EFFECT OF FRUIT DIPPING IN SODIUM HYPOCHLORITE AND OXALIC ACID THEN COATING IN BEES-CARNAUBA MIXED WAX ON PEEL BROWNING AND DECAY OF VIETNAMESE LONGAN FRUIT

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ABSTACT

The aim of this research was to evaluate the effect of sodium hypochlorite (SH) and oxalic acid (OA) dipping in combination with bees-carnauba mixed wax (MW) coating on decay and peel browning of Vietnamese longan fruit cv. Long. The experiments were firstly carried out by dipping fruits in 200 ppm SH solution for 2 min, and then dipping in 7.5% OA solution for 5 min. After drying in the shade, dipped fruits were coated in 4 and 6% MW for 30 seconds, and stored at 5±1°C for 30 days. Untreated fruits were used as control. Peel browning, peel color, polyphenol oxidase (PPO) activity, total microorganisms, percentage of fruit decay, fruit weight loss, and total soluble solids (TSS) content were monitored during the storage period. It was found that dipped fruits coated in 6% MW had delayed peel browning and fruit decay for 25 days in storage, and the TSS content remained unchanged. Moreover, the fruits maintained low PPO activity, low total microorganism levels and low weight loss. This result suggests that application of 200 ppm SH, 7.5% OA and 6% MW could be feasible for longan fruits storage on a commercial scale.

Keywords:sodium hypochlorite, oxalic acid, bees-carnauba mixed wax, peel browning, fruit decay

INTRODUCTION

Longan fruit (Dimocarpus longan Lour.) is a type of valuable fruit in Vietnam for domestic and export markets because of its delicious taste and excellent nutritional properties. However, the postharvest life of fruit is very short (3 to 4 days) at ambient temperatures due to fruit is desiccated, browned and rotted (Tongdee, 2001; Jiang et al., 2002; Apai, 2010). In recent years, there have been many researches on storage of 'Long' longan fruits and reported data indicates that shelf life of fruit can be prolonged by treating with carbendazim (Hoan et al., 2001), SO₂ fumigating (Thuy and Duyen, 2011), and treating with sodium metabisulfite (Hai et al., 2011). SO₂ reduces the activity of PPO on fruit peel, hence reducing peel browning (Wu et al., 1999; Tongdee, 2001). Carbendazim and SO₂ can inhibit the growth of fungi and decay (Hoanet al., 2001; Tongdee, 2001). Nevertheless, the negative effects of the toxic residue of SO₂ and carbendazimin humans and other reactions with sensitive individuals were reported. Therefore, chitosan coating was studied as an alternative treatment to sulfur compounds and carbendazim application (Huven and Thuy, 2011). This research showed that the shelf-life of longan fruit cv. Long can extend for 20 days, however fruits decay very high (11.4%). Thus, developing efficient methods and safety are needed for replacing SO₂ and carbendazim, reducing fruit decay and prolonging shelf-life longer than 20 days for 'Long' longan fruit. An alternative method is the use of SH and OA dipping

in association with MW coating. SH often has used by researches for surface sanitizing and sterilizing fruits and vegetables (Hong and Gross, 1998). Food surfaces contact with sanitizing solutions do not exceed 200 ppm available chlorine (the US government regulations: 21 CFR Part 178). Chlorine has no residual affect (Sawyer, 1978). Chlorine have been primarily used to inactivate or destroy pathogenic bacteria, fungi, viruses, cysts and other propagules of microorganisms concerning seeds, cuttings, etc. (Suslow, 2000). Khunpon et al. (2011) concluded that dipping in sodium chlorite has the potential to reduce exocarp browning in longan fruits cv. Daw by reducing the activity of PPO. Waxes prevent water loss thus reducing weight loss and shriveling; reduce respiration rate; protect the development of fungi; and maintain visual appearance in fruits and vegetables (Hagenmaier and Shaw, 1992; Thirupathi et al., 2006; Torres et al., 2009; Hu et al., 2011; Shahid and Abbasi, 2011). Oxalic acid delays browning due to reducing the pH of product thus minimising PPO activity and are generally recognized as safe (Suttirak and Manurakchinakorn, 2010). Oxalic acid prevented pericarp browning due to inhibited PPO activity in longan fruit (Boonin et al., 2006; Whangchai et al., 2006). In a previously research Hai et al., (2014a) found that MW coating maintained qualities of longan fruits cv. Long for 20 days, but during extended storage time (25 days) the fruits began to decay ($\geq 9.4\%$). In another research, Hai et al., (2014b) concluded that fruits soaked in

