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Full Length Research Paper

A novel 1-methylcyclopropene treatment for quality control in Nangou pears at ambient temperature

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1-Methylcyclopropene (1-MCP) has been shown to counteract ethylene responses in plants in many studies. The traditional 1-MCP treatment is conducted in an air-tight facility by fumigating with 1-MCP for 12 to 24 h. However, the facility for the treatment should be as tight as possible to prevent gas leakage and its availability could be a limitation for commercial use. The aim of this study was to analyze the effectiveness of a new 1-MCP treatment mode for delaying ripeness by using Nanguo pears as material and comparing with both traditional 1-MCP treatment (T2) and without 1-MCP treatment (CT). In the novel treatment, harvested pear fruits were put in plastic bag; a small self-sealing bag containing 1-MCP complex powder were put in the bag after being punctured with a pin. The powder released 1-MCP gas when in contact with the water vapor produced by the fruit itself through transpiration during storage. The treatment of fumigating with 1-MCP as usual and treatment without 1-MCP were used as the controls. All treated fruits were stored at about 20°C and analyzed every 7 days for up to 42 days. Compared to traditional 1-MCP treatment (T2), the new treatment (T1) showed similar results in terms of delayed ripening, suppressed respiration and ethylene climacteric. It also markedly retarded the rate of softening and ripeness index, delayed the peak of total phenol amount both in flesh and peel. It was noted that the novel 1-MCP treatment could achieve comparable effect with the traditional 1-MCP treatment. It has the advantage and practicality of not needing special facilities, and, therefore, the potential of providing an alternative way for 1-MCP treatment to delay fruit ripening and senescence process, and control quality changes during the storage of fruit.

Key words: 1-Methylcyclopropene, ripening, softening, pears.

INTRODUCTION

1-Methylcyclopropene (1-MCP) had been proven to have immense benefit for controlling the ripening and senescence of a number of fruits and vegetables (Blankenship and Dole, 2003; Watkins, 2006; Huber, 2008). 1-MCP is a gaseous ethylene antagonist-chemically similar to natural occurring substances-that inactivates the ethylene receptor in different fruits and other plant tissues for a varying number of days by single exposure to very low dose levels of this cyclic compound, preventing the signaling mechanisms that activate senescence- and ripening-associated genes (Sisler and Serek, 1997, 2003). As originally formulated, 1-MCP has been developed as a powder with 1-MCP complexed with γ-cyclodextrin which when mixed with water or buffer solution releases the 1-MCP gas (Lalel et al., 2003; Ahmad and Khana, 2007). For commercial use, plant materials are treated in enclosed environment, such as tightly built greenhouses, rooms, coolers, truck trailers, shipping boxes/containers, etc. However, the availability of the proper facilities to treat the fruit could be a limitation under certain commercial situations (Serek and Serek, 1994; Manganaris et al., 2007; Blankenship, 2003; Watkins, 2006; Huber, 2008).

More recently, preparations of 1-MCP designed for use as aqueous solutions or sprays have been developed, which have been designed to facilitate broader agricultural applications of the ethylene-action inhibitor, particularly, in post-harvest settings (Yuan et al., 2007; Elfving et al., 2007; Sun et al., 2008a, b, 2009). Sun and Donald (2008a, b, 2009) reported on the use of aqueous 1-MCP formulations to suppress the post-harvest ripening

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of tomato and avocado and climacteric fruits previously shown to respond well to ripening attenuation with gaseous 1-MCP. Following immersion in aqueous 625 µg L^{-1}, 1-MCP for 1 min, the efficacy of the aqueous formulation at delayed ripening of breaker-turning tomato fruit was comparable to that observed in response to exposure to gaseous 1-MCP at 500 nl L^{-1} for 9 h. Immersion in aqueous 1-MCP delayed and suppressed the ethylene and respiratory climacterics and also delayed softening, lycopene accumulation and increases in polygalacturonase activity indicating that the responses of ethylene-sensitive ripening parameters to aqueous 1-MCP were comparable to gaseous 1-MCP.

