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Responses of physiology and quality of sweet cherry fruit to different atmospheres in storage

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Abstract

Sweet cherry (*Prunus avium* L. cv Lapis) fruits were stored in modified atmosphere packaging (MAP) and controlled atmospheres (CA) of 5%O_2 plus 10%CO_2, or 70%O_2 plus 0%CO_2 at 1 °C, to determine the effects of different O_2 and CO_2 concentrations on physiological properties, quality attributes and storability during storage periods of 60 days. The results indicated that CA with 5%O_2 plus 10%CO_2 more significantly inhibited the enzymatic activities of polyphenol oxidase (PPO) and peroxidase (POD), reduced malondialdehyde (MDA) content, effectively prevented flesh browning, decreased fruit decay and extended storage life of sweet cherry fruit than did other treatments. CA with high O_2 concentration (70%O_2 + 0%CO_2) was more effective at inhibiting ethanol production in flesh and reducing decay than other treatments, but stimulated fruit browning after 40 days of storage. Meanwhile, the fruits stored in 5%O_2 plus 10%CO_2 had a higher degree of firmness, higher vitamin C and titratable acidity contents than those in MAP and CA with high O_2 level during all the storage periods. But soluble solids contents (SSC) were not significantly affected by different atmosphere treatments.

1. Introduction

The production of sweet cherry (*Prunus avium* L.) increases rapidly while the cultivation areas increase year by year in China, as the fruit has good quality with a very high market value. But sweet cherry fruits deteriorate rapidly after harvest, due to water loss, surface pitting, stem browning and decay (Dugan & Roberts, 1997; Mattheis & Roberts, 1993). Postharvest treatment with low O_2 and/or high CO_2 concentrations is an attractive alternative for controlling fungal decay, maintaining fruit quality and extending postharvest life of fruits (Ceponis & Cappellini, 1985; Ke, Rodriguez-Sinobas, & Kadar, 1991; Prusky, Perez, Zutkhi, & Ben-Arie, 1997). Sweet cherry is a unique fruit that has a higher tolerance to elevated CO_2 concentrations than most stone fruit crops (Porritt & Mason, 1965). High CO_2 concentrations were used to reduce losses from decay caused by many fungi in the fruit (De Vries-Paterson, Jones, & Cameron, 1991). In a previous study, we found that the growth of *Monilinia fructicola* on potato dextrose agar (PDA) was completely suppressed, and brown rot was not found in inoculation sites on sweet cherry fruits in 10–30%CO_2 after 30 days at 0 °C (Tian, Fan, Xu, Wang, & Jiang, 2001). Day (1996) reported that high O_2 mixtures (e.g. 70–100%) were particularly effective at inhibiting enzymatic discoloration, preventing anaerobic fermentation reactions, and inhibiting aerobic and anaerobic microbial growth. CA, with 70%O_2, effectively reduced ethanol production in flesh and maintained peel green colour of longan (*Dimocarpus longan*) fruits (Tian, Xu, Jiang, & Gong, 2002). However, there are no published data on the effects of high O_2 mixtures on physiology and quality of sweet cherry fruits. The objectives of this study were to investigate the effects of high O_2 or high CO_2 concentrations on physiological properties, quality and storability of sweet cherry fruits stored in modified atmosphere packaging (MAP), controlled atmospheres (CA), with high CO_2 concentration (5%O_2 + 10%CO_2), or CA with high O_2 concentration (70%O_2 + 0%CO_2) for 60 days at 1 °C.

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2. Materials and methods

2.1. Fruits

Sweet cherry (*P. avium* L. cv Lapis) fruits, with a commercial maturity according to acceptable colour standards, were harvested from an orchard in Qixia, Shangdong province, China. The fruits were precooled immediately and transported to Beijing in a refrigerated van at 2–5 °C on the day of harvest, and then stored at 1 °C until required for experimental purposes.

