MATERIALS AND METHODS

The fresh persimmon fruit used in this work was the first grade astringent type of P2 cultivar (*Diospyros kaki*) (Royal Project Foundation, 1999) brought from The Royal Project Foundation Orchard (Chiangmai, Thailand).

This study was conducted in 3 parts:

-Part I: Persimmon quality assessment during storage
-Part II: Mass transfer behavior during osmotic treatment and mathematical models development.
-Part III: Persimmons product development using osmotic dehydration as a pre-treatment

1. Part I: Persimmon Quality during Storage

The objective of this part was to investigate the quality changes occurring in persimmon (P2 astringent type) during cold storage.

1.1 Material

P2 persimmon cultivar with a degree of ripening of 70 % was selected. The size of the fruits was also uniform and their weights varied between 100-110 g. The fruits were washed, trimmed and cleaned with a dry cloth.

1.2 Packing method

Six fruits were place in an expanded polystyrene (EPS) tray and vacuum packed in a 0.06 mm thick Nylon-LDPE bag using a vacuum packing machine (VM201, Adionvac and Weesp) at pressure 76 mm.Hg for 45 sec. The packed fruits were stored at 4-6 ^{o}C for 6 weeks (Boonyakeait *et al*., 1997) (Appendix Figure 1). Weekly, one fruit was withdrawn for quality measurement.

1.3 Quality Measurements

1.3.1 Determination of Weight Loss

For determining weight loss during storage, twelve fruits were numbered and their weights were monitored over the storage period using electronic balance having least weight of 0.01 g. Weight loss was expressed as the percentage of initial weight

1.3.2 Total Soluble Solids Analysis

Total soluble solids (°Brix) content of the juice obtained from the fruit was determined using a hand held refractometer (Atago 2411w)

1.3.3 Color Analysis

Skin and flesh colors were measured using a Handy Colorimeter (BYK Gardner), and expressed in terms of CIE 'L*' (lightness), 'a*' (redness and greenness) and 'b*' (yellowness and blueness). The sensor was standardized using a white and black tile. For each storage time, fifteen persimmons were examined. The skin and flesh color of each fruit were measured on slices taken at three points: the top, middle and lower part of fruit.

1.3.4 Texture Analysis

Firmness was analyzed using a texture analyzer (TA.XT2 Stable Micro System) fitted with a cylinder probe having a diameter of 6 mm. A compression force was applied at a rate of 1.5 mm/s which compressed the sample to a depth 10 mm from the contact point. Once again, samples were taken from three points - at the top, middle and lower part of fruit. The firmness was expressed as the force in N required compressing the fruit through a distance of 10 mm. The average values of 10 replications for each storage time are reported.

1.3.5 pH and Titratable Acidity Analysis

The pH was measured using a pH-meter (1000euten Cyber scan) and titratable acidity (TA) was quantified by an AOAC (1990) procedure with NaOH titration method and expressed as percentage of malic acid.

1.3.6 Soluble Tannin Analysis

Soluble tannin on the above sample content was determined following the method from AOAC (1990).

1.3.7 Sensory Evaluation

Sensory evaluation was done by the Quantitative Descriptive Analysis (QDA) method using trained panels. Twelve panelists were selected using the following criteria: interested and willing to participate, available for all sessions and able to verbally communicate about the fruit with each other. The panel was composed of nine females and three males. Each panelist was extremely well trained for the purpose. The panelists regularly underwent basic taste tests to check their sensory performance. To generate a descriptive terminology, three persimmon samples stored over different periods of time were presented to panelists to provide a wide range of attributes of persimmon. The terms used in the descriptive analysis of peeled persimmon are listed in Table 1. Two pieces of peeled persimmon (about 20 g each) were served in white container, and the response was gathered using a 15 cm continuous unstructured scale (Appendix Figure 2).

Table 1 Terms used in descriptive analysis of peeled persimmon.