The aim of this study was to investigate whether a novel treatment of 1-MCP was sufficient to delay the ripening of Asian pears. This method of application was compared with the regular method of 1-MCP application and non-treated fruit.

MATERIALS AND METHODS

Nanguo pears (about 80% matured stage) were harvested from an orchard in Anshan city, Liaoning Province of China and were transferred to the laboratory at Shenyang Agriculture University within 5 h after harvesting. Fruits with uniform size and appearance were selected and treated as described. 1-MCP was obtained from Smart FreshTM, AgroFresh Inc., USA as a commercial powder (0.14%) product.

Treatments

Pears were divided into three groups with each group having a total of 210 pears. Each group was divided into three replicates with 70 pears in each replicate. Each individual replicate was given one of the following three treatments: (1) Treatment 1 (T1), the pears were put in plastic bags (average 5 kg per bag), then a self-sealing bags containing 1-MCP 0.2 g, Starch 1 g, Potassium hydroxide 0.5 g, drying agent for food 3 g with pinprick were put in the plastic bags (0.15 mm, about 0.25 m^3). The amount of 1-MCP would vary according to the volume of plastic bag and the amount of fruits. In this experiment, the 1-MCP concentration was 0.5 µL L^{-1} when released from the powder into the free headspace of the sealed bags; (2) Treatment 2 (T2), the pears were treated with 1-MCP as expatiated (Luo et al., 2009; Liu et al., 2009; Ahmad and Khana, 2007). The pears were placed in plastic tent (0.4 mm, about 0. 5 m^3) containing 1-MCP at 0.5 µL L^{-1} for 18 h at 20°C; (3) Fruits were put into the containers (0.4 mm, about 0.5 m^3) without 1-MCP treatment for 18 h as the controls (CT). After that, treated fruits (T2 and controls) were removed out of the vessels, and then put in the plastic bags (0.15 mm, about 0.25 m^3). All the treated fruits were stored at 20°C and analyzed every 7 days for up to 42 days.

Respiration rate and ethylene production

Respiration rate was analyzed as described by Srivastava and Dwivedi (2000) with minor modifications. Ten Nanguo pears were placed in a chamber with an airflow rate of 30 ml min^{-1}. The outflow air was connected to an ADC 225 MK3 infrared gas analyzer (IRGA) (Analytical development Co. Ltd., Hoddesdon, Hertfordshire, UK) and respiratory rate indicated by CO_2 production was measured. The IRGA was previously calibrated with standard CO_2. The results were expressed as ml h^{-1} kg^{-1} fresh weight (Srivastava et al., 2000).

Ethylene was measured by placing replication of 20 fruits in a 3 L glass jar hermetically sealed with a rubber stopper for 1 h. One ml of the holder atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a gas chromatography (CP-3800 GC, VARIAN) the conditions of chromatographic analyses were 1 m × 3 mm stainless steel column equipped with GDX-502, hydrogen flame ionic detector (FID), N_2 with a 4.0 ml min^{-1} current velocity, 60°C of column temperature and 270°C of detector temperature (Valero et al., 2003).

Quality evaluations

Firmness of pears was determined on the opposite side (blush and green) of the fruit after peeling. Firmness of 5 pears per treatment and 3 replicates in total was measured by using a sclerometer (China, GY-1, 3.5 mm diameter probe) and expressed in Newtons (N). Juice samples were obtained by squeezing half of the fruit slices from each replicate through four layers of cheesecloth. Soluble solids content (SSC) of the juice was measured with an Abbe Refractometer, model WYT-1(ShangHai Instrument, China). Titratable acidity (TA) was measured in juice pressed from the whole fruit by titrating 10 ml of juice with 0.1 ml^{-1} NaOH to pH 8.1 using 1% (v/v) phenolphthalein and results were expressed as g malic acid per liter (Guillen et al., 2007).

