Micro X-ray Computed Tomography and Image Analysis of Frozen Potatoes Subjected to Freeze-Thaw Cycles

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Abstract

Frozen products are subjected to freeze-thaw cycles during storage and shipping. These temperature fluctuations result in ice recrystallization, which causes undesirable quality changes, texture loss, and surface dehydration of frozen foods. The objective of this study was to investigate the effect of temperature fluctuations on ice crystal growth/decay in frozen potatoes. The potatoes, which were in a cuboid French fry shape, were frozen at -80 °C (control group) and fluctuated from -17 and -16 °C, -17 and -11 °C, and -17 and -7 °C for one and two weeks' duration. X-ray micro-computed tomography (CT) was used to observe the three-dimensional (3D) microstructure of frozen potatoes. Image analysis showed significant growth of ice crystals with increases in temperature fluctuations and duration of freezing. The total number of pores was reduced with increases in amplitude and duration of freeze-thaw cycles. These results demonstrate that micro-CT and image analysis can be used to analyze the microstructure of frozen materials and obtain valuable information for designing the freezing process.

1. Introduction

Freezing is one of the most important methods to extend the shelf life of foods. The freezing process can prevent microbial growth, reduce water availability, and decrease chemical and enzymatic reaction rates (Sun, 2016). Freezing is initiated by nucleation when the external temperature falls below the food's freezing point. Ice crystals start to grow and propagate as the temperature falls, and the solute concentration gradually increases in unfrozen regions as the vicinal water diffuses to the nucleation sites to crystallize. When the freezing temperature drops enough, and the maximum freeze concentration of solutes is reached, a non-equilibrium but highly viscous glassy state dominates the freezing process, and the corresponding temperature is defined as glass transition temperature (T_g). In the glassy state, the mobility of water and other molecules decreases significantly due to the high viscosity ($\sim 10^{13}$ poise) and low temperature during freezing. Therefore, a frozen product that remains in the glassy state is considered to be in the ideal storage condition.

The potato is the 4th most important global food crop after rice, wheat, and maize. More than 30% of the potato crop is processed as French fries worldwide (Saguy, 1995). For longer and more stable storage, raw French fries are usually frozen to avoid discoloration and blackheart in the tissues (Talbert & Smith, 1967). However, during the shipping and storage of frozen foods, damage to product quality may occur due to temperature fluctuations. These freeze-thaw cycles may result in textural changes, moisture migration, and ice recrystallization in the frozen materials (Adapa, Schmidt, Jeon, Herald, & Flores, 2000). The rate of ice crystal growth, the surface area, and the volume of pores in potatoes significantly increase as a function of temperature fluctuations during the freezing process (Szymońska & Wodnicka, 2005). Freezing and thawing would also annul the positive effects, such as inducing small ice crystals and

45 keeping foods in the glassy state, that prevail during quick freezing. Food components may
46 undergo a transition from a glassy to rubbery state or even defrost because of temperature
47 instability. Some ice may also melt, which leads to a gradual growth of the mean crystal size
48 caused by accretive and migratory recrystallization. The intracellular and intercellular diffusion
49 rate of water increases dramatically, which is caused by not only the glassy-to-rubbery state
50 transition, but also the dilution occurring from the melting of small ice crystals. As a result, the
51 moisture in the frozen product is lost and the microstructure of solid walls is damaged (Goff,
52 1997). Therefore, the temperature fluctuations that occur during the freezing process and storage
53 cause critical damage to frozen foods through quality degradation.

54 To improve shipping and storage conditions of frozen foods as well as to solve heat and fluid
55 transport models for designing freezing systems, there is a need to investigate ice crystal growth
56 and distribution in frozen food products. X-ray micro-computed tomography (CT) provides high-
57 resolution internal imaging of materials. It allows visualization and measurement of the complete
58 three-dimensional morphological configuration of objects without invasion. This technique is
59 broadly applied in the investigation of rocks, bones and metals (Salvo, Cloetens, Maire, Zabler,
60 Blandin, Buffière, & Josserond, 2003; Buffiere, Maire, Adrien, Masse, & Boller, 2010). In
61 addition, food materials such as fresh fruits, beef, and food proteins have been imaged with
62 micro-CT to determine their microstructures and other physical characteristics (Frisullo, Marino,
63 Laverse, Albenzio, & Del Nobile, 2010; Mousavi, Miri, Cox, & Fryer, 2007; Ta-Te Lin et al.,
64 2008). Ullah et al. (2014) performed a 2D image analysis for freeze-dried potatoes scanned with
65 micro-CT and investigated the ice morphology and pore size distribution. 3D image analysis was
66 used in the present study, which provides a direct observation of the whole structure of frozen
67 samples. Alam and Takhar (2016) also conducted micro-CT scanning of fried potatoes and used

Avizo 9.0 (FEI Houston, Hillsboro, OR, U.S.A) for image analysis by selecting the pores using manual thresholding based upon visual judgment.