7.5% OA in association with wax coating could be stored with good postharvest quality for 25 days, however the treated fruits had decay ($\geq 2.6\%$). In a recent research Hai *et al.*, (2014c) demonstrated that 200 ppm SH soaking in combination with wax coating prevented fruit decay (0%) after 25 days in storage, however treated fruits were browned and not acceptable for marketing purposes.

The aim of this research was to evaluate the effect of combination of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on decay and peel browning of fresh 'Long' longan fruit throughout the preservative period at 5° C.

MATERIALS AND METHODS

Plant material: In the 2014 harvesting crop of Hung Yen province, Vietnam, mature longan fruits cv. Long were harvested and transported to laboratory. Uniformity of shape, size and nondefected fruits were selected to do experiments.

Studying methods: Oxalic acid at concentration of 7.5% and sodium hypochlorite at concentration of 200 ppm were selected according to the findings of Hai *et al.* (2014b; 2014c). The feasible and optimal concentrations of mixed between bees wax and carnauba wax (4 and 6% MW) were selected and made according to Hai *et al.* (2014a).

The fruits were firstly dipped in 200 ppm SH solution for 2 min, and then dipped in 7.5% OA solution for 5 min. After drying for 1 h in shade, dipped fruits were coated in 4 and 6% MW for 30 seconds and dried for 8 h at room temperature while the control fruits were not dipped and coated. After that, the longan fruits were packed in polypropylene bags, 1 kg per bag (305 x 457 mm in size and 0.035 mm thick with 4 holes of 0.8 cm² per hole). Afterward, fruits were preserved at $5\pm1^{\circ}$ C in a cold room and sampled/analyzed at 5 day intervals. Each treatment had three replications. H₀ was undipped and uncoated fruits, and H₁ and H₂ dipped fruits were coated in 4 and 6% MW respectively.

Polyphenol oxidase (PPO) activity was analyzed. Firstly, PPO was extracted by homogenizing 10 g longan peel in 40 ml of 0.05 M potassium phosphate buffer (pH 6.2) containing 1 M KCl and 2% polyvinylpyrrolidone and after that centrifuging for 5 min at 13,500 rpm (Hermel-Z383K) at 4°C. The enzyme extract expressed as supernatant was collected (Huang *et al.*, 1990; Whangchai *et al.*, 2006). PPO activity was assayed followingthe methods of Jiang and Fu (1998) and Whangchai *et al.*, (2006) by using the reaction mixture of 0.05 M potassium phosphate buffer (pH 7.5) containing 0.2 M catechol (0.2 ml) and crude enzyme (0.5 ml). Tubes were incubated for 5 min at 30°C and absorbance was measured at 420 nm by visible spectrophotometer (Thermo Spectronic). The unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.01 in absorbance per minute.

The total microorganism populations on fruit surface were determined according to the method of Whangchai *et al.*, (2006). For each sampled of fruit, 300 g of fruit was extracted by immersing in 2,700 ml sterile distilled water and shaking 180 rpm for 30 min at room temperature. Afterward, 1 ml sample suspension was spread over a potato dextrose agar (PDA) medium. 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 ml⁻¹).

The total soluble solids (TSS) content was measured by a digital refractometer (PAL-1, Atago, Japan).Eighty fruits per treatment were used to determine TSS content. The flesh of each fruit was pressed by hand and approximately 0.3 ml juice of each fruit was placed onto the prism surface. The measurement was taken, and TSS content of each fruit (%) was displayed.

Peel browning expressed as browning index was estimated by observing the extension of total browned area on each fruit surface following scales: 1 = 0% (no browning); 2 = 1-25% (slight browning); 3 = 26-50% (moderate browning); 4 =51-75% (extreme browning), and 5 = 76-100%(extreme browning with poor quality) peel browning area. A browning index (BI) was calculated following formula: browning scale times the percentage of corresponding fruits in each scale. Fruit had BI ≥ 2.0 it was not acceptable (Jiang and Li, 2001; Hai *et al.*, 2011).