Sensory attribute	Description
Appearance	
Orange color	Light to dark orange
Taste	
Sweet	Taste of sucrose
Bitter	Taste of bitterness (caffeine)
Odor	
Grassy odor	Odor of green grass, newly cut
Flavor	
Grassy flavor	Green, newly cut grass
Texture	
Firmness	Mechanical textural property, related to the force required for deformation of the product by biting
After taste	
Astringency	Intensity of astringency after rinsing the mouth with water and wait for 30 s

1.4 Statistical analysis

The analysis of variance method was used for analyzing the data obtained in order to detect significance of differences at the 5% level (P=0.05), and the Duncan's Multiple range test was used to make a statistical comparison between the different storage time. The data were analyzed using the SAS 6.0 statistical data analytical software (Statistical Analysis Systems, Cary, NC, USA).

2. Part II: Mass Transfer Behavior during Osmotic Treatment and Mathematical Models Development

The objectives of this part were to study mass transfer behavior of P2 persimmon during osmotic treatment using ternary sucrose - NaCl solution and to develop mathematical models for mass transfer.

2.1 Mass Transfer Behavior during Osmotic Treatment

2.1.1 Material

Persimmons, P2 cultivar, astringent type, having 70% ripeness were packed in Nylon-LDPE bag under vacuum and stored at 4-6 ^{o}C for 6 weeks to remove the astringency before used. The fruits were selected for good, uniform size of weight 100-110 g with an equal degree of ripeness level with total soluble solid content from 15-16 °Brix in order to ensure maximum uniformity of the raw material. The average moisture content was 2.78 ±0.15 kg/kg, dry basis.

2.1.2 Raw Material Preparation

The fruit calyx were trimmed by scissor, washed and prepared in 2 different shapes, disks and cubes. The fruits were horizontally sliced into flat discs of 4.5 mm. and 10 mm. thickness for disks and cubes, respectively by using a vegetable slicing machine. The average diameter of the disk cut using a sharp cork borer was 50 mm. and the average dimension of the cube cut using a sharp knife was 10 mm. In addition, osmotic dehydration of the whole fruit was also studied. The skin was manually peeled with a knife. Photographs of the disk, cube and peeled whole fruit are shown in (Appendix Figure 3).

2.1.3 Osmotic Solution Preparation

Commercial food grade Sucrose and NaCl were used to prepare the osmotic solution. A second-order central composite design (CCD) with two variables was used to study the response pattern and determine the optimum combination of variables. Sucrose concentration (X_1: 30 to 60 g / 100g) and sodium chloride concentration (X_2: 0 to 10 g / 100 g), each at five levels as shown in Table 2, formed the two variables. The solutions were prepared by dissolving sucrose and NaCl in water in the proportion mentioned. These solutions were kept overnight at room temperature before use to ensure complete dissolution.

Table 2 Second-order central composite design (CCD)

Treatment no.	Code value		Sucrose Concentration (X_1,g/100g)	NaCl Concentration (X_2,g/100g)
	X_1	X_2		
1	-1	-1	34.40	1.47
2	1	-1	55.60	1.47
3	-1	1	34.40	8.53
4	1	1	55.60	8.53
5	-1.414	0	30.00	5.00
6	1.414	0	60.00	5.00
7	0	-1.414	45.00	0
8	0	1.414	45.00	10.00
9	0	0	45.00	5.00
10	0	0	45.00	5.00
11	0	0	45.00	5.00
12	0	0	45.00	5.00
13	0	0	45.00	5.00

2.1.4 Osmotic Treatment

The samples were placed in a stainless steel basket (5 mm. mesh) and fully immersed in a glass jar containing the osmotic solution at ratio 1:10 sample to osmotic solution (w/w). The temperature was maintained constant at 30 ^{o}C by using a water bath. The solution was continuously stirred with a flat-blade stirrer (Stuart Scientific, model SS3 Soft Start) to ensure that there were no external mass transfer effects. The stainless steel basket was used to keep the samples totally immersed in the osmotic solution and separate from the stirrer. Samples were taken from the solution after 15, 30, 60, 120, 240 and 360 min. for disks and cubes. In the case of the peeled whole fruit, the samples were taken from the solution after 3, 6, 12, 24, 36 and 48 hr. These samples were rinsed, blotted gently with a tissue paper and placed on a filter paper in order to remove adhering water.

