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EFFECTS OF PHENYLLACTIC ACID COMBINATION WITH WAX COATING ON POSTHARVEST QUALITY AND STORAGE LIFE OF VIETNAMESE SWEET ORANGE CV. CANH

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ABSTRACT

Effects 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; total microorganisms; total soluble solids content (TSS) and titrable acidity 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 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 acidity increased by PLA during the storage period. The shelf-life of the control fruit was only 10 d compared with 25 d for the PLA-treated fruit when fruit were stored at ambient temperature.

Keywords*:* phenyllactic acid, orange, total microorganisms, mixed bees and carnauba wax

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. Fresh fruit and vegetables represent an opportune niche for many undesirable fungi due to high water availability, and long term storage during transportation with *Fusarium, Penicillium, Alternaria, Botrytis* species and, others identi-fied as major fungal spoilers (Crowley *et al*., 2013).

Phenyllactic acid (PLA) is produced fermentatively by *Lactobacillus* spp. 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). 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.

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, Penicillium roqueforti, 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 8-wk storage at ambient conditions (Thuy, 2013).

The purpose of this study was to investtigate 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.

MATERIALS AND METHODS

Materials: Fruit of 'Canh' orange from a commercial orchard in the Thanh Oai district, Hanoi were harvested at 220-235 d after fruit set and were laid in a sponge-filled

box (20 kg fruit/box) and transported to the laboratory within 2-3 h of harvest.

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 'Canh' orange at the Vietnam Institute of Agricultural Engineering and Postharvest Technology.

Bees and carnauba wax in the ratio of 7% to 3% were mixed to use concentrations of mixed wax (MW) of 8% (Thinh, 2013). All measurements of each treatment were the average of three replications

Studying methods: *Isolation and identification microorganisms in 'Canh' orange and* Micro-organisms were classified according to Raper *et al.,* (1965), and John (1979);

***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 1.5, 2.0, 2.5 and 3.0% PLA in combination with 8% MW was spread onto the plate's surface. After that 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 days (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 and combined with 8% MW. A volume 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, each mixture was spread onto the surface of the petri plates 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 spores in the control (without PLA); T is number of germinated spores in plates treated with PLA and MW.

Efficacy test of phenyllatic acid combined with mixed wax to control Penicillium* sp. *in 'Canh' orange:

The inhibition effectiveness of PLA against *Penicillium* sp. on orange fruit was determined by the method of Lam *et al*. (2011). The 'Canh' oranges were harvested and 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) (0.2%) 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

Effect of phenyllatic acid combined with mixed wax coating on quality and storage time of 'Canh' orange fruit: Fruit were divided into 2 groups: Control group (un-treated), and treated group (fruit were soaked in PLA at concentration of 2.5% and in 0.2% CBZ for 5 min, then soaked fruit were coated in 8% MW for 1 min and storage at ambient temperatures (22 ± 2°C), RH (Relative Humidity) 80 ± 5%. Data collection:

Percentage of weight loss was calculated by weighing the whole fruits before (100%) and after storage. The percentage of decayed fruit was assessed as follows:

(Num. of decayed fruit/total fruit) x 100

The total microorganism populations on the surface of oranges, including yeasts, and molds, were analyzed according to the method of Whangchai *et al*., (2006) units (CFU/g).

The total aerobic bacteria count was determined according to the Vietnam Standards (TC VN 5165:1990) units (CFU/g).

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

The titrable acidity (TA) was determined as citric acid by titrating against 0.1 NaOH by following the method of the AOAC (2000).

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

RESULTS AND DISCUSSION

Isolation and identification: Isolation and identification of post-harvest rot of orange caused by *Aspergillus* sp. and *Penicillium* sp.

Antifungal acitivity of phenyllactic acid and MW 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 Fig 1b. 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. 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 *L. plantarum* strain against fungal spoilers such as *Aspergillus fumigatus* and *Penicillium camemberti* was small (6.5 - 12 mg/ml).

Fig. 1a 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 Fig. 1a, 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. Our results are consistent with the findings of Lavermicocca *et al.,* (2001) who found that spore germination of *Aspergillus niger* FTDC3227, *A. flavus* FTD3226 and *P. corylophilum* IBT6978 were inhibited by 98.6, 86.5 and 100%, respectively by 50 mg/ml of PLA 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).

