Pak. J. Biotechnol. Vol 15 (4) 939-946 (2018) ISSN Print: 1812-1837
www.pjbt.org ISSN Online: 2312-7791

EFFECT OF BEES, CARNAUBA MIXED WAX COATING ON SHRINKAGE PATTERN, WEIGHT LOSS, RESPIRATION AND SENSORY OF SWEET ORANGE CV. CANH

Nguyen Thu Huyen[1,2], Tanachai Pankasemsuk[2]

[1]Post-Harvest Technology Research Institute, Chiang Mai University, Thailand. [2]Vietnam Institute of Agriculture Engineering and Postharvest, Hanoi, Vietnam. **E.mail:***nhatduchuyen@gmail.com

Article received 03.1.2018, Revised 11.12.2018, Accepted 17.12.2018

ABSTRACT

The impact of bees, carnauba wax - mixed wax (MW) on shrinkage pattern, respiration, sensory weight loss of Vietnamese sweet orange cv. Canh was studied, by coating fruit with 4, 6, 8 and 10% MW and stored at ambient temperature (22±2ºC) RH 80±5% for 20 days, while uncoated fruit were used as control. The shrinkage pattern on the top, middle and bottom were collected during the storage time. The results presented that 8%MW coating at ambient temperature reduced the wrinkle, respiration, maintained sensory values, and weight loss compare with the control fruit. Thickness of orange cv. Canh coated at 10%MW was highest. No significant change on the top, middle, bottom was recorded due to treatment waxing. The number of lenticels and stomata was observed by Scanning Electron Microscope (SEM).

Key words: bees wax, carnauba wax, mixed wax, shrinkage pattern, wrinkle, orange cv. Canh

1. INTRODUCTION

Orange fruit (*Citrus sinensis* Osbeck) is one of the popular in Vietnam. Fruit has a thin and smooth peel. The pulp is orange color, very sweet, high vitamin C and low acidity. In Vietnam there was not any research to store orange cv. Canh fruit and problem of fruit after harvest and storage were high decay and weight loss, and low quality, fast wrinkled. There is a need to research effectiveness methods to prolong the shelf-life of orange cv. Canh. One of methods is that the use of MW coating to extend the shelf life, prevent shrivelling and maintain qualities of fruit. Waxes prevented shrinkage, reduced weight loss, extending storage life, decreasing rate of transpiration, (Hagenmaier and Shaw, 1992; Postharvest Handling Technical Bulletin, 2004). Coating oranges with a thin carnauba wax or shellac reduced shrivel and significantly prolong fruit market life (Postharvest Handling Technical Bulletin, 2004). Fracture peel of 'Chanh' orange application of high Chitosan concentration was thick (Hoan *et al*., (2001). Waxing improves the appearance of fruit skin, waxing aims to prevent shrinkage, weight loss, and to stretch the shelf life, of vegetable and fruit throughout preservative time (Postharvest Handling Technical Bulletin, 2004). Wax-hydrocolloid coatings of 'Nova' fruit resulted that stomata of 'Nova' fruit were less blocked by such coatings led to these coatings effectively reducing the disadvantages related to commercial wax coatings (Chen and Nussinovitch, 2000).

The main purpose of this study was to evaluate effects of 4, 6, 8 and 10% MW on shrinkage pattern, respiration and sensory

2. MATERIALS AND METHODS

2.1. Materials

Fruit samples: Orange fruit cv. Canh were harvested at 220-235 days after fruit set from a commercial orchard in ThanhOai district, Hanoi, Fruits were laid in spongy box (20 kg fruit/box) and transported to laboratory within 2-3 hrs. Fruit were then selected for uniformity of size, shape and non-defected fruit.

Bees-Carnauba mixed wax (MW): The bees wax and carnauba wax in the ratio of 7:3 was prepared following process of Thinh, (2013).

Fruits were coated in 4, 6, 8 and 10% MW for 1 min at room temperature, after drying in a room, coated fruit were laid on trays and stored at ambient temperature (22±2ºC) and RH 80±5% for 20 days and sampled/analyzed at 5 days intervals. While uncoated fruit were used as control.

A completely randomized design was used for the experiment. All measurements of each treatment were the average of three replications.

