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Physiological Responses of Banana Fruit to Two Structural Analogues of 1-MCP as Ethylene Action Inhibitors

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Abstract

Ethylene induced actions during fruit ripening have been found to be inhibited by 1-methylcyclopropene (1-MCP) and possibly its analogues by competing for the sites of binding on the ethylene receptor. The purpose of this study was to evaluate the effect of treatment with 1-PentCP or 1-OCP, two structural analogues of 1-MCP, on inhibiting ethylene action in banana (*Musa sapientum* L.) fruit. The banana fruit after being fumigated for 20 h at 20 ± 2°C with 0 (control), 0.4, 0.8 or 1.2 μL l^{-1} of 1-MCP, 1-PentCP or 1-OCP were stored in air environment at 20 ± 2°C for ripening assessment. Ethylene production, color change, firmness, ACS activity, ACO activity and ACC content of the fruits were measured every 5 days during storage. The results of our study showed that these two structural analogues, just as 1-MCP, exerted their effect in a concentration-depended manner. 1-OCP was found to inhibit ethylene-induced ripening of banana fruit at a low concentration relatively. Treatment for 20 h with 0.8 μL l^{-1} 1-OCP or 1.2 μL l^{-1} 1-PentCP all delayed ethylene climacteric peak of banana fruit by 10 days as compared with the untreated group, after which the banana fruit resumed normal ripening. Softening and color change of banana fruit was restrained by the two structural analogues. Increasing the concentration of 1-PentCP to 1.2 μL l^{-1} caused a marked delay in softening and color change, but increasing the concentration of 1-OCP to 1.2 μL l^{-1} or higher did not result in a further delay in softening and color change. The results indicated that treatment with 0.8 μL l^{-1} 1-OCP is sufficient to exert maximal delay of banana fruit ripening. Treatment with the most effective concentration of 1-OCP or 1-PentCP delayed ACC content, ACS activity and ACO activity of banana fruit as compared with the untreated group, but less effective than 1.2 μL l^{-1} 1-MCP. It is suggested that 1-OCP was found to be a more potent inhibitor of banana fruit ripening than 1-PentCP, but less potent than the mother compound 1-MCP. The two structural analogues are beneficial to fruit storage and enhance the fruit resistance to decay that can be promised to be applied in agricultural practice.

Keywords: Banana; Ethylene; 1-methylcyclopropene (1-MCP); Structural analogues; ACC synthase; ACC oxidase

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Introduction

Ripening and senescence of climacteric fruits are accompanied by autocatalytic increases in ethylene production and in respiration rate in the fruits [1,2]. Not only endogenous ethylene, but also exogenous ethylene can induce some ripening-related changes. As ethylene has been regarded as the 'ripening hormone', studies with various ethylene inhibitors have provided better understanding of its regulation of biosynthesis in cases such as fruit ripening [3,4]. Ethylene effect can be inhibited by either inhibitors of ethylene production or inhibitors of ethylene action. Inhibitors of ethylene production, such as aminoethoxyvinylglycine (AVG) that is an inhibitor of ACC synthase, can inhibit the production of ethylene [5,6]. Inhibitors of ethylene action bind to the receptor of ethylene and thereby restrain ethylene effect. Inhibitors of ethylene action are considered particularly effective for agriculture use since they protect the fruits and vegetables from both endogenous and exogenous ethylene. Although the practical use of inhibitors of ethylene action was introduced, none of these inhibitors was found suitable for use with edible agricultural produces because of their drawbacks [7]. For example, the heavy metal silver (silver thiosulfate) is an efficient inhibitor of ethylene action, but its toxicity limits its use [8,9]. The use of 2, 5-norbornadiene, an excellent means for controlling ethylene

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responses, is limited because of its strong chemical odors and corrosiveness [10].

During the last decade, commercial product of 1-MCP (1-methylcyclopropene) appeared as a stable powder has been demonstrated to inhibit ethylene action which easily released as a gas when the powder is dissolved in water. However, 1-MCP has been recently shown to control ethylene action by competing with ethylene for the binding site on the ethylene receptor level in plant tissues and thereby prevent ethylene-dependent responses [11]. Many researchers conducted in different places with various plant materials have shown that the effect of 1-MCP on postharvest physiological changes and quality of fruits and vegetables are strong and extensive. Not only dose 1-MCP provide the potential to maintain fruit and vegetable quality after harvest, but also it provides a powerful tool to gain insight into the fundamental processes that are involved in ripening and senescence [12]. Even so, the practical use of 1-MCP is still limited in some areas of agricultural practice because of the uneven color development of the treated fruits. And this problem was exacerbated because of the difference in the maturity range of the treated fruits presented in a commercial consignment [13,14].

