CHAPTER 3

Materials and Methods

3.1 Materials

3.1.1 Raw materials

Fresh 'Puk Mai Lie' papaya (unripe, half ripe and fully ripe) was purchased from local farmers in Chiang Mai, Thailand. The fruit were kept refrigerated at 5 ± 1ºC until used (not more than 5 days).

3.1.2 *Lactobacillus casei*

Lactobacillus casei TISTR 390 was obtained from Thailand Institute of Scientific and Technological Research (Bangkok, Thailand).

3.1.3 Impregnation media

- Commercial sucrose (Mitrphol brand, Thailand)
- Distilled water (Polestar, Chiang Mai, Thailand)

3.1.4 Packaging material

Polyethylene tetraphthalate/polypropylene/aluminium (PET/PP/Al) (Siam Pack, Thailand).

3.2 Chemicals and media

3.2.1 Chemical for analysis

- NaOH (Merck, Germany)

- Methylene blue indicator (BDH, UK)
- Toluene (RCI Labscan, Thailand)
- Calcium chloride (Merck, Germany)
- Calcium lactate (Merck, Germany)
- Trichloroacetic acid (Loba Chemie, India)
- Ascorbic acid (Fisher Scientific, UK)
- 2,6-dichlorophenol indophenol (Fluka, Austria)
- Sodium bicarbonate (Merck, Germany)

3.2.2 Media for microorganism

- MRS broth (Becton Dickinson, France)
- Maximum Recovery Diluent (Oxoid, England)
- Peptone (Merck, Germany)
- Microbiological agar (Criterion, USA)
- Plate Count Agar (Becton Dickinson, France)
- Potato Dextrose Agar (Becton Dickinson, France)
- Glacial acetic acid (Merck, Germany)
- Tartaric acid (BDH, UK)

3.3 Equipment

- Hot air oven (Memmert UF260plus, Germany)
- pH meter (Consort C830, CE Belgium)
- Hand refractometer (ATAGO, Japan)
- a_w meter (Series 3, AquaLab, USA)
- Colourimeter (CR-400, Konica Minolta, Japan)
- Texture analyzer (TA-XT. Plus, Stable Micro systems, Surrey, UK)
- Vacuum oven (Binder VD23, Germany)
- Refrigerated centrifuge (Rotina 46R, Hettich Zentrifugen, Germany)
- Freezer at –20^{o}C (Sanyo, Japan)
- Analytical balance (Denver, Germany)

- Water bath (Memmert, Germany)
- Incubator (Memmert, Germany)
- Autoclave (Gallenkamp, England)
- Blender
- Desiccator
- Hot plate stirrer (Heidolph, Germany)
- Vacuum seal (J-V002, Taiwan)
- Scanning Electron Microscope (Model JEOL JSM-5910LV, JEOL., Japan)

3.4 Methods

3.4.1 Raw material of papaya

'Puk Mai Lie' papaya fruit at three different stages of maturation, including unripe (full green), half ripe (more yellow than green) and fully ripe (fully yellow) were used as raw materials (Schweiggert et al., 2011). The fruit was obtained from a local market in Chiang Mai. The physical and chemical properties of papaya fruit were analysed. Experiments were run in triplicate.

Analyses of fresh papaya fruit

- Moisture content

Moisture content was examined according to the method of AOAC (2000) and Cheenkachorn et al. (2012). An empty dish and its lid were dried in an oven at 105±1ºC for 3 h and transferred to a desiccator to cool. Samples were weighted for approximately 2-3 g into the dish. The dish with the sample was placed in the hot air oven at 105±1ºC for 3 h. After drying, the dish with partially covered lid was transferred to the desiccator to cool. The dish and its dried sample were reweighted. The sample was dried until the final weight of samples was stable. The sample moisture content was calculated according to Equation 3.1.

Calculation for the moisture content:

$$\text{Moisture (\%)} = \frac{(W1-W2)}{W1}\ x\ 100 \qquad (3.1)$$

where, W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying

- pH value

The analysis of pH value of fresh papaya fruit was carried out based on the methods of Waghmare and Annapure (2013) and Tirkey et al. (2014) using a pH meter. For pH of fresh papaya, 10±0.3 g samples were homogenised with a blender and measured the homogenised samples with the pH meter. Determination of pH value using the pH meter was done by immersing a combined electrode in the homogenised sample and read the values.

