Review Article *Science, Engineering and Health Studies 2018, 12(3), 125-137*

Food drying at sub-zero temperature: Importance of glassy phase on product quality

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Received: June 11, 2018; Accepted: September 27, 2018

ABSTRACT

Large number of drying researches have been carried out in pharmaceutical and food sectors with particular interest in the quality assurance. Freeze-drying is known as one of the best drying method for maintaining original product qualities. However, it involves numbers of potential factors that lead to quality loss of the product. This review elucidated the mechanisms of freeze-drying with focus on the phenomena relating to the glassy phase. Freezing process involves formation of the ice crystal and freeze-concentrated phase, glassy phase, and the relaxation phenomena. This process determines ice crystal morphology, the degree of freeze-concentration and the crystallinity of the concentrated matters. Product temperature during freeze-drying affects various quality attributes, and the influence was dependent on how high is the temperature from the glass transition temperature. These explanations were made with the X-ray computed tomography (CT) images of freeze-dried materials. It is important to understand the mechanisms of the relaxation phenomena in order to optimize product quality. A method that can deal with the complicated interaction of the kinetics in a glassy matter could be a clue.

Keywords: food drying; glass transition; vacuum freeze-drying; atmospheric freeze-drying

1. FOOD DRYING: IMPORTANCE OF THE GLASS TRANSITION

Drying is one of the techniques since ancient times in order to control water in a material. It has long been known that the shelf stability of food is strongly related to the moisture content. Water in food is closely related to the physical properties of food, kinetics of quality change, bacteria growth rate during storage and so forth. Numbers of drying techniques have been implemented both in terms of culturally and industrially. Sun drying, smoking, salting, pickling, frying and roasting are all food drying techniques. For example, salting is a dehydration operation by using osmotic pressure difference through the cell membrane as a driving force of the mass transfer. Smoking lowers moisture content of the product surface to form a dried film layer. This layer preferably adsorb aroma component that effectively inhibit bacteria growth. Natural ham production process (i.e. salting, smoking and drying in a cool dark place) is not only to decrease moisture content but also to crystallize umami component and killing of virus.

In recent years, large number of drying researches have been carried out for pharmaceutical and food industries. A major focus is to get rational guidelines to meet quality requirements rather than the removal of moisture (Ciurzyńska and Lenart, 2011; Devahastin and Niamnuy, 2010; Duan et al., 2015;

ISSN Online 2630-0087 DOI: https://doi.org/10.14456/sehs.2018.12

Jangam, 2011; Ratti, 2001; Sagar and Kumar, 2010). In the case of powdered drugs, for instance, not only the expected medicinal effect of the ingredients but also the formability, solubility and so forth are quality parameter affected by the drying conditions. And these qualities are to be assured by GMP validations (Cogdill and Drennen, 2008; Yu, 2008). In the case of food production, safety, taste, texture, aroma, retention of nutrient components, cooking performance (instant food calibration and cracking etc.), appearance, color, shelf stability, and the like are counted as qualities. Food quality and safety are regulated by the quality standard system such as Codex, HACCP, etc. (Trienekens and Zuurbier, 2008) Many of these qualities are affected by the processing, and water transfer in food system is one of the most important quality parameter. Numbers of studies were dedicated to optimize processing conditions in order to maximize product qualities (Banga et al., 2003; Banga and Paul Singh, 1994; Baş and Boyacı, 2007; Devahastin and Niamnuy, 2010; Dufour, 2006; Nakagawa et al., 2016b).

In order to know the affinity of products to water, it is common to use water adsorption isotherms. It is often denoted “sorption isotherm” by food scientist and technologists. The sorption isotherm is obtained by plotting the moisture content against the ratio of water vapor pressure and saturated water vapor pressure (relative vapor pressure). When a food with certain moisture content is stored in a sealed container, the inside of the container reaches a constant water vapor pressure. This relative vapor pressure is denoted as the water activity; this has been considered as an important measure for predicting the shelf stability of food. Lowering the water activity prolongs the shelf stability; the activity value is essential rather than the moisture content. Therefore, even foods having the same water content, we predict that the one with lower water activity is more stable. This is a well-known concept being summarized in the "food stability map" by Labuza (Figure 1) (Labuza, 1970). The reaction rates of various phenomena are given in this map as a function of the water activity. Although this is practically useful map that indicates qualitative measures, it cannot be universally applicable because the absolute water activity (relative vapor pressure) value that controls various reaction kinetics depend on each food system and its composition (van den Berg, 1986). A misleading given by this map was cautioned, especially in case systems with different compositions are compared (Slade et al., 1991). At the Faraday Discussion Conference held in Cambridge in 1985, the arguments were settled in the limitation of the equilibrium treatment of water activity, the error of the old concepts such as bound water and unfrozen water, and the importance of the plastic nature of food components (Slade et al., 1991).