The peel color expressed as L* and b* value was measured by a digital colorimeter (Konica Minolta CR-300, Japan), and L* indicating lightness, ranged from black = 0 to white = 100; b* indicating chromaticity on a blue (-) to yellow (+) axis (MacGuire, 1992).

Loss of fruit's weight was weighed and calculated the whole fruits packed in polypropylene bags before and after preservation (taken as 100%).

Fruit decay was weighed and calculated according to formula below:

Fruit decay (%) = $\frac{\text{Number of decayed fruits}}{x \ 100}$

Total fruits

Data were statistically analyzed by using the statistical package for the social sciences (SPSS) software (version 20.0) and Duncan's Multiple Range Test ($P \le 0.05$) to analyze the significantly different of means between the treatments and control.

RESULTS AND DISCUSSION

Peel browning, peel color and PPO activity: Figure 1 illustrates peel browning expressed as browning index (BI) of treated and control fruits throughout the preservative time at 5°C. Fruit was not accepted for marketable if it has BI ≥ 2 . As shown in Figure 1, there was marked difference in BI between treated fruits and control throughout the preservative time (P \leq 0.05). By day 10 in preservation, control was not accepted for marketing because of BI > 2. In contrast, fruits in H₁ and H₂ treatments maintained BI lower than 2.0 for 20 and 25 days respectively (Figure 1). Untreated longan fruits peel browned after 5 days in storage at 2-7°C (Jaitrong, 2006; Apai, 2010). The BI of control in this study is consistent with the findings of Hai *et al.* (2011; 2014a; 2014b; 2014c). Overall, the BI of preservative period, and fruits in H₂ treatment had the longest preservative time and the best peel color for 25 days in preservation (Figure 1).

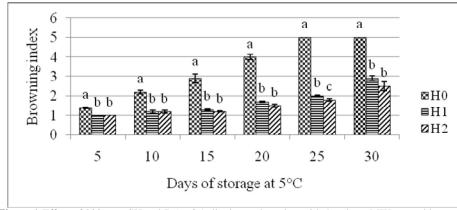


Figure 1:Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on peel browning expressed as browning index of longan fruit throughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

Longan peel browning augmented with preservative time (Whangchai et al., 2006; Khunpon et al., 2011). The results of this study are consistent with the finding on peel browning of treated longan fruits cv. Long of Hai et al. (2014b) but better than the findings of Hai et al. (2011; 2014a; 2014c). The result of this study demonstrates the effectiveness of preservative agents used in prevention of browned peel. Browning of longan fruit is the results of the oxidation of phenolic compounds by endogenous PPO (Jiang et al., 2002). The water loss in fruit leads to activate the activity of PPO (Su and Yang, 1996). The water loss in fruit could be prevented by wax coating (Thirupathi et al., 2006; Hu et al., 2011, Shahid and Abbasi, 2011). Oxalic acid delayed browning due to reducing the pH of product, thus minimising PPO activity (Suttirak and Manurakchinakorn, 2010). Oxalic acid prevented pericarp browning due to inhibited PPO activity in longan fruit (Boonin et al., 2006; Whangchai et al., 2006). Oxalic acid had effective controlling the peel browning of lychee fruit throughout postharvest preservation (Zheng and Tian, 2006).

Peel color, expressed as L* (lightness) and b* (yellowness) values, is an important factor to attract consumers. There were significant differences in L* and b* values between treated and control fruits (P \leq 0.05), with higher L* and b* values found in treated fruits throughout the preservative time. In contrast, control fruits had the lowest L* and b* values which reduced with augmenting of preservative time (Figure 2 and Figure 3). The results of this study are suitable for the reporting data on L* value and b* value of longan fruit (Jaitrong, 2006; Apai, 2010; Hai et al., 2011; Huyen and Thuy, 2011; Thuy and Duyen, 2011; Hai et al., 2014b). As seen in Figure 2 and Figure 3, the H_2 treatment maintained the highest L* and b* values throughout the preservative time. This result demonstrates the efficiency of SH and OA dipping in combination with MW coating in keeping the peel color of longan fruit by inhibiting the activity of PPO and preventing water loss from peel as described by Shi (1990); Son et al., (2000); Yoruk and Marshall (2003); Zheng and Tian (2006); Boonin *et al.*, (2006); Whangchai *et al.*, (2006); Thirupathi *et al.*, (2006); Shahid and Abbasi (2011); and Hai *et al.*, (2014a). This study shows that high L^* and b^* values correlate with low browning indexes (Figure 1, Figure 2, and Figure 3). Apai (2010) found that L^* value is negatively correlated with browning index.

