Pak. J. Biotechnol. Vol. 13 (4) 253 – 258 (2016)
www.pjbt.org

ISSN Print: 1812 – 1837
ISSN Online: 2312 - 7799

COMPARISON OF EFFECTIVENESS BETWEEN BEES-CARNAUBA MIXED WAX AND CHITOSAN WHEN COMBINATION WITH PHENYLLACTIC ACID ON POSTHARVEST QUALITIES OF ORANGE FRUIT CV. CANH DURING LOW TEMPERATURE STORAGE

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Article received 8.8.2016; Revised 8.12.2016; Accepted 14.12.2016

ABSTRACT

Comparison of effectiveness between bees-carnauba mixed wax (MW) and chitosan when combination with phenyl lactic acid (PLA) on postharvest qualities of orange fruit cv. Canh during low temperature storage was studied, by soaking fruit in 2.5% PLA before coating with 8% MW and 2% chitosan solution, and then storing at 5 ± 1°C for 60 days. While, untreated fruits were used as control. Percentage of weight loss and fruit decay, total sugar content, titratable acidity content, total soluble solids (TSS) content, ascorbic acid content, and total microorganisms were monitored during the preservative time. The results showed that 2.5% PLA in combination with 8% MW had a higher preservative effectiveness when compared to 2.5% PLA in combination with 2% chitosan and control fruits, which was expressed as lower percentage of weight loss and fruit decay, and lower total microorganisms for 60 days in storage. Moreover, fruits maintained nutrient ingredients expressed as total sugars, titratable acidity, TSS and ascorbic acid content.

Keyword: Orange fruit cv. Canh, bees-carnauba mixed wax, chitosan, phenyl lactic acid.

INTRODUCTION

Orange fruit (*Citrus sinensis* Osbeck) is one of the most popular fruit in Vietnam. The post-harvest life of fruit under ambient temperature is short due to fruit is decayed, lost weight and shed stalk. At present, there were not any stable technologies to prolong the shelf-life of orange fruit cv. Canh in Vietnam. For other cultivars are available in Vietnam such as 'Sanh' and 'Chanh' orange fruit, recent published works showed that the shelf life of fresh orange could extend from 30 to 40 days by soaking fruit in 0.07% carbendazim solution and then coating with 2% chitosan (Hoan *et al*., 2002). Carbendazim, thiabendazol, imazalil are inorganic fungicides, they are damaging the health of people and to the environment. Replacing these agents are needed to treat orange fruit before storing, one of methods is that the use of bio fungicide in combination with MW coating to extend the shelf life and maintain qualities of fruit.

Waxes reduce weight loss, prolong storage time, decrease rate of respiration and inhibit post-harvest disease of fruit, maintain attractiveness, increase freshness, reduce ethylene production (Hagenmaier and Shaw, 1992; Thirupathi *et al*., 2006; Hung, 2008; Shahid and Abbasi, 2011; Ladaniya, 2008). Shahid and Abbasi (2011) concluded that coating 'Blood Red' orange fruit at concentration of 5% bees wax along with 0.5% benlate performed better results in improving the overall quality and extending the shelf life of fruit. The Delta Valencia orange fruit were coated with carnauba based wax could store for 28 days at 5°C (Pereira *et al.*, 2013). Lavermicocca *et al.*, (2003) showed that the ability of phenyl lactic acid to act as a fungicide provides new perspectives for the possibility of using this natural antimicrobial compound to control fungal contaminants (*Aspergillus ochraceus, Penicillium roqueforti, P. citrinu*, etc.) and extend the self-life of food and/or feedstuffs. Vangiang orange fruit treated with 2% PLA followed by coating with wax (CP-01) maintained quality and appearance, while reducing the spoilage rate during 8 weeks in storage at ambient condition (Thuy *et al.*, 2013).

The purpose of this study was to compare effectiveness between MW and chitosan when combination with PLA on postharvest qualities of orange fruit cv. Canh during low temperature storage.

MATERIALS AND METHOD

Materials

Orange fruits cv. Canh of a commercial orchard in Thanh Oai district, Hanoi city were harvested at 220-235 days after fruit set and transported to a laboratory within 2-3 h. Fruits were then selected for uniformity of shape, size, and non-defected fruits, prior to use in this experiment.

