CAT contained 1.5 ml of 0.2 M phosphate buffer (pH 7.8), 1 ml of distilled water, 0.3 ml of 0.1 M hydrogen peroxide, and 0.2 ml of enzyme extract. The decrease in hydrogen peroxide was followed as a decline in absorbance at 240 nm. One unit of CAT activity was defined as the amount of enzyme, which caused the absorbance decrease of 0.1 at  $240 \text{ nm min}^{-1}$  at  $25^\circ\text{C}$ .

#### 2.3.5. Peroxidase (POD) activity

POD was extracted in 0.05 M phosphate buffer (pH 7.0). The homogenate was centrifuged at  $10000 \times g$  for 15 min at  $4^\circ\text{C}$  and the supernatant used to determine POD activity. POD activity was measured spectrophotometrically using the reaction mixture of equal volumes of guaiacol (0.05 M) and  $\text{H}_2\text{O}_2$  (0.05 M) solutions as substrate based on the method of Sheu and Chen (1991). The substrate solution (2.9 ml) was transferred into a cuvette and the reaction was started by adding 0.1 ml of crude enzyme extract. One unit of POD activity was defined as an increase in absorbance of 0.001 at  $420 \text{ nm min}^{-1} \text{ g}^{-1}$  of fresh weight under assay conditions.

#### 2.3.6. Malondialdehyde (MDA) content

Samples were homogenated in 10% trichloroacetic acid (TCA). The homogenate was centrifuged at  $10000 \times g$  for 15 min and the supernatant used as the extract of samples to determine MDA content. The assay of MDA content was measured according to Dhindsa, Plumb-Dhindsa, and Thorpe (1981) with modifications. The reaction mixture for MDA contained 2 ml of 0.6% thiobarbituric acid (TBA) in 10%

trichloroacetic acid (TCA) was mixed with 2.0 ml extract of sample in a test tube. The mixture solution was heated in a boiling water bath for 15 min, quickly cooled and then centrifuged at  $10000 \times g$  for 15 min. The supernatant was used to measure the absorbance at 532 and 600 nm. The concentration of lipid peroxides together with oxidatively modified proteins of plants were thus quantified in terms of MDA level using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  and expressed as  $\text{nmol g}^{-1}$  fresh weight.

#### 2.3.7. Membrane permeability

Membrane permeability, expressed by relative leakage rate, was determined by conductivity ( $L_t/L_0$ ) (Vieira Santos, Campos, Azevedo, & Caldeira, 2001). The fresh asparagus spear were sliced into small discs (10-mm diameter, 2-mm thick, 2 g total) and washed three times with deionized water. After drying with filter paper, the discs were placed in flasks with 20 ml deionized water and shaken for 30 min at  $25^\circ\text{C}$ . Electrolyte conductivity ( $L_t$ ) of the solution was determined with a conductivity meter (Model DDS-IIA, Shanghai Scientific Instruments). The flasks with solution were then heated at  $100^\circ\text{C}$  for 15 min, quickly cooled and the electrical conductivity of the solution measured again ( $L_0$ ).

#### 2.3.8. Surface color

The surface color of fresh asparagus for raw material and thermally treated samples was measured using a colorimeter (WSC-S, Shanghai Optical Instrument Company, Shanghai, China) with white background. The instrument was a tristimulus colorimeter which measures four specific wavelengths in the visible range, specified by the Commission Internationale del' Esclairage (CIE). Tristimulus data give a three-dimensional value for color in which equal distances approximate equally perceived color differences. The color of the sample can be described as  $x, y, z$  or  $L^*, a^*, b^*$ . The  $L^*, a^*,$  and  $b^*$  values are the three dimensions of the measured color which

give specific color value of the material. The  $L^*$  value represents the light–dark spectrum with a range of 0 (black) to 100 (white), the  $a^*$  value represents the green–red spectrum with a range of  $-60$  (green) to  $+60$  (red) while the  $b^*$  value represents the blue–yellow spectrum with a range of  $-60$  (blue) to  $+60$  (yellow). During the experiment, the external surface color of the three randomly selected caudexs of fresh asparagus was measured for every sample.

### 2.3.9. Statistical analysis

The experimental design was a completely randomized one with three replications. Statistical evaluation of the differences 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  $P < 0.05$ .

