CHAPTER 5

Effect of phenyllactic acid combination with mixed wax coating on quality and storage life of sweet orange cv. Canh.

Effect of phenyllactic acid (PLA) in combination with mixed bees wax and carnauba wax (MW) on postharvest quality and storage life of Vietnamese sweet orange cv. Canh were studied by soaking fruit in 1.5, 2.0, 2.5 and 3.0% PLA, and then coating with 8% MW fruit. Non-treated fruit were used as the control. Percentage of fruit decay and weight loss; sensory properties; total microorganisms; total soluble solids content (TSS); and titrable acid were monitored during the storage period. The results showed that PLA at a concentration of 2.5% in association with 8% MW coating had strong antimicrobial and antifungal activity against *Penicillium* sp. and *Aspergillus* sp.; and were able to completely inhibit the growth of green mold *Penicillium* sp. infection in sweet orange cv. Canh. Moreover, the percentage of fruit decay, weight loss, TSS content were reduced; and total microorganisms, titrable acid increased by PLA during the storage period. In addition, fruit maintained a higher sensory score for marketing purposes. The shelf-life of the control fruit was only 10 days compared with 25 days for the PLA-treated fruit when fruit were stored at ambient temperature.

5.1 Introduction

Orange fruit (*Citrus sinensis* Osbeck) is a non-climateric subtropical fruit, and it is one of the most valuable fruit in Vietnam for domestic and export markets because it has a delicious taste and excellent nutritional properties. In Vietnam, the production area of oranges and tangerines was around 58,300 ha, and the yield was over 736,100 tons (Vietnam General Statistics, 2014). Fresh fruit and vegetables represent an opportune nicheformany undesirable fungi due to high water availability, and long term storage during transportation, with *Fusarium*, *Penicillium*, *Alternaria* and *Botrytis* species,

others, identified as major fungal spoilers (Crowley *et al*. 2013).

Losses from postharvest disease caused by various pathogens account for nearly 50 % of the total wastage in citrus fruit, and infection and contamination occurs at different stages in the field and after harvest during marketing (Ladaniya, 2008). Decay from postharvest infection include *Penicillium* rot, *Aspergillus* rot, *Rhizopus* rot, and *Fusarium* rot (Ladaniya, 2008). Hatton *et al*. (1987) reported that mandarin fruit cv. Sunburst subjected to 66h of de-greening showed 10% decay, whereas fruit de-greened for 45h <2% decay. An extra discontinuous layer of wax applied artificially with sufficient thickness and consistency to prevent anaerobic respiration within the fruit, provides the necessary protection against decay-causing organisms (Pantastico, 1997).

Phenyllactic acid (PLA) is produced fermentatively by *Lactobacillus* sp. including *Lactobacillus plantarum*, and is an organic acid proven to have a broad inhibitory spectrum against microorganisms (Lavermicocca *et al.,* 2001). However, there have been few studies which applied PLA for preserving fresh products. PLA has mainly been applied in some processed foods such as milk products, bread and bakery goods (Magnusson, 2001; Li *et al*., 2007).

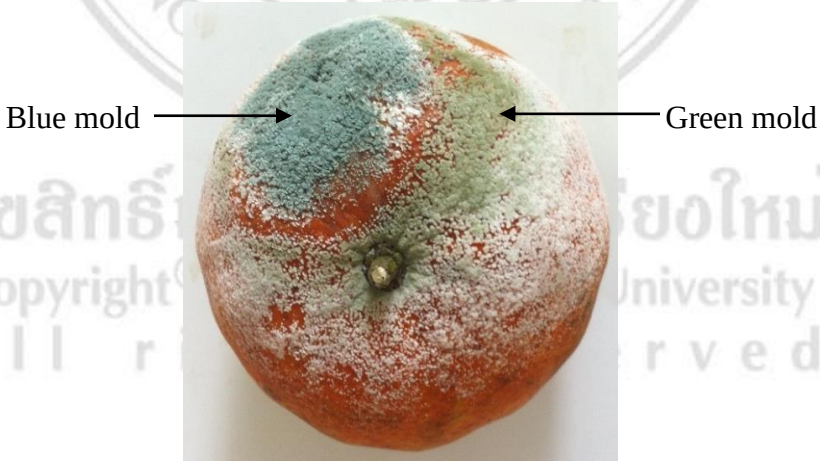


Figure 5.1 Blue mold and green mold of orange cv. Canh

Sathe *et al*. (2007) demonstrated the ability of *L. plantarum* CUK501 to inhibit growth of four different fungi on cucumbers for up to 8d compared to a non-treated control. Green mould, blue mould, caused by the fungus *Penicillium digitatum*, *Penicillium*

italicum are typically the worst postharvest disease of orange, tangerines. It attacks injured areas of the peel and first appears as a soft, watery, discoloured spot on the rind. White fungal growth soon appears on the fruits surface, and after the spot enlarges, green spores, blue spores are produced in the center part of the spot (Fig. 1.1). The rot eventually penetrates the peel and into the edible fruit segments. Under high RH conditions, the fruit collapses into a soft, decomposing mass (Postharvest Handling Technical Bulletin, 2004a).

Lavermicocca *et al.* (2003) shown that PLA inhibited a wide range of mold species isolated from bakery products, flour, and cereals, including some mycotoxigenic species, namely *Aspergillus ochraceus, Penicilliumro queforti, P. citrinu*, etc. The ability of PLA to act as a fungicide provides new perspectives for the possibility of using this natural antimicrobial compound to control fungal contaminants and extend the self-life of food and/or feedstuffs. A 2% concentration of PLA completely inhibited the growth of the green mold *Penicillium digitatum* in oranges. 'Vangiang' orange fruit treated with 2% PLA followed by coating with wax (CP-01) maintained quality and appearance, while reducing the spoilage rate during an 8wk storage at ambient conditions (Thuy, 2013).

