Postharvest Biology and Technology 147 (2019) 59–67

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Effect of pressurized argon combined with controlled atmosphere on the postharvest quality and browning of sweet cherries

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ARTICLE INFO

Keywords:
Sweet cherry
Pressurized argon
Controlled atmosphere
Browning
Quality

ABSTRACT

Sweet cherries are in demand in domestic and international markets due to their medical and health benefits. However, storage and transportation of these cherries are difficult due to their thin skin. In this study, we investigated the effects of pressurized argon (0.5 MPa for 1 h at 0 °C), storage in a controlled atmosphere (5% O_2 + 10% CO_2 + 85% argon), and their combination on the postharvest quality and browning of sweet cherries during 63 d of storage at 0 °C. Results showed that treatment with pressurized argon, controlled atmosphere, and their combination effectively reduced the fruit decay rate (13.33 (58.54%), 15.56 (68.29%), and 18.89 (82.93%), respectively) compared with the control fruit after 63 d of storage. The treatments also effectively maintained a high good fruit rate (28.89 (72.22%), 36.67 (91.67%), and 45.56 (113.89%), respectively) compared with the control fruit after 63 d of storage and delayed the decline of lightness, saturation, and hue angle. They also inhibited reduction in firmness, levels of total soluble solid, titratable acidity, and ascorbic acid content, thus maintaining better fruit quality. The accumulation of membrane lipid peroxide malondialdehyde and increase in relative permeability was significantly decreased. The decrease in fruit phenolic compound content and increase in polyphenoloxidase and phenylalanine ammonia lyase enzymatic activity were inhibited. Ultimately, the development of fruit browning was reduced, and the browning index was maintained (11.94, 6.94, and 3.10, respectively) at a low level after 63 d of storage. Combined treatment with pressurized argon and a controlled atmosphere yielded the best results and may be considered one of the ideal methods for preserving sweet cherries.

1. Introduction

Sweet cherries have a vivid color, rich flavor, and abundant nutrients. In addition, they have great value in medical treatment and health benefits, and are thus in demand in domestic and international markets (Wang et al., 2016). However, sweet cherries are juicy and have thin skin that cannot tolerate storage and transport well. In addition, their harvesting season has high temperatures, due to which they are extremely susceptible to dehydration, browning, and decay, thus losing their market value in 3–5 d at ambient temperature (Serrano et al., 2009; Yildiz et al., 2018). Thus, alleviating the browning of sweet cherries and improving fruit quality are essential for their storage and transport.

Pressurization with inert gas is a new technology for preservation. At certain pressures, inert gases can form a special “clathrate hydrate” structure with the intercellular water in fruit and vegetables (Davidson, 1973; Yoshioki, 2010; Makino et al., 2006a,b). These structures not only restrict the activity of molecules in fruit and vegetables but also reduce their enzymatic activity, thereby inhibiting the physiological and metabolic activity of fruit and vegetables, ultimately inhibiting their browning and ripening and maintaining their quality (Rahman et al., 2002a; Zhan and Zhang, 2005; Purwanto et al., 2001). Because this technology has the advantage of lacking toxic side effects, simple operation, and clear results, it has gained increasing attention in recent years. A study by Rahman et al. (2002b) showed that xenon pressurization at 0.3 MPa could reduce the respiration rate of persimmon fruit and inhibit the development of flesh browning and decay. Treatment with 0.4 MPa xenon significantly shortened the longitudinal relaxation time and transverse relaxation time in eggplants (Rahman et al., 2001). Browning appeared on 6 d of storage in samples without xenon treatment, whereas browning did not appear even after 17 d in samples treated with 0.4 MPa xenon. Oshita et al. (2000) found that

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https://doi.org/10.1016/j.postharvbio.2018.09.007
Received 6 June 2018; Received in revised form 10 September 2018; Accepted 11 September 2018
Available online 21 September 2018
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pressurization treatment of broccoli with 0.6 MPa xenon increased the viscosity of intracellular water and that there was no browning or dehydration during the storage period. A study by Zhang et al. (2008) found that treatment with mixed xenon and argon (Ar) pressurization inhibited increases in respiration rate, lignification, and browning in asparagus. Wu et al. (2012a) found that high-pressure nitrogen could inhibit increases in respiration rate, ethylene production, browning, and the loss of antioxidant activity in freshly cut pineapple, thereby extending its shelf life and maintaining good quality. Although studies on preservation using pressurized inert gas have yielded some results, many inadequacies still exist in the technologies for the preservation of specific fruit and vegetables. Integration of different preservation technologies is thus forming a new trend. Controlled atmosphere storage is a preservation technology in which respiration is inhibited and ripening is delayed in produce through changing the gas composition of the storage microenvironment. Currently, inert gas pressurization combined with controlled atmosphere storage is gradually gaining attention, but a vast majority of studies have concentrated on pressurization technologies combined with modified atmosphere storage technologies; in addition, studies are primarily focused on the quality of fruit and vegetables. In the storage of sweet cherries, only controlled atmosphere storage (Yu et al., 2009; Serradilla et al., 2013) and pressurization storage (Árbol et al., 2016) have been studied, whereas the integrated use of inert gas pressurization and controlled atmosphere storage has not been explored. Based on previous studies, in this study, we treated mid-to-late ripe “Lapins” sweet cherries (the test material) with pressurization with 0.5 MPa Ar for 1 h combined with a 5% O_2 + 10% CO_2 + 85% Ar atmosphere. The effects of argon pressurization, controlled atmosphere, and their combination on the browning and quality of sweet cherries during cold storage were studied, and their regulatory mechanisms in sweet cherry browning were revealed. This study thus provides a theoretical basis and technical guidance for controlling the browning of sweet cherries after harvesting.

2. Materials and methods

2.1. Materials and treatments

“Lapins” sweet cherries were used as test materials in this study. The samples of sweet cherry were obtained from twelve-year-old sweet cherry trees on the ‘Gisela 5’ rootstock, from a sweet cherry planting area in Jiang County, Shanxi Province, China (lat. 35°20′19″ N, long. 111°21′49″ W). Fruit trees were maintained with standard cultural, fertilizer, herbicide, and pesticide practices. Fruit were harvested at 80–90% commercial maturity stage (transverse diameter > 24 mm, per fruit weight > 9 g, bright red color). Within 1 h after manual harvesting, the fruit were moved to the postharvest facilities at Yuncheng University. Fruit with vivid color, uniform size, no disease-related or mechanical injury, and a similar degree of maturity were selected as the test material. The fruit were randomly divided into 4 groups (30 kg per group, 3 replicates per group, with 10 kg of fruit per replicate). The experimental design is shown in Table 1.

