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Respiratory pathway metabolism and energy metabolism associated with senescence in postharvest Broccoli (*Brassica oleracea* L. *var. italica*) florets in response to O_2/CO_2 controlled atmospheres

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ABSTRACT

Broccoli is a highly perishable vegetable with abundant nutritional and medical value. Recent studies indicated that O_2/CO_2 controlled atmospheres (CA) was an effective method for broccoli storage.

In this study, ATP (adenosine triphosphate) content, energy charge level, respiratory pathway and their key enzymes of broccoli (*Brassica oleracea* L. var. *italica*) were investigated, employed CA treatments of 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 at 10 °C. The 50% O_2 + 50% CO_2 treatment led to an obvious decrease in respiration rate of broccoli and it could inhibit the reduction of ATP level, energy charge level, SDH (succinic dehydrogenase), CCO (cytochrome oxidase), and boost the G-6-PDH (glucose-6-phosphate dehydrogenase) + 6-PGDH (6-phosphogluconate dehydrogenas) activity. Treatment of 50% O_2 + 50% CO_2 suppressed reduction of the TCA (tricarboxyfic-acid-cycle) and CCP (cytochrome oxidase pathway) rate, and increased the HMP (phosphopentose pathway), while had little impact on the EMP (embden-meyerhof-parnas) rate and PGI (phosphohexose isomerase) activity. Compared to 50% O_2 + 50% CO_2 treatment, treatment of 30% O_2 + 70% CO_2 had an opposite effect on broccoli. Results indicated that O_2/CO_2 controlled atmospheres could alter the proportion of respiratory pathway, and 50% O_2 + 50% CO_2 treatment could enhance the endurance capability of broccoli to high CO_2 concentration according to the changes of respiratory pathway and delay in its senescence though relatively higher ATP.

1. Introduction

Respiration plays an important role in metabolism of high plants (Taiz and Zeiger, 2009). In postharvest fruits and vegetables, respiration contributes energy and intermediates in maintaining life activities and physico-chemical reactions (Liang et al., 1998). Meantime, respiration consumes metabolic substances which lead to the senescence of fruits and vegetables.

Respiration of plants includes several pathways, such as embden-meyerhof- parnas (EMP), phosphopentose pathway (HMP or PPP), tricarboxyfic-acid-cycle (TCA), mitochondrial electron transport with cytochrome pathway (CCP) (Alisdair et al., 2004), and each pathway has peculiar functions in life activities.

Research results showed that respiratory pathway and respiratory intensity of plants varied with the changes of external environment and different treatment (Zhao et al., 2012). Two layer shading treatment could reduce the EMP rate of dormant large cherry buds, while increase the TCA and HMP pathway (Li et al., 2005). 6-Benzyladenine and gibberellins spraying could increase the respiratory intensity, the proportion of TCA and HMP in nectarine blossom buds, while had little effect on the proportion of EMP (Yang, 2004).

Recent studies have shown that senescence of postharvest plants is often associated with a high respiration and a low energy. There was a negative correlation between the amounts of ATP extent and senescence of longans, litchi, pears and apples (Jiang et al., 2007; Yi et al., 2010).

Broccoli is a perishable vegetable that is increasingly recognized as a nutritional source for vitamins, antioxidants, and anti-carcinogenic compounds in the daily diet (Podsedek, 2007). Many

Abbreviations: ATP, adenosine triphosphate; EMP, embden meyerhof-parnas; TCA, tricarboxyfic-acid-cycle; PPP or HMP, phosphopentose pathway; 6-P-F, fructose-6-monophosphate; SDH, succinic dehydrogenase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenas; CCO, cytochrome oxidase; DMPD, dimethyl-*p*-phenylenediamine; PGI, phosphohexose isomerase; CA, controlled atmospheres.

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methods of controlled atmospheres (CA) were used for broccoli storage. 10% O_2 + 5% CO_2 at 1–2 °C (Fernandez-Leon et al., 2013a) and 2% O_2 + 6% CO_2 at 4 °C (Paradis et al., 1996) were both available for broccoli storage, which could extend the shelf life to 21 d and 35 d, respectively. Our previous study showed that treatments of 60% O_2 + 40% CO_2 at 0 °C, and 50% O_2 + 50% CO_2 at 10 °C, prolonged storage life of broccoli by approximately 49 d, 31 d respectively (Li et al., 2014), which could reduce the respiration and ethylene level, and mediate the micro-environment of active oxygen metabolism in broccoli. However, there is little information available about the O_2/CO_2 CA storage in respiratory metabolism especially about respiration pathway and energy.

In this study, we aimed to utilize O_2/CO_2 CA to investigate the changes of ATP content, energy charge level, respiration rate, EMP, TCA, HMP, and CCP pathway and their key enzymes of broccoli during storage at 10 °C. The objective of this article is to elaborate the feasibility of O_2/CO_2 CA for broccoli storage through respiration metabolism and energy metabolism.

