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Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases

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Abstract

Noble gases that form clathrate hydrates when dissolved into water and restrict water molecule activity can prolong the shelf life of fruits and vegetables. Physiological and physical aspects of asparagus spears treated with mixtures of compressed (1.1 MPa absolute) argon (Ar) and xenon (Xe) (2:9 in partial pressure) were compared with controls under normal preservation in modified atmosphere packaging (5% O_2 and 5% CO_2) and cold storage (4 °C). The bract opening and respiration rates of asparagus spears were reduced slightly after the Ar–Xe treatment. A clear reduction between the compressed air and compressed mixture of Ar and Xe was only observed on the 6th and 15th days of storage for the bract opening rate and the 15th day of storage for the respiration rate. The climacteric peak was comparatively inconspicuous. The increase of crude fiber was reduced and both vitamin C and chlorophyll preserved. Based on comprehensive evaluation, asparagus spears treated for 24 h with mixtures of Ar and Xe could be kept in good quality for 12 days at 4 °C.

Keywords: Noble gas mixture; Modified atmosphere packaging; Asparagus spear; Storage; Shelf-life

1. Introduction

Green asparagus (*Asparagus officinalis* L.) is one of the important fresh vegetables in China in recent years because of its special flavor and nutritional value. However, it is a highly perishable vegetable, deteriorating rapidly after harvest. Asparagus spears typically have a shelf life of 3–5 days under normal postharvest handling at ambient temperatures (Baxter & Waters, 1991; Lipton, 1990). Physiological and compositional changes that reduce asparagus spear quality during storage include bract opening (‘feathering’), toughening, loss of water, chlorophyll degradation, and changes in ascorbic acid, carbohydrates, protein and amino acids (Chang, 1987; Siomos, Sfakiotakis, & Dogras, 2000). Undesirable changes can be reduced by rapid cooling after harvest, keeping at low temperatures (0–5 °C) and use of modified atmosphere storage (Lipton, 1990). However, Lipton (1990) and Siomos et al. (2000) reported that controlled atmospheres did not retard deterioration of asparagus during cold storage.

Nonpolar gases can form clathrate hydrates at certain temperatures and pressures (Wang, 2003). Noble gases such as argon (Ar), krypton (Kr) and xenon (Xe) can form clathrate hydrates in some vegetables and fruits more readily under higher pressure than the critical pressure point (Oshita, Seo, & Kawagoe, 2000; Powrie, Chiu, & Wu, 1990; Purwanto, Oshita, & Seo, 2001; Zhan & Zhang, 2005). In clathrate hydrates, water molecules form variously sized clathrates due to hydrogen bond. Gas molecules are caged in the clathrates. Researchers have found three kinds of hydrate structures: structure type-I, structure type-II and structure type-H (Belosludov et al., 2002; Ohgaki, Sugahara, & Suzuki, 2000; Purwanto et al., 2001). In our previous study (Zhan, 2005), structure type-I

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($8Xe \cdot 46H_2O$) was observed in cucumber samples under the pressure range of 0.4–1.1 MPa. When noble gases dissolve into water, hydrophobic hydration occurs and enzymatic reactions are inhibited, resulting in restrained vegetable metabolism. Mixtures of Ar and Xe can form clathrate hydrates that restrain the activity of intracellular water. It is therefore desirable to explore the possibility of using clathrate hydrates induced by compressed noble gases to preserve perishable vegetables.

A US patent was issued for the use of Ar in the preservation of cut and segmented fresh fruits (Powrie et al., 1990). Ar gas as a major component of the atmosphere in modified atmosphere packaging (MAP) has also been reported to reduce microbial growth and improve the quality retention of fresh produce like broccoli and lettuce (Day, 1996, 1998; Jamie & Saltveit, 2002). However, there is no information available regarding the effect of Ar and Xe combinations on the preservation of green asparagus spears.