The Ripening Index (RI) of peel was determined by a panel of three trained judges who scored the visual color of a 30 fruit sample per treatment on day 0 and 7, 14, 21, 28, 35 and 42 days of storage. Samples were scored independently according to the same 30 fruits during the entire period the samples were coded with a label to mask the treatment identity in order to minimize the test subjectivity and ensure test accuracy. Ratings were based on a 4-point scale, such as 0 = all green, no ripening; 1 = partly yellow (less than 30%); 2 = mainly yellow (30 to 70%); 4 = all yellow (70 to 100%), absolute ripening. Ripening Index = ∑(RI level × number of fruit at the RI level)/(4 × total number of fruit in the sample) ×100% (Jiang et al., 1999; Paull et al., 1996).

Total phenolics and polyphenol oxidase (PPO)

Total phenolics contents were determined according to the method of Moyer et al. (2002) with slight modifications. Fruits were extracted with 100% acetone and 70% acetone in turn. Extracts were diluted (1: 1000) before incubation at 40°C. The absorbance of the mixture at 755 nm was measured on a UV-Vis spectrophotometer (TU-1810DSPC, China). Total content of phenolics was indicated as mg of catechin equivalents per 100 g of fresh weight.

Polyphenol Oxidase (PPO) activity was assayed by measuring the rate of increase in absorbance at a given wavelength using TU-1810DSPC and double beam UV-Vis spectrophotometer (TU-1810DSPC, China) as previously described (Colak et al., 2005; Dincer et al., 2002; Kolcuoglu et al., 2007). For PPO, 5 g of tissue was homogenized in 20 ml of 0.1 M sodium phosphate buffer, pH 6, together with 1 g of polyvinylpolypyrrolidone (PVP). The homogenate was centrifuged at 20,000 g for 15 min. The supernatant was used for the PPO activity. The assay was performed using 0.5 ml of 100 mM 2-methylcatechol, 1.0 ml of 0.1 M sodium phosphate buffer (pH 6) and 1.5 ml of the supernatant. The increase in absorbance at 410 nm at 25°C was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme that caused a change of 0.01 in the absorbance in 1 min.

Statistical analyses

The experimental design was a randomized block, and the data

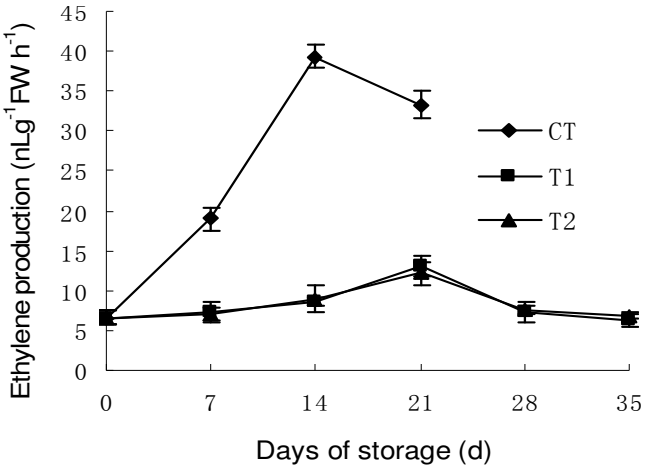

Figure 1. Ethylene production of Nanguo pears treated with 1-MCP in two different modes. 1-MCP released from solid (T1), gaseous (T2) or air (Control). In control the pears decayed after 21 days.

