CHAPTER 3
MATERIALS AND MEDTHODS

3.1 Fruit samples

Tangerine fruit cv. 'Sai Nam Phueng' were harvested at commercial maturity from a commercial orchard in Fang district, Chiang Mai province, Thailand, during the December 11, 2007 to March 10, 2009. Fruit were packed in carton box and transported by van or truck to the Postharvest Horticultural Laboratory, Department of Plant Science and Natural Resource, Faculty of Agriculture, Chiang Mai University. Fruit were selected for weight range of 90-130 g and defect-free.

3.2 Experiments

The experiment studied on commercial coatings and developed coating materials on quality of tangerine fruit cv. 'Sai Nam Phueng'. The experiments consisted of 5 treatment units as follow;

Experiment 1 Comparison of commercial coatings, polyethylene microemulsion and chitosan on the physico-chemical and physiological characters of tangerine fruit.

This experiment was divided into 2 phases. Coatings were applied by manual method.

For 1st phase, tangerine fruit were coated with different coatings and divided into 13 different treatments as follow;

T1-T9 = Fruit coated with commercial coatings
T10 = Fruit coated with polyethylene microemulsion
T11-T12 = Fruit coated with chitosan solutions (1.5 and 2.0%)
T13 = Non-coated fruit

The tangerines were stored at room temperature (23±3°C) and 56±5% relative humidity for 13 days.

For 2nd phase, the 5 commercial coatings, polyethylene microemulsion and 2.0% chitosan were selected for investigating the effects of temperatures and coating materials on the physico-chemical and physiological characters of tangerine fruit. There are 2 factors as follow;

Factor 1 = Storage temperatures
- 5°C and 90±2 relative humidity
- 10°C and 90±2 relative humidity
- Room temperature (23±3°C and 53±3 relative humidity)

Factor 2 = Coating treatments
- 5 commercial coatings (Citrashine, Sealkote, Rosy Plus, Zivdar and Perfect Shine)
- polyethylene microemulsion
- 2.0% chitosan
- Non-coated fruit

Components of 9 commercial coatings, which are usually used by packing houses, polyethylene microemulsion and chitosan solution (1.5 and 2.0%) were shown in Table 3.1.

Experiment 2 Comparison of commercial coatings by commercial method on the physico-chemical and physiological characters of tangerine fruit

In the experiment 2, the best coating (Zivdar), the bad coating (Citrashine) were selected from the experiment 1 and Fomesa (coating used by packing houses) were further studied by commercial coating method.

Tangerine fruit were coated by commercial method and divided into 4 different treatments as follow;

T1 = Fruit coated with Zivdar
T2 = Fruit coated with Fomesa
T3 = Fruit coated with Citrashine
T4 = Non-coated fruit

The coated tangerines were stored at room temperature (24±3°C) and 59±6% relative humidity or 5±2°C and 85±3% relative humidity.

Table 3.1 Coating materials, main components, and their sources

Name of commercial coating	Main components	Source of products
Citrashine	shellac-based wax formulated with purified natural secretion and water-emulsifying agents	Citrashine (Pty) Ltd., South Africa
Fomesa	10% oxidized polyethylene wax, 8% glycerol ester of wood rosin and 2% ammonium hydroxide	Fomesa Fruitech, S.L., Spain
Citrosol AK	18% w/v carnauba and rosin	Productos Citrosol, S.A., Spain
Supershine-C	18% w/v waxes, modified gum, rosin, oxidized polyethylene, and adjuvants	Tecnidex, Spain
Zivdar	18% w/v waxes, shellac, polyethylene wax, and imazalil	Safepack Products Ltd., Israel
Perfect Shine	carnauba wax, natural resin, fatty acid, fatty alcohol, ammonia	P.S. Wax Tech, Co. Ltd., Thailand
Sealkote	-	-
Rosy Plus	-	-
Wax (unknown)	-	-
polyethylene microemulsion	18.3% polyethylene wax, 4.66% oleic acid, morpholine and 80 ml H_2O	Prepared in laboratory
1.5 and 2.0% chitosan	1.5 or 2.0% chitosan in 1% citric acid	Prepared in laboratory

Components of commercial coatings were declared on the product labels

Experiment 3 Screening of developed coating materials for tangerine fruit cv. 'Sai Nam Phueng'

Tangerines were coated with variation of developed coating materials and stored at room temperature. This experiment was divided into 5 phases. The chemicals for preparation of coating materials were shown in Table 3.2. The

compositions and preparations of the coating materials were shown in Table 3.3 and 3.4. Figure 3.1, 3.2, 3.3, 3.4 and 3.5 were shown the developed coating materials.

