Chapter 3

Materials and Methods

Plant material

Mature mango fruit (*Mangifera indica* Linn. cv. Nam Dok Mai Si Thong) were harvested from Siyud Orchard (a commercial fruit grower), Chachoengsao province, Thailand. All fruits were transported to laboratory of postharvest technology institute, Chiangmai University. Fruits were selected for uniformity of shape, color and size, and blemished or diseased fruits were discarded.

3.1 Determine thermal properties and chemical composition of mango fruit cv Nam Dok Mai Si Thong.

3.1.1 Measurement of thermal properties

3.1.1.1 Measurement of specific heat and thermal conductivity

The specific heat was measured by Modulated Differential Scanning Calorimeter (MDSC model Q100 , TA instrument). Sample was prepared of middle pulp mango by 4.20 mm-diameter cork borrer. The cylindrical tissue sample in the cork borer was cut into small pieces by a razor blade to obtain 4.20 mm-diameter, <1 mm length. The sample disc was placed into hermatic aluminium pan and weight (10-11 mg) using a micro-balance. The sample containing the pulp mango tissue disc was sealed with the DSC sample sealer. The sealing procedure was completed with in 30 second to prevent serve moisture loss from the mango tissue. The encapsulated test sample was placed on the sample sensor. Use an empty hermatic aluminium pan and lid on the reference side. Measure the heat capacity of sample at 25 °C for 20 min. Record the specific heat (*Cp*) in unit of J/g °C. Created the following method:

1) Data storage OFF
2) Equilibrate at 25°C
3) Modulate ±0.5°C every 80 seconds
4) Data storage ON
5) Isothermal for 20 minutes

The thermal conductivity was measured by MDSC followed the method of ASTM E-1952-98 (ASTM, 1998). Thick sample was prepared of middle pulp mango by 6.0 mm-diameter cork borer, <4 mm length (Figure 3.1). Measure and record the diameter (d), length (L) and weight of the sample. Place a small drop of silicone oil on the DSC sample and reference sensor. Place a thin aluminum disk over each drop of oil. Carefully place the thick sample on the aluminum disk covering the sample sensor. Measure the apparent heat capacity of the sample at 25°C for 20 min. Record the apparent heat capacity (C) in the unit of mJ/ °C. Created the following method:

1) Data storage OFF
2) Equilibrate at 25°C
3) Modulate ±0.5°C every 80 seconds
4) Data storage ON
5) Isothermal for 20 minutes

The method was repeated by thin sample which prepared same as sample for measure specific heat. Record the heat capacity (Cp) in the unit of J/g °C. Calculate the experimental thermal conductivity of the polysterene sample using the following equation:

$$K_0 = \frac{\left(8LC^2\right)}{\left(C_p M d^2 P\right)} \qquad (3.1)$$

Where: L = sample length (mm)

C = apparent heat capacity (mJ/°C)

C_p = specific heat capacity (J/g°C)

M = thick specimen mass (mg)

d = thick specimen diameter (mm)

P = period of measurement (sec)

Calculate the calibration constant using the following equation:

$$D = (K_0 \times K_r)^{0.5} - K_r \tag{3.2}$$

Where: K_o = uncorrected thermal conductivity (W/m°C)

K_r = reference thermal conductivity (Polysterene)

Calculate the thermal conductivity of sample (W/m°C) by equation:

$$K = \left[K_0 - 2D + (K_0^{\,2} - 4DK_0)^{0.5}\right]/2 \tag{3.3}$$

Thermal properties from mango peel was prepared as described above. Six replications from 6 fruits were taken for each experiment and then the mean values were reported.

3.1.1.2 Measurement of density

Density of mango peel and flesh were measured by pycnometer (Figure 3.2). Volume of a sample can be measured by direct measurement of volume liquid displaced:

(i) determine the exact capacity of the pycnometer by weighing it when empty and likewise with distilled water.

(ii) Ten to fifteen mg of samples were placed in pycnometer. Density of sample were calculated from the equation:

$$\rho_s = \frac{m_s \times \rho_w}{m_A + m_s - m_B} \tag{3.4}$$

Where: ρ_s = density of sample (kg/m^3)

ρ_w = density of water at temperature (°C) (kg/m^3)

m_s = mass of sample in pycnometer (kg)

m_A = mass of pycnometer and water at temperature (°C)

m_B = mass of pycnometer, sample and water (kg)

Six replications from 6 fruits were taken for each experiment and then the mean values were reported.