- Total soluble solid

Total soluble solids (TSS) (%Brix) were measured using a hand refractometer (ATAGO, Japan) at 25ºC (Fuggate et al., 2010). Fresh papaya fruit was homogenised without distilled water. Some drops of papaya solution were placed on a fixed prism of the hand refractometer. The value of the line dividing the light and dark parts of the surface in the field of view was read. All measurements were carried out in triplicate.

- Water activity

Water activity of fruit samples was examined by a water activity meter (Series 3 AquaLab, USA). Papaya fruit sample in a small size of plastic cup was placed into the base of test chamber. The measuring head enclosed the sample and formed an airtight seal with the base. At least 3 samples were measured for each treatment (Rico et al., 2007 and Udomkun et al., 2014).

- Total acidity

For total acidity (TA), fresh papaya was used for 10±0.3 g of samples. The papaya fruit was mixed with 100 ml distilled water and homogenised. An amount of 10 ml of the sample solution and 2-3 drops of phenolphthalein were added into an Erlenmeyer flask. Sample was assessed by a titration method using

0.1 M sodium hydroxide until the pH was 8.2 (AOAC, 2006; Fuggate et al., 2010). The calculation of titratable acidity expressed as g citric acid (7.005) was determined using Equation 3.2.

$$\text{TA (\%)} = \frac{EqWt\ x\ M(NaOH) x\ V(NaOH) x\ V(made\ up)}{Wt(sample) x\ V(tritrand)} \quad (3.2)$$

Equivalent weight
Citric acid = 7.005
Acetic acid = 6.005
Malic acid = 6.706
Tartaric acid = 7.504
Lactic acid = 9.008

- Real porosity

Real porosity or total porosity (ε_r) of the fruit was calculated using apparent and real densities according to Equation 3.3 (Mújica-Paz et al., 2003b and Panarese et al., 2013):

$$\varepsilon_r = \frac{\rho_r - \rho_a}{\rho_a} \quad (3.3)$$

where, ρ_a = apparent density (kg/m^3)
ρ_r = real density (kg/m^3)

- Apparent density (ρ_a)

Apparent density (ρ_a) was determined in fruit pieces by toluene using a pycnometer method (Mújica-Paz et al., 2003b). Equation 3.4 was employed to determine apparent density.

$$\rho_a = \frac{m}{V} \quad (3.4)$$

Where, m = mass of fruit sample (g)
v = volume of fruit sample (m^3)

- Real density (ρ_r)

Real density (ρ_r) was analysed in fruit puree after the fruit pieces are previously homogenised and de-aired at a pressure of 260 mbar for 2 h (Mújica-Paz et al., 2003b; Rongkom et al., 2013). Real density was calculated using Equation 3.5.

$$\rho_r = \frac{m}{V} \quad (3.5)$$

Where, m = mass of fruit sample (g)

v = volume of fruit sample (m^3)

- Ripening index

Ripening index was calculated using the soluble solids to acidity ratio according to Equation 3.6 (Mújica-Paz et al., 2003a).

$$\text{Ripening index (Brix /Acid Ratio)} = \frac{Brix}{\%\ Acid\ (^{W}/_{W})} \quad (3.6)$$

- Colour

The colour values were expressed as L* (whiteness to darkness value), a* (redness to greenness value) and b* (yellowness to blueness value). The sample colour values were determined by a colourimeter (CR-400, Konica Minolta, Japan) (Fuggate et al., 2010 and Orikasa et al., 2014).

- Texture

Texture was evaluated by a Texture Analyzer (Moreno et al., 2013). Samples were compressed till 5 mm from top of the sample by using a probe number P/36R. The setting conditions were 1.50 mm/s pre-test speed, 1.50 mm/s test speed, 10 mm/s post-test speed and 5 g trigger force. The maximum compressing force (N) was recorded as the firmness value of the fruit samples. The texture of each treatment samples was determined for 15 times measurement.

Statistical analysis

Collected data was statistically analysed by completely randomised design using triplicate data. Differences between treatment means were compared by Duncan's new multiple range test ($p<0.05$) using SPSS software (SPSS version 17.0).