## 3. Results and discussion

### 3.1. SOD and CAT activities

The changing trend of SOD and CAT activities under both hypobaric and atmosphere cold storage conditions were basically the same. That was, SOD and CAT activities of asparagus increased in the beginning and then declined with the extension of storage time under both conditions (Figs. 2 and 3). Under hypobaric storage condition, these two indices reached the highest value on 20th day and then began to decrease slowly; but under atmosphere cold storage, these two indices reached the highest value on 10th day and then began to decrease rapidly. On 20th day, SOD activity in the former case was 176% of that in the latter case; and CAT activity in the former case was 194% of that in the latter case. Under atmosphere cold storage condition, the highest values of SOD and CAT were only 82 and 86% of that under hypobaric storage, respectively.

### 3.2. POD activity

The changing trend of POD activity under different storage conditions was the same, which increased slightly in first 5

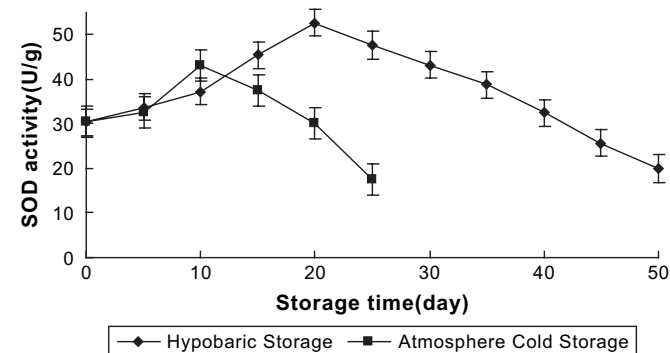


Fig. 2. The change of SOD activity during hypobaric and atmosphere storage (each point represents the mean of three replications. Vertical bars represent the standard deviation.)

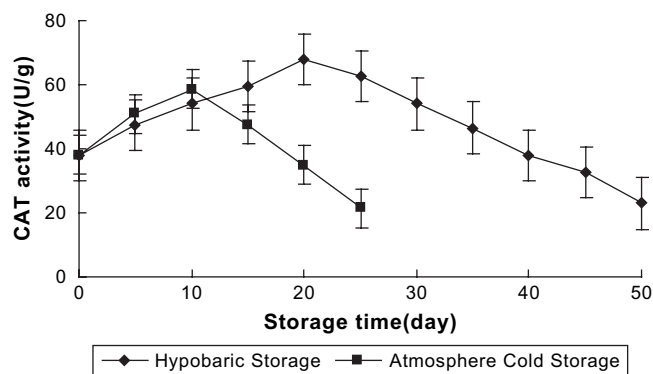


Fig. 3. The change of CAT activity during hypobaric and atmosphere storage. (each point represents the mean of three replications. Vertical bars represent the standard deviation.)

days of storage and then declined with the extension of storage time under both conditions (Fig. 4). In first 20 days storage, POD activity was a bit higher under atmosphere cold storage than under hypobaric storage, and then POD activity was lower under atmosphere cold storage. That was, the decrease rate of POD activity under hypobaric storage was slower than that under atmosphere cold storage during the latter stage of storage. This indicated that hypobaric storage could inhibit POD activity of asparagus in the beginning stage of storage.

POD is an important correlative enzyme with lignin synthesis of asparagus, which can accelerate the interlinking of lignin precursor during process when  $H_2O_2$  was decomposed (Catalina, Ahmed, & Milagros, 2001; Christensen, Bauw, & Welinder, 1998). This showed that hypobaric storage could control the progress of lignification of asparagus in the same stage.

### 3.3. $O_2^{\cdot-}$ and $H_2O_2$ contents

The changing trend of produce rate of  $O_2^{\cdot-}$  and  $H_2O_2$  contents under both hypobaric and atmosphere cold storage conditions were basically the same. It was “firstly rise, then decline, and then rise again” (Figs. 5 and 6). In first 10 days, produce rate of  $O_2^{\cdot-}$  was a bit higher under hypobaric storage than under atmosphere cold storage, and then produce

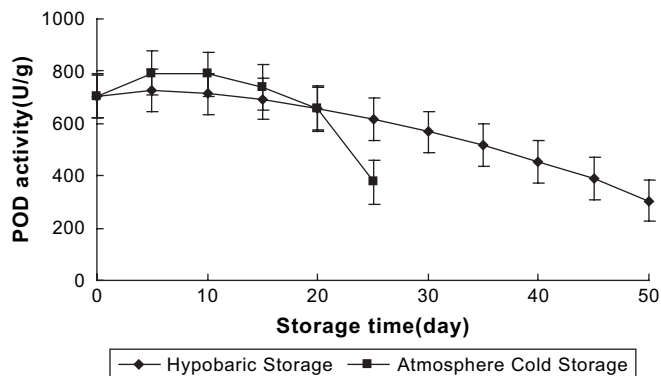


Fig. 4. The change of POD activity during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation.)