Xe, among three noble gases (Ar, Kr and Xe), had the best effect on forming clathrate hydrates under 1.5 MPa pressure in our previous study on preservation of asparagus and cucumber, but the high cost may limit its application (Zhan, 2005; Zhan & Zhang, 2005). The objectives of this research were to study the effect of mixed Ar and Xe on normal preservation of green asparagus based on respiration rate, weight loss, ascorbic acid, chlorophyll content and bract opening rate during storage to understand the microstructural changes during compressed Ar and Xe treatment of asparagus spears.

2. Materials and methods

2.1. Plant materials and handling

Fresh green asparagus (*A. officinalis* L. cv. 'UC800') spears were obtained from Nantong Hualin Agricultural Products Co., Ltd., Jiangsu Province, China. The spears were cut at ground level between 8:00 and 9:30 am, placed in crushed ice and transported to the laboratory within 3 h on the day of harvest. Straight, undamaged spears with closed bracts, 8–20 mm in diameter and 22 cm in length, were used in the tests. The experimental system to apply the compressed Ar and Xe mix is shown in Fig. 1.

The asparagus spears were divided into four groups: (1) compressed air treatment (CAT) at room temperature (15–20 °C) at 1.1 MPa for 24 h; (2) cold storage at 4 °C and 90% RH for 18 days (RCS); (3) MAP treatment at the initial gas concentration of 5% O_2 and 5% CO_2 at 4 °C for 24 h in 25 μm polyvinyl chloride (PVC) bags with 7.8×10^{-12} and 3.6×10^{-12} mol/s mm^2 kPa CO_2 and O_2 permeability (final gas concentration after 24 h: 4.8% O_2 and 5.2% CO_2), which is achieved by a MAP system (ADFM-V300 Type, Henzhong Machinery Co., Zhangjiaguang, China) and optimal for preservation of the same asparagus variety (An, Zhang, & Lu, 2004); and (4) treatment by mixed Ar and Xe at 2:9 (v:v) in partial pressure, which is suitable for vegetable preservation with reasonable processing cost (Zhan, 2005) at room temperature at 1.1 MPa for 24 h (CNG). Increasing the Ar:Xe ratio could reduce the ratio of formed clathrate hydrates, which might affect the preserving effect, although the processing cost would decrease significantly. Asparagus after treatments of CAT, RCS and CNG were stored in unsealed 25 μm PVC bags at 4 °C and 90% RH for 18 days, with the MAP samples stored under the same conditions in sealed bags.

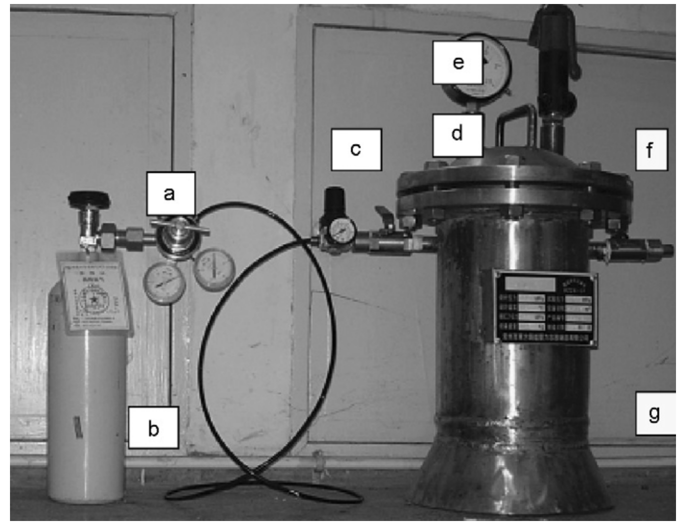

Fig. 1. Schematic diagram of apparatus to apply compressed Ar and Xe mix: (a) regulator, (b) mixed gas cylinder, (c) adjusting valve, (d) safety valve, (e) pressure gauge, (f) ball valve and (g) sample chamber.