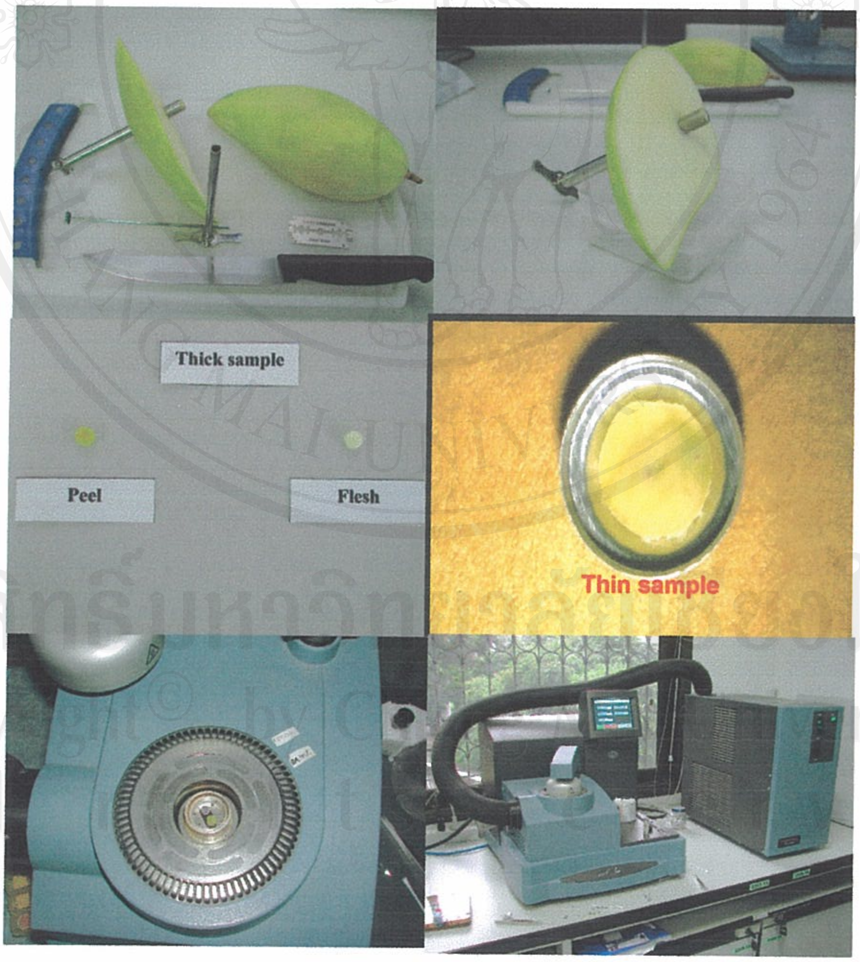

Figure 3.1 Sample preparation for determined specific heat and thermal conductivity of mango fruit.

Figure 3.2 Measurement of density by pycnometer.

3.1.1.4 Calculate thermal diffusivity

Thermal diffusivity is calculated by equation:

$$\alpha = \frac{k}{\rho_s C_p} \tag{3.5}$$

Where: α = thermal diffusivity (m^2/s)

k = thermal conductivity (W/m°C)

ρ_s = density of sample (kg/m^3)

C_p = specific heat (J/g°C)

3.1.2 Measurement of chemical properties

3.1.2.1 Moisture content

The oven drying method was used to determine the moisture content of mango peel and flesh. This method consisted of measuring the weight lost by fruits due to the evaporation of water. In fruit products containing an appreciable proportion of sugar, Ranganna (1977) suggested that it is advisible to dry at lower drying temperatures of 60 to 70 °C.

Ten to fifteen grams of each replication was spread over in a moisture can. The can was covered and then weighed as quickly as possible using an analytical balance in order to avoid moisture loss. The cover was removed and the mango samples dried in a hot air oven maintained at a temperature of 70 °C and at atmospheric pressure for heating duration of 24 hours. After drying the lid was replaced, the sample was cooled in a dessicator, and re-weighed. The moisture content was calculated as a percentage of wet basis.

$$\%\ moisture\ content\left(wet\ basis\right)=\frac{\left(w_t-w_t\right)}{w_{ii}}\times 100 \qquad (3.6)$$

When

w_t = weight of sample after drying (g)

w_i = initial weight (g)

3.1.2.2 Protein content

Sample preparation: peel and flesh were dried by vacuum oven at 70°C for 24 hours. Cool down in desicator. Chemical properties of peel and flesh mangos were measured by proximate analysis. Protein in mango flesh will be measured by Kjeldahl method (AOAC, 2000). The Kjeldahl method determines the total nitrogen present as -NH-in the food, i.e. true protein N, amino N and amide N. This is then converted into protein by multiplying this percentage of nitrogen by an appropriate conversion factor 100/X, where X is the percentage of nitrogen in the food protein.