The objective of this study was to quantify the 3D ice crystal morphology, size distribution, and volume fraction by performing micro X-ray computed tomography of frozen potatoes, which are subjected to simulated temperature fluctuations encountered during industrial storage and shipping. An image analysis was carried out by utilizing “auto-thresholding” and “watershed separation” methods implemented in commercial 3D image analysis software (Avizo 9.0, FEI Visualization Sciences Group, Hillsboro, OR, U.S.A). Using these methods allowed for performing objective segmentation and reduced strenuous analysis steps of manual segmentation, as employed in the study of Alam and Takhar (2016) for fried potato disks.

2. Materials and methods

Sample preparation and freezing method

Fresh potatoes of the Russet variety were purchased from a local grocery store. Potatoes of similar size were selected and cut into cuboidal (11.9 mm side) fries. The cut potatoes were blanched at about 85 °C for two minutes to remove starch from the surface and prevent browning from contact with atmospheric oxygen. The cut samples were divided into seven groups and put in Ziploc storage bags marked from S1 to S7 for further freeze processing. Each group contained about ten cuboidal potatoes. One group of samples (S1) was stored in a freezer at -80 °C and designated the control. Other groups were stored in the freezer at varying temperatures. Sample groups S2 and S3 experienced a temperature fluctuation between -17 °C and -16 °C for one and two weeks, respectively. Sample groups S4 and S5 were placed in the freezer with temperatures

fluctuating between -17 °C and -11 °C for one and two weeks, respectively. S6 and S7 were put in the freezer with temperatures varying between -17 °C and -7 °C for one and two weeks, respectively. Ullah et al. (2014) followed the procedure detailed in Syamaladevi et al. (2010) to determine that onset of ice melting at maximum freeze-concentration occurs at (T_m') of -13 °C for frozen potatoes. Therefore, the first range of temperature fluctuations (-17 to -16 °C) was selected with minimum variation below T_m' . The second and third ranges of fluctuating temperature amplitudes (-17 to -11 °C and -17 to -7 °C) were kept around T_m' , which were expected to yield larger crystals than fluctuation amplitudes below T_m' . The temperature of the freezers during storage was regulated by a programmable temperature controller (Model BCS-462, Embedded Control Concepts, Huntington Beach, CA). The temperature fluctuations were performed for one and two weeks. One freeze-thaw cycle took about 20 to 24 hours to complete. Thermocouples were placed in the middle of the plastic bags and freezer to record temperature profiles and monitor the operation of the freezer.

X-ray micro-CT imaging

After freezing, the samples were dehydrated using a vacuum freeze dryer (FreeZone 6, Labconco Co., Kansas City, MO) for 48 hours. Freeze drying removed moisture inside the material by sublimation and left a network of solid fibers and pores. The freeze-dried samples were scanned by a high-performance X-ray computed tomography (CT) scanner (Model Micro XCT-400 Xradia, Rockville, CA) at the University of Illinois, Urbana-Champaign, IL, U.S.A. The scanner settings used for obtaining the images were 1× magnification, 50 kV voltage, and 10 W power source. The exposure time was 2s per slice, and the sample was rotated from -180° to 180°, which resulted in 721 image slices for each sample. The total scanning time was about 90 minutes. Multiple replications for scanning each sample were not performed since micro-CT is

an expensive technique. A voxel size of 22 μm was used for image acquisition. The obtained images (16-bit TIFF) were reconstructed into a series of 2D images using the XMReconstructor software (Xradia Software, Xradia Inc., Pleasanton, CA).