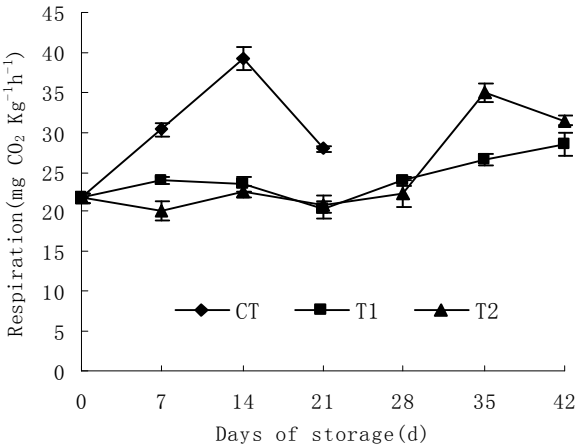

Figure 2. CO_2 production of Nangu pears treated with 1-MCP in two different modes. 1-MCP released from solid (T1), gaseous (T2) or air (Control). In control all pears decayed by 21 days.

were analyzed by ANOVA followed by Duncan's multiple-range test at a significance level of $P<0.05$. ANOVA was performed using the statistical software SPSS 12.0 (SPSS Inc., Chicago, USA).

RESULTS

Effects of 1-MCP treatment on the respiration rate and ethylene production

Generally speaking, the appearance of an ethylene peak is indicative of fruit ripening. As indicated in Figure 1, production rate of ethylene increased greatly with ripening and reached a climacteric peak after 14 days in the control pears, and then decreased gradually. However, this peak was delayed and reduced by 1-MCP in treated samples. During the entire storage period ethylene production was significantly reduced in both T1 and T2 treated fruits. There was no difference observed in pears treated by 1-MCP by either method.

The effects of 1-MCP on respiration rate were investigated and results shown in Figure 2. In the control fruits, the respiration rate increased with ripening and reached a peak after 14 days (climacteric peak) and then, followed by decrease and decay development after 21 days. This result was consistent with other researchers' report (Ji et al., 2009). Results on respiration rate showed that pears treated with 1-MCP showed lower respiration. However, the patterns are similar compared to the control pears. These results also confirmed that T1 (new 1-MCP treatment) were effective as the T2 (traditional 1-MCP treatment) in controlling pear fruit ripening.

Effects of 1-MCP treatment on the ripening index

With respect to color change, the most significant change was turning color index. The influence of 1-MCP on surface color of Nanguo pears is shown in Figure 3. In control fruits, Ripening Index (RI) increased rapidly from initial values of around 0 to 100% during 14 days. 1-MCP treatment effectively delayed the raise in RI, with RI remaining about 10% for 14 days and increasing to about 100% on 42 days. However, the RI of Nanguo pears in the control group were significantly higher than that in the treated fruits, and there was no distinct difference observed between the T1 and T2 during the entire storage.

Effects of 1-MCP treatment on the total phenol and polyphenoloxidase (PPO)

Many studies have reported that pears have browning disorders during storage. Nanguo pears are no exception. It was found that the occurrence of browning is due to the enzymatic oxidation of phenolic compounds by polyphenoloxidase (PPO) to o-quinones, which are very reactive and form brown colored polymers (Guiwen et al., 1995; Galeazzi and Sgarbieri, 1981). Therefore, it is important to study the content of total phenol and the activities of polyphenoloxidase (PPO). As indicated in Figure 4, total phenol content increased greatly with ripening and reached to a peak on the 14^{th} day in the control, and then decreased gradually. Interestingly, all fruits treated with 1-MCP showed a relatively lower total phenol content and both treatment fruits had the similar effects. Our results showed that Nanguo pears had less total phenol in flesh (45 to 70 μg g^{-1}) than in peel (115 to 158 μg g^{-1}).

PPO activities increased sharply in the control fruits with the prolongation of storage and reached a peak on the 14^{th} day, and then decreased gradually both in Peel and flesh. 1-MCP treatment effectively delayed the raise