The purpose of this study was to investigate the effects of soaking in phenyllactic acid in combination with a mixed bees wax and carnauba wax coating on postharvest quality and shelf-life of Vietnamese sweet orange cv. Canh during the storage at ambient temperatures.

5.2 Materials and methods

5.2.1 Materials

Phenyllactic acid (PLA) in this study was produced by fermentation using *Lactobacillus plantarum* C2 (Thuy, 2012). Other analytical chemicals were purchased from Sigma. Two fungal strains, *Aspergillus* sp. and *Penicillium* sp., were collected from orange cv. Canh at the Vietnam Institute of Agricultural Engineering and Postharvest Technology.

Fruit of orange cv. Canh from a commercial orchard in the ThanhOai district, Hanoi were harvested at 220-235 d after fruit set by clipping the fruit stalk just above the point of attachment to the fruit. The oranges were laid in a sponge-filled box (20 kg fruit/box) and transported to the laboratory within 2-3 h of harvest. Fruit were then selected for uniformity of shape, size and absence of defects.

The bees wax and carnauba wax in the ratio of 7:3 was prepared following process of Thinh, (2013). The bees wax and carnauba wax was melted by a magnetic stirrer at 80-85°C. After that 1.5% oleic acid, 0.08% palmitic acid, and water were added to the mixture during stirring and blending for 8% concentrations of mixed wax (MW) for 25-30 minutes. The MW of various concentrations were cooled down to ambient temperature before coated to the fruit. All measurements of each treatment were the average of three replications.

5.2.2 Methods

1) Isolation and identification microorganisms in orange cv. Canh

Microorganisms were classified according to Raper *et al.* (1965), and John (1979); observed using a E600-Nikon microscope (Japan) with the Image-Pro Plus program version 4.5 for Windows; and scanned using a JSM-5410LV electron microscope.

2) Antifungal activity of PLA and MW on mycelial growth of the fungi

Effects of PLA and MW on the growth of *Aspergillus* sp. and *Penicillium* sp. were tested *in vitro* on PDA (potato dextrose agar) medium. A volume of 200 µl of 1.5, 2.0, 2.5 and 3.0% PLA in combination with 200 µl of 8% MW was spread onto the plate's surface. After that, 10 µl suspensions containing *Aspergillus* sp. and *Penicillium* sp. (10^5cfu/ml) were put in the center of PDA plates. PDA plates without PLA and MW were used as controls. Then PDA plates were incubated at 25°C for 7 d (Skidmore and Dickinson, 1976). Fungal growth expressed as diameter was measured by using a digital caliper.

PLA at different concentrations (1.5%, 2.0%, 2.5% and 3.0%) was added in tubes

containing PDA (200 µl) and combined with 200 µl 8.0% MW. A volume of 10 µl of the test organisms at a spore concentration of 10^5cfu/ml was injected into the tubes. Tubes were incubated at ambient temperature(24 ± 2°C) for 24 h. Then, 10µl of each mixture was spread onto the surface of the petri plates containing 10 ml of PDA followed by incubation at ambient temperatures (24 ± 2°C) for 48 h (Skidmore and Dickinson, 1976). After incubation, the percentage of fungal inhibition was calculated by using the formula given below:

Percentage inhibition (%) = (C-T)/ C * 100

Where, C is number of germinated sporesin the control (without PLA); T is number of germinated sporesin plates treated with PLA and MW.

3) Efficacy test of phenyllatic acid combined with mixed wax to control *Penicillium* sp. in orange cv. Canh

The inhibition effectiveness of PLA against *Penicillium* sp. in orange cv. Canh fruit was determined by the method of Lam *et al*. (2011). The oranges were washed with fresh water, and after drying, fruit were punctured using a sterilized needle to produce five holes (3 mm deep, and 5 mm in diameter) and inoculated with 10 µl of a spore suspension of the *Penicillium* sp. strain at a concentration of 10^5cfu/ml in each hole. Next, the inoculated fruit were soaked in 1.5%, 2%, 2.5% and 3.0% PLA and carbendazim (CBZ) (500ppm) solutions for 5 min, and coated in 8.0% MW, and then stored for 6 d at ambient temperature (22 ± 2°C) and while untreated fruit were used as control. The growth of *Penicillium* sp. in fruit was determined, as follows:

% Percentage of holes decayed = (Number of decayed holes/Total hole) x 100

4) Effect of phenyllatic acid combined with mixed wax coating on quality and storage time of orange cv. Canh fruit.

Fruit were divided into 2 groups: Control group (non-treated), and treated group (fruit were soaked in PLA at concentration of 2.5% and inCBZ 500ppm for 5 min then soaked fruit were coated in 8% MW for 1 min and storage at ambient temperatures (22 ± 2°C), RH 80 ± 5%). Data collection:

The titrable acid (TA) was determined as citricacid by titrating against 0.1NaOH by

following the method of the AOAC (2000).

Total soluble solids (TSS) content in filtered juice was determined by using a digital refractometer (RFM-80) (Atago, Tokyo, Japan).

The percentage of decayed fruit was assessed as follows:

$$\text{Fruit decay (\%)} = \frac{\text{Num. of decayed fruit}}{\text{Total fruit}} \times 100$$

Percentage of weight loss was calculated by weighing the whole fruit kept in a tray before and after storage, as follows:

$$\text{Percentage of weight loss} = \frac{\text{Weight}_{\text{before}} - \text{Weight}_{\text{after}}}{\text{weight}_{\text{before}}} \times 100$$

The effect of different concentrations of PLA and MW on the sensory quality of fruit was tested 1, 5, 10, 15, 20 and 25 d after harvest at ambient temperatures. Fruit were peeled, separated into segments, and placed onto a disk. Each sensory experiment included a mixture of segments from five different fruit. Fruit taste was assessed by a committee consisting of five members. Each member evaluated the various samples, with anchorpoints of extremely liked and extremely disliked for each characteristic and sensory data were written on a point scale on the evaluation form. Panel discussions were performed in order to develop the sensory form.Each member was requested to rate peel color, odor, taste, and flavor on a hedonic scale from 1 to 9 points with 9=extremely liked; 1=extremely disliked; and 5=neither liked nor disliked (Hung, 2008).

