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Responses of postharvest broccoli (*Brassica oleracea* L. var. *italica*) florets to controlled atmospheres with varying CO_2/O_2 levels at different temperature

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Summary

Controlled atmospheres (CA) have been widely used in postharvest storage of fruits and vegetables. The gas compositions of common CA are consisted of O_2, CO_2 and N_2, postharvest storage condition with gas combination of O_2 and CO_2 was rarely studied. In this research, the effects of CA with different CO_2/O_2 levels, i.e. 70% O_2 + 30 % CO_2, 60 % O_2 + 40 % CO_2, 50 % O_2 + 50 % CO_2, 40 % O_2 + 60 % CO_2, 30 % O_2 + 70 % CO_2, and air (control) on the storage life and properties of broccoli during storage were investigated at different temperatures including 0, 10 and 20 °C. Results showed that the storage period of air treatments at 0, 10 and 20 °C was 21, 12, and 4d. Treatments with 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C maximum inhibited respiration rate and ethylene production, maintain chlorophyll and ascorbic acid content, reduced the accumulation of acetaldehyde and ethanol, and extended the storage life of broccoli florets to 49, 31, and 14d compared to 21 d at 0 °C, 12 d at 10 °C and 4 d at 20 °C in air-treated broccoli. These results indicated that an appropriate CA with O_2/CO_2 might be a potential strategy for postharvest storage of broccoli heads, and the appropriate proportion of O_2 and CO_2 might vary with different temperature.

Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is a vegetable increasingly recognized as a nutritional source for vitamins, antioxidants, and anticarcinogenic compounds in the daily diet (PODSEDEK, 2007). However, broccoli is a highly perishable product, its sensory quality after harvest encounters a rapid yellowing and undesirable off-flavor under inappropriate storage conditions (DESCHENE et al., 1991). In recent years, various methods have been tested to extend the shelf life and improve postharvest quality of broccoli, such as refrigeration (TOIVONEN, 1997), heat treatment (TETSUYA et al., 2005), ethanol vapor (HAN, 2006), 1-methycyclopropene (1-MCP) application (YUAN et al., 2010), modified atmosphere packaging (SERRANO et al., 2006), and controlled atmospheres (CA) (FERNÁNDEZ-LEÓN et al., 2013). Moreover, controlled atmosphere (CA) storage is also considered an effective technique for maintaining postharvest quality of broccoli (THOMPSON, 2010), which inhibited respiration rate and ethylene production, delayed tissue turgidity, chlorophyll loss and undesirable compositional changes, reduced the incidence and severity of decay, and alleviated some physiological disorders (THOMPSON et al., 2010; EASON et al., 2007; TOIVONEN et al., 2004; FERNÁNDEZ-LEÓN et al., 2013).

Numbers of studies noted that appropriate proportions of O_2 and CO_2 in CA condition of broccoli varied temperature, which demonstrated indicating that CA of broccoli is temperature-dependent (TOIVONEN et al., 2004; CANTWELL et al., 1999; JONES et al., 2006). For example, for fresh broccoli packaging storage, the recommended condition was 18.8 % O_2 + 2.5 % CO_2 and 9.5 % O_2 + 4 % CO_2 at 4 and 10 °C, respectively (ANNELIE et al., 2004). In broccoli CA storage, the optimized gas concentration was 10 % O_2 + 5 % CO_2 at 1-2 °C (FERNÁNDEZ-LEÓN et al., 2013), and 2 % O_2 + 6 % CO_2 at 4 °C (PARADIS et al., 1996). Our previous study showed storage with higher concentrations of O_2 and CO_2 (40 % O_2 + 60 % CO_2) atmospheres at 15 °C prolonged storage life of broccoli by approximately 4 fold (GUO et al., 2013). However, a combined effect of higher levels of O_2/CO_2 and temperatures on storage life of broccoli has not been tested yet. The objective of this study was to establish the suitable CA conditions under different storage temperatures for extending storage life and maintaining quality of broccoli.

Materials and methods

Raw material

Two batches of broccoli florets (*brassica oleracea* L. var. *italica*) were harvested from field of Shouguang vegetable station, Shandong Province on 12 April 2012 and 15 April 2013, respectively. Healthy broccoli florets with diameter ranging from 13 to 15 cm were selected and stored in a walk-in cooler at 5 °C for pre-cooling for 4 h.