2.1.5 Determination of Sample weight and thickness

The sample weight was determined gravimetrically and the sample thickness was determined by using a vernier caliper.

2.1.6 Determination of Moisture and Solid Content

A known weight of the sample was dried to constant weight in a vacuum oven and the loss weight equaled to the moisture content of the food. A clean and dry aluminum pan was weighed and its weight recorded. Then 4-6 g of the sample was weighed accurately. The sample was then dried in a vacuum oven at 60°C for 18 h. or to constant weight. When constant weight was reached the sample was removed and cooled in a desiccator. The weight of the aluminum pan together with the sample after drying was then recorded. The moisture and solid content was then calculated by assuming that the loss in weight of the sample was due to the loss of moisture alone. The results were expressed on the basis of initial dry matter.

2.1.7 Determination of Mass Transfer Parameters

Weight reduction (W_R), water loss (W_L) and solid gain (S_G) were expressed in kg/kg initial matter in order to account for initial weight differences between samples. Mass transfer parameters as a function of contact time in the solution were determined under the assumption that the solutes in the fruit samples did not diffuse into the solution. These were calculated as follows (Ade-Omowaye *et al*., 2002; Moreira and Sereno, 2003; Mavroudis *et al*., 2004):

$$W_R = (w_o - w_t) / w_o$$

$$S_G = (s_t - s_o) / w_o$$

$$W_L = W_R + S_G$$

where:

w_o = initial weight of sample

w_t = weight of osmosed sample at time t

s_o = initial solid weight of sample

s_t = solid weight of osmosed sample at time t

2.1.8 Determination of Water and Solute Diffusion Coefficients

The estimation of water (D_{ew}) and solute diffusion coefficient (D_{es}) during osmotic dehydration for regular geometries was based on the solution of Fick's second law. The following assumptions were made:

1) uniform initial water distribution;
2) negligible external resistance to mass transfer;
3) no shrinkage during osmotic dehydration;
4) isothermal process
5) apparent diffusion coefficient is constant

For a disk, which can be assumed to be an infinite flat plate of thickness 2*l*, being dehydrated from one side, the following are well known equations for the transfer of water and solute, respectively (Ade-Omowaye *et al*. ,2003):

$$MR = \frac{M_t - M_e}{M_o - M_e} = \frac{8}{\pi^2}\left[\sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 D_{ew} t(\pi / 2l)^2\right]\right] \quad (9)$$

$$SR = \frac{S_t - S_e}{S_o - S_e} = \frac{8}{\pi^2}\left[\sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 D_{es} t(\pi / 2l)^2\right]\right] \quad (10)$$

For a cube, the equation can be combined in three dimensions yielding the following equations for the transfer of water and solute, respectively (Park *et al*., 2002):

$$MR = \frac{M_t - M_e}{M_o - M_e} = \left[\frac{8}{\pi^2}\sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 D_{ew} t(\pi / 2l)^2\right]\right]^3 \quad (11)$$

$$SR = \frac{S_t - S_e}{S_o - S_e} = \left[\frac{8}{\pi^2}\sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 D_{es} t(\pi / 2l)^2\right]\right]^3 \quad (12)$$

where:

MR = the moisture ratio

SR = the solute ratio

M = the average moisture content (kg/kg) on dry basis

S = the average solid content (kg/kg) on dry basis

t = the immersion time

l = the half thickness of the disk or half length of the cube

subscripts *o, e* and *t* are the relevant concentrations initially, at equilibrium, and at any time respectively.

