

## Research Article

### Postharvest Propionic Acid and Hot Water Treatment for the Control of Black Spot Disease in Chinese Ya Pears

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**Abstract:** Black spot disease caused by *Alternaria alternata* (Fr.) is one of the main postharvest diseases of Chinese Ya pears and causes serious economic loss during storage. The fungi-toxicity of postharvest propionic acid and hot water treatment at different concentrations and temperatures was investigated *in vitro* and directly on Ya pears. The *in vitro* results showed that *A. alternata* was susceptible to propionic acid solutions at all tested temperatures. The toxicity of propionic acid increased linearly and the LC 99 decreased from 13.43 to 4.31 g/L with an increase in temperature from 20 to 45°C. The results in Ya pears showed that propionic acid and hot water treatment dramatically controlled black spot disease and 99% inhibition was achieved upon treatment with 9 g/L propionic acid solution for 30 min at 45°C. Furthermore, the treatment inhibited the respiration of fruits and did not cause any adverse effects on other quality parameters; however, slight surface injury was noted at 9 g/L. The safety of propionic acid treatment was further confirmed by residue analysis. The results of this study suggest that postharvest propionic acid and hot water treatment is an alternative method for the control of black spot disease in Ya pears.

**Keywords:** Black spot disease, chinese Ya pear, hot water treatment, propionic acid

## INTRODUCTION

Chinese Ya pear (*Pyrus pyrifolia*), a crisp, sweet and delicious fruit native to north China, has great commercial importance due to its large production and consumption demand. However, black spot disease caused by *Alternaria alternata* (Fr.) Keissler often occurs in Chinese Ya pears during storage, which limits its marketability and results in serious post harvest loss (Baudry *et al.*, 1993; Zhang *et al.*, 2003). Moreover, *A. alternata* is considered to be a pest of quarantine importance by some countries; thus, it hampers the export of Chinese Ya pears (Roberts, 2005). Currently, postharvest application of chemical fungicides is restricted due to increasing public concerns regarding the environment and human health (Waard *et al.*, 1993). Therefore, there is a need to develop an economical, effective and safe postharvest treatment measure.

Heat treatment has been widely investigated as an effective method for disinfestation of insect and fungal pests from fruits (Couey, 1989). Heat treatment can also inhibit ripening and chilling injury of many fruits during storage, thus extending their shelf life (Paull and Nancy, 2000). Considering that water is a more efficient medium than air and the cost of hot water treatment is much lower than that of hot air treatment, hot water treatment is becoming increasingly accepted commercially (Fallik, 2004). However, similar to what

was observed in research on mango (Prusky *et al.*, 1999), an extremely high temperature was required to control *A. alternata* in our preliminary test and this temperature could not be tolerated by Ya pears.

It has been shown that the addition of certain low-toxicity chemicals improves the efficacy of heat treatment for the control of fungal pathogens. For example, a combination of peracetic acid and hot water treatment provides an alternative treatment for the control of *Monilinia* spp. infection on peaches and nectarines (Sisquella *et al.*, 2013). Ethanol and heat treatments have been developed to reduce postharvest decay in mango and Chinese bayberries (Gutiérrez-Martínez *et al.*, 2012; Wang *et al.*, 2011). These successful practices suggest a new strategy for the control of postharvest disease in fruits.

Propionic acid has been used worldwide as a food additive for over 30 years. Because it is Generally Recognized as Safe (GRAS), propionic acid can be directly used in animal feed and food for humans and can even be added to drinking water (Burdock and Carabin, 2004). The inhibitory effect of propionic acid on postharvest disease has been determined. It was reported that fumigation with propionic acid vapors could be used for the control of *Monilinia fructicola*, *Penicillium expansum* and *Rhizopus stolonifer* on certain fruits, such as cherry, peach and apple (Sholberg, 1998). However, there is still lack of data on

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the control of *A. alternata* in pears, especially with respect to the performance of propionic acid in combination with heat treatment. Herein, we report the fungi-toxicity of propionic acid and hot water treatment on *A. alternata in vitro* and on pear fruits. The effect of postharvest treatment on fruit qualities and degradation dynamics of propionic acid residues were also investigated.

