Exogenous Carbon Monoxide Treatment Delayed the Ethanol Metabolism and Fruit Softening of Postharvest Jujube

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ABSTRACT---- ***To investigate the effect of exogenous carbon monoxide (CO) treatment on the ethanol metabolism and fruit softening of postharvest jujube during storage period, the winter jujube was fumigated with 10μmol·L^{-1}CO. The content variations of pyruvic acid, ethanol and acetaldehyde, and the activities of ADH and LDH of postharvest jujube were investigated. Meanwhile, the fruit firmness, the content changes of insoluble and soluble pectin, and the activities of PG, PE and Cx were also researched. The experimental results showed that treating jujube with 10μmol·L^{-1} CO might restrain the activities of PDC, ADH and LDH, and reduce the accumulations of pyruvate, acetaldehyde and ethanol content of postharvest jujube during storage period. In addition, CO treatment could also restrain the activities of PE, PG and Cx, reduce the hydrolysis of insoluble pectin and the increase of soluble pectin content, and postpone the decrease of jujube firmness. Therefore, exogenous CO treatment could restrain the ethanol metabolism of postharvest jujube during storage owing to anaerobic respiration and accordingly delayed fruit ferment and softening. It probably becomes an effective measure for jujube and other fruit preservation in the future.***

Keywords---- CO, jujube, ferment, softening

1. INTRODUCTION

Jujube is rich in vitamins, essential amino acids, trace elements and medicinal ingredients, possessing important therapeutic value and various health care effects. It was known as the rare fruit in the world and favored by consumers. As one of fresh food jujube varieties with thin skin, crisp flesh and special taste, it has a broad market prospect. However, winter jujube was prone to shrinkage, ferment, and rot because of its own particular physiological characteristics that restrain its preservation. Accordingly, commercial and economic value was discount [1]. Winter jujube is quite sensitive to the carbon dioxide concentration of modified atmosphere. Unsuitable carbon dioxide concentration might promote anaerobic respiration. Ethanol accumulation induced by ethanol metabolism during storage, flesh browning and softening during winter jujube senescence are several important reasons that decrease the preserving properties of winter jujube. Low temperature and controlled atmosphere are commonly methods to preserve winter jujube, but these methods could lead to ethanol accumulation owing to anaerobic environment forming gradually during jujube preservation, exacerbating the softening browning of fresh jujube. Meanwhile, the terminal oxidases such as PPO and flavin oxidase was with little sensitivity to low temperature, so controlling the ethanol metabolism of postharvest jujube through low temperature is rather difficult[2]. Therefore, to prolong the shelf life of postharvest jujube and improve its economic value, the exploration of effective method to inhibit ethanol metabolism and fruit softening is crucially important.

Some researchers found that treating jujube using 1-MCP or low temperature in combination with high oxygen atmosphere could effectively inhibit the ethanol and acetaldehyde accumulation of jujube flesh [3, 4]. In addition, $CaCl_2$ and GA_3 could restrain the activity decrease of some endogenous enzyme including PPO, POD and CAT, reduced MDA accumulation, and slow down the ethanol accumulation. As a result, the firmness of postharvest jujube was maintained and softening or browning of jujube was delayed [5].

Carbon monoxide is a stable diatomic gas molecule. At present, as another gas signal transducer molecule of plant apart from NO, it involved in various physiological and metabolic regulation [6]. Endogenous CO in the plant body might be generated via catalyzing heme catabolism by heme oxygenase (HO) [7]. it may exert its physiological function through

binding the protein that contains heme[6,8]. Recent research suggested that CO could enhance the anti-senescence ability of plant leaf, improve the SOD, POD and CAT activities of plant tissue, and reduce the MDA content of fresh cut Chinese rose flower[9,10]. In addition, CO fumigation could prevent the browning of fresh cut lotus root [11]. At present, the researches about the action mechanism and the effect of CO treatment on the physiological effects of postharvest fruits and vegetables were still in the initial stage. Our research team found that CO treatment could maintain the quality of postharvest jujube, and the shelf life was prolonged, but the concrete mechanism need to be further investigated [12]. Ethanol metabolism was close relation with ferment, browning and softening of postharvest winter jujube. Therefore, to effectively control the senescence of postharvest winter jujube during storage time, the investigation of CO on ethanol metabolism and fruit softening was significance.