PLA was produced by fermentative method, by using *Lactobacillus plantarum* C2 according to the method of Thuy *et al.*, (2012).

The optimal concentrations of mixed between bees wax and carnauba wax (8% MW) was selected after preliminary tests and made per Thinh (2013). The bees wax and carnauba wax in the ratio of 7 to 3 were melted and mixed well at 80-85°C for 30 min. Oleic acid (4.8%) and palmitic acid (0.5%) were added to the mixture before the mixture was blended (oleic acid and palmitic acid are solvent substances to dissolve waxes). Water was added to the mixture during stirring and blending for adjustment concentrations of 8% weight/volume.

Chitosan was produced by Vietnam Institute of Natural Products Chemistry was used in this study. Degree of deacetylation of chitosan ranges 85%. The molecular weight of produced chitosan is about 10,000 Daltons. The optimal concentrations of chitosan (2%) was selected after preliminary tests. Chitosan was prepared by dissolving 2% chitosan in dilute 1 liter solution acetic acid 1.5% (Binh and Dien, 1995).

The 360 fruits were soaked in 2.5% PLA for 5 min. After drying in the shade, the 180 soaked fruits were coated in 8% MW (MW+PLA) for 1 min, and the 180 soaked fruits were coated in 2% chitosan (CW+PLA) for 1 min at room temperature. All treated fruits were dried in the shade again, after that the treated and untreated fruits (control) were laid onto trays and stored at 5±1°C and RH 80±5% for 60 days and sampled /analyzed at 10 day intervals. A completely randomized design was used for the experiment. All measurements of each treatment were the average of three replications.

METHODS

The titratable acidity (TA) was determined as citric acid by titrating against 0.1NaOH (AOAC, 2000).

Vitamin C (ascorbic acid) was estimated using the detective dye 2.6 dichlorophenol-indophenol by standardizing 0.1% standard 2.6 dichlorophenol indophenol dye solution against 0.1% ascorbic acid solution (AOAC, 2000).

Total sugars were analyzed by using the procedure of Lane and Eynonas described in AOAC (2000).

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

The percentage of decayed fruit was assessed as follows: (Num. of decayed fruit/total fruit) x100

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

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

The total microorganism populations on fruit surface were determined as reported by Whangchai *et al*., (2006).

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

RESULTS AND DISCUSSION

Change in titratable acidity, total sugars, vitamin C, total soluble solids.

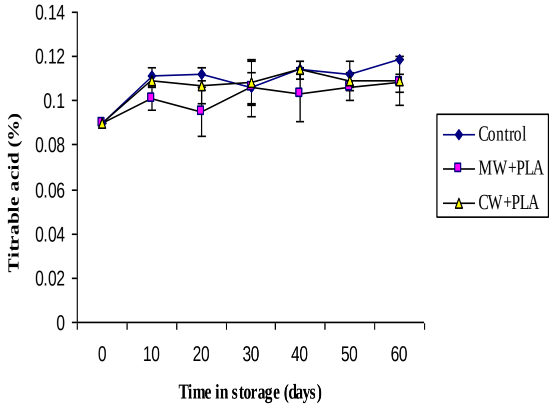

Fig.1 Changes in titratable acidity of treated and control orange cv. Canh fruit stored at low temperature (5 ± 1°C), 80 ± 5%RH

Changes in titratable acidity (TA) of treated and control fruit during the storage period are presented in Fig.1. Maximum titratable acidity (0.119%) was observed in control. Least value (0.108%) was recorded in 8% MW combination with 2.5% PLA. The TA increased slightly in all treatments and the control. Titratable acidity of the fruit treated with 8% MW + 2.5% PLA were 0.108% after 60 d in storage.

These results are in accordance with the finding of Thirupathi *et al*., (2006), who said that no significant change in constituents like vitamin C, acidity and sugar contents (In citrus fruit especially) were recorded due to treatment waxing. TA of Murcott tangor when fruit were treated with 0.2% chitosan alone increased faster and higher than control when stored for 9 weeks at 15ºC (Po-Jung *et al.*, 2007). There did not observe effects of either the commercial wax or edible coating on acidity levels in citrus fruit after 4 weeks of cold storage followed by 5 days at 20ºC (Hadar *et al.*, 2014).