The total microorganism populations on the surface of oranges, including yeasts, and molds, were analyzed according to the method of Whangchai *et al*. (2006). The sampled fruit were immersed in sterile, distilled water and shaken at 180 rpm for 30 min at ambient temperatures (24 ± 2°C). For each treatment, a sample (1 ml) of the suspension was spread over PDA. The PDA plates were incubated at 25°C for 72 h and the survival of microorganisms was expressed as the mean number of colony forming units (CFU/g).

The total aerobic bacteria count was determined according to the Vietnam Standards (TCVN 5165:1990). For each sample of fruit, 30 g of fruit pericarp was chopped up and extracted in 270 ml sterile distilled water by shaking at 180 rpm for 30 min at ambient temperatures (24 ± 2°C). Afterward, a 1 ml sample of the suspension was spread over a standard agar medium (1% peptone, 0.5% NaCl, 0.1% glucose, 2% agar). The agar plates were incubated at 24 ± 2°C for 72 h and the survival of aerobic bacteria was expressed as the mean number of colony forming units (CFU/g).

Determination of storage life: Using the above indices, the storage life of orange cv. Canh will be determined as unacceptable for marketable purposes as follows: when the percentage of fruit decay is above 10%, or/and when sensory score is ≤ 5.

Statistical analysis was carried out using Duncan's multiple range test was use to analyze the significant differences ($P \leq 0.05$) between treatments and the control.

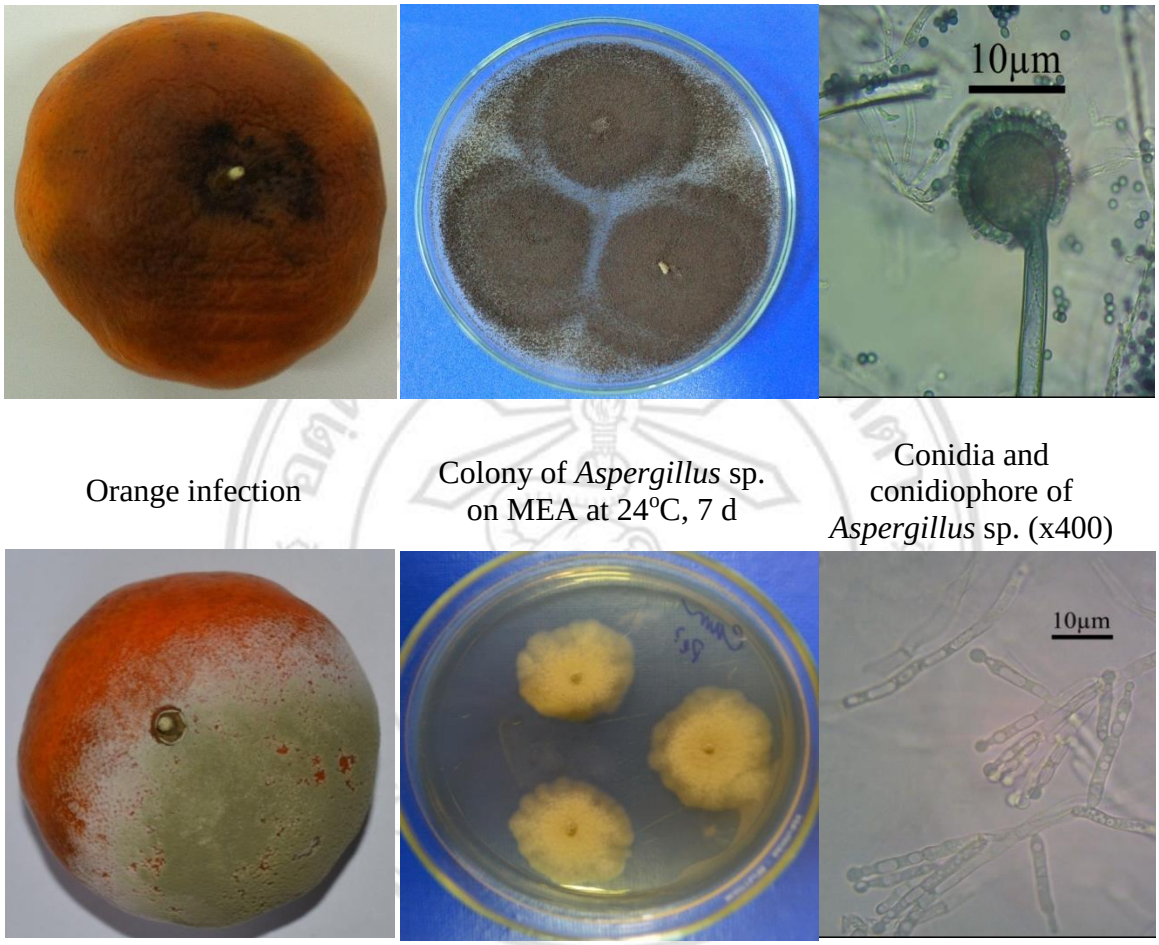
5.3 Results and Discussions

5.3.1. Isolation and identification

Aspergillus sp. colonies on Czapek's solution agar grew rapidly at room temperature (24-26^{o}C) reaching a diameter of 5 to 6 cm in 12 d; the strain produced dense purple-brown or purple black sporulation, andgray conidial structures. Its morphology included: conidial heads globose at first with a diameter up to 1mm but commonly 500 to 700µm shattering easily with columns deciduous; conidiophores un-colored or slightly brownish below the vesicles, usually 1.0 - 2 mm by 9-13 µm but up to 2.5mm long to 18-20 µm in diameter with walls up to 2.0 to 2.5 µm thick, smooth or occasionally showing a limited deposit of granular material (Raper *et al*. 1965).