Sterilization treatment: The material in each group was disinfected by immersion in 0.05 g L^{-1} chlorine dioxide solution for 30 s and air dried at 1 °C.

Table 1
The experimental design.

Experimental group	Pressurization conditions	Atmospheric ratio
Control	0	0
Pressurized Ar (P A)	0.5 MPa Ar, 1 h	0
Controlled atmosphere (CA)	0	5% O_2 + 10% CO_2 + 85% Ar
Pressurized Ar + controlled atmosphere (P A + CA)	0.5 MPa Ar, 1 h	5% O_2 + 10% CO_2 + 85% Ar

2.1.1. Pressurization treatment

Fruit were placed in a custom-built high-pressure argon reactor. The air originally inside the reactor was not evacuated. Ar was added to a pressure of 0.5 MPa and maintained for 1 h. The times of pressurization and depressurization were approximately 120 s and 180 s. The pressurization process was conducted at 0 ± 1 °C environmental conditions.

2.1.2. Controlled atmosphere treatment

Fruit were arranged in plastic baskets (44 cm × 33 cm × 11 cm) and then stacked inside an atmosphere box (length/width/height: 130 cm × 62 cm × 113 cm, total volume 0.4 m^3). In total, 12 baskets were stacked inside each atmosphere box, with each basket weighing 2.5 kg. A gas mixer (MAP Mix 9000, PBI-Dansensor (Far East) Limited., Copenhagen, Denmark) was used to mix the desired concentration of O_2, CO_2, and Ar from external gas tanks to achieve specified gas combinations (5% O_2, 10% CO_2, and 85% Ar), which were stored in buffer tanks and passed to the atmosphere box as a continuous flow (100 mL min^{-1}). Gas composition of atmosphere box was monitored regularly using a gas analyzer (Gas Analyzer CheckMate II, PBI-Dansensor (Far East) Limited., Copenhagen, Denmark) to keep the fruit in a predetermined gas environment. Controlled atmosphere treatments were carried out at conditions of 0 ± 1 °C and 90–95% relative humidity.

2.1.3. Control treatment

Fruit were arranged in plastic baskets (44 cm × 33 cm × 11 cm) and a 0.03 mm polyethylene (PE) fresh-keeping bag that was left unsealed. The fruit were stored at conditions of 0 ± 1 °C and 90–95% relative humidity.

Ten samples were collected at regular intervals during the storage process and 75 fruit were collected at each time. Of these, 45 fruit were used for determination of fruit skin color, flesh firmness, total soluble solid (TSS) content, titratable acids, ascorbic acid (AsA), and cell membrane relative permeability. In addition, the fruit flesh was flash frozen in liquid nitrogen and stored in an -80 °C freezer to be used for malondialdehyde (MDA) and polyphenol content determination and browning-associated enzymatic activity. The remaining 30 fruit were used for the determination of decay index, rate of good fruit quality, and fruit flesh browning index. Each treatment was replicated three times. The experiment was conducted twice. A similar result was observed in the two experiments; thus, the data from one experiment are presented.

2.2. Index determination

2.2.1. Browning index determination

The browning index was determined as described by Yang and Wang (2016). In total, 30 fruit were collected and cut horizontally along the equator. The browning index was evaluated in each fruit individually using a five-point hedonic scale based on the percentage of cut surfaces affected by browning symptom. Browning index was scored as follows:

0, no browning of flesh; 1, browning of ≤ 1/4 of flesh area; 2, browning of 1/4 - 1/2 of flesh area; 3, browning of 1/2–3/4 of flesh area; 4, browning of ≥ 3/4 of flesh area.

Flesh browning index (%) = Σ (browning score × number of fruit with that score)/(4 × total number of fruits) × 100%

Three independent replicates were conducted for each treatment.

2.2.2. Decay index determination

In total, 30 fruit were used for decay index assessment. Fruit decay index was assessed in each fruit individually using a five-point hedonic

scale based on the extent of the total decayed area. Decay index was scored as follows: 0, no decay of fruit; 1, presence of 1–2 lesions with an area no larger than 0.2 cm^2; 2, fruit with ≤ 1/4 decayed area; 3, fruit with 1/4 - 1/2 decayed area; 4, fruit with ≥ 1/2 decayed area.

Decay index (%) = Σ (decay score × number of fruit with that score)/(4 × total number of fruits) × 100%

Three independent replicates were conducted for each treatment.

2.2.3. Rate of good fruit

In total, 30 fruit were used for determining the good fruit rate. The rate of good fruit was evaluated in each fruit individually based on the absence (without) of decay or browning.

Rate of good fruit (%) = number of good fruits (without decay or browning)/total number of fruits × 100%

Three independent replicates were conducted for each treatment.

2.3. Fruit skin color determination

Fifteen fruit were used for skin color determination. Two sites relative to the equator of the fruit were selected. A Minolta Chroma meter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Tokyo, Japan) was used to evaluate the color of fruit skin. The color space is shown as Lightness (L* value), red-green (a* value), and yellow-blue (b* value). The hue angle (h^o value) shows the change in the color of fruit skin, with a lower h^o indicating more severe browning. L* represents the lightness of fruit skin, with higher L* indicating brighter skin color and less browning. saturation (C* value) represents the degree of color saturation, with higher C* indicating a more vivid fruit skin color and higher market value. Three independent replicates were conducted for each treatment.

2.4. Fruit quality index determination

2.4.1. Determination of firmness

In total, 15 fruit were used for determining firmness. A razor blade was used to remove the skin on the two surfaces relative to the equator of the fruit. A texture analyzer (Texture analyzer TA-XT Express-v3.1, Stable Micro Systems Ltd., London, UK) was used to measure the firmness of the fruit. The probe diameter was 0.002 m. Compression was applied at a speed of 0.005 m s^{-1} and a depth of 0.002 m. The firmness index was recorded as the largest peak value with units in N. Three independent replicates were conducted for each treatment.

2.4.2. TSS determination

In total, 15 fruit were used to determine the TSS using a handheld refractometer. The values were expressed as %. Three independent replicates were conducted for each treatment.

2.4.3. Determination of titratable acidity and AsA

Titratable acidity was determined as described by Yang et al. (2016) with slight modifications using 15 fruit. Briefly, 2.0 g of homogenized fruit flesh was collected and ground in a mortar and 0.02 L distilled water was added. The slurry was washed into a 0.05 L centrifuge tube, mixed by shaking, and centrifuged at 3000 g for 10 min. Of the supernatant, 0.02 L was decanted into an Erlenmeyer flask, and 0.1 mol L^{-1} NaOH was added until the pH was raised to 8.1. Titratable acids were calculated based on the malic acid content and expressed as % (mass fraction). Three independent replicates were conducted for each treatment.

