CHAPTER 3

Effect of bees wax, carnauba wax (mixed wax) coating on shrinkage pattern of sweet orange cv.Canh

The impact of bees wax and carnauba wax - mixed wax (MW) on shrinkage pattern of Vietnamese sweet orange fruit cv. Canh was studied, by coating fruit with 4, 6, 8 and 10% MW and stored at ambient temperature (22±2^{o}C) 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 period. The results showed that 8%MW coating at ambient temperature reduced the wrinkle compare with the control fruit. Thickness of oranges cv. Canh coated at 10%MW was highest. No significant change on the top, middle, bottom were recorded due to treatment waxing. The number of lenticels and stomata was observed by Scanning Electron Microscope (SEM).

3.1 Introduction

Orange fruit (*Citrus sinensis* Osbeck) is one of the popular fruit in Vietnam. Sweet orange cv. Canh is one of the best quality fruit and has a high economic value in Vietnam (MARD, 2014). Fruit has a thin peel, smooth, and the pulp is orange color, very sweet, high vitamin C and low acidity. In Vietnam there was not any research to storeorange cv. Canh fruit and then problem of fruit after harvest and storage: high weight loss, high decay 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, prolong storage life, decreasing rate of transpiration, (Hagenmaier and Shaw, 1992; Postharvest Handling Technical Bulletin, 2004a). Coating oranges with a thin carnauba wax or shellac reduced shrivel and significantly extend fruit market life (Postharvest Handling Technical Bulletin, 2003). 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 extend the shelf life, of fruit and vegetable throughout preservative time (Postharvest Handling Technical Bulletin, 2004). Deliberate disturbance by gums in the ordered structure of wax-hydrocolloid coatings, small changes in their roughness, and the fact that stomata of 'Nova' fruit were less blocked by such coatings led to these coatingseffectively reducing the disadvantages related tocommercial wax coatings (Chen and Nussinovitch, 2000). SEM analysis of 'Moro' oranges showed that the typical wax micro-structure present on control fruit surface, after treatment with TBZ was altered and tended to disappear when treatmentwas performed at 53 ^{o}C (Salvatore *et al*. 2013).

The main purpose of this study was to evaluate effects of 4, 6, 8 and 10% MW on shrinkage pattern of Vietnamese sweet orange cv. Canh during the storage period at ambient temperature.

3.2 Material and method

3.2.1 Materials

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

The bees wax and carnauba wax in the ratio of 7:3 was prepared following process of Thinh, (2013). The bees wax and carnauba wax was melted by a magnetic stirrerat 80-85°C. After that 1.5% oleic acid, 0.08% palmitic acid, and water were added to the mixture during stirring and blending for 4, 6, 8 and 10% concentrations of mixed wax (MW) for 25-30 minutes. The MW of various concentrations were cooled down to ambient temperature before coated to the fruit.

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±2^{o}C) and RH 80±5% for 20 days, and sampled/analyzed at 5 day intervals. While uncoated fruit were used as control (Control).

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

3.2.1 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. Length (10 mm) of the orange cv. Canh coated in 4, 6, 8, and 10% MW for 1 min each zone were marked by pen for the observation wrinkle of the coating surface. The fruit appearance especially the wrinkles on the fruit peel in every zone was recordedand counted (%). The shrinkage pattern was generated in a diagram. The changes in wrinkle was studied under a light microscope (Nikon, Japan, taken 20 times magnification).

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 (Zhermack, Italy) for the observation spots, wrinkle of the coated skin surface before and after storage by Scanning Electron Microscope (SEM), and were introduced by The (2014).

+ Sample preparation: orange fruit were divided into 2 groups: Control group (un-treated), and treated group: orange cv. Canh coated in 4, 6, 8, and 10% MW for 1 min were dried at room temperature, then all fruit were marked with a circle (diameter 10 mm) by pen.

+ The method of making pattern

Pattern were designed ready-made: circle, 2.0 cm in diameter, 2.5 mm in thick.

Pattern was putted onto the position marked on oranges.

Silicon was poured into the cavity to the surface of the pattern

Piece of paper marked with the name of research samples, and kept during 5 - 10 minutes to the Silicon for drying.