2.2. Respiration rate and weight loss

Each group consisting of three replicates of 12 spears (300 ± 50 g) were introduced into 2.0 l glass jars ventilated with humidified air at a flow rate of 30 ml/min. The jars were placed in a refrigerator at 2 °C. CO_2 was analyzed with a gas chromatograph (Shimadzu GC-14, Kyoto, Japan) equipped with a thermal conductivity detector (TCD). The flow rate of the carrier gas (He) was 30 ml/min and the column temperature was isothermal at 55 °C. Calibration was performed using commercially analyzed gas standards (Toivonen & DeEll, 2001). The respiration rate was expressed as mg CO_2/kg h.

Because water transpiration is one of the key factors that affect the series of physiological reactions that occur in vegetables, it is important to restrain the water transpiration rate. Weight loss was calculated according to the weight of each sample before and after storage, and expressed as a percentage of the initial sample weight (Zhang, De Baerdemaeker, & Schrevens, 2003; Zhang, Huan, Tao, & Wang, 2001; Zhang, Xiao, & Salokhe, 2006).

2.3. Ascorbic acid (vitamin C)

Ascorbic acid in asparagus was determined according to the AOAC method (AOAC, 1995). A 5 g sample of fresh asparagus was blended with 50 ml of metaphosphoric-acetic acid solution to extract ascorbic acid. The mixture was centrifuged, transferred to a volumetric flask, and then rapidly titrated with an indophenol solution until a light distinct rose pink color persisted for more than 5 s. Three replicates were conducted for each treatment.

2.4. Chlorophyll and crude fiber content

Chlorophyll was extracted from 5 g of macerated spears by homogenizing it in 20 ml of 80% acetone with a tissue homogenizer (DS-1, Shanghai, China) at a moderate speed (10,000 rpm) for 30 s. The homogenate was filtered through four layers of cheesecloth, centrifuged at 15,000 rpm for 15 min, and its absorbance (Abs) read at 647.0 and 664.5 nm on a UV–vis recording spectrophotometer (UV-754, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). The total chlorophyll content was calculated using the formula developed by Inskeep and Bloom (1985): chlorophyll $(\text{mg/g}) = 17.95 \times \text{Abs}647.0 + 7.9 \times \text{Abs}664.5$. The crude fiber content of the spears was determined every 3 days of storage according to Sosa-Coronel, Vest, and Herner (1976).

2.5. Bract opening rate and cell membrane permeability

During storage, the head of some asparagus spears may fan out. Bract opening rate is defined as the ratio of the number of asparagus spears that fan out to the total number of asparagus spears. It can be used to characterize the aging of asparagus spears.

The samples were cut into $0.8\,\text{cm} \times 0.8\,\text{cm} \times 0.4\,\text{cm}$ pieces, and 10 g samples were weighed and then washed with de-ionized water. After absorption, 50 ml of distilled water was added and the samples were kept at a constant temperature of 30 °C for 10 min. The cut samples were then immersed in boiling water for 15 min and the electrical conductivity determined with a conductivity meter (DDS-11A, Shanghai Huaguang Co., Shanghai, China). Membrane permeability was characterized by the electrical conductivity ratio of the pre-heated to post-heated samples.

2.6. Microstructure changes and statistical analysis

The study of asparagus spear microstructure was conducted to understand the changes during compressed Ar and Xe treatment. Microscopy techniques provide valuable detailed structural information about texture and other quality properties (Baxter & Waters, 1991). Light microscopy was performed on an XSP-8C light microscope (Optical Instrument Company, Ltd., Shanghai, China) at the beginning of the treatment, 12 h after treatment, 24 h after treatment and 2 min after pressure removal. The sections between 145 and 150 mm away from the top of each stalk were segmented off, fixed in formol-aceto-alcohol (FAA), dehydrated in a graded ethanol series solution, embedded in paraffin, sliced to a thickness of $18 \pm 2\,\mu\text{m}$, stained with Hematoxylin and sealed with Canada Balsam (Li, 1987).