At the beginning of this century, a research group led by Sisler et al. [10] firstly synthesized eight new structural analogous of 1-MCP, namely 1-butylcyclopropene (1-BCP), 1-pentylcyclopropene (1-PentCP), 1-Hexylcyclopropene (1-HCP), 1-heptylcyclopropene (1-HeptCP), 1-octylcyclopropene (1-OCP), 1-decatylcyclopropene (1-DCP), 1-ethylcyclopropene (1-ECP) and 1-propylcyclopropene (1-PCP), which contain a differently longer side chain at 1-position and are found to exert similar effect of 1-MCP to inhibit ethylene action [15]. An over-all description on these newly synthesized structural analogous of 1-MCP as inhibitors of ethylene action has been reported by Apelbaum et al. [16]. Some preliminary studies about the effect of some new structural analogues of 1-MCP on the ripening of avocado, pea seeds, citrus leaf and tomato fruit have been reported [4,17]. Between these new structural analogues, 1-MCP, 1-PentCP and 1-OCP possess the biggest difference in the 1-position side chain length of the main cyclopropene structure, which provide a good way that can be possibly employed to investigate the events and mechanisms. The study reported herein is mainly focused on the potency of 1-PentCP and 1-OCP to stall ethylene-induced processes in banana fruits as a representative of climacteric fruit. The aim of the present study is to learn more about the inhibitory nature of 1-PentCP and 1-OCP and to assess its ability to control the ripening of banana fruits so as to provide theory reference for seeking a more effective ethylene inhibitor and applying in agricultural practice.

Materials and Methods

Fruit and treatment

Mature green banana fruit (*Musa sapientum* L.) was purchased at local market in Shenyang, China during the commercial harvesting season and then the fruits were transported to the laboratory of College of Food Science, Shenyang Agriculture University on the same day. Damaged fruits and outliers in size and color were excluded and 10 groups of 20 green ripe stage fruits were selected from the qualified fruits for further treatment. Within 2 h of purchase the 10 selected group of 20 fruits each were separately sealed in ten plastic tents (50 cm × 50 cm × 50 cm) and exposed to 0 (control), 0.4, 0.8 or 1.2 µL l^{-1} of 1-MCP, 1-PentCP or 1-OCP for 20 h at 20 ± 2°C. The ten plastic tents were then vented and thereafter the fruits were stored in air environment at 20 ± 2°C and their ripening was assessed by measuring the following parameters: ethylene production, color change, firmness, ACS activity, ACO activity and ACC content. Each treatment was carried out in five replications, and all experiments were repeated three times.

Determination of ethylene production

Banana fruits were sealed in ten 3 L glass jars (five fruits per jar) for 1 h. The ethylene production rate (µl $kgFW^{-1}$ h^{-1}) was measured by withdrawing two 1 mL gas samples with a gas syringe from each jar through a septum stopper fitted in the jar lid [4]. Ethylene production was analyzed by using a gas chromatography (CP-3800 GC, Varian, USA) equipped with a flame ionic detector (FID) and a stainless steel column (1 m × 0.4 mm, GDX-502, Agilent, USA). The chromatographic analytic parameters were 60°C of column temperature, 270°C of detector temperature and N2 at 4.0 mL min^{-1} current velocity. The jars were left open between measurements for ventilation.

Assessment of color change

Color change of banana fruit was determined by using a Minolta Chromo-meter (CR-400, China). Peel color change of banana fruit was scored on a scale of 1 (green), 2 (breaker), 3 (<25% color change), 4 (25-50% color change), 5 (>50% but <100% color change), 6 (fully yellow), and 7 (yellow with black spots) [18].

Measurement of firmness

Fruit firmness (N) was determined by using a digital penetrometer (FT-327, Fruit Test™, Italy) fitted with a 5 mm diameter conical probe. The conical probe was pushed into the banana fruit through the skin to the depth of the head (10 mm) and expressed in Newtons (N) [19].