## MATERIALS AND METHODS

### Preparation of an inoculum of *A. alternata*:

*A. alternata* was obtained from diseased pears and was prepared as previously described (Liu *et al.*, 2012). The fungus was cultured on Potato Carrot Agar (PCA) medium (2 potato, 2 carrot, 1.5% agar) in the dark at 25°C for 10 days. Sterile water (1 mL) was injected into each plate and the spores and mycelia were suspended with a sterile glass-coated bar. The suspension was then transferred into a 5 mL tube and the spores were counted with a hemocytometer to adjust the concentration to  $10^6$  spores/mL via the addition of sterile water.

**In vitro treatments:** Propionic acid (99.5% purity) was purchased from the Guoyao company (Beijing, China). A series of propionic acid solutions were obtained by adding calculated volumes of propionic acid to preheated sterile water. Propionic acid solutions (50 mL volumes) were transferred into sterile 100-mL screw-capped glass bottles. The bottles were then placed in water baths at 25, 30, 35, 40 and 45°C, respectively and allowed to equilibrate for 2 h.

Plugs (7 mm in diameter) of PDA were cut with a sterile whole punch from 10-day-old cultures and placed in the bottles prepared above. After treatment for 30 min, each plug was transferred to the center of a new PDA plate. The radial growth of *A. alternata* was measured after 5 days of incubation at 25°C and the results are expressed as percent inhibition. Inhibition rate = the average diameter of the treated fungus/the average diameter of the untreated fungus  $\times 100$ . Six replicates were performed for each concentration and temperature and untreated plugs were used as controls.

**Inoculation of Ya pears:** Ya pears (*Pyrus pyrifolia*) were purchased from a local orchard that did not apply fungicides one month prior to harvest. The fruits were then wounded and artificially inoculated as previously described (Liu *et al.*, 2012). The inoculum density of *A. alternata* was  $10^6$  spores/mL and eight small wounds were made in each fruit. The inoculated fruits were incubated at 25°C and 90% relative humidity for 4 h before treatment.

**Treatment of Ya pears:** According to the inhibition results, the inoculated fruits were treated with 0, 4.5, 6,

7.5 and 9 g/L, respectively propionic acid solutions at 45°C. The treatments were performed in a thermostatic water bath (BK 710, Yamato, Japan) containing 50 L of water and fruits treated with water at 25°C for 30 min were used as controls. After immersion for 30 min, the fruits were incubated at 25°C and 70% relative humidity for 7 days to record the black spots. Three replicates were performed for each treatment and 40 fruits were treated in one replicate.

Six groups of healthy Ya pears were also treated under the conditions described above for the evaluation of fruit quality. After the treatments, the fruits were stored in an incubator at 25°C with 70% relative humidity. Potential phytotoxic responses were determined after 13 days of storage and fruit quality parameters including weight loss, firmness, soluble solids content, titratable acidity and respiration were examined after 1, 4, 7, 10 and 13 days of storage, respectively.

**Quality evaluation:** Fruit firmness was measured using a firmness tester with a 2-mm diameter plunger (QA Supplies, FT-02, USA). Soluble solids were quantified using a handheld refractometer (G-won, GMK-701R, Korea). Titratable acidity was measured using an acidity meter (G-won, GMK-855, Korea). The respiration of fruits was determined using a gas chromatograph (Agilent, 6890N, USA) as described by Liu *et al.* (2010). Pitting was the major injury observed and the amount of surface covered by pitting was rated as none (0), very slight (5% or less), slight (5-10%), moderate (10-25%), severe (25-50%) and very severe (>50%). Fruits with moderate, severe and very severe ratings were considered to be unmarketable.

**Residue determination:** To evaluate the safety of the combined treatment, the degradation dynamics of propionic acid residues on Ya pears treated with 9 g/L propionic acid were investigated. Samples were prepared from the treated fruits stored for 1, 3, 5 and 7 days, respectively at 25°C and 70% relative humidity. The propionic acid residues in the peel and pulp were then determined by gas chromatography (6890N, Agilent, USA) according to the national standards of China (GB/T 5009.120-2003, 2003). Three replicates were measured separately.

**Data analysis:** Probit analysis was performed using PoloPlus (Leora Software 2003, USA) and the slope, LC 50 and LC 99 values of each treatment were calculated. Tukey's multiple range tests ( $p \leq 0.05$ ) was performed for mean separation of all parameters using the SPSS package, version 16.0 (SPSS Inc., Chicago, IL, USA). Standard deviations of the mean were also calculated.

