Research Article

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Physiological and quality responses of Chinese 'Suli' pear (*Pyrus bretschneideri* Rehd) to 1-MCP vacuum infiltration treatment

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Abstract

BACKGROUND: 1-Methylcyclopropene (1-MCP) is a potent inhibitor of ethylene action and can maintain the quality and extend the shelf life of fresh produce. But the effectiveness of application of 1-MCP may depend upon its capacity to diffuse into the flesh tissue. However, to our knowledge, no study has been performed on the quality maintenance of pear fruit treated with 1-MCP vacuum infiltration. The objectives of this study were to examine the effect of 1-MCP vacuum-infiltration treatment on post-harvest quality and physiology of 'Suli' pears (*Pyrus bretschneideri* Rehd).

RESULTS: 1-MCP treatment led to decreased losses in flesh firmness and titratable acidity (TA), delayed change in skin colour and reduced polyphenol oxidase (PPO) activity and accumulation of malondialdehyde (MDA) during cold storage. It also inhibited flesh browning and maintained a higher taste score after shelf life. These effects were higher when fruits were vacuum-infiltrated with 1-MCP. However, 1-MCP had little effect on total soluble solids (TSSs) and respiration rate of pear.

CONCLUSION: Overall, our results indicated that 1-MCP vacuum infiltration treatment was effective in controlling fruit ripening changes.

Keywords: pear; 1-MCP; vacuum infiltration; quality

INTRODUCTION

Recently, the use of 1-methylcyclopropene (1-MCP) has proved to be an effective inhibitor of ethylene action, and to prolong storage life in various fruit and vegetable products since 1-MCP blocks the ethylene receptors and inhibits their hormonal action.[1] Exposure to 1-MCP can induce beneficial effects in fruit quality, such as delays in physico-chemical changes related to ripening and reductions in decay and weight loss, thereby extending the storage life of some climacteric and non-climacteric fruit.[2]

Pear fruit have been used in studies on the effects of 1-MCP on ethylene biosynthesis, fruit softening, and superficial scald.[3] 1-MCP treatment could delay pear fruit degreening and softening, reduce respiration and ethylene production,[4–6] and be used to replace diphenylamine (DPA) as a post-harvest treatment to control scald in 'Rocha' pear.[7]

However, the effectiveness of 1-MCP to delay ripening and senescence of fruits and vegetables depends upon the concentration applied, method and timing of application, temperature, plant maturity, and commodity.[2,8] Absorption through fruit material is the limiting factor to 1-MCP activity because tissue structure and cuticle resistance limit gas diffusion.[9] Absorption of 1-MCP is also affected by fresh weight, dry matter, insoluble dry matter, and water content of the target plant.[9] Valero *et al*.[10] showed that packaging can influence the effect of 1-MCP on plum fruit because different packaging conditions had different 1-MCP gas diffusion. Hayama *et al*.[11] have suggested that the 1-MCP application under sub-atmospheric pressure forced the exchange of gas inside the flesh to 1-MCP. However, to our knowledge, no study has been performed on the quality maintenance of pear fruit treated with 1-MCP vacuum infiltration. To enable 1-MCP to infiltrate into pear fruit or ensure pear to absorb 1-MCP actually, we designed a method of vacuum infiltration to force 1-MCP to infiltrate pear fruit.

The objectives of this study were to investigate the physiological and quality responses of pear to post-harvest 1-MCP vacuum infiltration treatment in order to maintain pear fruit quality during cold storage.