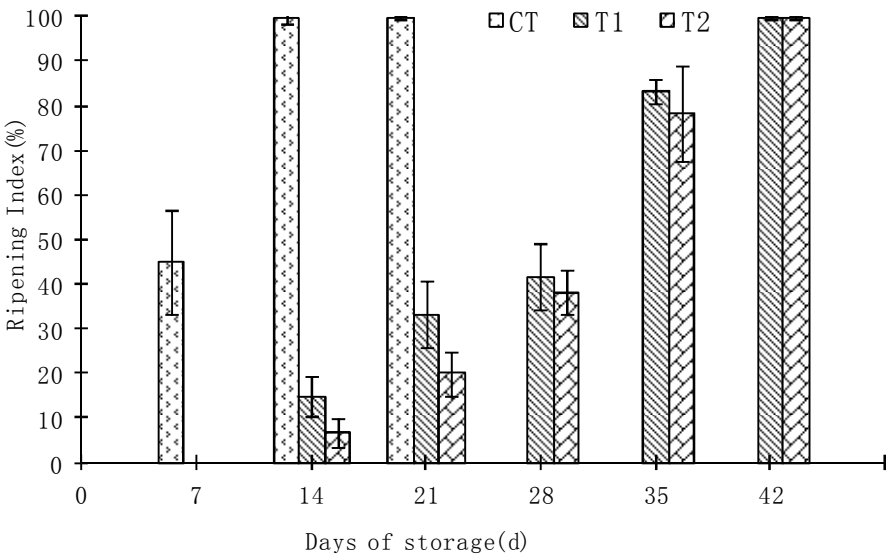

Figure 3. Ripening index in Nanguo pears treated with 1-MCP at two different modes, 1-MCP released from solid (T1), gaseous (T2) or air (Control). In control all pears decayed by 21 days.

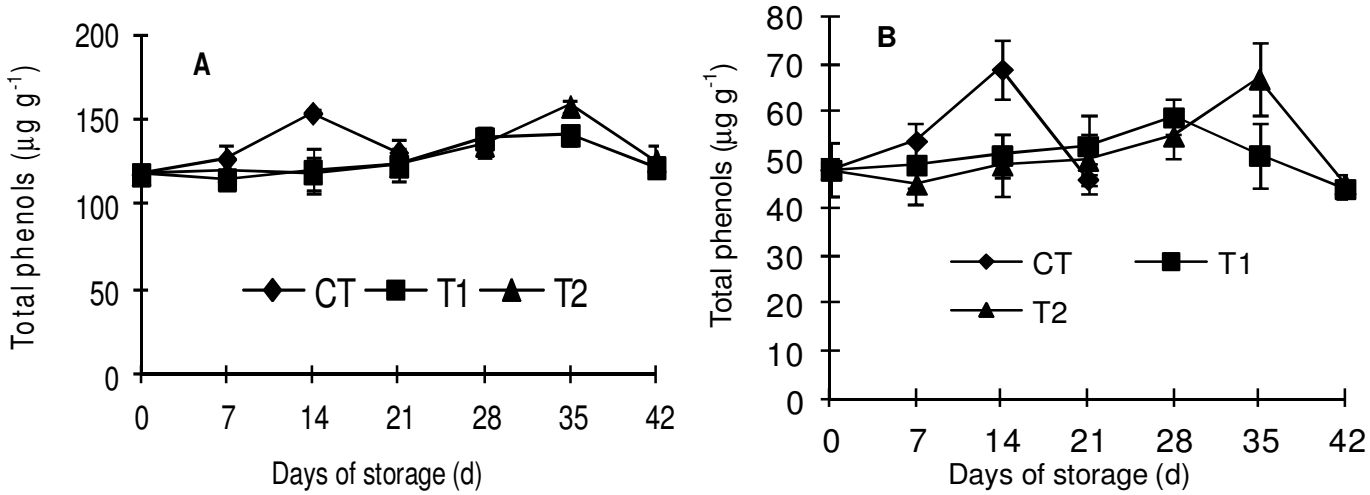

Figure 4. Total phenols content in Nanguo pears treated with 1-MCP at two different modes. 1-MCP released from solid (T1), gaseous (T2) or air (Control). (A) Total phenols content in peel; (B) Total phenols content in flesh.

in PPO activity, with PPO activity increasing to the peak after 35 days. These results also showed that T1 (solid 1-MCP) were effective as the T2 (gaseous 1-MCP) in inhibiting the PPO activity from increasing (Figure 5).