Changes in total sugars are indicated in the Fig. 2. Total sugars content of control fruit decreased faster than other treatments at low temperature and long storage period. Minimum total sugars were recorded in control, maximum total sugars (5.53%) was recorded in 2.5% PLA association with 8% MW after 60 d in storage. There were no significant differences in total sugars among the treated fruit ($P \leq 0.05$), and which were significantly different from the control fruit ($P \leq 0.05$). After 60 days storage, the total sugars content of treated fruit tended to decrease slightly (Fig. 2). Therefore, the changes in total sugars content tended to decrease with the long storage period at low temperature. Total sugars of orange cv. Canh fruit in our study was similar to the reported data of David *et al.*, (2008) who found that 'Navel' orange fruit coated with shellac and imazalil and stored at 5°C for 6 weeks have a sweet content it reduced slightly from 111.2 to 108.2mg/100ml. Coating 'Mucott' mandarin with carnauba and imazalil reduced lightly sweetness for 6 weeks at cold storage and 1 week 20°C (David *et al.*, 2011).

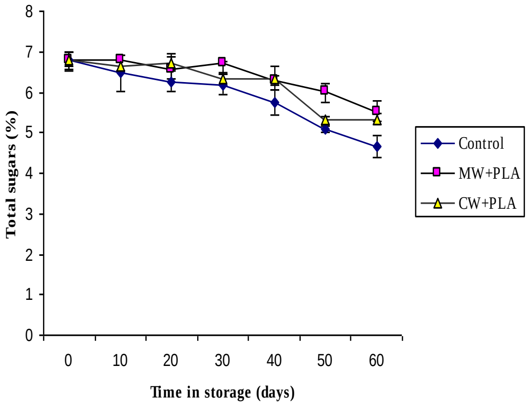

Fig.2 Changes in total sugars of treated and control orange cv. Canh fruit stored at low temperature (5 ± 1°C), 80 ± 5%RH

Fig. 3 shows the changes in vitamin C content of treated and control fruit during the preservative period. The vitamin C content of all treatments and control groups notably decreased after 20 d of storage. Ascorbic acid is the major antioxidant compound found in citrus fruit. Dipping orange fruit in 2.5% PLA association with 8% MW coating showed the highest vitamin C content (23.52 mg/100g) and the highest preservative time at 5°C. There were significant differences in ascorbic acid among the treatments and the control fruit ($P \leq 0.05$). This result explains that MW + PLA treatment was more effective in preventing the reduction of vitamin C content, compared with the CW + PLA treatment and the control.

The results of our study contrast with the results of Po-Jung *et al*., (2007) who reported that 0.1% low molecular weight chitosan coating maintained ascorbic acid in 'Murcott' tangor fruit higher than control after 56 days of storage at 15°C. The 'Kinnows' coated with polyethylene based wax and shellac based wax which showed maximum ascorbic acid content during 105 days storage at 5°C (Babar, 2007). Vitamin C had significantly higher ascorbic acid as compared to fruit stored under ambient conditions (Thakur, 2002).

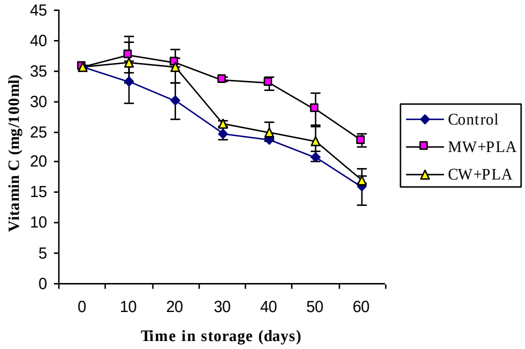

Fig. 3 Changes in vitamin C of treated and control orange cv. Canh fruit stored at low temperature (5 ± 1°C), 80 ± 5%RH

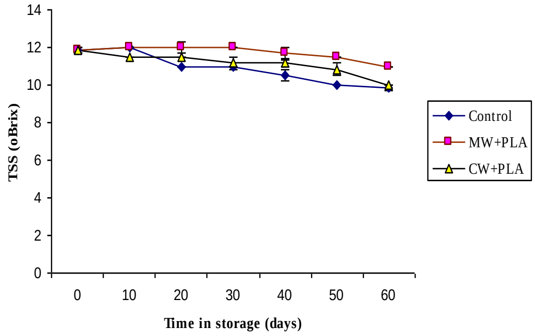

Fig. 4 Changes in TSS of treated and control orange cv. Canh fruit stored at low temperature (5 ± 1°C), 80 ± 5%RH

Changes in total soluble solids (TSS) content are illustrated in Fig. 4. The TSS content of control reduced lightly throughout the preservative time. While, maximum TSS content (11.00° brix) was recorded in fruit coated in 8% MW and dipping 2.5% PLA, minimum TSS content (9.83°brix) was measured in control fruit. In this research, TSS contents of fruit in all treatments and the control decreased after 60 d in storage perhaps due to respiration and metabolism sugars into alcohol.