MATERIALS AND METHODS

Materials and treatments

'Suli' pears were picked from a commercial orchard in Fengyang, Anhui Province, China. The pear fruit were harvested at their commercial ripeness stage (firmness around 59 N, total soluble solids (TSSs) 7.8°Brix), and immediately after each harvest, fruit

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were randomly divided into three lots with each treatment of 120 fruit. These included stored under regular cold storage (0 °C, 90–95% relative humidity) served as control, treated with normal atmospheric pressure 1-MCP (1 μL L^{-1} and 100 kPa), and 1-MCP vacuum-infiltrated treatment (1 μL L^{-1} and 10 kPa), followed by the cold storage. Each treatment consisted of three replications of 40 fruit. Fruit were placed into an airtight glass desiccator for 1-MCP treatment. The 1-MCP powder (0.07% active ingredient; Lytone Enterprise, Inc. Taiwan) liberates 1-MCP when added to 40 °C water and the concentration of 1 μL L^{-1} 1-MCP was calculated according to the manufacturer's recommendations. For vacuum-infiltrated treatments, the concentration of 1 μL L^{-1} 1-MCP was prepared in a sealed plastic bag and the pressure were performed at 10 kPa by using a vacuum pump in a 12-L desiccator containing the fruit. Once the desired vacuum pressure (indicated on a manometer as MPa) was reached, the freshly prepared 1-MCP was immediately injected into the desiccator through the lid, then the vacuum conditions were maintained for 10 min to allow 1-MCP to equilibrate in the desiccator, followed by repressurisation in air to infiltrate 1-MCP into the peel and pulp to allow equilibrium in desiccator for up to 24 h. During the treatment, all the desiccators were kept at room temperature. Immediately after treatment, the treated fruit were then packaged in polyethylene (PE) film and stored at 0 °C for up to 120 days for the storage experiment and held at 20 °C for 7 additional days to simulate shelf life. Fruit firmness, skin colour, total soluble solids and titratable acidity was evaluated at intervals of 30 days, while respiration rate, MDA and PPO were measured at intervals of 10 days throughout the 4-month storage period. Internal browning and taste scores were evaluated after 7 days at 20 °C following cold storage.

Quality evaluations

Fruit quality was evaluated in terms of firmness, surface colour, total soluble solids (TSSs), titratable acidity (TA), internal browning and taste scores. For each analysis five fruit were randomly selected from each of three replicate groups. Flesh firmness was measured using a hand-held penetrometer (plunger diameter 8 mm) and the mean values of the firmness were expressed as newtons (N). Sections of pear skin were removed at the equator on either side of the fruit to allow two independent readings from each pear. Fruit peel colour was measured using a hand-held colorimeter (CR-10; Minolta Co., Ltd., Osaka, Japan) using the standard CIE illuminant in the $L^*a^*b^*$ mode. Measurements were taken on opposite sides of each fruit, and the mean value was calculated. Colour changes from green to yellow were indicated by calculating the hue angle (h°), from arctan b^*/a^*.[5] TSSs were determined by measuring the refractive index of the same juice with a hand refractometer and the results expressed as °Brix.[12] For TA measurement, 100 mL of distilled water were added to 10 g of liquidised fruit pulp. Afterwards, the mixture was strained and 10 mL of the solution were graded with 0.01 mol L^{-1} sodium hydroxide, utilising two drops of phenolphthalein in alcohol solution at 1% as indicator, and the results were expressed as %.

For determination of internal browning (IB), five fruits were cut longitudinally and the area of the fruit flesh that was affected by a brown core was compared to the total area. The IB assessment was based on five stages, according to the browning area, as follows: no browning (0), slight browning (I, <30% of the area), moderate browning (II, about 30–70% of the area) and severe browning (III, >70% of the area, with only the cortex fraction just underneath the peel not showing browning). The browning index is the sum of the browning score of the five pears divided by 15, and multiplied by 100%, and I, II and III refer to the number of pears in the various browning classes. A browning index value of 0% means no browning; 100% means maximal browning.