2.2. Storage conditions

Storage conditions were as follows: MAP, polyethylene film bags (0.04 mm thick, 220 × 300 mm for 500 g fruit, 13–18%O_2 + 2–4%CO_2); CA-I, 5%O_2 + 10%CO_2; CA-II, 70%O_2 + 0%CO_2. Controlled atmosphere cabinets (105 × 55 × 100 cm), with CO_2 absorber (Soda lime containing ethyl violet as indicator) and ethylene absorber (Odoroxidant Media, M-06318), were linked with an atmosphere analyzer (FC-701, Italy), Initial O_2 and CO_2 levels in the cabinets were established by a flow-through system, mixing N_2 (100%) and O_2 (99.5%), or N_2 and CO_2 via pressure regulators, then automatically controlled and regulated by the analyzer. There were 100 kg of fruit in each CA cabinet. There were 40 MAP bags (each with 500 g), in which the concentrations of O_2 and CO_2 were measured by an atmosphere analyzer (CYES-II, Shanghai, China) every 10 days. All treatments were at 1 °C with approximately 95% RH.

2.3. Determination of activities of POD and PPO

A flesh sample of 20 g from 20 fruits was ground with 20 ml of 0.2 M sodium phosphate buffer (pH 6.4) and homogenized using a Kinematica tissue grinder (Crl-6010, Kriens-LU, Switzerland) at 4 °C. After filtration of the homogenate through one layer of cheesecloth, the filtrate was centrifuged at 13,000*g* for 30 min. polyphenol oxidase and perioxidase (PPO) and (POD) activities were measured in the supernatant by monitoring the rate of dopachrome formation at 398 and 460 nm, respectively, using a UV-160 spectrophotometer (Shimadzu, Japan) according to Galeazzi, Sgarbieri, and Constantinides (1981) and Putter (1974) with slight modification.

The assay of PPO activity was performed using 3 ml 0.5 M 4-methylcatechol in 0.2 M sodium phosphate buffer (pH 6.4) and 100 μl of the crude enzyme sample. POD activity was assayed using 2 ml of 0.05% guaiacol (0.2 M potassium phosphate buffer, pH 6.4) incubation medium, containing 500 μl of the crude enzyme for 15 min at 30 °C, after which 1 ml of 0.08% H_2O_2 was added to give a final volume of 3.5 ml. The reactions were carried out for 10 s and 1 min at 25 °C, respectively, and the change in absorbance at 398 and 460 nm with time was recorded. The initial velocity was calculated from the slope of the linear part of the curve obtained. The straight line section of the activity curve was used to express the enzyme activity. Enzymatic activities were defined as an increase in one absorbance unit per minute under the conditions of the assay. Experiments were conducted three times each, in duplicate.

2.4. Determination of MDA content

MDA content was measured according to the method of Heath and Packer (1968) with slight modification. A 1.5 ml aliquot of supernatant was mixed with 4 ml of 15% trichloracetic acid containing 0.5% thiobarbituric acid. The mixture was heated at 100 °C for 18 min, and quickly cooled, then centrifuged at 10,000*g* for 10 min. The absorbance of the supernatant was measured at 532 and 600 nm, respectively. The unspecific turbidity was corrected by subtracting A_{600} from A_{532}. The concentration of MDA was calculated using an extinction coefficient of 155 m/M/cm as follows: MDA content (nmol/g) = $[(OD_{532} - OD_{600}) \times 4\ \text{ml} \times (40\ \text{ml}/1.5\ \text{ml})]/(1.55 \times 10^{-1} \times 20\ \text{g})$.

2.5. Ethylene and ethanol measurement

Contents of ethylene and ethanol were determined by gas chromatography (Shimadzu, GC-9A, Japan). Ethylene and ethanol contents in fruit were determined by head space gas chromatography (Tian et al., 2002). A fruit sample of about 100 g was put in an Omni-mixer (Omni International Inc. Waterbury, USA) and an equal amount of 20% trichloroacetic acid was added, and the mixture then homogenized for 2 min in an ice water bath. A 5 g sample of the mixture was sealed in a 10 ml vial, and then incubated in a thermostated bath at 40 °C for 60 min.

