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Research Article

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Ethylene-regulation of fruit softening and cell wall enzymes activities during peach storage

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ABSTRACT

'Yanhong' peach fruits were treated with 1 mg/g ethrel and 2 μl/l 1-methylcyclopropene and stored at room temperature for 12 days. Ethylene production, firmness, and the activities of polygalacturonase, endo-1,4-β-glucanase, β-galactosidase, α-L-arabinofuranosidase and endo-β-mannanase in 'Yanhong' peach were monitored during storage. Control fruit displayed a typical climacteric pattern of ethylene production and the early softening of peach fruits started before the climactic stage. Ethrel promoted the decrease in firmness and onset of ethylene production. In contrast, 1-methylcyclopropene treatment delayed the decline in fruit firmness and onset of ethylene production. The activities of polygalacturonase, endo-1,4-β-glucanase, β-galactosidase, α-L-arabinofuranosidase and endo-β-mannanase in both epicarp and mesocarp tissue were enhanced by exogenous ethrel and inhibited by 1-methylcyclopropene during storage. Additionally, most activities of β-galactosidase, α-L-arabinofuranosidase and endo-β-mannanase were associated with the epicarp than that in mesocarp throughout fruits storage. These results suggest that softening of 'Yanhong' peach fruits was associated with these cell wall enzymes activities which were regulated by ethylene. Thus, application of 1-methylcyclopropene can extend the postharvest life of 'Yanhong' peach fruits.

Keywords: Peach, softening, cell-wall-degrading enzymes, ethrel, 1-methylcyclopropene

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INTRODUCTION

Fruit softening is one of the major ripening-related phenomena that directly affect the ability to transport and store fruit, as well as influencing the susceptibility of fruit to infection by postharvest pathogens [1]. Softening is partly explained by cell wall breakdown due to the coordinated action of several enzymes such as polygalacturonase (PG), pectinmethylesterase (PME), β-galactosidase (β-Gal), endoglucanase (EGase), expansins (Exp) and xyloglucan endotransglycosylase/hydrolase (XTH) [2].

Climacteric fruit display an ethylene production burst and a peak in respiration at the onset of ripening. Ethylene acts as a key signal for the initiation and coordination of their ripening events, including fruit softening [2]. A highly potent inhibitor of ethylene action, 1-methylcyclopropene (1-MCP), has been shown to reduce ethylene production and to delay softening in many fruit, such as apples, apricots, plums, avocados and peaches [3]. Furthermore, application of 1-MCP has been reported to affect the trends in activities of PG and EGase enzymes during activities of cell wall enzymes in avocado and plum fruit and to completely suppress the fruit ripening[4, 5]. The use of 1-MCP on post- harvest science is both providing the potential to maintain fruit quality after harvest and supplying a powerful tool to gain insight into the fundamental processes that are involved in ripening and senescence [6].
"*Yanhong*" peach is one of the most well-liked fruits in south-west of China because of its flavor, dietary value,

attractive color and medicinal worth. However this variety is highly perishable, mainly due to rapid softening once harvested and further microbial spoilage, limiting its market availability to a small geographical area. Application of 1-MCP is known to modulate the physiology of peach fruit softening during ripening and most of them focused on the changes in mesocarp of fruits [7]. The effects of 1-MCP on the softening, ethylene biosynthesis and cell wall hydrolysis enzymes during '*Yanhong*' peach storage in the epicarp and mesocarp have not been investigated and warrant further investigation. The aims of the present study were to (1) investigate the role of exogenous application of 1-MCP and ethrel in regulation of softening and ethylene production, and (2) activities of PG, EGase, β-Gal, α-L-arabinofuranosidase (α-l-Af) and endo-β-mannanase (β-Man) in epicarp and mesocarp tissues of '*Yanhong*' peach during storage.

EXPERIMENTAL SECTION

Fruit material and treatments: Peach (*Prunus persica* [L.] Batsch *cv*. Yanhong) fruits were harvested at commercial maturity stage from a commercial orchard in Nanming region in Guiyang, China, in 2010. After their immediate transfer to the laboratory, fruits that were free of mechanical injury, insects, and diseases were selected, then fruits were divided into three groups, each containing forty fruit per replicate (40×3), and were immediately exposed to the following treatments: (a) ethrel treatment, peach fruit were dipped in 1 mg/g ethrel (>98 %, Sangon Biotech Shanghai Co., Ltd, China) solution, soaked for 10 min, and then air-dried; (b) for 1-MCP treatment, fruits were closed up in a desiccator under 2 µl/l 1-MCP (4.5 %, Jiancheng Biotech Lanzhou Co., Ltd, China) for 12 h and ventilate for 30 min; (c) the control group (CK). Then fruits were placed directly into plastic bags (0.04mm thick) and stored in a room held at room temperature (25±2 °C) with 70~80% relative humidity for 12 days. Fruits were sampled at 0, 2, 4, 6, 8, 10 and 12 days after the start of the experiment.

