Effects of Peroxyacetic Acid, Peroxycitric Acid, Sodium Bicarbonate, Potassium Sorbate, and Potassium Metabisulfite on the Control of Green Mold in *Sai Nam Phueng* Tangerine Fruit

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ABSTRACT

The efficiency of peroxyacetic acid (PAA) at concentrations of 0.01 and 0.02% w/v; peroxycitric acid (PCA) and potassium metabisulfite (KMS) at concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05% w/v; and sodium bicarbonate (SBC) and potassium sorbate (KS) at concentrations of 1.0, 1.5, 2.0, 2.5 or 3.0% w/v on controlling green mold on 'Sai Nam Phueng' tangerine fruit was investigated. Fruit inoculated with Penicillium digitatum sp. were dipped into test sanitizing solutions for 3 min and stored at 25±2°C, 95±3% RH for 5 days. The evaluation was based on disease incidence, disease severity (wound diameter) and percent of sporulation compared with control 1 (neither washed nor dipped into distilled water) and control 2 (dipped into distilled water for 3 min). The lowest concentration of each solution that provided the best result was subsequently used to search for the most effective dipping time (1, 3, or 5 min). The pretreated fruits were then stored at 25±2°C, 95±3% RH for 5 days. The solution efficiencies were compared to choose the optimal solution for green mold disease control and then tested again at 5±2°C, 65±3% RH for 25 days. The results indicated that the treated fruit showed significantly less severe symptoms of infection of green mold than the control fruits. The most effective solution to control green mold was the mixed solution of 1.5% KS and 0.02% PAA w/v and dipping time of 5 min which could reduce disease incidence from 94.00% of control 1 and 100.00% of control 2 to 53.33% of treated fruit when stored at 25±2°C, 95±3% RH for 5 days and could reduce disease incidence from 97.33% of control 1 and 100.00% of control 2 to 45.33% of treated fruit when stored at 5±2°C, 65±3% RH for 25 days.

Keywords: Green mold, *Penicillium* sp., Tangerine, Antimicrobial substances

INTRODUCTION

A common postharvest fungus disease of citrus fruit is green mold caused by *Penicillium digitatum*. Controlling postharvest diseases with synthetic fungicides leaves residues on fruit. Currently, Generally Recognized As Safe (GRAS) substances are alternative methods to control postharvest diseases (Sangchote, 2008). Sodium bicarbonate (SBC) and potassium sorbate (KS) are GRAS antimicrobial substances that effectively control green mold caused by *P. digitatum* on oranges and lemons (Smilanick et al., 1999, 2008; Palou et al., 2002; Montesinos-Herrero et al., 2009). Treating with potassium metabisulfite (KMS) controls postharvest decay on seed-corn caused by *Aspergillus parasiticus* (Pongpratoom, 1999). The food industry has long used these three types of GRAS antimicrobial food additives because they are safe for consumers and inexpensive. Other interesting antimicrobial substances are peroxyacetic acid (PAA) and peroxycitric acid (PCA). Both substances are strong oxidizing agents popularly used in the food industry. PAA is a mixture of acetic acid and hydrogen peroxide and PCA is a mixture of citric acid and hydrogen peroxide, with stabilizing agents added to both in commercial formulations. Acetic acid, citric acid, and hydrogen peroxide are GRAS substances. Treatments with PAA have been reported to control postharvest decay caused by *Penicillium digitatum* on tangerines (Pukdee and Sardsud, 2007) and *Monilinia laxa* and *Rhizopus stolonifer* on stone fruits (Mari et al., 1999; 2004). However, no one has reported treating *Sai Nam Phueng* tangerine fruit with PCA to control green mold. The objective of this study was to compare the efficiency of SBC, KS, KMS, PAA, and PCA to control green mold in *Sai Nam Phueng* tangerine fruit.

MATERIALS AND METHODS

Fruit

Sai Nam Phueng tangerine fruit (*Citrus reticulata* Blanco) were harvested at commercial maturation stage in May 2009 from a commercial orchard in Phrao District, Chiang Mai Province, Thailand. Fruits were selected for uniform size (diameter of 6.0-6.8 cm), with no wounds or infection. Fruits were then washed with water, air-dried, and placed at random in plastic baskets.

Inoculum

The identified *P. digitatum* was obtained from the Department of Biology, Faculty of Science, Chiang Mai University and then cultured on potato dextrose agar (PDA) for 7-10 days at 25±2°C. Spores were harvested and suspended in sterile distilled water. The concentration was determined by using a haemacytometer and the final concentration was adjusted to 105-106 spores/ml.