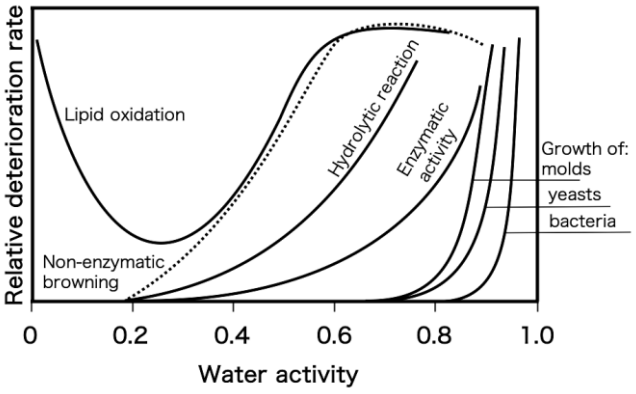

Figure 1 Food stability map (redrawn with permission (Slade et al., 1991)).

Natural polymers and oligomers such as proteins, polysaccharides, etc. are major food components. For those molecular assemblies, water acts as a plasticizer. The viscosity of the plasticized matrix increases as decreasing temperature. The temperature at which the viscosity of the polymer plasticized by water reaches at about 10^{12} Pas is defined as the glass transition temperature. At temperatures below the glass transition point, the molecular motion in the glass phase is

greatly restricted and various reaction rates are also decreased. In glass systems, it will not reach true equilibrium within a short period. Considering the lifespan of food products, dynamic properties control the system rather than equilibrium physical properties. Although the above-mentioned food stability map is a reasonable expression of reaction rates in products that are nearby equilibrium, the reactions that occur during processing cannot be well predicted. When drying a product, the system approaches the glass transition point as decreasing the moisture content. Along with this, the molecular mobility decreases in the system, and consequently affects the kinetics of physicochemical phenomena. Furthermore, drying is a non-isothermal process in which heat and mass simultaneously transfer, therefore it is not simple to predict the variations of kinetics. Kumagai estimated Gibbs energy change of water and adsorbent (i.e. food polymers) before and after the water sorption (Kumagai et al., 1997a; Kumagai et al., 1997b). The change in Gibbs energy on the adsorbent has a greater impact than that of water, suggesting that the property change of solid by plasticizer (i.e. water) is a major attribute of the kinetics of physicochemical phenomena. An important quality attribute linking to drying process could largely be affected by the phenomena in the glassy phase that forms in a product. Following sections review on topics of dehydration and product quality with particular focus on drying at sub-zero temperature.

2. GLASSY PHASE FORMATION DURING FREEZING

When a product is frozen, 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. It is known that the degree of the freeze-concentration is kinetically controlled; the glassy phase formed in a rapidly frozen system is not perfectly freeze-concentrated because of the formation of the non-crystalized water (i.e. vitrified water) (Ablett et al., 1992; Kawai et al., 2006; Levine and Slade, 1988; Ohkuma et al., 2008; Sahagian and Goff, 1994). This imperfect freeze-concentrated phase is gradually concentrated due to the increase in crystalized water, namely due to the relaxation of the freeze-concentrated phase. The rate of the relaxation is dependent on the temperature. When a glassy system is placed over T_g, the fraction of the amorphous phase exponentially decreases with ($T - T_g$) (Levine and Slade, 1988; Slade et al., 1993). This kinetics is known as Williams-Landel-Ferry (WLF) kinetics, where the state behavior is governed by the mobility of the matrix plasticized by water. At the end of the relaxation, that phase is maximally freeze-concentrated and results in the reduction of the water content to W'_g at T'_g. Nakagawa et al. employed X-ray computed tomography technique to observe a frozen sugar solutions (i.e. rapidly frozen by liquid nitrogen and placed at –5ºC for selected period) by using monochromatized X-ray from the synchrotron radiation, and confirmed the progress of the freeze-concentration as a function of time placed over T_g (Nakagawa et al., 2018a). They reported that the trend was obvious in the order of T'_g of the solution; the sucrose-water system required nearly 20 hours for the completion of the relaxation, whereas dextrin-water systems required much longer periods. Obvious ice crystal size increase was also confirmed as the progress of the relaxation. However, ice crystal sizes continued to increase even after the completion of the freeze-concentration due to Ostwald ripening. Ice crystals in a multiphase system can recrystallize to minimize interphase surface area and result in ripening of ice crystals (Kurz and Fisher, 1986; Ratke and Voorhees, 2013; Searles et al., 2001a, b). These mechanisms are commonly used in freeze-drying