Peel or flesh was weighed accurately 3-5 g and transfer to a digestion tube. Add 10.0 g of K_2SO_4:$CuSO_4$ (10:1) and 20 ml of concentrated sulphuric acid. Place the tube in the preheated digester at 400°C for about 2 hours or until a clear solution is obtained. After digestion, remove the tubes from the digester, cool and diluted sample in the distillation unit. Place a conical flask containing 40 ml of 4% boric acid (containing indicator) under the condenser outlet. Dispense the alakali (50 ml of 32% NaOH) and distil for 4 min. Titrate the ammonium borate solution formed with either 0.1 N sulphuric acid to a purplish-grey end-point (Fig 3.3)

Calculate the nitrogen content and hence the protein content of the sample given that:

$$\% \, Nitrogen = \frac{0.14 \times A \times B}{weight \; of \; sample \; in \; grams} \tag{3.7}$$

And

$$\% \, protein \; in \; sample = \% \, Nitrogen \; x \; conversion \; factor \tag{3.8}$$

where conversion factor for fruits is 6.25

3.1.2.3 Ash

Peel or flesh was weighed accurately 5-10 g into ignited and tared silica dish. Place in muffle furnace (CARBONITE; CWF 1100, England) at 525°C for 24 hours (free from carbon). Cool in dessicator and weigh.

Calculation

$$\% \; Ash = \frac{ash \; wt.(g.)}{sample \; wt.(g.)} \times 100 \tag{3.9}$$

Figure 3.3 Protein Analyzer (Gerhardt; Germany)