Table 3.2 Chemicals for preparation of coating materials

Chemicals	Source of products
Zein from maize	Sigma-Aldrich Chemical Co., Missouri, USA
Polyethylene wax	Honeywell Specialty Chemicals, New Jarsey, USA
Polyethylene wax: IMERZOL OPE-35 M	Syntec Additive Co, Ltd., Thailand
Polyethylene: High density polyethylene (HDPE)	Syntec Additive Co, Ltd., Thailand
Carnauba wax: CAWAX-201	Syntec Additive Co, Ltd., Thailand
Candelillac wax	Strahl & Pitsch, New York, USA
Shellac wax	EXCELACS CO., LTD. , Bangkok, Thailand
Shellac wax	Gammaco, Bangkok, Thailand
Gum arabic	Sigma-Aldrich Chemical Co., Missouri, USA
Propylene glycol : Propane-1,2-Diol	Unilab, Auckland, New Zealand
Isopropanol : Propan-2-OL	Lab-Scan, Bangkok, Thailand
99.9% Ethanol	Merck, Darmstadt, Germany
Morpholine	RFCL Limited, New Delhi, India
Citric acid	Univar, New South Wales, Australia
Ammonia	J.T. Baker, New Jersey, USA
Oleic acid	Panreac, Barcelona, Spain
Chitosan	from crab carapace

Table 3.3 Compositions and preparations of the coating materials in experiment 3

Coating materials	Preparation methods
20% polyethylene	57.14 ml of 35% polyethylene (IMERZOL OPE-35M) plus 45.86 ml of water
18% polyethylene	51.43 ml of 35% polyethylene (IMERZOL OPE-35M) plus 51.43 ml of water
17.5% polyethylene + 0.5% shellac	50 ml of 35% polyethylene (IMERZOL OPE-35M) plus 10 ml of 5% shellac-ethanol solution, and plus 40 ml of water
17% polyethylene + 1% shellac	48.57 ml of 35% polyethylene (IMERZOL OPE-35M) plus 20 ml of 5% shellac-ethanol solution, and plus 31.43 ml of water
16% polyethylene + 2% shellac	45.71 ml of 35% polyethylene (IMERZOL OPE-35M) plus 40 ml of 5% shellac-ethanol solution, and plus 14.29 ml of water
15% polyethylene + 1% shellac	42.86 ml of 35% polyethylene (IMERZOL OPE-35M) plus 20 ml of 5% shellac-ethanol solution, and plus 37.14 ml of water
14% polyethylene + 1.54% shellac	40 ml of 35% polyethylene (IMERZOL OPE-35M) plus 40 ml of 5% shellac-ethanol solution, and plus 50 ml of water
15% polyethylene + 8% zein	75 ml of 50 ml of 20% polyethylene (IMERZOL OPE-35M) plus 25 ml of 8% zein solution
16% polyethylene + 1% gum arabic	80 ml of 50 ml of 20% polyethylene (IMERZOL OPE-35M) plus 20 ml of 20% gum arabic solution
19% polyethylene + 5% glycine	95 ml of 50 ml of 20% polyethylene (IMERZOL OPE-35M) plus 5 ml of 100% glycerine
18% carnauba	69.23 ml of 26% carnauba (CAWAX-201) plus 30.77 ml of water
17.5% carnauba + 0.50% shellac	67.31 ml of 26% carnauba (CAWAX-201) plus 10 ml of 5% shellac-ethanol solution, and plus 22.69 ml of water
17% carnauba + 1% shellac	65.35 ml of 26% carnauba (CAWAX-201) plus 20 ml of 5% shellac-ethanol solution, and plus 14.65 ml of water
16% carnauba + 2% shellac	61.53 ml of 26% carnauba (CAWAX-201) plus 38.46 ml of 5% shellac-ethanol solution, and plus 50 ml of water

Table 3.3 (continued) Compositions and preparations of the coating materials in experiment 3