2.1.9 Measurement of Osmotically Treated Products Quality

Osmotically treated product qualities from treatment of (-1,-1), (0, 0) and (1, 1) code value for sucrose and NaCl solutions (see Table 2) were determined.

2.1.9.1 Texture Analysis

Firmness was analyzed using a texture analyzer (TA.XT2 Stable Micro System). A test of compression force was carried out at a constant probe speed. Firmness measurements were taken as the first peak force value obtained during the test to penetrate the fruit and expressed as the force in N. Mean values were calculated from the results of 30 samples for disk and cube, and 10 sample for whole fruit.

The persimmon disk was analyzed at the outer cortex region for 5 points with a steel needle probe of diameter 4 mm. This was conveyed into the persimmon disk at a speed of 5 mm/s, to a penetration depth from the surface of sample of 2.5 mm. A cylindrical probe of diameter 6 mm was used to penetrate the surface of the persimmon cube. This was set up by using a penetration depth of 2.5 mm. at a speed of 5 mm/s. For the whole fruit, the compression force with a cylindrical probe of diameter 6 mm. was used at a speed of 1.5 mm/s to compress through the fruit for 10 mm from the contact point. The measurements were undertaken in three points: at a top, middle and bottom of fruit.

2.1.9.2 Color Analysis

Color determination was carried out on the surface of osmotically samples using a Handy Colorimeter (BYK Gardner, mentioned above) in terms of CIE ‘L*’ (lightness), ‘a*’ (redness and greenness) and ‘b*’ (yellowness and blueness). The sensor was standardized using a white and a black tile. Sensor

was standardized with a white tile and black tile to measure the color. The surface color measurements were done at five different points for 30 samples of disks and cubes, and 15 samples whole fruit samples.

2.1.9.3 Microstructure observation

Microstructure was examined using confocal scanning laser microscopy (CSLM). CSLM was conducted with a Leica TCS NT confocal imaging system (TCS NT version1.6.551) (Appendix Figure 4 a)). Cube samples for observation were sliced to 3 mm. thickness from the surface area with a razor blade.

A section of sample was labeled by directly staining with 0.1% congo red solution (Appendix Figure 4 b)) for 10 min without any further preparation. The cell walls (cellulose) were stained with congo red dye and examined using an excitation wavelength of 568 nm and detecting wavelengths above 590 nm, highlighted the cell wall structure. Each sample was mounted on a glass slide and viewed on the microscope (Appendix Figure 4 c)).

2.1.9.4 Water activity

Water activity (a_w) was measured using a Thermoconstanter Hygrometer (Novasina).

2.1.9..5 Sucrose content

Sucrose content was quantified by HPLC using a Polyspher CHCA column at 90°C eluting with deionised water, with refractometric detector (Forni *et al*., 1992).

2.1.9.6 NaCl content

NaCl content was determined as total chloride using the direct tritration method with $AgNO_3$ (AOAC, 1995).

2.2 Development the Mathematical Models

The response variables Y (Weight reduction (W_R), water loss (W_L), solid gain (S_G), water diffusion coefficient (D_{ew}) and solute diffusion coefficient (D_{es})) were separately correlated with the factor variables: sucrose concentration (X_1) and sodium chloride concentration (X_2). Response surface graphical technique was used to determine the effect of sucrose and sodium chloride concentrations on responses. A quadratic model was chosen for description of the response variables:

$$Y = \sum a_i X_i + \sum a_{ii} X_i^2 + \sum a_{ij} X_i X_j \qquad (13)$$

where a_i represent the linear, a_{ii} the quadratic, and a_{ij} the interaction effect of the factors.