2. Material and methods

2.1. Plant materials

Broccoli (*Brassica oleracea* L. var. *italica*) heads were freshly harvested from Shouguang Vegetable Station in Shandong Province on 15 April 2014. The broccoli heads were individually sealed in polyethylene bag (35 × 30 cm, 0.35 mm thick) with small holes and immediately transported to the laboratory of Shandong University of Technology. Before experiments, broccoli heads were pre-cooled at 5 °C for 4 h in a walk-in cooler. Broccoli heads with uniform size in 15–18 cm diameter and 0.5–0.6 kg without pest and mechanical injury were selected for experiments.

2.2. Postharvest treatments

Broccoli heads were disinfected with 1% (v/v) sodium hypochlorite for 2.5 min, then rinsed with tap water and air-dried. Broccoli heads were randomly divided into 6 groups with 45 broccoli heads per group, and each group divided into 3 parts for replications with 15 broccoli heads per replication. Each group broccoli heads of 3 replications were respectively placed into three 0.5 m^3 sealable containers, 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, respectively. Energy, respiration pathway and enzymes were determined using 3 broccoli heads from each group every 4 d, and a number of florets from 3 broccoli heads were collected for these parameters measurements. Fresh weight of broccoli was used for measuring parameters. During storage, the gases concentration was regularly checked using an FBI-Dansensor CheckPoint O_2/CO_2 (MR-07825-00, FBI-Dansensor America Inc.). Broccoli heads were stored at 10 °C and 80–90% relative humidity (RH).

2.3. 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 (Maria del et al., 2011; Dank et al., 1999).

2.4. Determination of ***contents of*** ATP and energy charge

Extractions and assays of ATP, ADP and AMP were conducted using the procedure describing by Ozogul et al. (2000). Briefly, 3 g broccoli florets were ground in liquid nitrogen, followed by homogenized with 25 mL of 0.6 mol L^{-1} perchloric acid. The homogenate was centrifuged at 16,000 × *g* for 12 min at 4 °C. 1 mol L^{-1} KOH was added into 10 mL of filtered supernatant quickly, neutralizing to pH 6.5–6.8. The solution was filtered through a 0.45 μm filter and then used for ATP, ADP and AMP measurements. Waters 600 analytical high performance liquid chromatography (HPLC, Waters Corporation, USA) equipped with an UV (ultraviolet) detector and a Megres™ C18 column (4.6 mm × 250 mm) was used at 254 nm. HPLC separation was achieved using continuous gradient elution by the method of Liu et al. (2006). ATP, ADP and AMP in broccoli samples were identified by comparison with retention times of standards. Amounts of ATP, ADP and AMP were calculated according to the external standard program. Energy charge was calculated by [ATP + 1/2ADP]/ [ATP + ADP + AMP].

2.5. Determination of the respiration rate and the proportion of four types of respiration pathways

Liquid-phase oxygen measurement system (Chlorolab-2, Hansatech Company, UK) was employed to detected respiration rate and the proportion of four types of respiration pathways. Meantime, liquid-phase oxygen measurement system equipped with constant temperature flow device could maintain the certain temperature at 10 °C to confirm the results accuracy of respiration rate. Briefly, 1 g broccoli florets was sampled from 3 broccoli heads with approximate 1.0 mm diameter and put into reaction cup with 2.5 mL deionized water and kept 3 min for temperature balance, then the total decreasing rate of respiration was tested, the value marked as Rt. Thereafter, 0.1 mL of 0.1 mol L^{-1} sodium fluoride (NaF) was added to the reaction cup and the decreasing rate of respiration was tested for calculating EMP proportion, the value marked as Ve. Similarly, 0.1 mL of 0.1 mol L^{-1} Na_3PO_4, 0.1 mL of 0.5 mol L^{-1} malonic acid and 0.1 mL of 0.1 mol L^{-1} NaN_3 were added to the reaction cup, for HMP, TCA and CCP measurement, respectively. The HMP, TCA and CCP values were marked as Vh, Vt, and Vc, respectively. The total respiration rate was calculated by the depletion of O_2 in certain time and expressed as μmol O_2 min^{-1} g^{-1}. The proportion of EMP, HMP, TCA and CCP were expressed as Pe, Ph, Pt and Pc, and their propotions were calculated as following, $Pe = (Rt - Ve)/Rt \times 100\%$, $Ph = (Rt - Vh)/Rt \times 100\%$, $Pt = (Rt - Vt)/Rt \times 100\%$, and $Pc = (Rt - Vc)/Rt \times 100\%$, respectively.