A 0.5 ml sample of the head space gas was removed by syringe and injected into the gas chromatograph equipped with a flame ionization detector (FID) and glass column (2 mm × 4 m). The experimental conditions were: 85 °C oven temperature, 130 °C injector temperature, 250 °C detector temperature. The components were identified individually by comparing retention times against standards, concentrations being determined by a regression equation calculated on four samples of standard concentrations. Experiments were conducted, three times each, in duplicate.

2.6. Measurements of firmness, TSS, vitamin C and titratable acidity contents

Flesh firmness (using 20 fruits) was determined on opposite peeled cheeks of the fruit using a Fruit Firmness Tester (FT-327, Italy), equipped with a 8-mm

plunger tip. Total soluble solids (TSS), titratable acidity and vitamin C contents of the fruit were determined using the same methods as previously reported (Tian et al., 2002).

2.7. Determination of fruit decay and browning

Thirty fruits in each treatment were assessed, once every 10 days. Fruit decay and browning rate were determined in 30 fruits with 3 replications. Flesh browning severity was determined as: 0, no browning; 1, slight browning <1/3; 2, 1/3–2/3 browning; 3, >2/3 browning. The browning index was calculated using the following formula:

$$\text{Browning index} = \sum(\text{browning scale} \times \text{percentage of corresponding fruit within each class}).$$

2.8. Statistical analysis

All data were processed by an analysis of variance as a one-factor general linear model procedure (ANOVA). The treatment means were separated using the least significant difference method. Differences at $P = 0.05$ were considered as significant.

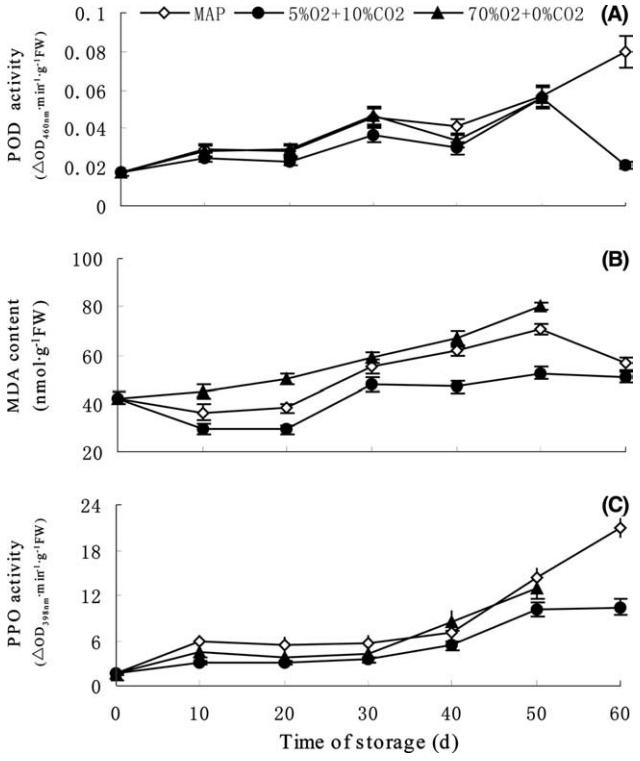

Fig. 1. Changes in activities of POD and PPO, and MDA contents of sweet cherries stored in different atmospheres at 1 °C during storage periods. Bars represent standard errors of the mean.

3. Results and discussion

3.1. Effects of different atmospheres on POD activity and MDA content

POD activity of sweet cherry fruits increased slightly and changed from 0.02 $\Delta OD_{460\ nm}$/min/g FW at harvest to 0.06 $\Delta OD_{460\ nm}$/min/g FW after 50 days of storage (Fig. 1(A)). In general, the change of POD activity was related to ripening processes, and it increased with advancing senescence of fruits. In this experiment, the fruits stored in CA with $5\%O_2 + 10\%CO_2$ senesced slowly and showed better quality (Fig. 4) than in MAP and CA with $70\%O_2 + 0\%CO_2$ Similarly, POD activity of sweet cherries in CA with high CO_2 level was lower than in other treatments, particularly by the end of storage (Fig. 1(A)).

