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The effect of CO₂ concentration on sweet cherry preservation in modified atmosphere packaging

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Abstract: The effect of CO₂ concentrations on the preservation of sweet cherries in modified atmosphere packaging was greatly different. In the present paper, an accurate gas-regulating storage device was used to set the concentrations of CO₂ at 0%, 5%, 10%, 15%, 20% and 25%, respectively (O₂ was set at 5% and the remaining parts were filled with N₂) to store sweet cherries. Then the quality change of sweet cherries was determined approximately from –1 to +1 °C and from 80 to 85% relative humidity. The results showed that all six air treatments had a certain inhibitory effect on deterioration of sweet cherries. In particular, the 10% CO₂ group could reduce rotting rate, maintain firmness, delay the change of soluble solids and vitamin C, reduce the activity of polyphenol oxidase and peroxidase. This group always had good firmness, nutrition and taste after 120 days of storage. So the 10% CO₂ concentration provides suitable gas storage conditions of sweet cherries in modified atmosphere packaging.

Keywords: cherries; gas composition; controlled atmosphere; quality

Sweet cherry is the iconic fruit of Yantai in Shandong province. The annual output is over 200 000 tons that accounts for more than 50% of the total national production (Zhang 2016). Sweet cherry has a high nutritional value and is mainly sold in the form of fresh fruit. Sweet cherry is always transported to the market at high temperatures which could easily lead to rot after harvest. The fruit is a highly perishable product due to its high respiration rate and rapid softening process at room temperature. That ultimately causes the colour changes, weight loss, browning and changes of nutrients (Pasquariello et al. 2015; Dong & Wang 2017). Those greatly limit the market supply range and supply period of sweet cherry (Conte et al. 2009; Jiang et al. 2009). In general, sweet cherries are stored in a refrigerator approximately from –1 to +1°C. The shelf life is only a month or so, and fruits are more likely to become soft and decayed in transit (Ali et al. 2014).

In recent years, many scholars have done many researches on sweet cherries in order to increase the

preservation period of cherries by using chemical preservation, coating preservation and modified atmosphere packaging (MAP) (Dang et al. 2010; Zhao et al. 2010; Yan et al. 2015). Among them, MAP has gradually attracted people's attention because of good preservation effect, low cost, long storage period and ease of sales (Lara et al. 2015). MAP uses a high-barrier composite packaging material to seal food in the artificial mixed gas environment. The way changes the storage environment of food to inhibit the growth of microorganisms, prevent enzymatic reaction and slow down the oxidation rate (De Paiva et al. 2017). The method extended the shelf life of a product greatly. There have been many reports on the study of MAP on sweet cherry. Yang (2019) investigated the effects of pressurized argon, storage in a controlled atmosphere, and their combination on the postharvest quality and browning of sweet cherries. Colgecen & Aday (2015) researched the efficacy of the combined use of chlorine dioxide and passive modified atmosphere packaging on sweet

cherry quality. Manuel et al. (2013) evaluated the effect of different controlled atmospheres on changes in the microbial population of 'Ambrunés' sweet cherries throughout storage. But the effect of different CO₂ concentration on the quality of sweet cherry at modified atmosphere packaging has been little reported.

Therefore, the fixed initial volume concentration of O₂ and different concentrations of CO₂ were used to store sweet cherries at MAP for revealing the effect of different CO₂ concentrations on sweet cherry post-harvest storage quality. The method provides a novel way of improving the quality of sweet cherries.

MATERIAL AND METHODS

Material and treatments

In the experiment, 'Pioneer' sweet cherry was selected from the cherry orchard in Zhanggezhuang, Fushan district, Yantai city. Cherries were transported to laboratory immediately after harvest. After water precooling, 200 g of cherries were loaded into an air-conditioned box (length/width/height: 220 × 130 × 60 mm), which was made from plastic. The box was covered with polyethylene film. An air baling machine was used to package (extraction time 2 s, inflation time 2 s, heat sealing time 2 s, heat sealing temperature 165 °C). The packaging method was active MAP. Gas ratios in the experimental group were 0%, 5%, 10%, 15%, 20% and 25% CO₂. [Jiang (2011) and Yang (2019) have found that 5% O₂ had the best preservation effect]. The rest was filled with N₂. The control group was filled with air. Twenty-one boxes were prepared for each treatment and there were about 25 cherries in each box. Cherries were put at approximately -1 to +1 °C after gas packaging and the rotting rate, hardness, soluble solids, polyphenol oxidase (PPO), peroxidase (POD) and vitamin C were measured.