Image processing and analysis

Figure 1 shows the image analysis steps performed using Avizo 9.0 (FEI Visualization Sciences Group, Hillsboro, OR). First, the original image slices obtained from micro-CT scanning were masked to remove the background (Figures 2a and b). Then the contrast and brightness of the images were adjusted to obtain a clear distinction between the pores and solids in the frozen samples (Figure 2c). The auto-thresholding module of Avizo was used to compute a two-level threshold of the grayscale images. The frozen potatoes subjected to a temperature fluctuation from -17 to -7 $^{\circ}\text{C}$ (S7) are presented as an example to describe the image processing steps. Two intensity values of 5201 and 20805 were obtained for sample S7 using auto-thresholding in Avizo. These two intensity values marked pores and solids respectively in the S7 images. These intensity values served as reference values for further segmentation (Figure 2d). Pores inside the samples were selected by inputting the first intensity level (0-5201). After this selection, the pixels inside the first intensity level were highlighted and assigned a value of 1. Thus, the grayscale images were transformed into binary images (Figure 2e). Next, some noise filtering was applied to remove small islands and fill 3D cavities less than 15 pixels in size. This noise filtering did not affect the porous and solid regions of the image. Watershed segmentation was used to improve porous and solid regions marked using auto-thresholding (Haris, Efstratiadis, Maglaveras, & Katsaggelos, 1998). The watershed algorithm is analogous to the water drops, which follow a distance map to separate a landscape into different catchment basins (Orbert, Bengtsson, & Nordin, 1993). The “separate objects” feature of Avizo 9.0 was used to compute

watershed lines on a distance map. This allowed for separation of the connected pores to obtain the distribution of sizes (Figure 2f). If this separation is not performed, all connected pores appear as one large pore, which does not yield pore size distribution. Label analysis was performed to obtain the volume distribution of pores. The equivalent pore diameter (D_{eq}) values were calculated from the volume of pores (V_{3D}) using the following equation:

$$D_{eq} = \sqrt[3]{\frac{6 * V_{3D}}{\pi}}$$

Pore size distribution was depicted by plotting a histogram of the label analysis results. Solid walls were also segmented with a similar method, using the second intensity level 5201-20805 obtained using auto-thresholding (Figure 2g). A 3D view of the solid fraction was rendered for direct visualization of the inner microstructure of samples (Figure 2h).

Porous properties, such as the number of pores, equivalent diameter, pore volume, and pore size distribution were analyzed using Origin Pro (Ver 9.1, OriginLab Corp., Northampton, MA). A Shapiro-Wilk test was conducted to test the normality of pore size distribution using R (3.2.2, R Core Team., Vienna, Austria). Porosity (Φ) of various samples was calculated as: $\Phi = V_{pore}/V_{total}$. Here, V_{pore} represents the total volume of pores, and V_{total} represents the total volume of a sample. Tortuosity was obtained using the centroid path tortuosity method, which computes the ratio of the length of a flow channel connecting two pores to the straight line distance between the two pores (Shanti, Chan, Stock, De Carlo, Tornton & Faber, 2014).

3. Results and discussion

During rapid freezing at -80°C , small crystals are formed, and solid walls in potatoes are expected to be closer to their native structure (Ullah, Takhar, & Sablani, 2014).

By comparing the pore sizes of the control sample (frozen at -80°C) and samples subjected to freeze-thaw cycles, deductions about crystal growth were made. Therefore, the micro-CT images of samples subjected to freeze-thaw cycles can be analyzed to determine changes in pore size distribution and their relationship to ice crystal sizes. These properties are needed to simulate heat and fluid transport phenomena and to improve frozen product quality during shipment and storage.

Pore counts and pore size distribution

Figure 3 depicts the 2D images (xy and xz plane) and 3D rendering for all of the micro-CT scanned samples. The raw potatoes consisted of numerous interconnected micropores (Alam and Takhar 2016). After freezing at -80°C , both 2D and 3D views show small compact pores, which are either natively present in raw potatoes or were formed by very small ice crystals due to rapid freezing, as the -80°C water and solute molecules are expected to have limited mobility. However, the ice crystals become larger when the amplitude of temperature fluctuation and freezing time increase (Figure 3 S2 to S7). This can be explained by ice recrystallization occurring during the freeze-thaw cycles. Recrystallization is caused by the diffusion of unfrozen water in potato tissue to the crystal sites. Especially when the freezing temperature is not stable, some smaller ice crystals melt and move to the surface of larger crystals to minimize free energy, which leads to a gradual growth of mean crystal size in a sample. As the temperature fluctuations, peak temperature value, and freezing time increase, the ice crystals are enlarged by the intensified effect of recrystallization. The solid wall is also disrupted when the smaller ice crystals migrate to the larger ones, which facilitates mass and moisture loss during freezing and