Table 1: Probit analysis of the fungi-toxicity of propionic acid against *A. alternata* at different temperatures *in vitro*

Temperature (°C)	Slope±S.E. <sup>1</sup>	Hetero	LC <sub>50</sub> (g/L) (95% CI)	LC <sub>99</sub> (g/L) (95% CI)
20	51.11±3.28	1.87	12.10 (11.96, 12.23)	13.43 (13.16, 13.85)
25	47.35±3.05	2.09	11.10 (10.97, 11.24)	12.43 (12.15, 12.87)
30	23.99±1.43	1.57	7.80 (7.65, 7.94)	9.75 (9.40, 10.26)
35	22.49±1.59	1.54	6.70 (6.55, 6.84)	8.50 (8.14, 9.07)
40	18.79±1.27	0.67	4.15 (4.07, 4.22)	5.52 (5.32, 5.77)
45	18.18±1.37	2.10	3.21 (3.08, 3.34)	4.31 (4.02, 4.85)

<sup>1</sup>: Mean±S.E.; CI: Confidence interval

## RESULTS

**Effect of propionic acid concentration and water temperature on *A. alternata* *in vitro*:** Propionic acid treatment of *A. alternata* *in vitro* was separately conducted with the same dipping time (30 min) and at different temperatures (20, 25, 30, 35, 40 and 45°C, respectively). Propionic acid treatment reduced the growth of *A. alternata* at all tested temperatures and the influence of temperature on the efficacy of COS fumigation was distinctive. The LC 50 value decreased 10-30% as the temperature increased (every 5 from 20 to 45°C). The LC 99 value showed the same trends as LC 50, indicating that there was significant synergy between propionic acid and hot water treatments (Table 1).

The probit regression lines were parallel between 20 and 25°C, 30-35°C and also between 40 and 45°C, indicating that propionic acid had the same fungitoxicity in these three temperatures ranges (Table 1). Therefore, despite the linear decline of LC 50 and LC 99 values among all the temperatures tested, the temperature influence on fungi-toxicity could be divided into three groups: normal conditions (20-25°C), in which the fungi-toxicity of propionic acid was low; warm conditions (30-35°C), in which the fungi-toxicity of propionic acid was intermediate; and hot conditions (40-45°C), in which the fungi-toxicity of propionic acid was high.

**Effect of propionic acid treatment on the incidence of black spot disease in pears:** To evaluate the effect of propionic acid treatment on the incidence of black spot disease in pears, the inoculated pears were treated with a series of propionic acid solutions at 45°C and results are shown in Fig. 1. Hot water treatment alone resulted in slight control of black spot disease. As the concentration of propionic acid increased, less infection was observed. Compared with the control treatment, in which 95% of the fruits were infected, the disease was 99% controlled by the treatments including 9 g/L propionic acid.

**Effect of propionic acid treatment on the quality of Ya pears:** Treatment with different concentrations of propionic acid did not significantly affect fruit weight loss, fruit firmness, soluble solids or total acidity during 13 days of storage; however, respiration effects and

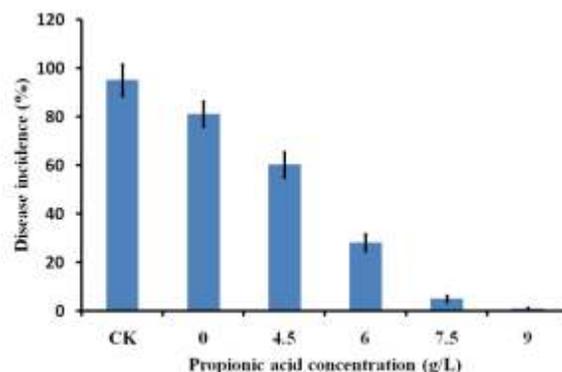


Fig. 1: Effect of postharvest treatment on black spot disease incidence in Ya pears artificially inoculated with *A. alternata*. Error bars indicate S.E.

