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Research paper

Observation of glassy state relaxation during annealing of frozen sugar solutions by X-ray computed tomography

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ABSTRACT

Glassy phase formation in a frozen product determines various properties of the freeze-dried products. When an aqueous solution is subjected to freezing, a glassy phase forms as a consequence of freeze-concentration. During post-freezing annealing, the relaxation of the glassy phase and the ripening of ice crystals (i.e. Ostwald ripening) spontaneously occur, where the kinetics are controlled by the annealing and glass transition temperatures. This study was motivated to observe the progress of glassy state relaxation separate from ice coarsening during annealing. X-ray computed tomography (CT) was used to observe a frozen and post-freezing annealed solutions by using monochromatized X-ray from the synchrotron radiation. CT images were successfully obtained, and the frozen matrix were analyzed based on the gray level values that were equivalent to the linear X-ray attenuation coefficients of the observed matters. The CT images obtained from rapidly frozen sucrose and dextrin solutions with different concentrations gave clear linear relationships between the linear X-ray attenuation coefficients values and the solute concentrations. It was confirmed that the glassy state relaxation progressed as increasing annealing time, and this trend was larger in the order of the glass transition temperature of the maximally freeze-concentrated phase. The sucrose-water system required nearly 20 h of annealing time at −5 °C for the completion of the glassy phase relaxation, whereas dextrin-water systems required much longer periods because of their higher glass transition temperatures. The trends of ice coarsening, however, did not perfectly correspond to the trends of the relaxation, suggesting that the glassy phase relaxation and Ostwald ripening would jointly control the ice crystal growth/ripening kinetics, and the dominant mechanism differed by the annealing stage.

1. Introduction

Formation of the glassy phase in a frozen product greatly links to the key properties of the frozen or freeze-dried products such as stability of biological component, storage stability, cake firmness, reconstitution ability etc. [1–7]. For better operating a process that involves hydration and/or dehydration, a special care must be taken for glassy state controls for giving a desirable property to the product [8,9]. When a product that contains water is subjected to freezing, a glassy phase forms as a consequence of freeze-concentration, and the water content in the glassy phase can be related to the temperature. The glass transition temperature (T_g) of the maximally freeze-concentrated glassy phase is commonly denoted as T'_g, and the water content of this phase is denoted as W'_g. When a product is rapidly frozen, the glassy phase is not perfectly freeze-concentrated because of the formation of the non-crystalized water (i.e. vitrified water) [10–14]. Annealing over T_g reduces the fraction of the amorphous phase, so it achieves completion of the freeze-concentration and results in the reduction of the water content to W'_g at T'_g. This is a relaxation of the glassy state, and the relaxation rates increase exponentially with $(T - T_g)$ [12,15]. This non-Arrehenius kinetics is known as Williams-Landel-Ferry (WLF) kinetics, where the state behavior is governed by the mobility of the matrix plasticized by water. Ice crystals, that coexist with the glassy phase, can recrystallize above T'_g to minimize interphase surface area and result in ripening of ice crystals. This phenomenon is known as Ostwald ripening [16,17], and this can be used to modify ice microstructures in terms of size and uniformity in a frozen product [18]. Post-freezing annealing is a commonly used technique in freeze-drying (lyophilization) process in order to reduce primary drying time and also to alter crystallinity and/or aggregation degree of the components [1,6,10,11,19–23]. During freeze-drying, water is mainly removed from the frozen layer by sublimation. The removal of water coincidently forms dried layer, and the

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subsequently sublimated water vapor must transfer from the frozen interface through the dried cake layer with porous microstructures. The mass transfer resistance in the cake layer is thus controlled by the modification of the ice crystal sizes and morphologies [18,24–26]. Water removal during freeze-drying is not only by ice sublimation but also by vaporization from the freeze-concentrated amorphous phase. The viscous flow of the freeze-concentrated phase may cause the loss of the dried layer structure (i.e. collapse) depending on the temperature, evaporation rate and dried layer strength [15,27–32]. The degree of the relaxation directly relates to the viscosity of the glassy state. Furthermore, it would affect the rate of the water evaporation from the glassy state and the resultant strength of the dried layer. The temperature that causes collapse of a freeze-drying matrix could be determined as a result of the interactions among these parameters.

X-ray computed tomography (CT) is a powerful tool to visualize microstructure of a material without sample destruction, and to quantify structural information based on the visualized images [33–35]. A CT image is composed of volume elements, and the gray levels in an image correspond to the attenuation of X-ray that pass through each volume element. X-ray attenuation is mainly determined by the applied X-ray energy, the material density and composition. When an image is acquired with monochromatized X-ray (i.e. (mono-energetic photons), the gray levels directly indicate the density and composition of the observed area [36–38]. Our concern in this study is to observe the glassy state transition during annealing by using CT with monochromatized X-ray; this can be realized by using the synchrotron light source. In a frozen system, the ice crystal phase is separated from the glassy phase, and the attenuation levels of X-ray through these phases are different from each other. The obtained CT image is a reflection of the phase compositions, so the glassy state relaxation and ice crystal ripening are expected to be quantified based on a CT image.