Replica (Copy of silicon) was removed from the pattern gently.

+ Replica was gilded by JFC-1200 (Japan)

+ Replica were observed by scanning electron microscope JSM-5410LV (Japan), (taken: 15, 35, 50 time magnification).

+ Data analyze:

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).

The images was observed by software SEM-Afore 5:21 and scanning electron microscope JSM-5410LV (Figure 3.1.a).

2) Determination of physical images of the coating layer

Thickness of the coating layer was measured by E600-Nikon (Japan) (Fig 3.1.b)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 Scanning Electron Microscope (SEM) (taken 400 times magnification) (Fig. 3.1.a).

Rectangular pieces (10 mm x 5 mm x 1 mm) of the orange cv. Canh coated in MW for 1 min were cut with a razor blade for the observation of the coating surface. Samples were immediately immersed in liquid nitrogen, dried to the critical point, mounted on aluminum stubs and stored in liquid nitrogen until analysis. For the analysis, the samples were sputter-coated with 10 nm of gold before being viewed on SEM (Jacobi and Gowanlock, 1995; Celano *et al*., 2009). The number of lenticels and stomata was observed.

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

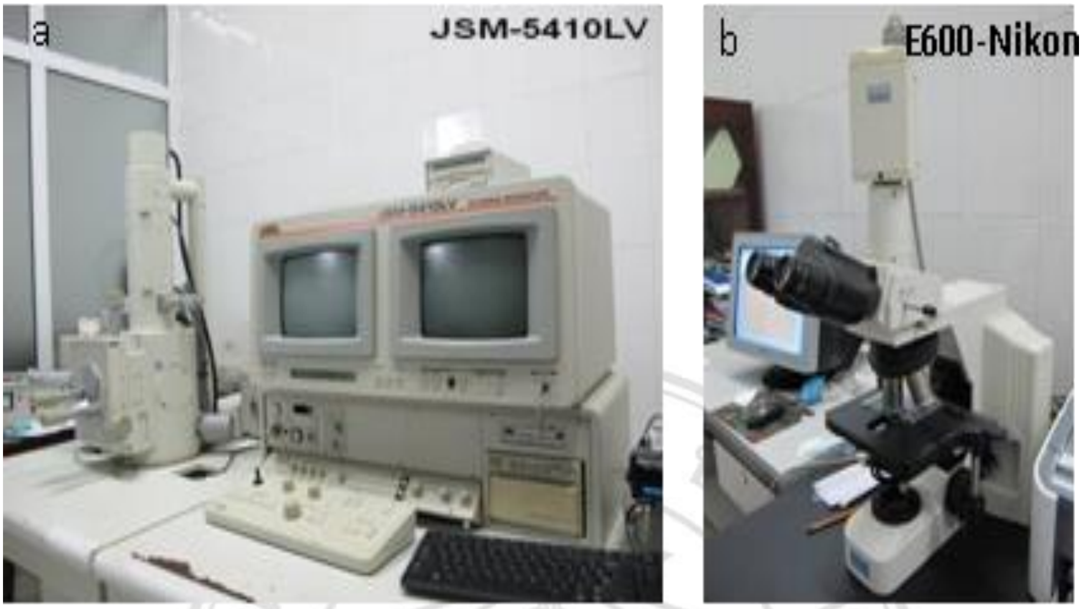


Figure 3.1 Scanning electron microscope JSM-5410LV (a) and E600-Nikon (b)

3.3 Results and discussion

3.1 Change in shrinkage pattern

The changes in shrinkage pattern of orange cv. Canh peel during the storage period at ambient temperature 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 d in storage interval, whereas shrinkage pattern in control was observed to be highest. Increase in wrinkle might be due to the water loss of the fruit which the control was the highest in water loss. The maximum wrinkles (14.0%) was observed in control, minimum wrinkles (0%) was recorded in 8% MW after 20 d 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 ($P \leq 0.05$) (see appendix D). Overall, mixed wax improves the appearance of fruit skin, preventing shrinkage of orange cv. Canhpeel during storage 20 d.