2. MATERIALS AND METHODS

2.1. Material

Jujubes (*Ziziphus jujuba* Mill. cv. Dongzao) were purchased from Yaodu District, Linfen City. They were picked at physiologically mature stage, and the related characters were as followed: mature-green stage, with the white and green peel, the firmness of approximately 9.0 Kg/cm^2, and the soluble solids content 11.0 brix. 100 kg of jujube was selected to acquire uniform shape, size and colour, and then they were boxed and transported to the laboratory quickly.

CO (99.99%) was purchased from Beijing Huaneng Special Gases Co., Ltd. (Beijing, China). Sinopharm Chemical Reagent Co., Ltd. (Shanghai China) supplied, phenylmethylsulfonyl fluoride, polygalacturonic acid and dinitrosalicylic acid (biochemical reagent). Other reagents (analytical grade) were purchased frome Alfa Aesar Company (Tianjin, China).

2.2. Fruit Treatment

In view of formal screening experiment result, after precooling at 0-4℃for 24 h, 10 μmol L^{-1} CO treated jujubes for 1 hour showed the best effect. In the next experiment, the jujubes were fumigated with CO gas at 10 μmol L^{-1} CO for 1 hour under ambient temperature (about 20 ± 2℃). Approximately 3 kg of jujubes was placed in a glass container with a lid. CO gas was injected into the glass container through a port of the lid. The jujubes were fumigated with CO for 1 h. No fumigated Jujubes were also sealed in a glass container for 1 h, and they were used as control samples. After treatment, all of the samples were placed in plastic bags and stored under ambient temperature with 85% relative humidity. Fruit firmness, pyruvate, ethanol, acetaldehyde, Non-water soluble pectin and water soluble pectin, and related enzymes (containing PE, PG, Cx, PDC, ADH, and LDH) activities of the jujubes were measured periodically.

In order to be sure CO gas in the container could have uniform spatial distribution, an experiment was conducted to verify the homogenous dispersion of CO in the container. Specific program was as followed. 10 μmol/L CO was injected into the container. 30 s later, the CO concentration of the container was measured online using an infrared CO analyser (GXH-3011A, Beijing Huayun Analysis Instrument Co., Ltd., Beijing, China). The CO concentration tests were performed at the bottom, middle, top, and diagonal areas of the container, and all positions showed the same concentration. Thus, CO may be concluded to have achieved equilibrium in the container.

2.3 Contents of ethanol, acetaldehyde and pyruvate

The contents of ethanol and acetaldehyde were determined according to the method of SUN et al. (2007) with slight modifications [1]. 10 g of flesh fruits was homogenized in 10 mL of cold 0.1 mol·L^{-1}HCl. 4 of homogenate was sealed in a 10 mL glass vial, and then incubated at 70℃for 30 min. A 2-mL sample of the headspace gas was withdrawn with a gastight syringe, and injected into a gas chromatograph (Agilent 7820, U.S.A) and an FID detector, with column temperature of 85℃, and injection temperature of 200℃. The carrier gas was nitrogen, with a flow rate of 30 mL·min^{-1}. The results were expressed as the average of three replications. The pyruvate content in fruits was measured by colorimetry (Northwest Agricultural University 1986) [13].

2.4 Measurements of ADH, PDC, and LDH activities

The activities of alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) were measured according to the methods described by SUN et al. (2007) [1]. The change of 0.01 in absorbency per gram per min was one unit (U) of activity, expressed as U·g^{-1} FW min^{-1}. The activities of pyruvate decarboxylase (PDC) were measured according to the methods described by Botondi et al. (2012) [14].