The content of MDA is often used as an indicator of lipid peroxidation, resulting from oxidative stress (Smirnoff, 1995). In this study, we found that MDA content increased with storage time, and was higher in high O_2-treated fruits than in other treated fruits (Fig. 1(B)). This may be attributed to high O_2 injury, because the browning rate of the fruits, at high O_2 level, increased rapidly after 30 days (Figs. 2(A) and (B)). Kader and Ben-Yehoshua (2000) found that mature-green tomatoes, exposed to 80 or 100 kPa O_2 for more

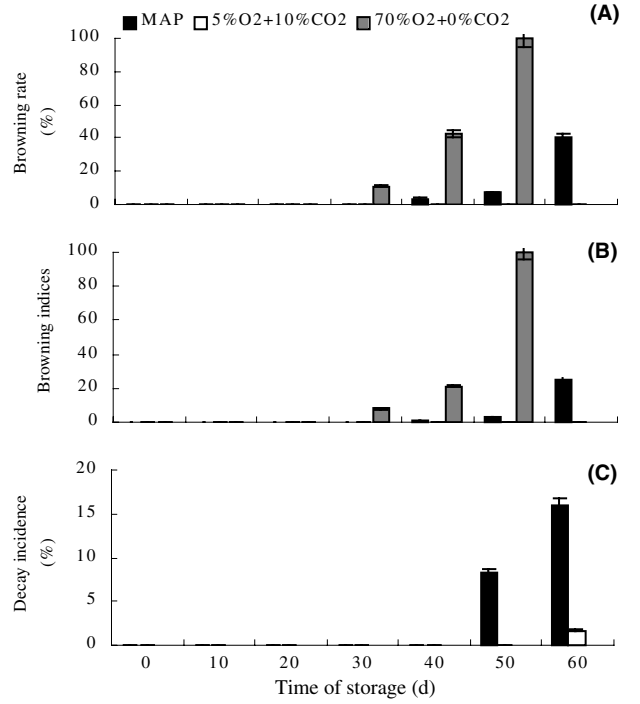

Fig. 2. Effects of different atmospheres on flesh browning and decay of sweet cherry fruits kept at 1 °C during storage periods. Bars represent standard errors of the mean.

than 5 days, exhibited dark-brown spots on their skin. The explanation for O_2 toxicity is the formation of superoxide radicals (O_2^-), which are destructive to some components of cell metabolism (Gerschman, 1964). The fruits stored in $5\%O_2 + 10\%CO_2$ atmosphere had the lowest contents of MDA, throughout all the storage periods (Fig. 1(B)). CA with $5\%O_2 + 10\%CO_2$ reduced lipid peroxidation, as indicated by less accumulation of MDA and by more effectiveness at delaying fruit senescence and maintaining quality in comparison with MAP or CA with high O_2 concentration.