Measurement of ACC content

A 0.2 g sample of fresh banana tissue from 3 banana fruits each treatment was extracted in 1 mL of 80% (v/v) ethanol (AR, 95%) for at least 16 h at 4°C and the samples were centrifuged at 12000 rpm for 10 min. The supernatant were evaporated under vacuum. The dry residue was dissolved in a known amount of distilled water (KY-HS.Z68.5, Beijing Tongde Venture Science and Technology Co., LTD, China) (half to one time the original fresh weight of the tissue, according to the anticipated concentration of ACC) and ACC contents were measured by a modification of the method of Lizada and Yang [20]. To 0.1 mL of the above aqueous extract in a test tube was added 1 µmol of $HgCl_2$, and sufficient water to bring the volume to 0.8 mL. The test tubes were sealed with serum caps and 0.2 mL of an ice-cold mixture (2:1, v/v) of 5% NaOCl and saturated NaOH was injected. The assay tubes were held in an ice bath and after 3 min a gas sample was withdrawn for measurement of ethylene content by gas

chromatography (CP-3800 GC, Varian, USA). The efficiency of ACC conversion to ethylene in each sample was determined by adding a known amount of ACC as an internal standard to a replicate assay tube. The identity of ACC in banana tissue was verified by chromatography with authentic ACC on Whatman No. 3 paper (Sinopharm Chemical Reagent Co. Ltd, China) developed with butanol-acetic acid-water (4:1:5, v/v) as described by Lizada and Yang [20].

Measurement of ACS and ACO activity

ACS (1-aminocyclopropane-1-carboxylate synthase, EC 4.4.1.14) enzyme was extracted by the method of Kato and Hyodo [21] with slight modifications. A 1 g of banana pulp collected from 3 banana fruits for each treatment was homogenized in a mortar with 1 ml extraction buffer, pH 7.2, containing 0.1 M Tris-(hydroxymethyl)-ainomethane (BR, ≥ 99.5%), 5 mM dithiothreitol (DTT, AR, 99.0%), 30 mM L-ascorbic acid sodium salt (AR, 99.0%) and 30% glycerro (AR, 99.0%). The homogenate was centrifuged at 12 000 rpm for 30 min at 4°C and the supernatant was assayed for ACS activity by a modification of the method of Kato et al. (1999). ACS activity was measured using a high-resolution gas chromatography (Agilent Hewlet Packard 6890 Series, USA) equipped with ZB-624 capilar colon, at 200°C flame ionization detector and expressed as nmol ACC formed h^{-1} gFW^{-1}.

ACO (1-aminocyclopropane-1-carboxylate oxidase, EC 1.14.17.4) enzyme was extracted and its activity assayed by a modification of the methods of Pathak et al. [22]. A 1 g of banana pulp collected from 3 banana fruits for each treatment was extracted in sealed conical flasks with 3 ml reaction mixture consisting of 1 mM ACC, 0.4 M mannitol (AR, 99.0%), 30 mM Na-ascorbate (AR, 99.0%), 0.1 mM $FeSO_4$ (AR, 99.0%), 20 mM $NaHCO_3$ (AR, ≥99.5%) and 0.1 M tricine (AR, ≥ 99.0%), pH 7.5. After standing for 1 h, 5 mL of gas from the head-space of the sealed conical flasks was removed by a gas syringe for the ACO assay. The gas was injected onto the gas chromatography (CP-3800 GC, Varian, USA). The GC settings were: injector temperature 120°C; detector temperature 250°C; oven temperature 68°C; carrier gas N_2; flow rate through column was 30 mL min^{-1}. The ACO activity was expressed as nmol C_2H_4 produced h^{-1} gFW^{-1}.

Synthesis and quantification of 1-PentCP and 1-OCP

1-MCP was obtained from National Engineering Technology Research Center for Preservation of Agricultural Products (CPAP, Tianjin, China) in a ready to use form. Both 1-PentCP and 1-OCP were synthesized according to the methods described by Al Dulayymi et al. [23,24] by a decompression distillation unit (Sinopharm Chemical Reagent Co. Ltd, China) at the Department of Food Science (Shenyang Agricultural University, China). For quantification, 1-PentCP and 1-OCP were injected (1 mL gas sample) into a Varian 3300 gas chromatograph fitted with a 23% SP-1700 on 80/100 chromosorb P AW column and a FID. Cyclohexane was used as a calibration standard for both compounds.