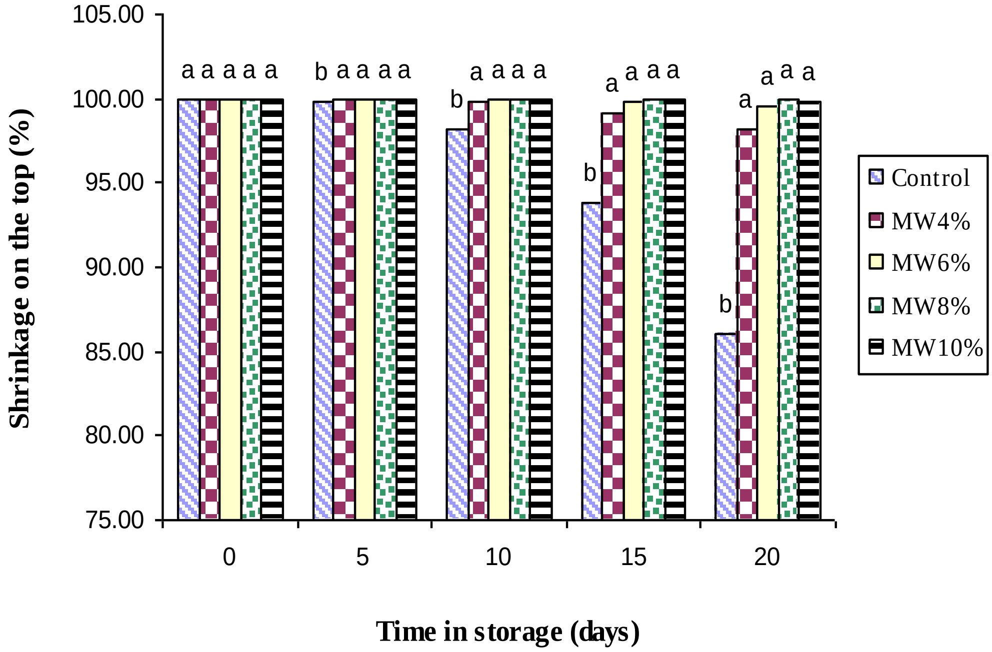


Figure 3.2 Effect of bees-carnauba wax (MW) coating on shrinkage pattern on the top oforange cv. Canhpeel stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

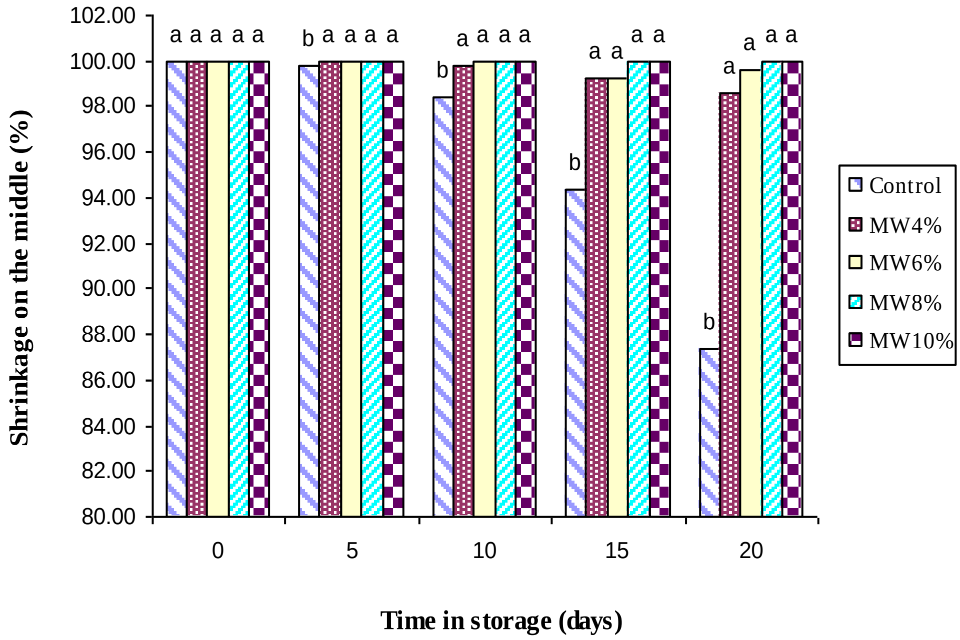


Figure 3.3 Effect of bees-carnauba wax (MW) coating on shrinkage pattern on the middle of orange cv. Canh peel stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

