CHATPER 3 MATERIALS AND METHODS

This chapter describes the details of the experimental set-up, test material as well as the experimental procedures and statistical analysis used in this study.

3.1 Materials

Fresh carrots (*Daucus carota* var. sativa) with an initial moisture content of about 8.4-11 kg/kg (d.b.) were purchased from a local market and stored at 4 °C. Before starting of each experiment carrots were removed from a refrigerator to attain room temperature. Carrots were then peeled and diced into cubes with the dimensions of 1×1×1 cm; only the cortex tissue was used because different parts of carrots might have different microstructures, which might affect the results. The moisture content of fresh carrots was determined by drying a sample at 103±2 °C for 16 h in a hot air oven (Memmert, model 800, Schwabach, Germany) according to AOAC official method 984.25 (AOAC, 2000).

3.2 Experimental Set-up

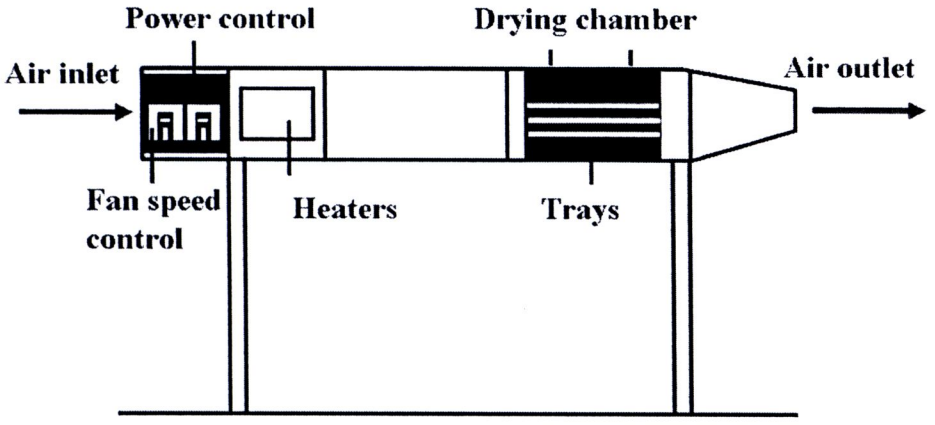

Figure 3.1 A schematic diagram of a hot air dryer

3.2.1 Hot Air Dryer

A schematic diagram of a hot air dryer used in the present study is shown in Figure 3.1. The dryer consists of a stainless steel drying chamber, which is connected to an electric heater rated at 6.6 kW, which was controlled by a proportional-integral-derivative (PID) temperature controller (Fenwal, model AR33L, Tokyo, Japan) with an accuracy of ±1 °C. Carrot cubes were placed on a tray screen with the dimensions of 30×40 cm. The air velocity over the tray in the drying chamber was fixed at 0.8 m/s.

3.2.2 Low-Pressure Superheated Steam Dryer

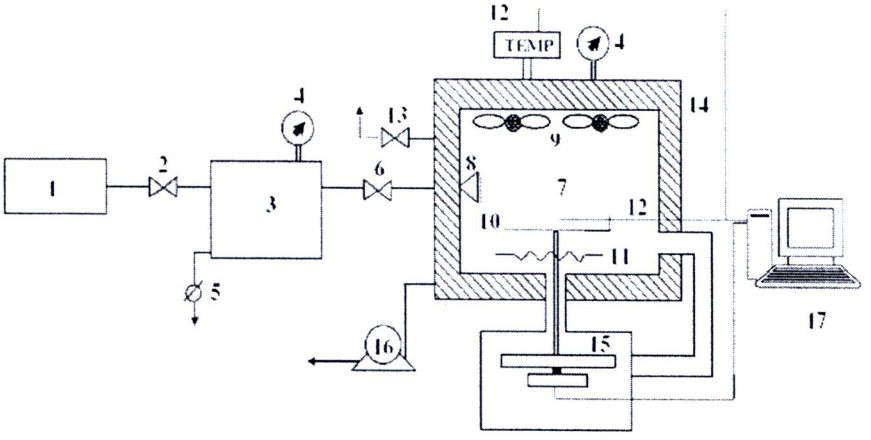

Figure 3.2 A schematic diagram of low-pressure superheated steam dryer and associated units. (1) boiler; (2) steam valve; (3) steam reservoir; (4) pressure gauge; (5) steam trap; (6) steam regulator; (7) drying chamber; (8) steam inlet and distributor; (9) electric fans; (10) sample holder; (11) electric heater; (12) on-line temperature sensor and logger; (13) vacuum break-up valve; (14) insulator; (15) on-line weight indicator and logger; (16) vacuum pump; (17) PC with installed data acquisition card