Determination of firmness and ethylene production: Fruit firmness was determined at four equatorial regions on the flesh of three peaches using a hand penetrometer. Ethylene production was measured by placing 3 fruits in a 2.5 L crisper sealed with an air- tight lid equipped with a rubbers loop, and left at room temperature for 2 h. Then 1ml gas sample was withdrawn from the headspace by syringe to determine ethylene levels using a gas chromatograph. After the measurement of flesh firmness and ethylene production, fruits were peeled, cored, and diced. Epicarp and mesocarp pieces were immediately frozen in liquid nitrogen and separately stored at -80 °C for enzyme analysis.

Enzyme assay: For the extraction of PG and EGase activities, 0.1 g of epicarp or mesocarp was homogenized with ice-cold ethanol. The insoluble material was incubated in 1.8 mol L^{-1} NaCl/50 mmol L^{-1} sodium acetate buffer (pH 5.5) at 4 °C for 20min and centrifuged at 4°C for 10 min at 16,000 g. The supernatant collected and used to assay PG and EGase activity. PG activity was assayed by the method described by Cao *et al*. [8]. The formation of reducing groups was estimated against D-galacturonic acid as the standard after measuring the absorbance at 540 nm. For the assessment of EGase activity, the DNS method, with carboxymethylcellulose as the assay substrate, was used to determine the amount of reducing sugars released, with glucose as a standard [8].

For the extraction of β-MAN, 0.2 g of epicarp and mesocarp pieces was ground separately in 500 µl of 0.1 mol L^{-1} Hepes buffer (pH 8.0). The extract was centrifuged at 4°C for 10 min at 13,000 g, and the supernatant collected and used to assay β-MAN activity. β-MAN activity was determined using a gel-diffusion assay[9]. The proteins were quantified by the method of Bradford [10].

β-Gal and α-l-Af activities were assayed according to Goulao *et al*. by measuring the hydrolysis of *p*-nitrophenyl-β-D-galactopyranoside or *p*-nitrophenyl-β-D-arabinofuranoside, in 50 mmol L^{-1} sodium acetate pH5.0, respectively[11]. The released *p*-nitrophenol was measured spectrophotometrically at 415 nm, after incubations of 30 min and 3 h at 37°C, respectively. Activity was reported as the amount of *p*-nitrophenyl glycoside released according to a comparison with a standard curve constructed using *p*-nitrophenol (Sigma).

Statistical analysis: Data were analyzed by analysis of variance (ANOVA) with SPSS 16.0 statistical software. Significant differences were performed by Duncan's new multiple range tests. Differences at $P< 0.05$ were considered as significant. All the experiments were repeated three times.

RESULTS AND DISCUSSION

Effects of 1-MCP or ethrel treatment on fruit firmness: Fruit firmness is one of the most common physical parameters used to assess the progress of fruit softening and ripening [3]. As shown in Fig. 1, the control '*Yanhong*' peach fruit softened rapidly during storage, especially within the first 6 d. The decline in firmness was about 62.6 % within the first 6 d which was considered as the phase of rapid change in fruit texture. Ethrel-treatment accelerated softening of fruit markedly, and fruit exhibited a sharp decline in fruit firmness by 58.8 % within first 2 days as compared to the control fruits. This result is in agreed with the report on 'stony hard-flesh 'Manami' peach [12]. However, fruit softening was greatly inhibited by 1- MCP treatment within the first 6 d of storage, and then fruit began to soften sharply, with firmness still higher than that of the control fruit on day 12. Similarly, delayed fruit softening in 1-MCP-treated fruit has been reported in the stone fruit such as peach [13] and plum [5].