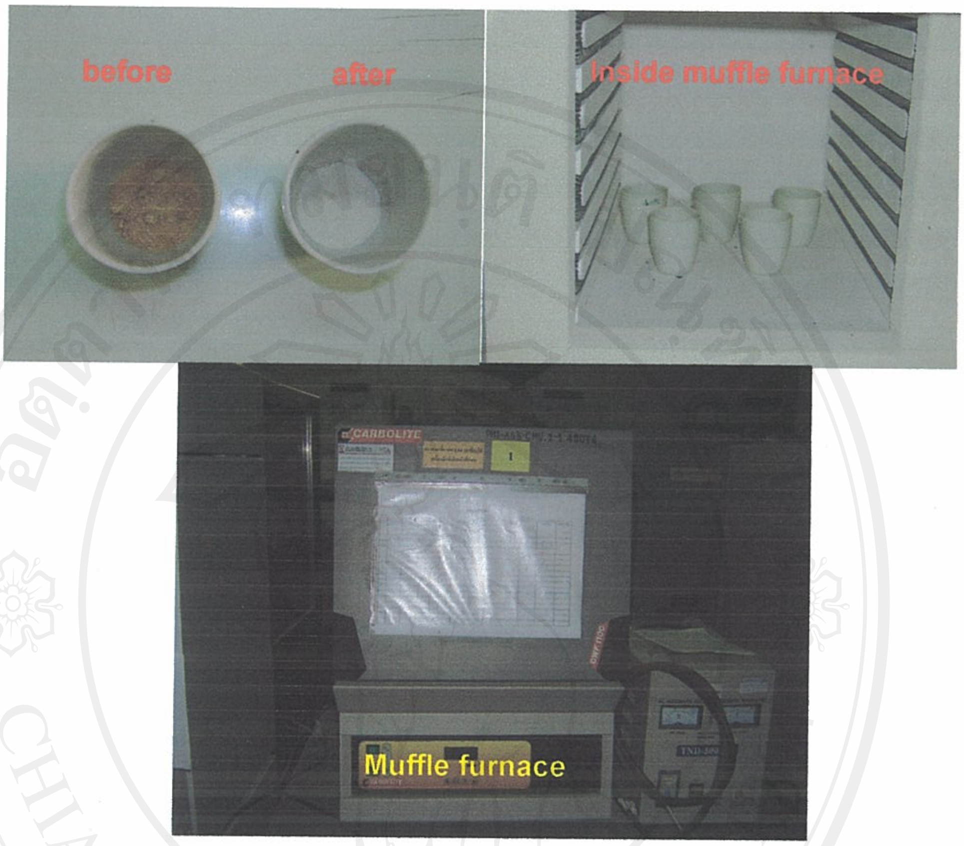

Figure 3.4 Muffle furnace

3.1.2.4 Fat content

Five grams of dried sample was weighed on filter paper and enclose in second filter paper folded in such fashion as to prevent escape of meal (see figure 3.5). Leave second paper open at top like a thimble. Piece of absorbent cotton may be placed in top of thimble to distribute solvent as it drops on sample. Place thimble in cup and assemble apparatus (Fat Analyser, FOSS; AVANTI 2055, SWEDEN) as shown in figure 3.5. Place ca. 80 ml of petroleum ether into cup. Heat on electric hot plate at 135°C, 25 min for boiling, 50 min for rinsing, 10 min for solvent recovering and 5 min for drying sample. After process was finished, remove cup with crude fat extact dried at 105°C in hot air oven for 30 min. cool down in dessicator and reweigh again.

Calculation

$$\% \, Fat = \frac{wt. \, of \; crude \; extact}{wt. \, of \; sample} \times 100 \qquad (3.10)$$

3.1.2.5 carbohydrate

Carbohydrate of sample were calculate from equation:

$$\%\text{Carbohydrate} = 100 - \%\text{Fat} - \%\text{ash} - \%\text{ protein} - \%\text{ moisture content} \qquad (3.11)$$

Figure 3.5 Fat Analyser, FOSS; AVANTI 2055, SWEDEN

3.1.3 Calculated thermal properties from chemical composition

3.1.3.1 Determination specific heat of mango peel and mango flesh from equation (2.2) to (2.8)

3.1.3.2 Determination thermal conductivity of mango peel and mango flesh from equation (2.11) to (2.18)

3.1.3.3 Determination thermal diffusivity of mango peel and mango flesh from equation (2.21) to (2.24)

3.1.3.4 Comparative thermal properties from data experiment and calculated value of chemical composition

$$\%Error = \frac{\left|data_{\exp} - data_{cal}\right|}{data_{cal}} \times 100 \tag{3.12}$$

3.2 Prediction of internal temperature in mango fruit cv. Nam Dok Mai Si Thong using thermal properties.

3.2.1 Validation of the simulation mode.

A simulation model must be validated before it can be used with confidence. In this study, the simulation model was validated by experiments method.

Model validation by experimental method. Heating test with hot water was performed using a water bath (Heto, Denmark) in which a constant temperature at 48±0.5°C. The thermocouples were securely placed at location m=1, m=3, and m=5, and the fruit was placed in a stainless steel basket before immersing into a water bath (Heto, Denmark), controlled at 48.0±0.5°C (Fig 3.8). The temperature at 3 positions in the fruit as well as that of the heating medium were recorded every 1 minute by a time–temperature recorder (CMC 821, Ellab, Denmark), until the temperature at location m=1 reached 46.5°C and held further 10 minutes at this temperature. The same procedure was set up for cooling experiment where the temperature of the incubator (SANYO, MIR-553, Japan) was held at 13.0±0.5°C, the experiment was ended when the temperature at the same location reached 13.0°C.

Mango fruits cv Nam Dok Mai Si Thong was used in this test. The average fruit diameter was 70.4-73.3 × 10^{-3} m. All tests were conducted in tripicate (3 fruits for 1 replication).

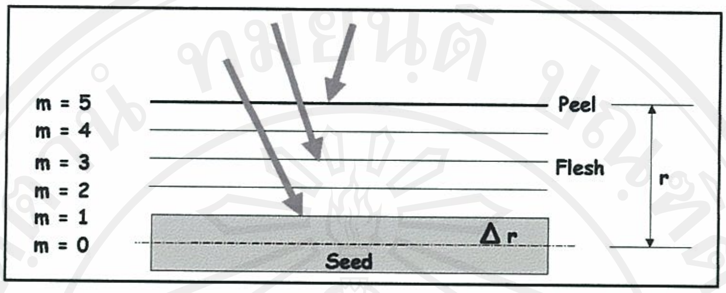

Figure 3.6 Temperature measurement locations.