The experiment was designed randomly with three replicates. Data analysis was carried out by an analysis of variance (ANOVA), with mean separation by paired t-tests and significant differences established at $P < 0.05$.

3. Results and discussion

3.1. Weight loss of asparagus

When noble gas formed gas hydrates in the experiment, water molecules were confined by hydrogen bonds, which decreased the loss of water. The weight loss of asparagus during storage is shown in Fig. 2. The weight loss of the MAP treatment was the lowest among the four treatments, practically null during storage apparently because of high relative humidity in the bag. The weight loss of the CNG, CAT and RCS treatments increased steadily during storage to a maximum of 10% after 18 days, without differences among the three treatments. For the first 12 days, RCS had the highest weight loss, but the clear difference between CAT and CNG was observed only after the 12th day of storage. Since weight loss lower than 6% in asparagus spear storage can be considered acceptable (An et al., 2004), besides MAP treatment, CNG treatment also could reach the end of tolerance at day 12.

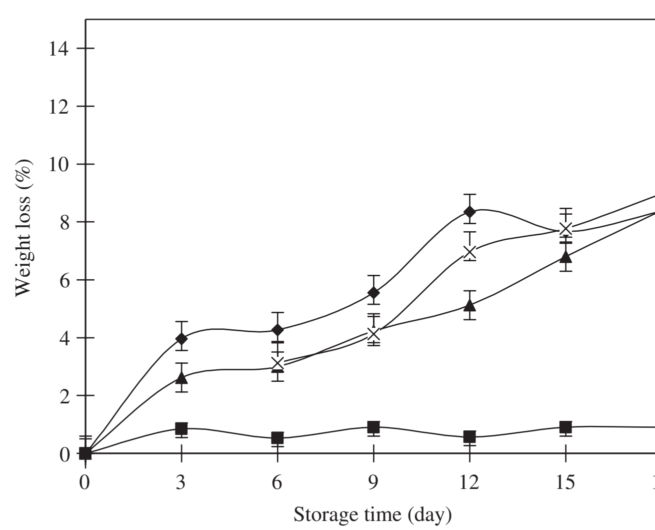

Fig. 2. Weight loss of asparagus spears during storage at 4 °C and 90% RH after the four treatments: (×) CAT; (◆) RCS; (■) MAP; (▲) compressed Ar and Xe mix at 2:9 partial pressure [CNG]. The mean and standard deviation values were obtained by three replicates.

3.2. Bract opening and respiration rates of asparagus

The bract opening of asparagus spears is an indicator of aging. Fig. 3 shows that CNG, CAT and MAP treatments reduced the tract opening rate of asparagus spears after the 6th day of storage compared to the RCS treatment. Moreover, CNG could lead to the same effect as MAP treatment at the end of storage. According to the commercial standards in Southern China, a bract opening rate below 50% is acceptable (An et al., 2004). Therefore, CNG, CAT and MAP treatments at day 15 could be considered at the end of tolerance compared to the control RCS treatment reaching the end of tolerance after 9 days of storage. This result agreed with the single Xe treatment effect in broccoli (Oshita et al., 2000), but differed from preservation using He, Ar and Ne in mushroom (Ozdemir, Varoquaux, Tournemelle, & Yildiz, 2004) and broccoli or lettuce (Jamie & Saltveit, 2002). A possible reason is that the pressure used in our study (1.1 MPa) was similar to the former (0.4–1.2 MPa) and different from the latter (0.099 MPa or atmosphere pressure), implying that critical pressure may be needed when forming clathrate hydrates in some vegetables and fruits (Zhan, 2005).