The molybdenum blue colorimetric method was used for AsA determination. Briefly, 2 g of sweet cherries was weighed, and 0.075 L oxalic acid EDTA was added. The mixture was ground into a slurry, carefully transferred to a 0.01 L centrifuge tube, and centrifuged at 12,000 g for 10 min. Next, 0.001 L of the supernatant was collected, and its absorbance was read at 760 nm as described by Gao (2006). As A content was expressed as grams of AsA per kg of fresh weight. Three independent replicates were conducted for each treatment.

2.5. Determination of MDA content and cell membrane relative permeability

Cell membrane relative permeability was determined as described by Yang et al. (2013). Disks (0.1 cm thick) of the equatorial region tissue were excised using a stainless-steel cork borer (1.0 cm diameter). After rinsing three times (2 min–3 min) with deionized water, 30 small discs (0.1 cm thick, 1.0 cm diameter) were incubated in 0.03 L of deionized water at 25 °C, followed by shaking for 30 min. The electrical conductivity (P_0) of the suspended solution was measured using a conductivity meter (Conductivity Meter DDS-11 A, Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai, China). The samples were then boiled for 15 min and quickly cooled to room temperature, and the total electrolytes of the solution (P_1) were then measured again. Cell membrane relative permeability was calculated according to the formula: $P_0/P_1 \times 100\%$. Cell membrane relative permeability was expressed as a percentage (%). Three independent replicates were conducted for each treatment.

MDA content was determined as described by Dhindsa et al. (1981), with slight modifications. Briefly, 3 g of sweet cherries was weighed, and 0.006 L of pre-cooled 10% (w/v) trichloroacetic acid was added. The mixture was ground into a homogenous slurry and centrifuged at 12,000 g for 20 min at 4 °C. Next, 0.002 L of the supernatant was mixed with 0.003 L of 0.67% (w/v) thiobarbituric acid. The mixture was heated to 100 °C for 20 min, quickly cooled, and then centrifuged at 10,000 g for 20 min at 4 °C. Absorbance of the supernatant was determined at 450, 532, and 600 nm. MDA concentration was calculated according to the formula: [6.45 × (A532 - A600) - 0.56 × A450] × Vt/(Vs × m), where Vt and Vs are the total volume of the extract solution and the volume of the extract solution contained in the reaction mixture solution, respectively, and m is the mass of the samples. MDA content was expressed as millimole of MDA per kg of fresh weight. Three independent replicates were conducted for each treatment.

2.6. Determination of polyphenol content

Polyphenol content was determined using the Folin phenol method. Briefly, 0.7 g of sweet cherries was weighed, and 0.001 L of pre-cooled 60% ethanol was added. The mixture was ground into a homogenous slurry and centrifuged at 12,000 g for 30 min at 4 °C. Next, 0.00025 L of the supernatant was collected and placed in test tubes, and 0.00025 L of Folin phenol reagent was added. After mixing and allowing to stand for 3 min, 0.0005 L of 12% sodium carbonate solution was added, mixed by shaking, made up to 0.005 L, and placed at 20 °C in the dark for 2 h. Absorbance was read at 765 nm as described by Kaur and Kapoor (2002). Polyphenol content were expressed as grams of gallic acid equivalent per kg of fresh weight. Three independent replicates were conducted for each treatment.

2.7. Determination of browning-associated enzymatic activity

Briefly, 2 g of fruit flesh from 15 fruit was homogenized with 0.004 L of 100 mmol L^{-1} potassium phosphate buffer (pH 6.8) containing 1.0 mmol L^{-1} ethylenediaminetetraacetic acid disodium salt dihydrate, 5% (w/v) polyvinyl polypyrrolidone, and 1% (v/v) Triton X-100. The homogenate was centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was used for peroxidase (POD) and polyphenoloxidase (PPO) enzyme assays.

PPO enzymatic activity was determined as described by Zhang et al. (2010); 0.003 L of the reaction mixture contained 100 mmol L^{-1} sodium phosphate buffer (pH 6.4), 0.0005 L enzyme extract, and

100 mmol L^{-1} catechol. Enzyme activity was determined by monitoring the increase in the absorbance at 398 nm. One unit of enzymatic activity was defined as a change of 1 in the absorbance at 398 nm per minute per kilogram of fruit flesh (fresh weight) and was expressed as U kg^{-1}. Three independent replicates were conducted for each treatment.

POD enzyme activity was determined as described by Yang et al. (2013); 0.003 L the reaction mixture contained 50 mmol L^{-1} sodium phosphate buffer (pH 7), 0. 0005 L enzyme extract, 10 mmol L^{-1} guaiacol, and 10 mmol L^{-1} H_2O_2. Enzyme activity was determined by monitoring the increase in the absorbance at 470 nm. One unit of enzymatic activity was defined as a change of 1 in the absorbance at 470 nm per minute per kilogram of fruit flesh (fresh weight) and was expressed as U kg^{-1}. Three independent replicates were conducted for each treatment.

Phenylalanine ammonia lyase (PAL) enzymatic activity was determined as described by Assis et al. (2001), with slight modifications. Briefly, 2 g of fruit flesh from 15 fruit was homogenized with 0.004 L of 100 mmol L^{-1} borate buffer (pH 8) containing 5 mmol L^{-1} β -mercaptoethanol, 2.0 mmol L^{-1} ethylenediaminetetraacetic acid disodium salt dihydrate, and 5% (w/v) polyvinyl polypyrrolidone. The homogenate was centrifuged at 12,000 g for 20 min at 4 ℃. The supernatant was used for PAL enzyme assays; 0.003 L of the substrate contained 60 mmol L^{-1} l-phenylalanine in 0.1 mol L^{-1} borate buffer (pH 8) and was pre-incubated at 37 ℃ for 10 min. The reaction started when 0.0008 L enzyme extract was mixed with the warm substrate solution and ran for 60 min before stopping by adding 0.0001 L of 6 mol L^{-1} HCl. One unit of enzymatic activity was defined as a change of 1 in the absorbance at 290 nm per minute per kilogram of fruit flesh (fresh weight) and was expressed as U kg^{-1}. Three independent replicates were conducted for each treatment.