Results were expressed as the browning index (%) calculated using the following formula:[13]

$$\text{browning index} = \frac{[\text{I} + (2 \times \text{II}) + (3 \times \text{III})]}{3(0 + \text{I} + \text{II} + \text{III})} \times 100.$$

Fruit were subjectively evaluated for taste scores after 7 days of shelf life. A taste panel of 10 people graded the fruit on a nine-point hedonic scale (1, extremely poor; 3, poor; 5, acceptable, limit of marketability; 7, good; and 9, excellent).[14]

Measurement of respiration rate

A static method was used to assess respiration rate.[15] Before assessment, approximately 1000 g of the pears were removed from the packages and exposed to ambient conditions for 1 h so that the CO_2 accumulated in the tissue diffused into air, and then the sample was placed in a desiccator at 1 °C with 10 mL 0.4 mol L^{-1} NaOH in a Petri dish. The Petri dish was taken out after 1 h and the NaOH was used for titration with 0.1 mol L^{-1} oxalic acid ($C_2H_2O_4$) immediately. The respiration rate was calculated according to the change of concentration of CO_2, expressed as the production rate of CO_2, in mg kg^{-1} h^{-1}.

Determination of malondialdehyde content

The malondialdehyde (MDA) content was determined with the thiobarbituric acid reaction updated from the method as described by Tao *et al.*[16] In brief, 1.0 g of tissue was homogenised in 5 mL of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 10 000 × *g* for 5 min. To a 1-mL aliquot of the supernatant, 4 mL of 20% (w/v) trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid was added. The mixture was heated at 95 °C for 15 min and cooled immediately, and the absorption of the supernatant was read at 450, 532 and 600 nm, respectively. MDA contents (nmol g^{-1} FW) were calculated by the following formula:[17]

$$\text{MDA} = 6.45(A_{532} - A_{600}) - 0.56A_{450}/\text{FW}$$

where FW is fresh weight.

Determination of polyphenol oxidase

In each treatment, 10 g flesh from 10 fruit was collected and homogenised in 25 mL of ice-cold extraction buffer (100 mmol L^{-1} sodium phosphate buffer, pH 6.4) containing 0.5 g polyvinyl polypyrrolidone (PVPP). Homogenates were centrifuged at 15 000 × *g* for 30 min at 4 °C and the resulting supernatants were used for assay. Crude enzyme extraction solution (0.5 mL) was incubated with 3 mL of buffered substrate (100 mmol L^{-1} sodium phosphate, pH 6.4 and 500 mmol L^{-1} catechol) and these were monitored by measuring the change of absorbance at 398 nm for 25 s. The specific activity was expressed as U mg^{-1} protein, where 1 unit was defined as increase 1 ΔOD_{398} min^{-1} mg^{-1} protein.[18]

Data analysis

Data were subjected to one-way ANOVA using the statistical package SPSS (version 11.0), and the means were separated at $P < 0.05$ level using Duncan's multiple range test.