Statistical analysis

The data were carried out by one-way analysis of variance with the statistical software SPSS 13.0 for Windows (SPSS Inc., Chicago, USA). Unless noted otherwise, only results significant at $p<0.05$ were discussed.

Results

Effects of different ethylene inhibitors on ethylene production

The responses of banana fruit to varying concentration of treatment with ethylene inhibitors revealed that ethylene-induced effect was essentially eliminated. In untreated banana fruit, the measured maximum ethylene peak appeared on day 5 after harvest. However, to some extent, treatment with 1-MCP, 1-PentCP and 1-OCP for 20 h at 20 ± 2°C markedly delayed ethylene-induced ripening of banana fruit in a concentration dependent manner. Treatment of banana fruit with 0.4, 0.8 or 1.2 µL l^{-1} 1-MCP delayed ethylene-induced ripening by 5, 10 and 10 days, respectively, as compared with untreated banana fruits. Higher concentrations of 1-MCP (1.2 µL l^{-1}) did not further delay ripening (**Figure 1A**).

Similar results were obtained with 1-PentCP and 1-OCP

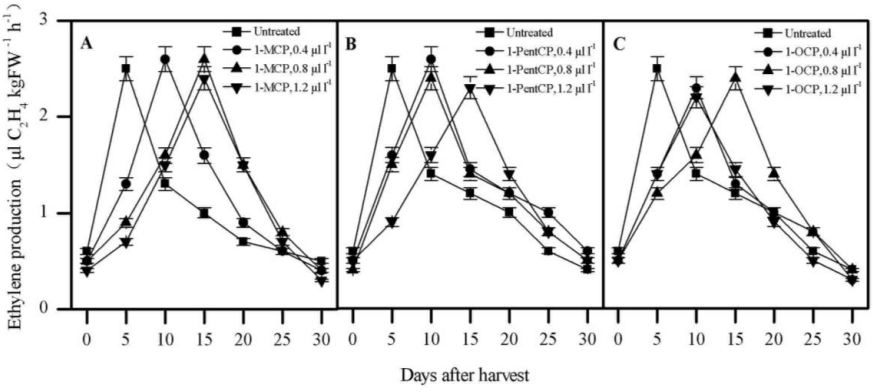

Figure 1 Effects of treatment with different ethylene inhibitors on ethylene production in banana fruit. The fruit were treated for 20 h at 20 ± 2°C with the indicated concentrations of 1-MCP, 1-PentCP or 1-OCP and then stored at 20 ± 2°C for ripening assessment. Each value is a mean for five replications, and vertical bars indicate standard errors.

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treatments. Both treatments with 0.4 and 0.8 µL l^{-1} 1-PentCP delayed ethylene-induced ripening of banana fruit by 5 days, and increasing the concentration to 1.2 µL l^{-1} further delayed ripening by 10 days, as compared with untreated banana fruits (**Figure 1B**). Treatment with 0.4, 0.8 or 1.2 µL l^{-1} 1-OCP delayed ethylene-induced ripening by 5, 10 and 5 days, respectively, as compared with untreated banana fruits. In this case also, increasing the concentrations of 1-OCP to 1.2 µL l^{-1} did not further delay ripening (**Figure 1C**). It should be noted that all the fruits treated with 1-MCP, 1-PentCP or 1-OCP resumed normal ripening after recovery from the inhibition. This fact is importance when considering the putative inhibitors for practical use.

Effects of treatment with different ethylene inhibitors on firmness and color change

The enhancement of softening and color development of banana fruit depend on exogenous and endogenous ethylene concentration and duration of exposure [4,7]. In varying degrees, treatment with various concentrations of the three different ethylene inhibitors extended the period to reach fruit softening and prolonged the time needed to complete color change in banana fruit at 20 ± 2°C (**Figure 2**).