3.4.2 The effect of ripening stages and fruit sizes on the physicochemical properties of vacuum impregnated papaya

Fresh papaya fruit at different maturity stages, including unripe, half ripe and fully ripe, that was free from defects was selected to be used in the experiment. The selected fruit was washed with tap water to remove surface contamination and drained for about 10 min at room temperature. The fruit was then hand-peeled, seeded and the layer of flesh fruit was scraped off with a stainless steel sharp knife. The edible portions of papaya were cut into pieces, including cubic shapes of approximately 1 x 1 x 1 cm^3 (Nimmanpipug and Therdthai, 2013) and 2 x 2 x 2 cm^3 (Khurnpoon et al., 2010) and a slice shape of 0.5 x 2 x 7 cm^3 using the knife. The peeling and cutting were done carefully to prevent tissue damage.

For a vacuum impregnation process, the papaya pieces/slices were immersed under a sucrose solution that had an a_w similar to the papaya fruit (Rongkom et al., 2013) using a ratio of 1 to 5 (w/w) (Nimmanpipug and Therdthai, 2013; Piromvard et al., 2010). During the impregnation process, the papaya fruit was undergone a vacuum pressure of 50 mbar for 10 min (Gras et al., 2003; Piromvard et al., 2010) and a restoration time (a relaxation time) of 10 min at atmospheric pressure (Gras et al., 2003; Piromvard et al., 2010). The vacuum impregnated papaya was separated from the solution using a strainer and analysed for its physicochemical properties. Each treatment was carried out in triplicate.

Analyses of vacuum impregnated papaya

Physical properties

- Apparent density (ρ_a) was determined according to the procedure in the section 3.4.1
- Real density (ρ_r) was measured based on the procedure in the section 3.4.1
- Real porosity or total porosity (ε_r)

Real porosity or total porosity (ε_r) of the fruit was calculated using apparent and real densities according to Equation 3.7 (Mújica-Paz et al., 2003b and Panarese et al., 2013):

$$\varepsilon_r = \frac{\rho_r - \rho_a}{\rho_a} \quad (3.7)$$

Where, ρ_a = apparent density (kg/m^3)

ρ_r = real density (kg/m^3)

- Effective porosity (ε_e)

Effective porosity (ε_e) was measured based on Equation 3.8 (Betoret et al., 2003; Rongkom et al., 2013):

$$X - \gamma = \varepsilon_e \left(1 - \frac{1}{r}\right) - \frac{\gamma}{r} \quad (3.8)$$

Where, γ = volumetric deformation of the sample (m^3/m^3 initial sample)

X = the amount of liquid incorporated into the sample (m^3 liquid/m^3 sample)

R = compression ratio (atmospheric pressure/vacuum pressure)

- Water loss

Water loss was determined according to Equation 3.9 (Mújica-Paz et al., 2003a; Nimmanpipug and Therdthai, 2013):

$$WL = \frac{(Ww_0) - (w_t - Ws_t)}{(Ws_0 + Ww_0)}\ x\ 100 \quad (3.9)$$

Where, WL = water loss (%)

W_{wo} = the weight of water in the fruit (kg)

W_{so} = the weight of solids initially present in the fruit (kg)

W_t = the weight of the fruit at the end of treatment (kg)

W_{st} = the weight of solids at the end of treatment (kg)

- Solid gain

Solid gain was calculated based on Equation 3.10 (Mújica-Paz et al., 2003a):

$$SG = \frac{(W_{st} - W_{so})}{(W_{so} + W_{wo})} \, x \, 100 \qquad (3.10)$$

Where, SG = solids gain (%)

W_{wo} = the weight of water in the fruit (kg)

W_{so} = the weight of solids initially present in the fruit (kg)

W_{st} = the weight of solids at the end of treatment (kg)

- Weight reduction

Weight reduction was calculated based on the following Equation 3.11 (Mújica-Paz et al., 2003a):

$$WR = WL - SG \qquad (3.11)$$

Where, WR = weight reduction (%)

SG = solids gain (%)

WL = water loss (%)

- Volume of fruit impregnated with external solvent (X value)

Volume of fruit impregnated with external solvent (X value) was examined using Equation 3.12 (Rongkom et al., 2013):

$$X = \frac{(M_f - M_i)}{\rho_s V_o} \quad (3.12)$$

Where, X = Impregnated sample volume fraction(m^3 liquid/m^3 sample)

M_f = final mass of fruit sample (kg)

M_i = initial mass of fruit sample (kg)

ρ_s = density of impregnation solution (kg/m^3)

V_o = initial volume of the sample (ml)

- Sample volume deformation (γ value)

Sample volume deformation (γ value) was determined based on Equation 3.13 (Rongkom et al., 2013).