2.2. Method

1**) Determination of shrinkage pattern:** Physical changes of orange peel expressed as shrinkage pattern was measured during the storage. The fruit was divided in three zone as upper, middle and lower zones. The fruit appearance especially the wrinkles on the orange cv. Canh coated in 4, 6, 8, and 10% MW for 1 min each zone was recorded and counted (%). The changes in wrinkle was studied under a light microscope (Nikon, Japan, taken 20 times magnification) after 20 days sotrage. Shrinkage pattern were determined by method replica: orange cv. Canh coated in 4, 6, 8 and 10% MW for 1 min were marked by silicone for the observation spots, wrinkle of the coated skin surface before and after storage by Scanning Elec-

tron Microscope (SEM) and were introduced by the (2014). Statistical analysis was carried out using Duncan's multiple range test and used to analyze the significant differences ($P \leq 0.05$) convex of spot distances of treatments and the control on replica (concave spots distance of treatments and the control on orange cv. Canh skin).

2) Determination of physical images of the coat -ing layer: Thickness of the coating layer was observed by E600-Nikon (Japan) with Image-Pro Plus program version 4.5 for Windows. The sample was prepared by cutting cross-sections of the coated surface. Thickness at three different zones (top, middle, bottom) of orange cv. Canh fruit was recorded by Park *et al*. (1994). The surface of the coating layer was measured by JSM-5410LV SE-M (taken 400 times magnification). For the analysis, the samples were cut and sputter-coated with 10nm of gold before being viewed on SEM (Jacobi and Gowanlock, 1995; Celano *et al*., 2009). The number of lenticels and stomata was observed.

3) Percentage of weight loss was calculated by weighing the whole fruit putted into tray before and after storage, as follows:

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

4) Respiration rate was measured according to the method of Jiang and Li, (2001). The 1000 g fruit was sealed in a glass chamber for 2 hours at ambient temperature and 5°C, then a 5 ml gas sample was withdrawn with a gas-tight hypodermic syringe and analyzed by gas chromatography (Agilent Technology 7820A).

5) Sensory evaluation was tested on the day of harvest on the day 5, 10, 15 and 20 at ambient temperature (22±2°C). Members were requested to rate overall acceptable: such as peel color, odor, taste, flavor on a hedonic scale from 1 to 9 point by a board of tasters as follows: 9=extremely like; 1= extremely dislike and 5=neither like nor dislike (Hung, 2008). We also performed open discussion group in order and establish the sensory form.

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

3. RESULTS AND DISCUSSION

3.1. Change in shrinkage pattern: The changes in shrinkage pattern of orange cv. Canh peel during the storage time at ambient temperature of MW and control are shown in Figure 3.1, 3.2, 3.3. The shrinkage pattern of 8% and 10% MW was not appeared when fruit were tested after 20 days in storage interval, whereas shrinkage pattern in control was observed to be highest. Increase in wrinkles might be due to the water loss of the fruit which the control was the highest in water loss. The maximum wrinkles (14.0%) were observed in control, minimum wrinkles (0%) were recorded in 8-10% MW after 20 days storage at ambient temperature (22 ± 2°C). There were no significant differences in shrinkage pattern on the top, middle, and bottom among the treated fruit ($P \leq 0.05$), which were all significantly different from the control fruit ($P \leq 0.05$). Overall, mixed wax improves the appearance of fruit skin, preventing shrinkage of orange cv. Canh peel during storage 20 days.

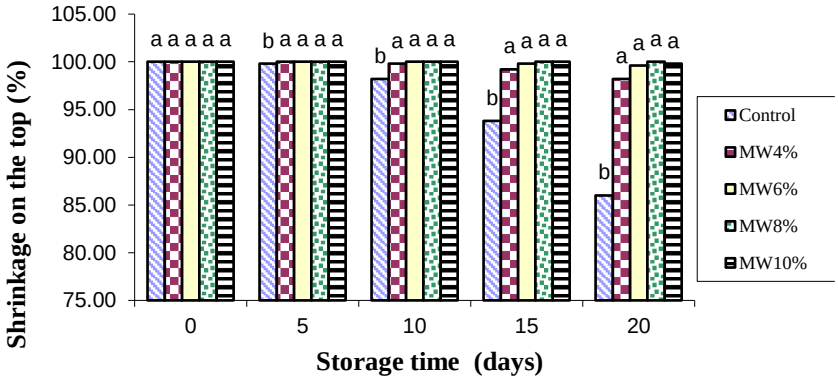


Figure 3.1 Effect of mixed wax coating on shrinkage pattern on the top of orange cv. Canh peel stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