degrades the product quality. A reduction in the number of ice crystals is also observed due to the merger of smaller crystals to larger crystals when the fluctuations in temperature increase (Figure 4). In the control sample (S1), the total number of pores was counted as 10,549 and a thick fibrous network was observed in the 2D and 3D views. For samples (S2 and S3) obtained by fluctuating temperature between -17 and -16 °C for one and two weeks, the total number of pores were 3,932 and 3,848 respectively. This occurred due to an increase in ice crystal size, which ruptured the walls of smaller pores. Even fewer pores were observed when the samples (S4 and S5) were stored at a fluctuating temperature between -17 and -11 °C for one week (N=3074) and two weeks (N=2216). Finally, at the temperature fluctuation between -17 and -7 °C, only 1,275 and 851 pores remained in samples S6 and S7 respectively. The average pore volumes are also shown in Figure 4. The average pore size becomes larger as the temperature fluctuations and freezing time increase. This phenomenon is also observed in the 2D and 3D views of samples (Figure 3). Smaller crystals were formed when the control group (S1) was stored in a -80 °C freezer because of the low and stable freezing temperature. The pore volume increased as a result of solid structure disruption due to ice migration from smaller crystals to larger ones. The results quantitatively indicate that the freeze-thaw cycles facilitate ice recrystallization and disrupt product structure due to the growth of ice crystals.

Ullah et al. (2014) performed a 2D image analysis of the microstructure of potatoes subjected to different temperature fluctuation ranges. Table 1 and Figure 5 present the comparison of the equivalent diameter of pores and ice crystal growth in Ullah's study and the present results. Different image processing methods are attributed to the difference in the pore diameter values. We determined the equivalent diameter by selecting the pores using 3D image analysis and equating pore volumes to equivalent sphere volumes, while Ullah et al. (2014) analyzed the 2D

pore area and obtained diameter values from micro-CT images. Our results show a trend similar to that of Ullah et al. (2014) -- when the amplitude of temperature fluctuations increases, ice crystals become larger in size. Ice crystal growth percentage was determined using the following equation:

$$\% \text{ Ice crystal growth} = \frac{(\text{Final } X_{50} - \text{Initial } X_{50})}{\text{Initial } X_{50}} \times 100$$

where X_{50} represents the median equivalent diameter of ice crystals and the initial X_{50} is the equivalent diameter of the control sample (S1), which is assumed to be equal to the native pore diameter in raw potato samples. The ice crystal growth rate confirmed that both temperature fluctuations and freezing time duration accelerate crystal growth. The amplitude of temperature fluctuations seemed to have a stronger effect on the growth rate in comparison to the increase in freezing time (Figure 6). The crystal growth also represents the rate of recrystallization, which is not only correlated with the molecular mobility of water and solute molecules, but also depends on the difference between surrounding temperature and T_g ($T - T_g$) (Syamaladevi, Manahiloh, Muhunthan, & Sablani, 2012). Ice crystals in the glassy state ($T < T_g$) have relatively smaller size due to the limited molecular diffusivity compared to the rubbery state. The increased temperature fluctuations aggravate the ice crystal growth and enlarge the pore volume, which damages the solid walls and degrades the product quality. Similar observations have also been reported for other frozen materials (Ablett, Clarke, Izzard, & Martin, 2002; Do, Sagara, Tabata, Kudoh, & Higuchi, 2004; Syamaladevi Manahiloh, Muhunthan, Sablani, 2012).

Figures 7a-g show the quantitative analysis of pore size distribution for all samples. The histograms present the non-normality in distributions, which was confirmed by performing a Shapiro-Wilk test ($p < 0.001$) using R (R Foundation, 2016). The possible reason for non-

normality is the resolution limit of the X-ray micro-CT scanner. The voxel size for these tests was 22 μm , which means that pores smaller than 22 μm could not be quantified using image analysis. Therefore, the average pore volumes exhibit the distributions with right-sided skewness. The pore volume for the control group ranges between 0 and 4 mm^3 . The distributions clearly show a broader distribution (b-g) as the temperature fluctuation and freezing time increase, which spreads the range toward 10 mm^3 when the sample was frozen between -17 to 7 $^{\circ}\text{C}$ for two weeks. In addition, the count of pore numbers shows a remarkably decreasing trend as the pore volume increased.

Label analysis and porous characterization

Label analysis results also indicate a considerable difference in pore connection as a function of temperature fluctuations and freezing time. In Figure 8, connected sets of pores are labeled with the same color. The control group (S1) shows more connections between pores (Figure 8a), which confirms the observation that raw potatoes have compact and interconnected pores. More disruption of solid networks with increasing temperature fluctuations leads to fewer connections between the voids and enlarges the volume of pores. **Therefore, smaller pores melt and merge together to form larger pores (Figures 8b & c).** Figure 8 elucidates that small ice crystals merge to form larger ones because of ice recrystallization.