To validate the computer model, predictions were compared with experimental data. The goodness of fit was evaluated by Root Mean Square Erroe (RMSE, °C) defined as (Lawson ans Hanson, 1974):

$$RMSE = \sqrt{\left(\frac{1}{p}\sum_{i=1}^{p}\left[T_{mea}(i)-T_{sim}(i)\right]^2\right)} \qquad (3.13)$$

Where T_{sim} is the simulated tempertature (°C) and T_{mea} is the measured temperature (°C) at selected locations in the fruit, and p for the number of data points collected over a selected period of time.

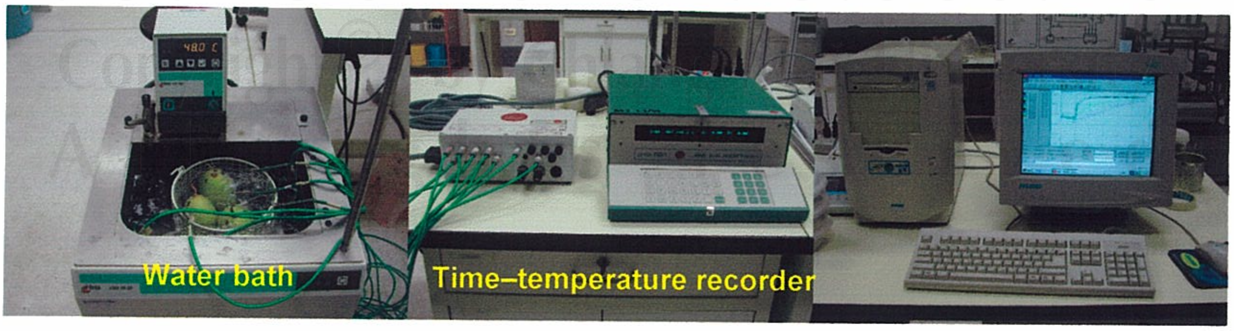

Figure 3.7 Heating test with hot water.

Figure 3.8 Cooling experiment.

The geometry of mango can be characterized as a cylindrical shape, whose diameter measured from the widest part of the fruit. Temperature distribution in fruit is thus a function of radial position and treatment time. In conventional heating, hot air and/or water is the source of thermal energy. Thermal energy is transferred from the heating medium to the fruit surface ($r=r_0$) by convection as described by following boundary heat flux equation (Dincer, 1997):

$$-k\left(\frac{\partial T}{\partial r}\right)_{r=r0} = h\left(T_{a,t}-T_{s,t}\right) \tag{3.14}$$

Where k = thermal conductivity of fruit (W/m °C),

h = the heat transfer coefficient of heating/cooling medium (W/m^2 °C)

r = the radial coordinate originate from the fruit center (m),
r_0 = the fruit radius (m)
t = the treatment time (s)
T_a = the fruit temperature (°C)
T_s = the heating medium temperature(°C).

According to Eq. (3.14), heat flux from the heating medium to the fruit surface is proportional to the surface heat transfer coefficient, h and the temperature difference between the heating medium and the fruit surface ($T_{a,r}$-$T_{s,t}$). with forced convection heat transfer coefficient can be estimated based on boundary layer similarity for a cylindrical (Campbell, 1997; Dincer, 1997)

$$Nu = C\,\mathrm{Re}^{m}\,\mathrm{Pr}^{\frac{1}{3}} \tag{3.15}$$

$$h = \frac{Nu k_f}{D} \tag{3.16}$$

Where D = the cylindrical diameter (m),
k_f = the thermal conductivity of the medium (W/m °C)
Nu = the dimensionless Nusset number.
Re = the Renolds number
Re = $D v \rho / \mu$
Pr = the Prandtl number;
Pr = $C_p \mu / k$
C , m = constants that depend on Re ($40<\mathrm{Re}<4\text{x}10^3$),
C=0.683 m=0.466
v = the heating medium speed (m/s)
ρ = the density of medium (kg/m^3)
μ = the viscosity of medium (kg/m s).

Thermal resistance at the fruit surface can be considered as *1/h*. Increasing air or water speed increases the value of h, and reduces thermal resistance at the fruit surface.

Once the thermal energy is transferred to the fruit surface, it moves into the fruit interior by conduction. Fruit temperature as a function of time at any location within cylindrical fruit is govered by a general energy balance equation (Holdsworth, 1997).

$$\rho c_P \frac{\partial t}{\partial T} = K\left(\frac{\partial^2 T}{\partial r^2} + \frac{1}{r}\frac{\partial T}{\partial r}\right) + Q \qquad (3.17)$$

Where C_p = the specific heat of the fruit (kJ/kg °C)
Q = the heat generation within fruit (W/m^3)
ρ = the fruit density (kg/m^3).