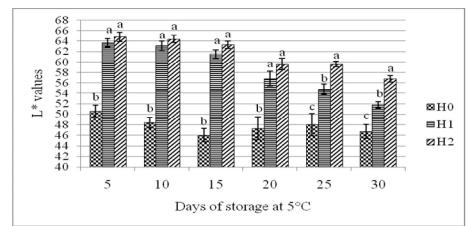


Figure -2:Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on peel color expressed as L* value (lightness) of longan fruit throughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

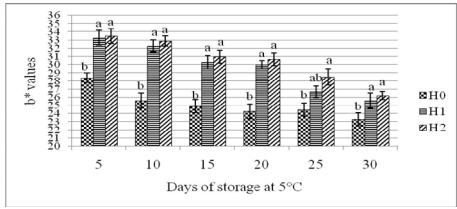


Figure -3:Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on peel color expressed as b* value (yellowness) of longan fruit throughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

The PPO activity in the peel of treated fruits and control throughout the preservative time were determined and results are shown in Figure 4. There was marked difference in PPO activity between treated fruits and control throughout the preservative time ($P \le 0.05$) and PPO activity ranged from 2.7 to 4.4 unit/mg protein for control fruits, 1.7 to 2.8 unit/mg protein for H₁ treatment and 1.6 to 2.1 unit/mg protein for H₂ treatment after 25 days in preservation. The results of this study are suitable for reporting data on PPO activity in longan peel (Whangchaiet al., 2006; Apaiet al., 2009; Hai et al., 2011). Overall, PPO activity of treated fruits and control augmented with the augmenting of preservative period, and treated fruits maintained lower PPO activity than control fruits throughout the preservative time. Tissue browning peel of longan fruit is dependent upon PPO activity (Kader, 2002). The water loss in fruit leads to activate the activity of PPO (Su and Yang, 1996). Wax coating could prevent moisture loss in fruits (Thirupathi et al., 2006). OA delayed browning as a result of reducing the pH of product, therefore minimising the activity of PPO (Suttirak and Manurakchinakorn, 2010). The optimal pH for maximal action of PPO in longan fruit is 6.5 (Jiang, 1999). The PPO action is little at pH < 4 as a result of copper loss at the active site (Suttirak and Manurakchinakorn, 2010). OA binds with copper to form an inactive complex and the action of PPO is inhibited (Yoruk and Marshall, 2003). OA prevented pericarp browning due to inhibited PPO activity in longan fruit (Boonin et al., 2006; Whangchai et al., 2006). The result in this study explains that treatments markedly prevented PPO activity in longan peel throughout the preservative

time when compared with control.

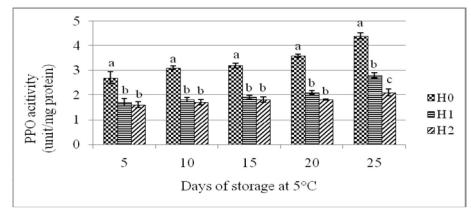


Figure-4: Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on PPO activity of longan fruitthroughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