Experimental treatments

For each temperature treatment (0, 10 and 20 °C), pre-cooled broccoli florets were divided into 6 groups with 30 broccoli heads per group, and each group divided into 3 parts for replications with 10 broccoli heads per replication. Each group broccoli florets of 3 replicates were placed into three 0.5 m^3 sealable plastic containers, respectively, and each group of broccoli heads was connected to a constant flow (0.05 m^3 min^{-1}) of air (control), 70 % O_2 + 30 % CO_2, 60 % O_2 + 40 % CO_2, 50 % O_2 + 50 % CO_2, 40 % O_2 + 60 %CO_2, and 30 % O_2 + 70 % CO_2. The samples were stored at RH 80-90 % and 0, 10 and 20 °C, respectively, until the end of their storage periods. During storage, gas composition was regularly checked using an FBI-Dansensor CheckPoint O_2/CO_2 (MR-07825-00, FBI-Dansensor America Inc.). The broccoli heads in each treatment during storage were randomly selected for evaluation of storage period and physiological attributes as described below.

Storage period

The end of storage period of broccoli heads was designated as the time when approximately 30 % yellow coloration appeared on the surface of the product (YUAN et al., 2010; XU et al., 2006) or off-flavor occurred (JORGE et al., 1995; DANK et al., 1999).

Determination of respiration rate and ethylene production

Three broccoli heads from each treatment, were firstly kept at their storage temperature for 8 h after removal from CA conditions in order to eliminate interference of treatments on internal gases composition, and then individually sealed in a 3.86-L plastic container equipped with septa for 1 h at 0, 10, or 20 °C, according to their storage temperature. Five milliliters of headspace gas was withdrawn from each container, and CO_2 and C_2H_4 levels were detected

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using a gas chromatograph (Varian CP-3800, Agilent Technologies, Lexington, MA, USA) equipped with a thermal conductivity detector (TCD, CO_2) and a hydrogen flame ionization detector (FID, ethylene). The temperature of column oven, TCD, FID and injector were set to 50, 130, 150 and 180 °C, respectively. Respiration rate and ethylene production were expressed as mg kg^{-1} h^{-1} and μL kg^{-1} h^{-1}, respectively.

Determination of acetaldehyde and ethanol contents

Acetaldehyde and ethanol levels were measured with static headspace gas chromatography (Varian CP-3800, Agilent Technologies, Lexington, MA, USA) following the method of JI et al. (2010) and the FID was set at 250 °C. Acetaldehyde and ethanol contents were both expressed as mg kg^{-1}.

Determination of chlorophyll content and yellowing rate

Chlorophyll content was measured according to MORAN (1982) with slight modifications. Briefly, 0.1 g of broccoli florets were ground with 10 mL ethanol (95 %), and then extracted in the darkness for 24~36 h until broccoli tissue became white. The supernatant was measured at 652 nm with UV-1750 spectrophotometer (Shimadzu). The total chlorophyll content was expressed as mg kg^{-1} FW.

Yellowing rate was measured according the surface yellow area of treated broccoli heads. Ten broccoli heads from each treatment were pictured using canon camera (Canon PC1468, Canon Inc., Japan), and the average yellowing rate of each treatment was calculated using Photoshop software (Adobe Photoshop CS5 software package).

Determination of ascorbic acid (AA) contents

Ascorbic acid (AA) content was analyzed with dinitrophenylhydrazine (DNPH) method (TERADA et al., 1978). Briefly, 0.5 g broccoli florets of each treatment was homogenated with 20 mL mixture acid (6 % metaphosphoric acid cantaining 2 mol L^{-1} acetic acid) and then centrifuged at 15,000 × g for 20 min. Thereafter, the supernatant was filtrated through filter paper. One mL of filtrated supernatant was mixed with 0.05mL of 0.2 % 2, 6-dichlorophenolindophenol and then maintained at room temperature for 1 h. One mL of 2 % thiourea and 0.5 mL of 2 % DNPH were added to the mixture and then heated at 60 °C for 3 h. 2.5 mL of 95 % H_2SO_4 was added to the mixture and cooled on the ice prior to reading the absorbance at 540 nm. Ascorbic acid content was expressed as g kg^{-1} FW.

Statistical analyses

These experiments were conducted on a completely randomized design, and each treatment comprised three replicates. Data were analyzed using SPSS 13.0 statistical software and significant differences among treatments were determined by least significant differences (LSD) test at $P \leq 0.05$.

Results

Storage period

Combinations of O_2/CO_2 prolonged the storage period of broccoli florets heads under different temperature (Tab. 1). Treatments of 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C showed maximum efficacy, by which the storage periods of broccoli heads were extended to 49, 31, and 14 d, respectively, compared to the storage period of air treatments at 0 °C for 21 d, 10 °C for 12 d and 20 °C for 4 d.

In common conditions, broccoli heads treated with higher O_2 such as 70 % O_2 + 30 % CO_2 behaved as yellow surface, while treated with higher CO_2 such as 30 % O_2 + 70 % CO_2 behaved as off-flavors.

Tab. 1: Storage period of broccoli florets treated with varied O_2/CO_2 concentration under different temperature. Different little letters indicates that there is significant difference among treatments.