Five days after treatment, higher concentration (0.8 µL l^{-1} or 1.2 µL l^{-1}) of 1-MCP and 1-PentCP were required to achieve a further delay of softening for banana fruit. The color value decreased significantly in fruit treated with higher concentrations of 1-MCP (0.8 µL l^{-1} or 1.2 µL l^{-1}) and 1-PentCP than with lower concentrations (0.4 µL l-1). But treatment with higher concentration (1.2 µL l-1) of 1-OCP did not further delay softening and decrease color value. Also, no significant difference ($p<0.05$) in color value was evident in banana fruit treated with 0.4 µL l^{-1} and 0.8 µL l^{-1} of 1-MCP (**Figure 2**).

As for 15 days after treatment, banana fruits treated with the three ethylene inhibitors, continued to show good delaying effect on fruit softening and color changing. The most effective concentration of the inhibitor 1-MCP (1.2 µL l^{-1}) and 1-PentCP (1.2 µL l^{-1}) required to delay ethylene-induced softening in fruits was higher than that of 1-OCP (0.8 µL l^{-1}). However, the most effective concentration of 1-PentCP required to delay color change in fruits was 1.2 µL l^{-1}. But as for 1-MCP and 1-OCP, there was no significant difference ($p<0.05$) between the effect of treatments with 0.8 µL l^{-1} and 1.2 µL l^{-1} on delaying color change (**Figure 2**).

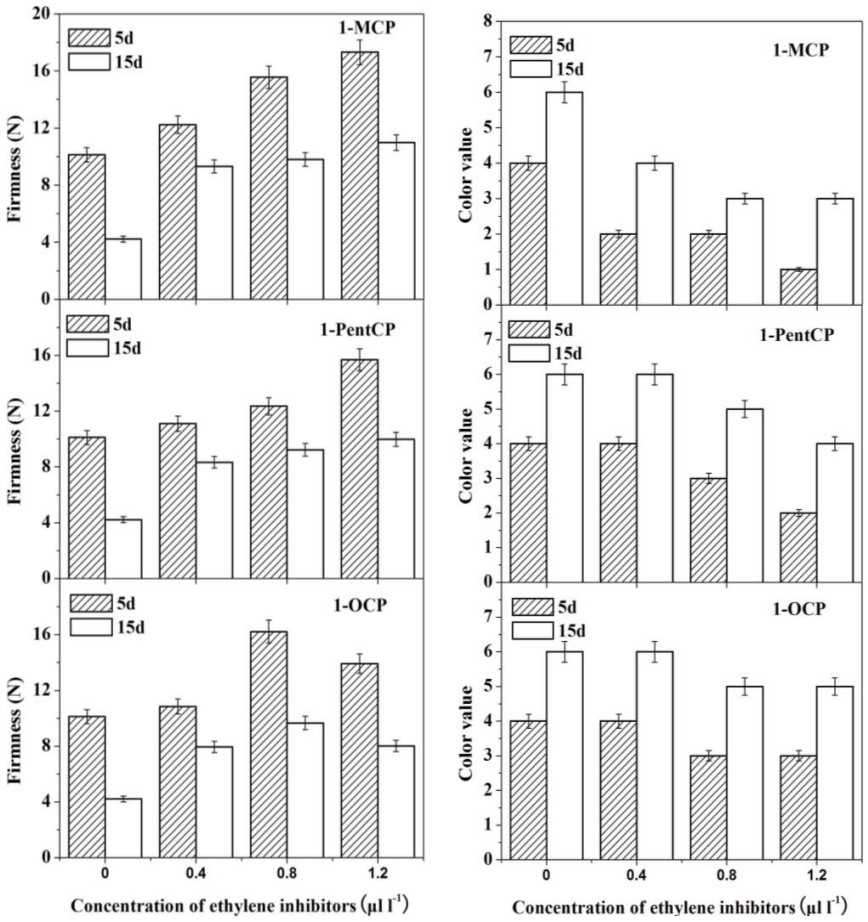

Figure 2 Effects of treatment with different ethylene inhibitors on firmness and color change in banana fruit. The fruit were treated for 20 h at 20 ± 2°C with the above indicated concentrations of 1-MCP, 1-PentCP and 1-OCP, afterwards stored at 20 ± 2°C for ripening assessment. Results of day 5 and 15 after treatment for each analogue are presented. Each value is a mean for five replications, and vertical bars indicate standard errors.