2.8. Statistical analysis

The experiments were performed using a completely randomized design. All statistical analyses were performed with SAS statistical software (version 8; SAS Institute, Cary, NC, USA). The data were analyzed by one-way ANOVA. The mean separations were performed by Duncan's multiple range tests. Differences at $P < 0.05$ were considered significant. Data were presented as mean ± SE.

3. Results and analysis

Fig. 1A shows that no browning appeared in the sweet cherries on the first 14 d of storage. Symptoms of browning first appeared in the control fruit at 21 d of storage, and the browning index showed an increasing trend as the time of storage increased. In fruit treated with pressurization, controlled atmosphere, or both, the appearance of flesh browning was delayed, and an increase in the browning index was inhibited. The difference from controls was significant from day 21 of storage to the end of the experiment ($P < 0.05$). At the end of the experiment on day 63 of storage, the browning indices of fruit treated with pressurization, controlled atmosphere, or both were decreased by 46.94 (79.72%), 51.94 (88.21%), and 55.83 (94.81%), respectively, over the control fruit. Treatment of fruit with both pressurization and controlled atmosphere reduced the browning index better than either treatment alone, with the difference reaching significance on day 56 of storage until the end of the experiment ($P < 0.05$).

Fig. 1B shows that there was no decay of fruit in the early stages and that there was an increasing trend in the decay index at the middle to late stages. Treatment of fruit with both pressurization and controlled atmosphere delayed fruit decay and inhibited the increase in decay index, with the difference from controls reaching significance from day 28 of storage until the end of the experiment ($P < 0.05$). At the end of the experiment on day 63 of storage, the decay index of fruit treated with pressurization, controlled atmosphere, or both was decreased by 13.33 (58.54%), 15.56 (68.29%), and 18.89 (82.93%), respectively, over the control fruit. Treatment of fruit with both pressurization and controlled atmosphere reduced the decay index better than either treatment alone, with the difference reaching significance on day 56 of storage until the end of the experiment ($P < 0.05$).

Fig. 2 shows that the rate of good fruit was 100% at the early stages of storage and that it showed a decreasing trend at the middle to late stages. Treatment of fruit with both pressurization and controlled atmosphere delayed and inhibited the decrease in the rate of good fruit, with the difference from controls reaching significance from day 28 of storage until the end of the experiment ($P < 0.05$). At the end of storage, the rate of good fruit among fruit treated with pressurization, controlled atmosphere, or both was increased by 28.89 (72.22%), 36.67 (91.67%), and 45.56 (113.89%), respectively, over the control fruit.

As shown in Fig. 3A, the L* value of fruit showed an overall decreasing trend, with a rapid decrease in the early and middle stages of storage and a slower decrease at the late stages. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibited the decrease in L* value, and the inhibitory effect was particularly significant at the middle or late stages, with the difference from controls reaching significance from day 21 of storage until the end of the experiment ($P < 0.05$). At the end of storage, the L* values of fruit treated with pressurization, controlled atmosphere, or both were increased by 3.77 (13.44%), 4.53 (16.17%), and 8.46 (30.15%) over the control fruit, respectively. Treatment of fruit with both pressurization and controlled atmosphere was better than either treatment alone, with the difference reaching significance on day 35 of storage until the end of the

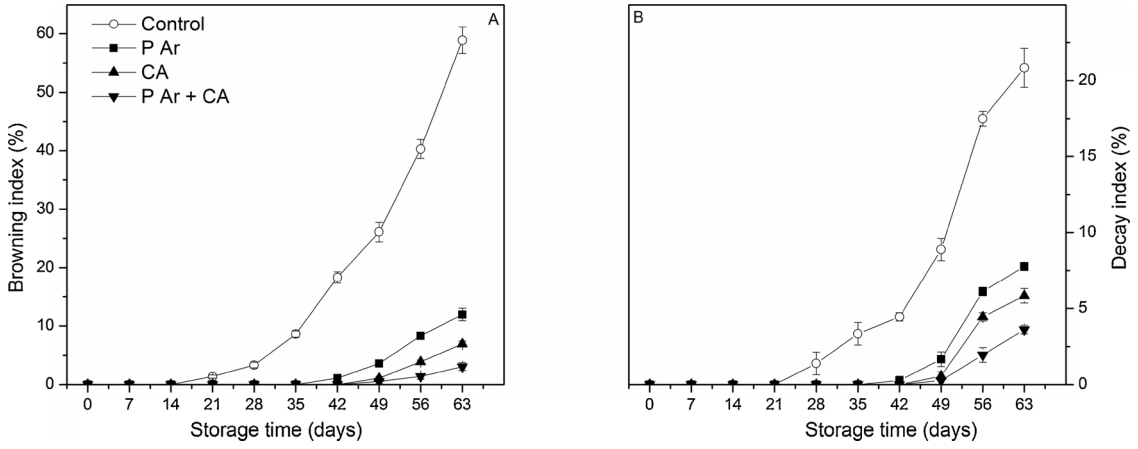

Fig. 1. Effect of argon pressurization, controlled atmosphere, and their combination on the browning index (A) and decay index (B) of sweet cherry during storage. Vertical bars represent S.E. of means, n = 3.

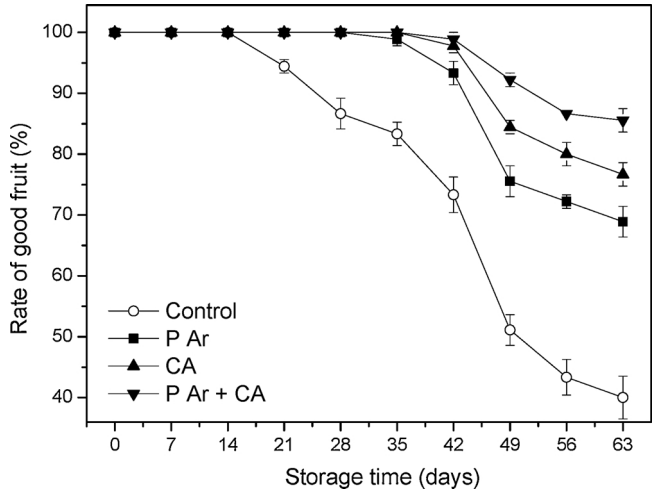

Fig. 2. Effect of argon pressurization, controlled atmosphere, and their combination on the good fruit rate of sweet cherry during storage. Vertical bars represent S.E. of means, n = 3.