Treatments	Storage period (d) Storage temperature (°C)		
	0	10	20
70 % O_2 + 30 % CO_2	35c	8f	3e
60 % O_2 + 40 % CO_2	49a	28b	8c
50 % O_2 + 50 % CO_2	42b	31a	10b
40 % O_2 + 60 % CO_2	35c	24c	14a
30 % O_2 + 70 % CO_2	21e	20d	8c
Air	28d	12e	4d

Respiration rate and ethylene production

Respiration rate in air and 70 % O_2 + 30 % CO_2-treated broccoli heads rapidly increased with the peaks under 0, 10, and 20 °C appeared at 14, 8, and 2 d, respectively (Fig. 1). Among treatments, 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C, strongly suppressed respiration rate, which was maintained at a relatively lower level compared to that in other treated broccoli.

Ethylene productions of all treatments were in parallel with the changes of respiration rate (Fig. 2). Broccoli treated with 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C, showed a lowest and steady level of ethylene production.

Acetaldehyde and ethanol contents

Overall, acetaldehyde and ethanol contents in all broccoli florets showed a gradual increase trend with extension of storage time (Fig. 3, Fig. 4). Differently, among all treatments, acetaldehyde and ethanol contents in broccoli florets treated with 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C, showed a the lowest level during whole storage (Fig. 3, Fig. 4).

Chlorophyll content and yellowing rate

Chlorophyll contents of all treated broccoli florets declined during storage time, and lower temperature optimum delayed the chlorophyll loss (Fig. 5). For example, during 0-14 d of storage time, the chlorophyll content of broccoli florets treated with 40 % O_2 + 60 % CO_2 declined by 6.0 % compared to 13.8 % and 16.4 % at 0,10 and 20 °C, respectively. In general, the chlorophyll content of broccoli florets treated with 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C, showed the lowest decline trend, compared with the other treatments at their respective storage temperature. Yellowing rate of broccoli florets of all treatments showed a reverse trend of that of chlorophyll content (Fig. 5 and 6).

Ascorbic acid (AA) contents

Changes of AA content of broccoli florets were familiar with chlorophyll content (Fig. 5 and 7). Among the treatments of their temperature, the least loss of AA content of broccoli florets was 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C. Furthermore, lower temperature could significantly delay the loss of AA content. During 0-14 d of storage time, the AA content of broccoli florets treated with 40 % O_2 + 60 % CO_2 at 0, 10, and 20 °C, was declined by 5.7 %, 13.6 %, and 19.0 %, respectively.