3.2. Effects of different atmospheres on PPO activity and browning

PPO activity in the flesh of sweet cherry was quite low at harvest and increased with storage time in all treatments. The fruits in MAP shown a more rapid increase in PPO activity than in CA conditions, particularly before 30 days of storage (Fig. 1(C)). Browning was found in the fruits stored in $70\%O_2 + 0\%CO_2$ after 30 days; then browning rate increased rapidly and reached 100% in 50 days (Fig. 2(A)), accompanying the rapid increase in browning index (Fig. 2(B)). At the end of the storage, browning rate of the fruits stored in MAP reached 40.2%, but no browning was found in sweet cherries kept in $5\%O_2 + 10\%CO_2$ (Figs. 2(A) and (B)). Tissue browning, due to oxidation of phenolic compounds by polyphenol oxidase (PPO), results from loss of compartmentalization within the cells when exposed to physical and/or physiological stresses. There are different opinions about the relationship between PPO activity and flesh browning. Whitaker and Lee (1995) considered that enzymatic browning was primarily responsible for the browning of fruits and vegetables. But Cheng and Crisosto (1995) indicated that PPO activity was not a limiting factor in enzymatic browning. Many reports have demonstrated that concentrations of O_2 and CO_2 obviously influenced enzymatic activity of fruits and vegetables. Day (1996) hypothesized that high O_2 levels may cause substrate inhibition of PPO or, alternatively, high levels of colourless quinones formed may cause feedback inhibition of PPO. Browning potential of sweet cherry fruits was not significantly related to PPO activity in this experiment because, when the fruits in high O_2 level showed higher browning rate than in other treatments, no significant difference of PPO activity was found among all the treatments at this time. However, PPO activity in the peel of longan fruits rapidly increased before browning occurred (Tian et al., 2002). This indicated that increasing PPO activity might stimulate browning, and enzymatic browning was only an indirect consequence of browning, and therefore its cause should be sought in other oxidative or senescent-related processes (Larrigaudiere, Lentheric, & Vendrell, 1998). Increased O_2 concentrations around and within the horticultural commodity result in higher levels of free radicals that can damage plant tissues (Fridovich, 1986). Sensitivity to O_2 toxicity varies among species and developmental stages (Kader & Ben-Yehoshua, 2000). CA with $70\%O_2 + 0\%CO_2$ more effectively prevented peel browning of longan fruits before 30 days storage than did MAP or CA with $5\%O_2 + 10\%CO_2$ (Tian et al., 2002). But the results of this experiment demonstrated that high O_2 treatment was not suitable for sweet cherry.

3.3. Effects of different atmospheres on ethanol content and ethylene production

Ethanol contents of sweet cherry fruits increased rapidly from 7.1 nmol/g at harvest to 22.1, 17.0 and 11.8 nmol/g in MAP, CA with $5\%O_2 + 10\%CO_2$ and/or CA with $70\%O_2 + 0\%CO_2$, respectively, after 10 days (Fig. 3(A)). The highest value of 22.8 mol/g of ethanol content was found in the fruits stored in MAP for 50 days of storage. Sweet cherries kept at $70\%O_2$ level always maintained the lowest level of ethanol, particularly before 30 days, compared to the fruits in other treatments (Fig. 3(A)) Similarly, a high O_2 level ($70\%O_2 + 0\%CO_2$) was also effective at decreasing ethanol production in the flesh of longan fruit (Tian et al., 2002). These results further confirm that high O_2 concentration is beneficial to the inhibition of ethanol production (Day, 1996). Elevated O_2 levels may affect synthesis and accumulation of some volatile compounds, including fermentive metabolites such as acetaldehyde (AA), ethanol and ethyl acetate (Solomos, Whitaker, & Lu, 1997). Since the first metabolite formed under anaerobic conditions is AA, which is converted to ethanol by alcohol dehydrogenase (ADH) (Pesis & Marinansky, 1992), it

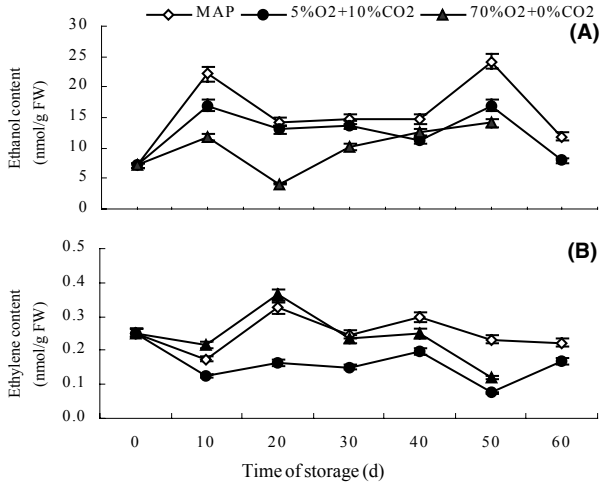

Fig. 3. Effects of different atmospheres on ethylene and ethanol contents in pulp of sweet cherry fruits kept at 1 °C during storage periods. Bars represent standard errors of the mean.