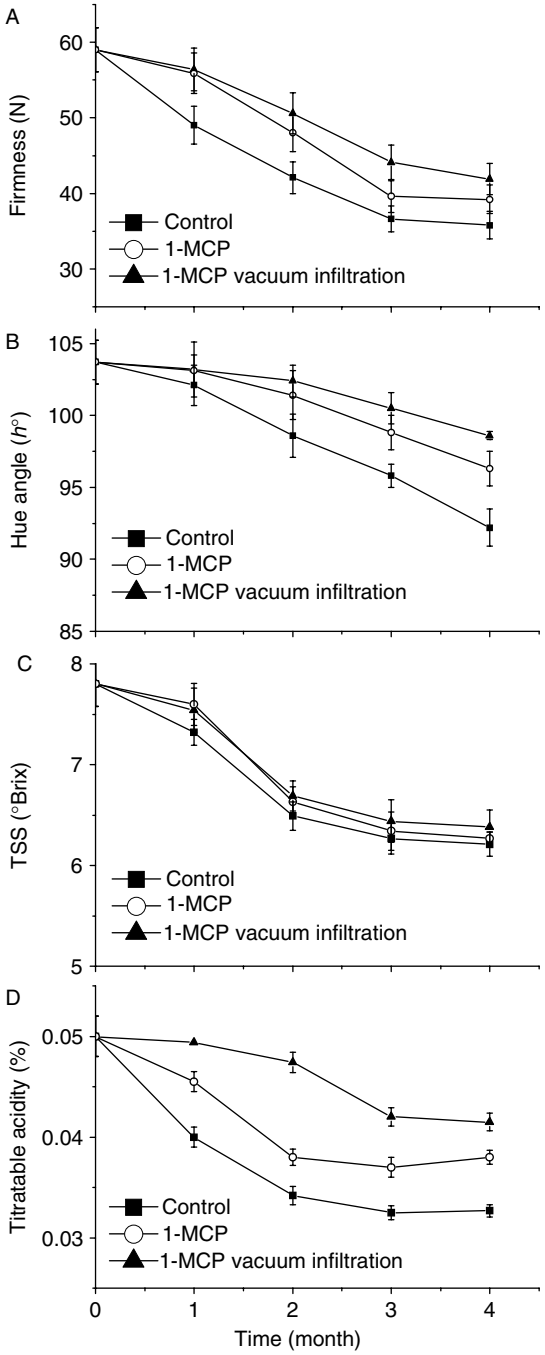

Figure 1. Changes in flesh firmness (A), skin colour (hue angle) (B), total soluble solids contents (C), and titratable acidity contents (D) of pears stored under cold storage, or treated by with 1 μL L^{-1} 1-MCP and 1 μL L^{-1} 1-MCP vacuum infiltration. Error bars indicate the standard error of each mean value.

Table 1. Effect of 1 μL L^{-1} 1-MCP and 1 μL L^{-1} 1-MCP vacuum-infiltration treatment on internal browning and taste scores of pears after 7 days at 20 °C following cold storage

Treatment	Internal browning (%)*	Taste score (1–9)†
Harvest time	0.00 ± 0.00^{d}	8.67 ± 0.29^{a}
After 4-month cold storage + 7 days at 20 °C		
Control	8.60 ± 0.04^{a}	5.02 ± 0.31^{c}
1-MCP	1.90 ± 0.23^{b}	7.56 ± 0.26^{b}
1-MCP vacuum-infiltration	1.30 ± 0.21^{c}	8.02 ± 0.08^{b}

* The internal browning assessment was based on five stages, according to the browning area. A browning index value of 0% means no browning; 100% means maximal browning.
† Taste scores are based on a nine-point hedonic scale, where 1 is extremely poor and 9 is excellent.
Mean values within the same column followed by the same letter are not significantly different ($P > 0.05$).

RESULTS

Effects of 1-MCP vacuum infiltration on fruit quality

Firmness

The firmness of fresh pear was 59 N at harvest. The firmness of control and treated fruit decreased continuously with time (Fig. 1A). The decreasing rate in 1-MCP treated pear firmness was smaller than that in the control samples during the entire storage period. The 1-MCP treated pears remained significantly firmer ($P < 0.05$) than the control pears after 4 months of storage, indicating that pear softening was greatly inhibited by 1-MCP. However, the firmness increases in the pears treated by vacuum infiltration with 1-MCP were more than those with 1-MCP treatment alone.

Surface colour

1-MCP-treated fruit had a delay in the development of skin colour when compared with the untreated fruit (Fig. 1B). The loss of the green colour in pear skin was expressed as lower hue angle (h°). In control fruit, hue angle decreased rapidly after 1 month of storage, indicating a loss in green colour, but application of 1-MCP clearly slowed the change in hue angle. Green colour was retained best in fruit treated by 1-MCP vacuum infiltration, because the hue angle decreased slowly during all of the storage period. A significant difference ($P < 0.05$) was observed between 1-MCP vacuum-infiltrated fruit and the fruit treated by 1-MCP alone or control fruit after 4 months of storage.

Total soluble solids

Total soluble solids in untreated fruit and fruit treated with 1-MCP decreased quickly within the first 2 months, and then decreased slightly (Fig. 1C). Although there were no significant differences in TSSs content among the three treatments, the fruit treated by 1-MCP, especially 1-MCP vacuum infiltration, showed higher TSSs values after 120 days of storage.