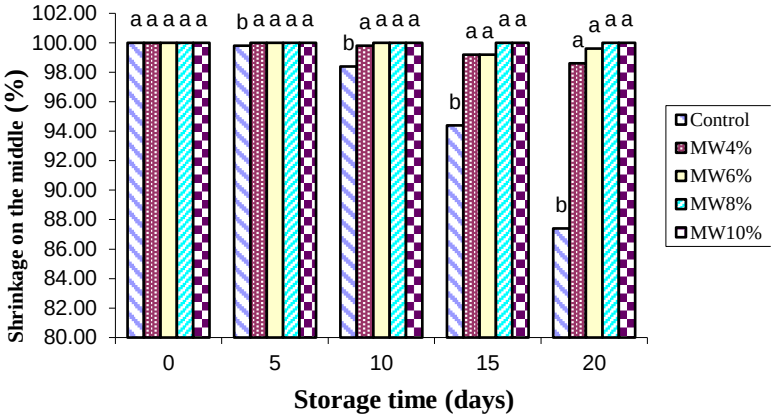


Figure 3.2 Effect of mixed wax coating on shrinkage pattern on the middle of orange cv. Canh peel stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

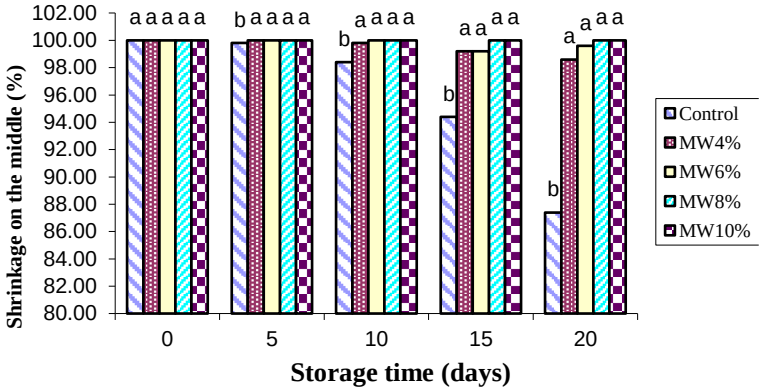
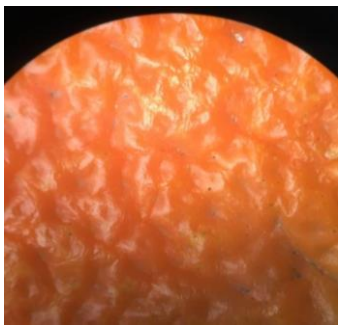


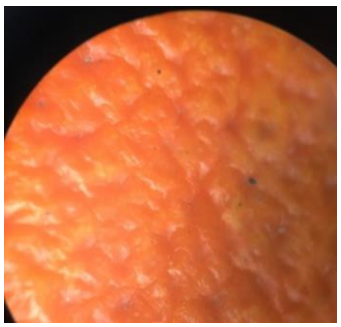
Figure 3.3 Effect of mixed wax coating on shrinkage pattern on the bottom of orange cv. Canh peel stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

This result was like Postharvest Handling Technical Bulletin, (2004) which reported that coating oranges with a thin carnauba wax or shellac reduced wrinkle and significantly extended fruit market life. The same as Hoan *et al*., (2001) reported that fracture peel of 'Canh' orange application of different Chitosan 2.25% and 2.5% was 15.0 to 25.7%, respectively.