process (i.e. annealing, or post-freezing annealing) in order to reduce total drying time and to foster crystallinity and/or aggregation degree of the components (Abdul-Fattah et al., 2007; Ablett et al., 1992; Goshima et al.; Kasper and Friess, 2011; Milton et al., 1997; Nakagawa et al., 2016a; Nakagawa et al., 2009; Nakagawa et al., 2018a; Pikal, 2004; Sahagian and Goff, 1994). It is widely recognized that a slow freezing leads formation of larger ice crystals and a rapid freezing leads smaller ice crystal formation (Nakagawa, 2011; Nakagawa et al., 2006, 2007). In a freezing system, ice crystal growth and related relaxation phenomena coincidently occur. Phenomena that we expect during post-freezing annealing are included in a freezing step (Nakagawa et al., 2018a; Nakagawa et al., 2018b). Freezing rate relates to the combinations of the ice crystal growth rate and the relaxation kinetics, and consequently determines resultant physicochemical properties of a frozen product.

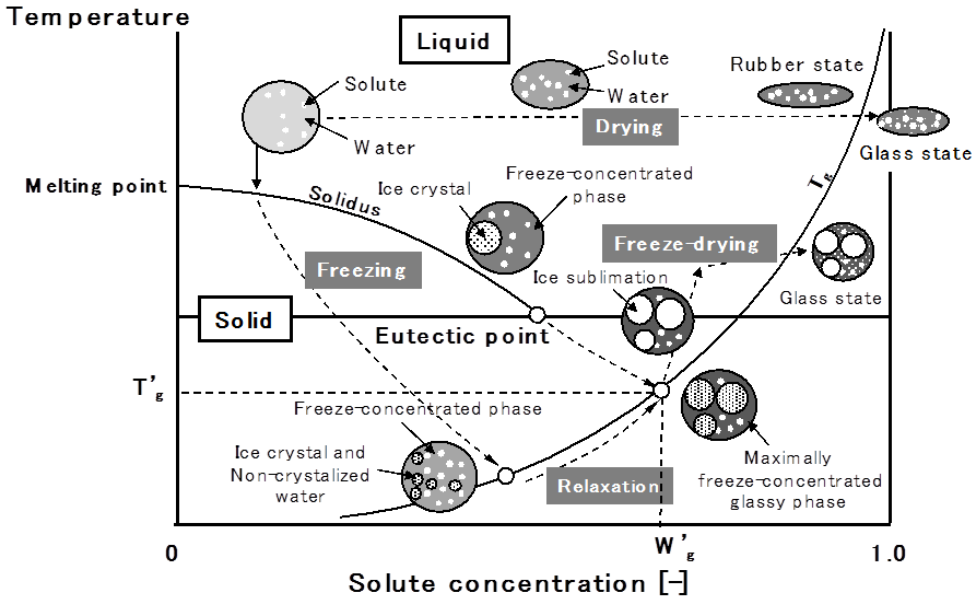

Figure 2 Progress of freezing and drying tracked on the phase diagram.