Effects of different treatments on ACC content

Because of the better effect of treatment with 1.2 µL l^{-1} 1-MCP, 1.2 µL l^{-1} 1-PentCP or 0.8 µL l^{-1} 1-OCP as observed in the experiments, only the results of ACC content, ACS activity and ACO activity in banana fruit treated with these concentrations of the three ethylene inhibitors were presented. The untreated fruit showed low level of ACC content on day 0 (0.2 nmol gFW^{-1}) which increased about 3.0 fold on day 5 (0.6 nmol gFW^{-1}) and reached a peak of 7.5 fold on day 10 (1.5 nmol gFW^{-1}), but declined thereafter (**Figure 3**). ACC content of banana fruits treated with 1-MCP at the most effective concentration (1.2 µL l^{-1}) gradually increased during storage from day 0 to day 15, but the peak value (0.8 nmol gFW^{-1}) was 1.88 fold less than that of untreated fruit (1.5 nmol gFW^{-1}). In addition, the peak appearing time of ACC content of banana fruits treated with 1.2 µL l^{-1} 1-MCP was probably delayed for 5 d (**Figure 3**). In banana fruit treated with 1-PentCP at the most effective concentration (1.2 µL l^{-1}), ACC content showed two lower peaks (0.7 nmol gFW^{-1}) on day 10 and 20, which was 2.14 fold less than that of untreated fruits (1.5 nmol gFW^{-1}). Also, during the whole storage period, a dramatical fluctuation in ACC content was observed **(Figure 3)**. In 0.8 µL l^{-1} 1-OCP treated fruits, a sharp increase was observed up to day 10 and then a slight decline in ACC content appeared. The peak value (0.7 nmol gFW^{-1}) was also 2.14 fold less than that of untreated fruit (1.5 nmol gFW^{-1}) on day 10 **(Figure 3)**.

Effects of different treatments on ACS activity

While the activity of ACS and ACO determines the ethylene production, they are two key enzymes which play an important role in the ethylene biosynthetic pathway [25]. The ACS activity of untreated fruit increased slowly from day 0 to day 15, remaining steady until day 20 then decreased (**Figure 4**). The maximum activity of ACS (0.7 nmol ACC h^{-1} gFW^{-1}) in fruits treated with 1.2 µL l^{-1} 1-MCP appeared on day 20 followed by a gradual decline. In fruits treated with 1.2 µL l^{-1} 1-PentCP, a gradual increase in ACS activity was observed, but its activity peak (1.0 ACC h^{-1} gFW^{-1}) was 1.43 fold higher than that of fruits treated with 1.2 µL l^{-1} 1-MCP (0.7 ACC h^{-1} gFW^{-1}). However, the ACS activity in fruits treated with 0.8 µL l^{-1} 1-OCP increased gradually before day 20 then decreased **(Figure 4)**. Not only treatment with 1.2 µL l^{-1} 1-MCP postponed the time that reached the peak of ACS activity, but it was the highest concentration in this experiment. Therefore, the most effective inhibitory action of the three ethylene inhibitors was 1.2 µL l_{-1} 1-MCP.

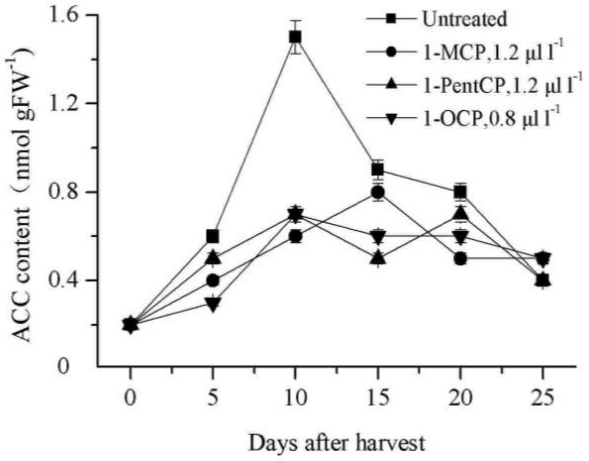

Figure 3 Effects of treatment with different ethylene inhibitors on ACC content in banana fruit. The fruit were treated for 20 h at 20 ± 2°C with the indicated concentrations of 1-MCP, 1-PentCP or 1-OCP, then stored at 20 ± 2°C for ripening assessment. Results with the most effective concentration for each compound are presented. Each value is a mean for five replications, and vertical bars indicate standard errors.