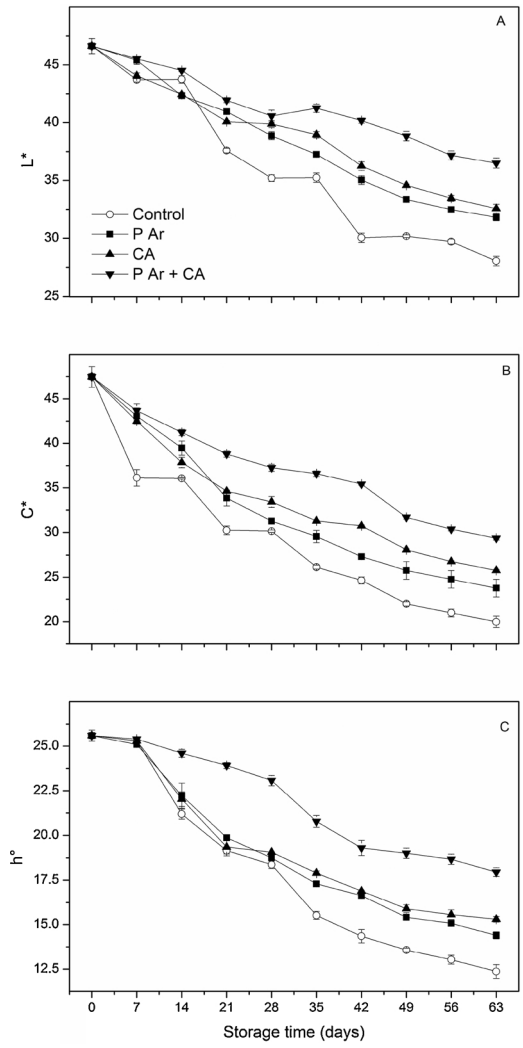

Fig. 3. Effect of argon pressurization, controlled atmosphere, and their combination on L* (A), C* (B), and h° (C) values of sweet cherry during storage. Vertical bars represent S.E. of means, n = 3.

experiment ($P < 0.05$). At the end of storage, the L* values of fruit treated with pressurization or controlled atmosphere alone were increased to 4.68 (14.73%) and 3.92 (12.04%), respectively, over the control fruit.

As shown in Fig. 3B, the C* value of fruit showed an overall decreasing trend, with a rapid decrease in the early and middle stages of storage and a slower decrease at the late stages. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibit the decrease in C* value, and the inhibitory effect was particularly significant at the late stage, with the difference from controls reaching significance from day 21 of storage until the end of the experiment ($P < 0.05$). The difference between fruit treated with both pressurization and controlled atmosphere and control fruit reached statistical significance on day 21 of storage until the end of the experiment ($P < 0.05$). The difference between pressurization, controlled atmosphere alone, and their combination also reached statistical significance ($P < 0.05$). At the end of storage, the C* values of fruit treated with pressurization, controlled atmosphere, or both were increased by 3.77 (18.86%), 5.76 (28.84%), and 9.38 (46.94%), respectively, over the control fruit.

As shown in Fig. 3C, the hº value of fruit showed a decreasing trend, with a slow decrease in the early and late stages of storage and a rapid decrease in the middle stage. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibit the decrease in hº value, and the inhibitory effect was particularly significant with the combined treatment; the difference between fruit treated with both pressurization and atmosphere and the other groups was significant on day 14 of storage until the end of the experiment ($P < 0.05$). The difference between pressurization or atmosphere alone and the controls reached statistical significance on day 35 of storage ($P < 0.05$). At the end of storage, the hº values of fruit treated with pressurization, controlled atmosphere, or both were increased by 2.03 (16.44%), 2.93 (23.72%), and 5.58 (45.15%), respectively, over the control fruit.

As shown in Fig. 4A, the firmness of sweet cherries showed an overall decreasing trend with a rapid decrease in the early and middle stages of storage, and a slower decrease in the late stage. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibit the decrease in firmness, with the difference from controls reaching statistical significance on day 28 of storage until the end of the experiment ($P < 0.05$). The inhibitory effect with both pressurization and controlled atmosphere was even better, with the difference from either treatment alone reaching statistical significance on day 35 of storage until the end of the experiment ($P < 0.05$). At the end of storage, the firmness of fruit treated with both pressurization and controlled atmosphere was increased by 1.28 (10.78%), 1.33 (11.97%), and 1.92 (47.33%) over atmosphere alone, pressurization alone, and control conditions, respectively.

As shown in Fig. 4B, TSS content showed an overall decreasing trend, with a slow decrease in the early stage of storage and a rapid decrease in the middle and late stages. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibit the decrease in soluble solids, with the difference from controls reaching statistical significance on day 21 of storage until the end of the experiment ($P < 0.05$). The inhibitory effect with both pressurization and controlled atmosphere was even better, with the difference from either treatment alone reaching significance on day 42 of storage until the end of the experiment ($P < 0.05$). At the end of storage, TSS in fruit treated with both pressurization and atmosphere were increased by 1.15 (8.69%), 1.18 (9.04%), and 2.09 (22.60%) over the conditions of atmosphere alone, pressurization alone, and the control conditions, respectively.

As shown in Fig. 4C, the fruit titratable acids showed an overall decreasing trend, with a slow decrease in the early and late stages of storage and a rapid decrease in the middle stage. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibit the decrease in titratable acids, with a significant difference from the controls on day 28 of storage until the end of the experiment ($P < 0.05$). The difference from either treatment alone also reached statistical significance on day 42 of storage until the end of the