2.5 Firmness, non-water pectin, water pectin

Firmness was determined by a penetrometer (mode GY-4, Mudanjiang, China) with a 8-mm-long probe with a diameter of 5 mm. 10 fruits were used and two points per fruit were selected to determine the firmness of each replicate sample.

Pulp (5 g) was homogenized in 40 ml of 85 ℃ ethanol (95%), and the homogenate was incubated for 10 min at 85 ℃, then centrifuged (10 000 ×g). The residue was re-extracted with 25 ml of 63% ethanol at 85℃for a further 10 min. The residue was again centrifuged, the water-soluble pectin extracted from the ethanol washed residue. The extract is used to determined water-soluble pectin; the residue is use for determination non-water pectin. The non-water pectin, water pectin was determined by the method of Ketsa and Daengkanit (1999) [15].

2.6 PG, PE, andCx

PG activity was determined according to the modified method of Amnuaysin et al, 1998 [16]. Flesh tissue (20 g) from 10 fruits were homogenized in 0.02 M sodium phosphate buffer pH 7.0, 0.02M EDTA, 1% TritonX-100, 0.02M cysteine-HCl and 1mM phenyl-methylsulfonyl fluoride(PMSF). Then, homogenate was centrifuged at 15,000 × g for 30 min at 4℃. Clear supernatant was used for enzyme activity analysis. 0.5 ml of enzyme extract was mixed with 0.3ml of 1% polygalacturonic acid (PGA, 50mM sodium acetate pH4.5) and 0.2 ml of 0.2M sodium acetate buffer pH4.5 in a total volume of 1ml and then incubated at 37℃ for 1h. To measure the amount of reducing sugar released, 0.5ml of dinitrosalicylic acid (DNS) was added to the reaction mixture. After the reaction mixture was boiled for 5min and cooled to room temperature, absorbance was measured at wavelength of 520nm (UV-1100, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China). One unit of PG activity was expressed as nmol of galacturonic acid per min per mg protein.

PE Activity was determined in pulp tissues following the method as described by Zaharah *et al* (2013) with some modification[17]. The fruit pulp tissue (13.0g) was homogenized with 13.0 mL cold solution, containing 12% polyethyleneglycol and 0.2% sodium bisulphite ($NaHSO_3$). The supernatant was immediately stored at −80℃for determination of protein content. Following centrifugation at 4℃for 40min at 15,000 × g, the pellet was washed with a 13.0 mL aqueous solution of $NaHSO_3$ (0.2%) and recentrifuged at 4℃ for 40 min at 15,000 × g. The pellet was stored at −80℃for determination of PE. The pellet was resuspended in 15mL cold solution containing NaCl(7.5%,w/v) + EDTA (0.75%, w/v) at pH 6.5 and incubated at 4℃for 10 min. Following centrifugation at 15000 × g for 15min, a 20 mL of jujube pectin solution (1%, w/v) at pH 7.5 was mixed with 5 ml enzyme extract solution. The reaction mixture was titrated against 0.01 N NaOH and maintained at pH 7.4, while incubating at 30℃ for 10 min. During the titration and incubation time, the reaction mixtures were continuously and slowly shaken by hand. The total amount of 0.01 N NaOH to maintain pH 7.4 was used to calculate the PE activity. The PE enzyme activity was expressed as mM NaOH mg protein$^{-1}h^{-1}$. Cellulase activity was measured according to the method of Abeles and Takeda (1990) [18].

2.7. Statistical analysis

The data were processed by analysis of variance using DPS7.05 statistical software (Refine Information Tech. Co., Ltd., Hangzhou, China). The treatments were compared at P = 0.05 using Tukey's test, which indicates the multi-comparison value in each case. The data were expressed as mean ± standard deviation (SD).