2.6. Determination of phosphohexose isomerase (PGI) activity

The PGI activity is reflected by the content of substrate 6-P-F (Zhang, 2008). The activity of PGI was measured using the procedure describing by Brown and Wary (1968). In brief, 1 g broccoli florets was ground in an ice bath with 5 mL Tris–HCl buffer (0.05 mol L^{-1}, pH 7.4), followed by centrifugation at 5000 × *g* for 30 min and filtration. 0.5 mL of filtered supernatant, 1.0 mL of 10 mmol L^{-1} glucose-6-monophos phate (6-P-G) were mixed and maintained at 30 °C for 5 min. Thereafter, 2 mL 10% trichloroacetic acid was added to the mixture and centrifuged at 5000 × *g* for 30 min. One milliliter of the supernatant, 6 mL of 3% HCl, and 2 mL of 0.1% resorcinol were mixed and maintained at 80 °C for 8 min. The optical density was measured at 520 nm with UV-1750 spectrophotometer (Shimaozu Co., LTD., Suzhou, China). 6-P-F content was expressed as mg g^{-1}.

2.7. Determination of succinic dehydrogenase (SDH) activity

The measurement of SDH activity was carried out according to the method of Zhang (2008). An enzyme solution was prepared with 0.4 mol L^{-1} sucrose, phosphate buffer (0.1 mol L^{-1}, pH 7.7), 0.1% resorcinol, and 0.01 mol L^{-1} ethylenediamine tetraacetic acid

Table 1
Storage period of broccoli florets treated with varied O_2/CO_2 concentration at 10 °C. Different little letters indicates that there is significant difference among treatments. Significant differences among treatments were determined by LSD test at $P \leq 0.05$. Each mean value represents $n = 8$.

Treatments	Storage period (d) (10 °C)
70%O_2 + 30%CO_2	8[f]
60%O_2 + 40%CO_2	28[b]
50%O_2 + 50%CO_2	31[a]
40%O_2 + 60%CO_2	24[c]
30%O_2 + 70%CO_2	20[d]
Air (CK)	12[e]

disodium salt (EDTA). A reaction liquid was prepared with 10 mL 0.1% gelatin, 2 mL of 0.5 mg L^{-1} 2, 6-dichloroinphenol, 2 mL of 3 mg L^{-1} phenazine methosulfate, and 3 mL of 0.1 mol L^{-1} phosphate buffer. One gram of broccoli florets were ground in an ice bath with 5 mL Enzyme solution and centrifuged at 10,000 × *g* for 15 min at 0 °C, and the deposition was dissolved with 5 mL of 0.5 mol L^{-1} Tris buffer for SDH activity measurement. 0.1 mL dissolved solution, 1.7 mL reaction liquid, and 0.4 mL of 0.2 mol L^{-1} succinic acid sodium, were added to tubes and kept at 37 °C for 15 min. Thereafter, 0.3 mL of 0.25 mol L^{-1} HCl was added for ceasing reaction. The absorbance was determined at 600 nm and the SDH activity was expressed as nkat mg^{-1} p.

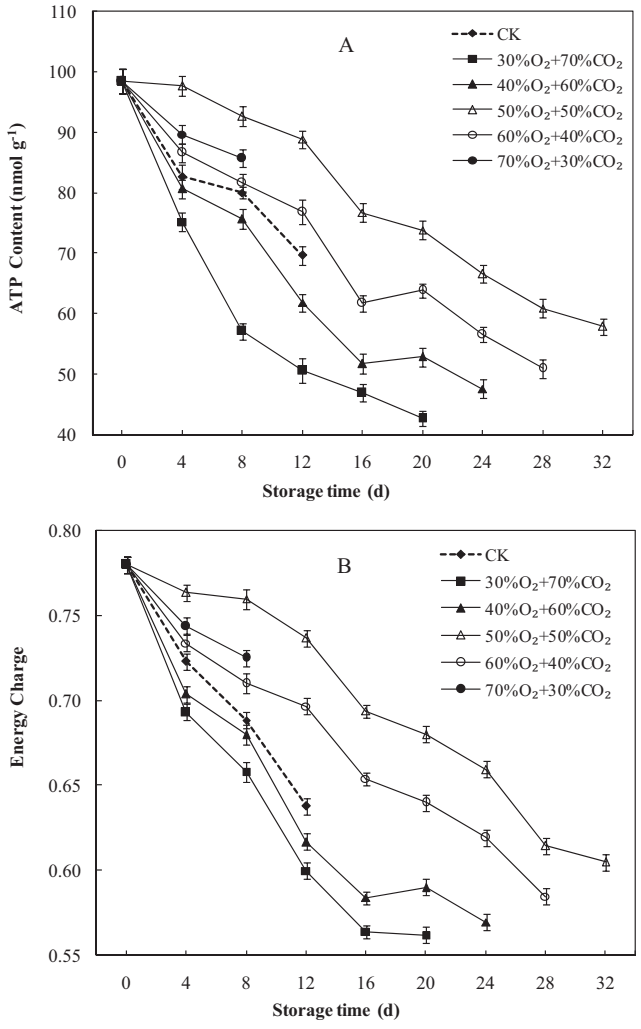

Fig. 1. ATP content and energy charge level of broccoli heads treated with varying O_2/CO_2 levels at 10 °C. Vertical bars indicate the standard deviations for each treatment. Air treatment was used as control. Energy charge was calculated by [ATP + 1/2ADP]/ [ATP + ADP + AMP].