Index determination

Rotting rate. If spots, sag, flesh softening or sour smell appeared on sweet cherries, they could be considered as rotting fruit. Calculation of the rotting rate:

$$\text{Rotting rate} = \frac{\text{number of rotting fruit}}{\text{initial number of all fruits}} \times 100\% \quad (1)$$

Respiration rate. The respiration rate of sweet cherries was measured according to the procedure described by Fagundes et al. (2014) with some modifications. Briefly, fruits were placed in a closed jar container at 20 °C for 3 h, 1 mL of the accumulation of CO₂ in the headspace atmosphere was withdrawn with a gas syringe and in-

jected into a gas chromatograph (Shimadzu GC-14A; Shimadzu, Japan) with a thermal conductivity detector and a filter unit containing chromosorb. Helium was chosen as the carrier gas at a flow rate of 50 mL min⁻¹. Temperatures were fixed at 55 °C, 55 °C and 110 °C for the oven, injector and detector, respectively. Results were expressed as mg kg⁻¹ h⁻¹.

Firmness

In total, 15 fruits were used for determining their firmness. A razor blade was used to remove the skin on the two surfaces relative to the equator of the fruit. A texture analyzer (CT3-10K-230; Brookfield, USA) was used to measure the firmness of the fruit. The probe diameter was 0.002 m. Compression was applied at a speed of 0.010 m s⁻¹ and a depth of 0.006 m. The firmness index was recorded as the largest peak value with units in N. Three independent replicates were conducted for each treatment.

Soluble solids

Soluble solids were established with a hand-held refractometer (PR-101α; Atago, Japan). The cover of the instrument was opened and wiped with clean gauze. Two drops of distilled water were dropped on the prism surface and a zero-set was performed. Then the cover was opened and wiped clean again. Two drops of cherry juice were put on top of the prism and the value was read.

Enzyme activity

An aliquot of 2 g of fruit flesh 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 pyrrolidone 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 polyphenol oxidase (PPO) and peroxidase (POD). PPO activity was measured following the method of Assis et al. (2001). One unit of enzyme activity was defined as the amount of the enzyme that caused an increase in absorbance of 1 at 420 nm per min. Additionally, POD activity was determined according to the previous method (Jing et al. 2013). One unit of POD activity was defined as a change of 1 in the absorbance at 470 nm per min per kg of fruit flesh (fresh weight)."

Vitamin C

Vitamin C content was established with the People's Republic of China National Standard GB 5009.86-2016

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(The determination of ascorbic acid in foods). An aliquot of 100 g of samples and 100 g of 20 g L⁻¹ oxalic acid solution were put into the crusher to mash into homogenate quickly. Approximately 10–40 g of the homogenate of samples (accurate to 0.01 g) were put in a beaker and transferred to a 100-mL volumetric flask with oxalic acid solution, diluted to scale, shaken well and then filtered. If the filtrate had colour, 0.4 g clay per 1 g sample were added for decolourization before filtration. 10 mL of the filtrate were put in a 50 mL conical flask accurately and titrated with calibrated 2,6-dichloroindophenol solution until the solution was pink and did not fade for 15 s. A blank test was done at the same time. Then vitamin C content was calculated.

Data analysis

The data were analyzed by one-way ANOVA (analysis of variance). The mean separations were performed by Duncan's multiple range tests. Differences at $P < 0.05$ were considered significant. Data were presented as mean \pm SE.

RESULTS AND DISCUSSION

Effects of different CO₂ concentrations on the rotting rate. Figure 1 shows that the rotting rate of sweet cherry decreased significantly after the gas was filled. There was hardly any decay within 30 days and the rotting rate increased later. The 10% CO₂ group was the lowest. When stored for 120 days, the rotting