Titratable acidity

The effect of 1-MCP on TA is presented in Fig. 1D. In fruit treated with 1-MCP vacuum infiltration, TA remained almost constant during the first 2 months, and decreased slightly thereafter. Significant differences in TA were observed between the untreated controls and fruit treated with 1-MCP during the whole storage period.

Internal browning

The flesh browning index of all fruit increased after 4 months of storage plus 7 days of shelf life (Table 1). Pears treated by 1-MCP vacuum infiltration developed minor incidents of internal browning disorders. Fruit treated with 1-MCP alone showed slight browning, and the highest browning index was observed in control fruit, increasing to 8.60 ± 0.04%. Browning indices of pears treated by 1-MCP vacuum infiltration were significantly lower than those treated by 1-MCP alone and control fruit.

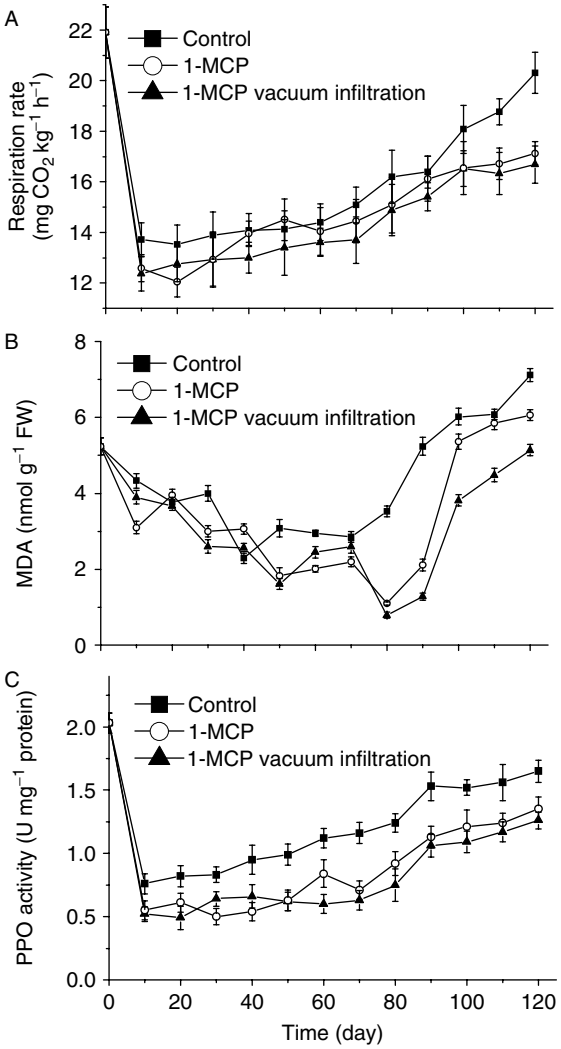

Figure 2. Changes in respiration rate (A), MDA content (B), and PPO activity (C) of pears stored under cold storage, or treated by with 1 μL L^{-1} 1-MCP and 1 μL L^{-1} 1-MCP vacuum infiltration. Error bars indicate the standard error of each mean value.

Taste scores

Compared to values at harvest, the decreases in taste scores of the pears were observed in all treatments after 7 days shelf life following 4 months of cold storage (Table 1). The taste score was significantly higher for 1-MCP-treated pears than for the control samples, and pears treated by with 1-MCP vacuum infiltration showed higher taste scores (a score of 8.02 ± 0.08) than those treated by 1-MCP alone (7.14 ± 0.26), but there was no significant difference in taste scores between both of 1-MCP treatments ($P > 0.05$).

Effects of 1-MCP vacuum infiltration on respiration

The respiration rate of pear was 21.9 mg CO_2 kg^{-1} h^{-1} at harvest. A sharp decline in respiration rate was observed immediately after treatment, mainly because fruit was transported from ambient temperature to cold storage temperature (Fig. 2A). Respiration rate underwent a slight increase during cold storage. The respiration rate of 1-MCP treated fruit did not show a distinct climacteric peak but only a gradual rise towards the end of the storage period. The respiration rate of pears that were not treated wtih 1-MCP began to increase after 90 days, indicating the start of climacteric respiration. 1-MCP-treated fruit had lower respiration rates than the control at all times except for at 50 days, although the differences were not significant ($P > 0.05$). These results suggest the 1-MCP treatment remained effective for inhibiting respiration rate.