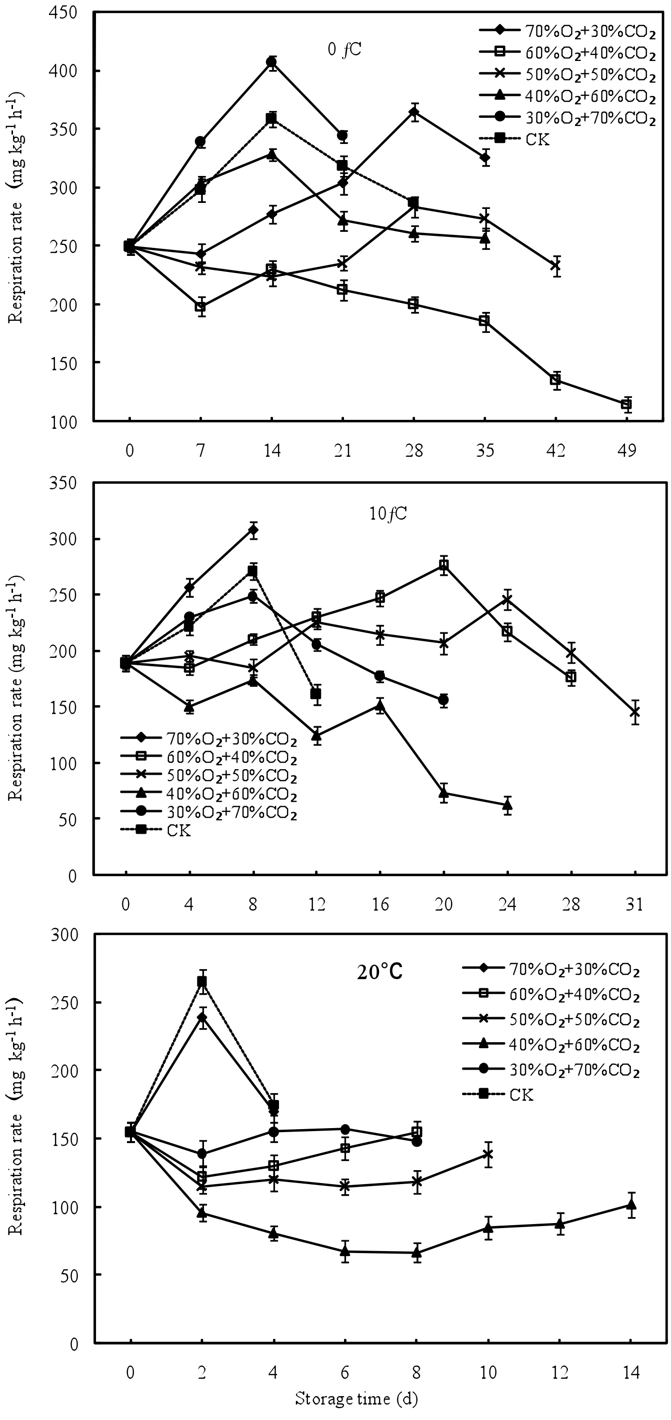

Fig. 1: Respiration rate of broccoli heads treated with varying O_2/CO_2 levels at different temperature. Vertical bars indicate the standard deviations for each treatment (n=5). Air treatment was used as control.

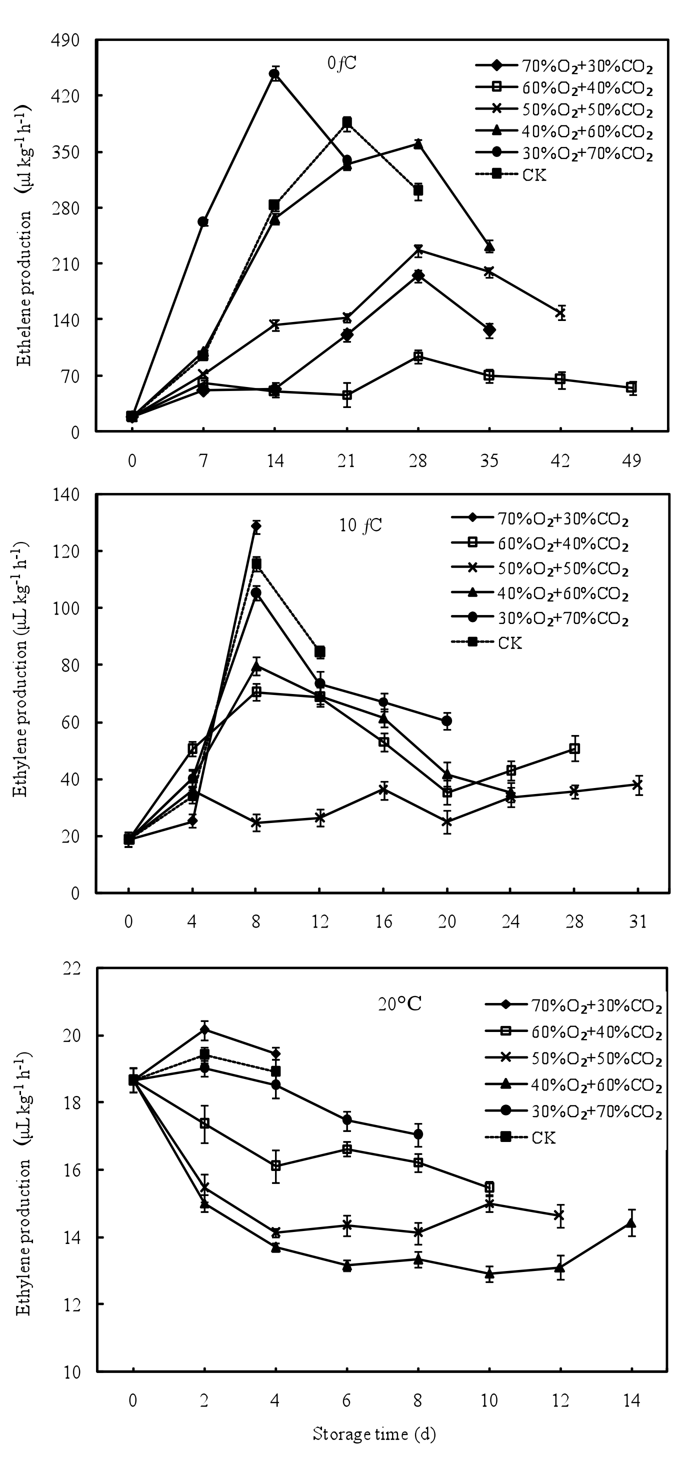

Fig. 2: Ethylene production of broccoli heads treated with varying O_2/CO_2 levels at different temperature. Vertical bars indicate the standard deviations for each treatment (n=5). Air treatment was used as control.