Coating materials	Preparation methods
15% carnauba + 1% shellac	57.69 ml of 26% carnauba (CAWAX-201) plus 20 ml of 5% shellac-ethanol solution, and plus 22.31 ml of water
14% carnauba + 1.54% shellac	53.85 ml of 26% carnauba (CAWAX-201) plus 40 ml of 5% shellac-ethanol solution, and plus 36.15 ml of water
6% candelilla microemulsion + 14% polyethylene	30 ml of 20% candelilla microemulsion plus 70 ml of 20% polyethylene (IMERZOL OPE-35M)
8% candelilla microemulsion + 12% polyethylene	40 ml of 20% candelilla microemulsion plus 60 ml of 20% polyethylene (IMERZOL OPE-35M)
10% candelilla microemulsion + 10% polyethylene	50 ml of 20% candelilla microemulsion plus 50 ml of 20% polyethylene (IMERZOL OPE-35M)
zein solution (4, 8, and 12%)	4, 8 or 12 gram of zein was dissolved in 35% ethanol plus 35% isopropanol-water solution (by volume: 36.8 ml of 95% ethanol, 35 ml of 100% isopropanol, and 8.2 ml of water), propylene glycol (PG) was added as a plasticizer which resulted in glossy zein-based coatings
20% gum arabic	20 gram of gum arabic (Siagma, Missouri, USA) was dissolved in 100 ml water
2% chitosan	2 gram of chitosan from crab carapace was dissolved in 1% citric acid solution
1% chitosan + 1% oleic acid	100 ml of 1% chitosan (dissolved in 1 citric acid solution) plus 1 ml of oleic acid
1% chitosan + 2% oleic acid	100 ml of 1% chitosan (dissolved in 1 citric acid solution) plus 2 ml of oleic acid

Table 3.3 (continued) Compositions and preparations of the coating materials in experiment 3

Coating materials	Preparation methods
1% chitosan + 3% oleic acid	100 ml of 1% chitosan (dissolved in 1 citric acid solution) plus 3 ml of oleic acid
1% chitosan	1 gram of chitosan from crab carapace was dissolved in 1 citric acid (Univar, New South Wales, Australia) solution

Table 3.4 Groups of coating materials in experiment 3

Group of coating materials	Coating materials	Storage condition	Duration (days)
Group A	4% zein 8% zein 12% zein 18% polyethylene 17% polyethylene + 1% shellac 16% polyethylene + 2% shellac	24±3°C and 74±7% relative humidity	12
Group B	18% carnauba 17% carnauba + 1% shellac 16% carnauba + 1.28% shellac 18% polyethylene 17% polyethylene + 1% shellac 8% zein	21±3°C and 72±6% relative humidity	15
Group C	15% carnauba + 1% shellac 14% carnauba + 1.54% shellac 17.5% carnauba + 0.5% shellac 15% polyethylene + 1% shellac 14% polyethylene + 1.54% shellac 17.5% polyethylene + 0.5% shellac	22±3°C and 73±6% relative humidity	15

Table 3.4 (continued) Groups of coating materials in experiment 3

Group of coating materials	Coating materials	Storage condition	Duration (days)
Group D	15% polyethylene + 2% zein 16% polyethylene + 1% gum arabic 19% polyethylene + 5% glycerine 17.5% polyethylene + 0.5% shellac 20% gum arabic 8% zein 2% chitosan (in 1% citric acid) 20% polyethylene	20±4°C and 67±7% relative humidity	12
Group E	6% candelilla + 14% polyethylene 8% candelilla + 12% polyethylene 10% candelilla + 10% polyethylene 1% chitosan + 1% oleic acid 1% chitosan + 2% oleic acid 1% chitosan + 3% oleic acid 1% chitosan 20% candelilla 20% polyethylene	(27±4°C) and 57±8% relative humidity	9

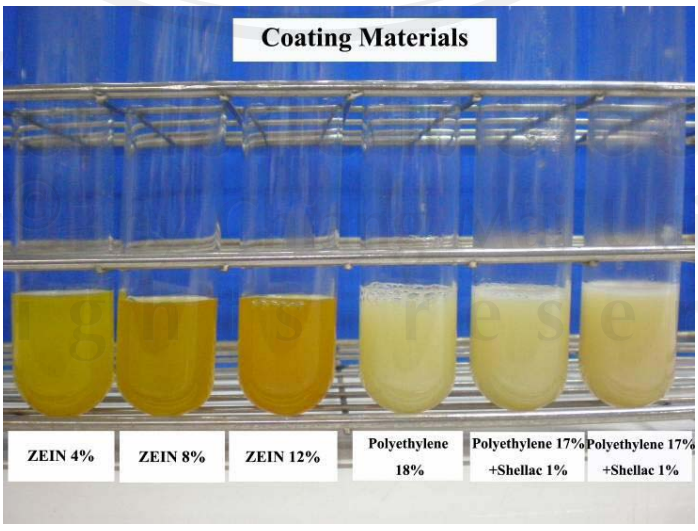

Figure 3.1 Characteristics of 6 developed coating materials (Group A) in experiment 3