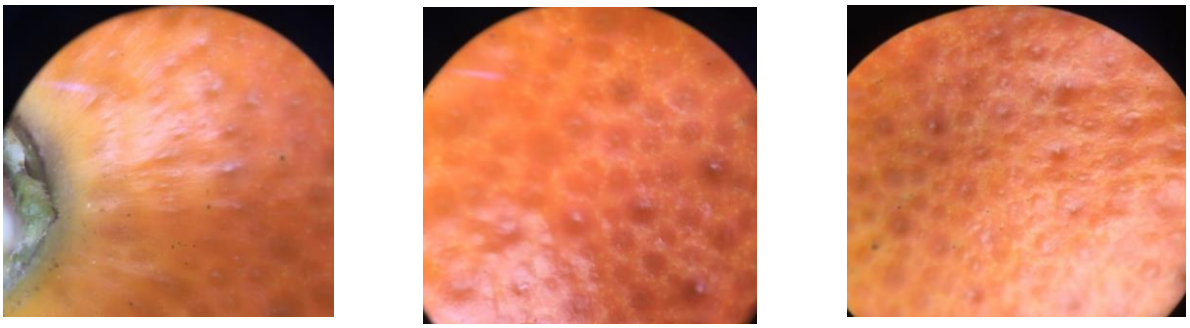
Control on the top



Control on the middle



Control on the bottom



MW on the top MW on the middle MW on the bottom

Figure 3.4 Wrinkle of orange cv. Canh by microscope (taken 20 times) after 20 days

Table 3.1 The distance of convex spots on the top, bottom of mixed wax coated (4, 6, 8 and 10%) of orange cv. Canh fruit stored at 22 ± 2°C, 80 ± 5%RH

The distance of convex spots (µm)	Top			Bottom		
	0 day	20 days	Δ*	0 day	20 days	Δ*
Control	1181.3±148	443.6±51	637.7c	1497±208	970.4±181	526.6d
MW 4%	1609.7±208	986.6±169	623.1c	1295.0±66	961.3±81	333.7c
MW 6%	1756.5±171	1132±147	624.5c	1115.4±48	922.4±172	193.0b
MW 8%	1181.5±151	1012.3±89	169.2a	1247.0±159	1106.5±103	140.5a
MW 10%	1374.7±32	1057±157	317.7b	1151.2±205	944±148	207.2b

Note: *: Δ = The distance of convex spots (20days) - The distance of convex spots (0 day)

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

The changes distance of convex spots on replica of orange fruit cv. Canh during the storage time at ambient temperature and control are presented in table 3.1, 3.2. Maximum distance of convex spots was recorded in control on the top, minimum distance of convex spots was observed in 8% MW after 20 d storage at ambient temperature (22 ± 2 °C). There were significant differences in distance of convex spots on the top, and bottom among the coating fruit, and control fruit ($P \leq 0.05$). Number of convex spots of orange cv. Canh in control appears more than in MW by SEM before and after storage 20 days (table 3.2).

3.2. Change of physical images of the mixed wax coating layer: Table 3.3 shows thickness of the coating layer increased with the increase of MW concentration. Thickness of coating layer depended on the concentration of MW emulsion. Coating thickness was affected by concentration of mixed wax during storage (Table 3.3 and Fig. 3.5). The coating layer was thicker as increasing mixed wax concentration.