2.8. De termination of total activity of glucose-6-phosphate dehydrogenase (G-6-PDH) + 6-phosphogluconate dehydrogenas (6-PGDH)

The activity of G-6-PDH + 6-PGDH was carried out following the description of Brown and Wary (1968). In brief, 1 g broccoli florets was ground on ice bath with 5 mL pre-cooled potassium phosphate buffer (0.05 mol L^{-1}, pH 6.8, contained 0.25 mol L^{-1} sucrose, 0.005 mol L^{-1} EDTA, 1 mg L^{-1} bovine serum albumin). Thereafter, the ground mixture was centrifuged at 10,000 × *g* for 15 min and the supernatant was discarded. Five milliliter of 0.05 mol L^{-1} pH 7.6 Tris–HCl buffer was added to the deposition, and 0.1 mL dissolved solution was pipetted to 0.9 mL reaction solution (containing 5 mmol L^{-1} 6-P-G, 5 mmol L^{-1} $MgCl_2$, 5 mmol L^{-1}, pH 7.4 Tris–HCl). The absorbance was immediately recorded at 340 nm and the total activities of G-6-PDH + 6-PGDH were expressed as μmol NADP g^{-1} min^{-1}.

2.9. Determination of cytochrome oxidase (CCO) activity

The activity of CCO was measured using the procedure describing by Huang and Wang (1984). Samples of 3 g broccoli florets were ground in an ice bath with 7.5 mL of 0.05 mol L^{-1} pH 7.4 phosphate buffers, then centrifuged at 3000 × *g* for 10 min at 4 °C and filtrated. 0.3 mL of filtered supernatant, 0.04% cytochrome C, and 3 mL double distilled water were mixed and preheated at 37 °C for 2 min. Thereafter, 0.3 mL of 0.4% dimethyl-*p*-phenylenediamine (DMPD) was added and maintained at 37 °C for 3 min until red color appeared. 0.1 mol L^{-1} HCl was used for adjusting pH to 5.6–6.0. Finally, 12.6 mL mixture containing tetrachloroethylene and absolute ethyl alcohol (the ratio was 1:3) was added and absorbance was measured at 510 nm. The CCO activity was expressed as nkat g^{-1}.

2.10. Statistical analyses

Experiments were performed with a random way and data expressed as mean standard deviation. Data were analyzed using

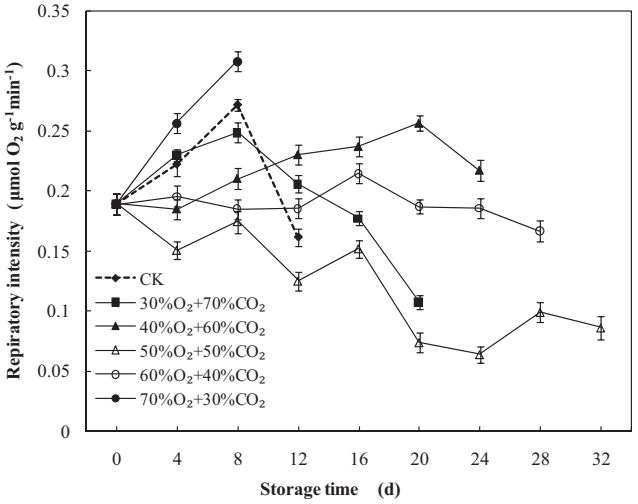

Fig. 2. Respiration rate of broccoli heads treated with varying O_2/CO_2 levels at 10 °C. Vertical bars indicate the standard deviations for each treatment. Air treatment was used as control.

SPSS 13.0 statistical software and significant differences among treatments were determined by LSD test at $P \leq 0.05$.

3. Results

3.1. Storage period

Proper combinations of O_2/CO_2 CA prolonged the storage period of broccoli heads (Table 1). Compared to 12 d storage period of air treatment, broccoli heads treated with 50% O_2 + 50% CO_2, 60% O_2 + 40% CO_2, and 40% O_2 + 60% CO_2 extended their shelf life to 31 d, 28 d and 20 d, respectively.

3.2. Contents of ATP and energy charge level

ATP contents and energy charge level of all treatments decreased as storage time progressed (Fig. 1). They decreased rapidly within the beginning of storage time and thereafter declined slightly. Compared to others, the ATP contents and energy charge level of 50% O_2 + 50% CO_2 treatment declined more slowly and exhibited higher values during postharvest storage.

3.3. Respiration rate

The changes of respiration rate of all treatments showed similar treads during storage period, all increased at early storage and then decreased (Fig. 2). Respiration rate of 50% O_2 + 50% CO_2 treatment showed a lower and stable level, while that of 30% O_2 + 70% CO_2 and CK treatments rose dramatically at the beginning of storage time (Fig. 2). However, respiration rate of broccoli heads treated with 40% O_2 + 60% CO_2 increased significantly during the later storage period.