Respiration of vegetables continues during postharvest handling and storage, and must be restrained to preserve freshness. Fig. 4 shows the effects of the four treatments on the respiration of asparagus (An et al., 2004). The climacteric respiration peak of the control RCS treatment appeared on the 3rd day, the MAP treatment showed a smaller respiration peak at day 12, while the CAT treatment reached a diminished climacteric peak after 15 days of storage. The CNG treatment did not show a climacteric peak during the 18 days of storage in this study,

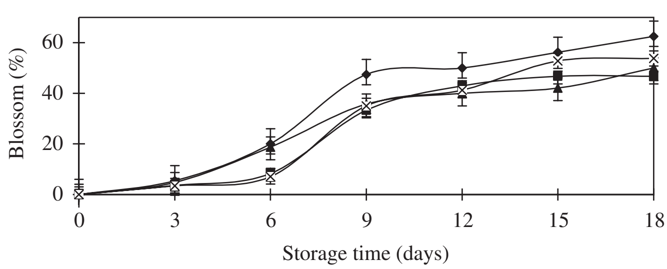

Fig. 3. Bract opening rate of asparagus spears during storage at 4 °C and 90% RH after the four treatments: (×) CAT; (♦) RCS; (■) MAP; (▲) compressed Ar and Xe mix at 2:9 partial pressure [CNG]. The mean and standard deviation values were obtained by three replicates.

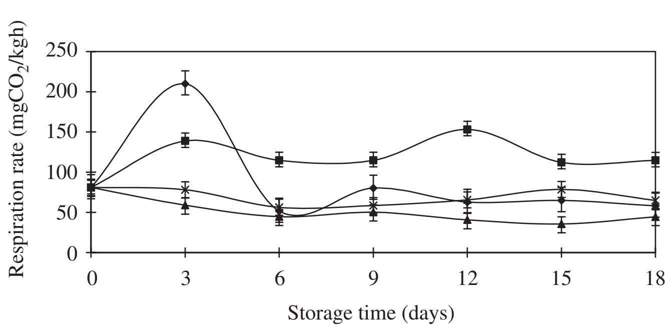

Fig. 4. Respiration rate of asparagus spears during storage at 4 °C and 90% RH after the four treatments: (×) CAT; (♦) RCS; (■) MAP; (▲) compressed Ar and Xe mix at 2:9 partial pressure [CNG]. The mean and standard deviation values were obtained by three replicates.

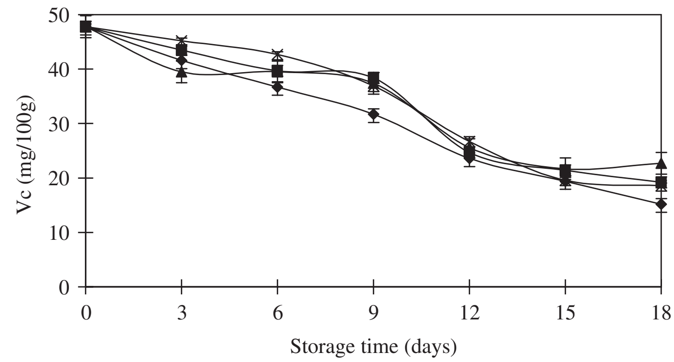

Fig. 5. Vitamin C changes of asparagus spears during storage at 4 °C and 90% RH after the four treatments: (×) CAT; (♦) RCS; (■) MAP; (▲) compressed Ar and Xe mix at 2:9 partial pressure [CNG]. The mean and standard deviation values were obtained by three replicates.