Effects of 1-MCP treatment on the firmness, total soluble solids and titratable acidity in Nan Guo pear during storage

The progress of ripening is generally defined by a combination of several physiological and biochemical changes. In the case of Nanguo pears, ripening is associated with a sharp early decrease in flesh firmness and an abrupt increase in total soluble solids and titratable acidity.

Texture loss is the most noticeable change occurring in fruit and vegetable during storage. A typical softening process was observed in the control pears. The firmness of control pears decreased from 19.25 to 3.5 N with a reduction of 81.8% during 21 days at room temperature (20℃), showing a substantial softening of tissues (Table 1). Both treatments 1 and 2 that made use of 1-MCP showed a beneficial result on firmness retention of pear

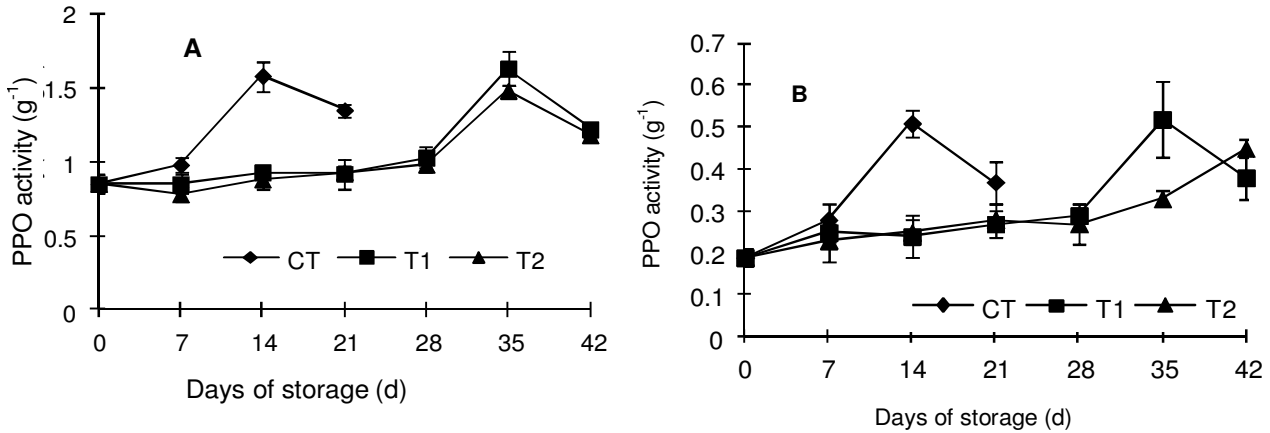

Figure 5. Polyphenoloxidase activity in Nanguo pears treated with 1-MCP in two different modes, 1-MCP released from solid (T1), gaseous (T2) or air (Control); (A) Polyphenoloxidase activity in peel; (B) Polyphenoloxidase activity in flesh.

Table 1. Effects of 1-MCP treatment on firmness, total soluble solids and total titratable acidity in Nan Guo pear during storage.

Treatment type	Fruit firmness (N)			Total soluble solids (%)			Total titratable acidity (%)		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
Control	17.96 ± 0.84^{a}	9.8 ± 0.54^{A}	3.5 ± 0.61^{A}	13.4 ± 0.35^{a}	14.9 ± 0.38^{a}	13.7 ± 0.42^{a}	0.32 ± 0.04^{a}	0.313 ± 0.07^{a}	0.283 ± 0.04^{a}
Treatment1	18.46 ± 0.5^{a}	17.63 ± 0.25^{B}	15.96 ± 1.16^{B}	12.7 ± 1.15^{a}	13.6 ± 0.21^{b}	13.6 ± 0.72^{a}	0.36 ± 0.05^{a}	0.344 ± 0.03^{a}	0.337 ± 0.05^{a}
Treatment2	18.74 ± 0.69^{a}	17.82 ± 0.52^{B}	17.11 ± 1^{B}	13.2 ± 0.72^{a}	13.5 ± 0.83^{b}	13.8 ± 1.02^{a}	0.36 ± 0.04^{a}	0.35 ± 0.03^{a}	0.343 ± 0.04^{a}