may be beneficial for ethanol to be converted into AA through ADH in high O_2 concentrations. Accumulation of ethanol is usually related to off-flavours in fruit, but the ethanol levels causing off-flavours differ greatly among commodities. Ethanol contents reaching 6.5 nmol/g in apricot caused off-flavours (Folchi, Pratella, Tian, & Bertolini, 1995), but 110 nmol/g ethanol content showed an acceptable taste in sweet cherry fruits stored at $0.3\%O_2 + 0\%CO_2$ at 0 °C for 24 days (Tian, 2000). This may be due to the high soluble solids contents in sweet cherry fruits, since soluble solids contents (SSC) plays an important role in determining the ethanol level that causes off-flavours (Ke et al., 1991).

Ethylene production of fruit flesh was very low and fluctuated from 0.12 to 0.36 nmol/g during the storage periods (Fig. 3(B)). But, CA with $5\%O_2 + 10\%CO_2$ was more effective at inhibiting ethylene production than other treatments ($P = 0.01$). Chavez-Franco and Kadar (1993) reported that high CO_2 atmospheres significantly inhibited ethylene biosynthesis in 'Bartlett' pears. Ethylene production in sweet cherries in CA with $70\%O_2$ level was high early in storage, but then decreased rapidly (Fig. 3(B)). Klaustermeyer and Morris (1975) considered that superatmospheric O_2 levels increased ethylene production and the incidence and severity of pink rib and C_2H_4-induced russet spotting on lettuce. In contrast, exposure to 80 or 100 kPa O_2 reduced ethylene production rates and delayed ripening of mature-green and breaker tomatoes at 20 °C (Kader & Ben-Yehoshua, 2000). These results indicated that the response of crops to high O_2 might depend on O_2 concentration.

3.4. Effects of different atmospheres on fruit decay

After 50 days, 8.3% of disease incidence occurred in MAP-stored fruits, and no decay was found in CA-stored fruits (Fig. 2(C)). Decay incidence reached 16% in MAP-stored fruits, and only 1.6% decay rate shown in the fruits in CA with $5\%O_2 + 10\%CO_2$ after 60 days of storage. CA conditions, both with high O_2 and high CO_2, more significantly inhibited fruit decay than did MAP. CA with high CO_2 concentrations could significantly reduce storage decay in a number of different fruits (De Vries-Paterson et al., 1991; Prusky et al., 1997; Sitton & Patterson, 1992). Brown rot was not found in inoculation sites on sweet cherries in $10–30\%CO_2$ for 30 days at 0 °C (Tian et al., 2001). But CA with $70\%O_2$ was not significantly effective at controlling decay of sweet cherries (Fig. 2(C)). Amanatidou, Smid, and Gorris (1999) reported that exposure to 80–90 kPa O_2 generally did not inhibit microbial growth strongly, but caused a significant reduction in the growth rate of some of the microorganisms. When high O_2 or high CO_2 concentration was used alone, the inhibitory effect on microbial growth was highly variable. Stronger and much more consistent inhibition of microbial growth was obtained when the two gases were used in combination (Amanatidou et al., 1999).

3.5. Effects of different atmospheres on quality attributes

Firmness of sweet cherries stored in MAP slightly decreased with storage time. The fruits in CA conditions shown an increase in firmness after 20 days storage, and then the fruits in $5\%O_2 + 10\%CO_2$ still retained a higher degree of firmness than that in other conditions during all the periods (Fig. 4(A)). But the fruits in $70\%O_2 + 0\%CO_2$ showed a rapid decrease in firmness after 40 days, and showed browning at this time. CA with high CO_2 level more effectively maintained fruit firmness than did other treatments.