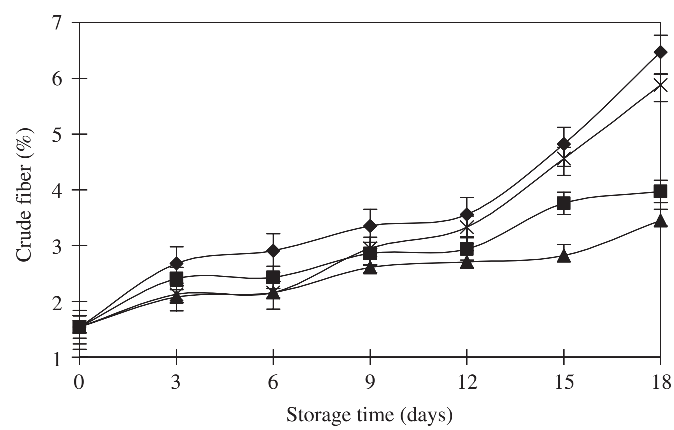

Fig. 6. Crude fiber changes of asparagus spears during storage at 4 °C and 90% RH after the four treatments: (×) CAT; (♦) RCS; (■) MAP; (▲) compressed Ar and Xe mix at 2:9 partial pressure [CNG]. The mean and standard deviation values were obtained by three replicates.

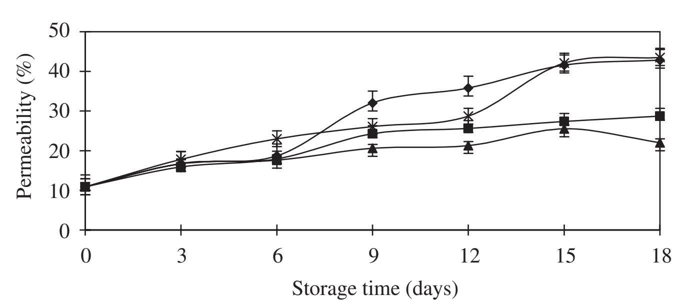

Fig. 7. Cell membrane permeability of asparagus spears during storage at 4 °C and 90% RH after the four treatments: (×) CAT; (♦) RCS; (■) MAP; (▲) compressed Ar and Xe mix at 2:9 partial pressure [CNG]. The mean and standard deviation values were obtained by three replicates.

suggesting that CNG can lead to a very low respiration rate during the storage of asparagus spears.

3.3. Vitamin C and crude asparagus fiber

Vitamin C content decreased with the storage time for all treatments (Fig. 5). The CNG treatment did not reduce the loss of vitamin C significantly compared with the other three treatments.

Fig. 6 shows the effects of the four treatments on the crude fiber content of asparagus, which increased during storage. The fiber content of the CNG treatment was consistently lower than the rest of the treatments. The MAP treatment limited physiological reactions due to the high concentration of carbon dioxide (An et al., 2004), while the increase in crude fiber was higher for the control RCS treatment during cold storage.

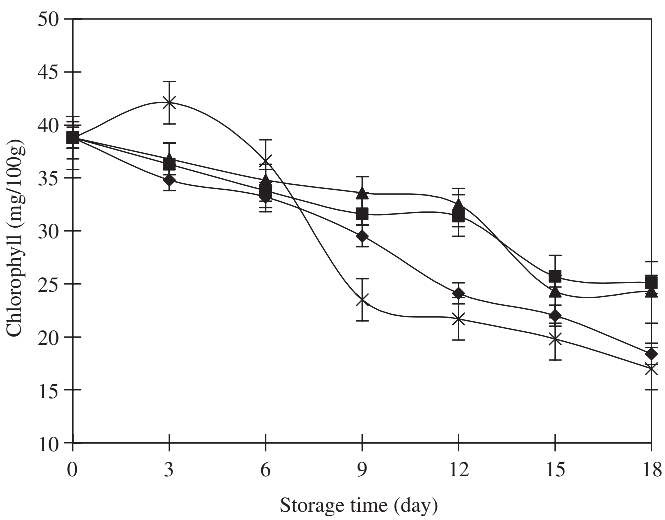

Fig. 8. Total chlorophyll of asparagus spears during storage at 4 °C and 90% RH after the four treatments: (×) CAT; (♦) RCS; (■) MAP; (▲) compressed Ar and Xe mix at 2:9 partial pressure [CNG]. The mean and standard deviation values were obtained by three replicates.