Effects of PAA, PCA, SBC, KS, and KMS concentrations on the control of green mold in *Sai Nam Phueng* tangerine fruits

Experiment 1. *Sai Nam Phueng* tangerine fruits were wound-inoculated by puncturing the fruit with a sterile needle that had been dipped in a spore suspension (10^6 spores/ml) of *P. digitatum* at two sites midway between the stem and stylar end. The inoculated fruits were kept for 3 h at 25±2°C before being dipped separately into PAA at concentrations of 0.01 and 0.02% w/v; PCA or KMS at concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05% w/v; and SBC or KS at concentrations of 1.0, 1.5, 2, 2.5 and 3% w/v for 3 min. The dipped fruits were placed in plastic baskets, air-dried, kept on plastic trays, and then put in cardboard boxes. The control fruits were divided into two groups; the first group was neither washed nor dipped into distilled water (control 1) and the second group was dipped into distilled water for 3 min (control 2). The treated fruits and controls were stored at 25±2°C, 95±3% RH for 5 days and evaluated daily for the development of disease, based on disease incidence, severity (wound-diameter) and percent of sporulation index. Sporulation index describes the percentage of the fruit surface covered with green mold spores, where 0% = no sporulation on the surface; 1 = 1-20%; 2 = 21-40%; 3 = 41-60%; 4 = 61-80% and 5 = > 80% (Inkha et al., 2009). Each treatment was repeated three times, with 10 fruits per replication.

Experiment 2. *Sai Nam Phueng* tangerine fruits were wound-inoculated by puncturing the fruit with a sterile needle that had been dipped in a spore suspension (10^6 spores/ml) of *P. digitatum* at two sites midway between the stem and stylar end. The inoculated fruits were kept for 3 h at 25±2°C before being dipped into the lowest concentration of each solution (from Experiment 1) that provided the best result to search for the most effective dipping time (1, 3, or 5 min). The control fruits were divided into two groups, the first group was neither washed nor dipped into distilled water (control 1) and the second group was dipped into distilled water for 5 min (control 2), similar to Experiment 1. The treated fruits and controls were stored at 25±2°C, 95±3% RH for 5 days and evaluated daily for the development of disease, based on disease incidence, severity (wound-diameter), and percent of sporulation appearing on the peel surface. The disease incidences were compared to choose the optimal solution for controlling green mold disease. Each treatment was repeated three times, with 10 fruits per replication.

Experiment 3. The efficiency of the optimal solution (from Experiment 2) for controlling green mold disease was examined again with the same methods as in Experiment 1 and 2 and stored at 5±2°C, 65±3% RH for 25 days. Each treatment was repeated three times, with 10 fruits per replication.

Statistical analysis

All statistical analyses were performed using SPSS version 16. Data were analyzed in completely randomized design by using analysis of variance and mean separation with Turkey's test at P=0.05.

RESULTS AND DISCUSSION

Effects of PAA, PCA, SBC, KS, and KMS concentrations on the control of green mold in *Sai Nam Phueng* tangerine fruits

The lowest concentration of each solution that was significantly effective in controlling green mold on *Sai Nam Phueng* tangerine fruit, caused by *P. digitatum* compared to control 1 (neither washed nor dipped into distilled water) and control 2 (dipped into distilled water) were 0.02% PAA, 1.5% SBC and 1.5% KS w/v (Table 1). PCA and KMS at concentrations of 0.01-0.05% w/v had little effect. During storage for 5 days at 25±2°C, 95±3% RH, dipping inoculated fruits into 0.02% PAA, 1.5% SBC and 1.5% KS w/v reduced disease incidence from 92.0% of control 1 and 100.0% of control 2 to 86.0, 85.3 and 66.7%, respectively; reduced disease severity (wound diameter) from 8.6 cm of control 1 and 8.9 cm of control 2 to 7.8, 7.5 and 6.1 cm, respectively; and reduced percent of sporulation from 49.3% of control 1 and 58.00% of control 2 to 45.3, 41.3 and 22.0%, respectively (Table 1).

Table 1. Effects of various PAA, PCA, KMS, SBC and KS concentrations on green mold disease incidence, severity and percent of sporulation on inoculated *Sai Nam Phueng* tangerine fruit after storage at 25±2°C, 95±3% RH for 5 days.