Discussion

The optimal atmospheres proportion in CA storage of broccoli was different when storage temperature was diverse. It has been reported that the proper gas concentration was 10 % O_2 + 5 % CO_2 at 1-2 °C (Fernández-León et al., 2013), while combination of 2 % O_2 and 6 % CO_2 was the most appropriate for broccoli preservation when stored at 4 °C (Paradis et al., 1996). High O_2 (30 %-80 %) has a favorable effect on preservation of fruits and vegetables, which could extend shelf life of 'Yaoshan' pears (Li et al., 2012), and high CO_2 could inhibit the accumulation of off-flavor substances during iceberg lettuce storage (Heimdal et al., 1995). However, adverse effects also were found in other postharvest crops. For instance, 60-100 % O_2 treatment accelerated the decay of Chinese bay-berry, strawberry and blue berry (Zheng et al., 2008), and increased the loss of green color and chlorophyll pigments (Laxman et al., 2012). For high CO_2 treatments, studies of Yasutaka (Yasutaka et al., 1990) showed that treatment with 20 % O_2 + 60 % CO_2 + 20 %

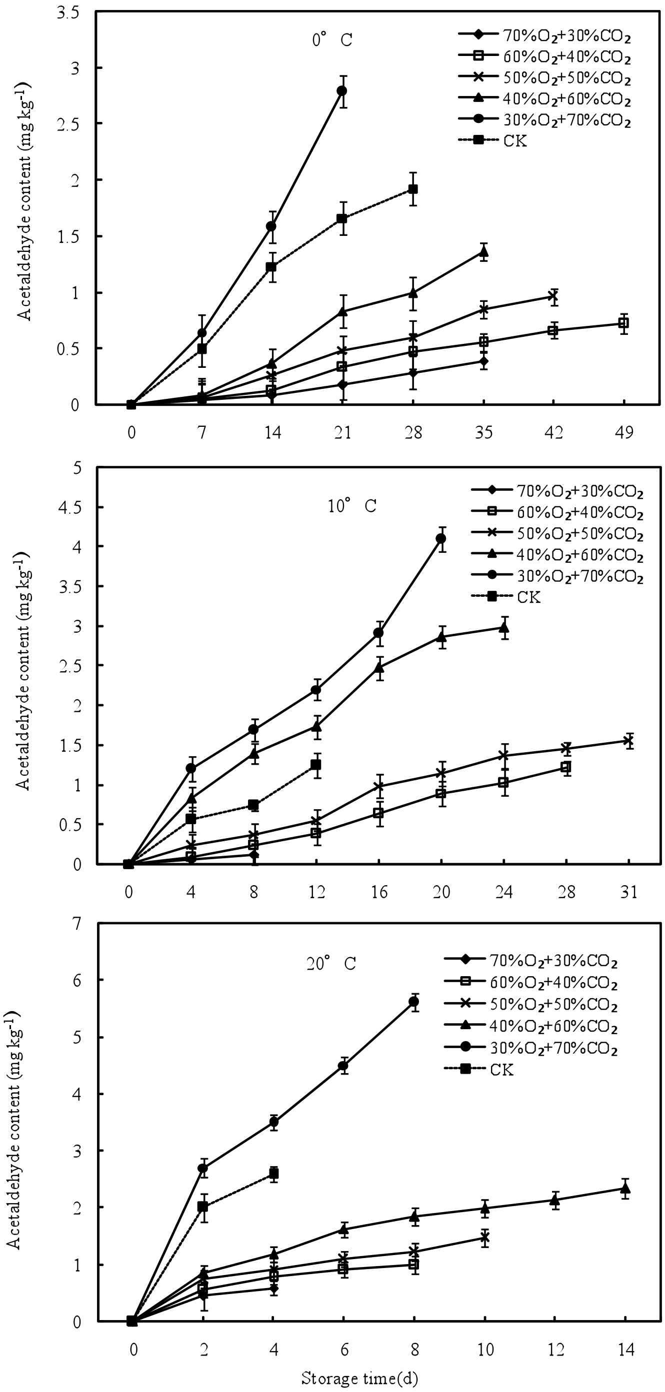

Fig. 3: Acetaldehyde contents of broccoli heads treated with varying O_2/CO_2 levels at different temperature. Vertical bars indicate the standard deviations for each treatment (n=5). Air treatment was used as control.