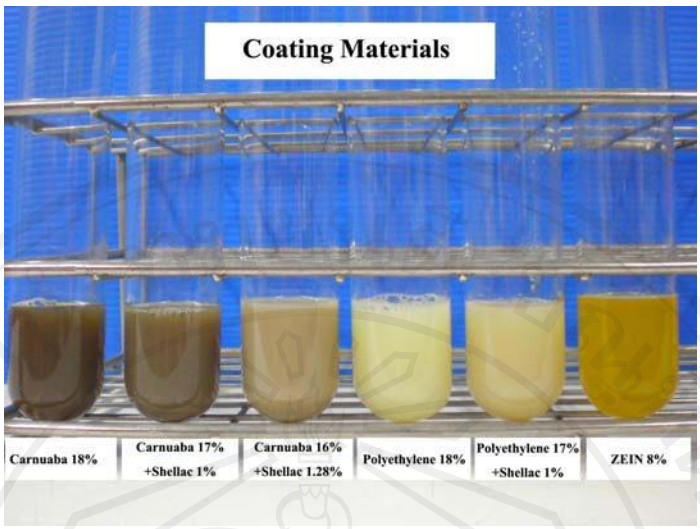

Figure 3.2 Characteristics of 6 developed coating materials (Group B) in experiment 3

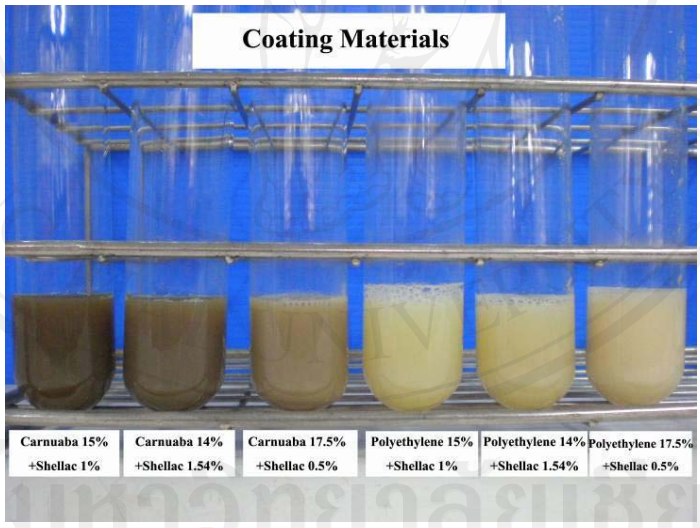

Figure 3.3 Characteristics of 6 developed coating materials (Group C) in experiment 3

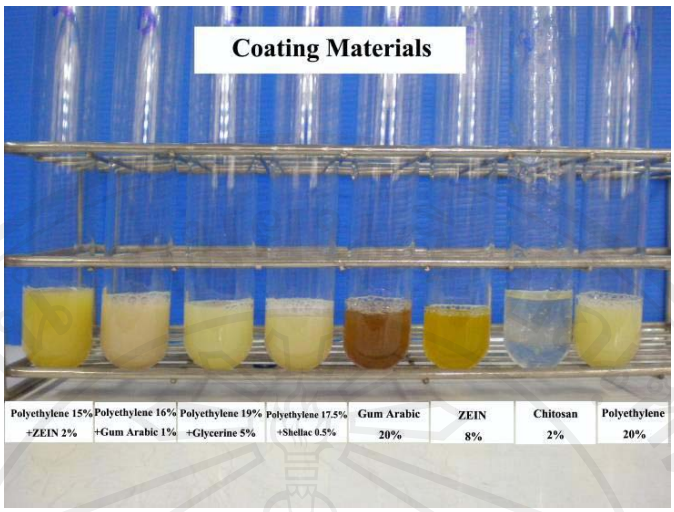

Figure 3.4 Characteristics of 8 developed coating materials (Group D) in experiment 3

Figure 3.5 Characteristics of 9 developed coating materials (Group E) in experiment 3

Experiment 4 Comparison of 4 developed coating materials and commercial coating (Zivdar) on the physico-chemical and physiological characters of tangerine fruit

The four best treatments were selected from experiment 3, with a little modification in the coating materials such as polyethylene microemulsion and shellac microemulsion. Coatings were applied manually with four developed coatings and Zivdar. This experiment was divided into six different treatments as follow;

T1 = Formulation A (8% candelilla microemulsion + 12% polyethylene)

T2 = Formulation B (17.5% polyethylene + 0.5% shellac)

T3 = Formulation C (17.5% polyethylene + 0.5% shellac microemulsion)

T4 = Formulation D (17.5% polyethylene microemulsion + 0.5% shellac microemulsion)

T5 = Zivdar

T6 = Non-coated fruit (control)

The tangerines were stored at room temperature (27±3°C) and 56±11% relative humidity.

The compositions and preparations of the 4 developed coating materials as shown in Table 3.5 and Figure 3.6.