Effects of 1-MCP vacuum infiltration on MDA content

The tendency of MDA content proved to be similar in all treated fruits. The MDA content in all fruit gradually decreased during the first 80 days of storage and then sharply increased (Fig. 2B). However, MDA content of fruit after vacuum infiltration with 1-MCP was always lower than that in the controls and of fruit treated wtih 1-MCP alone from 80 to 120 days of storage and significantly lower from 100 to 120 days of storage. 1-MCP treatment could inhibit the accumulation of MDA, and thus attributed to inhibiting the senescence of pear fruit.

Effects of 1-MCP vacuum infiltration on PPO activity

PPO activity in the flesh of pear was low under cold storage conditions, and then increased slightly with the storage time for all treatments (Fig. 2C), which was similar to the trend of respiration rates (Fig. 2A). PPO activities of fruit treated by 1-MCP were significantly lower than those of control fruits after 120 days of storage ($P < 0.05$).

DISCUSSION AND CONCLUSION

Fruit are often attractive to the consumer because of their aesthetic qualities of flavour, colour and texture.[19] The general objective in fruit storage is to slow down the ageing processes related to these three quality characteristics. Cold temperature and 1-MCP treatment, an inhibitor of ethylene action, are widely used for this purpose in many fruit species.[2] In the present study, 1-MCP application is a useful tool for maintaining pear fruit quality attributes, because 1-MCP-treated pears retained a green skin colour and flesh firmness, and inhibited the decrease of titratable acidity. Similar effects of 1-MCP have been reported for 'Bartlett' pear,[6] 'Rocha' pear,[7] 'd'Anjou' pears,[20] Japanese pears[19] and in other fruit such as avocados[21] and citrus sinensis,[22] for delaying fruit ripening, retaining firmness, titratable acidity and green peel colour, or reducing softening and chlorophyll degradation.

In comparison to European pears, where a soft fruit at consumption is desired, high-quality Chinese pears have high firmness and green skin colour.[23] The reduced changes in the skin colour, firmness and titratable acidity showed the effectiveness of 1-MCP in retarding fruit ripening. However, 1-MCP vacuum infiltration treatment showed a better effect on maintaining the high quality of pears than 1-MCP treatment alone.

In contrast, 1-MCP treatment had little effect on TSSs content. A similar effect had been reported for 'Pedro Sato' guava, where 1-MCP had no influence on changes in soluble solids concentration during storage.[24] Koukounaras and Sfakiotakis suggested that 1-MCP treatment did not significantly affect soluble solids concentration of 'Hayward' kiwifruit either during cold storage or shelf life.[25] In general, less ripe fruit have higher TA and lower TSSs. The increase in TSSs associated with a decrease in TA would be a favourable consumer-related ripening change. A typical climacteric pattern of ripening is characterised by an increase in the sugar content during ripening, and an increase in TSSs is presumably related to conversion of starch to sugars. The depletion of TSSs could be explained by respiratory activities of

pear fruit, and on the basis that 'Suli' pear has not been found to ripen within 120 days of storage.

When 1-MCP -reated fruit showed higher quality attributes, no significant differences were observed in terms of respiration rate. The respiration rate of control fruit and 1-MCP-treated fruit did not show a distinct climacteric peak, but only a gradual rise, towards the end of the experimental period, was found in pears that were not treated with 1-MCP. This is in agreement with the study by Trinchero *et al*.[6] and indicated that all treated fruit did not develop the onset of senescence at the end of the experiment.