Fruit decay and total microorganisms: There was significant difference in decay between treated fruits and control throughout the preservative time $(P \le 0.05)$. The control fruits began to decay (2.8%) after 10 days in preservation, and thereafter decay accelerated with increased preservative time (after 25 and 30 days it was 58.9 and 98.8% respectively). In contrast, treated fruits did not decay throughout the first 25 days and they began to decay (5.2% for H₁ treatment and 4.7% for H₂ treatment) after 30 days in storage (Figure 5). High percentage of fruit decay in control longan fruits was found by Hoan et al., (2001); Apai (2010); Hai et al., (2011); Thuy and Duyen (2011); Huyen and Thuy (2011); and Hai et al., (2014a; 2014b; 2014c). Results from this study demonstrate the effectiveness of preservative agents used in controlling decay in longan fruit throughout the preservative time. The results of this study are suitable for the findings of Hai et al. (2014c) and better than the study of Huyen and Thuy (2011) who found that the best treatment of 2% chitosan coating in longan fruit had 11.4 and 20.8% fruit decay after 20 and 30 days in storage respectively. This research shows that low fruit decay and browning index interrelate (Figure 1 and Figure 5). Postharvest longan fruit susceptibly decays due to fruit is infected with bacteria, fungi and yeasts (Jiang et al., 2002). SH was included in treatment due to its fungicidal property (Cerioniet al., 2009). SH is used to prevent microbial inoculation (Sawyer, 1978). Hot water dipping with SH (200 ppm) at 52°C for 3 to 4 min was recommended for fungal disinfection of mango fruit (APEDA, 2007). Fresh-cut cilantro treated with SH had no decay by day 4 in storage (Kim et al., 2007). Waxing creates a barrier to resist the pathogens of fungi and bacteria to penetrate into the product (Postharvest Handling Technical Bulletin, 2004). Mango fruits were coated in carnauba wax decreased fruit decay throughout preservative time (Baldwin et al., 1999).

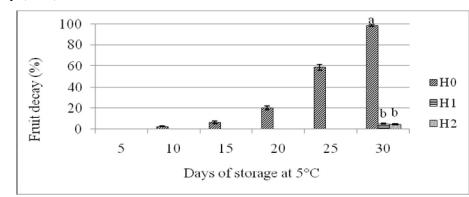


Figure 5: Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on fruit decay of longan fruit throughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

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There was marked difference in total microorganism populations on longan fruit surface between control and treated fruits ($P \leq 0.05$). Overall, total microorganisms tended to increase with the time spent in storage (control fruits increased from 2.0 to 14.1 x 106 CFU ml-1 and treated fruits increased from 0.2 to 2.2 x 106 CFU ml⁻¹ after 25 days in storage) (Figure 6). Microorganism populations on longan fruit surface markedly increased after 3 days in storage at 25°C (Whangchai et al., 2006). As shown in Figure 6, the control fruits had much higher total microorganism than treated fruits throughout the preservative time. This result justifies that the treatments in this study significantly reduced total microorganism populations on the surface of longan fruit cv. Long. This research also shows that low total microorganism populations, fruit decay and browning index interrelate (Figure 1, Figure 5, and Figure 6). About 106 species of microorganisms have been isolated from longan fruit, comprising 36 bacteria, 63 mold and 7 yeast species (Lu et al., 1992). Major post harvest pathogens of longan fruit are Enterobacter srtohrnrd sp. and Acinetobacte sp. (Lu et al., 1992) for bacteria; and Botryodiplodia sp. (Jiang, 1997), Penicillium sp., Rhizopus sp., Alternaria sp. (Lu et al., 1992), Lasiodiplodia sp., and Cladosposporim sp. (Sardsud et al., 1994) for

mold. Sawyer (1978) reported that SH is used to prevent microbial inoculation. Using 200 ppm of hypochlorite solutions reduced total bacterial, Pseudomonas spp., yeasts and moulds, etc. in cantaloupes (Ukuku, 2006). Total bacterial count and veast and mould count were found to be nil (below detectable level) in the SH treated litchi fruits during the storage period (Kumar et al., 2012). During refrigerated preservation, prewashing strawberries in SH (200 µg/mL) solution showed lower microbial loads (P<0.05) than untreated, ultrasonicated, UV-C irradiated or waterwashed samples (Alexandre et al., 2012). Sanitizing tomato fruit with SH before packaging significantly reduced microbial spoilage (Bhowmik and Pan, 1992). Chlorine wash reduced final microbial count by 2.7 log units on fresh-cut iceberg lettuce when compared to controls (Beltran et al., 2005). All treatments containing SH significantly reduced initial aerobic plate count, coliform/E. coli counts on fresh-cut cilantro compared to those washed with tap water alone (Kim et al., 2007). Fruits and vegetables are waxed primary to prevent the growth of mold (Thirupathi et al., 2006). Waxing creates a barrier to resist the pathogens of fungi and bacteria to penetrate into the product (Postharvest Handling Technical Bulletin, 2004).