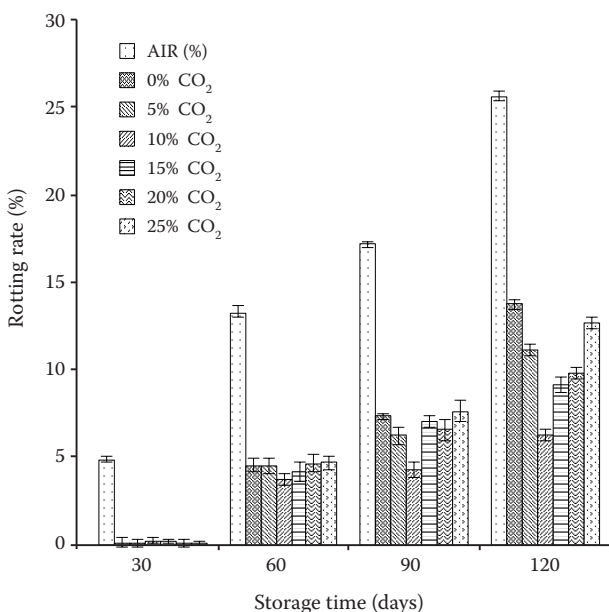


Figure 1. Rotting rate of sweet cherries at different CO₂ concentrations

rate was only 6.45%, which was significantly different from the other groups ($P < 0.05$). The results showed that controlled atmosphere could significantly decrease the rotting rate of fruits. The most significant effect was observed in the 10% CO₂ group. The reason was that the high concentrations of CO₂ could inhibit the growth of microorganisms on the fruit surface (Tian et al. 2001). But excessively high CO₂ concentrations made the concentration of H⁺ and HCO₃⁻ in the fruit cell too high to cause the cell death and loss of antibacterial ability (Gonçalves et al. 2004).

Effects of different CO₂ concentrations on respiration rate. The respiration rate of sweet cherry in various coating treatments is presented in Figure 2. Fruits in the control showed a rapid decrease in respiration rate during storage and this decreasing trend continued to the end of storage. In the first two days, the respiration rate dropped rapidly. The reason was that a rapid drop in temperature limited the activity of respiratory enzymes. Among them, the 10% CO₂ group was more significant than the other groups ($P < 0.05$). After 30 days, the respiration rate of all treatments dropped very low until the end of storage.

Effects of different CO₂ concentrations on firmness. Figure 3 shows that the firmness of cherries declined during storage with different initial concentrations of CO₂. The control group showed a significant decline compared to the treatment groups ($P < 0.01$). Among treatment groups, the 10% CO₂ group was more significant than the other groups ($P < 0.05$). It proved

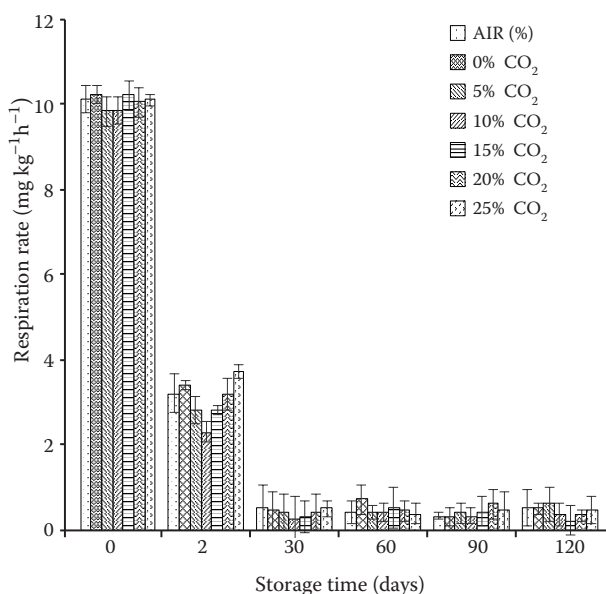


Figure 2. Respiration rate of sweet cherries at different CO₂ concentrations

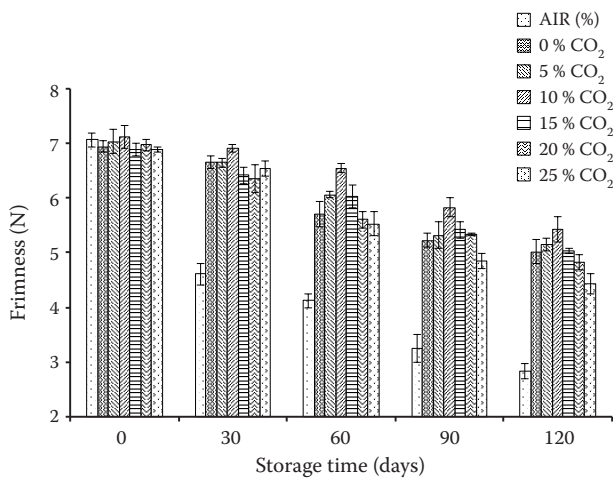
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Figure 3. Firmness changes of sweet cherries at different CO₂ concentrations