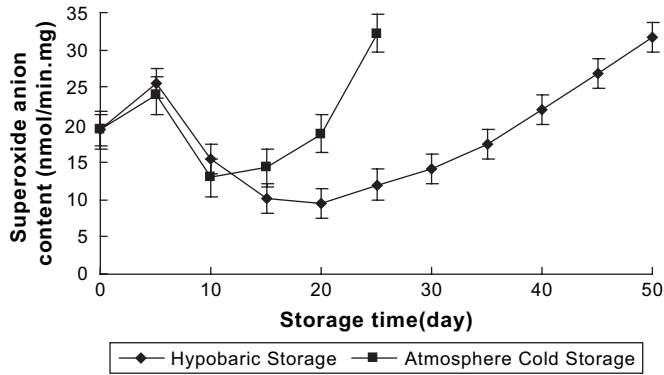


Fig. 5. The change of  $O_2^{\cdot -}$  content during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation).

rate of  $O_2^{\cdot -}$  was higher under atmosphere cold storage; but  $H_2O_2$  content was higher under atmosphere cold storage than under hypobaric storage during all the storage time. Compared with atmosphere cold storage condition, produce rate of  $O_2^{\cdot -}$  and  $H_2O_2$  contents under hypobaric storage condition were not significantly different in first 10 days. Under atmosphere cold storage, produce rate of  $O_2^{\cdot -}$  and  $H_2O_2$  contents of asparagus decreased to the lowest value on 10th day (it was in accordance with the highest value of SOD and CAT activities under that condition, Figs. 2 and 3), and then it started to increase rapidly. The produce rate of  $O_2^{\cdot -}$  of asparagus on 25th day was 1.67 times as much as that in the initiation, and it was 2.69 times as much as that under hypobaric storage condition; while  $H_2O_2$  content of asparagus was 3.41 times as much as that in the initiation, and it was 4.70 times as much as that under hypobaric storage condition. Under hypobaric storage, produce rate of  $O_2^{\cdot -}$  and  $H_2O_2$  contents of asparagus firstly decreased to the lowest value on 20th day (it was in accordance with the highest value of SOD and CAT activities under that condition, Figs. 2 and 3), then it started to increase slowly, and finally it began to rise rapidly again from 35th day. The increase rate of produce rate of  $O_2^{\cdot -}$  and  $H_2O_2$  contents

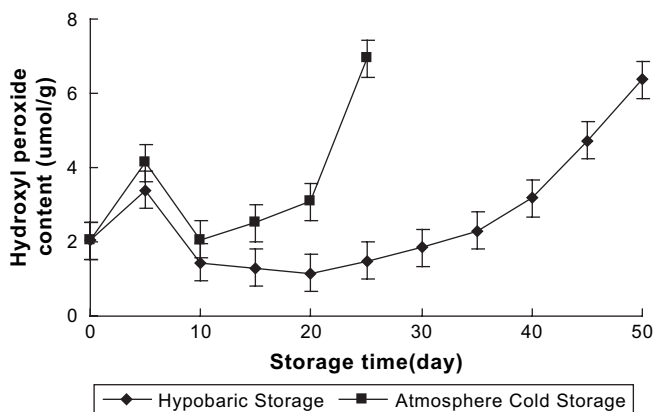


Fig. 6. The change of  $H_2O_2$  content during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation).

under hypobaric storage was significantly different from that under atmosphere cold storage condition ( $P < 0.05$ ).

With the extension of storage time under both storage conditions, produce rate of  $O_2^{\cdot -}$  and  $H_2O_2$  contents increased, but SOD, CAT and POD activities decreased. This showed that higher SOD, CAT and POD activities could keep produce rate of  $O_2^{\cdot -}$  and  $H_2O_2$  contents in a lower level. Furthermore, SOD, CAT and POD could eliminate  $O_2^{\cdot -}$  and  $H_2O_2$ .

### 3.4. MDA content

MDA is a product of membrane lipid peroxidation. It can reflect the injury degree of cell membrane system. The content of MDA increased with the extension of storage time under both hypobaric and atmosphere cold storage conditions (Fig. 7). MDA content of asparagus under hypobaric condition was not significantly different from that under atmosphere cold storage during first 5 days ( $P > 0.05$ ). Under the atmosphere cold storage, MDA content started to increase slowly after 5th day and then began to rise rapidly after 10th day. On 25th day, MDA content was 3.75 times as much as that in the initiation and it was 1.72 times as much as that under hypobaric storage condition. Under the hypobaric storage condition, MDA content increased slowly. The increase rate of MDA content under hypobaric storage was significantly different from that under atmosphere cold storage ( $P < 0.05$ ). As a result, hypobaric storage reduced the accumulation of MDA. This indicated that hypobaric storage could inhibit membrane lipid peroxidation of asparagus.