Table 3.5 Compositions and preparations of the developed coating materials in experiment 4

Coating materials	Preparation methods
Formulation A (8% candelilla microemulsion + 12% polyethylene)	40 ml of 20% candelilla microemulsion plus 60 ml of 20% polyethylene (IMERZOL OPE-35M)
Formulation B (17.5% polyethylene + 0.5% shellac)	87.5 ml of 20% polyethylene (IMERZOL OPE-35M) plus 5 ml of 5% shellac-ethanol solution, and plus 2.5 ml of water
Formulation C (17.5% polyethylene + 0.5% shellac microemulsion)	87.5 ml of 20% polyethylene (IMERZOL OPE-35M) plus 2.23 ml of 22.44% shellac microemulsion, and plus 10.27 ml of water
Formulation D (17.5% polyethylene micro-emulsion + 0.5% shellac micro-emulsion)	92.11 ml of 19% polyethylene microemulsion plus 2.23 ml of 22.44% shellac microemulsion, and plus 5.66 ml of water

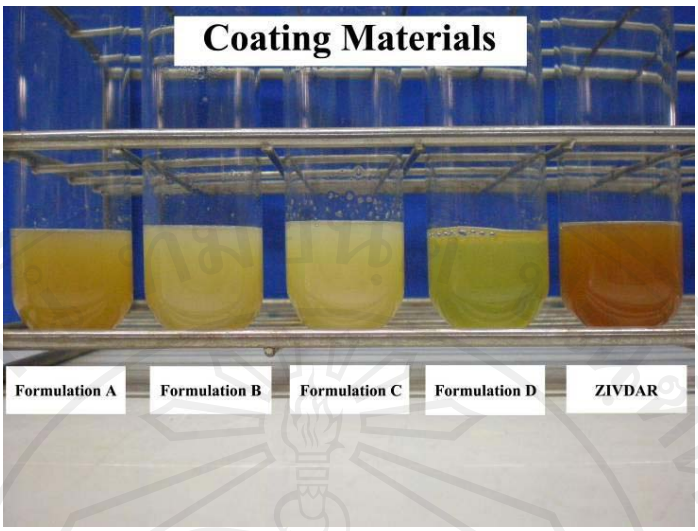

Figure 3.6 Characteristics of 4 developed coating materials and Zivdar that provide in experiment 4

Experiment 5 Scanning electron microscope (SEM) observation and permeability of coatings

9 commercial and 4 developed coating materials were studied by mean of their coating characteristics on tangerine fruit surface using scanning electron microscope (SEM) observation.

Zivdar and 17.5% polyethylene microemulsion + 0.5% shellac microemulsion were investigated their O_2 and water vapor permeability.

3.3 Coating application

3.3.1 Manual method

Tangerine fruit were cleaned with dried cotton cloth and coated with different coating materials, and non coated fruit were used as control. Coatings were applied manually (average 0.2 g of wax/fruit), spread evenly over the fruit surface using latex gloved hands, and air dried at room temperature (Figure 3.7 and 3.8). The tangerines were packed in cardboard boxes and stored at room temperature or low temperature.

Figure 3.7 Coating application by manual method

Figure 3.8 Appearance of coated tangerine fruit with coating materials and non-coated fruit

3.3.2 Commercial practice in packing house

Tangerine fruit were harvested at commercial maturity and selected for defect-free. Fruit were washed with water and rotating on soft brush. Fruit surfaces were dried by hot air (45°C) before coating with commercial coatings (Zivdar, Fomesa and Citrashine) then dried again by hot air (40°C), and non-coated fruit were used as control (Figure 3.9). Fruit were packed in carton box and transported by truck (~3 hours) to the Postharvest Horticultural Laboratory, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University. The tangerines were repacked in cardboard boxes and stored at room temperature or low temperature.

Figure 3.9 Cleaning and coating application by commercial method

3.4 Quality evaluation

3.4.1 Determination of weight loss: Weight loss was determined with samples of ten fruit per treatment. The fruit from each treatment were weighed on day 0, 1, 4, 7, 10 and 13 of storage. All treatments were weighed using a balance (EK-600H, Sartorius, USA). The percentage of weight loss was calculated from the difference between the initial and final weight, using the following equation:

$$\%\text{Weight loss} = \frac{(W_1 - W_2) \times 100}{W_1}$$

Where: W_1 = initial weight

W_2 = final weight

3.4.2 Measurement of the gloss: Reflectance measurements of fruit shine were measured in gloss units (G.U.) with the micro-TRI-gloss reflectance meter (BYK Gardner Inc., Silver Spring, MD, USA) fitted with a shield having 19 mm diameter hole (Figure 3.10). For each experiment, ten measurements per fruit were made on each of ten numbered tangerine on day 1 (Hagenmaier and Baker, 1994b).