Table 2: Effect of hot water treatment and different concentrations of propionic acid on the surface injury of Ya pears

Concentrations (g/L)	CK	0	4.5	6	7.5	9
Surface injury (%)	0±0	0±0	0±0	0±0	1.5±0.7	6.5±2.3

some surface injuries were noted (Fig. 2 and Table 2). Compared with the control fruits treated at 25°C, the respiration of all fruits treated at 45°C decreased by approximately 20% after treatment (Fig. 2E).

No surface injury was observed in the fruits treated with 0, 4.5 and 6 g/L, respectively propionic acid at 45°C. Very slight surface injury (1.5%) was noted in the fruits treated with 7.5 g/L propionic acid and slight surface injury (6.5%) occurred in the fruits treated with 9 g/L propionic acid, indicating that the tolerance limit of Ya pears is 9 g/L propionic acid at 45°C.

**Degradation dynamics of propionic acid residues on Ya pears during storage:** Because propionic acid was recognized as a GRAS chemical, it was exempt from the legal residue limit in the USA and had limits of 4-30 and 2.5 g/kg in the EU and in China, respectively (EFSA, 2011; GB2760, 2011). To confirm the safety of the combined treatment, the degradation dynamics of propionic acid and propionate residues on Ya pears treated with 9 g/L propionic acid were determined (Fig. 3). After 1 day of storage, the propionic acid residues in the peel and pulp were both below 2.5 g/kg and the amount of residue in the peel was 50% greater than that in the pulp. Residues in both parts decreased rapidly during storage and no residues were detected in the fruits stored for 5 days.