### 3.5. Membrane permeability

Membrane permeability was expressed by relative leakage rate. The relative leakage rate increased with the extension of storage time under both hypobaric and atmosphere cold storage conditions (Fig. 8). But the index under hypobaric condition was not significantly different from the index under atmosphere cold storage in first 10 days. Under atmosphere cold storage, relative leakage rate started to increase rapidly after 15th day. On 25th day, relative leakage rate was 3.66 times as high as that in the beginning and it was 1.32 times as high as that under

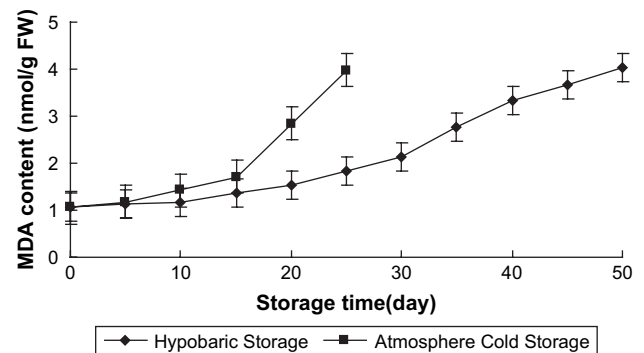


Fig. 7. The change of MDA content during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation).

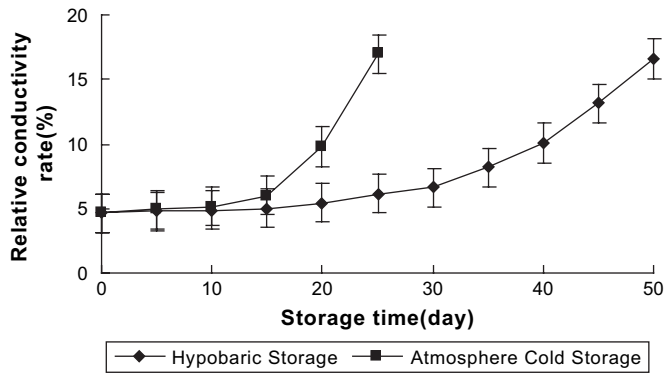


Fig. 8. The change of relative conductivity rate during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation).

hypobaric storage. The increase rate of relative leakage rate was significantly ( $P < 0.05$ ) lower under hypobaric storage than under atmosphere cold storage. Therefore, hypobaric storage could inhibit the increase of relative leakage rate. This showed that hypobaric storage could protect the integrity of membrane system and delay the senescence of asparagus.

### 3.6. $L^*$ , $a^*$ and $b^*$ values

The changing trend of  $L^*$ ,  $a^*$  and  $b^*$  values under both hypobaric and atmosphere cold storage conditions were basically the same. They all increased with the extension of storage time under both storage conditions (Figs. 9–11). Under the atmosphere cold storage,  $L^*$ ,  $a^*$  and  $b^*$  values increased slowly during first 20 days, and then  $L^*$ ,  $a^*$  and  $b^*$  values began to rise rapidly after 20th day. Under the hypobaric storage condition,  $L^*$ ,  $a^*$  and  $b^*$  values started to rise rapidly from 25th day. The increase rate of  $L^*$ ,  $a^*$  and  $b^*$  values under hypobaric storage was significantly different from that under atmosphere cold storage ( $P < 0.05$ ). Therefore, hypobaric storage could inhibit the increase of  $L^*$ ,  $a^*$  and  $b^*$  values.

The change of  $L^*$ ,  $a^*$  and  $b^*$  values showed that the color of asparagus became light, and the green color of asparagus decreased or the yellow color of asparagus increased with the extension of storage time under both hypobaric and

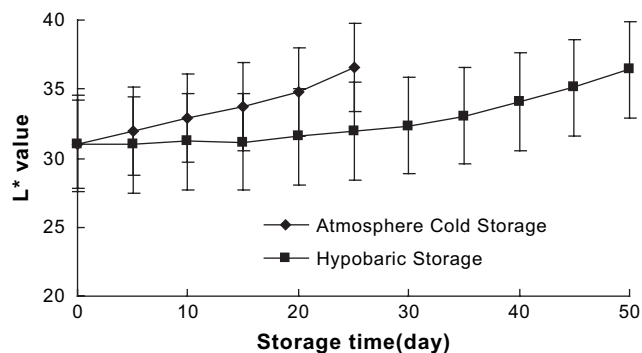


Fig. 9. The change of  $L^*$  value during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation).