In this study, an attempt was made to observe a frozen and post-freezing annealed solutions by X-ray computed tomography using monochromatized X-ray from the synchrotron radiation. A motivation was to observe the progress of glassy state relaxation during annealing separate from ice coarsening. The transition phenomena that occur in the frozen matrix were analyzed based on the value of the linear X-ray attenuation coefficient estimated from CT images. Experiments were carried out with frozen dextrin and sucrose solutions. Optical microscopic observations were separately carried out to support the obtained results from the X-ray CT analysis.

2. Materials and methods

2.1. Materials

Millipore-purified water was used for the sample preparation. Sucrose was purchased from Wako Pure Chemical Industries (Osaka, Japan). Dextrins were donated by Matsutani Chemical industry Co., Ltd. (Hyogo, Japan). Dextrins of which dextrose equivalent (DE) equal to 11 and 25 were used in this study, and they are respectively denoted as Dex11 and Dex25. Glass transition temperatures of maximally freeze-concentrated aqueous solutions of these dextrins (T'_g) were measured by DSC, and that for Dex11 and Dex25 were around −11 °C and −18 °C, respectively.

2.2. Frozen sample preparation

Sucrose and dextrin aqueous solutions were prepared with concentrations between 10 and 80 %(w/w). These solutions were filled in a plastic microtubes (1.5 mL, diameter 10 mm, height 30 mm), and set them in an aluminum thermoblock with holes that fit for 1.5 mL microtubes. The thermoblock was pre-cooled in liquid nitrogen so as to freeze inner solution rapidly with keeping its direction of freezing from the bottom to the top. Frozen samples with a concentration of 20% (w/w) were subsequently annealed at −5°C in an electric cooling devise for selected duration (0–35 h). Frozen samples were then stored in a deep freezer at −90 °C until measurement.

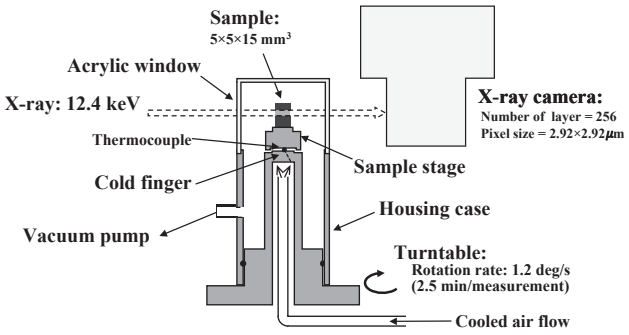

Fig. 1. Schematic illustration of X-ray CT measurement stage.

2.3. Synchrotron X-ray computed micro tomography

X-ray computed tomography was carried out at synchrotron facility SPring-8 (BL19B2, Hyogo, Japan). Monochromatized X-ray was used for this measurement in order to obtain X-ray attenuation linear coefficient from the tomograms. The X-ray energy was adjusted to 12.4 KeV by a double Si-crystal monochromator (net plane: (1 1 1)) and a pair of X-ray mirrors coated with Rh (mirror angle: 3 mrad) that remove harmonics contaminants from monochromatized X-ray.

The set-up of the measurement stage was schematized in Fig. 1. The measurement stage was equipped with a turntable with a cold finger of which temperature was controllable by an external air blowing device in the range of −80 to −10 °C. Frozen sample was taken out from the microtube by cutting the tube with a craft cutter and fixed on the sample stage made by stainless steel pre-cooled at −70 °C with respecting the direction of freezing. Frozen sample was trimmed within the size of 5 × 5 × 15 mm before the fixation. All of this operation was rapidly carried out on a dry ice plate, and a cryostat specimen matrix (Tissue-Tek® O.C.T. Compound, Sakura Finetek Co. Ltd., Japan) was used for the fixation. The sample stage with a frozen sample was immediately placed on the cold finger pre-cooled at around −80 °C. The sample stage on the turntable was then fully covered with a housing case, and the inner space was evacuated by connecting a vacuum pump in order to avoid frosting during measurement. The top of this housing component was made by acrylic resin with a thickness of 0.5 mm. The X-ray passed through this acrylic window and the sample, and then exposed to X-ray camera that was set 100 mm behind the housing component. An X-ray imaging unit (AA40, Hamamatsu Photonics K.K., Japan) and CCD camera (C4880-41S, Hamamatsu Photonics K.K., Japan) were used for image acquisition. 256 transmission images were acquired for one set of measurement by rotating the turntable from 0° to 180° at a speed of 1.2°/s. This set-up can achieve freeze-drying a solution by increasing the stage temperature, but, in the present study, the temperature was kept at low not to accelerate freeze-drying. The exposure time of X-ray was set to 0.12 s for taking an image, and the pixel size in the image was 2.92 μm square. Intensity of each pixel in a transmission image was converted into transmission rate, R, by dividing the intensity values in the transmission image with sample by those in the image without sample. A horizontal cross sectional image (i.e. tomogram) was reconstructed from the transmission image by the filtered back projection method. The pixel intensity values that construct tomogram were equivalent to the linear X-ray attenuation coefficient. All the images were measured in triplicate with different sample lots.

2.4. Microscopic observation of frozen samples

Frozen samples that contain slight amount of rhodamine dye were separately prepared for microscopic observation with exactly same