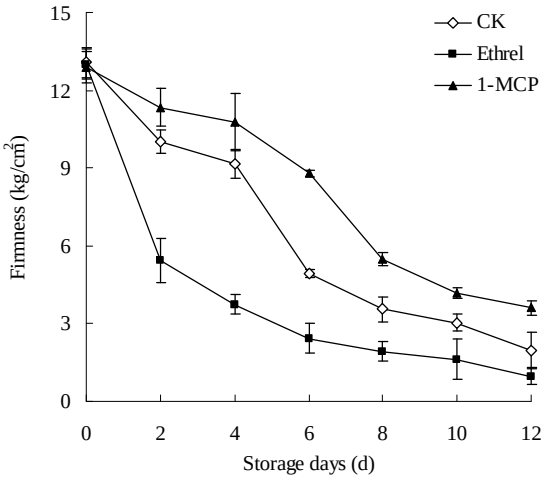

Fig. 1 Effects of 1-MCP or ethrel treatment on firmness of '*Yanhong*' peach fruit
Each value is presented as means ± SE (n = 3)

Effects of 1-MCP or ethrel treatment on ethylene production: Peach is a typical climacteric fruit. Peach fruit ripening is closely related to ethylene production [14]. The result showed ethylene production rate exhibited a typical climacteric pattern during '*Yanhong*' peach fruit storage (Fig. 2). Ethylene production in the control fruits increased immediately at the beginning of storage, and reached a maximum on day 6 of storage, and then declined sharply, and a slight increase was observed until day 12 during the storage period. The peak of ethylene lagged behind the fruit softening (Fig.1). These results agree with the previous report on peach fruits [15, 16]. Murayamaa *et al*. [16] suggested that the early softening before the climactic stage during ripening in peaches might be independent of ethylene. The changed pattern of ethylene production in ethrel-treated fruits was similar to that of the control fruits (Fig. 2). However, the ethylene production in ethrel-treated fruits increased more rapidly and reached a maximum at 4 d, ~2 days earlier than in the control fruit, and the ethylene production value in ethrel-treated fruits was higher than that in control (Fig.2). 1-MCP treatment significantly delayed the onset of ethylene production; a delayed peak was observed on day 8 of storage and was significantly lower than those of the control fruits (Fig. 2). The reduction in ethylene production of 1-MCP-treated fruits may be due to 1-MCP interfering with the autocatalytic production of ethylene, as ethylene binding sites have been irreversibly blocked by 1-MCP [5].

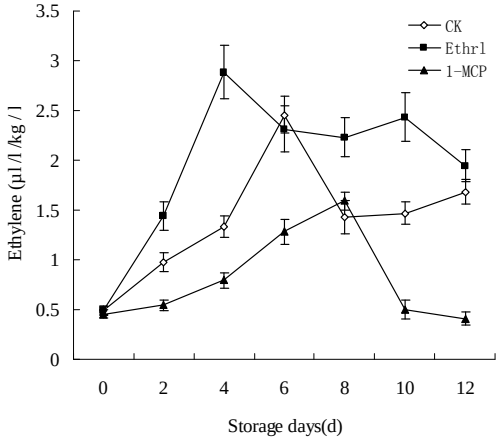

Fig. 2 Effects of 1-MCP or exogenous ethrel treatment on ethylene production of "*Yanhong*" peach fruit
Each value is presented as means ± SE (n = 3)

Effects of 1-MCP or ethrel treatment on the PG and EGase activities: Cell wall degradation is the main factor involved in fruit softening and texture changes. The cell wall hydrolytic enzymes play a key role in cell wall degradation and softening of fruit [5].