3. RESULTS AND ANALYSIS

3.1 Pyruvate, acetaldehyde and ethanol contents

As shown in Figure 1A, the pyruvic acid content of postharvest winter jujube firstly increased and then decreased during storage time. From the first day, CO treatment significantly reduced the production of pyruvic acid of jujube flesh tissue. And the pyruvic acid content treated with CO was 12% lower than that of control sample at 30 day. Similar to the change trend of pyruvic acid, the acetaldehyde content of postharvest jujube also first increased and then decreased. And CO treatment apparently restrained the variation of acetaldehyde content. Figure 1C displayed the ethanol content changes of postharvest jujube. The ethanol content showed increasing trend in the first 20 days, and then slightly decreased from 20 to 30 days. And treated jujube with CO exhibited low ethanol content compared to control sample. The ethanol content of treated jujube was 23%~27% lower than that of control sample during the whole storage time.

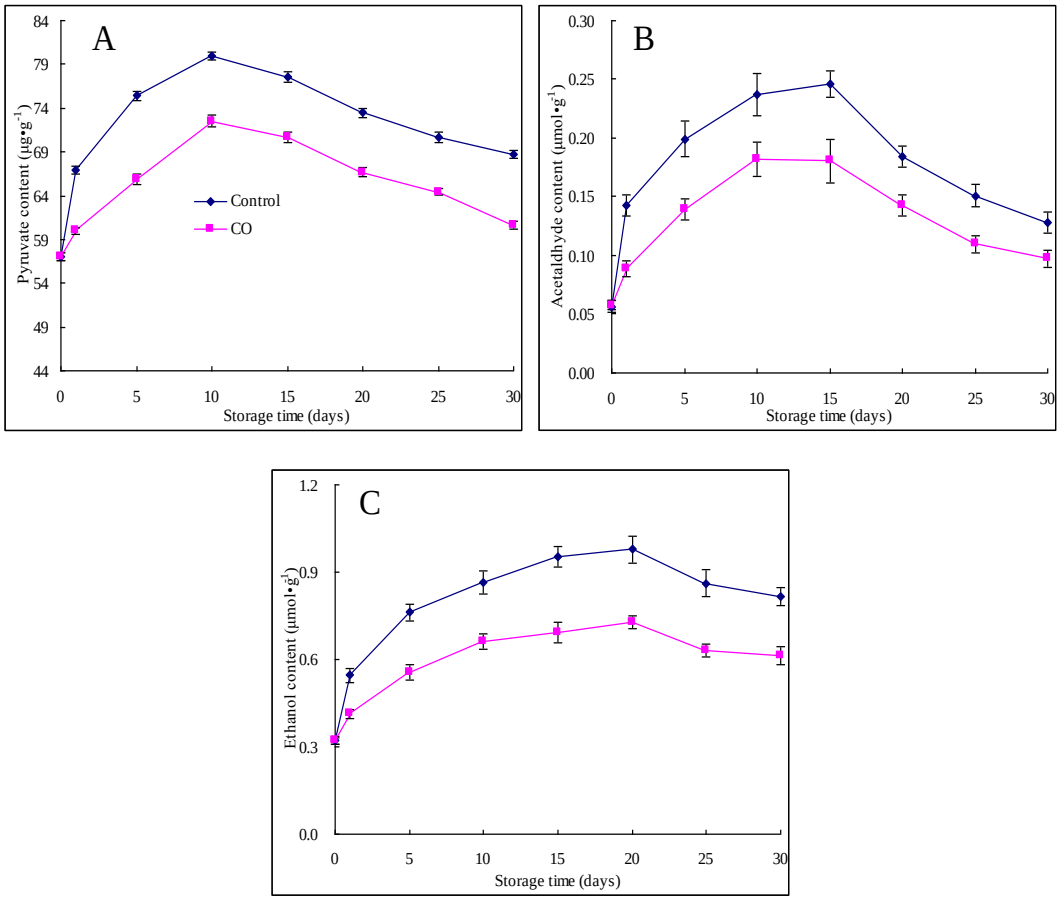
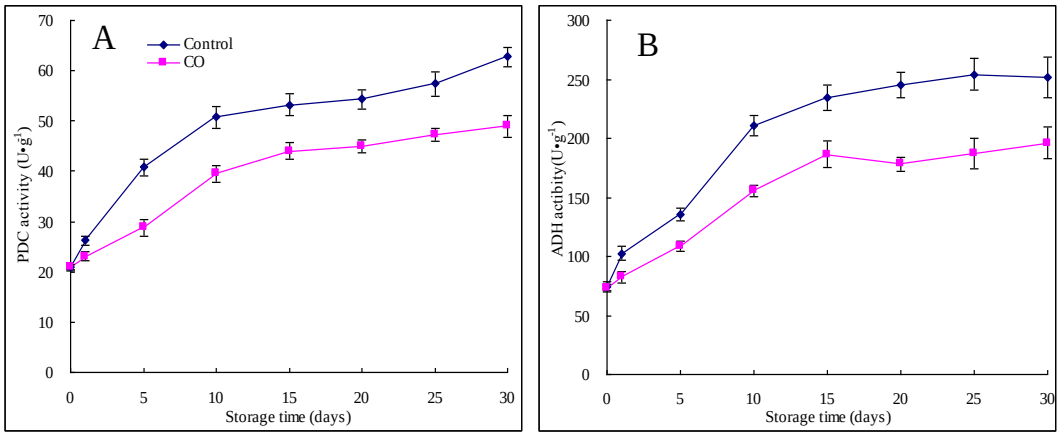