Penicillium sp. colonies on MEA (malt extract agar) solution agar grew rapidly at room temperature (24-26^{o}C) reaching 35 to greater than 70mm in diameter. Its morphology included relatively sparse, strictly velutinous, margins subsurface, irregular; mycelium usually inconspicuous; condiogenesis moderate; conidiophores borne from surface or aerial hyphae with thin smooth walls, bearing terminal penicilli when fully developed

terverticillate but frequently biverticillate or irregular, conidia ellipsoidal to cylindroidal 6-8 (-15) x 2.5-5 (-6) µm smooth walled, bone in disordered chains (John, 1979).



Orange infection | Colony of *Aspergillus* sp. on MEA at 24°C, 7 d | Conidia and conidiophore of *Aspergillus* sp. (x400)

Green mold infection | Colony of *Penicillium* sp. on MEA at 24°C 7 d | Conidia and conidiophores of *Penicillium* sp. (x400)

Figure 5.2 Symptoms and signs of post-harvest rot of orange cv. Canh caused by *Aspergillus* sp. and *Penicillium* sp.

5.3.2 Antifungal acitivity of phenyllactic acid and mixed wax on mycelial growth of two fungal strains

The inhibitory ability of PLA in combination with MW against *Aspergillus* sp. and *Penicillium* sp. is shown in the Table 5.1. The growth of both fungi was indirectly related to the concentration of PLA. The control fruit had the highest fungal development during the storage period. PLA concentrations of 2.5 and 3% were the

most effective in inhibiting fungal growth. A PLA concentration of 1.5% significantly inhibited *Penicillium* sp. (d = 4.3 cm), *Aspergillus* sp. (d = 4.6 cm). *Penicillium* sp. was more sensitive to PLA than *Aspergillus* sp., total growth inhibition occurred at PLA concentrations of 2.5 and 3.0%, respectively.

Table 5.1 Antifungal activity of phenyllatic acid and mixed wax against mycelial growth *Aspergillus* sp. and *Penicillium* sp. after 7d

PLA concentration (%)[1]	Mycelial growth diameter (cm)	
	Aspergillus sp.	*Penicillium* sp.
Control	5.0 ± 0.05a	6.3 ± 0.06a
1.5%	4.6 ± 0.04a	4.3 ± 0.05a
2.0%	3.8 ± 0.05a	3.1 ± 0.07b
2.5%	0.6 ± 0.02b	0 ± 0.00c
3.0%	0 ± 0.00b	0 ± 0.00c

Note:[1]PLA was applied in combination with 8% mixed wax
Means followed by the same letter(s) within a column are not significant different as determined by Duncan's multiple-range test P< 0.05.

Thuy*et al.* (2013) reported that the highest inhibitory activity of PLA against three strains of fungi including *Aspergillus niger*, *A.flavus*, and *Penicillium digitatum*, occurred at concentrations of 50, 50 and 40mg/ml, respectively. According to Lavermicocca *et al*. (2001), the antifungal compounds produced by LAB display growth inhibition against common fungal strains such as *Aspergillus niger, A. terreus, A. flavus, A. nidulans, Penicillium roqueforti, P. corylophilum, P. expansum.* Prema *et al*. (2010) showed that *inhibitory concentration*of PLA produced by a *L. plantarum* strain against fungal spoilers such as *Aspergillus fumigatus* and *Penicillium camemberti* was small (6.5 - 12 mg/ml).

Figure 5.3 shows the inhibition of *Aspergillus* sp. and *Penicillium* sp. germination in various concentrations of PLA combined with 8% mixed wax after 48h at 22 ± 2°C. High concentrations of PLA totally inhibited the growth of the microorganisms. As seen in Figure 5.3, PLA at a concentration of 1.5% inhibited *Aspergillus* sp. and *Penicillium* sp. germination by 65 and 72%, respectively. Total inhibition of

Penicillium sp. and *Aspergillus* sp. occurred at PLA concentrations of 2.5 and 3%, respectively.