Treatments	Disease incidence (%)	Disease severity (cm)	Sporulation (%)
Control 1 (not washed)	92.00ab±4.00	8.63ab±0.35	49.33b±3.06
Control 2 (distilled water 3 min)	100.00a±0.00	8.90a±0.10	58.00a±2.00
0.01%PAA, 3 min	90.67ab±5.03	8.10ab±0.44	47.33b±2.31
0.02%PAA, 3 min	86.00b±4.00	7.83c±0.40	45.33b±1.16
C.V. (%)	4.11	4.17	4.47
Control 1 (not washed)	92.00b±4.00	8.63a±0.35	49.33b±3.06
Control 2 (distilled water 3 min)	100.00a±0.00	8.90a±0.10	58.00a±2.00
0.01%PCA, 3 min	98.00ab±2.00	8.93a±0.12	56.67a±1.16
0.02%PCA, 3 min	94.67ab±3.06	8.70a±0.50	54.00ab±2.00
0.03%PCA, 3 min	96.00ab±2.00	8.80a±0.20	49.33b±3.06
0.04%PCA, 3 min	95.33ab±3.06	8.73a±0.12	53.33ab±2.31
0.05%PCA, 3 min	95.33ab±3.06	8.53a±0.15	52.67ab±3.06
C.V. (%)	2.84	2.98	4.63
Control 1 (not washed)	92.00b±4.00	8.63a±0.35	49.33cd±3.06
Control 2 (distilled water 3 min)	100.00a±0.00	8.90a±0.10	58.00ab±2.00
0.01%KMS, 3 min	99.33a±1.06	9.10a±0.20	61.33a±3.06
0.02%KMS, 3 min	98.67a±1.16	9.00a±0.20	60.00ab±2.00
0.03%KMS, 3 min	98.67a±1.16	8.90a±0.20	55.33abc±1.16
0.04%KMS, 3 min	96.67ab±1.16	9.03a±0.23	53.33bc±1.16
0.05%KMS, 3 min	95.33ab±1.16	8.63a±0.15	45.33d±4.16

C.V. (%)	1.85	2.44	4.72
Control 1 (not washed)	92.00ab±4.00	8.63ab±0.35	49.33abc±3.06
Control 2 (distilled water 3 min)	100.00a±0.00	8.90a±0.10	58.00a±2.00
1.0%SBC, 3 min	91.33ab±2.31	8.43abc±0.40	50.67ab±3.06
1.5%SBC, 3 min	85.33b±4.16	7.53d±0.31	41.33bc±7.02
2.0%SBC, 3 min	87.33b±4.62	7.90bcd±0.26	41.33bc±5.78
2.5%SBC, 3 min	87.33b±1.16	7.83cd±0.15	37.33bc±7.02
3.0%SBC, 3 min	84.00b±3.46	7.80cd±0.10	36.67c±4.16
C.V. (%)	3.61	3.25	12.10
Control 1 (not washed)	92.00ab±4.00	8.63a±0.35	49.33ab±3.06
Control 2 (distilled water 3 min)	100.00a±0.00	8.90a±0.10	58.00a±2.00
1.0%KS, 3 min	83.33bc±3.06	7.90a±0.17	39.33b±3.06
1.5%KS, 3 min	66.67d±4.62	6.10b±0.61	22.00c±7.21
2.0%KS, 3 min	68.67cd±3.06	6.57b±0.15	24.67c±3.06
2.5%KS, 3 min	66.67d±2.31	6.00b±0.10	17.33c±2.31
3.0%KS, 3 min	57.33d±11.72	5.40b±0.89	18.00c±3.46
C.V. (%)	6.97	6.23	11.65

Note: Mean separation within column groups is by Turkey's test at P=0.05.