Fig.1 Effect of CO treatment on pyruvate content (A), acetaldehyde(B) and ethanol (C) contents of postharvest jujube.

3.2 PDC, ADH and LDH activities



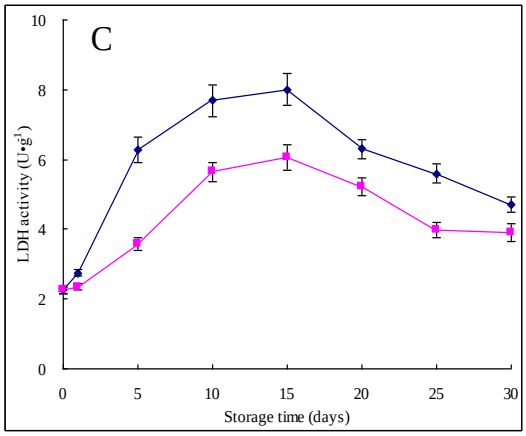


Fig.2 Effect of CO treatment on PDC (A), ADH (B), and LDH (C) activities of postharvest jujube.

3.3 Firmness, non-water soluble pectin content and water soluble pectin content

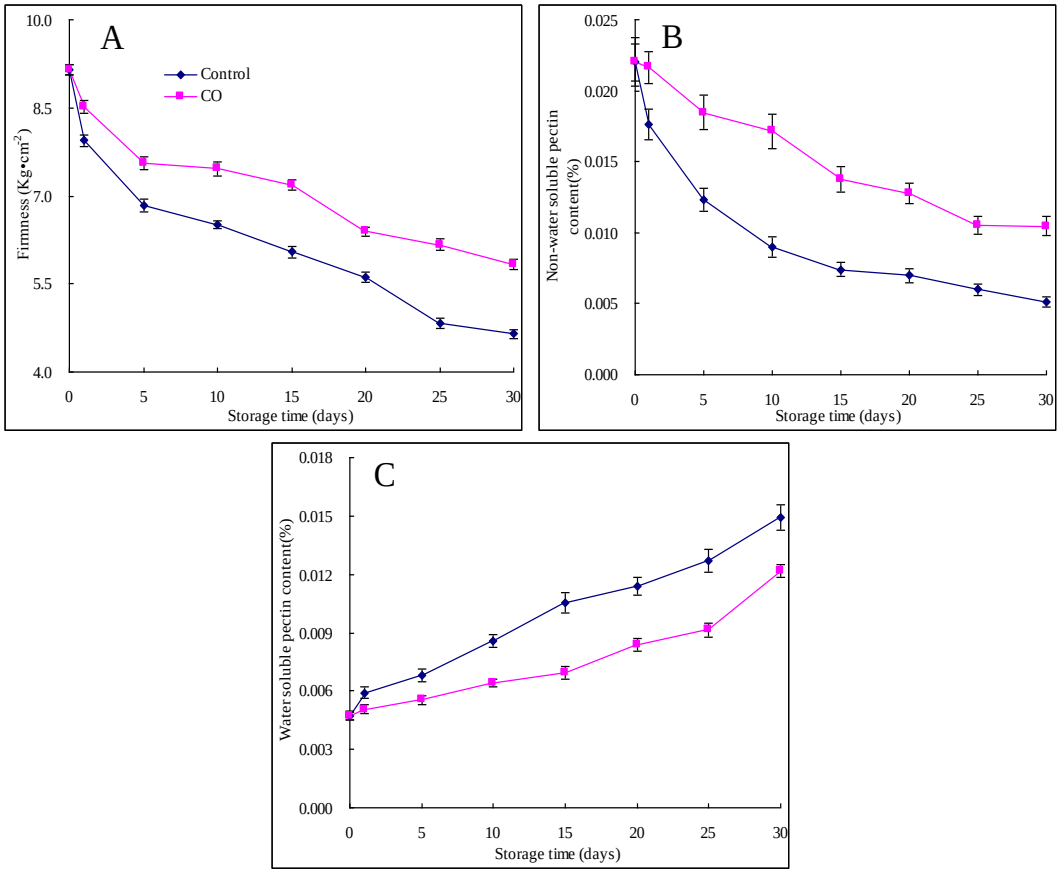


Fig.3 Effect of CO treatment on firmness(A), non-water soluble pectin content(B), and water soluble pectin content(C) of postharvest jujube.

As shown in Figure 3A, the firmness of postharvest jujube decreased with storage time extension. The firmness of treated jujube obviously decreased lower than that of control samples, and on day of 30, it was 5.83Kg·cm^{-2}, which was 20.24% higher than that of control samples. The soluble pectin content of postharvest jujube increased during jujube storage

time (Figure 3B). Treated jujube showed slowly increasing trends compared to control sample, and its soluble pectin content was 18.12% lower than that of control sample at 30 day. Contrary to the increasing trend of the soluble pectin content of postharvest jujube, the insoluble pectin decreased gradually. However, the insoluble pectin decline speed of treated jujube was lower than that of control samples. On day of 30, the content of insoluble pectin was 2.06 times of control samples (Fig. 3C).