3.4. EMP pathway and 6-P-F content

The changes of EMP pathway in broccoli heads varied with different treatments (Fig. 3A). Treatments of 30% O_2 + 70% CO_2 and 70% O_2 + 30% CO_2 led to a significant rise of the proportions of EMP pathway during stage, and their average values were 23.42% and 23.11%, respectively. Compared with CK, the EMP proportion of 50% O_2 + 50% CO_2 and 40% O_2 + 60% CO_2 showed lower and stable level during the whole storage, the average proportions were 17.31% and 15.90%, respectively.

The changes of 6-P-F contents with all treatments were roughly similar to those of EMP pathway proportion during the whole

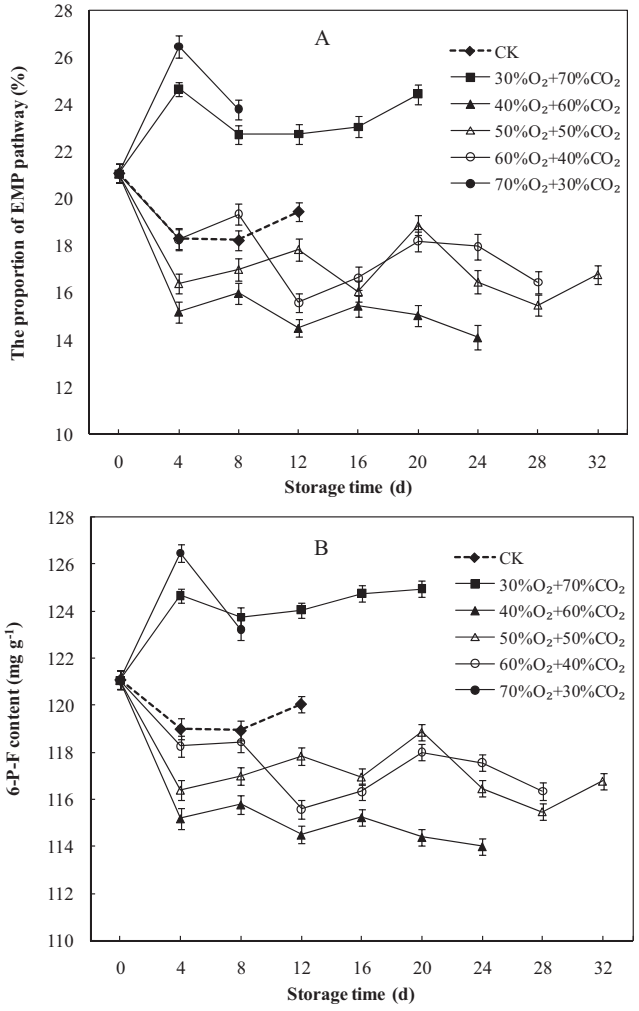

Fig. 3. EMP pathway and 6-P-F content of broccoli heads treated with varying O_2/CO_2 levels at 10 °C. Vertical bars indicate the standard deviations for each treatment. Air treatment was used as control. The EMP proportion was calculated as below: Pe = (Rt − Ve)/Rt × 100%. Here, Pe means the EMP proportion, Rt means the total respiration rage, Ve means the respiration rate of EMP which was determined by adding 0.1 mol L^{-1} NaF to reaction cup.