TSS of sweet cherry fruits was not significantly affected by the atmosphere treatments, and changed very slightly under all storage conditions (Fig. 4(B)). Many researchers have reported that CA with high CO_2 and low O_2 or with high O_2 atmospheres did not significantly

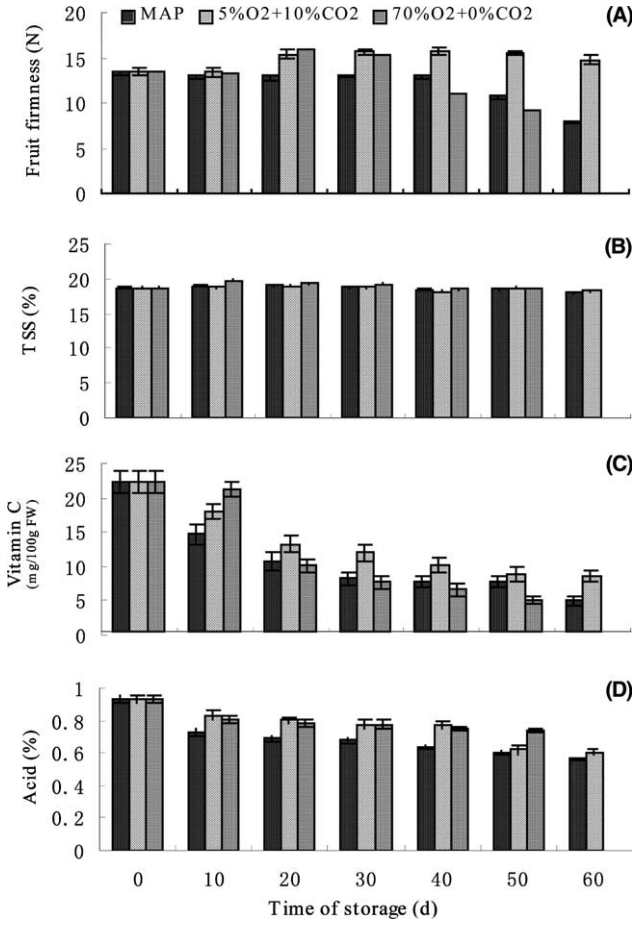

Fig. 4. Effects of different atmospheres on firmness, TSS, vitamin C and titratable acidity contents of sweet cherry kept at 1 °C during storage periods. Bars represent standard errors of the mean.

influence TSS of apples and pears (Ke et al., 1991), 'Bing' and 'Rainier' cherries (Neven & Drake, 2000) or longan (Tian et al., 2002).

Vitamin C contents decreased rapidly with storage time (Fig. 4(C)). Sweet cherries in 5%O_2 + 10%CO_2 had a relatively higher vitamin C content than that in other treatments ($P = 0.05$). But high O_2 atmosphere showed a beneficial effect in maintaining vitamin C content only before 10 days, after which it soon decreased. Titratable acidity of sweet cherries slightly decreased with storage time (Fig. 4(D)), and was significantly higher in CA-stored fruits than in MAP ($P = 0.05$). Although very little information was available on effects of elevated O_2 levels on contents of vitamins, minerals, acidity, and phytonutrients of fresh fruits and vegetables, Day (1996) found that high O_2 MAP had beneficial effects on the retention of ascorbic acid and the degree of lipid oxidation.

4. Conclusion

In comparison with MAP and CA with high O_2 concentration, CA with high CO_2 concentration more significantly inhibited activities of PPO and POD, reduced MDA content, effectively controlled flesh browning and delayed fruit senescence, as well as decreased fruit decay and extended storage life of sweet cherry fruit. The fruits in 5% O_2 plus 10% CO_2 showed higher degrees of firmness, and higher contents of vitamin C and titratable acidity than that in MAP or 70%O_2 plus 0%CO_2 after 60 days of storage. High O_2 level was significantly effective at inhibiting ethanol production in the flesh and reducing decay of sweet cherry fruits, but easily stimulated browning by high-O_2 injury. Soluble solids contents were not significantly affected by different atmosphere treatments.

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