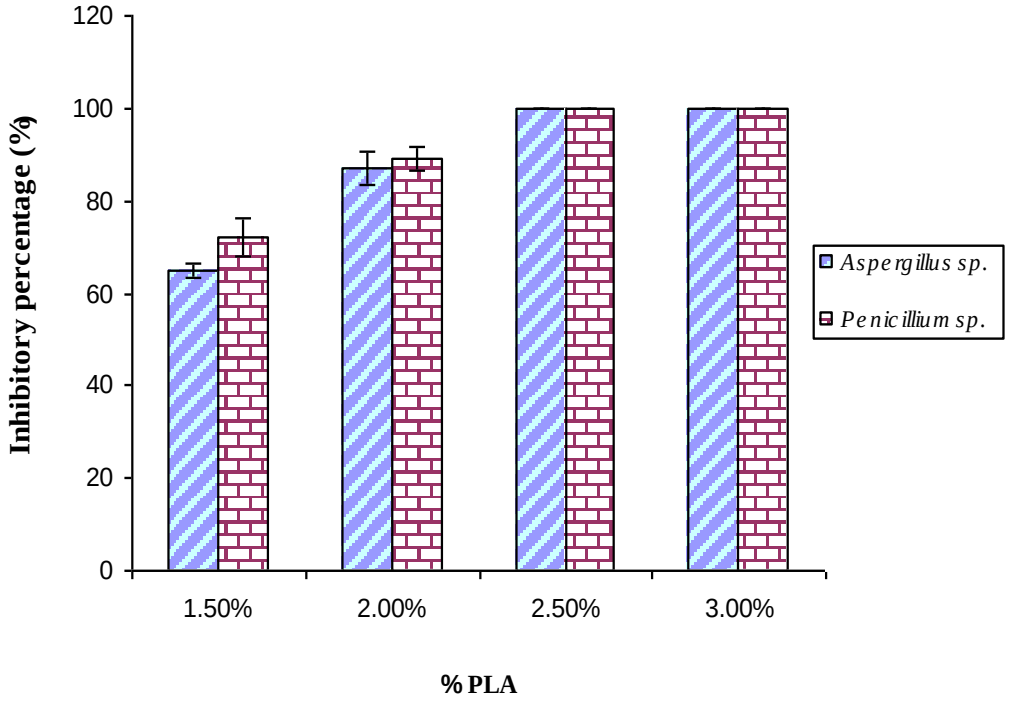


Figure 5.3 Inhibitory activity of PLA combined with 8% mixed wax against *Aspergillus* sp. and *Penicillium* sp. germination after 48h

Our results are consistent with the findings of Lavermicocca *et al.* (2001) who found that spore germination of *Aspergillus niger* FTDC3227, and *A. flavus* FTD3226 and *P. corylophilum* IBT6978 were inhibited by 98.6, 86.5 and 100%, respectively by 50 mg/ml ofPLA obtained from the fermentation process of *L. plantarum* 21B. El-Mougy *et al.* (2012) reported that carnauba wax had no inhibitory effect against *Geotricum candidum* (sour rot), *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). On the other hand, Gonçalves *et al*. (2010) found that nectarines covered with 9% carnauba wax inhibited the conidial germination of *Monilinia fructicola* and *Rhizopus stolonifera* by 50 and 90%, respectively. Henik *et al*., (2013) concluded that the combination of *Eugenia caryophyllata* crude extract and *Candida utilis* TISTR 5001 decreased natural development of green mold rot incidence by 90% and severity by 86%.

5.3.3 Efficacy test of PLA and MW to control and *Penicillium* sp. in orange

Table 5.2 Effect of PLA applied with mixed wax on *Penicillium* sp. mold incidence in orange cv. Canh

Time in storage (days)	Disease incidence (%)					
	Control	1.5% PLA	2.0% PLA	2.5% PLA	3.0% PLA	500ppm CBZ
1	0	0	0	0	0	0
2	42.0a	5.0b	0	0	0	0
3	85.0a	12.0b	0	0	0	0
4	90.0a	35.0b	5.0c	0	0	0
5	100.0a	70.0b	12.0c	0	0	0
6	100.0a	85.0b	25.0c	0	0	0

Note: *Means followed by the same letter(s) within a row are not significant different as determined by Duncan's multiple-range test P< 0.05.*

Fungal growth in inoculated wounds in the fruit surface is shown in Table 5.2. The percentage of infected wounds per fruit was monitored daily. As shown in Table 2, 2.5 and 3.0%, PLA completely inhibited *Penicillium* sp. The inhibitory activity of PLA in association with MW at these concentrations was equal to that of CBZ. This result confirmed that a concentration of 2.5% or higher of PLA totally controlled *Penicillium* sp. spoilage in orange cv. Canh. Our results are in accordance with the report of Thuy*et al.* (2013) who showed that concentrations of 2.0 and 2.5% PLA completely inhibited *P. digitatum* in 'VanGiang' orange, and was equivalent in effectiveness with the conventional fungicides imazalil and thiabendazole. Wang *et al.* (2012) showed that the shuffled strain F3A3 of *L. plantarum* had an excellent ability to prevent the fungal spoilage caused by *P. digitatum* KM08 in kumquat. However, *L. plantarum* IMAU10014 exhibited limited ability to prevent fungal spoilage. The yeast *Debaryomyces hansenii* reduced the incidence of *Penicillium* rot (Mehrotra *et al.*, 1996) and sour rot of orange fruit (Mehrotra*et al*., 1998). A water suspension of yeast cells applied to wounds on the fruit surface prior to inoculation with spore suspensions of pathogens reduced disease by 80-90% (Mehrotra *et al*., 1998).