The present study shows that 1-MCP treatment significantly inhibited the accumulation of MDA, which is a secondary end product of polyunsaturated fatty acid oxidation,[26] and suppressed PPO activities, as also reported for 1-MCP-treated avocado.[21]

In our work the retardation of the processes mentioned above was more pronounced when pear was treated with 1-MCP vacuum infiltration.

Changes in internal browning and taste score of pears after a 7 days shelf life are presented in Table 1. Internal browning in pears was inhibited effectively when fruit were treated with 1-MCP after shelf life, and browning indices of pears treated by 1-MCP were significantly lower than that of control fruit, perhaps due to reduced polyphenol oxidase (PPO) activities.[21]

The taste score was significantly higher for 1-MCP-treated pears than for the control samples; these data confirm that exposure to 1-MCP were advantageous to the taste of pears. Nevertheless, although no significant difference in taste scores between both 1-MCP treatments, it seemed that pears treated by vacuum infiltration of 1-MCP retained the fruit taste better during the shelf life (Table 1).

1-MCP has been shown to compete with ethylene for the binding site on the ethylene receptor in plant tissue, which prevents ethylene from exerting its physiological action.[27] Therefore, the effectiveness of inhibition of ripening and senescence of fruit and vegetables is a function of the 1-MCP concentration applied, up to saturation of the binding sites.[8]

No significant difference was found in respiration rate between the control and 1-MCP treated pears, probably due to the low content of ethylene receptors since respiration peak was not observed during 120 days of storage.

Although 1-MCP is gaseous at ambient temperature and penetrates into fruit tissues easily and uniformly,[28] the rate of uptake will also be impacted by factors affecting gas diffusion. Absorption through fruit material is the limiting factor to 1-MCP activity because tissue structure and cuticle resistance limit gas diffusion.[9] Among important factors affecting gas diffusion through fresh produce is tissue morphology and cuticular resistance. In most fruit and bulky storage organs, the peel or periderm is the major barrier to gas diffusion, so that most of the gas exchange takes place through gas-filled pores or lenticels.[29] Absorption of 1-MCP is also affected by fresh weight, dry matter, insoluble dry matter, and water content of the target plant.[9]

Vacuum infiltration could increase the amounts of 1-MCP that infiltrate into pear fruit and enable 1-MCP to reach (close to) the ethylene binding site. Thus, 1-MCP efficacy might be improved. A similar effect was found in the study by Hayama *et al*.[11] where it was shown that 1-MCP application under sub-atmospheric pressure forced the exchange of gas inside the flesh to 1-MCP-containing air.

In addition, the contact between 1-MCP and fruit might play an important role in influencing the effect of 1-MCP. The contact could be affected by mode of packaging and temperature. Valero *et al*.[10] showed that packaging can influence the effect of 1-MCP on plum fruit because different packaging conditions induced different rates of 1-MCP gas diffusion. Individualisation of fruit could be achieved when plum fruit are packaged in small perforated boxes and gas diffusion could reach the entire fruit surface, but the treatment in bulk obstructed 1-MCP access to the whole fruit surface primarily because each fruit was partially covered by neighbouring fruit. Vacuum infiltration is more effective possibly because of the improvement of 1-MCP gas diffusion.

Ku and Wills[30] and Sisler and Serek[1] had suggested that 1-MCP was better able to reach and attach to the ethylene receptor sites at the higher temperature. The treatment at ambient temperature would seem to offer an advantage to commercial operators to treat produce immediately after harvest in a chamber that is not temperature controlled and then cool the broccoli for storage or transport.[30]

Although low pressure is potentially beneficial for delaying senescence of a fresh product,[31,32] no significant difference were observed between vacuum control without 1-MCP and 1-MCP vacuum infiltration treatment in the present work (data not shown) because of a shorter low-pressure treatment time.

In conclusion, the present study clearly indicates that 1-MCP vacuum infiltration may provide a feasible technique for extending the post-harvest life and remaining quality of pear. To the best of our knowledge, this is the first study reporting the application of 1-MCP vacuum infiltration on fleshy fruit. However, the exact mechanism warrants further investigation.

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