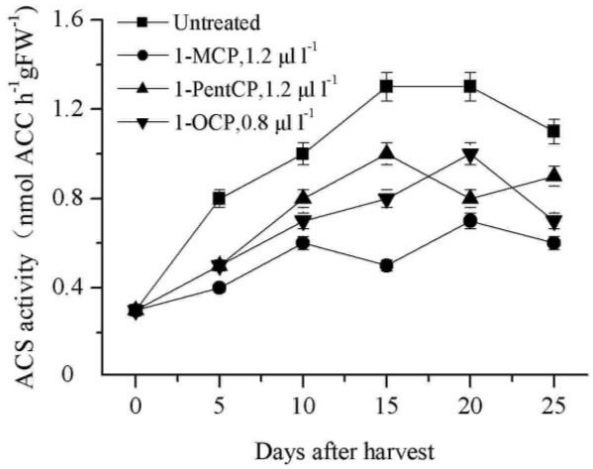

Figure 4 Effects of treatment with different ethylene inhibitors on ACS activity in banana fruit. The fruit were treated for 20 h at 20 ± 2°C with indicated concentration of 1-MCP, 1-PentCP or 1-OCP, afterwards stored at 20 ± 2°C for ripening assessment. Results with the most effective concentration for each compound are presented. Each value is a mean for five replications, and vertical bars indicate standard errors.

Effects of different treatments on ACO activity

ACO is the major rate-limiting enzyme during the ripening process of fruit [26]. The results indicate ACO activity (**Figure 5**) showed a different change pattern compared with ACS activity (**Figure 4).** In untreated fruit, ACO activity increased sharply to match the peak appearing time of ACO activity (1.5 nmol C_2H_4 $h^{-1}gFW^{-1}$) from day 0 to day 10, thereafter decreased gradually **(Figure 5)**. Among fruits treated with 1.2 µL l^{-1} 1-MCP, ACO activity gradually increased during storage from day 0 to day 15 but the peak value (1.1 nmol C_2H_4 $h^{-1}gFW^{-1}$) was 1.36 fold less than that of untreated fruit (1.5 nmol C_2H_4 $h^{-1}gFW^{-1}$). Also, the peak appearing time of ACO activity was probably delayed for 5 d. In banana fruit treated with 1.2 µL l^{-1} 1-PentCP, ACO activity showed a lower peak (0.9 nmol C_2H_4 $h^{-1}gFW^{-1}$) on day 10, which was 1.67 fold less than that of untreated fruits (1.5 nmol C_2H_4 $h^{-1}gFW^{-1}$). From day 10 on, a slight fluctuation in enzyme activity was observed **(Figure 5).** In 0.8 µL l^{-1} 1-OCP treated fruits, a gradual increase was observed up to day 10 and then a slight decline in enzyme activity appeared. ACC content and ACO activity showed almost a similar pattern, possibly due to the fact that ACO activity was directly related to internal ACC content **(Figures 3 and 5)**.

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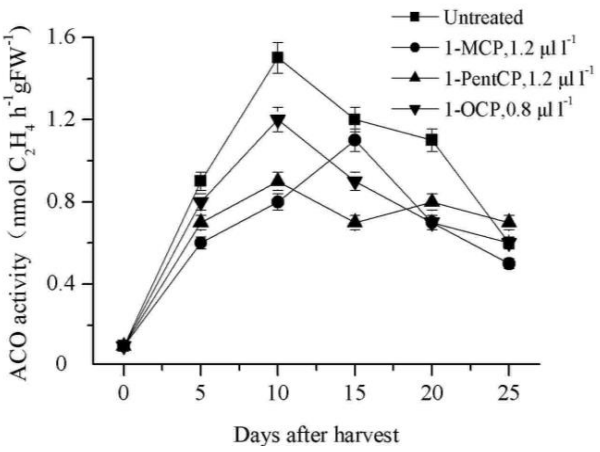

Figure 5 Effects of treatment with different ethylene inhibitors on ACO activity in banana fruit. The fruit were treated for 20 h at 20 ± 2°C with indicated concentration of 1-MCP, 1-PentCP or 1-OCP, afterwards stored at 20 ± 2°C for ripening assessment. Results with the most effective concentration for each compound are presented. Each value is a mean for five replications, and vertical bars indicate standard errors.