5.3.4 Effects of PLA in combination with MW coating on quality and storage time of orange fruit cv. Canh

1) Change in titrable acidity and TSS

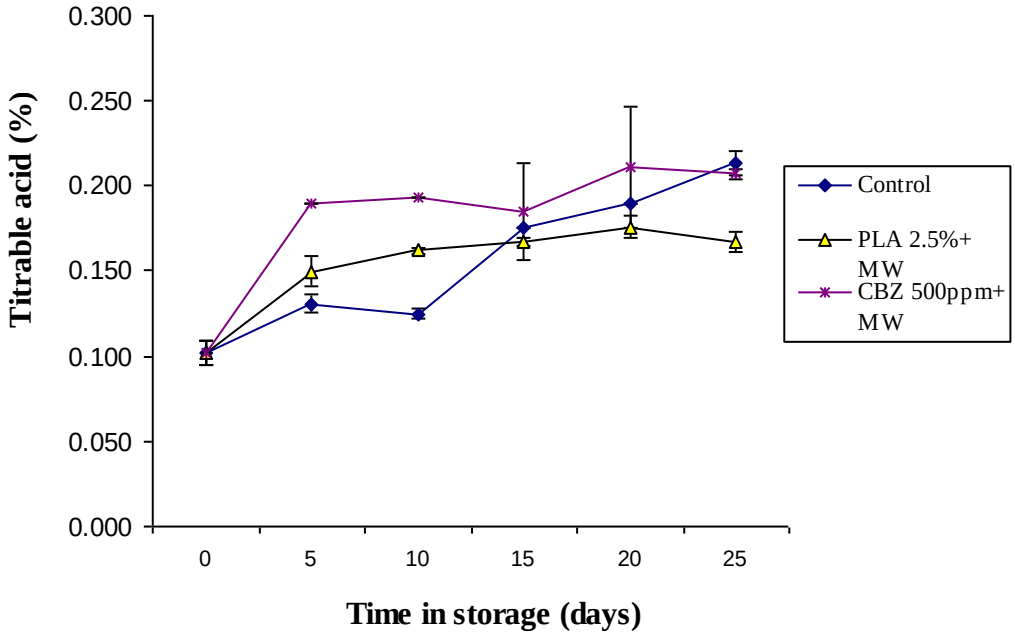


Figure 5.4 Effect of PLA combined with MW 8 % on titrable acid of orange cv. Canh fruit stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

Changes in titrable acid (TA) are shown in figure 5.4. The TA increased slightly in all treatments and the control, and which is not significantly difference after 10 d in storage ($P \leq 0.05$) (see appendix D). Titrable acid of the fruit treated with 2.5 PLA in combination with MW were not different after 25 d in storage, and were the least changed (0.167 %). The fruit treated with 2.5% PLA in combination with MW had the lowest Titrable acid content (0.167%), and TA content of the fruit treated with CBZ 500pm in combination with MW was 0.207%. The results are in line with finding of Shahid and Abbasi, (2011), who showed that the effect of bees wax coating and benlate on sweet orange cv Blood red at room temperature (12-19^{o}C), titrable acid increased faster in control than in wax coated orange cv. Canh.

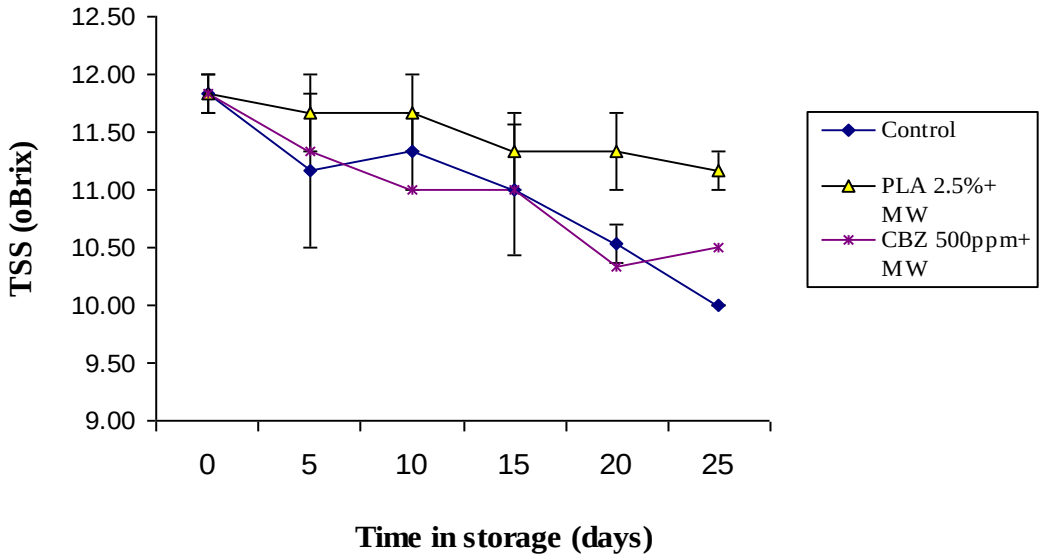


Figure 5.5 Effect of PLA combined with MW 8 % on TSS of orange cv. Canh fruit stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

Changes in total soluble solids (TSS) are shown in Fig. 5.5. There was no significant difference in TSS content of PLA+ MW treated fruit and the control fruit ($P \leq 0.05$). After 25 d in storage at ambient temperatures, the TSS of the control fruit decreased from 11.83 to 10.0^{o}brix. The TSS content of fruit treated with 2.5 PLA in combination with MW decreased from 11.83 to 11.17^{o}brix. The TSS for fruit treated with CBZ 500ppm in combination with MW was 10.5^{o}brix. Our results are consistent with the reported data on TSS content of orange cv. Canh fruit (Feng *et al*. 2014), the TSS content of 'Pokan' mandarin fruit decreased after 7d citral (95%) treatment in combination with a commercial wax coating (SP-1).

2) Change in fruit decay, weight loss

The percentage of decay in the treated and control fruit during the storage period is shown in Figure 5.6. The control and 2.5 PLA in combination with MW treated fruit had 20.71 and 0% fruit decay respectively after 25 d in storage. The percentage of fruit decay increased to 6.06% by day 25 for fruit treated with 2.5% PLA in combination with MW. There were no significant differences in fruit decay between 2.5% PLA, and CBZ 500ppm in combination with MW, and each treatment was significantly

different from the control fruit ($P \leq 0.05$) after 25d storage.