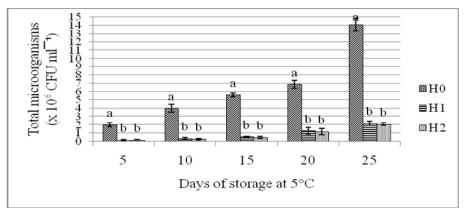


Figure 6: Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on total microorganisms in longan fruit surface throughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

Weight loss and TSS content: The weight of treated fruits and control was weighed throughout the preservative time and results are shown in Figure 7. There was marked difference in the loss of treated fruits weight and control fruits weight throughout the preservative time, and between H₁ and H₂ treatments by day 5 and after 20 days in preservation ($P \le 0.05$). The percentage of weight loss of treated fruits and control augmented with

augmenting of preservative period. As seen in Figure 7, the H_2 treatment maintained the lowest weight loss during the storage period. After 30 days in storage, the weight loss of the control, H_1 treatment, and H_2 treatment was 11.9, 8.6, and 6.3% respectively. This result demonstrates that H_2 treatment had the best efficiency on decreasing the loss of weight in longan fruit cv. Long throughout the preservative time. The result of this research is

suitable for the reporting data on weight loss of longan fruit (Jiang and Li, 2001; Apai*et al.*, 2009; Huyen and Thuy, 2011; Hai *et al.*, 2014a; 2014b; 2014c) and is lower than the researched result of Hoan *et al.*, (2001) who found that weight of treated longan fruits cv. Long were lost about 10% after 20 days in preservation. Vegetables and fruits are waxed for purpose is to inhibit the loss of weight (Thirupathi *et al.*, 2006). Waxing tomato fruits delay weight loss (Torres*et al.*, 2009). Mango fruits were coated in carnauba wax markedly decreased water loss compared to uncoated fruits (Baldwin *et*

al., 1999). Sta-Fresh 2952 wax (60g/l) had efficient in reducing the loss of weight in pineapple fruits (Hu *et al.*, 2011). Sweet orange fruit cv. Blood Red was coated in 5% bees wax presented the minimal loss in fruit's weight (Shahid and Abbasi, 2011). Orange, kinnow, lemon and grape fruit were coated in Fruitex, Britex-561 and SB 65 wax alleviated weight loss (Farooqi *et al.*, 1998). Waxing pear fruits cv. Xiang Sui and Pien Pu decreased the loss of fruit's weight at all preservative temperatures (Sornsrivichai *et al.*, 1990).

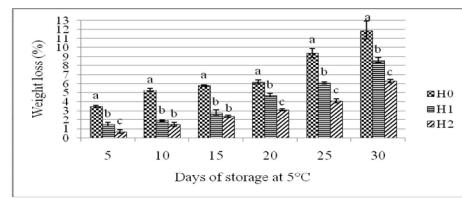


Figure 7: Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on weight loss of longan fruit throughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

There was not different in TSS content between treated and control fruits after 25 and 30 days in preservation ($P \le 0.05$), and it also was close to the TSS content of fruit at harvested time (19.9%). After 25 days in preservation, the TSS content of control fruit was 22.4% and treated fruits were from 21.6 to 22.3% (Figure 8). The result of this research is suitable for the reporting data on TSS content (Hoan *et al.*, 2001; Hai *et al.*, 2011; Huyen and Thuy, 2011; Thuy and Duyen, 2011; Hai *et al.*, 2014a; 2014b; 2014c). This result can assume that the preservative agents used in this study did not affect the TSS content of fruits throughout preservative time. The TSS content of treated and control fruits slightly augmented with the raising of preservative time may be as a result of dehydration.

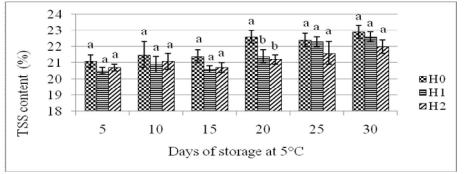


Figure 8: Effect of 200 ppm SH and 7.5% OA dipping and coating with 4 and 6% MW on TSS content of longan fruit throughout preservative time. Vertical bars represent standard errors. Columns with different letters of each storage period indicate significant differences ($P \le 0.05$).

CONCLUSIONS

Application of 200 ppm SH and 7.5% OA dipping in association with 6% MW coating could delay peel browning and fruit decay for 25 days at 5°C. This treatment can be feasible for longan fruits storage on a commercial scale.

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