3.4 PE, PG and Cx activities

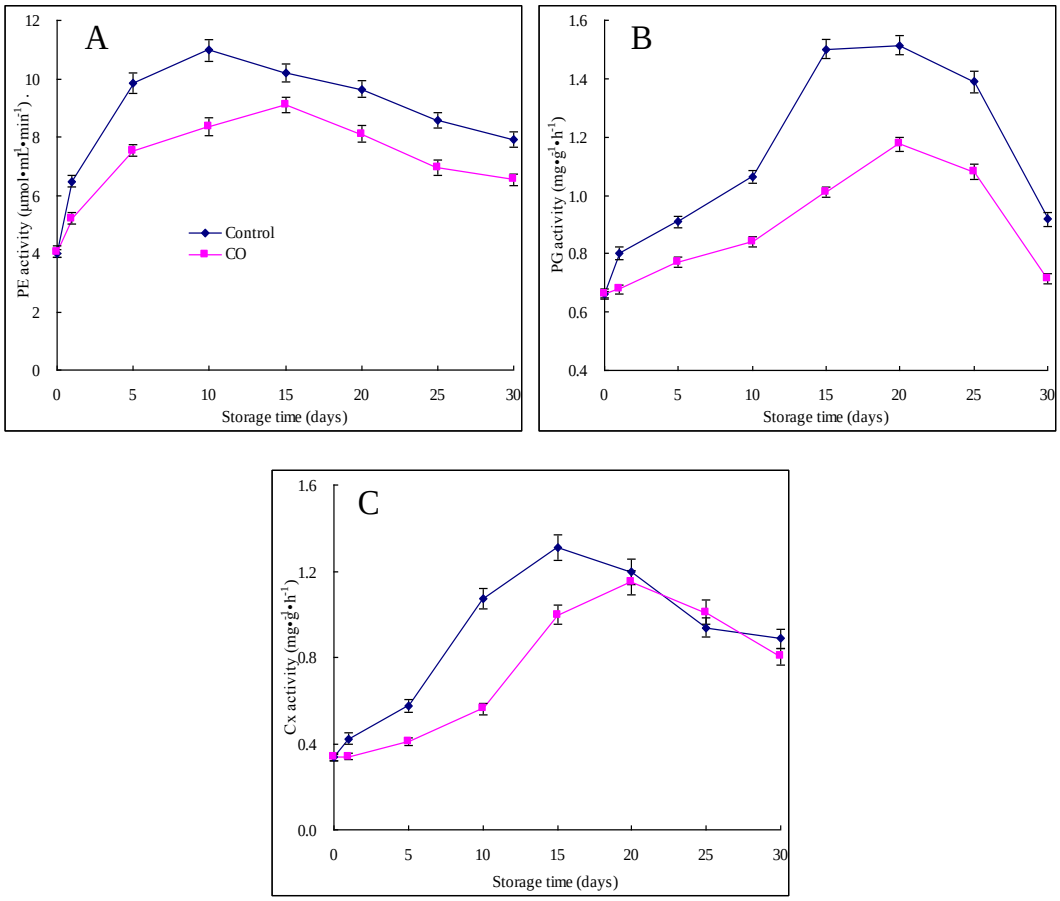


Fig.4 Effect of CO treatment on PE (A), PG (B), and Cx activities (C) of postharvest jujube.

As shown in Figure 4A, the PE activity of postharvest jujube firstly increased, and then decreased. The peak values of control and treated sample respectively appeared on day of 10 and 15. Treated jujube delayed 5 days to reach the maximum of PE activity compared with control sample, and its maximum was 17.05% lower than that of control sample. In addition, the PE activity of treated sample was lower than that of control sample during the whole storage time. Figure 4B described the PG change trend of postharvest jujube. Compared with the PE activity, in the first 10 days, the PG activity of postharvest jujube slowly rose, and it fast decreased from 20 to 30 days. The peak values of control samples and treated samples all appeared at 20 day, the peak value of treated samples was significantly 22.36% lower than that of control treatment, and there was significantly difference between them ($P<0.05$). As shown in Figure 4B, before 20 days, the Cx activity of treated jujube were lower than that of control sample, and there was significant difference between them from 1 to 15 days ($P<0.01$). Moreover, the maximum Cx activity of treated jujube was postpone 5 days compared to that of control sample.