The porosity of samples obtained with increase in freeze-thaw fluctuations (S2-S7) shows larger magnitude than that of the control group, which indicates that freezing and thawing cycles enlarge the size and volume of ice crystals. Tortuosity is the ratio of path length to the shortest distance between connected pores and is a measure of the degree of waviness of connected pore pathways. Figure 9 presents porosity and tortuosity of frozen samples as a function of the increase in amplitude and duration of temperature fluctuations. A higher tortuosity value implies

a greater resistance of the sinuous pore network to fluid flow (Vallabh, Banks-Lee, & Seyam, 2010). Figure 9 shows that tortuosity decreases as porosity increases. The possible reason is that wall disruption caused by the growth of ice crystals simplifies porous pathways' waviness. The increase in porosity corresponds to the increase in freeze-thaw temperature fluctuations and storage time duration.

From the image analysis results, it can be concluded that the temperature fluctuations during freezing, storage, and transportation are expected to disrupt the microstructure of frozen potatoes depending upon the temperature magnitude in comparison to T_g , as well as the amplitude and duration of fluctuations.

4. Conclusion

The X-ray micro-computed tomography and analysis of acquired images offers valuable results to show pores formed by ice crystals during freeze-thaw cycles. The 2D and 3D image analyses clearly indicated that the size of ice crystals is influenced by the amplitude and duration of temperature fluctuations. The increase in crystal size due to temperature fluctuations and freezing time is caused by ice recrystallization during the freezing process. The ice crystals continuously grow as the amplitude of temperature fluctuations increases, while the number of pores left by ice crystals decreases as the smaller crystals merge and migrate to the larger ones. As a result, solid walls and cell structures are ruptured, which influences the moisture/solute diffusion and subsequently degrades frozen food quality. The detailed quantitative information obtained from micro-CT image analysis can be used for modeling and designing transport phenomena of freeze-thaw processes and improving textural attributes of frozen products.

269 **Acknowledgements**

270 Thanks to USDA-NIFA for providing financial support under Award No. 2015-67017-23074.

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Figure 1 The flow chart depicting image processing and analysis steps.

Figure 2 Image processing steps of micro X-ray CT data of a frozen potato (S7). a) Grayscale image orthoslice. b) Image masking. c) Contrast and brightness adjustment. d) Auto-thresholding (the phases' thresholding intensities are 5201 and 20805). e) Segmentation of pores by using the thresholding intensity range from 0-5201. f) Separation objects using the watershed algorithm. g) Segmentation of the solids using the thresholding intensity range from 5201-20805. h) 3D rendering of solid structure.

Figure 3 Masked grayscale orthoslices (xy plane and xz plane) and 3D images of potatoes frozen at different freezing conditions (S1-S7).

Figure 4 Average pore volume and numbers of pores for potatoes subjected to different freezing conditions.

Figure 5 Equivalent diameter comparison of present study and Ullah et al. (2014) study.

Figure 6 Ice crystal growth rate versus freezer temperature during storage of potatoes.

Figure 7 Pore size distribution (pore volume of pores) in potatoes subjected to different freezing conditions. The y-axis is plotted on $\log_{10}$ scale.

Figure 8 The 3D images of S1(A), S5(B) and S7(C) showing pores as a solid network (connected set of pores are labeled with the same color).

Figure 9 Tortuosity and porosity of potatoes subjected to different freezing conditions.

Table 1 Comparasion of image analysis results in present study and Ullah's study.

Present study	Equivalent diameter (μm)	Average pore area (μm^2)- A_{3D}	Ice crystal growth %	Ullah study	Equivalent diameter (μm)	Ice crystal growth %
S1 (-80 °C)	112.66	1.51×10^6		-80 °C	284.34	
S2 (-17 to -16 °C 1 week)	167.06	2.81×10^6	48%	-17 to -16 °C	431.89	52%
S3 (-17 to -16 °C 2 weeks)	223.35	2.97×10^6	98%			
S4 (-17 to -11 °C 1 week)	364.84	2.96×10^6	224%	-17 to -11 °C	593.07	109%
S5 (-17 to -11 °C 2 weeks)	508.01	4.14×10^6	351%			
S6 (-17 to -7 °C 1 week)	795.88	9.07×10^6	606%	-17 to -7 °C	605.03	113%
S7 (-17 to -7 °C 2 weeks)	832.84	9.32×10^6	639%			