Relative to externally applied energy, the heat of respiration is small over the period of quarantine heating. Thus Q = 0. Dividing by ρC_p and substituting α for $k/\rho C_p$, Eq (3.17) becomes:

$$\frac{\partial T}{\partial t} = \alpha\left(\frac{\partial^2 T}{\partial r^2} + \frac{1}{r}\frac{\partial T}{\partial r}\right) \qquad (3.18)$$

Where α = the thermal diffusivity (m^2/s)

Conductivity heat transfer within fruit, as represented by the right hand side of Eq. (3.17), is slow due to the relatively small value of thermal diffusivity for fruit ($\alpha \approx 1.00 \times 10^{-7}$ m^2/s as compared to the $1.5\text{-}17 \times 10^{-5}$ for metal). As a result, the heating rate at the fruit center can be very slow, especially for large fruits such as mango. The internal thermal resistance can be represented by a value of $\Delta r/k_{pf}$. The influence of surface and internal thermal resistances on the heating rate can be described by the non-dimensional Biot number (Bi) defined as (Incropera and dewitt, 1996):

$$Bi = \frac{\text{internal resistance}}{\text{Surface resistance}} = \frac{h\Delta r}{k_{pt}} \tag{3.19}$$

Where *Bi* is less than 0.1, the surface thermal resistance dominates the rate of heat transfer and the internal temperature gradient are small. When the Bi number is large (e.g., 10), internal thermal resistance plays the most important role and the internal temperature gradients are significant.

When provided with initial fruit temperature, heating medium temperature and speed, fruit diameter and thermal conductivity, Eq. (3.19) in combination with Eq. (3.16) can be solved numerically. In developing the simulation model in this study Eqs (3.16) and (3.19) were replaced by finite difference equations at different depths. The fruit was divided by 5 equally space planes labeled m = 0, 1, 2, 3, 4 and 5 as shown in Figure 3.7. The distance between plane 0 and plane 1 equaled to half of the thickness of the seeds.

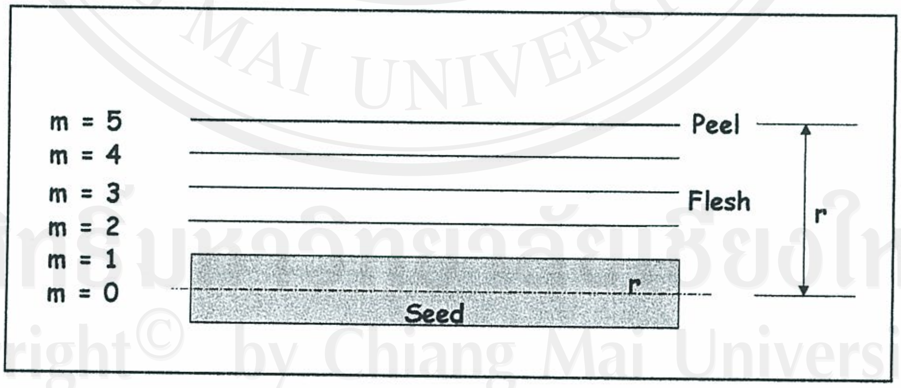

Figure 3.9 The distance between plane 0 and plane 1 equaled to half of the thickness of the seed.

In equation (3.18), temperature (T) is a function of the independent variables of radial length (r) from the center of fruit. The temperature at any point may be denoted by $T_{m,n}$.

The explicit form of finite difference of Eqn (3.18) for interior m,n of the fruit is

$$\frac{1}{\alpha}\left(\frac{T_{m,n+1}-T_{m,n}}{\Delta t}\right)=\left(\frac{T_{m+1,n}+T_{m-1,n}-2T_{m,n}}{(\Delta r)^2}\right)+\frac{1}{m\Delta r}\left(\frac{T_{m+1,n}-T_{m,n}}{\Delta r}\right)$$

(3.20)

Where

$T_{m,n}$ = temperature at m and $n\,\Delta t$

$T_{m,n+1}$ = Temperature at m and $(n+1)\,\Delta t$

And temperature at ; $5 < m < 0$, $(n+1)\,\Delta t$

$$T_{m,n+1}=(F_0+\frac{F_0}{m})T_{m+1,n}+\left(1-2F_0-\frac{F_0}{2m}\right)T_{m,n}+(F_0)T_{m-1,n} \qquad (3.21)$$