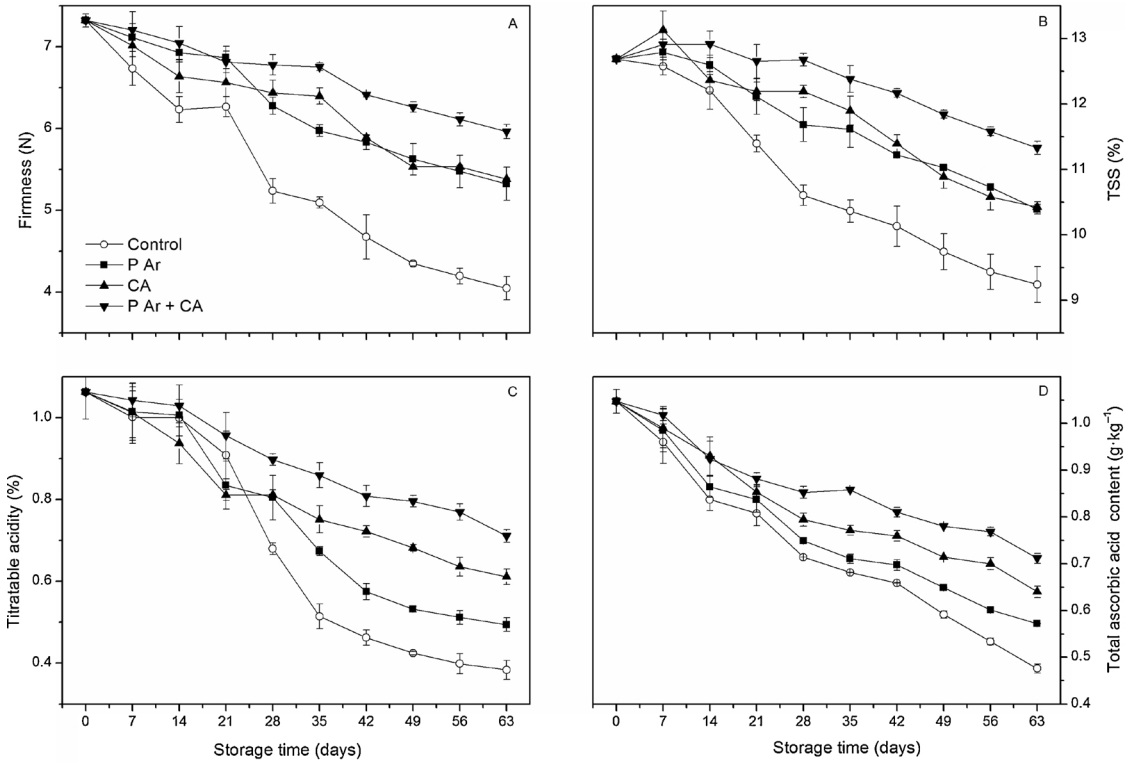

Fig. 4. Effect of argon pressurization, controlled atmosphere, and their combination on firmness (A), TSS (B), titratable acidity (C), and AsA (D) contents of sweet cherry during storage. Vertical bars represent S.E. of means, n = 3.

experiment ($P < 0.05$). At the end of storage, the titratable acids in fruit treated with pressurization, controlled atmosphere alone, or both were increased significantly by 0.11 (28.88%), 0.23 (59.36%), and 0.33 (85.48%), respectively, over the controls ($P < 0.05$).

Fig. 4D shows an over decreasing trend in AsA. Treatment of fruit with pressurization, atmosphere, or both could inhibit the decrease in AsA, with a significant difference from the controls on day 28 of storage until the end of the experiment ($P < 0.05$). A statistically significant difference was also observed among the three groups ($P < 0.05$). The inhibitory effect of combined pressurization and controlled atmosphere treatment was the best. At the end of the storage, the AsA in fruit treated with both pressurization and controlled atmosphere was increased significantly by 0.24 (49.50%), 0.07 (11.18%), and 0.14 (24.36%), over pressurization alone, atmosphere alone, and the controls, respectively ($P < 0.05$).

As shown in Fig. 5A, MDA content showed an increasing trend, with slow increases at the early and late stages and a rapid increase at the middle stage. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibit the increase in MDA content, with a significant difference from the controls on day 14 of storage until the end of the experiment ($P < 0.05$). The difference among the three groups also reached statistical significance on day 35 storage ($P < 0.05$). MDA content was ultimately maintained at the lowest level with the combined pressurization and controlled atmosphere treatment, and the MDA content at day 35, until the end of storage, averagely decreased by 2.81 (30.46%), 1.50 (18.94%), and 1.04 (14.16%) compared to the controls, pressurization alone, and controlled atmosphere alone, respectively.

As shown in Fig. 5B, relative membrane permeability showed an overall increasing trend, with rapid increases at the early and middle

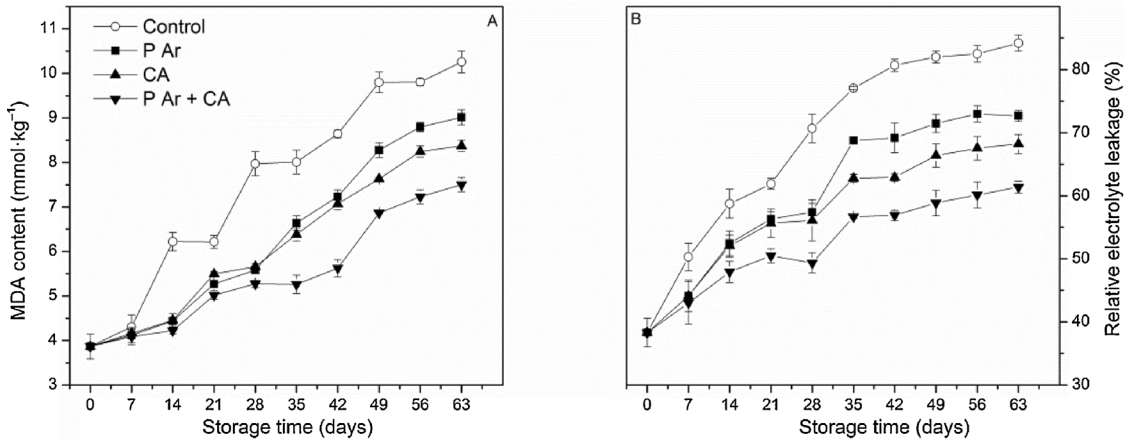

Fig. 5. Effect of argon pressurization, controlled atmosphere, and their combination on MDA (A) content and cell membrane relative permeability (B) of sweet cherry during storage. Vertical bars represent S.E. of means, n = 3.

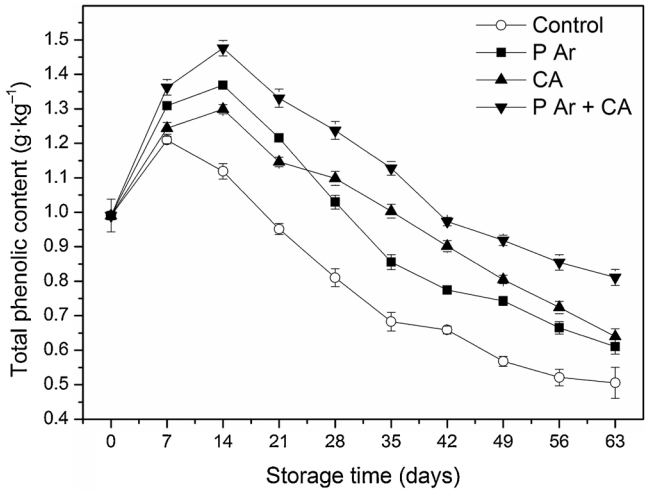

Fig. 6. Effect of argon pressurization, controlled atmosphere, and their combination on polyphenol content of sweet cherry during storage. Vertical bars represent S.E. of means, n = 3.

stages and a slower increase at the late stage. Treatment of fruit with pressurization, controlled atmosphere, or both could inhibit the increase in relative membrane permeability, with a significant difference from the controls from day 14 of storage until the end of the experiment ($P < 0.05$). The difference among the three groups also reached statistical significance on day 35 of storage ($P < 0.05$). Relative membrane permeability was ultimately maintained at the lowest level with combined pressurization and controlled atmosphere treatment, and the membrane permeability at day 35, until the end of storage averagely decreased by 22.49 (27.67%), 12.22 (17.22%), and 6.78 (10.32%) compared to the controls, pressurization alone, and controlled atmosphere alone, respectively.