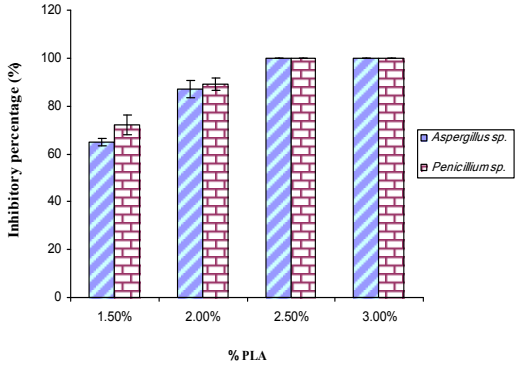

Fig-1a: Inhibitory activity of PLA combined with 8% mixed wax against *Aspergillus* sp. and *Penicillium* sp. germination after 48h

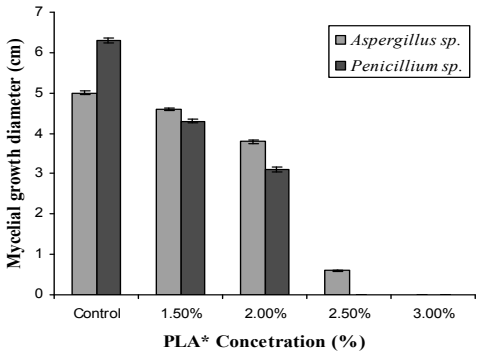

Fig.-1b: Antifungal activity of phenyllatic acid and MW and MW against mycelial growth *Aspergillus* sp. and *Penicillium* sp. after 7d; *PLA was applied in combination with 8% mixed wax

Efficacy test of PLA and mixed wax to control and *Penicillium* sp. in orange Canh: Fungal growth in inoculated wounds in the fruit surface is shown in Tab.-1. The percentage of infected wounds per fruit was monitored daily. As shown in Tab.-1, 2.5 and 3.0%, PLA completely inhibited *Penicillium* sp. The in-hibitory 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 'Canh' orange.

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 'Van Giang' orange, and was equivalent in effectiveness with the conventional fungicides imazalil and

thiabendazole.Wang *et al.,* (2013) 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).

Tab-1: Effect of PLA applied with mixed wax on *Penicillium* sp. mold incidence in orange'Canh'

Time (day)	Disease incidence (%)					
	Control	1.5% PLA	2.0% PLA	2.5% PLA	3.0% PLA	0.2%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.*

Effects of PLA in combination with MW coating on quality and storage time of orange Canh

Change in titrable acids and TSS: Change in titrable acids (TA) are shown in Fig-2 at ambient temperatures. The TA increa-sed slightly in all treatments and the control, which is not signify-cantly difference after 10 d in storage ($P \leq 0.05$). Titrable acids 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 TA content (0.167%), and TA content of the fruit treated with CBZ (0.2%) in combi-nation 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 acidity incre-ased faster in control than in wax coated orange.

Changes in total soluble solids (TSS) are shown in Fig.-3. There was no significant diff-errence 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

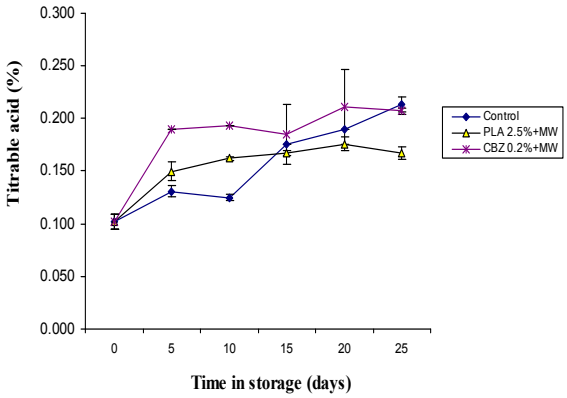

Fig.-2: Effect of treatment of 'Canh' orange fruit by PLA combined with MW on titrable acids

from 11.83 to 11.17 ^{o}brix. The TSS for fruit treated with CBZ in combination with MW was 10.5^{o}brix. Our results are consistent with the reported data on TSS content of oranges fruit (Feng *et al*., 2014), the TSS content of 'Pokan' mandarin fruit decreased after 7d citral (95%) treament in combination with a commercial wax coating (SP-1).

Change in fruit decay, weight loss: The percentage of decay in the treated and control fruit during the storage period is shown in Fig. -4 at ambient temperatures. The control and 2.5% PLA in combination with MW treated fruit had 20.71 and 0% fruit decay respectively after 25 days in storage.