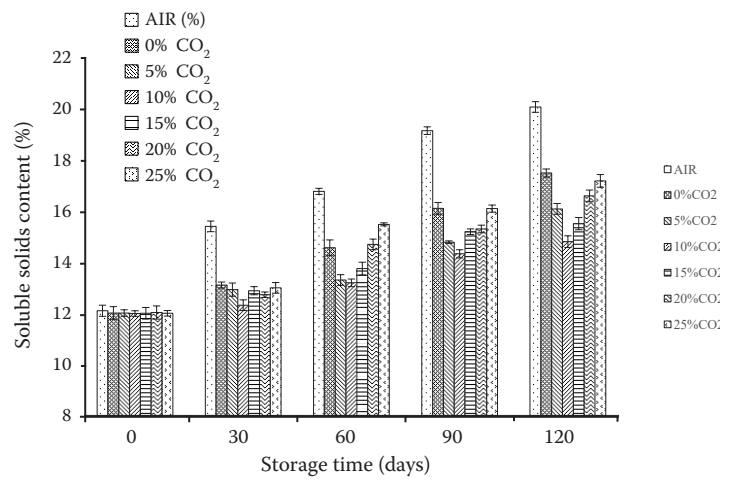


Figure 4. Soluble solids content of sweet cherries at different CO₂ concentrations

that the air-controlled storage could maintain the fruit firmness. The main reason was that CO₂ was the competitive inhibitor of ethylene to reduce the physiological effect of ethylene, where high concentrations of CO₂ were toxic to fruits (Pérez et al. 1999).

Effects of different CO₂ concentrations on soluble solids content. The soluble solids content of cherries with different air conditioning packages showed a rising tendency. The control group rose faster than the other groups ($P < 0.05$) (Figure 4.). The rise range of 10% CO₂ group was the smallest. It indicated that the rise of soluble solids content could be significantly inhibited by 10% CO₂ atmosphere packaging. The reason was that CO₂ infiltrated into cells and dissolved into cytoplasm, which increased the acidity of cells to inhibit the activity of respiratory enzymes. However, an excessive concentration of CO₂ poisoned the fruit cells resulting in decomposition of soluble solids (Gonçalves et al. 2004).

Effects of different CO₂ concentrations on PPO and POD. The PPO activity of cherries with different air conditioning packaging presented a gradually increasing trend. In the early stage of storage, the activity of PPO in each group increased significantly ($P < 0.05$), and then the activity of PPO in the middle and late stage of storage was basically stable ($P < 0.05$) (Figure 5A). The change of the 10% CO₂ group was lowest. The POD activity also increased gradually in the early stage of storage and then it decreased (Figure 5B). The POD activity in the control group increased significantly compared to treatment groups ($P < 0.05$). The CO₂ concentration between treatment groups had a low effect.

The results indicated that the 10% CO₂ group could reduce the activity of PPO effectively. The ripening and senescence of fruits were closely related to reactive ox-

xygen. If reactive oxygen was not cleared in time, plant organs and tissues would be under oxidative stress that resulted in cell metabolism disorders to contribute