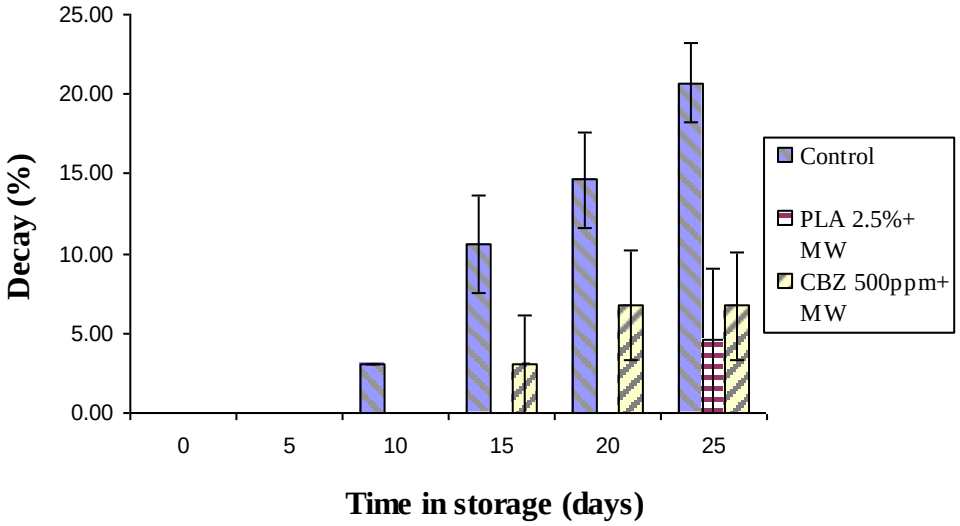


Figure 5.6 Effect of PLA combined with MW 8 % on decay of orange cv. Canh fruit stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

Our results are consistent with the finding of Thuy *et al.* (2013) who found that decay percentages of PLA treated fruit were lower than those of non-treated fruit, and Lan *et al.* (2012) who reported that the antifungal strain *Weissel lacibaria* 861006 inhibited the growth of *P. oxalicum* on the surface of grapes for up to 6 d.

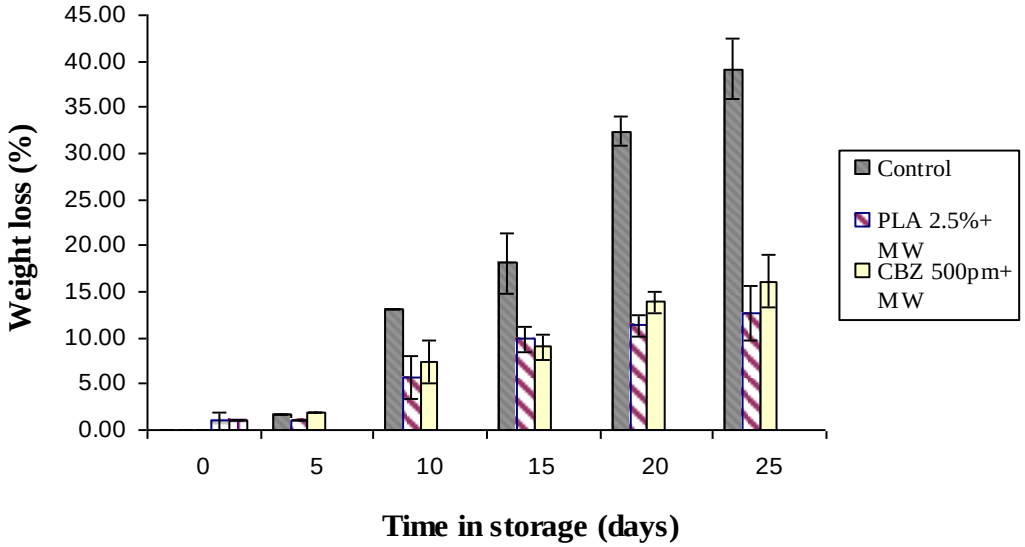


Figure 5.7 Effect of PLA combined with MW 8 % on weight loss of orange cv. Canh fruit stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

Changes in weight loss percentage of treated and control fruit during the storage period are shown in Figure 5.7. In general, weight loss percentage was low after 25 d in storage. There was a slight and non-significant increase in average weight loss, weightloss was 5.7% for fruit treated with 2.5% PLA, respectively, in combination with MWafter 10 d. After 25 d weight loss was 12.65% for fruit treated with 2.5% PLA, in combination with MW. For control fruit, after 25 din storage, the percentage of weight loss was 39.19%. There were no significant differences in weight loss among the treated fruit ($P \leq 0.05$), which were all significantly different from the control fruit ($P \leq 0.05$). This study indicates that high weight loss appeared to be related to high decay.

Nase and Hossein (2007) showed that weight loss was significantly decreased to 5% in 'Valencia' and 'Siavarz' oranges treated with hot water, fungicide and wax, compared to control. Henik *et al*., (2013) reported that the combination of *Candida utilis* TISTR 5001 and *Eugenia caryophyllata* crude extract significantly reduced the natural development of green mold of 'SaiNumPung' tangerine fruit and had no effect to fruit quality, weight lossed the lowest.

3) Change in sensory values and total microorganisms

Table 5.3 indicates the sensory score of treated and control fruit during the storage period. Generally, the hedonic score of treated PLA fruit was higher than that of non-treated fruit during the storage period. The sensory score of treated and control fruit was not different after 5days in storage. When the storage time was extended, there was a significant difference in sensory score between treated and control fruit ($P \leq 0.05$). Concentrations of PLA of 2.5% in combination with MW maintained higher sensory scores than other treatments and the control after 25d in storage. These results demonstrate that 2.5 % PLA in combination with MW were the most effective in maintaining the sensory quality of orange 'Canh' fruit during storage.

This result agree with Thuy *et al.* (2013) reported that 'Van Giang' orange fruit treat with 2% PLA followed by coating with CP-01 maintained a good quality expressed as external characteristics, taste, flavor, firmness, etc. during 8 wk in storage at ambient temperatures.