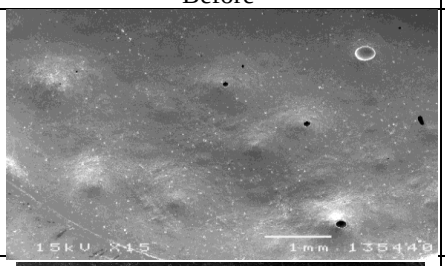
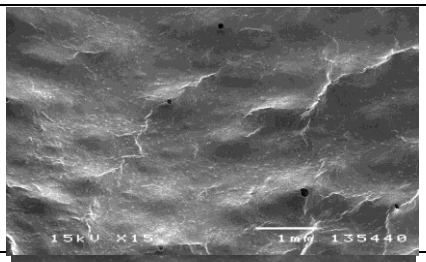
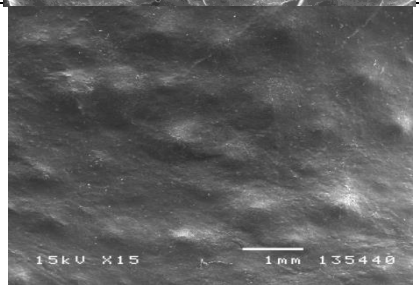
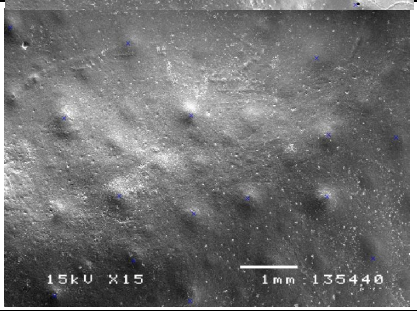
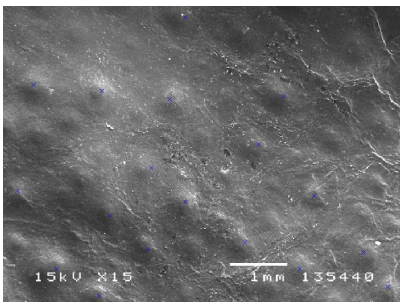
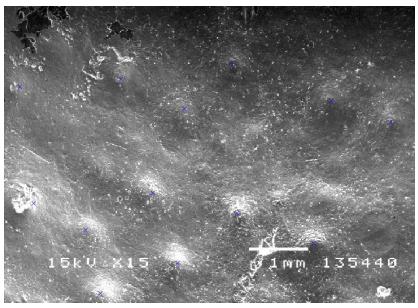
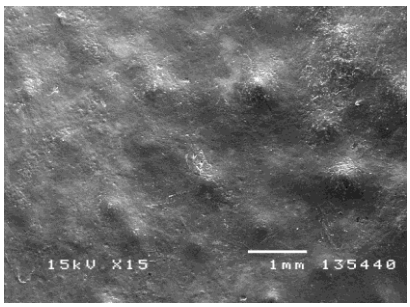
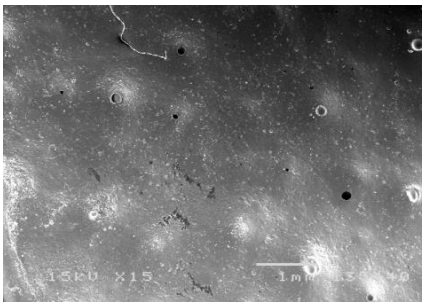
A coating thickness of 10.65 and 12.42 µm was found in the MW on the middle at the concentrations of 8 and 10%, respectively. Maximum thickness (12.82 µm) was recorded on the top in 10% MW. Least thickness value (5.14 µm) was observed on the middle in 4% MW. No significant variation existed in the thickness of difference treatments of orange cv. Canh fruit between on the top, middle, and bottom ($P \leq 0.05$).

Tab. 3.3 Effect of MW coating on thickness on the top, middle, bottom of orange cv. Canh

Thickness (µm)*	Bee-carnauba mixed wax concentration (%)			
	4	6	8	10
Top	5.25 ± 0.62	8.21 ± 0.32	10.12 ± 0.47	12.82 ± 0.97
Middle	5.14 ± 0.26	8.54 ± 0.33	10.65 ± 0.22	12.42± 0.72
Bottom	5.42 ± 0.47	8.86 ± 0.70	10.48 ± 0.47	12.21 ± 0.66

Similar SEM results were observed orange 'Moro', Salvatore *et al*. (2013) concluded that resulting in a complex network made up of wax reticulum, K-sorb and micro-particles of thiabendazole of 'Moro' oranges, which partially hid the stomata, while the stomata were clearly visible in the control. The wax-hydrocolloid combination creates fewer blockages of stomata of Nova' fruit than the coating without gum and the commercial coating (Chen and Nussinovitch, 2000).

Table 3.2 Convex spots of control and MW orange cv.Canh (before; after 20days storage) by SEMx15

Sample	Before	After
Control		
MW 4%		
MW 6%		
MW 8%		
MW 10%		

Depended on images attained the surface skin by SEM, lenticels and stomata was observed (Fig. 3.5). Occluding lenticels and thickness of wax layer based on a concentration of mixed wax emulsion. Coating with 8% MW completed fill in lenticels making a continuous wax layer (Fig. 3.5.b). The images of skin surface by SEM showed the surface and lenticels has oil bags and were covered with MW caused the lowest loss of water (Fig. 3.5.b), while the control fruit that lost more water its lenticels were not entirely covered with MW layer (Fig. 3.5.a). It was very important to mention that if we covered the skin surface of Canh orange with MW, prevented wrinkles (Table 3.1), the loss of water and extended storage life, so 8% MW coating could be suitable for presservation orange cv. Canh. Overall, thickness of mixed wax coating impacts wrinkle and completes fill in lenticels of orange cv. Canh during storage 20 days.