Where $F_0=\dfrac{\alpha\Delta t}{(\Delta r)^2}$

Eqn (3.20) is invalid at the center of fruit where $\dfrac{1}{r}\dfrac{\partial T}{\partial r}$ becomes an indeterminate quantity. However, using L'Hospital's rule, Eqn (3.21) can be transformed into the following form

$$\lim_{r\to 0}\left(\frac{1}{r}\frac{\partial T}{\partial r}\right)=\left(\frac{\partial^2 T}{\partial r^2}\right)_{r=0} \qquad (3.22)$$

Eqn. (3.18) can be transformed into the following form

$$\left(\frac{\partial T}{\partial t}\right)=\alpha\left(2\frac{\partial^2 T}{\partial r^2}\right) \qquad (3.23)$$

Finite difference for temperature at $r = 0$ ($m = 0$) and $(n+1)\,\Delta t$

$$T_{0,n+1} = 4F_0T_{1,n} + (1-4F_0)T_{0,n} \tag{3.24}$$

Finite difference for the surface points with specified convection boundary condition ($m = 5$)

$$T_{m,n+1} = \frac{BiT_{a,n+1} + T_{m-1,n+1}}{1+Bi} \tag{3.25}$$

Where $Bi = \dfrac{h\Delta r}{k_{pf}}$

3.3 To determine the relationship between thermal properties of mango and chilling injury during storage at 5 and 13 °C.

3.3.1 Determination of chilling injury in mango fruit during storage at 5 and 13°C

Chilling injury in mango fruit was evaluate from chilling index, membrane permeability and CO_2 production.

3.3.1.1 Chilling injury index

Chilling injury (CI) was evaluated by means of an arbitrary scale of visual symptoms, based on necrotic surface and intensity of browning: 0 = no pitting or no injured, 1 = slight injured (a few scattered injury), 2 = moderately injured covering up to 30% of the fruit surface, 3 = severely injured or pitting (extensive injured covering > 30 % of the fruit surface. The chilling index is calculated to the following equation:

$$chilling\ injury\ index = \Sigma_0^3 \frac{(injury\ level)\times(number\ of\ fruit)}{Total\ number\ of\ fruit} \tag{3.26}$$

3.3.1.2 Measurement of membrane permeability

Membrane permeability was determined based on electrolyte leakage. For each treatment, the five samples disc (10 mm-diameter x 4 mm length) of the mango flesh or mango peel were rinsed with deionized water for 3 seconds to eliminate the electrolyte at the cut surface two times. The sample were placed into a breaker containing 30 ml of 0.4 M mannitol. After incubate at 25 °C for 3 hours, electric conductivity were measured in a suspending solution with a conductivity meter (Sartorious Professional Meter pp-20, Germany) as initial reading. These sample were boiling of 121 °C, pressure 15 lb/inch^2 for 30 min. After boiling conductivity of solution were measure again (as 100% leakage). The percentage of ion leakage were calculated from the equation:

$$\%Electrolyte\ leakage = \frac{initial\ conductivity}{final\ conductivity} \times 100 \qquad (3.27)$$

3.3.1.3 Measurement of CO_2 production

CO_2 production of fruit was measured by closed system. Fruit was weight in air and in water. Fruit was place in plastic box which volume has 2500 ml. for 1 hours. Sampling gas by syringe and inject in gas chromatography (Thermo Finigan Model TRACE GC, Germany). The condition of gas chromatography used Hayesep Q and Molecular sieve column at 80°C; Helium (He) was the carrier gas with TCD detector. CO_2 production were calculate from equation:

$$CO_2\ production = \frac{difference\ in\ CO_2 \times free\ volume\ container\ (mls) \times 321.75}{time\ sealed\ (\min s) \times wt\ (kg) \times (273 + stored\ temp\ °C)} \qquad (3.28)$$

3.3.2 Quality assessment

The parameter used to monitor the quality of the mangoes before and after storage were skin and flesh color, pH, total soluble solid, % titratable acidity, texture and percent of mass loss.