$$\gamma = \frac{(V_t - V_0)}{V_0} \quad (3.13)$$

Where, Vo = initial volume of samples (m^3)

Vt = final volume of samples (m^3)

- Texture was followed the procedure given in the section 3.4.1
- a_w was followed the procedure given in the section 3.4.1
- Colour was followed the procedure given in the section 3.4.1

Chemical properties

- pH was followed the procedure given in the section 3.4.1
- Moisture content was followed the procedure given in the section 3.4.1
- Total acidity was followed the procedure given in the section 3.4.1
- Total soluble solid was followed the procedure given in the section 3.4.1

Statistical analysis

The effect of the ripening stages (unripe, half ripe and fully ripe) and fruit sizes (1 x 1 x 1 cm^3, 2 x 2 x 2 cm^3 and 0.5 x 2 x 7 cm^3) were analysed as

factorials in completely randomised design. Duncan's multiple range test was used to identify difference at 95% significant level using SPSS for Windows version 17.0.

3.4.3 The effect of impregnation solution ratio, vacuum time and relaxation time on the physicochemical characteristics of vacuum impregnated papaya

One ripening stage and one papaya piece size from the previous section were further studied in this section. In this section, vacuum impregnation ratios of 1:5 (w/w) (Piromvard et al., 2010) and 1:10 (w/w) (Yousefi et al., 2013) together with vacuum times of 5 (Moreno et al., 2013; Perez-Cabrera et al., 2011) and 10 min (Gras et al., 2003; Piromvard et al., 2010; Rongkom et al., 2013) at 50 mbar and relaxation times of 10 (Perez-Cabrera et al., 2011; Piromvard et al., 2010) and 30 min (Occhino et al., 2011) were examined for their effects on vacuum impregnated papaya. After separating the papaya pieces from the impregnation solution with a strainer, the papaya samples were subjected to physicochemical analyses. Each treatment was prepared in triplicate.

Analyses of vacuum impregnated papaya

Physical properties - apparent density, real density, real porosity, effective porosity, water loss, solid gain, volume of fruit impregnated with external solvent (X value), weight reduction and sample volume deformation (γ value) were determined according to the methods in the section 3.4.2. Texture, a_w and colour were done by following the procedures given in the section 3.4.1

Chemical characteristics – pH, moisture content, total acidity and total soluble solids were determined based on the methods in the section 3.4.1

Statistical analysis

Factorials in completely randomised design were used to study the effect of impregnation solution ratios and periods on parameters of vacuum impregnated papaya. Duncan's multiple range test was used to identify difference at 95% significant level using SPSS for Windows version 17.0.

3.4.4 The effect of drying methods and drying temperatures on the physicochemical of partially dried papaya

The optimum condition of vacuum impregnation in the previous section was continued to be applied in this section. Vacuum impregnated papaya was then dried using a hot air oven or a vacuum oven at drying temperatures of 40 (Betoret et al., 2003), 50 (Schulze et al., 2014; Udomkun et al., 2014) or 60ºC (Udomkun et al., 2014) until the a_w of the papaya sample was less than 0.6. The final product of papaya was evaluated for their physicochemical characteristics. Each treatment was done in triplicate.

Analyses of intermediate moisture papaya

- Yield

Yield of intermediate moisture papaya was calculated based on Equation 3.14.

$$\text{Yield (\%)} = \frac{weight\ of\ dried\ papaya}{weight\ of\ fresh\ papaya} \text{ x } 100 \qquad (3.14)$$

Physical characteristics

- Shrinkage

Shrinkage was done by applying the method of Udomkun et al. (2014) and calculated by following Equation 3.15.

$$V = V_f \frac{M_{f+s} - M_f - M}{\rho_s} \qquad (3.15)$$

Where V_f was the volume of the flask (cm^3), M_{f+s} was the weight of the flask plus the sample and the fluid (g), M_f was the weight of the flask (g), M was the weight of the sample (g), and ρ_s was the density of toluene (g/cm^3). Shrinkage (S) was expressed by the percentage change of the sample volume as compared with its original volume and determined based on Equation 3.16.

$$S = \frac{V_0 - V}{V_0} \, x \, 100 \quad (3.16)$$

Where V_o was the original volume of the sample (cm^3) and V was the volume of the sample after drying (cm^3).