Our results are consistent with the reported data on TSS content of oranges fruit of David *et al*., (2013) and Shahid and Abbasi (2011) who concluded that the possible reason in reduction of TSS content in 'Blood Red' orange in 5% bee wax + 0.5% benlate was due to these retarding the hydrolysis of starch into sugars and the conversion of polysaccharides in to disaccharides and monosaccharide by changing the biochemical activities. The TSS content of W. Murcott Afourer mandarins that were stored for 4 weeks at 5 ◦C and then moved to 20 ◦C for 2 weeks maintained better than stored fruit at 20◦C for 2 weeks (David *et al*., 2013).

Change in decay, weight loss and total micro-organisms:

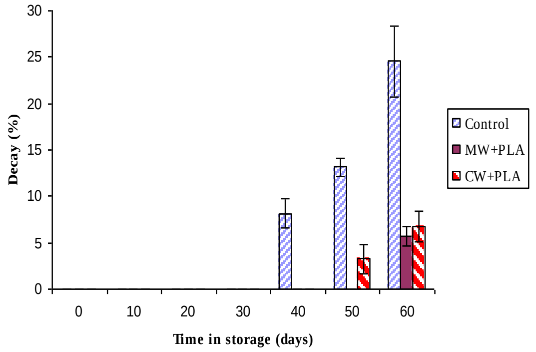

Fig. 5 Changes in decay of treated and control orange cv. Canh fruit stored at low temperature (5 ± 1°C), 80 ± 5%RH

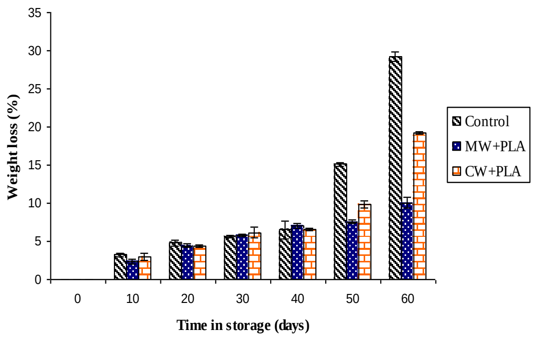

Fig. 6 Changes in weight loss of treated and control orange cv. Canh fruit stored at low temperature (5 ± 1°C), 80 ± 5%RH

The percentage of fruit decay of treated and control fruit during the storage time were determined and results are presented in Fig. 5. The rate of fruit decay increased rapidly when fruit was stored for long time. The highest fruit decay rate incidence was found in control, then decay rate was 6.75% in the CW combination with PLA treatment and lowest decay rate was found 5.69% in 8% MW + 2.5% PLA treatment after 60 days in storage. There was not significant variation in decay of orange cv. Canh fruit between treatments ($P \leq 0.05$) but were all significantly different from the control fruit ($P \leq 0.05$).

Postharvest decay of oranges was also reduced by the use of appropriate pre-postharvest and post-harvest fungicides, proper sanitation of the wash water and appropriate storage temperature and RH condition (Postharvest Handling Technical Bulletin, 2003). A 2% concentration of PLA completely inhibited the growth of the green mold *Penicillium digitatum* in 'Vangiang' orange (Thuy *et al.*, 2013). The application of L. enzymogenes 3.1 T8 in association with chitosan reduced the number of diseased plants by 50-100% relative to the *Pythium* control of stored cucumber (Jianglian and Shaoying, 2013). Henik *et al.*, (2013) illustrated that a combination *Eugenia caryophylata* crude extract and *Candida utilis* TITR 5001 reduced natural development of green mold rot of 'Sai nam Pung' tangerine.