Skin and flesh color were measured with Hunter Lab Model Color Quest XE (Hunter Associates Laboratory, Virginia, USA) equipped with 3.75 mm diameter measuring area. Measurements were reported in term CIE (Commission Internation de I' Eclairage) L* a* b* C* and hue angle are expressed as:

L* = whiteness/darkness, ranged from 0 to 100

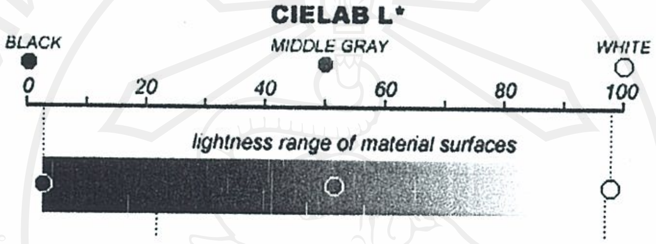

Figure 3.10 Value range in relation to lightness scales

Where

95-99 = Near white

86-94 = Very light valued

77-85 = Light valued

68-76 = Moderately light valued

59-67 = Mid valued

50-58 = Moderately dark valued

41-49 = Dark valued

32-40 = Very dark valued

31-27 = Near black

a* = redness for positive value and greenness for the negative one

b* = yellowness for positive and blueness for negative value

C* = intensity of color

where

100-88	=	Very intense
87-75	=	Intense
74-62	=	Moderately intense
61-50	=	Moderately dull
49-37	=	Dull
36-25	=	Very dull
24-13	=	Near neutral
12-0	=	Neutral

Hue (h°) = an angle measuring position around the color wheel or actual color

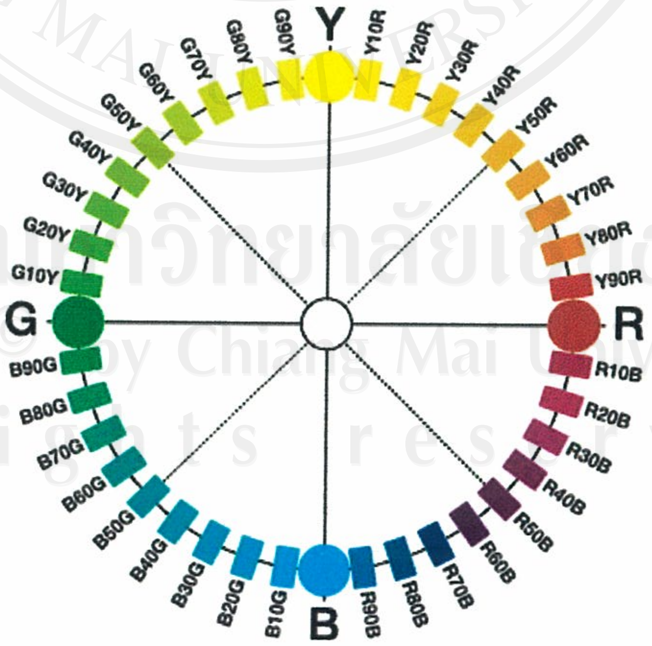

Figure 3.11 Color chart of natural color syatem (McGuire, 1992).

Calibration was performed against a standardized white and black calibration plate according to the manufacture specifications.

pH of the flesh was measured using pH meter (Satorious Model Professional PP 20, Satorious, Germany).

The total soluble solids (TSS) during the storage is a measure of the sugar and organic acid contents of mango juice including other soluble components. TSS was determined every 5 days by using digital hand refractometer (Atago PR101 scale 0-4, Japan)

Titratable acidity measures the total available amount of Hydrogen ionss in the solution. Five milliliters of mango juice was then titrated with standard Sodium Hydroxide (0.1 N NaOH) solution at end point 8.2 by using automatic titrator (Schott Model TitroLine easy, Germany). Titratable acidity was expressed in term of meq of citric acid per milliliter of juice extract.

A texture analyzer (TA. XT plus, England) was used to measure the textural properties of mango fruits. The test method was defined in the Texture Exponent software which is the data acquisition, control and analysis software for material testing. The test consisted of measuring the force required to push at a constant speed of 1.5 mm sec^{-1} a 6 mm cylindrical probe into the flesh of peeled, which penetrated deep to 5mm and the moving rate was 10mm sec^{-1}. Results were expressed in terms of the maximum force record (Fmax) in N.

Every mango was weighed individually every 5 days during the storage period of 25 days and percentages of weight loss was the calculated.

3.3.3 Measurement of thermal properties

The specific heat, thermal conductivity and density of mango peel and mango flesh were determined as described in 3.1.1.1 -3.1.1.4.

The collected data were subjected to analysis of variance (ANOVA) using SPSS® program (SPSS, Illinois, USA). Duncan multiple-range tests were used to locate significant differences between treatment means with the level of significant set at 0.05.