Figure 3.10 Micro-TRI-gloss reflectance meter (BYK Gardner Inc., MD, USA)

3.4.3 Determination of internal O_2 and CO_2 contents: Ten fruit were used per treatment for each coating. The internal gas was withdrawn by a syringe (previously flushed with helium gas to remove oxygen) with the needle inserted through the blossom end into the internal space of fruit submerged in water (Hagenmaier, 2001). The O_2 and CO_2 concentrations were measured with a gas chromatograph (Model GC-8A, Shimadzu, Japan) equipped with a thermal conductivity detector, fitted with a CTR-1 column (2 m × 6 mm o.d.) (Alltech, Deerfield, IL., USA), consisting of an outer column (Parapak Type N; 80-100 Mesh, Shimadzu, Tokyo, Japan). The column temperature was at 65°C and the thermal conductiving detector was at 110°C. Helium was used as the carrier gas at a flow rate of 150 ml/min. Peak areas obtained from standard gas mixtures and were determined before and after analysis of samples. Oxygen concentration was calculated from the O_2-Ar peak area after correction for 0.9% Ar in atmosphere (Hagenmaier, 2001).

3.4.4 Measurement of respiration rate: Respiration rates were measured every three days, 8 fruit (about 1 kg) were kept in plastic chamber (17.5 × 27.0 × 11.5 cm^3), with continuous air flow (100 ml/min) at 23±3°C. A 1 ml gas sample was withdrawn with a plastic-tight syringe and analyzed for CO_2 by gas chromatograph (Model GC-8A, Shimadzu, Japan) fitted with a Parapak Type N (80-100 Mesh, Shimadzu, Tokyo, Japan), consisting of an outer column (CTR-1 column; 2 m × 6 mm o.d., Alltech, Deerfield, IL., USA) and helium was used as a carrier gas. The column temperature was at 65°C and the thermal conductive detector was at 110°C. The respiration rate was expressed as milligrams of CO_2 per kilogram of fruit per hour (mg CO_2/kg/hr).

3.4.5 Determination of ethanol content: Ethanol in tangerine juice was measured using ethanol assay kit (Diagnostic Chemical Limited, Charlottetown, Canada) as described by Bonnichsen and Theorell (1951). The pooled juice of ten fruit was extracted using a juice maker. Ten microliters of juice was mixed with 1.5 ml of buffer-NAD-ADH-buffer mixture and incubated for 20 minutes at 25±2°C. The

absorbance was measured at 340 nm within 30 min. The concentration of ethanol was calculated from a standard curve. Ethanol was measured in triplicate determinations.

3.4.6 Determination of acetaldehyde: Frozen tangerine juice was thawed and a 5 ml was put in 50 ml screw-cap test tube, then homogenized with n-butanol (Sigma, Missouri, USA) and 40% methanol (Sigma, Missouri, USA), which was closed with a plastic cap. The solution was filtered with syringe filter (0.45 μm, Sartorius, Goettingen, Germany). A headspace sample was taken with a 1 ml glass syringe for determining acetaldehyde concentration using a gas chromatograph (6890N, Agilent Technologies, California, USA) equipped with a flame ionization detector (FID) and a capillary column HP-innowax (30 m × 0.25 mm × 0.25 micron, Agilent Technologies, California, USA) The helium was used as the carrier gas. The injector and detector temperatures were 200 and 250°C, respectively (Guzel-Seydim *et al*., 2000).

3.4.7 Enzyme extraction

For each replicate, 5 g of tissue was obtained from five tangerines (3 segments per fruit) and homogenized in 10 ml of 100 mM 2-(N-morpholino) ethane-sulfonic acid (MES) buffer (Fluka, Lyon, France) (pH 6.5) containing 2 mM dithithreitol (Fluka, Lyon, France) and 1% (w/v) polyvinylpyrolidone (Fluka, Lyon, France). The homogenate was centrifuged at 12,000 × g for 20 min at 4°C (Centrifuge, Universal 32 R, CE, Wisconsin, USA). The supernatant was decanted and set on ice as crude enzyme extract (Ke *et al*., 1994).

3.4.8 Enzyme assays and protein determination

3.4.8.1 Pyruvate decarboxylase (PDC) activity: PDC activity was assayed through coupling with ADH reaction by mixing 0.45 ml of 100 mM MES buffer (pH 6.5), 0.1 ml of 5 mM thiamine pyrophosphate (Sigma-Aldrich, Missouri, USA), 0.1 ml of 50 mM $MgCl_2$ (Merck, Darmstadt, Germany), 0.05 ml of 1.6 mM NADH (Fluka, Lyon, France), 0.1 ml of commercial ADH solution (containing 13.5 enzyme units) (Sigma-Aldrich, Missouri, USA), 0.1 ml of 50 mM pyruvate (Fluka, Lyon,

France), and 0.1 ml of enzyme extract. PDC oxidation was measured by recording the decrease in absorbance at 340 nm over time using a spectrophotometer (Thermo Spectronic, Model Genesys 10UV-Scanning, CE, USA). Enzyme activities were expressed as unit mg/protein (Ke *et al*., 1994).