The adequacy of the model was checked by estimating the average relative error E and the determination coefficient R^2.

$$E\ (\%) \quad = \quad \frac{1}{N} \sum_{i=1}^{N} \left| \frac{V_E - V_P}{V_E} \right| 100 \qquad (14)$$

where N is the number of experimental data, V_E is the experimental value and V_P is the value calculated from the model. Values of E less than or equal to 10% are considered to fit the experimental data satisfactorily (Lomauro *et al*., 1985).

2.3 Statistical analysis

The response variables obtained from the experiments were analyzed using SAS 6.0 statistical data analytical software (Statistical Analysis Systems, Cary, NC, USA). The RSREG (response surface regression) procedures were used for testing significance and developing appropriate models for predicting each response. The fitted quadratic equation was expressed as surface plot using Statistica 5.0 software in order to visualize the relationships between the response and factor levels.

The analysis of variance method was used for the data obtained to detect significance of differences at the 5% level (P=0.05), and Duncan's new multiple range test was used to make a statistical comparison between the treatments. The data were analyzed using the SAS 6.0 statistical data analytical software

3. Part III: Persimmons Product Development using Osmotic dehydration as a Pre-treatment

The objective of this part was to develop value-added persimmon products using osmotic dehydration as a pre-treatment which would be found to be acceptable by consumers. The final products were osmodehydrated whole fruit and osmodehydrofrozen cubes.

3.1 Raw Materials Preparation

For producing osmodehydrated and osmodehydrofrozen products, persimmon fruits having ripeness of 70% and 60%, respectively were packed in Nylon-LDPE bag under vacuum and stored at 4-6 ^{o}C for 4 weeks to remove the astringency. For osmodehydrated samples, the fruits selected were 80% ripe and the juice had 17-19 °Brix, whereas for osmodehydrofrozen product, the ripeness level was 70% and the juice was 15-16°Brix. The sizes of the fruits for osmodehydrated

product were also uniform and their weights varied between 110-120 g. The fruits were trimmed the calyx by scissor, washed and hand peeled. The fruits for osmodehydrofozen product were horizontally sliced into flat discs of 10 mm. by using a vegetable slicing machine. The average dimension of the cube cut using a sharp knife was 10 mm.

3.2 Optimization Condition for Osmotic Treatment

Response surface graphical optimization technique was used to determine the workable optimum condition for osmotic treatment, before further processing by microwave-vacuum drying or cryogenic freezing.

In order to determine the optimal concentration range of the osmotic medium, the criterion used in the case of the whole fruit was to select the concentrations which gave a high ratio of *WL/SG* which is a good indicator of the extent to which the osmotic solution succeeds in maximising water loss and minimising solid gain. In the case of persimmon cube, the crierion used was a high value D_{ew} in order to achieve a high rate of water removal and a low value of D_{es} to minimize solid gain.

Relevant contour plots were superimposed and the regions which best satisfied the above constraints were selected. The optimized sets were verified by conducting experiments under those conditions. Responses were monitored and the results were compared with model predictions. The optimum conditions were also judged by the sensory acceptance of panels.

3.3 Combined Osmotic and Microwave-vacuum Dehydration

Peeled whole fruits were placed in 2% $CaCl_2$ solution for 2 hrs before osmotic dewatering to improve the texture of the product. Further, 100 ppm of sorbic acid and sodium metabisulphite were also added to the solution in order to prevent fermentation during the osmotic process. The fruits were osmosed to 30%, 35% and

40% solid content (the immersion time was determined from the drying curve). Drying of fresh and osmotically treated fruits was conducted in a microwave vacuum dryer (Marchcool Industry Co., Ltd). The fruits were placed in the mictrowave chamber (Appendix Figure 5) and subjected to the following continuous programs: 640 W for 10 min, 320 W for 15 min and 160 W for 10 min. at vacuum pressure gage of -600mmHg. Water activity values lower than 0.75 were achieved in all cases and the texture was soft similar to the fresh fruit.