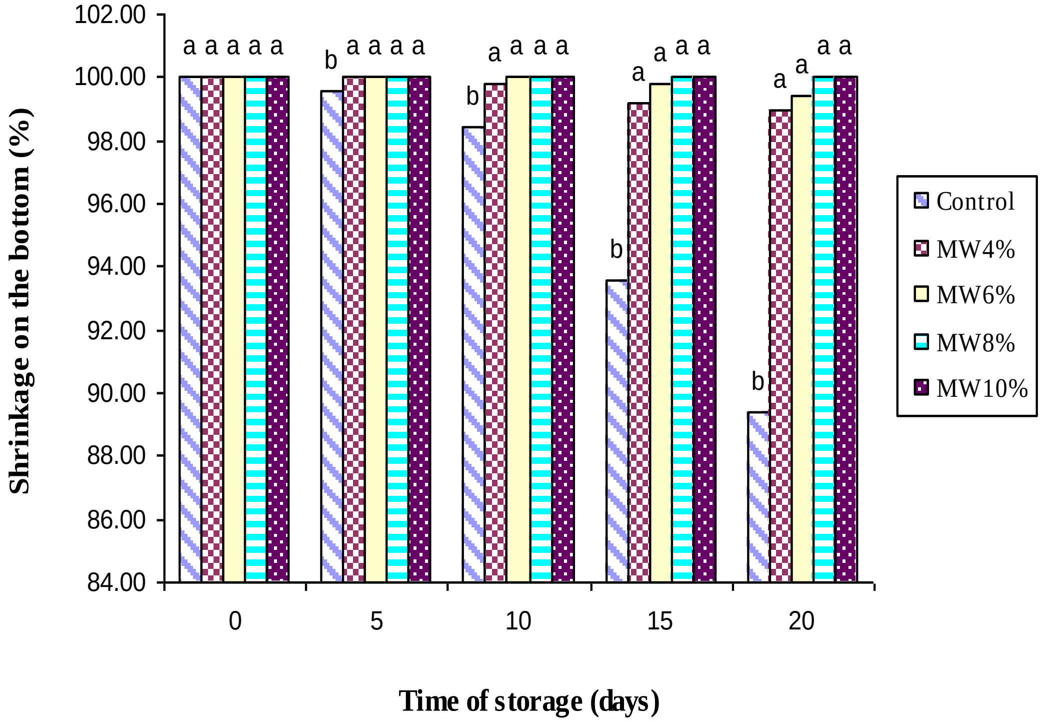
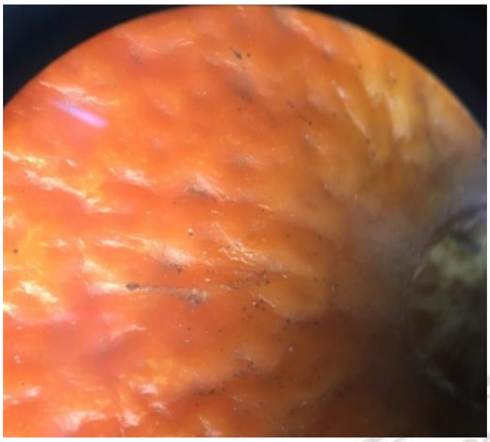


Figure 3.4 Effect of bees-carnauba wax (MW) coating on shrinkage pattern on the bottom of orange cv. Canh peel stored at ambient temperature (22 ± 2°C), 80 ± 5%RH

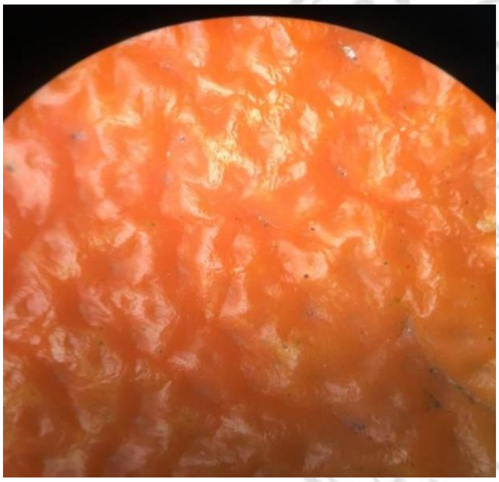
This result was agreed by Postharvest Handling Technical Bulletin, (2003) they reported that coating oranges with a thin carnauba wax or shellac reduced shrivel and significantly extended fruit market life. Hoan *et al*., (2001) reported that fracture peel of ‘Chanh’ orange application of different Chitosan 2.25% and 2.5% was 15.0 to 25.7%, respectively.