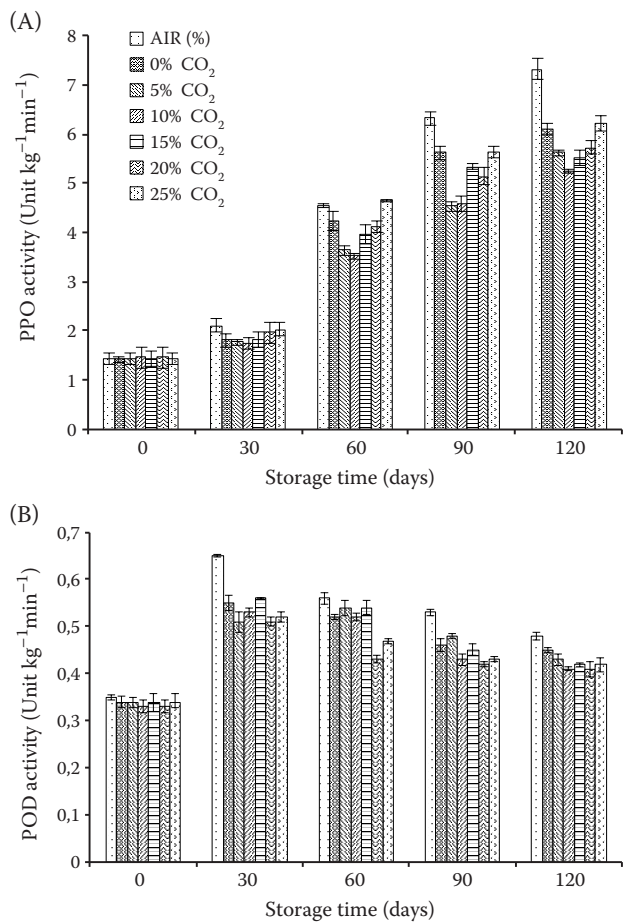


Figure 5. Enzyme activity of sweet cherries at different CO₂ concentrations: (A) polyphenol oxidase (PPO); (B) peroxidase (POD)

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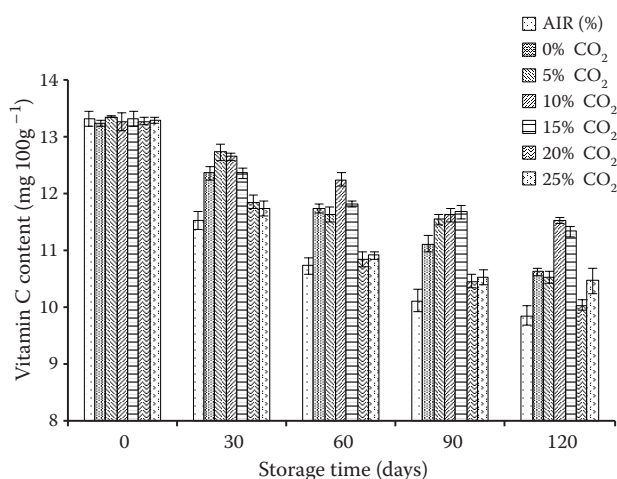


Figure 6. Vitamin C content of sweet cherries stored at different CO₂ concentrations

to cell senescence and death (Pérez et al. 1999). PPO could oxidize polyphenols to form peroxides which could accelerate fruit senescence. High concentration of CO₂ reduced PPO activity and delayed senescence. POD is a kind of enzyme with high activity in plants. Its activity changes constantly during plant growth and development. In general, the activity is higher in aged tissues, but weaker in young tissues. POD converts carbohydrates into lignin to increase the degree of fruit lignification. So POD was used as a physiological indicator of tissue aging. High concentration of CO₂ reduced POD activity to decrease intracellular reactive oxygen species and add the anti-aging ability of fruits (Asada 1999).

Effects of different CO₂ concentrations on vitamin C. The vitamin C content of cherries in different air conditioning packages decreased gradually (Figure 6). The declining trend of cherries in the 10% CO₂ group was the smallest ($P < 0.05$), while the decline was obvious in other groups ($P < 0.05$), especially in the control group ($P < 0.01$). The 10% CO₂ group significantly inhibited the reduction of vitamin C and maintained the quality of cherries. The reason was that low CO₂ concentration did not inhibit the effect while high CO₂ concentration increased acidity to improve enzyme activity and accelerate the decomposition of vitamin C. These results were in agreement with Tian et al. (2004).

CONCLUSION

MAP was a very effective way to extend the shelf life of sweet cherries. MAP significantly reduced the rotting rate, maintained firmness, delayed the change of

soluble solids and vitamin C, and reduced the activity of polyphenol oxidase and peroxidase. However, the effects of different initial CO₂ concentrations on physiological and biochemical indexes were significantly different in MAP. With the increase of CO₂ concentration, the preservation effect on sweet cherries rose at first and then it decreased. The reason was that low CO₂ concentration could not inhibit the effect on microorganisms while excessively high CO₂ concentration made the concentration of hazardous substances too high to cause the cell death and loss of antibacterial ability. Especially, the 10% CO₂ group significantly inhibited the reduction of soluble solids and vitamin C, significantly reduced the activity of PPO and POD, which had a good effect on keeping fruits fresh. Therefore, the initial gas combination of 5% O₂ and 10% CO₂ was suitable for modified atmosphere packaging of sweet cherries.

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