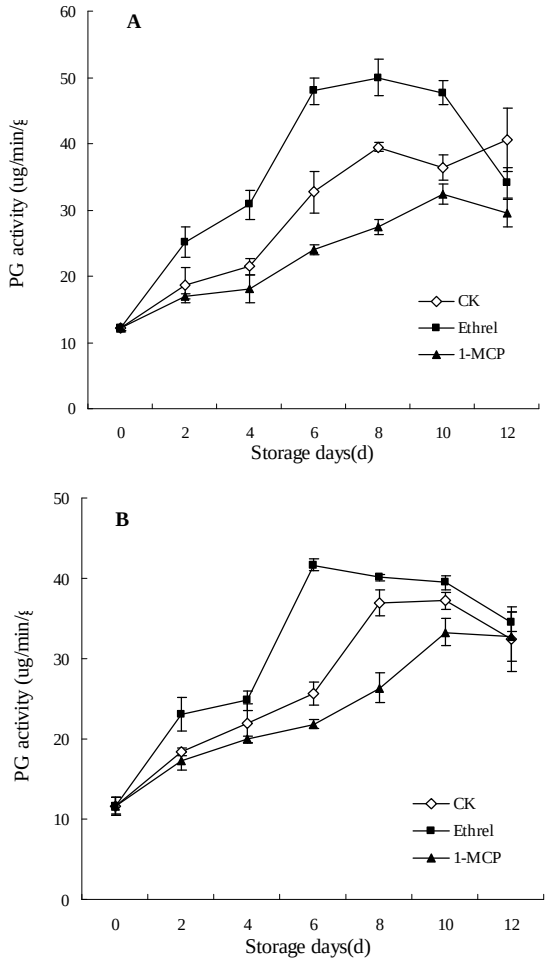

Fig. 3 Effects of 1-MCP or ethrel treatment on PG activity in the epicarp (A) and mesocarp (B) of '*Yanhong*' peach fruit
Each value is presented as means ± SE (n = 3)

The activities of PG and EGase in epicarp and mesocarp showed a notable 'up-down' trend during storage (Fig.3 and Fig. 4). Some PG and EGase activity could be detected at day 0. With the extend storage time, PG and EGase activities continued to rise until the late of storage. This suggests that these enzymes participate in mid- and late-stage softening events. Ethrel promoted the increase of PG and EGase activities both in epicarp and mesocarp tissues compared to the control fruit (Fig. 3 and Fig. 4). However, application of 1-MCP significantly reduced and delayed the increase of PG and EGase activities (Fig. 3 and Fig. 4). Similarly, reduced activities of PG and EGase enzymes have been reported in 1-MCP treated persimmon fruit [17] and Japanese plum [5], respectively.

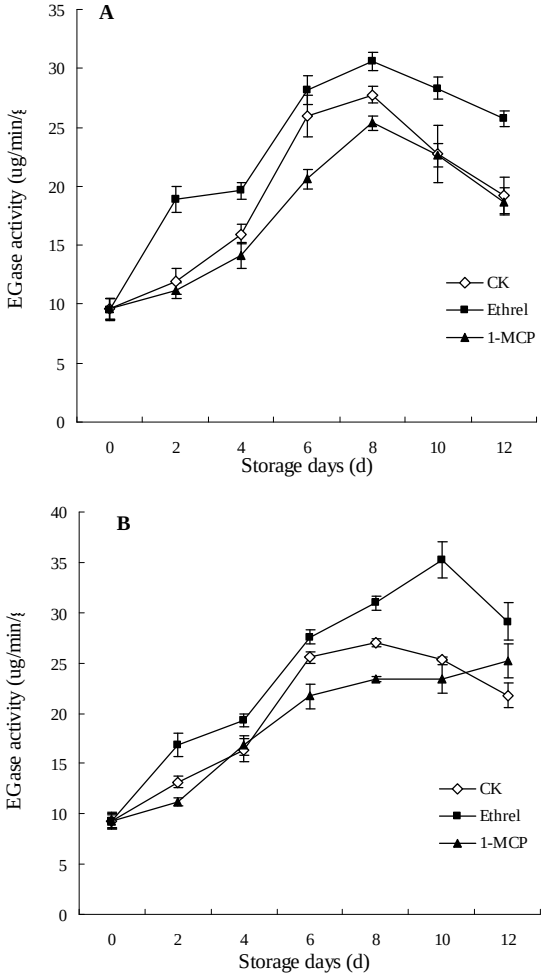

Fig. 4 Effects of 1-MCP or ethrel treatment on EGase activity in the epicarp (A) and mesocarp (B) of '*Yanhong*' peach fruit
Each value is presented as means ± SE (n = 3)

Effects of 1-MCP or ethrel treatment on the β-Man activities: Mannans are a component of fruit cell wall, β-MAN which hydrolases these polymers, has showed to be involved in fruit ripening and softening [18]. During '*Yanhong*' peach storage, the activities of β-Man in epicarp and mesocarp indicated a clear "up-down" pattern and with the highest value on day 6 (Fig. 5). However, most β-Man activity was associated with the epicarp than that in the mesocarp during storage. This corresponds with the result of Bewley et al. [18] on