3. VACUUM FREEZE-DRYING

Freeze-drying process involves two dehydration mechanisms: sublimation of ice crystals and evaporation from the freeze-concentrated phase (i.e. glassy phase). The later mechanism closely links to the product quality of the resultant dried product. The driving force of the water removal is produced by the vapor pressure difference between the product surface and the sublimation (evaporation) interface. In a vacuum freeze-drying operation, products are placed under vacuum condition within the pressure range of several Pa-several hundred Pa in order to realize appropriate driving force for the mass transfer. The water removal consequently forms dried layer, and the pore microstructures of the dried layer correspond to the mass transfer resistance for the subsequently sublimated water vapor that transfers from the frozen interface. The pores that form as a consequence of water removal are the replicas of the ice microstructures in the original solution. The mass transfer property during freeze-drying is thus controlled by modifying the ice crystal morphologies in terms of size and uniformity (Andrieu and Vessot, 2017; Nakagawa et al., 2006, 2007; Searles et al., 2001a). The product temperature

during drying is determined by the interaction between the mass and heat transfer flux, so it is affected by the numbers of factors such as thermal and mass transfer property both in terms of product and device, vacuum level (pump and leak flow rate), etc. Vacuum freeze-drying is commonly operated with a product temperature in the range at around –30 ~ –10ºC. This temperature must carefully be controlled in order to achieve successful freeze-drying to realize desired product quality.

Water is removed also by vaporization from the freeze-concentrated amorphous phase with the temperature over T_g. Since the movement of the water in the glass phase is largely restricted in the glass state, the apparent vapor pressure is also observed to be smaller as it approaches the glass transition line. As shown in Figure 3, isobaric contour can be obtained by connecting points where the relative pressure becomes equal on the state diagram based on the water sorption isotherm measured at different temperatures. As the distance from the glass transition line increases, the relative pressure of water vapor becomes equal to that of bulk water and it is observed remarkably low near the glass transition line. When moisture content in a product decreases due to drying, it will come across the isobaric contours, as shown in Figure 3. When approaching the glass transition line, the drying rate is slowed by the decrease of the apparent vapor pressure.

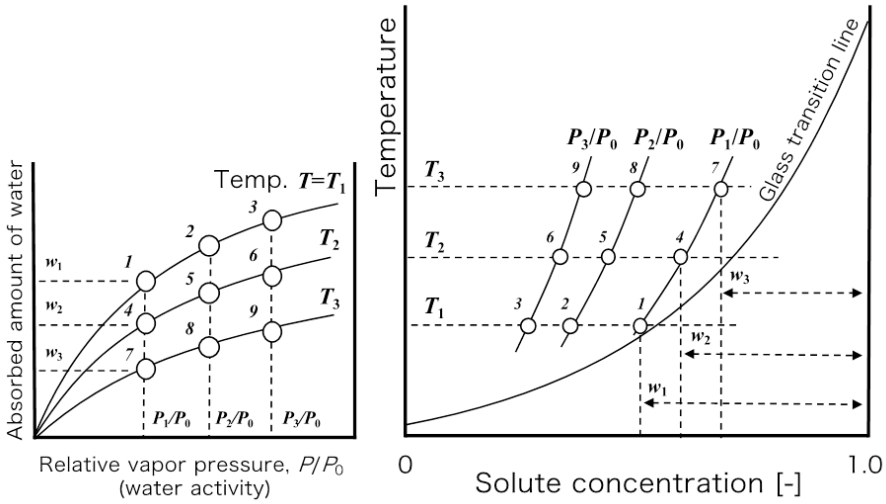

Figure 3 Converting water sorption isotherm into the isobaric contours.

When freeze-drying, the operation is carried out with a product temperature close to the glass transition temperature, therefore, the product temperature must be increased over T'_g in order to remove water from the glassy phase. However, when the temperature is increased at a critical level, the viscous flow of the glassy phase (i.e. rubbery phase) causes the loss of the dried layer structure. This phenomenon is called as collapse, and it occurs depending on the temperature, evaporation rate and dried layer strength (Carpenter et al., 1997; Levi and Karel, 1995; Meister and Gieseler, 2009; Nakagawa et al., 2018b; Pikal and Shah, 1990; Slade et al., 1993; Slade et al., 1991; To and Flink, 1978). The degree of the relaxation directly relates to the viscosity and the apparent vapor pressure of the glassy state. The collapse temperature in a freeze-drying run is determined by a complicated combination of the physicochemical factors that relate to both freezing and drying.

4. ATMOSPHERIC FREEZE-DRYING

Freeze-drying can also be realized under atmospheric condition by using low humidity air.