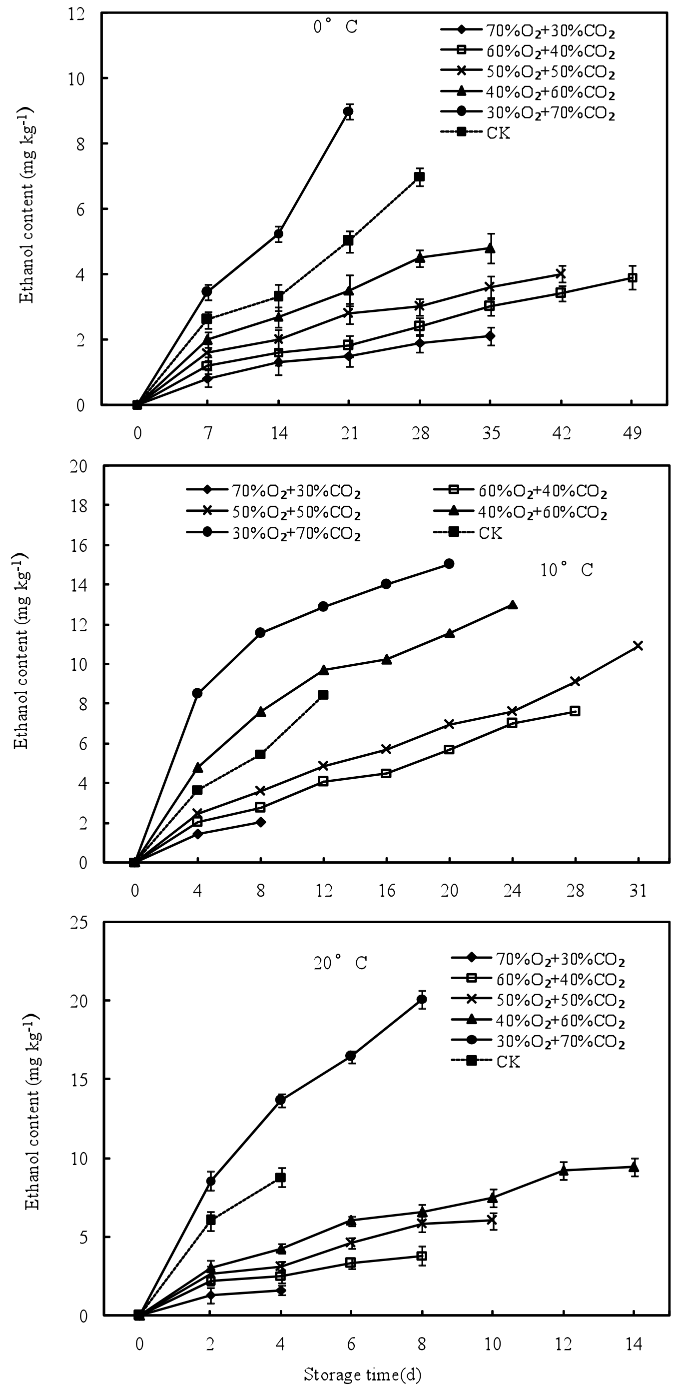

Fig. 4: Ethanol contents of broccoli heads treated with varying O_2/CO_2 levels at different temperature. Vertical bars indicate the standard deviations for each treatment (n=5). Air treatment was used as control.

N_2 for 24 h significantly reduced respiration rate and ethylene production, and delayed off-flavor and decay in broccoli, tomato, and peach. However, KADER (1995) pointed out that inappropriate high CO_2 might result in CO_2 injury or physiological disorder such as off-flavor, which involved in the accumulation of acetaldehyde, ethanol and a tiny part of other volatiles (JAMES et al., 2000; KE et al., 1990). Present study exhibited that high proportion of O_2/CO_2 such as 70 % O_2 + 30 % CO_2 accelerated chlorophyll loss and yellowing. On the other side, extreme high CO_2 in CA condition such as 30 % O_2 + 70 % CO_2 rapidly accumulated acetaldehyde and ethanol, indicating that inappropriate high CO_2 could result in off-flavor of broccoli florets. Only appropriate combinations of O_2 and CO_2 including 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C, provided the most effective ways to maintain quality and reduce off-flavor, as indicated by reductions in chlorophyll loss and the acetaldehyde and ethanol. Results implied that the similar proportioning with high levels of O_2 and CO_2 in CA storage under a wide range of temperature (0-20 °C) might be an appropriate strategy for broccoli storage.