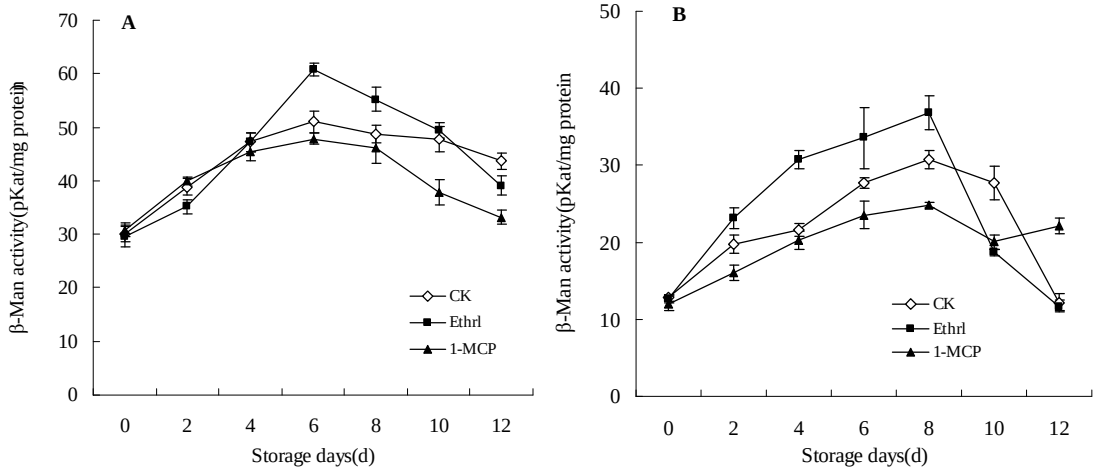

Fig. 5 Effects of 1-MCP or ethrel treatment on β-Manl activity in the epicarp (A) and mesocarp (B) of '*Yanhong*' peach fruit
Each value is presented as means ± SE (n = 3)

tomato fruit. Bewley *et al*. [18] explained that the high activity of the enzyme in the tomato skin to some extent could be due to the larger amount of cell wall material with which it is associate, compared to the pericarp region. Because the ripening and softening of climacteric fruits is controlled by endogenous ethylene biosynthesis, it has been presumed that the activities of ripening-regulated cell wall hydrolases are ethylene-regulated. However, up to date, no information is available on the effects of ethylene on the β-Man during fruit ripening and softening. In this work, 1-MCP treatment reduced the activity of β-MAN in the epicarp and mesocarp during peach storage (Fig. 5). In contrast, ethrel promoted the increase of β-MAN in both tissues (Fig. 5). This indicated that the activity of β-MAN could be regulated by ethylene.

Effects of 1-MCP or ethrel treatment on the β-Gal activities: β-Gal has been characterized in association with the removal of galactosyl residues from cell wall polymers during fruit softening [19].

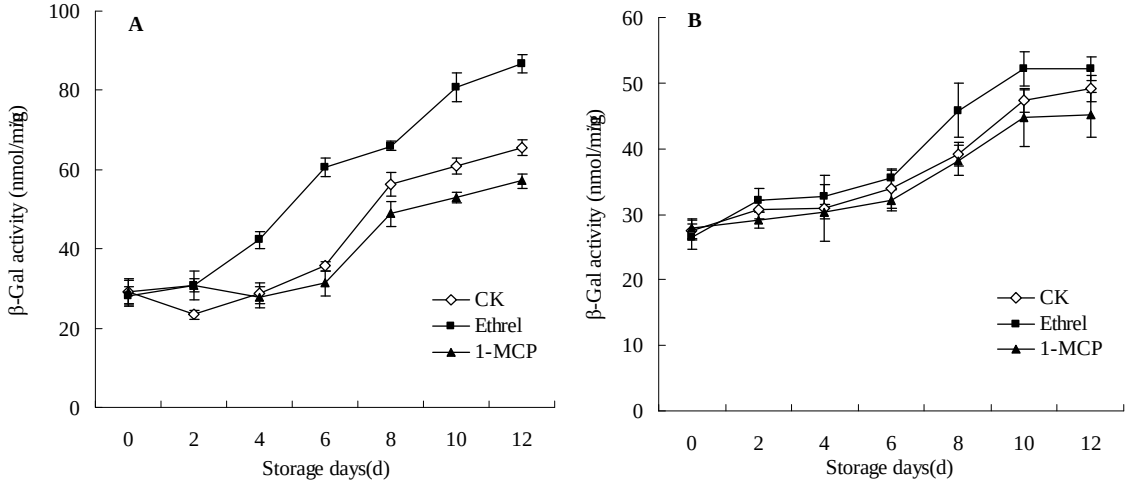

Fig. 6 Effects of 1-MCP or ethrel treatment on β-Gal activity in the epicarp (A) and mesocarp (B) of '*Yanhong*' peach fruit
Each value is presented as means ± SE (n = 3)