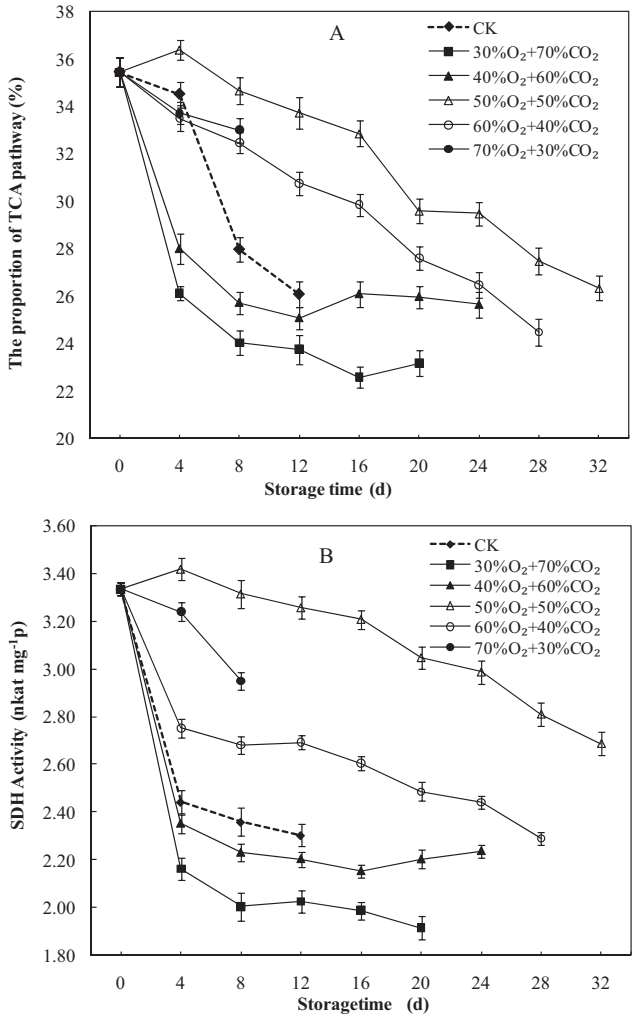

Fig. 4. TCA pathway and SDH activity of broccoli heads treated with varying O_2/CO_2 levels at 10 °C. Vertical bars indicate the standard deviations for each treatment. Air treatment was used as control. The TCA proportion was calculated as below: Pt = (Rt − Vt)/Rt × 100%. Here, Pt means the TCA proportion, Rt means the total respiration rage, Vt means the respiration rate of TCA which was determined by adding 0.1 mL of 0.5 mol L^{-1} malonic acid.

storage (Fig. 3B). The 6-P-F average contents of broccoli florets treated with 30% O_2 + 70% CO_2, 40% O_2 + 60% CO_2, 50% O_2 + 50% CO_2, 60% O_2 + 40% CO_2 and 70% O_2 + 30% CO_2 were 123.86, 115.74, 117.42, 117.70 and 123.57 g kg^{-1}, respectively.

3.5. TCA pathway and SDH activity

Basically, the proportion of TCA pathway showed a downward tendency (Fig. 4A). Compared to others, the TCA proportion of 50% O_2 + 50% CO_2 treatment declined more slowly and exhibited higher during post-harvest storage. It showed a reduction of 12%, while the reduction of 30% O_2 + 70% CO_2, 40% O_2 + 60% CO_2 and 60% O_2 + 40% CO_2, was 32%, 26% and17%, respectively.

The changes of SDH activity of all treatments was in parallel to those of TCA pathway proportion during the whole storage (Fig. 4B). SDH activity of broccoli florets treated with 50% O_2 + 50% CO_2 fell more slowly and showed a higher and stable level, while that of 40% O_2 + 60% CO_2 and 30% O_2 + 70% CO_2 declined significantly during the whole storage. As a whole, SDH activity of broccoli florets treated with 50% O_2 + 50% CO_2 declined 19%, while that of 30% O_2 + 70% CO_2 treatment fell 43% during the whole storage.

3.6. HMP pathway and G-6-PDH + 6-PGDH activity

The HMP proportion of 50% O_2 + 40% CO_2 and CK increased during the whole storage (Fig. 5A). Furthermore, HMP proportion of broccoli treated with 50% O_2 + 50% CO_2 behaved the highest level, while that treated with 70% O_2 + 30% CO_2 exhibited the lowest.

G-6-PDH and 6-PGDH are the key regulator enzymes of HMP pathway, which directly impact on the activation of HMP pathway. G-6-PDH + 6-PGDH activities of broccoli florets treated with 50% O_2 + 50% CO_2 showed the highest level among all treatments throughout storage, followed by 40% O_2 + 60% CO_2 (Fig. 5B). However, those of 30% O_2 + 70% CO_2 and 70% O_2 + 30% CO_2 treatment behaved the lowest level.

3.7. CCP pathway and CCO activity

The changes of CCP proportion of all treatments showed similar downward treads during storage period (Fig. 6A). However, the CCP proportion of 50% O_2 + 50% CO_2 behaved higher level throughout the storage, which declined by only 7% during 31 d of storage, while