Discussion

The storage period of climacteric fruits is terminated by softening associated with modulating levels of ethylene evolution at the onset of ripening. Thus, the shelf life of climacteric fruits is shortened by both endogenous and exogenous ethylene action [27,28]. 1-MCP is considered to be an effective ethylene inhibitor which can prevent ripening by irreversibly occupying the site of binding at the ethylene receptor level [12,29]. For example, 1-MCP has been shown to inhibit ethylene-induced effect in tomato [30,31] banana [30,32], plum [33], and apple [34] fruits, and in numerous ornamentals [30,35-37]. Moreover, the structural analogues of 1-MCP -- 1-ECP and 1-PCP -- were thought to be putative inhibitors of ethylene action and thereby the responses to ethylene in various plant systems were similar to that of treatment with 1-MCP [4,11].

The objective of this research reported herein was to assess the potency of the selected newly synthesized compounds of 1-PentCP and 1-OCP, which were expected to interact with the ethylene receptor and inhibit ethylene-induced responses in plants in a similar way as 1-MCP. In this research on the ripening of banana fruit, three concentrations (0.4 µL l^{-1}, 0.8 µL l^{-1} or 1.2 µL l^{-1}) of the three ethylene inhibits were used, and the experiments were conducted at the temperature of 20 ± 2°C that was known to hasten ripening. This was done in order to evaluate the potency of the structural analogues under extreme condition. The pattern of ethylene production during ripening in banana fruit is different from that in other climacteric fruits and similar to that of avocado [22]. In this study, ethylene-induced ripening of banana fruit treated with 0.4 and 0.8 µL l^{-1} 1-PentCP, judged by fruit firmness as compared with untreated banana fruits, was delayed by 5 days, and 1.2 µL l^{-1} 1-PentCP further delayed ripening by 10 days **(Figure 1B),** but 1.2 µL l^{-1} 1-OCP did not further delay ripening **(Figure 1C)**. Firmness and color are very important from the point of view of the consumer's acceptance in banana fruit. In our study, 0.8 µL l^{-1} or 1.2 µL l^{-1} 1-PentCP further delayed the softening and color change of banana fruit, but treatment with 1.2 µL l^{-1} 1-OCP did not further delay softening and decrease color value on day 5 after treatment. But up to day 15 after treatment, the most effective concentration of 1-PentCP (1.2 µL l^{-1}) required to delay softening was higher than that of 1-OCP (0.8 µL l^{-1}) **(Figure 2)**. However, the most effective concentration of 1-PentCP required to delay color change was 1.2 µL l^{-1} **(Figure 2)**. But as for 1-OCP, there was no significant difference between the effect of treated with 0.8 µL l^{-1} and 1.2 µL l^{-1} on delaying color change ($p<0.05$) **(Figure 2)**.

The peak of ACS activity in fruits treated with 1.2 µL l^{-1} 1-PentCP was 1.43 fold higher than that of fruits treated with 1.2 µL l^{-1} 1-MCP. However, the ACS activity in fruits treated with 0.8 µL l^{-1} 1-OCP increased gradually before day 20 then decreased **(Figure 4)**. ACO activity of banana fruit treated with 1.2 µL l^{-1} 1-PentCP showed a lower peak on day 10, which was 1.67 fold less than that of untreated fruits. In addition, ACO activity and ACC content showed almost a similar pattern, possibly due to the fact that ACO activity was directly related to internal ACC content (**Figures 3 and 5**). Treatment with the two structural analogues of 1-MCP seemed to have effect on ACC accumulation. Apparently treatment of 1-PentCP and 1-OCP inhibited effectively the first step of ethylene biosynthesis i.e., ACC accumulation **(Figure 3**).

Results in our study show that the two analogues 1-PentCP and 1-OCP exerted their effect in a concentration-dependent manner within the range of the concentrations tested and by blocking ethylene binding at the ethylene receptor. The structural analogue 1-OCP was found to be a more potent inhibitor of ethylene action than 1-PentCP, but less potent than the mother compound 1-MCP. Similar results have been reported that the newly synthesized inhibitors are in general less potent than 1-MCP [38]. As both analogues compounds of 1-MCP notably delayed the banana fruit ripening at low concentrations under extreme conditions that stimulate fruit ripening, this result suggests both 1-PentCP and 1-OCP be considered as candidates for practical application for agriculture.

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