To determine the effectiveness of the lowest concentration of solutions that could control green mold, fruit were dipped into 0.02% PAA, 1.5% SBC, 1.5% KS, and mixtures of 1.5% SBC with 0.02% PAA and 1.5% KS mixed with 0.02% PAA w/v for 1, 3, or 5 min and stored at 25±2°C, 95±3% RH for 5 days. The combination of 1.5% KS mixed with 0.02% PAA w/v with a dip time of 5 min was more effective than 0.02% PAA, 1.5% SBC, 1.5% KS and 1.5% SBC mixed with 0.02% PAA at controlling green mold on *Sai Nam Phueng* tangerine fruit, based on reduced disease incidence, disease severity, and percent of sporulation from both control 1 and control 2 (Table 2 and Figure 1). Therefore, the addition of 1.5% KS to 0.02% PAA has the potential to improve 1.5% KS effectively. KS is effective up to pH 6.5, with increasing effectiveness as the pH decreases (Smilanick et al., 2008). In our study, adding 0.02% PAA decreased the pH value of 1.5% KS from 8.82 to 5.15 (Table 2), thus leading to an increase in its effectiveness. However, 1.5% KS solution at a high pH still controlled green mold on *Sai Nam Phueng* tangerine fruit (Table 2 and Figure 1). The mode of action of KS was shown to be through the alteration of the morphological structure of the cell, genetic material changes, cell membrane alteration, and inhibition of enzyme or transport function (Sofos, 1989).

Table 2. Effects of 0.02% PAA, 1.5% SBC, 1.5% KS, 1.5% SBC + 0.02% PAA and 1.5% KS + 0.02% PAA dipping for 1, 3 or 5 min on green mold disease severity on inoculated Sai Nam Phueng tangerine fruit after storage at 25±2°C, 95±3%RH for 5 days.

Treatments	pH	Disease severity (cm)
Control 1 (not washed)	-	8.20abc±0.10
Control 2 (distilled water 5 min)	6.80	8.73a±0.31
0.02%PAA, 1 min	2.48	8.23abc±0.15
0.02%PAA, 3 min	2.48	8.25ab±0.44
0.02%PAA, 5 min	2.48	8.30ab±0.06
1.5%SBC, 1 min	8.44	8.10abc±0.26
1.5%SBC, 3 min	8.44	8.10abc±0.06
1.5%SBC, 5 min	8.44	8.10abc±0.31
1.5%KS, 1 min	8.82	6.73de±0.15
1.5%KS, 3 min	8.82	7.03cde±0.12
1.5%KS, 5 min	8.82	6.33ef±0.42
1.5%SBC+0.02%PAA, 1 min	7.27	7.87abcd±0.29
1.5%SBC+0.02%PAA, 3 min	7.27	7.50bcde±0.10
1.5%SBC+0.02%PAA, 5 min	7.27	7.47bcde±0.57
1.5%KS+0.02%PAA, 1 min	5.15	6.50e±0.70
1.5%KS+0.02%PAA, 3 min	5.15	5.20fg±0.95
1.5%KS+0.02%PAA, 5 min	5.15	4.60g±0.36
C.V. (%)	-	5.35

Note: Mean separation within column groups is by Turkey's test at P=0.05.

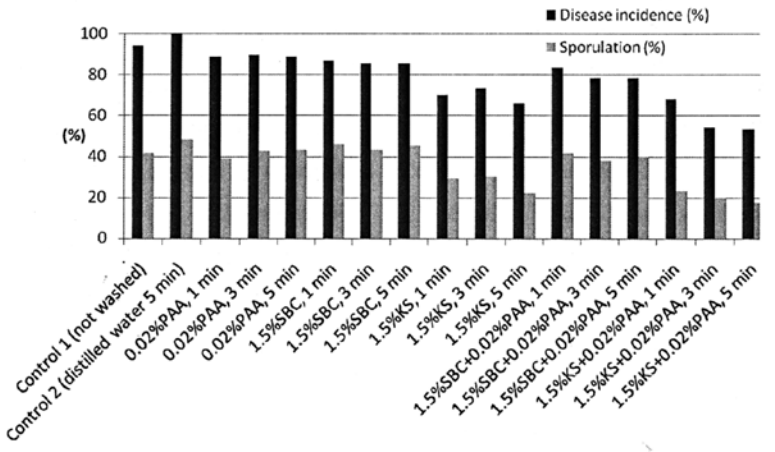

Figure 1. Effects of 0.02% PAA, 1.5% SBC, 1.5% KS, 1.5% SBC + 0.02% PAA and 1.5% KS + 0.02% PAA dipping for 1, 3 or 5 min on green mold disease incidence and percent of sporulation on inoculated *Sai Nam Phueng* tangerine fruit after storage at 25±2°C, 95±3% RH for 5 days.