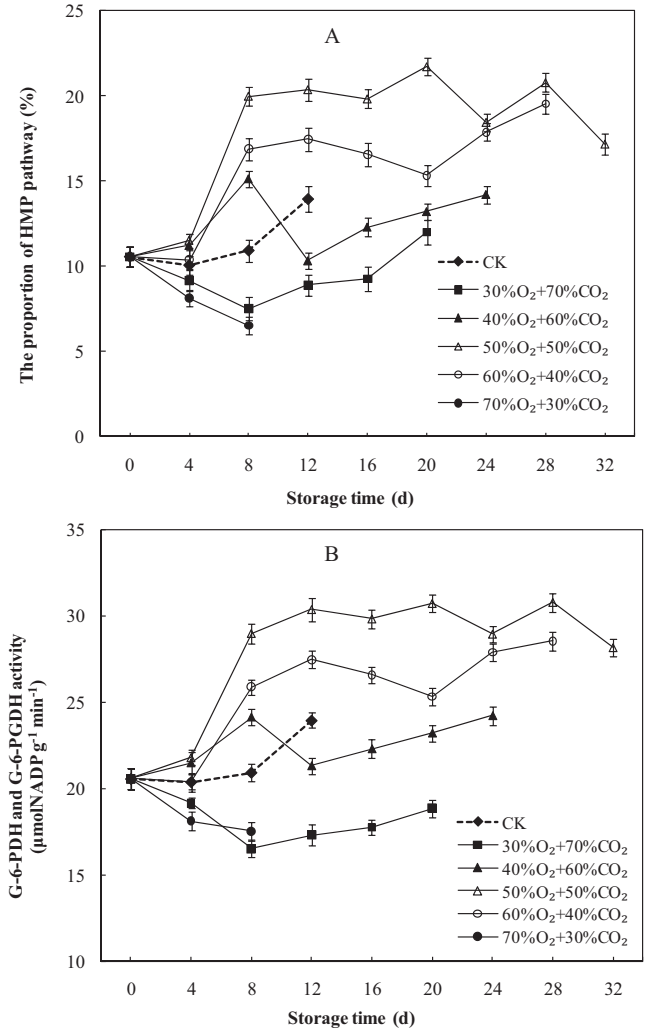

Fig. 5. HMP pathway and G-6-PDH and 6-PGDH activity of broccoli heads treated with varying O_2/CO_2 levels at 10 °C. Vertical bars indicate the standard deviations for each treatment. Air treatment was used as control. The HMP proportion was calculated as below: Ph = (Rt − Vh)/Rt × 100%. Here, Ph means the HMP proportion, Rt means the total respiration rage, Vh means the respiration rate of HMP which was determined by adding 0.1 mL of 0.1 mol L^{-1} Na_3PO_4.

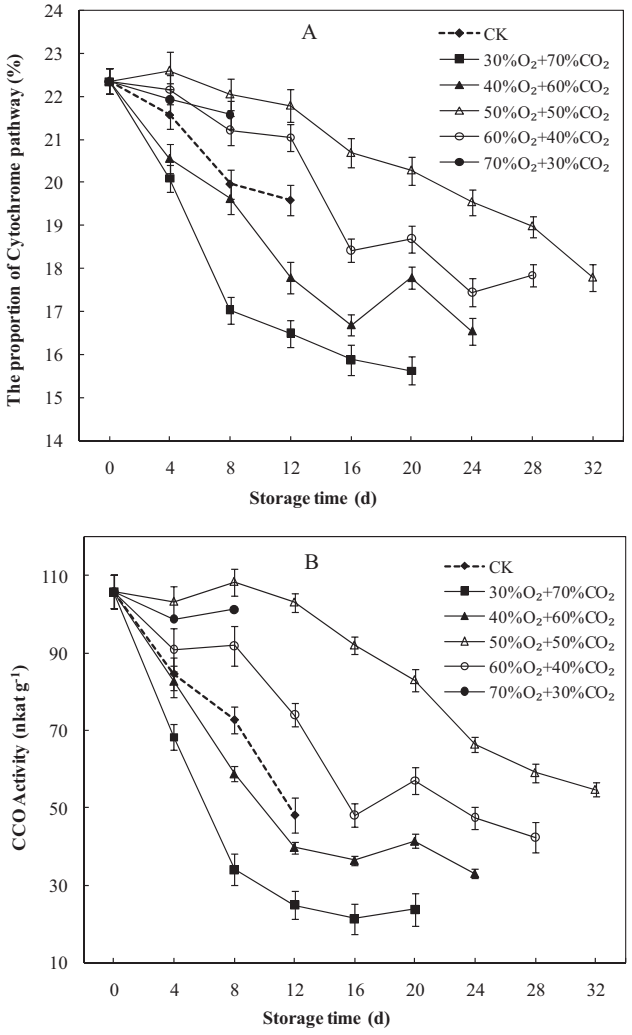

Fig. 6. CCP pathway and CCO activity of broccoli heads treated with varying O_2/CO_2 levels at 10 °C. Vertical bars indicate the standard deviations for each treatment. Air treatment was used as control. The CCP proportion was calculated as below: Pc = (Rt − Vc)/Rt × 100%. Here, Pc means the CCP proportion, Rt means the total respiration rage, Vc means the respiration rate of CCP which was determined by adding 0.1 mL of 0.1 mol L^{-1} NaN_3.

that of 30% O_2 + 70% CO_2 declined by 29% during the first 16 d of storage (Fig. 6A).

The change trends of CCO activity with all treatments were roughly similar to those of CCP rate during the whole storage (Fig. 6B). In general, CCO activity of 50% O_2 + 50% CO_2 treatment showed higher, while that of 30% O_2 + 70% CO_2 and 40% O_2 + 60% CO_2 treatments behaved the lowest level.