Fig. 6 indicates the percentage of weight loss in treated and control orange cv. Canh fruit during the storage period at 5ºC. The control had the highest weight loss (29.15%) after 60 days in storage, and the lowest weight loss was found in 8% MW+2.5% PLA (10.06%). Generally, percentage of weight loss of MW association with PLA coated fruit and the control fruit increased with increasing of storage times and temperature storage. There was significant difference in percentage of weight loss of treatments and the control fruit during the preservative time ($P \leq 0.05$). This result demonstrates that 8% MW coating in combination with 2.5% PLA soaking had the best effectiveness on reducing the weight loss, compared with the CW+PLA treatment and the control at low temperature. Fruits and vegetable are waxed for propose to inhibit weight loss (Thirupathi *et al*., 2006).

Our results are in similarity with the reported data on weight loss of orange fruit (Babar, 2007, Po-jung *et al.*, 2007, Hadar *et al*., 2014). Weight loss of commercial polyethylene wax and CMC/ chitosan bilayer edible coatings of 'Or' mandarin slightly reduced 4 weeks of cold storage followed by 5 days under shelf-life conditions at 20◦C (Hadar, 2014). Low molecular weight chitosan (0.2%) coating improved the water content for 'Murcott' tangor stored at 15ºC for 56 days (Po-Jung *et al*., 2007). The weight loss of Valencia orange treated with imazalil and carnauba significantly were reduced at pre-storage and post-storage of 26 days at low temperature and 7 days of shelf life at 20ºC (Ncumisa, 2012).

Tab. 1 Changes in total microorganisms of treated and control orange cv. Canh fruit stored at low temperature (5 ± 1°C), 80 ± 5%RH

Treatments	Day of storage (0 day)	Day of storage (60 days)
Control	3.4×10^5 a	8.8×10^6a
MW+PLA	4.2×10^2 b	3.7×10^3b
CW+PLA	4.4×10^2 b	3.9×10^3 b

Note: unit: CFU/g;

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

The total microorganisms of treated and control fruit during the preservation time were determined and results are presented in Tab.1 at cold temperatures. Total microorganisms of treated and control fruit increased with increasing preservation time. The total microorganism count of treated with PLA in combination with MW was 3.7 x 10^3CFU/g, and control was (8.8 x 10^6 CFU/g) (Tab. 1) at 60d in low storage. This result show that soaking in 2.5% PLA in combination with mix wax, chitosan coating could delay the increase of total microorganisms on orange cv. Canh compared with the control after 60 days storage at low temperature.

Lam *et al.,* (2011) reported that dipping in thiabendazol (150ppm for 150') for HamYen orange fruit inhibited the growth of blue, green mold *Penicilium digitatum* and *P. italicum*. Valencia orange treated with Imazalil and carnauba significantly reduced the development of fungus at pre-storage and post-storage (Ncumisa *et al.*, 2012). Addition of postharvest fungicides to the wax, emulsion to control *Penicillium* rot in stored citrus fruit also proved helpful (Thirupathi *et al*., 2006). Decay and total microorganisms raised slowly at PLA concentrations in association with MW at cold temperatures.

CONCLUSION

The 8% MW in combination with 2.5% PLA treatment had the highest effectiveness to prolong shelf-life and maintain postharvest qualities in orange fruit cv. Canh for 60 days at 5±1°C. This treatment can be feasible for orange fruits storage on a commercial scale.

REFERENCE

AOAC, Official Method of Analysis, 17th. AOAC International. Gaithersburg, Maryland, USA. Chapter 37: 1-7 (2000).

Babar, E. B., Postharvest Management of Kinnow (*Citrus reticulata* Blanco) Mandarin to extend shelf life, Ph.D. T*hesis*, Faisalabad Pp80-82 (2007).

Binh, T. Q. and L. D.Dien, Research and application of chitosan for preservation Orange in Vietnam. *In KC08-07* program. (in Vietnamese) Pp.149-16 (1995)

David O., C. Sue, M. Bruce, S. James and, L.A. Mary, Commercial packing and storage of Navel orange alters aroma volatile and reduce flavor quality. *Post Harvest Biology and Technology* **47:** 159-167 (2008).

David,O., C.Sue, M.Bruce, S.James and L.A.Mary Storage temperature and time influences sensory quality of mandarins by altering soluble solids, acidity and aroma volatile composition. Postharvest Biology and Technology **59:** 187–193 (2011).