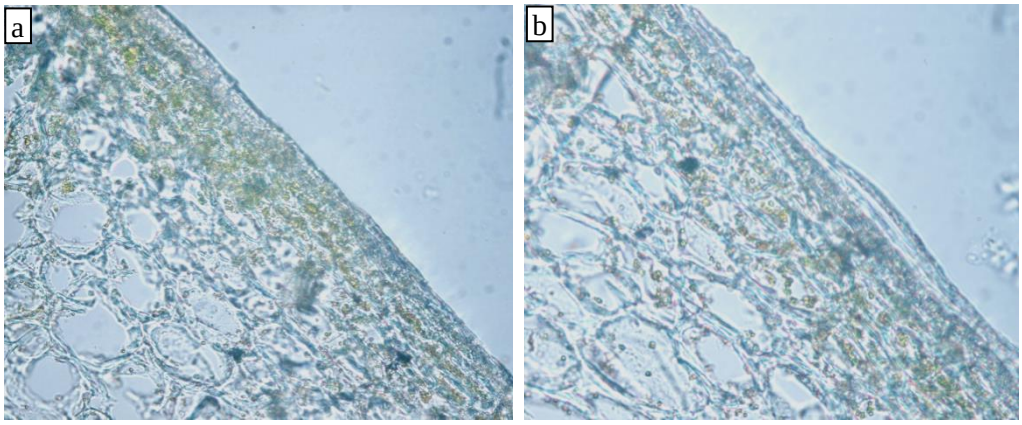


Figure 3.5 Cross-section image of surface skin coated by SEM: control (a) and 8%MW (b)

Hagenmaier and Shaw, (1992) reported that a major disadvantage of wax coatings is the develoopment of off-flavors from their use so that the layer coating must not be too thick. Valencia orange rind sample on SEM had a lot of fracturing of the applied wax (imazalil, carnauba and PE) on the fruit surface and the surface of the control fruit which served as untreated, displayed a natural wax layer with visible stomata spores and consisted of a crystallised undisturbed amorphous structure (Ncumisa, 2012). SEM analysis of 'Moro' oranges presented that the typical wax micro-structure present on control fruit surface, after treatment with thiabendazole was altered and tended to disappear when treatment was performed at 53 ^{o}C (Salvatore *et al*. 2013).

3.3. Change in fruit weight loss: Changes in weight loss of MW and control fruit during the storage period are presented in figure 3.6. Weight loss percentages of all MW treatment were increased with the storage time. The control fruit were loosed weight higher and faster than the MW coated fruit (Fig. 3.6). The significant differences of weight loss percentage between the uncoated and coated fruit were found after 5 days storage. Among the MW coated treatments, only the 4% MW treatment had significant higher weight loss percentage than another MW treatment (13.29% and 8.37%-10.94%, respectively). This result show that MW coating could slow down the rate of water of the fruit.

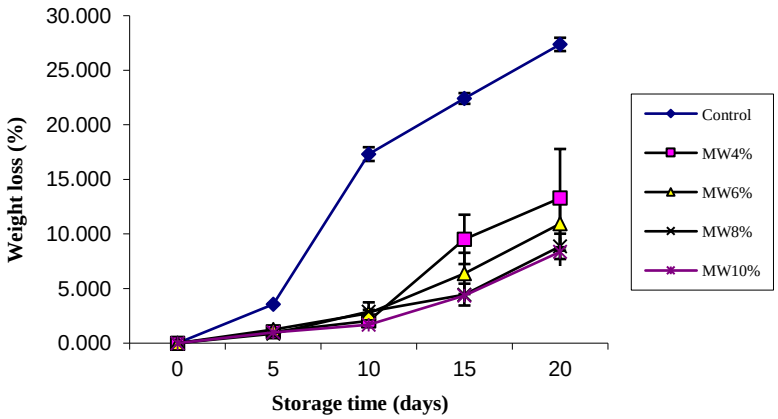


Figure 3.6 Effect of mixed wax coating on weight loss of orange cv. Canh fruit stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

This result is similar with the reported data on weight loss of oranges fruit of Ron *et al*. (2005) who showed that the loss of weight from the 'Mor' mandarin coated either with the commercial 'Tag' wax and with the new modified 'Tag' wax was only half of that lost by the control fruit. Coating the orange fruit with wax could prevent water loss at ambient temperature (Binh and Dien, 1995; Hoan *et al.*, 2001; Thang *et al.*, 2013, and Ladaniya, 1997).