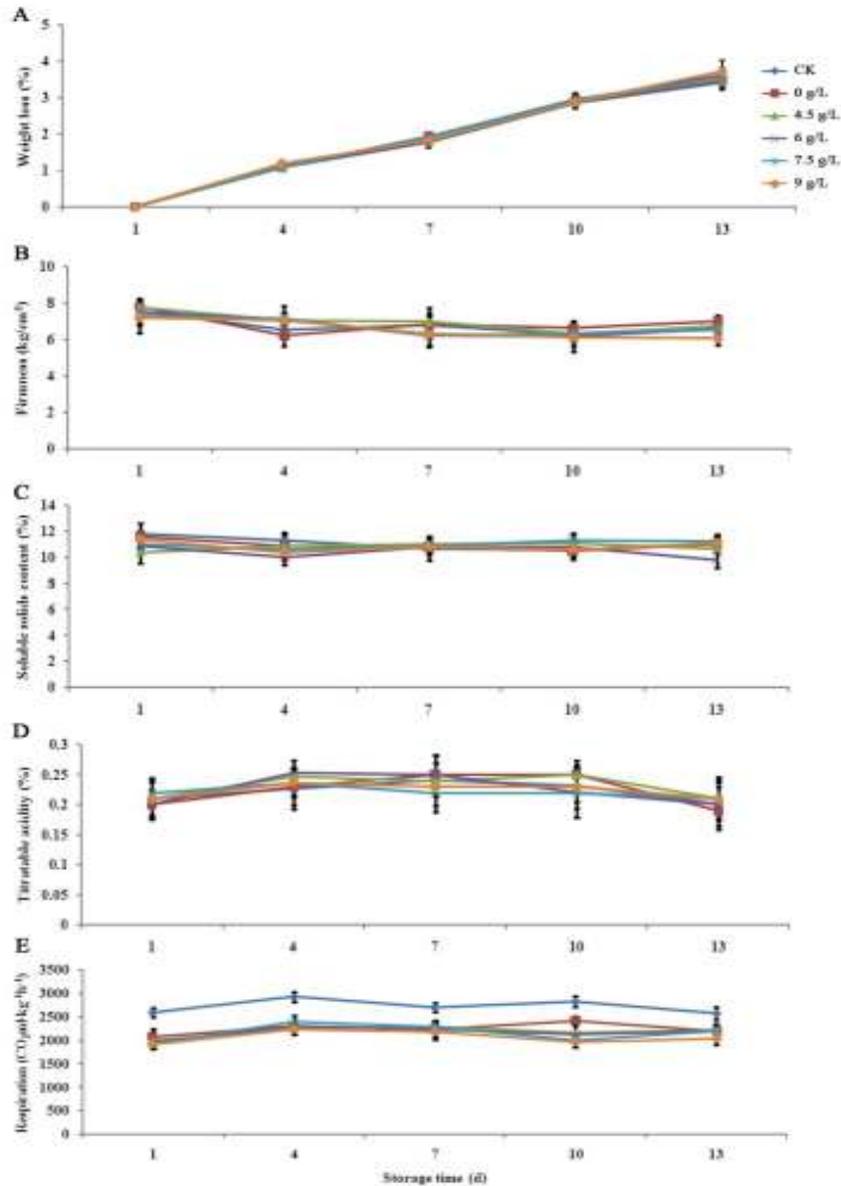


Fig. 2: Effect of postharvest treatment on the internal quality parameters of Ya pears, (A) weight loss, (B) firmness, (C) soluble solids content, (D) titratable acidity, (E) respiration. All data are presented as the average of three independent replicates; Error bars indicate S.E.

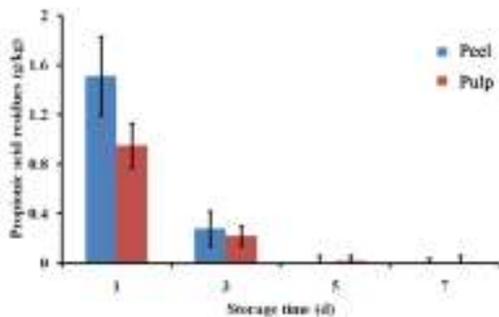


Fig. 3: The residual dynamics of propionic acid in Ya pears after treatment with 9 g/L propionic acid for 30 min at 45°C. Error bars indicate S.E.

## DISCUSSION

Due to the serious loss of fresh fruits caused by postharvest disease and the increasing social concern regarding chemical fungicides, there is a constant need to develop new antifungal technologies. Many alternative measures for controlling postharvest disease in fruit have been reported in recent years, including hot water (Fallik, 2004) and propionic acid (Sholberg, 1998). However, to our knowledge, this is the first study investigating the use of propionic acid and hot water treatment to control black spot disease caused by *A. alternata* in Ya pears.

Similar to ethanol (Gutiérrez-Martínez *et al.*, 2012; Karabulut *et al.*, 2004a), peracetic acid (Sisquella *et al.*, 2013) and many other alternative chemicals (Karabulut *et al.*, 2004b; Palou *et al.*, 2009), the effectiveness of propionic acid solutions for controlling postharvest disease was improved at higher temperatures (Table 1), indicating a synergistic effect between hot water and propionic acid. However, the concentration of propionic acid required to achieve 99% inhibition in inoculated pears was double the concentration required *in vitro*. One important reason for this result may be the fact that the temperature of the pears was much lower than the temperature of the propionic acid solutions during treatment. In fact, according to our preliminary temperature monitoring results, the average temperature of the pulp at a 2-cm depth below the peel was approximately 35°C during the treatment. Thus, it is reasonable that the concentration of propionic acid required to achieve 99% inhibition in inoculated pears was 9 g/L, considering that 8.5 g/L propionic acid caused 99% inhibition at 35°C in the *in vitro* analysis. Moreover, based on these results, we suggest that a short period of pretreatment at a high temperature would be useful for improved control of black spot disease; indeed, such a treatment was successful for disinfecting mango fruits that were infested with pest insects (Jacobi *et al.*, 2001).

It is well known that heat treatment can alter various biochemical and physiological processes and therefore disrupt fruit ripening (Lurie, 1998; Paull and Nancy, 2000). Our results show that the respiration of pears was dramatically inhibited after the treatments. Although there were no significant differences in other internal quality parameters observed during further storage, we presumed that postharvest treatment with propionic acid and hot water would delay ripening and benefit the storage of Ya pears, considering that respiration is an important biomarker of fruit ripening. Moreover, there were no significant changes between the different propionic acid solution concentrations, indicating that the respiration inhibition effect was primarily due to heat treatment and propionic acid had a neutral effect during the treatment.

In conclusion, postharvest propionic acid and hot water treatment was effective for controlling black spot disease both *in vitro* and on Ya pears. The treatment inhibited the respiration of fruits, maintained the internal quality and left no obvious residues. The concentration limit of propionic acid was also determined to be 9 g/L to avoid surface injury. Considering the fumigation toxicity of propionic acid against some stored pest insects (Germinara *et al.*, 2007; Sholberg, 1998), further research is required to develop protocols targeting postharvest disease and pest insects at the same time.

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