A schematic diagram of a low-pressure superheated steam dryer as well as its accessories is shown in Figure 3.2. The dryer consists of a stainless steel drying chamber with the inner dimensions of 45×45×45 cm; a steam reservoir, which received steam from a boiler and maintained its pressure at around 200 kPa (gage); and a liquid ring vacuum pump (Nash, ET32030, Nuremberg, Germany), which was used to maintain the vacuum in the drying chamber (fixed at 7 kPa in this study). A steam trap was installed to reduce excess steam condensation in the reservoir. An electric heater, which was controlled by

a proportional-integral-derivative (PID) temperature controller (Omron, model E5CN, Tokyo, Japan) with an accuracy of ±1 °C, was installed in the drying chamber to control the steam temperature and to minimize the condensation of steam in the drying chamber during the start-up period. Two electric fans were used to disperse the drying medium throughout the drying chamber. The change of the mass of a sample was detected continuously using a load cell (Minebea, Ucg-3kg, Nagano, Japan).

3.2.3 Vacuum Dryer

For a vacuum drying (VD) experiment the same experimental set-up as that of an LPSSD experiment was used but without the application of steam to the drying chamber.

3.3 Drying Experiments

To conduct each hot air drying (HAD) experiment approximately 100-120 cubes of carrot (about 100-120 g) were used. The air velocity over the tray in the drying chamber was fixed at 0.8 m/s. Drying experiments were conducted at 60 and 80 °C (Hiranvarachat et al., 2011). Samples were taken at every 60-min interval to determine the moisture content (method 984.25; AOAC, 2000), physical characteristics (shrinkage and hardness) and microstructure. That particular experiment was then terminated and a new experiment was performed until the next sampling time was reached; this step was repeated until carrots reached the moisture content of 0.1 kg/kg (d.b.). All experiments were performed in duplicate.

To conduct each low-pressure superheated steam drying (LPSSD) experiment approximately 35-40 cubes of carrot (about 35-40 g) were used. Drying was performed at an absolute pressure of 7 kPa and temperatures of 60 and 80 °C. The same sampling procedure to that used in the case of the hot air drying experiments was used. For a vacuum drying (VD) experiment the same experimental set-up as that of an LPSSD experiment was used but without the application of steam to the drying chamber.

3.4 Measurement of Volumetric Shrinkage

Five carrot cubes taken at any sampling time were used for a volumetric shrinkage measurement. Shrinkage is expressed in terms of the percentage change of the volume of carrot as compared with its initial volume as shown in Eq. (3.1)

$$\% \text{ Shrinkage} = \left(\frac{V_0 - V}{V_0} \right) \times 100 \tag{3.1}$$

where V_0 and V are the initial volume of carrot and the volume of carrot at each sampling time, respectively. The carrot volume was determined by *n*-heptane displacement (Devahastin et al., 2004). It is noted that the maximum uncertainty in the shrinkage measurement was ± 6.5%.

3.5 Textural Analysis

A texture analyzer (Stable Micro System, TA.XT.Plus, Surrey, UK) was used to evaluate the texture of a carrot cube through a compression test. The test involved applying a direct force to a sample, which was placed on a planar base. A 2-mm diameter rod probe was set to travel at a crosshead speed of 2 mm/s until the sample was penetrated. The maximum force in the force-deformation curve was noted and used to indicate the hardness of the sample.

3.6 Quantification of Sample Microstructure

Scanning electron microscopy (SEM) images were selected to be analyzed in this study instead of light microscopy (LM) images, which were used by Kerdpiboon and Devahastin (2007). This is because SEM images could be obtained in much less time than LM images; it is noted that many sample preparation steps are required prior to being able to capture each LM image. SEM also introduces fewer artifacts since the sample sectioning step is not required (Aguilera and Stanley, 1999); the sample could be fractured for scanning instead of being microtome sectioned as in the case of LM.