- a_w was followed the procedure given in the section 3.4.1
- Colour was followed the procedure given in the section 3.4.1
- Real porosity was followed the procedure given in the section 3.4.1
- Sample volume deformation was followed the procedure given in the section 3.4.2
- Texture was followed the procedure given in the section 3.4.1

Chemical properties

- Moisture content was followed the procedure given in the section 3.4.1
- pH value

The analysis of pH value of partially dried papaya was carried out based on the methods of Waghmare and Annapure (2013) and Tirkey et al. (2014) using a pH meter. For pH of intermediate moisture papaya fruit pieces, 2.5±0.3 g of samples mixed with 100 ml distilled water were homogenised with a blender. Determination of pH value using the pH meter was done by immersing a combined electrode in the homogenised sample and read the values.

- Total soluble solid

Total soluble solids (TSS) (%Brix) were measured using a hand refractometer (ATAGO, Japan) at 25ºC (Fuggate et al., 2010). For intermediate moisture papaya fruit pieces, 2±0.3 g of samples were homogenised with 20 ml distilled water and placed a small quantity of the sample solution (1-2 drops) on a fixed prism of a hand refractometer. The value of the line dividing the light

and dark parts of the surface in the field of view was read. All measurements were carried out in triplicate.

- Total acidity

For total acidity (TA), intermediate moisture papaya fruit pieces were used for 2.5±0.3g of samples. The papaya fruit was mixed with 100 ml distilled water and homogenised. An amount of 10 ml of the sample solution and 2-3 drops of phenolphthalein were added into an Erlenmeyer flask. Sample was assessed by a titration method using 0.1 M sodium hydroxide until the pH was 8.2 (AOAC, 2006; Fuggate et al., 2010). The calculation of titratable acidity expressed as g citric acid (7.005) was determined using Equation 3.17.

$$\% \text{ TA} = \frac{EqWt \; x \; M(NaOH) x \; V(NaOH) x \; V(made \; up)}{Wt(sample) x \; V(tritrand)} \qquad (3.17)$$

Equivalent weight

Citric acid	= 7.005
Acetic acid	= 6.005
Malic acid	= 6.706
Tartaric acid	= 7.504
Lactic acid	= 9.008

- Vitamin C

Standard ascorbic acid solution – dissolve 0.05 g pure ascorbic acid in 45 ml of 5 % trichloroacetic acid then adjust to 50 ml in volumetric flask.

Standard indophenol solution - dissolve 0.05 g of 2,6 dichlorophenol indophenol (sodium salt) in 50 ml distilled water with 42 mg sodium bicarbonate. Adjust to 200 ml with distilled water in volumetric flask and then filtrate with Whatman no. 4.

An amount of 2±0.3 g samples was homogenised for 3 min with 100 ml of 5% trichloroacetic acid. The sample solution was filtered with a filter paper (Whatman no. 4). Next, 10 ml of the filtered solution was transferred into 125 ml Erlenmeyer flask. Determination of vitamin C was established by titrating the solution with 2,6-dichlorophenol indophenol, until colour of the reagent turn into

light pink colour (end point) (AOAC, 2000; Cardoso et al., 2011; Šumić et al., 2013).

$$\text{Vitamin C (mg/100 ml)} = \frac{V1\ x\ W2\ x\ V3\ x\ 100}{V2\ x\ w1} \quad (3.18)$$

Where, V1 = volume of trichloroacetic acid (ml)

V2 = volume of sample (ml)

V3 = titrated volume of 2,6-dichlorophenol indophenol (sample) (ml)

V4 = titrated volume of 2,6-dichlorophenol indophenol (standard vitamin C) (ml)

W1 = weight of sample (g)

W2 = end point volume of vitamin C react with 2,6-dichlorophenol indophenol (mg/ml)

* W2 = mg of standard Vitamin C / V4

For the standard vitamin C, 2.00.± 0.01 mg of the standard needed 18.57± 0.06 ml of 2,6-dichlorophenol indophenol.

Sensory evaluation

Sensory evaluation was carried out using a nine point hedonic scale (ranking from 9-like extremely to 1-dislike extremely) for colour, hardness, aroma, flavour and overall preference of intermediate moisture papaya by 50 untrained panellists. All panellists were under-graduate and post-graduate students in the Faculty of Agro-Industry, Chiang Mai University (Perez-Cabrera et al., 2011).

Statistical analysis

The statistical evaluation was performed with SPSS for Windows version 17.0. The collected data was analysed using factorials in completely randomised design for two drying methods and three drying temperatures. Duncan's multiple range test was used to identify difference at 95% significant level. Results from the sensory test were assessed by completely randomised design (CRD).