Changes in total microorganisms on the fruit during the storage period are shown in Table 5.4. There was a marked difference in total microorganisms between treated and control samples after 25d storage. Overall, total microorganisms oftreated and control fruit increased with increasing storage time. After 25d in storage, the total microorganism count of fruit treated with 2.5% PLA in combination with MW was 1.5 x 10^3CFU/g, andit was much lower than that of control fruit (1.2 x 10^6CFU/g) (Table 5.4). Therefore, it was confirmed that PLA has a strong inhibitory effect against microorganisms not only *in vitro* (Table 5.1) but also in a practical trial. This result indicates that soaking in 2.5% PLA in association with a MW coating can delay the increase of total microorganisms on orange cv. Canh after 25 d storage at ambient temperature when compared with the control fruit. Total microorganisms, total aerobic bacteria, and decay increased slowly at high PLA concentrations in combination with MW at ambient temperatures.

Strain 43E of *Candida famata* (*Torulopsis candida*) when used together with 0.1g TBZ/L at a concentration of 10^6 cells/ml gives significantly better disease control then either TBZ or the yeast alone (Arras *et al*., 1997). The strain of commonly used yeast in wineries, *Saccharomyces cerevisiae* was found to inhibit the growth of *Aspergillus niger* in 'Nagpur' mandarins and acid limes (Naqvi, 1998). The fungus *Muscodo ralbus*, a bio-fumigant that produces certain low molecular weight volatiles has been used to fumigate whole rooms of lemons to control pathogens during storage, and was effective against green mold and sour rot (Mercier and Smilanick, 2005).

Table 5.3 Effect of PLA combined with MW on sensory values in orange cv. Canh

Treatments	Day of storage					
	0	5	10	15	20	25
Control	8.5a	6.1a	5.3a	4.5a	4.0a	3.0a
PLA2.5%+MW	8.5a	7.5a	7.7b	7.0b	6.5b	5.5b
CBZ500ppm+MW	8.5a	8.0a	7.4b	7.0b	6.5b	5.0b

Note: *Means followed by the same letter(s) within a column are not significant different as determined by Duncan's multiple-range test P< 0.05.*

9 = extremely liked; 1= extremely disliked; and 5 = disliked

Table 5.4 Effect of PLA combined with MW on total microorganisms and total aerobic bacteria in orange cv.Canh

Day of storage	Type	Control	PLA 2.5% +MW	CBZ 500ppm + MW
0	Microorganisms	$2.3x\ 10^5$ a	5.8×10^2 b	$5.4\ 10^2$ b
	Aerobic bacteria	5.1×10^2 a	1.5×10^1 b	1.9×10^1 b
5	Microorganisms	$2.7x\ 10^5$ a	4.6×10^2 b	$6.9\ 10^2$ b
	Aerobic bacteria	5.3×10^2 a	1.9×10^1 b	2.7×10^1 b
10	Microorganisms	$4.9x\ 10^5$ a	6.4×10^2 b	$8.2\ 10^2$ b
	Aerobic bacteria	7.2×10^2 a	2.3×10^1 b	2.3×10^1 b
15	Microorganisms	$8.8x\ 10^5$ a	7.3×10^2 b	$1.4\ 10^3$ b
	Aerobic bacteria	6.2×10^2 a	3.4×10^1 b	1.6×10^2 b
20	Microorganisms	1.0×10^6 a	8.8×10^2 b	$1.7\ 10^3$ b
	Aerobic bacteria	1.2×10^3 a	1.9×10^2 b	1.9×10^2 b
25	Microorganisms	$1.2x\ 10^6$a	1.5×10^3b	1.9×10^3 b
	Aerobic bacteria	4.8×10^3 a	3.9×10^2 b	3.2×10^2 b

Note: unit: CFU/g

- *Means followed by the same letter(s) within a row are not significant different as determined by Duncan's multiple-range test P< 0.05*

Table 5.5 shows the effect of PLA and 8% mixed wax on shelf life of orange cv. Canh fruit at 22 ± 2°C, 80 ± 5%RH.orange cv. Canh dipping 2.5% PLA and coating with 8% mixed wax could be kept for 25 days compared to 10 days of non-coated fruit, same as CBZ 500ppm combination with 8% MW. Although storage life of fruit coated with 8% MW wax could extend to 25 days but the percentage of fruit decay were low (4.54%), sensory score were 5.3. So waxing usually coupled with anti-fungus storage to extend shelf life. This result agree with Henik *et al*., (2013) reported that the combination of *Eugenia caryophyllata* crude extract and *Candida utilis* TISTR 5001 could extended

shelf life of 'SaiNumPung' tangerine fruit for 3 weeks at 25°C. A fungicide, bio-pesticide could also be added to the wax to provide added protection against decay and increase postharvest life (Postharvest Handling Technical Bulletin. 2004a).

Table 5.5 The effect of PLA combined with MW on shelf life of orange cv. Canh at 22 ± 2°C, 80 ± 5%RH

	Shelf-life (day)	Cause of shelf life limitation
Control	10	Percentage of fruit decay: 3.03%, sensory score: 5.5 (≤ 5.0)
PLA 2.5%+MW	25	Percentage of fruit decay : 4.54%, sensory score : 5.3 (≤ 5.0)
CBZ 500ppm +MW	25	Percentage of fruit decay : 6.73%, sensory score : 5.0 (≤ 5.0)

5.4 Conclusion

Aspergillus sp. and *Penicillium* sp. on orange cv. Canh were isolated and identified. Soaking fruit in 2.5%PLA and coating in 8% MW totally inhibited both fungal strains, and controlled the development of *Penicilium* sp. spoilageon orange cv. Canh. In addition, this treatment maintained the postharvest quality of fruit expressed as titrable acid, TSS, and sensory values; and reduced total microoganisms, weight loss, and fruit decay after storage for 25 d at ambient temperatures, compare with CBZ 500ppm and control.

Figure 5.8 Effect of PLA combined with MW 8% on storage time 25 days of orange cv. Canhstored at ambient temperature (22 ± 2°C), 80 ± 5%RH (a, b)