As shown in the Fig. 6, the total phenolic compound content of the fruit showed an increasing trend at the early stages of storage, reached maximum values at days 7 and 14, and then showed a decreasing trend. Treatment of fruit with pressurization, controlled atmosphere, or both promoted the increase in total phenolic compound content and inhibited its decrease, with the difference from controls reaching statistical significance on day 14 of storage until the end of the experiment ($P < 0.05$). A significant difference was also observed among the three groups (except for the difference between pressurization alone and controlled atmosphere at the end of storage) ($P < 0.05$). The total phenolic compound content was ultimately maintained at a high level with the combined pressurization and controlled atmosphere treatment, and the total phenolic compound content at day 14, until the end of storage, was averagely increased by 36.40 (52.94%), 18.35 (22.51%), and 13.91 (15.21%) compared to the controls, pressurization alone, and controlled atmosphere alone, respectively.

As shown in the Fig. 7A, POD activity showed an overall trend of first increasing, then decreasing, and then increasing again. Treatment of fruit with pressurization, controlled atmosphere, or both inhibited the increase in POD activity and reached statistical significance compared to the controls from day 7 of storage until the end of the experiment ($P < 0.05$). The inhibitory effect of the combined treatment was the best, with a significant difference compared to either treatment from day 35 of storage until the end of the experiment ($P < 0.05$).

As shown in Fig. 7B, PAL activity presented an overall increasing trend, with a slow increase in the early and late stages and a rapid increase in the middle stage. Treatment of fruit with pressurization, controlled atmosphere, or both inhibited the increase in PAL activity with statistical significance compared to the controls from day 21 of storage until the end of the experiment ($P < 0.05$). The inhibitory effect of the combined treatment was the best, with the difference from

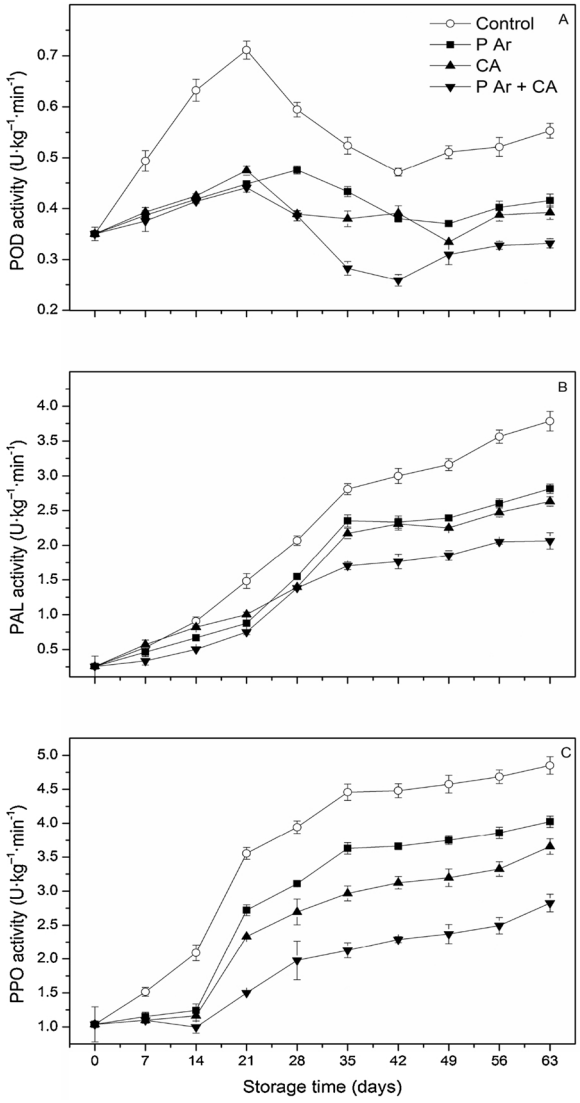

Fig. 7. Effect of argon pressurization, controlled atmosphere, and their combination on POD activity (A), PAL activity (B), and PPO activity (C) of sweet cherry during storage. Vertical bars represent S.E. of means, n = 3.

either treatment alone reaching statistical significance from day 35 of storage until the end of the experiment ($P < 0.05$).

As shown in Fig. 7C, PPO activity showed an overall increasing trend. Treatment of fruit with pressurization, controlled atmosphere, or both inhibited the increase in PPO activity, with a statistically significant difference from the controls from day 7 of storage until the end of the experiment ($P < 0.05$). The difference between the three treatments also reached statistical significance from day 35 of storage until the end of the experiment ($P < 0.05$). The inhibitory effect of the combined treatment was the best, with PPO activity eventually reaching low levels at all stages of storage.

4. Discussion

Sweet cherries are extremely susceptible to browning, decay, and other negative changes after harvesting, resulting in loss of nutritional value (Pasquariello et al., 2015; Paiva et al., 2017). The present study found that pressurization or controlled atmosphere alone could decrease fruit browning index and decay rate (Fig. 1), increase the rate of good fruit (Fig. 2), inhibit the decreases in L*, C*, and h^{o} values of the fruit skin (Fig. 3), and maintain high levels of firmness, TSS, titratable acids, and AsA content (Fig. 4). The preservative effect of the

combination of pressurization and controlled atmosphere was better than either treatment alone. This freshness preservation mechanism may be explained as follows. (1) After the pressurization treatment, residual Ar exists in microscopic air pockets in the fruit, forming low-oxygen microenvironments in the tissue interior. These microenvironments have been confirmed in tissue micrographs of cucumber (Zhan and Zhang, 2005). In addition, the low-oxygen external environment produced by subsequent atmospheric treatment slows the aerobic metabolism within the fruit. (2) Under pressurized conditions, Ar enters the fruit tissue and forms "clathrate hydrates" with water molecules, which can increase the viscosity of the juice in sweet cherries, reduce the fluidity of water, and inhibit enzymatic activity, thereby helping to reduce the degradation rate of fruit substances. Similar preservative efficacy has been found with broccoli (Oshita et al., 2000), persimmon (Rahman et al., 2002a, 2002b), cucumber (Meng et al., 2014), asparagus (Zhang et al., 2008), green pepper (Meng et al., 2012), cabbage (Makino et al., 2006a,b), and other produce.