Mechanism of atmospheric freeze-drying is basically equivalent to vacuum freeze-drying, where the ice sublimation and evaporation from the glassy phase (rubbery phase) are the dehydration mechanisms. The water vapor pressure difference between the frozen zone and the ambient gas is the driving force of the mass transfer. The air humidity must be kept low enough to operate at below sub-zero temperature. Heat-pump system is commonly used to produce low humidity air. Circulating air was condensed at a condenser with a temperature in the range at around –30 ~ –60ºC, where the water vapor is saturated in this air at the condensed temperature. This air is heated up to produce dehumidified air for drying. Numbers of studies on atmospheric freeze-drying process were achieved and greatly motivated to optimize this heat-pump drying system in terms of the energy consumption and drying rate (Alves-Filho, 2002; Chou and Chua, 2001; Claussen et al., 2007a; Claussen et al., 2007b; Jangam, 2011; Jangam and Mujumdar, 2011; Wolff and Gibert, 1990). Drying temperature applied to atmospheric freeze-drying is usually at around –10 to 0ºC. The product temperature during drying could be several degree lower than the circulating air temperature depending on the drying rate. This means that the product temperature during practical atmospheric freeze-drying run is much higher than in the case of vacuum freeze-drying. When drying agricultural products, a typical freeze-drying run places the products at above the glass transition temperatures (T'_g). The T'_g value, for instance, of strawberry is reported in the range from –33 to –41ºC; apple at around –42ºC; peach at around –36ºC (Slade et al., 1991). Considering the product temperatures during drying, atmospheric freeze-drying could lead product deformation (i.e. shrinkage and/or micro-collapse) as a consequence of the glass-rubber/glass-liquid transitions. In such cases, the dried cake layer, that commonly forms during a typical freeze-drying run, does not clearly separate from the frozen zone. This consequently affects the product quality.

5. FREEZE-DRIED MICROSTRUCTURE OBSERVATION BY X-RAY COMPUTED TOMOGRAPHY (CT)

X-ray CT is known as a strong tool to observe microstructure without any destructions (N'Diaye et al., 2013; Ndiaye et al., 2015; Schladitz, 2011). The author's research group installed a freeze-drying set-up to the X-ray CT stage in order to realize *in situ* observation of the microstructure formation during freeze-drying. As schematized in Figure 4, the measurement stage was equipped with a turntable with a drying stage of which inner space could be evacuated by external vacuum pump. Frozen samples were prepared and placed onto the sample stage pre-cooled at around –70ºC, then the inner space was evacuated just after setting the housing case. The cooling air was stopped to increase the stage temperature in order to start freeze-drying. The stage temperature was kept always lower than –10ºC. The temperature during freeze-drying was passively controlled by the balance between the drying rate and the heat sources (i.e. conductive heat from the sample stage and radiative heat from the ambience). The top of the housing component was made by acrylic resin, the X-ray could pass through this window and the inner sample, and exposed to X-ray camera behind the component. 256 transmission images were acquired for one set of measurement by rotating the turntable 180ºC at a rotation rate of 1.2 deg/min. Cross sectional images were reconstructed from the transmission image by the filtered back projection method. This measurement was carried out at synchrotron facility SPring-8 (Beamline: BL19B2, Proposal Number: 2016A1528, Hyogo, Japan).

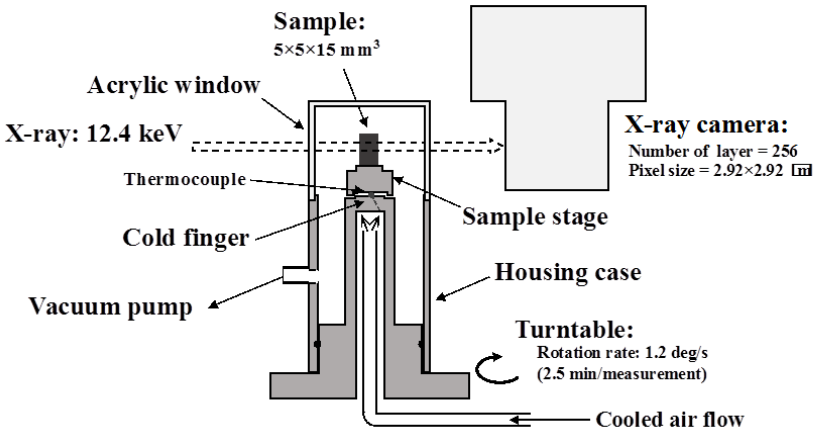

Figure 4 Schematic illustration of the *in situ* X-ray CT measurement stage for freeze-drying.