3.4 Combined Osmotic and Cryogenic Freezing

Cubes (fresh and osmotically treated samples) were sealed in polyethylene plastic bag before freezing. Samples were frozen at -40 °C in cryogenic freezer (Appendix Figure 6) and stored at -18 °C for 30 days. Samples were withdrawn from storage condition weekly. Thawing was carried out at 4 °C for 10 h.

3.5 Measurement of Persimmon Product Quality

3.5.1 Moisture and Solid Content

A known weight of the sample was dried to constant weight in a vacuum oven and the loss weight equated to the moisture content. A clean and dry aluminum pan was weighed and its weight recorded. Then 4-6 g of the sample was weighed accurately in the pan and the weight recorded. The sample was then dried in a vacuum oven at 60°C for 18 h. or to constant weight. When constant weight was reached the sample was removed and cooled in a desiccator. The weight of the aluminum can and food sample after drying was then recorded. The moisture and solid content of the food was then calculated by assuming that the loss in weight of the sample was due to the loss of moisture only.

3.5.2 Texture Analysis

Firmness was analyzed using a texture analyzer (TA.XT2 Stable Micro System). A compression test was carried out at a constant speed. Firmness measurement was taken as the peak force value observed during the penetration test and expressed in Newtons. For whole fruit osmodehydrated product, the mean values were calculated from the results obtained on 10 samples. The compression test was carried out with a cylindrical probe having diameter 6 mm. penetrating at a speed of 1.5 mm/s to for a depth of 10 mm from the contact point. The measurements were made at three points: at the top, middle and bottom parts of fruit. The same probe was also used to penetrate the surface of the thawed persimmon cubes, but the penetration depth was 2.5 mm. and the speed was 5 mm/s. Mean values were calculated from the results obtained on 30 cube samples.

3.5.3 Color Analysis

Color analysis was carried out on the surface of samples using a Handy Colorimeter (BYK Gardner) and expressed in terms of CIE 'L*' (lightness), 'a*' (redness and greenness) and 'b*' (yellowness and blueness). As described earlier, the sensor was standardized with white and black tiles. The surface color measurements were averaged over five different points on each of the 15 samples in the case of whole osmodehydrated fruits and each of the 30 samples in the case of cube.

3.5.4 Water Activity

Water activities (a_w) of osmodehydrated persimmon samples were measured using a Thermoconstanter Hygrometer (Novasina).

3.5.5 Drip loss

To estimate the drip loss of the fruit cubes during thawing, the frozen persimmon cubes were each put into a tare beaker of 100 ml capacity and covered with some glass and then thawed at 4°C for 24 h. Afterwards, the cubes were all put on a sieve for 15 min to allow the exuded water to drip off and then they were weighed. The difference between this weight and their initial weight was defined as the drip loss of the cubes. Triplicates of all the differently treated cubes were determined.

3.5.6 Microbial Analysis

Aerobic plate count, yeasts and molds of osmodehydrated product were conducted using standard techniques (B.A.M., 2001). 25 grams of samples were homogenized with 225 ml of sterile peptone stock solution in a stomacher (MIX1, AES Laboratory) for 2 min. One milliliter of this suspension was placed in tubes containing 9 ml of sterile peptone stock solution. Serial decimal dilutions to 10^{-5} were made. Total aerobic counts were determined on Plate Count Agar (PCA) incubated at 35 °C for 48 h. The yeasts and moulds were counted on Potato Dextrose Agar (PDA) after 5 days of incubation at 25 °C.

3.5.7 Sensory Evaluation

Flavor, texture, color and overall acceptability were evaluated by a 30 member untrained panel using 9 point hedonic scale (9: like extremely and 1: dislike extremely). Each panelist received samples attributed with three-digit random numbers.

3.6 Statistical analysis

The analysis of variance method was used for the data obtained to detect significance of differences at the 5% level (P=0.05), and Duncan's new multiple

range test was used to make a statistical comparison between the treatments. The data were analyzed using the SAS 6.0 statistical data analytical software.