3.4 Change in respiration: The changes in respiration rate of bee-carnauba mixed wax and the control orange cv. Canh fruit during the storage time. The fruit surface coating was relatively impermeable to O2 and CO2 and water (Thirupathi *et al*., 2006). This result was also confirmed that the MW had this property. The respiration rate of the

MW coated fruit was lower than the uncoated fruit (Fig. 3.7). Respiration rate pattern of the uncoated fruit tended to increase while the MW coated fruit tended to decrease. This should be the effect of MW which prevented O_2 to penetrate in to the fruit and prevented CO_2 to penetrate out the fruit. Thus, low O_2 and high CO_2 content in the fruit could reduce fruit respiration rate.

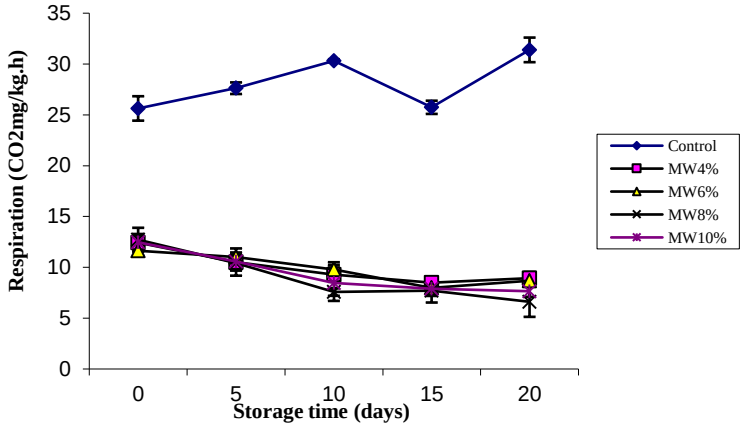


Figure 3.7 Effect of mixed wax coating on respiration of orange cv. Canh fruit stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

Consistent with our result, Thang *et al*. (2013) showed that respiration rate of Vinh oranges coated with mixed bees and PE wax reduced during storage at 22-29°C. Our results are in accordance with the report of Ladaniya *et al*., (1997) on respiration rate of 'Nagpur' mandarins. Respiration rate of TienGiang oranges coated with chitosan reduced after 25 days storage at 29°C (Binh and Dien, 1995). Commercial fruit wax has also been shown to reduce the respiration rate of coated fruit (Hagenmaier and Shaw, 1992). This result show that bees-carnauba mixed wax coating could reduce respiration rate.

3.5 Changes in sensory: Sensory score of treated and control fruit during the storage period are indicated in Table 3.4. The overall acceptable of MW coated fruit were higher than the control fruit through the storage time (Table 3.4) while there were no significant differences among the MW coated fruit. The MW coated fruit still had high quality and got the high score (7.0-7.3) after 10 days of storage. After that the overall quality reduced in all MW coated fruit and end up with neither like nor dislike quality (5.3-5.5) after 20days of storage.

Thirupathi *et al*., (2006) showed that the fruit were left without waxy cuticle, the water quickly begins to evaporate, resulted increase shelf life, and freshness. Our results are consistent with the reported data on sensory values of oranges fruit (Thang *et al*., 2013) who reported that Vinh orange coated with bees and PE wax had better form, good quality scores and special taste than control. Hagenmaier (2000) reported that flavor scores of 'Valencia' oranges at all storage conditions (at 15-25°C and 9-16 days) were highest (8.0-10.5).

Table 3.4 Effect of mixed wax on sensory in orange cv. Canh at 22 ± 2°C, 80 ± 5%RH

	Storage time (days)				
Treatments	0	5	10	15	20
Control	8.5	5.3a	5.3a	4.5a	3.5a
4%MW	8.5	7.3b	7.0b	6.5b	5.5b
6%MW	8.5	7.5b	7.2b	6.0b	5.3b
8%MW	8.5	7.8b	7.3b	6.5b	5.5b
10%MW	8.5	8.0b	7.2b	6.0b	5.5b

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=neither liked nor disliked

Thirupathi *et al*., (2006) showed that the fruit were left without waxy cuticle, the water quickly begins to evaporate, resulted increase shelf life, and freshness. Our results are consistent with the reported data on sensory values of oranges fruit (Thang *et al*., 2013) who reported that Vinh orange coated with bees and PE wax had better form, good quality scores and special taste than control. Hagenmaier (2000) reported that flavor scores of 'Valencia' oranges at all storage conditions (at 15-25°C and 9-16 days) were highest (8.0-10.5).