An increase in β-Gal activity was observed in both epicarp than that in mesocarp during peach storage, especially after day 6 of storage (Fig. 6). Treatments with ethrel dramatically increased β-Gal activity, whereas the opposite response was found with 1-MCP treatment (Fig. 6). A similar result was also found in pear and avocado treated with 1-MCP during fruit ripening and softening [20, 21]. Like β-Man, β-Gal activities were much higher in the epicarp than that in the mesocarp in all time-point assayed. Mean β-Gal activity was about 1.3 fold higher in mesocarp

tissues in contrast to fruit epicarp tissues.

Effects of 1-MCP or ethrel treatment on the α-l-Af activities: The glycoside hydrolase α-l-Af cleaves the glycosidic bonds between l-arabinofuranoside side chains and various oligosaccharides. During the ripening process, the activity of arabinosidases has been associated with softening in several fruits [19]. In the present work, α-l-Af activity increased during '*Yanhong*' peach storage (Fig.7). This result is in accordance with previous results on peach[22], apple [19] and pear [23]. Many α-l-Af isoforms have been characterized from pear [23]; one of them has been shown to be regulated by ethylene, functioning by releasing arabinosyl residues from the pectic fraction[19]. During '*Yanhong*' peach storage, α-l-Af activities in epicarp and mesocarp were notably inhibited by 1-MCP treatment, but somewhat promoted by ethrel (Fig.7). This indicated that α-l-Af is sensitive to ethylene and has an important role in softening the fruit texture [19]. In addition, α-l-Af activities were also higher in the epicarp than that in the mesocarp during peach storage. However, Di Santo *et al*.[24] showed that total *α-l-Af* activity was higher in the inner mesocarp and lower in the epicarp during 'Springcrest' peach ripening. These differences may be due to genetic variations between cultivars and/or to changes in the protein content per unit fresh weight, which certainly occurs as cells expand and accumulate water and solutes in vacuoles [24].

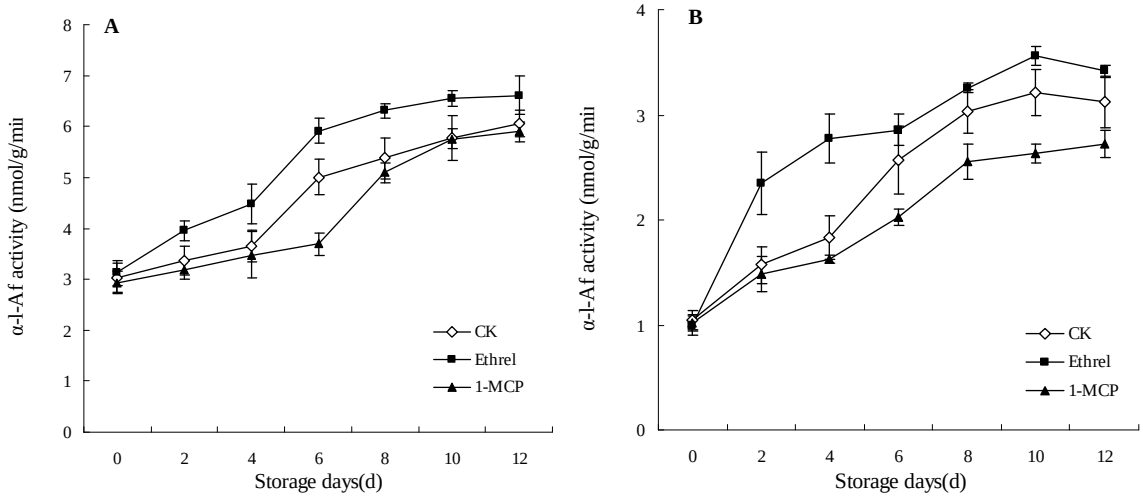

Fig. 7 Effects of 1-MCP or ethrel treatment on α-l-Af activity in the epicarp (A) and mesocarp (B) of '*Yanhong*' peach fruit
Each value is presented as means ± SE (n = 3)

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