Breaking the regional structure of cells promotes a large amount of contact between substrates and enzymes and is an important factor in the browning of fruit (Zhu et al., 2009). Under normal conditions, substrates and enzymes are distributed in different regions of cells, and enzymes do not promote browning. However, during adverse conditions such as senescence and low temperature, active oxygen metabolites are imbalanced. An excess of active oxygen attacks the cell membrane, leading to cell membrane lipidation, destruction of membrane structure, breakdown of the regional structure of cells, and promotion of a large amount of contact between substrates and enzymes leading to browning of flesh and finally resulting in loss of economic and nutritional value (Chomkitichai et al., 2014; Yang et al., 2009). In the present study, as the time of storage was increased, the browning index of the fruit (Fig. 1) increased along with a rapid increase in the MDA content and relative cell membrane permeability (Fig. 5). Furthermore, correlation analysis showed that the browning index of sweet cherries was highly correlated with MDA content and relative cell membrane permeability. In addition, the correlations were significantly positive with coefficients of 0.7906* and 0.7252*, respectively (Supplementary table A). This indicates that MDA content and relative cell membrane permeability are closely correlated with sweet cherry browning. Similar results were also obtained in longans (Wang et al., 2015), peaches (Rui et al., 2010), white mushrooms (Ding et al., 2016), and other fruit and vegetables. A study by Wang et al. (2015) revealed that regional destruction of fruit tissue presented as increased MDA content and relative cell membrane permeability. A mechanistic study of post-harvest peach fruit browning by Rui et al. (2010) showed that increased relative cell membrane permeability is a direct factor in the browning of peach fruit. Appropriate physicochemical procedures can inhibit the processes of cell membrane lipidation and the increase in cell membrane permeability. The present study found that individual use of pressurization or controlled atmosphere could inhibit the increases in MDA content and relative cell membrane permeability and showed that the inhibitory effect of the combined treatment was better than that of either treatment alone (Fig. 5). Dissolution of inert gases in water forms hydrophobic hydrate structures, which may help inhibit membrane peroxidation and stabilize the cell membrane structure and function. Similar inhibitory effects have also been found in freshly cut green pepper (Meng et al., 2012) and pineapple (Wu et al., 2012a). Using transmission electron microscopy, Wu et al. (2012a) observed the cell membrane structure of freshly cut pineapple that was stored for 14 d using high-pressure Ar treatment and found that the cell wall had an intact structure that was not degraded and that the cell membrane still adhered to the cell wall after high-pressure Ar treatment. The middle layers of the cell were clearly visible, indicating that high-pressure Ar treatment can promote maintenance of the intact cell membrane structure in freshly cut pineapple and delay its ripening.

Enzymatic oxidation of phenolic compounds is essential in fruit browning. PAL, PPO, and POD are essential enzymes that cause fruit browning (Supapvanich and Promyou, 2013). These enzymes can oxidize phenolic compounds in fruit into anthraquinone compounds with deeper colors, thereby leading to fruit tissue browning. The present study found that as the browning index increased (Fig. 1A), the phenolic compound content decreased (Fig. 6), PPO and PAL enzymatic activity increased, and POD first increased and then decreased (Fig. 7). Correlation analysis showed that the browning index exhibited a strongly significant negative correlation with phenolic compound content ($r = -0.8166$*) and a significant positive correlation with PPO ($r = 0.6802$*) and PAL ($r = 0.8319$*) enzymatic activity (Supplementary Table A). This indicates that phenolic compound content, PPO, and PAL are closely related to sweet cherry browning. The present study found that pressurization or controlled atmosphere alone could inhibit the decrease in phenolic compound content and the increase in PAL and PPO enzymatic activity and that the inhibitory effect of the combination was better than that of either treatment alone (Figs. 6 and 7B,C). A possible reason for this is that (1) pressurization and controlled atmosphere reduced O_2 and increased CO_2 concentrations, thus inhibiting enzymes from promoting oxidative browning; (2) the inert gas, Ar, could exert an active biochemical effect, competing with oxygen molecules for the active sites of enzymes and inhibiting the activity of some enzymes that are important in the browning process (Spencer, 2005; Spencer and Humphreys, 2003); (3) pressurization causes inert gases and water molecules to form inert gas hydrates, which can affect the hydrophobic residues on enzyme proteins of fruit and vegetables, changing the structure of the enzyme active center, and thus reducing enzymatic activity and slowing the enzymatic reaction rate (Zhang et al., 2001; Liu et al., 2008). Similar inhibitory effects of inert gases on the browning mechanism have also been found in freshly cut apples (Wu et al., 2012b) and mushrooms (Lagnika et al., 2011). In the present study, the correlation between POD activity and browning index was not significant ($P < 0.05$) (Supplementary table A), which indicates that POD does not play a role in sweet cherry browning but may play a role in antioxidant enzymes. However, this mechanism is still unknown and is a subject for further studies.

5. Conclusions

Treatment with pressurized Ar, controlled atmosphere, and their combination reduced the fruit decay rate, increased the high rate of fruit with good quality, maintained high L* and C*, and h^o, and inhibited reduction in firmness, TSS, titratable acidity, and AsA content, ultimately maintaining better fruit quality. Treatment with pressurized Ar, controlled atmosphere, and the combination of the two inhibited increases in MDA content and cell membrane permeability, while maintaining higher phenolic compound content and decreasing PPO and PAL enzymatic activity, ultimately maintaining low browning index and inhibiting fruit browning. Treatment with both pressurization and controlled atmosphere yielded better results than either treatment alone, indicating that this combination is an ideal method for the preservation of sweet cherries.

Acknowledgements

This study was supported by the Science and Technology Innovative Projects of Shanxi Higher Education Institutions (2015182), the Key Problem of Scientific and Technical Project of Shanxi Province (20150311020-4), PhD Research Startup Foundation of Yuncheng University (YQ-2016013 and YQ-2014025), Yun Cheng University Research Projects (XK-2015009 and XK-2015023), and Shanxi Key Subjects Construction (FSKSC).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2018.09.

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