4. Discussion

Respiration is one of a main measurable indicator in metabolism intensity of postharvest fruits and vegetables. High respiration rate could quickly lead to consumption of metabolic substrates, speed up maturity or senescence and shorten shelf life of fruits and vegetables (Lin and Zhao, 2007). In recent years, CA has been utilized to reduce the respiration rate and to store the postharvest of broccoli. For example, the respiration rate decreased by 13% and 21% respectively in broccoli heads treated with 10% O_2 + 5% CO_2, and 5% O_2 + 95% N_2 throughout the whole storage time (Chairat et al., 2008; Fernandez-Leon et al., 2013b). In our study, the respiration rate of broccoli heads treated with 50% O_2 + 50% CO_2 reduced 36.57% during storage, which indicated that proper proportion of O_2/CO_2 controlled atmosphere is a potential strategy in reducing the respiration rate of broccoli heads.

Respiratory metabolism was essential for living beings, which was determined by diversified respiratory enzymatic activities and ratio of different respiratory pathways. SDH is thought to be the key enzyme of TCA pathway, which catalyses the oxidation of succinic acid reversibly into fumaric acid (Soto et al., 2012). CCO is thought to be the key enzyme of CCP, which catalyses the transfer of electrons from ferrocytochrome c to molecular oxygen and plays a key role in aerobic metabolism and energy production during oxidative phosphorylation (Kan et al., 2011; Soto et al., 2012). Various proportions of O_2/CO_2 CA had a different effect on respiratory pathways of postharvest fruits and vegetables (Fernandez-Leon et al., 2013b, Paradis et al., 1996). Besides, different respiratory pathways have their particular role in the process of storage. EMP pathway, which oxidizes glucose to pyruvate, is a basic respiratory pathway, and subsequently followed by TCA and CCP. TCA and CCP were essential for energy provision of postharvest fruits and vegetables during storage (Alisdair et al., 2004; Wu et al., 1998). HMP is another respiratory way of fruits and vegetables, which supply intermediate reaction product. At the same time, high level of HMP enhanced the endurance capability of plants in adversity such as drought, heavy metal, salinity and cold (Van et al., 1988; Slaski et al., 1996; Zhu 2002).

Insufficient ATP may account for browning, disease occurrence and the reduction of disease resistance of harvested litchi (Yi et al., 2010). Several researches have showed that energy deficiency has close associations with senescence of postharvest fruits and vegetables such as longan (Chen et al., 2014), litchi (Wang et al., 2013), lettuce (Braidot et al., 2014) and pear (Jiang et al., 2007; Saquet et al., 2001). Zhou et al. (2014) reported that depletion of ATP and low energy prevented the maintenance of membrane integrity and function. Low level of ATP and energy depletion appeared to lose membrane potential, to block electron transferring at the terminal of the respiratory chain, to activate apoptotic signaling pathway, thus the senescence of the body began along with cell death (Partridge et al., 1994). In our study, 50% O_2 + 50% CO_2 treatment kept broccoli relatively high energy and retarded its senescence. Low ATP contents and low energy charge level are associated with senescence along with lower TCA and CCP and quality deterioration in broccoli CA stored.

Broccoli treated with 50% O_2 + 50% CO_2 had lower ratio EMP, higher TCA and CCP, ATP content and energy charge level, which demonstrated that most of pyruvate was oxidized through the TCA and CCP and more energy was produced. In addition, HMP rate was higher, which illustrated that it had stronger capability of stress resistance. On the contrary, broccoli treated with 30% O_2 + 70% CO_2 had higher EMP and lower TCA and CCP, which indicated that less production of energy and weak resistance to stress, and fermentation pathway, which produces ethanol, acetaldehyde, and lactate, might be occurred. Higher EMP could lead to more consumption of metabolic substrates, combined with less energy level for lower TCA and CCP, which could accelerate senescence of broccoli head. Moreover, accumulation of acetaldehyde and ethanol accounts for physiological disorder such as off-flavor (Ke and Kader, 1990; Mattheis and Fellman, 2000). Our previous study showed that the treatment of 50% O_2 + 50% CO_2 was proper to preserve the broccoli in maintaining physio-biochemical properties such as chlorophyll and ascorbic acid contents and reducing the accumulation of acetaldehyde and ethanol (Li et al., 2014), while with the treatment of 30% O_2 + 70% CO_2 had higher acetaldehyde and ethanol, which were matched with our studies presented here.

In conclusion, the 50% O_2 + 50% CO_2 treatment reduced the respiration rate, inhibited reduction of ATP content, energy charge level, the TCA, CCP proportion, and increased HMP proportion, reduced the metabolic substrates consumption. Accordingly, it delayed the senescence of broccoli and extended the storage period. However, the respiration rate of broccoli treated with 70% O_2 + 30% CO_2 was higher, and the HMP rate was lower, which increased the metabolic substrates consumption and declined the stress resistance. The 30% O_2 + 70% CO_2 treatment boosted EMP rate and reduced the TCA, CCP, which might stimulate the occurrence of fermentation pathway. The results revealed that proper O_2/CO_2 controlled atmospheres is a potential strategy for postharvest broccoli storage.

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