Babar (2007) suggested that percentage of fracture of ‘Kinnows’ fruit coated with polyethylene was higher than shellac wax because the rate of surface fracture development in the polyethylene base might be occurred due to its relatively larger particle size in emulsion as compared to the shellac based wax. Perez *et al*. (2002) described that the surface fracture in wax coated ’Fortune’ mandarin ranged from 2.0 to 2.5%, which was two times higher in mandarin stored at 9°C as compared to mandarin stored at 20°C.

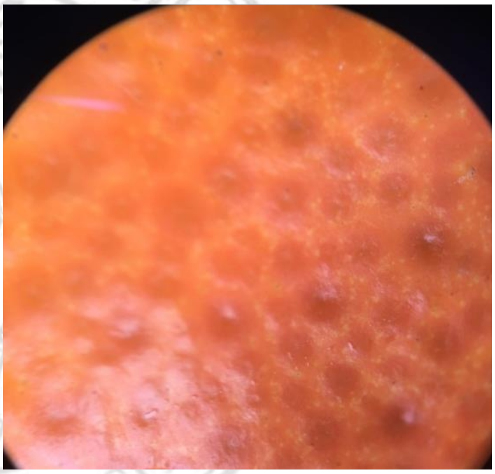


Control on the top

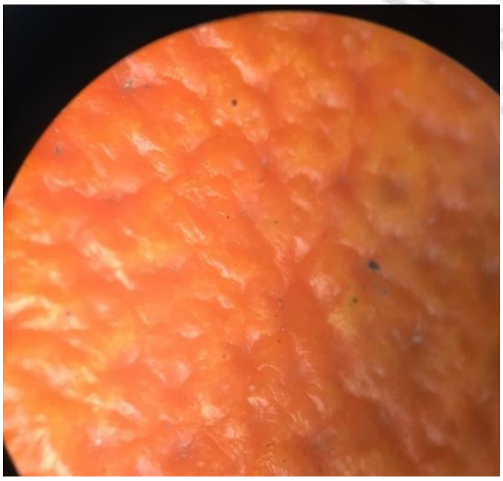
MW on the top



Control on the middle



MW on the middle



Control on the bottom

MW on the bottom

Figure 3.5 Wrinkle of orange cv. Canh by microscope (taken 20 times) (Nikon)

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)

The changes distance of convex spots on replica of orange fruit cv. Canh during the storage period at ambient temperature and control are shown in table 3.1, figure 3.6; 3.7. 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 treated fruit, and control fruit (P ≤ 0.05). Number of convex spots of orange cv. Canh in control appears more than in MW by SEM before and after storage 20 d (Figure 3.6, 3.7).