In conclusion, the optimum proportion of O_2 and CO_2 to the storage

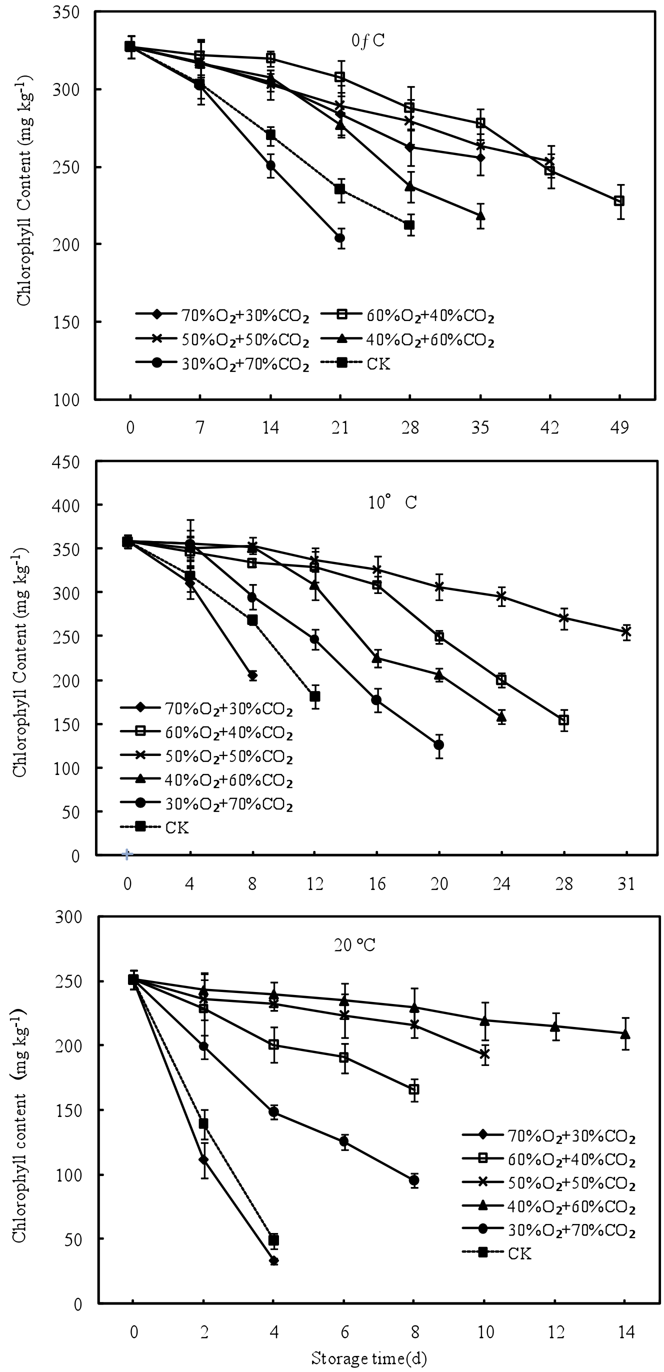

Fig. 5: Chlorophyll content of broccoli heads treated with varying O_2/CO_2 levels at different temperature. Vertical bars indicate the standard deviations for each treatment (n=5). Air treatment was used as control.