3.4. Cell membrane permeability and asparagus chlorophyll

During vegetable storage, cell membrane permeability increases, and mineral ions spread fast. Thus, the quality of vegetables can be characterized by measuring cell membrane permeability. As indicated in Fig. 7, the control asparagus tissues under RCS treatment reached a significantly higher permeability than the other treatments after 9 days, while the CNG and MAP treatments increased permeability very slowly compared with normal cold storage.

Since asparagus spear is a green vegetable, color is one of the major sensory indices. Fig. 8 shows the effects of four treatments on chlorophyll during storage, which decomposed, causing the color to turn yellow and the product to reduce in value. During the first 6 days of storage, no significant differences were observed, but after 9 days, the MAP and CNG treatments preserved chlorophyll better than the control RCS treatment and CAT asparagus spears.

3.5. Microstructure changes

Fig. 9 shows asparagus microstructure under pre- and post-CNG treatment of compressed (1.1 MPa) Ar and Xe (2:9, v:v, in partial pressure). The structure remained

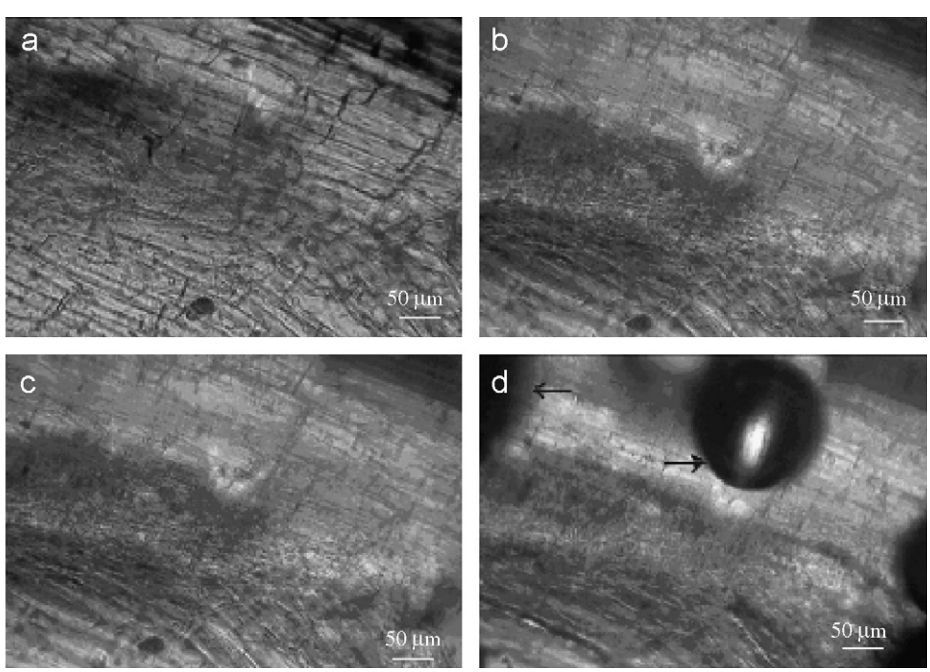

Fig. 9. Micrographs of asparagus tissue samples at different periods of treatment under compressed Ar and Xe mix: (A) beginning of the CNG treatment, (B) 12 h after the CNG treatment, (C) 24 h after the CNG treatment, (D) 2 min after the pressure removal.

unchanged after treatment processes for 24 h, but as the pressure was removed, many micropores appeared. This indicated that the Ar and Xe remained in the structure as micropores after the treatment process, suggesting a positive connection with keeping asparagus spears fresh.

4. Conclusion

Treating asparagus spears with a 1.1 MPa mixture of Ar and Xe (2:9, v:v) for 24 h at 4 °C extended their shelf-life. In the storage of asparagus spear, CNG was superior to cold storage with respect to all the indices. Compared to the MAP treatment with optimal initial gas composition (5% O_2 and 5% CO_2), the CNG treatment restrained the respiration peak, crude fiber formation and cell membrane permeability. The microstructure indicated that Ar and Xe remained in the product as micropores. Further research is needed to cover the economic aspects for practical applications.

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