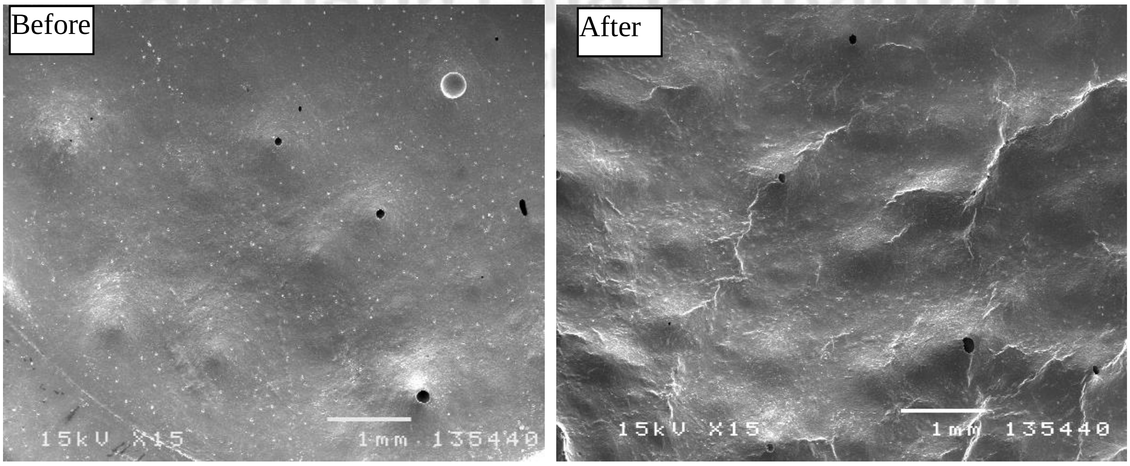


Figure 3.6 Convex spots of control orange cv. Canh (before, after storage) by SEMx15

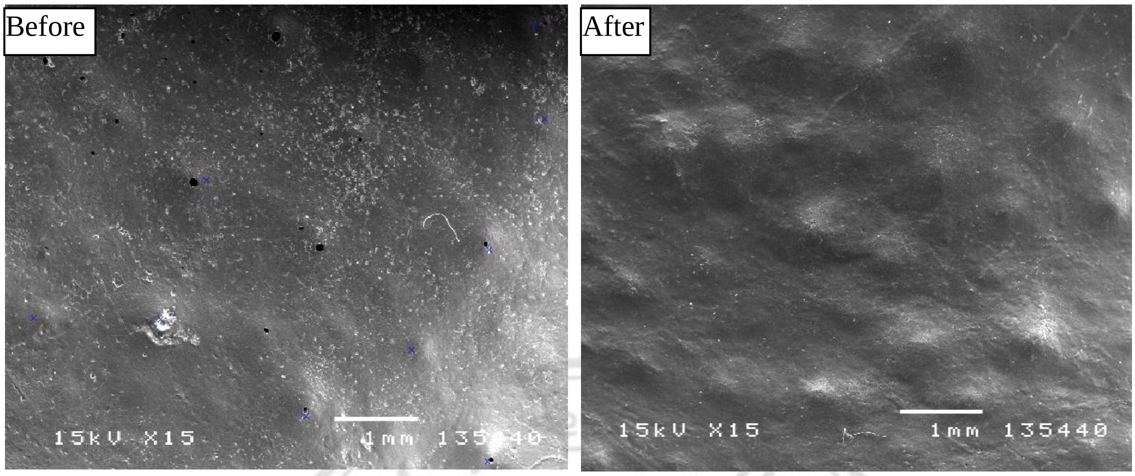


Figure 3.7a Convex spots of 4%MW orange cv. Canh(before; after storage) by SEMx15

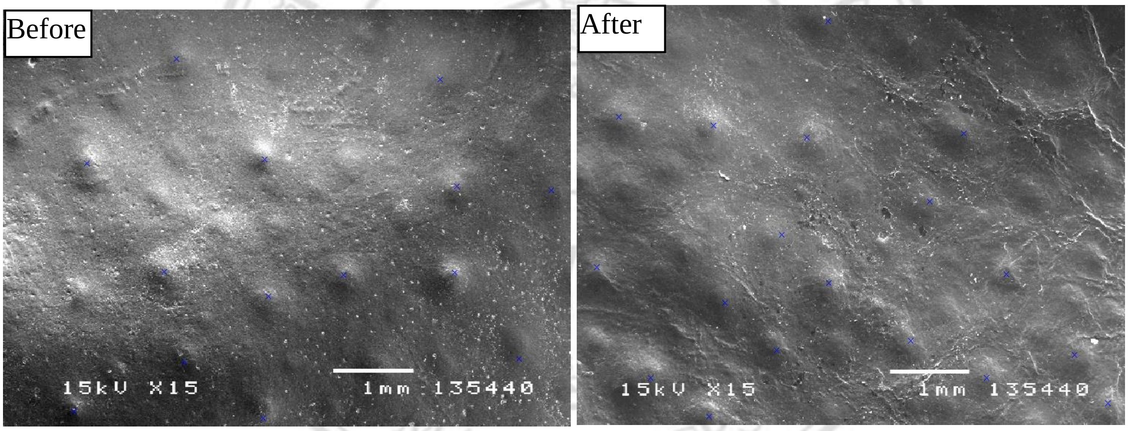


Figure 3.7.b Convex spots of 6%MW orange cv. Canh (before; after storage) by SEMx15