3.4.8.2 Alcohol dehydrogenase (ADH) activity: ADH activity was measured by mixing 0.8 ml of 100 mM MES buffer (pH 6.5), 0.05 ml of 1.6 mM NADH (Fluka, Lyon, France), 0.1 ml of crude enzyme extract, and 0.05 ml of 80 mM acetaldehyde (Riedel-de Haen, Hanover, Germany). ADH, NADH oxidation was measured by recording the decrease in absorbance at 340 nm over time (5 min) using a spectrophotometer (Thermo Spectronic, Model Genesys 10UV-Scanning, CE, Wisconsin, USA). Enzyme activities were expressed as unit/mg protein (Ke *et al*., 1994). One unit of enzyme activity was defined as the amount of the enzyme, which caused a change of 0.001 in absorbance per minute.

3.4.8.3 Protein determination: Soluble protein content was detected by calculating specific enzyme activity using the method described by Bradford (1976). The sample were measured at 595 nm using spectrophotometer (Thermo Spectronic, Model Genesys 10UV-Scanning, CE, Wisconsin, USA) and protein concentrations was determined for each sample with a bovine serum albumin (BSA) (Sigma-Aldrich, Missouri, USA) standard curve.

3.4.9 Sensory evaluation

3.4.9.1 Estimation of flavor

Ten untrained panelists (7 females, 3 males, aged between 25 and 31 years) evaluated flavor of tangerine fruit by tasting, using a score of 1 to 4 where 4 = excellent, 3 = slightly off-flavor, 2 = moderately off-flavor and 1 = extremely off-flavor. Fruit taste was rated “unacceptable” when the score was below 3.

3.4.9.2 Fruit visual appearance

Tangerine fruit were rated for visual quality, wilting and shriveling, using a scale of 1 to 5 in which 5 = excellent, 4 = good, 3 = fair, 2 = poor and 1 = unusable. Fruit appearance was rated “unacceptable” when the score was below 3.

3.4.10 Measurement of peel color: Tangerine fruit peel color was measured with a Chroma meter (Model CR-300, Minolta, Tokyo, Japan). Ten fruit were used for each treatment. Each fruit was marked (middle of fruit) by a pen on the peel (2 positions) before measuring peel color. Fruit were measured on subsequent occasions at the same spot (as far as was possible). Tangerine color was obtained coordinates, CIE 1976 (L*, a*, b*). CIE refers to the Commision Internationale de I'Éclairage (1978). In the CIE 1976 (L*, a* and b*) color space, abbreviated CIBLAB, the lightness coefficient, L*, ranges from black (0) to white (100). The a* value is positive for red and negative for green. The b* value is positive for yellow and negative for blue. A more appropriate measure of color can be obtained chroma (C*) and hue angle (H°), and index somewhat analogous to color saturation or intensity. The hue angle should remain positive between 0° and 360° of the color wheel (0° = purple-red, 90° = yellow, 180° = blue-green, 270 = blue) (McGuire, 1992) (Figure 3.11).

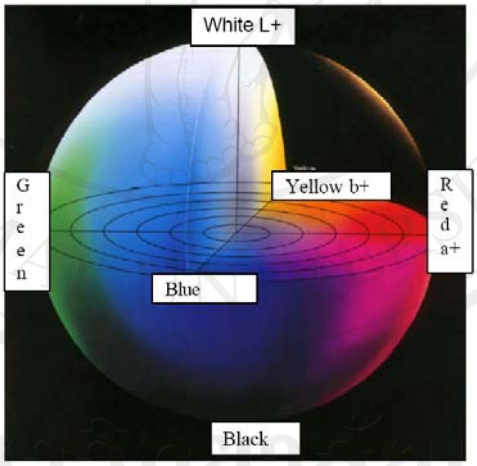

Figure 3.11 Representation of a color solid for L* a* b* color space (Minolta, 1994)

3.4.11 Measurement of total soluble solids (TSS): Three fruit of three replications per treatment were squeezed with a hand-press juicer. The juice was measured for total soluble solids content with a digital refractometer (Model PR-101, Atago, Tokyo, Japan). The values were expressed in percentage of total soluble solids.