4. DISCUSSIONS

4.1 Effect of CO on the ethanol metabolism of postharvest winter jujube

Anaerobic respiration occurred in postharvest jujube under hypoxic conditions. The sugar, acid and other substrates of jujube tissue could not fully oxidized into carbon dioxide, leading to the accumulation of acetaldehyde and ethanol. This is named ferment of postharvest fruit. Jujubes belong to sensitive fruit to ethanol and excess of ethanol accumulation may cause cell poisoning. As a result, the softening and browning, flavor loss and quality deterioration of postharvest harvest emerge [2, 19]. The experimental results showed that the contents of pyruvate, acetaldehyde and ethanol of postharvest jujube increased during jujube storage time. This was probably the gas exchange in the outer and inner of fruit was hindered with the ripening and senescence of fruit and the horny layer thickening of fruit peel. Accordingly, the oxygen level in inner tissue decreased. Thus, the aerobic respiration proportion via the path of tricarboxylic acid cycle (TCA) descended, while the proportion of anaerobic respiration enhanced. Therefore, the alcohol fermentation and lactic acid fermentation of postharvest was easy to arise [20]. Exogenous CO treatment could reduce the variations of pyruvate, acetaldehyde and ethanol content, thereby inhibiting the anaerobic respiration of postharvest jujube. Further research indicated that CO also restrained the activities of PDC, ADH, and LDH. So CO treatment might relieve ethanol metabolism and lactic acid fermentation of postharvest jujube by inhibiting the activities of PDC, ADH, and LDH. This result was similar to Sun who treated postharvest jujube with NO. Sun et al found that NO treatment could restrain the activities of ADH and LDH, slow down the contents of ethanol, acetaldehyde and pyruvate, and reduce the damage to fruit because of ethanol accumulation[1]. NO could directly interact with the cysteine located at the active center of ADH or LDH, inhibiting the activities of ADH and LDH of postharvest. However, different from NO, CO could not interact with cysteine owing to its stable chemical property. Accordingly, it could not directly react with ADH or LDH [1, 21]. Certain research found that CO as an upstream signal molecule of NO signal played an important role in plant physiological metabolism[22]. Therefore, CO probably performed its active function through regulating NO. Our unpublished data also suggested that treating jujube with cPTIO as NO specific inhibitor might restrain the production of pyruvate, acetaldehyde and ethanol of postharvest jujube.

4.2 Effect of CO on fruits softening of postharvest winter jujube

During fruit senescence, pectinase (PE, PG, PME) and cellulase (Ease,β-Glucosidase activity et al）catalyzes the hydrolysis of pectin and cellulose. Thus, fruit softening was initiated [23]. The experimental results showed that the ethanol content of postharvest jujube was related with fruit firmness, soluble pectin content, insoluble pectin content, PE activitiy, PG activity and Cx activity, and the correlation coefficients were -0.85, -0.93, 0.73, 0.90, 0.85 and 0.92, respectively ($P < 0.05$). Apparently, jujube softening was closely associated with ethanol metabolism and this result was similar to Sun *et al* [1]. In addition, Speirs also found that the softening of 6 species of tomato was significantly correlated with ADH activity, ethanol content and acetaldehyde [24]. During jujube storage time, exogenous CO treatment could suppress the activities of PE, PG and Cx of postharvest jujube, restrain the decrease of insoluble pectin content, reduce the increase of soluble pectin content, and inhibit the decline of flesh firmness. Though the exact mechanisms were not clear at present, we might infer as followed. CO might probably restrain ethanol metabolism and lactic acid fermentation, thereby inhibiting fruit softening and senescence owing to anaerobic fermentation during jujube storage period. Besides respiratory metabolism, fruit ferment and softening were related with ethylene metabolism [25]. Exogenous CO treatment could inhibit the ethylene biosynthesis of jujube during storage (unpublished), and indirectly restrain postharvest jujube ferment and softening. Certainly, the specific mechanism needs to be further investigated in future.

5. CONCLUSIONS

Treating jujube with $10\mu mol \cdot L^{-1}$ CO might restrain the activities of PDC, ADH and LDH, and reduce the accumulations of pyruvate, acetaldehyde and ethanol content of postharvest jujube during storage period. In addition, CO treatment could also restrain the activities of PE, PG and Cx, reduce the hydrolysis of insoluble pectin and the increase of soluble pectin content, and postpone the decrease of jujube firmness. Therefore, exogenous CO treatment could restrain the ethanol metabolism of postharvest jujube during storage owing to anaerobic respiration and accordingly delayed fruit ferment and softening.

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