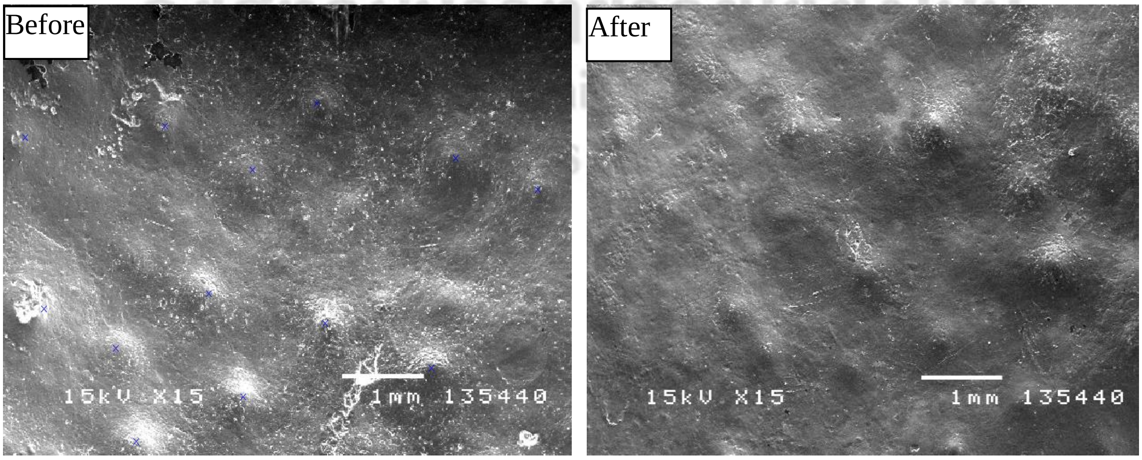


Figure 3.7.c Convex spots of 8%MW orange cv. Canh (before; after storage) by SEMx15

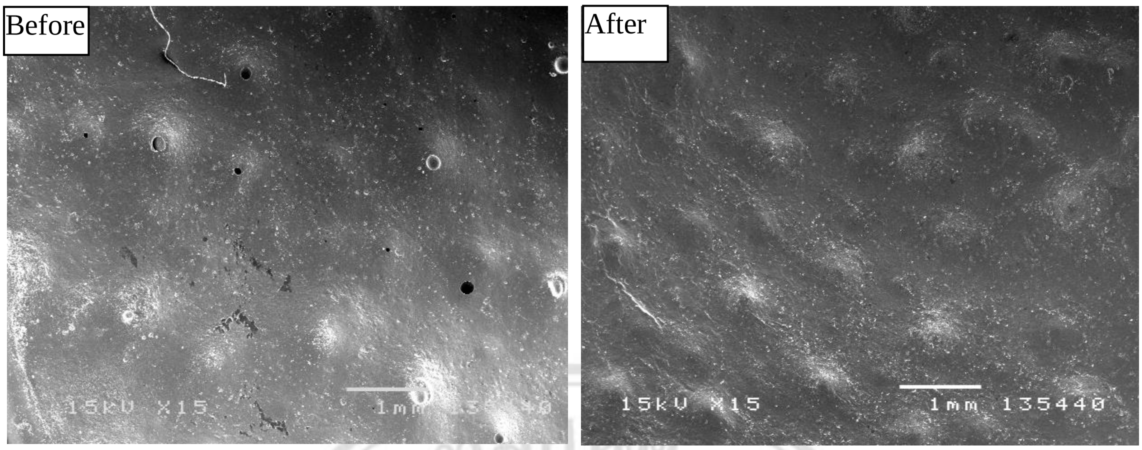


Figure 3.7.d Convex spots of 10%MW orange cvCanh (before; after storage) bySEMx15