David O., C.Sue, S.James and L.A.Mary, Mandarin flavor and aroma volatile composition are strongly influenced by holding temperature. Postharvest Biology and Technology **82:** 6–14 (2013).

Hadar, A., Z. Yana, P. Ron and P. Elena, Effect of carbon methyl cellulose and chitosan blilayer edible coating on postharvest quality of citrus fruit. Postharvest biology and technology **87:** 21-26 (2014).

Hagenmaier, R.D. and P.E. Shaw, Gas permeability of fruit coating waxes. *J. of Amer. Soc. Hort. Sci.* **117**: 105-109 (1992).

Henik,S., S.Somsiri and K.Netnapis, Control of pos -tharvest green mold of citrus fruit with yeasts, medicinal plant, and their combination. Postharvest Biology and Technol.**79:** 24-31 (2013).

Hoan N.C., L.H.Hai, H.K.Phuong and N.K.Dung, Study on improvement and transferring postharvest technology of orange, lychee fruits to farmers and producers. *Yearbook Research Results of Postharvest Technology Institute*, Pp. 201-213 (2002).

Hung, C. V., Study on technology and equipment for preliminary treatment and storage some fresh fruit, flowers, and vegetables. *The Final Report of Scientific Theme VIAEP* Pp.34 (2008).

Ladaniya M.S., Citrus Fruit biology, technology and evaluation, ICAR research Complex for Goa Ela, Old Goa 403402, Goa, India, Pp. 333-373 (2008).

Lam N.D., T.T.M. Nga and T.T. Mai, Hot water, sodium carbonate and thiabendazole for control of postharvest green and blue molds (*Penicillium digitatum* and *P. italicum*) of "Ham Yen" oranges. Proceedings of the National Conf. Agricultural Engineering and Food Preservation-Processing, Hanoi. *The Science and Tech. Publishing House*. Pp. 206-212 (2011)

Lavermicocca,P., F.Valerio and S.Viscoti, Antifungal activity of phenyllactic acid against molds isolated from bakery product. *Appl. Environ. Microbiol.* 69: 634-640

Ncumisa S.N., Imazalil and wax coating application in citrus packhouse to control green mold and preserve fruit quality. *Master Thesis*, Stellenbosch University Pp. 92 (2012)

Pereira, G.S., F.M.C.Machado and J.M.C.Costa, Quality of Delta Valencia orange grown in semiarid climate and stored under refrigeration after coating with wax. *Food Science Technology* Campinas 33(2): 276-281 (2013).

Po-Jung C., F.Sheu and L.Hung-Ren, Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quantity and shelf life. *Food Chem.*100:1160-1164 (2007).

Shahid, M. N. and N.A.Abbasi, Effect of bee wax coatings on physiological changes in fruits of sweet orange cv. 'Blood red'. *Sarhad J. of Agric.* **27**: 385-394 (2011).

Thakur, K.S., B.B.L. Kaushal and R.M. Sharma, Effect of different post harvest treatments and storage conditions on the fruit quality of Kinnow. *J. Food Sci. Technol.* **39**(6): 609-618 (2002).

Thinh, D.C., U. Jamnong and J. Adisak, The Suitable Coating Wax for Vietnamese Mango Fruit cv.Cat Hoa Loc. 13*th ASEAN Food Conference,* Singapore (2013).

Thirupathi, V., S.Sasikala and Z.L. Kennedy, Preservation of fruits and vegetables by wax coating. *Science Tech Entrepreneur*. Eds. Mittal, H.K. et al., NSTEDB, DST. Delhi Pp1-10 (2006).

Thuy B.K., N.D.Lam and N.TH.Tram, Production of phenyl lactic acid by *Lactobacillus plantarum* through fermentation process and anti-microbial effects in food. *13th ASEAN Food Conference*, Singapore (2013).

Thuy B. K., N. D. Lam, V. K. Thoa, Control of fungi and bacteria by phenyl lactic acid and Its treatment efficacy in preservation of some fresh produce and food, *Workshop on Agricultural Engineering and Postharvest technology for Asia sustainability*, Pp. 339-345 (2012).

Whangchai, K., K. Saengnil and J. Uthaibutra, Effect of ozone in combination with organic acids on the control of postharvest decay and pericarp browning of longan fruit. *Crop Protection* **25**: 821-825 (2006).