3.6.1 Quantification of Fractal Dimension

The fractal dimension of each sample image was calculated using the box counting algorithm (Quevedo et al., 2002) via the use of the ImageJ software (version 1.42q,

National Institutes of Health, Bethesda, MD). An image was first converted to a binary image and the edges of the cell spaces were segmented and detected. This was in turn used to calculate the fractal dimension.

To compare the changing values of the fractal dimension of a sample during drying the normalized change of the fractal dimension are reported (Kerdpiboon and Devahastin, 2007):

$$\Delta FD/FD_0 \tag{3.2}$$

$$\Delta FD = FD - FD_0 \tag{3.3}$$

where FD_0 and FD are the fractal dimension of an image of a fresh sample and that of a sample at any instant during drying, respectively.

3.6.2 Quantification of Cell Diameter

Prior to observing the microstructure of a sample via SEM the sample was prepared in the following manner. First, the sample was preserved with a fixing solution, which was made of 50% (v/v) ethyl alcohol solution, 40% (v/v) formaldehyde and glacial acetic acid at a ratio of 90:5:5. The sample was then washed with distilled water 3 times before being dehydrated with ethanol. The ethanol concentration used was increased in step, starting from 30%, 50%, 70%, 90% and 100% (v/v) (dehydration at a concentration of 100% (v/v)

was performed 3 times). The time used for dehydrating the sample at each ethanol concentration was approximately 30 min. Next, the sample was dried by a critical point dryer (Balzer, model CPD 020, Liechtenstein); ethanol was replaced by liquid CO_2 in order to maintain the cell structure of the sample. Each sample was ruptured at the center in liquid nitrogen before being mounted and then coated with gold. Finally, the microstructure of the sample was observed along the cross-section at the center of the cube. Only the microstructure along the cross section at the center of the cube was arbitrarily chosen to be observed since it was not possible to show or analyze via the use of image analysis the whole surface cross section at one time. The microstructural images were obtained using a scanning electron microscope (JEOL, model JSM-5410 LV, Tokyo, Japan) at 200× magnification at image sizes of 1332×1000 pixels.

Each SEM image of carrot was analyzed using ImageJ image analysis software (version 1.37c, National Institutes of Health, Bethesda, MD), which is capable of analyzing and calculating the fractal dimension of a three-dimensional image. The images were converted to binary images using thresholding-based segmentation technique, which was also used to distinguish cell spaces (or, in other words, the cell volume, the cellular dimension) from cell walls and intercellular spaces. The cell diameter was evaluated with the assumption that each and every cell was spherical. The average cell diameter was calculated from the histogram of a cell diameter distribution curve.

To compare the changing values of the average cell diameter of a sample during drying the normalized change of the average cell diameter are reported:

$$\overline{\Delta D} / \overline{D_0} \tag{3.4}$$

$$\overline{\Delta D} = \overline{D_0} - \overline{D} \tag{3.5}$$

where $\overline{D_0}$ and $\overline{D}$ are the average cell diameter of a fresh sample and the average cell diameter of a sample at any instant during drying, respectively.

3.7 Statistical Analysis

Correlations between $\Delta FD/FD_0$ and $\overline{\Delta D} / \overline{D_0}$ and X/X_0 and apparent physical changes were assessed by the Pearson's correlation coefficient. Pearson's correlation coefficient is a measure of a linear relationship between two variables and the correlation coefficient may take on any values between -1 and + 1. The sign of this correlation coefficient (+, –) represents the direction of a relationship. A positive correlation coefficient indicates that the values of both variables change in the same direction, while a negative coefficient indicates that the values of both variables change in the opposite directions. In addition, the experimental data were analyzed using the analysis of variance (ANOVA) via the use of SPSS® software (version 13; SPSS, Inc., Chicago, IL). The results are presented as mean values with standard deviations. Duncan's multiple range tests were employed to establish differences between mean values at a confidence level of 95 %. All experiments were performed in duplicate unless specified otherwise.