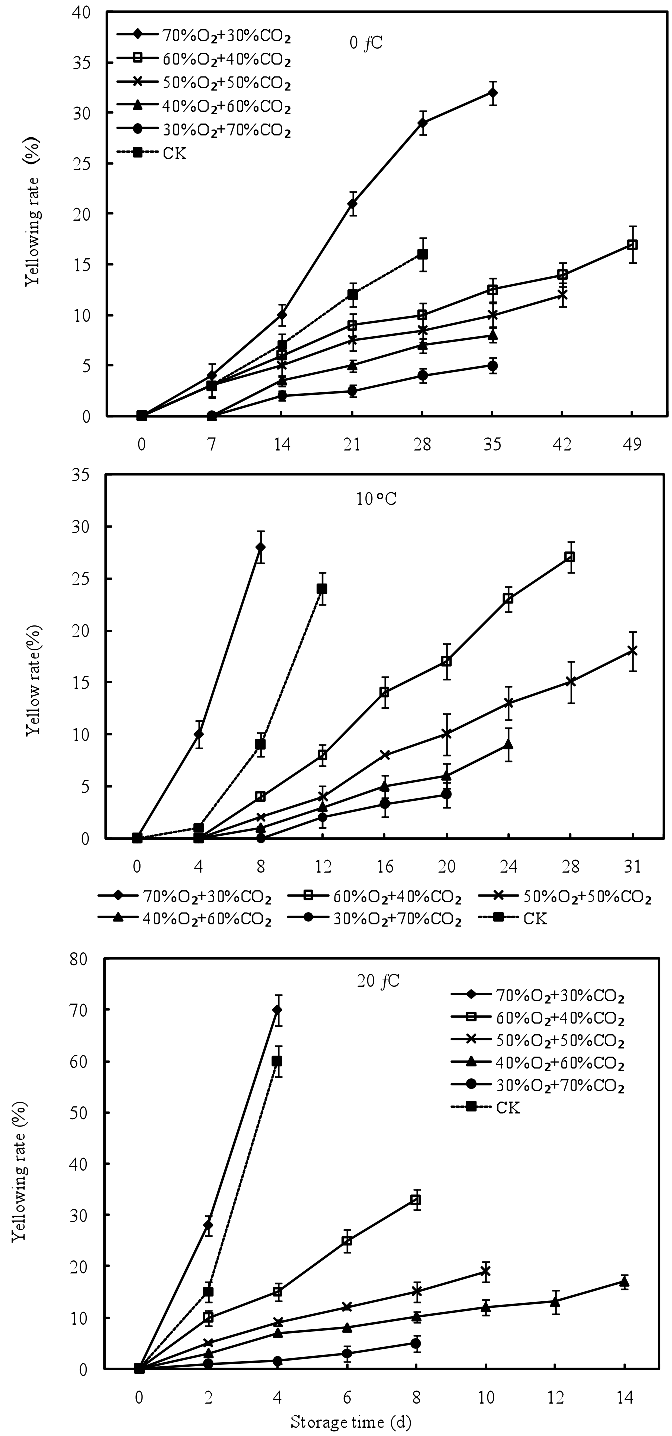

Fig. 6: Yellowing rate of broccoli heads treated with varying O_2/CO_2 levels at different temperature. Vertical bars indicate the standard deviations for each treatment (n=5). Air treatment was used as control.

and of broccoli varied with temperature. The best treatments of O_2/CO_2 were found to be 60 % O_2 + 40 % CO_2 at 0 °C, 50 % O_2 + 50 % CO_2 at 10 °C, and 40 % O_2 + 60 % CO_2 at 20 °C. These treatments could inhibit respiration rate and ethylene production, maintain chlorophyll and ascorbic acid content reduce the accumulation of acetaldehyde and ethanol, and extended the shelf life of broccoli to 49, 31, and 14, respectively. Furthermore, temperature played a critical factor on the preservation period of broccoli florets, with treatment of 60 % O_2 + 40 % CO_2 at 0 °C showed the best efficacy in extending storage life among all treatments at 10 and 20 °C.

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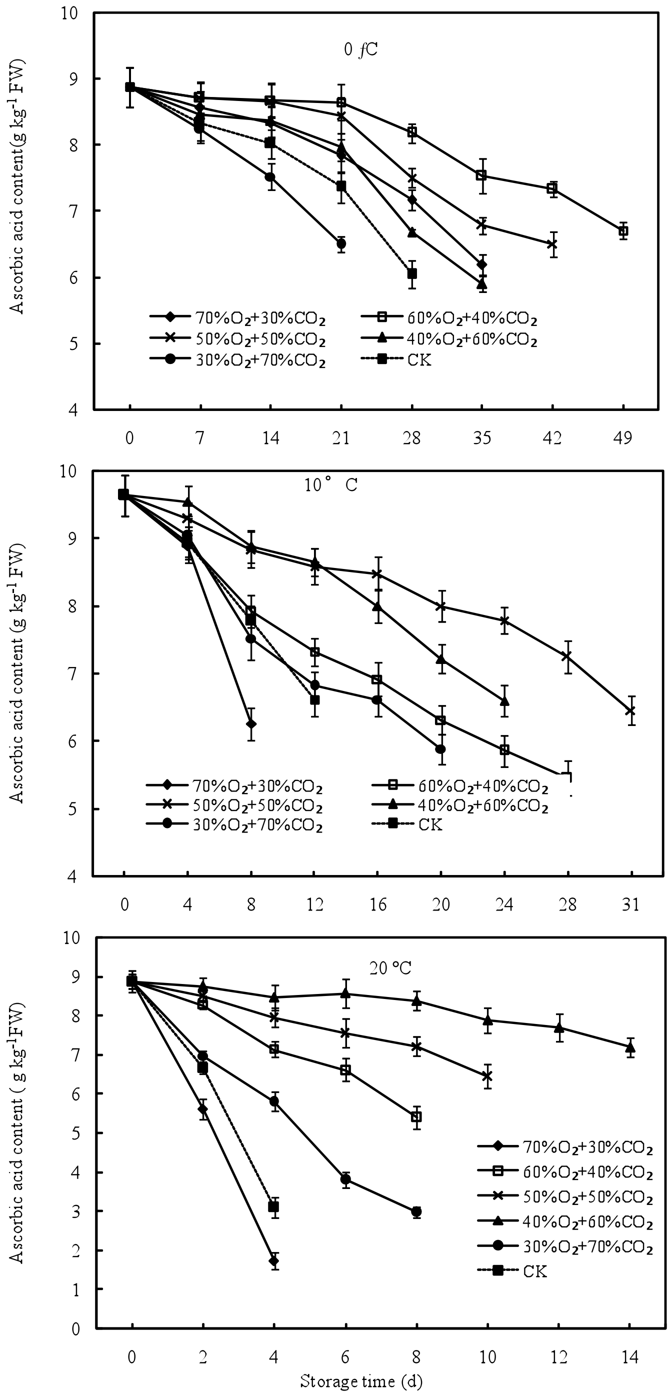

Fig. 7: Ascorbic acid contents of broccoli heads treated with varying O_2/CO_2 levels at different temperature. Vertical bars indicate the standard deviations for each treatment (n=5). Air treatment was used the control.

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