4. Conclusion

Coating orange fruit cv. Canh in 8% MW and storage at ambient temperature (22±2°C), 80 ± 5%

RH can reduced shrinkage pattern, respiration, weight loss, and maintained high score sensory throughout the 20 days in storage time.

REFERENCE

Babar, E.B., Postharvest Management of Kinnow (*Citrus reticulata* Blanco) Mandarin to extend shelf life, *Doctor thesis*, FAISALABAD Pp. 80-82 (2007).

Binh, T.Q. and L.D. Dien, Research and application of chitosan for preservation Orange in Vietnam. *In KC08-07* program.pp.149-161 (1995) (in Vietnamese).

Celano, G., A. Minnocci, L. Sebastiani, M. D'auria, and C. Xiloyannis, Changes in the structure of the skin of kiwifruit in relation to water loss. *Journal of Horticultural Science and Biotechnology*, 841: 41-46 (2009).

Chen, S. and A. Nussinovitch, Permeability and roughness determinations of wax hydrocolloid coatings, and their limitations in determining citrus fruit overall quality. *Food hydrocolloids* 15: 127-137 (2001)

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

Hagenmaier, R. D., Evaluation of polyethylene-candelilla coating for 'Valencia' oranges. *Postharvest Biology and Technology.*19: 147-154 (2000).

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 Tech. Institute* 201-213 (2002).

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

Jiang Y. and Y. Li, Effects of chitosan coating on postharvest life and quality of Longan fruit. Food Chemistritry 74: 139-143 (2001).

Ladaniya, M.S., R.K. Sonkar, and H.C. Dass, Evaluation of heat-shrinkable film wrapping of 'Nagpur' mandarin (*Citrus reticulata* Blanco) for storage. *Journal of Food Science Technology* 34: 324–327 (1997)

Ncumisa S.N., Imazalil and wax coating application in citrus pack house to control green mold and preserve fruit quality. *Master thesis*, Stellenbosch University http://scholar.sun.ac.za. Pp. 92 (2012)

Park, H.J., M.S. Chinnan, and R.L. Shewfelt, Edible comzein film coatings to extend the storage life of tomatoes. *Food Proc. Pres.* 184: 317-331 (1994).

Postharvest Handling Technical Bulletin, Waxing fruits and vegetables. *Technical Bulletin* No. 33: 11 (2004)

Ron, P., Batia, W., Lea, C., Avinoam, D. and B. Avi, Effects of polyethylene wax content and composition on taste, quality, and emission of off-flavor volatiles in 'Mor' mandarins. *Postharvest Biology and Technology* 38(3): 262-268 (2005).

Salvatore D.A., A. Fadda, A. Barberis, A. Palma, A. Angioni and M. Schirra, Combined effects of potassium sorbate, hot water and thiabendazole against green mould of citrus fruit and residue levels. *Food Chemistry* 141 (2013) 858–864 (2013)

Thang, P.C., N.D. Lam and L.M. Tuan, Study to replace carnauba wax component in the CP-01 fruit coating by beeswax and the testing results on Vinhorange preservation. *Workshop on Agricultural Engineering and Postharvest Technology for Asia Sustainability P*p. 307-312 (2013)

The L.T.014, Study of morphological wrinkles and eplastic charateristics of the skin surface of adults Vietnamese, Master thesis, Army Medical Academy, Vietnam, Pp. 115 (2014).

Thinh, D.C., 2014. Development of coating formular for shelf life and storage life extension of 'Cat Hoa Loc' Mango. *Doctor thesis*, Chiang mai Uni. Pp. 165 (2014).

Thirupathi, V., Sasikala, S. and Z.J. Kennedy, Preservation of fruits and vegetables by wax coating. *Science Tech Entrepreneur*. [Online]. Available: http://scribd.com (2006).