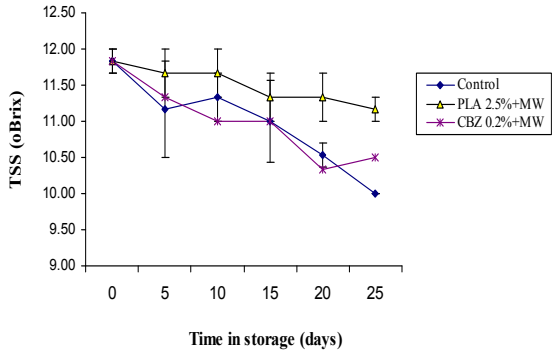

Fig.-3: Effect of treatment 'Canh' orange fruit by PLA combined with MW on TSS

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 (0.2%) in combination with MW, and each treatment was signifycantly different from the control fruit ($P \leq 0.05$) after 25d storage. 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. Lan, *et al*., (2012) who reported that the antifungal strain *Weissella cibaria* 861006 inhibited the growth of *P. oxalicum* on the surface of grapes for up to 6 days.

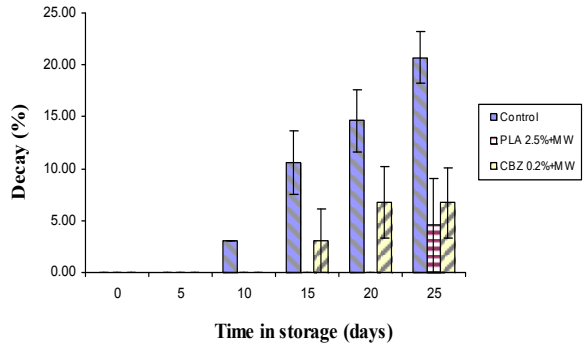

Fig.-4: Effect of treatment 'Canh' orange fruit by PLA combined with MW on decay percentage

Changes in weight loss percentage of treated and control fruit during the storage period are shown in Fig. -5. After 25 d weight loss was 12.65 % for fruit treated with 2.5% PLA in combination with MW. For control fruit, after 25 d in storage, the percentage of weight loss was 39.19%. There were no signifycant differences in weight loss among the treated fruit ($P \leq 0.05$), which were all signifycantly 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 'Sai Num Pung' tangerine fruit and had no effect to fruit quality and reduced weight loss.

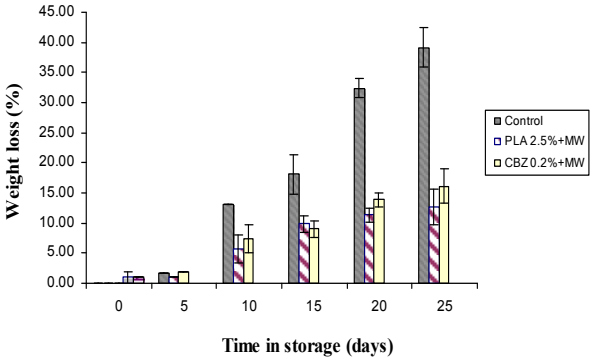

Fig.-5: Effect of treatment 'Canh' orange fruit by PLA combined with MW on weight loss

Change in total microorganisms: Changes in total microorganisms on the fruit during the storage period are shown in Tab.-2 at ambient temperatures. There was a marked difference in total microorganisms between treated and control samples after 25d storage. Overall, total microorganisms of treated 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, and it was much lower than that of control fruit (1.2 x 10^6 CFU/g) (Tab.-2). Therefore, it was confirmed that PLA has a strong inhibitory effect against microorganisms not only *in vitro* (Fig.-1a, Fig.-1b) but also in a practical trial. This result indicates that soaking in 2.5% PLA in association with MW coating can delay the increase of total microorganisms on orange fruit '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 signifycantly better disease control then either TBZ or the yeast alone (Arras *et al*., 1997).

Tab.- 2: *Effect of PLA combined with MW on total microorganisms (TM) and total aerobic bacteria (TAB) in orange 'Canh'*

Treatments	*Day of storage* (0)		*Day of storage* (25 day)	
	TM	*TAB*	*TM*	*TAB*
Control	$2.3x\ 10^5$ a	5.1×10^2 a	$1.2x\ 10^6$a	4.8×10^3 a
PLA 2.5% +MW	5.8×10^2 b	1.5×10^1 b	1.5×10^3b	3.9×10^2 b
CBZ 0.2% +MW	$5.4\ 10^2$ b	1.9×10^1 b	1.9×10^3 b	3.2×10^2 b

Note: unit: CFU/g; - 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.

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 *Muscodor albus*, a biofumigant 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).

CONCLUSION

Aspergillus sp. and *Penicillium* sp. were isolated and identified from orange cv. Canh. Soaking fruit in 2.5% PLA and coating in 8% MW totally inhibited both fungal strains, and controlled the development of *Penicilium* sp. and *Aspergillus* sp. spoilage on 'Canh' orange. In addition, this treatment maintained the postharvest quality of fruit expressed as titrable acid, TSS, and reduced total microoganisms, weight loss, and fruit decay after storage for 25 d at ambient temperatures, compare with CBZ 0.2% and control.

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