3.4.5 The effect of calcium solutions on the physicochemical properties of partially dried papaya

One drying method and drying temperature from the previous section was utilised in this section. The impregnation solution in this section was added with calcium solution, including calcium chloride and calcium lactate (Khurnpoon et al., 2010; Luna-Guzmán and Barrett, 2000; Occhino et al., 2011), at various addition levels of 1, 2 or 3% (w/w) (Beirão-da-Costa et al., 2008). A control treatment without any addition of calcium solution was also prepared. The impregnation process was carried out at 25 (Luna-Guzmán and Barrett, 2000) or 45ºC (Beirão-da-Costa et al., 2008). After drying the papaya samples, the samples were analysed for their physicochemical properties. Each treatment was carried out in triplicate.

Analyses of intermediate moisture papaya

- Yield was followed the calculation given in the section 3.4.4

Physical characteristics

- Shrinkage was followed the procedure stated in the section 3.4.4
- a_w was followed the procedure given in the section 3.4.1
- Colour was followed the procedure given in the section 3.4.1
- Real porosity was followed the procedure given in the section 3.4.1
- Sample volume deformation was followed the procedure given in the section 3.4.2
- Texture was followed the procedure stated in the section 3.4.1

Chemical characteristics

- Moisture content was followed the procedures written in the section 3.4.1
- pH was followed the procedure stated in the section 3.4.4
- Total acidity was followed the procedure given in the section 3.4.4

- Total soluble solid was followed the procedure given in the section 3.4.4

Sensory properties was followed the procedure in the section 3.4.4

Statistical analysis

All data were the means of triplicate. Determination of significant differences among treatment means was done by applying Duncan's multiple range tests ($p < 0.05$) based on factorials in completely randomised design. Analysis of variance was carried out using SPSS for Windows version 17.0. Results from the sensory test were treated as completely randomised design (CRD).

3.4.6 Supplementation of a lactic acid bacterium in partially dried papaya.

In this section partially dried papaya was supplemented with a lactic acid bacterium of *Lactobacillus casei*. Another set of partially dried papaya samples without the addition of lactobacilli was also prepared to be used as a control treatment.

Revival of lyophilised or freeze dried *L. casei* cultures was done by sterilising the culture ampoule with 70% alcohol dampened cotton. A file cut was made on the ampoule at the mid-point of the cotton wool plug. The ampoule was then wiped again with 70% alcohol dampened cotton. Carefully, the ampoule was broken gently at the scored area. Into the freeze dried culture, an amount of 0.3 to 0.4 ml MRS liquid medium was aseptically added. Afterwards, all of the suspension was transferred into 9 ml MRS liquid medium and mixed well. The culture was incubated at 37ºC for 24 h. On the following day, 5 ml of the recovered culture was inoculated into 45 ml of MRS broth and incubated again at 37ºC for 16 h. To prepare a stock culture, 1 ml of the cultured MRS broth containing *L. casei* was mixed with 1 ml 70% sterile glycerol in a sterile tube. After a thorough mixing, the stock culture was stored at −18ºC until used.

Preparation of impregnation liquid was started by inoculating 1 ml of *L. casei* culture into 9 ml of MRS broth. The culture of *L. casei* was grown

overnight at 37ºC for 24 h (Betoret et al., 2003). On the following day, 5 ml of the recovered culture was inoculated into 45 ml of MRS broth and incubated at 37ºC for 18 h. The culture were harvested by centrifugation at 4,000 rpm at 4ºC for 20 min (Paéz et al., 2012) and washed twice with 0.1% sterile peptone water based on Paéz et al. (2012). The centrifuged culture was aseptically inoculated into an impregnation medium, which was sucrose mixed with calcium solution from the previous section. The impregnation condition was followed the results from the section 3.4.3, while the drying condition was according to the finding in the section 3.4.4. The partially dried papaya containing lactobacilli was analysed for their physicochemical and microbial properties. Each treatment was done in triplicate.