3.4.12 Measurement of titratable acidity (TA): Titratable acidity (TA) was determined by diluting 10 ml of fruit juice to 100 ml with distilled water and titrated with 0.1N NaOH (Univar, New, South Wales, Australia) to a pH end point of 8.2 using a pH meter (Model CG842, Schott, Hofheim, Germany). Each treatment was replicated three times. TA was expressed as percent citric acid per 100 ml fruit juice, using the following equation:

$$\%TA = \frac{\text{normality of NaOH (0.1 N)} \times \text{equi.wt. of citric acid (0.070)} \times \text{vol. NaOH} \times 100}{\text{volume of sample}}$$

3.4.13 Calculation of TSS/TA ratio: The ratios of TSS to TA were calculated as the average of the ratios.

3.4.14 Measurement of pH: The pH of diluted juice (1 : 9) was measured by pH-Meter (Model CG 842/14 pH, Schott, Hofheim, Germany) previously calibrated with buffer solutions of pH 4.0 and 7.0. The pH measurement was done for 3 replicates per treatment and the value was registered once it had stabilized.

3.4.15 Measurement of ascorbic acid: Ascorbic acid content was determined by 2, 6-dichlorophenol-indophenol titration method by standardizing 0.04% 2, 6-dichlorophenol-indophenol dye solution against 0.1% ascorbic acid solution. Transfer three 1.0 ml aliquots ascorbic acid standard solution to each of three 50 ml Erlenmeyers flask. Titrate rapidly with 2, 6-dichlorophenol indophenol dye solution until light but distinct rose pink ≥ 15 seconds.

Ascorbic acid content was estimated by diluting 10 ml of juice with 90 ml of 0.4% oxalic acid (Univar, New South Wales, Australia). Mix thoroughly by shaking to ensure uniform test portion, and filter through filter paper Whatman® No.1 (Whatman Internaltional Ltd., Maidstone, England). Titrate 3 test solution aliquots each treatments until light but distinct rose pink ≥ 15 s. The results were expressed in milligrams of ascorbic acid per 100 ml fruit juice (Ranganna, 1986).

3.4.16 Scanning electron microscope (SEM) observation

Sample preparation and SEM observation

Tangerine peel (0.5 cm × 1.0 cm) was fixed in a primary fixative solution for anatomical preparation as described by Bozzola and Russell (1999) with some modification. The peel specimens were fixed with primary fixative containing 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 at 4°C for 2 hours and post-fixed in the same buffer for 2 hours. Then, the specimens were dehydrated stepwise by exposure to ethanol-buffer mixture (30, 50, 70, 80, 90, and 100%) allowing 15 minutes in each, and critical point dried (CPD) with liquid CO_2. For Scanning electron microscope (SEM), the dried specimen was mounted on specimen studs and sputter coated with gold. Coated samples were stored in a desiccator until assessed. Finally, the specimens were viewed with a SEM (JEOL, JSM-5410LV, JOEL Ltd., Tokyo, Japan) at 5 kv.

3.4.17 Permeability of coatings

3.4.17.1 Determination of oxygen permeability

The liquid coating was brushed onto plastic films of known high permeability (cast polypropylene film (CPP)). The coating thickness for each sample was measured with a micrometer caliper. A typical coating was 25 μm thick. A film specimen with 14 cm diameter was fixed between the upper and lower chambers. Oxygen in both chambers was removed under vacuum for 8 hours. After 8 hours, oxygen was flowed into the upper chamber. The amount of oxygen that permeated through the film in the lower chamber then determined. The test was done at 23°C and 0% relative humidity. Oxygen permeability was determined with oxygen permeation analyzer (Illinois 8000, Illinois instruments, Illinois, USA). Samples analyzed according to ASTM method D-3985-02 (ASTM, 2002).

3.4.17.2 Determination of water vapor permeability

The liquid coating was brushed onto kraft paper which known high permeability. The coating thickness for each sample was measured with a micrometer caliper. A typical coating was 308 μm thick. The humidity of the chamber was kept at 90% and 38°C. The water vapor permeance of coated films was measured with the

water vapor permeability tester (Lyssy L80-4000, Zollikon, Switzerland) according to ASTM E398 (ASTM, 2000).

3.4.18 Statistical Analysis

The experimental design of modified atmosphere packaging test was a completely randomized design (CRD) with factorial arrangement. CRD was used in the experiments 1, 2, 3 and 4. One-way analysis of variance was used to determine treatment effects and comparisons were made at $P \leq 0.05$ using the least significant difference test to separate means.

The experiment 1 (phase 2) was a factorial 3×8, in which the factors were storage temperatures and coating materials. The data were subjected to ANOVA tests. The difference between means was determined by Tukey's multiple range test at a 95% confidence interval. The statistical analysis was performed with SPSS version 6 (SPSS software; SPSS Inc., Chicago, IL).