Values are mean ± standard deviation, “a” means with the same letters mean no significant difference at P≤0.05, and “A” means with the same letters mean no significant difference at P≤0.01.

fruits during the entire storage period (Table 1) and the two treatments methods showed no significant ($p \leq 0.05$) difference on keeping fruit firmness. Similar results were obtained by Guillen et al. (2007), who studied the effect of dips in a 1-Methylcyclopropene-generating solution on ‘Harrow Sun’ plums stored under different tempe-rature regimes. They found that the immersion in 1-MCP-generating solutions was effective in de-laying softening under all of the storage regimens. Sugar and acid contents are two major determinants of flavor characteristics in many fruits, including pears. The control and two treatment fruits all showed that TA content decreased slowly as storage was prolonged. TA was higher in 1-MCP-treated pear fruit than untreated fruits stored at room temperature (20°C) for 21 days (Table 1). Both treatments affected TA similarly. No significant differences in SSC content between control and 1-MCP-treated pears were observed (Table 1). On the whole, the data showed that treatment 1 might be as effective as treatment 2 in controlling ripening of Asian pears.

DISCUSSION AND CONCLUSION

Many studies have shown that the exposure of fruits, vegetable and ornamentals to gaseous 1-MCP can be a useful tool for maintaining the quality the quality and extending the post-harvest life of horticultural products (Watkins, 2006). The 1-MCP is an inhibitor of ethylene action that acts at the

receptor level. In this way, the 1-MCP delays ripening in climacteric fruits and blocks all the ethylene dependent processes, especially, firmness and TA loss in the treated pears (Trinchero et al., 2004; Rizzolo et al., 2005; Eccher-Zerbini et al., 2005).

Recently, Guillen et al. (2007) found that dipping the fruits in a water solution of the new 1-MCP-generating formulation is effective in counteracting ethylene responses and extending the post-harvest life of plums and eliminates the need for a closed system for treating the fruit. The results showed that 1-MCP treatment can delay the surface color change and firmness loss in tomato and avocado fruit treated in solutions of chlorinated water and 1-MCP (3.70 mmol m^{-3}, 200 μgL^{-1}) (Sun et al., 2009; 2008a). 1-MCP solution treatments can make a much more versatile tool for post-harvest management of fruit, especially, as pre- or post-harvest sprays and dips. Yet, this kind of aqueous 1-MCP treatment may lead to diseases caused by micro-organisms and the spread of pests, thereby; resulting in the decay of fruit and vegetables during the post-harvest process and as such, a more convenient treatment should be developed.

In this study, the 1-MCP complexed power was directly put in the plastic package containing Nanguo pears after picking. The inclusion complex of 1-methylcyclopropene (1-MCP) and α-cyclodextrin can release the 1-MCP gas when encountering the water produced by the fruit itself through transpiration and the respiration process. This novel 1-MCP treatment could be applied conveniently to controlled atmosphere storage, modified atmosphere and any closed atmosphere without any other treatment process. In this research, it was found that direct treatment with 1-MCP can delay and decrease the peak of respiration and ethylene as much as, the traditional gaseous 1-MCP treatment.

In all, the result showed that this treatment which directly put the 1-MCP powder into the fruit package could achieve the similarly effect as the gaseous treatment. This study suggests that this treatment with 1-MCP constitutes a viable alternative to the use of gaseous 1-MCP for controlling the ripening of pear fruits. It seems evident that optimum effect will require further research on a range of fleshy fruits. In addition, the 1-MCP release characteristics, optimal level of treatment, storage temperature and humidity should be determined to increase the utility of this kind of application.

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