In this study, the pH did not seem to have an influence on the control of green mold caused by *P. digitatum*, despite a low pH environment (4.0-5.5) stimulating the growth of *P. digitatum* (Pelser and Eckert, 1977). Dipping inoculated fruits into either 1.5% KS solution at pH 8.82 or 1.5% KS mixed with 0.02% PAA solution at pH 5.15 was more effective than 0.02% PAA at pH 2.48, 1.5% SBC at pH 8.44 and 1.5% SBC mixed with 0.02% PAA at pH 7.27 to control green mold on *Sai Nam Phueng* tangerine fruit (Table 2). Moreover, in this laboratory experiment, the effectiveness of 1.5% SBC was similar to 0.02% PAA but inferior to 1.5% KS to control green mold on tangerine fruit. In contrast, Smilanick et al. (2008) reported that 1.0% SBC was more effective than 1.0% KS to control green mold on Valencia oranges and Karabulut et al. (2001) reported that 2.0% SBC was more effective than 2.0% KS to control postharvest decay caused by *Candida oleophila* on sweet cherries. In general, different types of antimicrobial substances have specific toxicity to different types of pathogens and the effectiveness of antimicrobial substances still depends on species and cultivars. For example, SBC was effective on oranges and lemons but much less effective on mandarins (Smilanick et al., 1999, 2008; Palou et al., 2002), which was confirmed by our study with *Sai Nam Phueng* tangerines. KS had been reported to control green mold on Ortanique tangor (a tangerine-sweet orange hybrid), Fino lemons and Valencia oranges but did not control green mold on Nadorcott and Clemenules mandarins (Montesinos-Herrero et al., 2009).

After storage for 25 days at 5±2°C and 65±3% RH, control 1 and control 2 had disease incidence up to 97.3 and 100.0%, disease severity (wound-diameter) of 8.6 and 9.0 cm, and percent of sporulation 12.7 and 13.3%, respectively. Fruits treated with 1.5% KS or 1.5% KS mixed with 0.02% PAA w/v for 5 min had disease incidence of 52.7 and 45.3%, disease severity (wound-diameter) of 4.6 and 3.9 cm, and percent of sporulation of 0.0 and 0.7%, respectively (Table 3 and Figure 2). At low-temperature (5±2°C, 65±3% RH) storage, it was found that the percent of sporulation and disease severity of green mold caused by *P. digitatum* after treating fruits with 1.5% KS or 1.5% KS mixed with 0.02% PAA showed less severe symptoms of green mold than both control fruits.

Table 3. Effects of 1.5% KS and 1.5% KS + 0.02% PAA dipping for 5 min on green mold disease severity on inoculated Sai Nam Pueng tangerine fruit after storage at 5±2°C, 65±3%RH for 25 days.

Treatments	Disease severity (cm)
Control 1 (not washed)	8.63a±0.32
Control 2 (distilled water 5 min)	8.97a±0.25
1.5%KS, 5 min	4.57b±0.25
1.5%KS+0.02%PAA, 5 min	3.90b±0.76
C.V. (%)	6.86

Note: Mean separation within column groups is by Turkey's test at P=0.05.

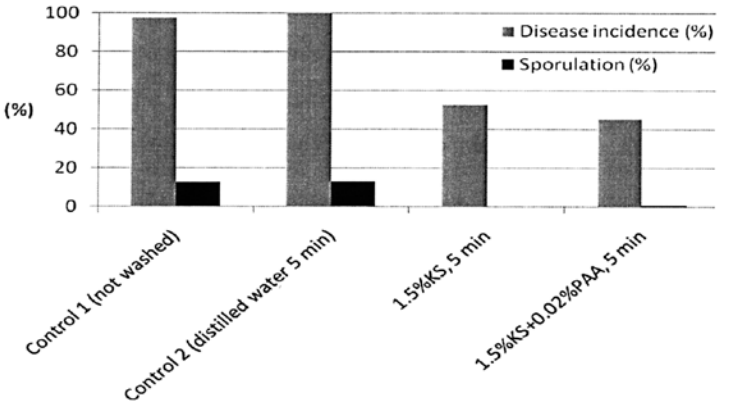

Figure 2. Effects of 1.5% KS and 1.5% KS + 0.02% PAA dipping for 5 min on green mold rot disease incidence and percent of sporulation on inoculated *Sai Nam Phueng* tangerine fruit after storage at 5±2°C, 65±3% RH for 25 days.

CONCLUSION

Treating *Sai Nam Phueng* tangerine fruit by dipping in 1.5% KS mixed with 0.02% PAA solution for 5 min was the best method to control green mold caused by *P. digitatum* by reducing disease incidence, severity, and percent of sporulation, whether stored at 25±2°C and 95±3% RH or 5±2°C and 65±3% RH.

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