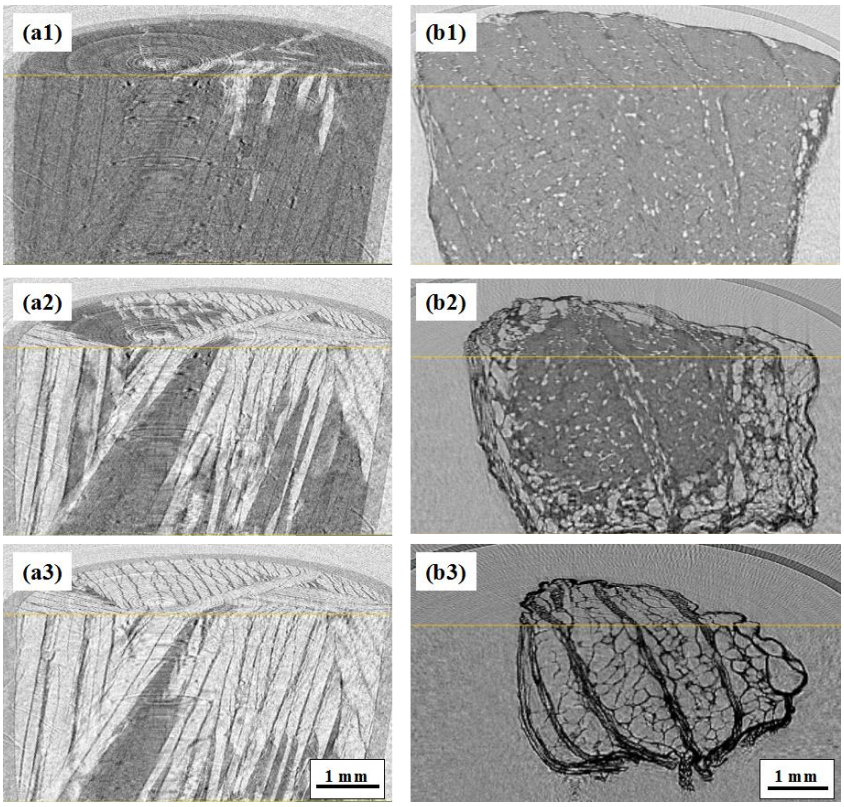

Figure 5 3D X-ray CT images during vacuum freeze-drying; (a1) dextrin solution dried for 20 min; (a2) dried for 60 min; (a3) dried for 90 min; (b1) broccoli stalk dried for 50 min; (b2) dried for 120 min; (b3) dried for 180 min.

X-ray CT images during vacuum freeze-drying of a dextrin solution and a slice of broccoli stalk are shown in Figure 5. The present CT image is acquired with monochromatized X-ray, so the gray levels directly indicate the density and composition of the observed area (Hubbell, 1999; Kinahan et al., 2003; Midgley, 2004). As seen in these images, ice and freeze-concentrated phases could be recognized with the gray scale contrast; shallow gray region: ice crystal; dark gray region: freeze-concentrated phase. The removal of the ice crystals and pore microstructure formations were clearly observed by this system. When the dextrin solution was dried, the ice crystals were removed from the system by maintaining the original microstructures made by the freeze-concentrated phase (Figure 5a). On the other hand, the broccoli slice was obviously shrunk as the progress of drying (Figure 5b). Microstructure of frozen broccoli was not only composed with ice and freeze-concentrated phases but also with original plant cell structures. Pore microstructures in the dried broccoli could largely be derived from the plant cell structures, whereas this significant shrinkage suggests that the drying was progressed at largely above the glass transition temperature.

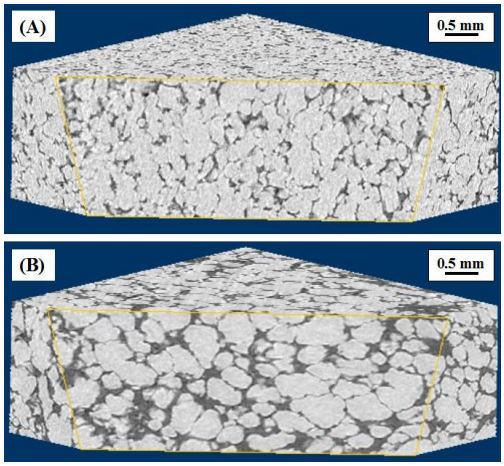

Figure 6 3D X-ray CT images during freeze-drying of sliced apple; (A) freeze-dried under vacuum condition; (B) freeze-dried under atmospheric condition.