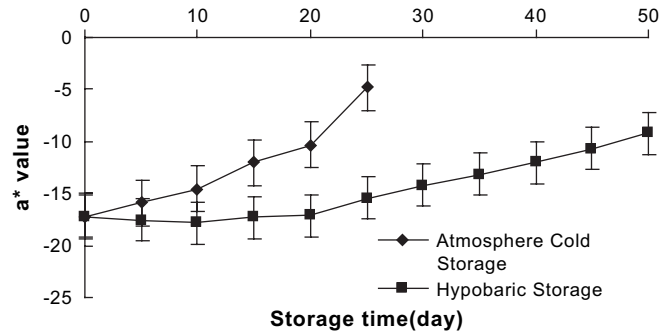


Fig. 10. The change of  $a^*$  value during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation).

atmosphere cold storage conditions. This changing trend of asparagus was closely related to the decrease of SOD, CAT and POD activities, the increase of MAD,  $O_2^{\cdot-}$  and  $H_2O_2$  contents and relative conductivity rate. It proved that the decrease of SOD, CAT and POD activities, lead to the accumulation of  $O_2^{\cdot-}$  and  $H_2O_2$  and the increase of lipid peroxidation product MDA. This caused the injury of membrane permeability and the damage of membrane integrity, the final outcome was chlorosis and senescence of asparagus. But hypobaric storage could delay the decrease of green color or the increase of yellow color of asparagus. That was, hypobaric storage could inhibit the chlorosis and senescence of asparagus.

## 4. Conclusions

- (1) With the extension of storage time under both hypobaric and atmosphere cold storage conditions, SOD, POD and CAT activities decreased,  $O_2^{\cdot-}$  and  $H_2O_2$  contents and  $L^*$ ,  $a^*$  and  $b^*$  values increased. Moreover, higher SOD, POD and CAT activities can keep  $O_2^{\cdot-}$  and  $H_2O_2$  contents in lower levels.
- (2) The three-stage hypobaric storage process developed in this research could significantly increase the activities of superoxide dismutase (SOD) and catalase (CAT), delay the decrease of SOD and CAT activities, lower the accumulation rate of malondialdehyde (MDA), superoxide anion ( $O_2^{\cdot-}$ )

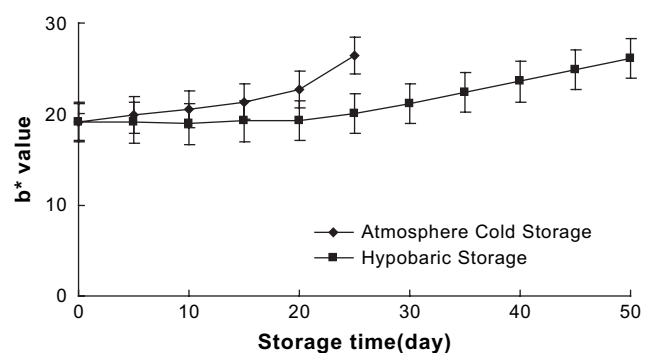


Fig. 11. The change of  $b^*$  value during hypobaric and atmosphere storage. (Each point represents the mean of three replications. Vertical bars represent the standard deviation).

and hydrogen peroxide ( $H_2O_2$ ), inhibit the increase of relative conductivity, delay the degeneration of surface color, and control the increase of peroxidase (POD).

- (3) The decrease of SOD, CAT and POD activities could lead to the accumulation of  $O_2^{\cdot-}$  and  $H_2O_2$  and the increase of lipid peroxidation product MDA and relative leakage rate. This indicated that membrane lipid peroxidation was enhanced and that it could change membrane permeability and damage membrane integrity. As a result, the injury of membrane system could accelerate chlorosis and senescence of asparagus. But hypobaric storage could inhibit the increase of  $L^*$ ,  $a^*$  and  $b^*$  values, delay the decrease of green color or the increase of yellow color of asparagus. Therefore, hypobaric storage could inhibit chlorosis and senescence of asparagus. By this technique the storage life of 50 days was achieved as compared with the storage life of only 25 days in atmosphere cold storage.

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