Physical characteristics – a_w, colour, real porosity and texture were followed the procedures in the section 3.4.1

Chemical properties

- Moisture content was followed the procedures written in the section 3.4.1
- pH was followed the procedure stated in the section 3.4.4
- total acidity was followed the procedure given in the section 3.4.4
- total soluble solid was followed the procedure given in the section 3.4.4

Microbial properties

- Total microbial count
- Lactic acid bacteria

A random amount of 2 g intermediate moisture papaya containing lactic acid bacteria was digested with 18 ml sterile Maximum Recovery Diluent, respectively, by a stomacher for 2 min. Serial dilutions were prepared accordingly in 9 ml Maximum Recovery Diluent. This was carried out by transferring 1 ml homogenate sample of previous dilution to 9 ml of sterile diluent. From the appropriate dilution, 1 ml of homogenate samples was poured in duplicate plates. A medium of Plate Count Agar was applied to determine

viable counts of aerobic mesophilic bacteria (total microbial count), while de Man Rogosa and Sharpe agar (MRS) was employed for viable enumeration of lactic acid bacteria. Petri dishes were aerobically incubated at 37ºC for 48 h. After the incubation time, the growth colonies were counted (Mårtensson et al., 2002, Tirkey et al., 2014 and Yoon et al., 2006).

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to investigate the location of the probiotic cells in the fruit tissue after impregnation and drying process at 15 kV accelerating voltage. The sample tissues were initially fixed in 2.5% glutaraldehyde by immersion. The samples were placed in buffered 2.5% glutaraldehyde and post fixed in buffered 1% osmium tetroxide. The samples were dehydrated by immersion in ethanol solution and then dried to the critical point dryer. A transverse section from a slice, which was taken from the middle section of a cylinder, was excised, mounted in stainless steel stubs, gold coated and observed by a scanning electron microscopy (Krasaekoopt and Suthanwong, 2008; Wirjantoro et al., 2015).

Statistical analysis

Samples in this section were prepared in triplicate. The statistical analysis was performed with SPSS for Windows version 17.0. Completely randomised design was employed to statistically analysis the collected data. Differences between mean values were assessed by Duncan's multiple range test. Significant difference was determined at $p<0.05$.

3.4.7 Survival of lactic acid bacteria during storage of partially dried papaya

Partially dried papaya supplemented with a lactic acid bacterium was packed in polyethylene tetraphthalate/polypropylene/aluminium (PET/PP/Al) with a size of 4 x 5 inch to avoid moisture gain into the product (Germer et al., 2012). The package was sealed in vacuum condition. The sample products were then stored at three storage temperatures of either at room temperature, 4ºC and

–18ºC for 3 months. Physicochemical and microbial qualities of the papaya samples were regularly evaluated during the storage period. At room temperature, representative samples were analysed on 0, 1, 2, 3 and 4 weeks of storage, while at 4 and -18ºC, papaya samples were examined on 0, 2, 4, 6, 8, 10 and 12 weeks of storage. Each treatment was prepared in triplicate.

Analyses of partially dried papaya containing *L. casei*

- Yield was followed the procedure given in the section 3.4.4

Physical characteristics - a_w, colour and texture were followed the procedures in the section 3.4.1

Chemical properties

- Moisture content was followed the procedures written in the section 3.4.1
- pH was followed the procedure stated in the section 3.4.4
- Total acidity was followed the procedure given in the section 3.4.4
- Total soluble solid was followed the procedure given in the section 3.4.4
- Vitamin C was analysed based on the procedure in the section 3.4.4

Microbial analyses

- Total microbial count was followed the procedure given in the section 3.4.6
- Lactic acid bacteria was followed the procedure given in the section 3.4.6
- Yeast and mould

A random amount of 2 g intermediate moisture papaya containing lactic acid bacteria was digested with 18 ml sterile Maximum Recovery Diluent by a stomacher for 2 min. Serial dilutions were prepared accordingly in 9 ml Maximum Recovery Diluent. This was carried out by transferring 1 ml homogenate sample of previous dilution to 9 ml of sterile diluent. From the appropriate dilution, 1 ml of homogenate samples was poured in duplicate plates. Potato Dextrose Agar at pH 3.5 using 10% tartaric acid was utilised for

the count of yeasts and moulds. Petri dishes were aerobically incubated at 25ºC for 3-5 days to enumerate yeast and mould. After the incubation time, the growth colonies were counted (Mårtensson et al., 2002, Tirkey et al., 2014 and Yoon et al., 2006).

Statistical analysis

Triplicate samples of intermediate moisture papaya samples were assessed. Results were analysed with SPSS for Windows version 17.0 and completely randomised design (CRD) test was used to determined significant differences among results at 95% significant level. Means were compared using Duncan's multiple range test.