X-ray CT images of freeze-dried apple slices dried under atmospheric and vacuum conditions are compared in Figure 6. As in the case of the broccoli slice, these microstructures could be derived both from the ice crystal and plant cell structures. Obvious shrinkage was confirmed when freeze-dried under atmospheric pressure, whereas not significant when vacuum freeze-drying was applied (image not shown). Interestingly, as seen in Figure 6, the pore wall thickness and the mean pore sizes for the atmospheric freeze-dried sample was significantly larger and denser. Both of them were dried over the glass transition temperature of apple (T'_g around –40ºC); the product temperatures during atmospheric and vacuum freeze-drying runs could be approximated at around –5ºC and –20ºC, respectively. Structural deformations due to the drying would occur in both of the cases, however it was enormous when dried greatly above the glass transition temperature.

Figure 7 compares the images of vacuum freeze-dried gelatinized starch solutions. After frozen at –40°C, 2 or 48 hours of annealing at –5°C was applied, then cooled again to –40°C and then subjected to vacuum freeze-drying. Comparing the X-ray CT images of the dried samples, clearly ordered porous structures seen in the 2 hours annealed sample could not be seen in the 48 hours annealed sample. By measuring the X-ray diffraction of these samples, a change in peak shape derived from crystallization of starch was confirmed by applying the 48 hours of annealing (at the angle around 2θ = 13ºC and 17ºC). This suggests the retrogradation of starch progressed during the annealing, and this was reflected in the pore microstructures of the freeze-dried sample. This structural deformation both in terms of crystalline and microscopic structures could be strong ties with the product quality such as rehydration property, sensory property and so on.

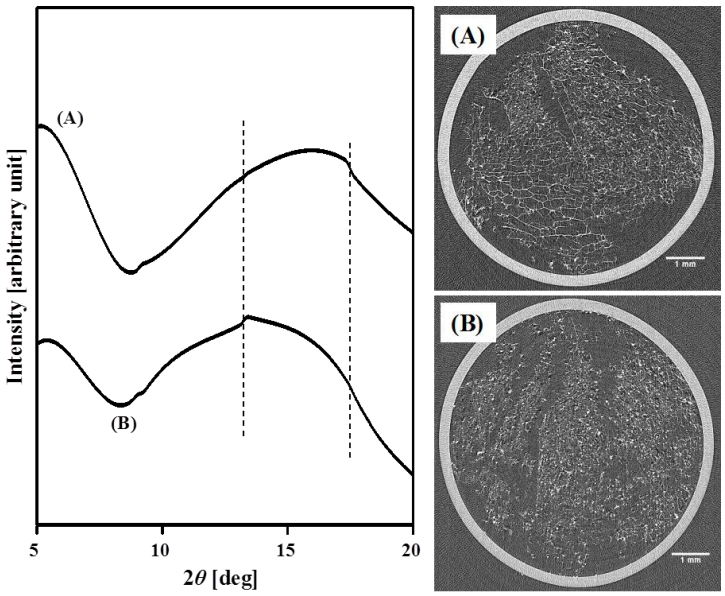

Figure 7 X-ray diffraction patterns and 2D X-ray CT images of freeze-dried starch; (A) annealed for 2 hours; (B) annealed for 48 hours.

6. CONCLUSIONS

Mechanisms of freeze-drying was elucidated in this review with focus on the glassy phase formation in frozen matters. Although freeze-drying is known as one of the best drying method in terms of quality assurance, it involves numbers of potential factors that affect resultant product qualities. Many of them relate to the kinetics in the glassy phase. Freezing conditions determines not only the ice crystal size but also the degree of freeze-concentration. Drying above the glass transition temperature potentially leads product shrinkage, and the degree of the shrinkage is larger as increasing the temperature difference between drying temperature and the glass transition temperature. This also affects pore microstructures of the freeze-dried products, the author observed the increase in the pore thickness by increasing the drying temperature. Post-freezing annealing also links to the crystallinity of the product as seen in the case of freeze-dried starch solutions. It is important to understand the mechanisms of these phenomena and optimize process parameters based on the knowledge. A method that can deal with the complicated interactions of the kinetics in a glassy matter could be a clue to give desired properties to the end-products.

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