3.2 Change of physical images of the mixed wax coating layer

Table 3.2 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 3.2 and Fig. 3.8). 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. 3.2 Effect of MW coating on thickness on the top, middle, bottom of orange cv. Canh

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

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

Similar SEM results were observed on ‘Cat HoaLoc’ mango treated with bees and carnauba wax (BC wax) (Thinh, 2014) that thickness of mango with BC wax coatingwas 10.15, 12.65 and 16.24 µm at the BC wax concentrations of 6, 8 and 10%, respectively. The wax-hydrocolloid combination creates less blockageof stomata of Nova’ fruit than the coating without gum and thecommercial coating (Chen and Nussinovitch, 2000). Salvatore *et al*. (2013) concluded that resulting in a complex network made up of wax reticulum, K-sorb and micro-particles of TBZ of ‘Moro’ oranges, which partially hid the stomata, while the stomata were clearly visible in the control.

Based on images obtained from the surface skin by SEM, lenticels and stomata was observed (Fig. 3.8). Occluding lenticels and thickness of wax layer depended on a concentration of mixed wax emulsion.Coating with 8% MW completed fill in lenticels making a continuous wax layer (Fig. 3.8.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. 3.8.b), while the control fruit that lost more water its lenticels were not entirely covered with MW layer (Fig. 3.8.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 preservation orange cv. Canh. Overall, thickness of mixed wax coating impacts wrinkle and completes fill in lenticels of orange cv. Canh during storage 20 d.

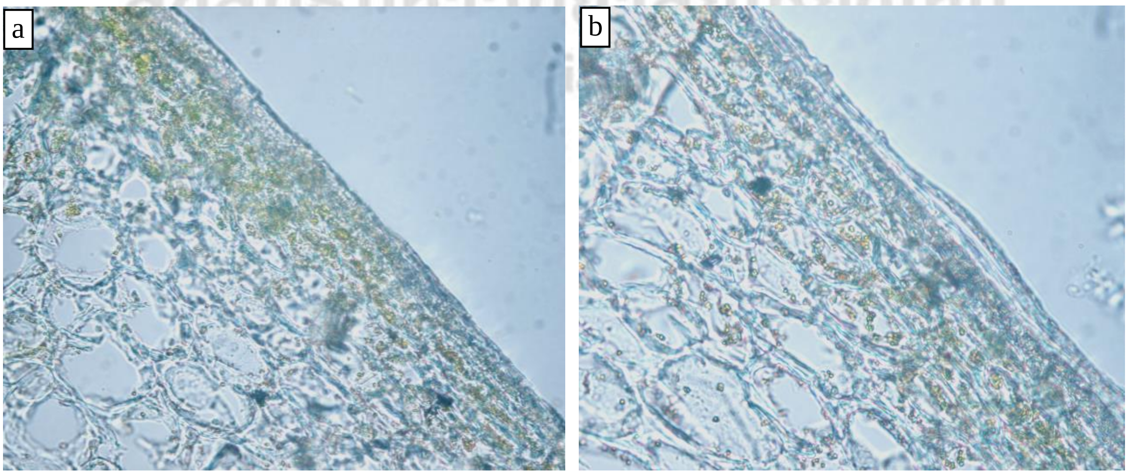


Figure 3.8Cross-section image of surface skin coated by SEM: control (a) and 8%MW

Hagenmaier and Shaw, (1992) reported that a major disadvantage of wax coatings is the development of off-flavors from their use so that the layer coating must not be too thick. It may be noted that too thick a coating is able to cause physiological disorder by reducing internal gas composition in fruit (Smith and Stow, 1984; Park *et al.*, 1994). 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 untreated fruit which served as control, showed a natural wax layer with visible stomata pores and consisted of a crystallised undisturbed amorphous structure (Ncumisa, 2012). High concentration bees and carnauba wax coated fruits completely filled in lenticels and was also to seen reduce respiration rate and delay the ripening of mango more than low concentration MW (Thinh, 2014).

3.4 Conclusion

Coating orange fruit cv.Canh in 8%MW and storing at ambient temperature (22±2°C), 80 ±5%RH reduced shrinkage pattern throughout the 20 days in storage. Thickness of orange cv. Canhcoated 10%MW was highest. Lenticels and stomata was observed by SEM.