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Fractal Analysis of Ice Crystals in Frozen Food

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Fractal analysis of the morphology of ice crystal particles in frozen food was attempted for frozen soybean curd (tofu). From a microscopic image analysis of the ice crystal particles, it was found that the perimeter of the ice crystal particles could be recognized as a fractal. Effects of the storage time and storage temperature on the fractal dimension (d_p) of the perimeter of the ice crystal particles were also investigated. As storage time was increased, the d_p value tended to decrease. When the storage temperature was increased, the value of d_p decreased more rapidly. The changes corresponded to the visual observation of the shape change for the ice crystal particles during storage reported by many researchers, suggesting that the fractal dimension d_p could be used as a quantitative indicator reflecting the surface roughness of ice crystal particles.

KEYWORDS: Fractal; ice crystal; image analysis; recrystallization

INTRODUCTION

Properties of ice crystals such as shape, size, and distribution play an important role in determining textural and physical properties of many frozen products (*1*). Understanding these properties of ice crystals is critical not only for quality control of frozen foods but also for proper design and development of many freeze-related processes (e.g., freeze-drying and freeze-concentration). Previously, there have been many studies that have investigated the structure of ice crystals formed in various kinds of foods. Thus, it is well-known that rapid freezing rather than slow freezing gives smaller size ice crystals in frozen foods (*2*, *3*). Also, it has been reported that ice crystals grow in size during storage by recrystallization, depending on the storage time and temperature (*4*−*10*).

However, most of the studies mentioned above dealt only with the size and number of ice crystals that formed; but the shape of ice crystals should also be considered, as it is also one of the factors affecting the textural and physical properties of frozen foods. For example, the shape of the ice crystals which are formed may be related to the water-holding capacity of the food after thawing. Or, in the freeze-drying process, the drying characteristics and vapor permeability of food could be influenced by morphology of ice crystals. Finally, the water resorbing behavior of freeze-dried products may be also related to the shape of the ice crystals formed in such foods. Therefore, the information about the shape of the ice crystals should be investigated because it could offer another way of improving the quality of frozen foods and freeze-related operations. There have been a few previous studies that focused on the shape of ice crystals in food (*1*, *4*, *11*, *12*), but most of these used only visual observation of the ice crystals. There are almost no studies that quantitatively investigated the shape of ice crystals in foods. To systematically understand the effect of ice crystal shape in foods, it is necessary to develop a proper method for measuring their shapes. However, it is difficult to characterize the shape of ice crystals in frozen food because the ice crystals often make complicated, and eventually irregular, shapes (*1*, *3*−*10*).

Recently, fractal analysis has been attracting attention as a quantitative analytical method to characterize many kinds of disordered shapes in nature when they are self-similar (*13*, *14*). Using this concept, the degree of irregularity can be estimated by a noninteger fractal dimension. In general, the higher the value of the fractal dimension, the more rugged the object is. The concept of fractal analysis has also been applied to the characterization of the structure of food. Peleg and Normand (*15*) estimated the ruggedness of the instant coffee particle shape by fractal analysis. Suzuki and Yano (*16*, *17*) showed that the surface of several powdered food products could be recognized as a fractal. Several studies performed fractal analysis of food protein aggregates (*18*−*24*) and fat crystal networks (*25*). However, there are no studies that applied fractal analysis to the characterization of ice crystal geometry in frozen food.

In this study, using a frozen soybean curd (tofu) (which has been used previously as a model food product for observation of ice crystal structure in several studies (*9*, *11*, *12*, *26*)), a fractal analysis of the morphology of ice crystal particles was attempted to investigate the application of fractal analysis on the morphology of ice crystal particles by using an image analysis technique. Additionally, the change of fractal dimension during storage at subzero temperature was also investigated.

MATERIALS AND METHODS

Theory for Fractal Analysis of Ice Crystal Particles. Examination of ice crystal particles in frozen food is often carried out by microscopic observation of the cross-section of samples (*3*, *5*, *6*, *9*). A schematic

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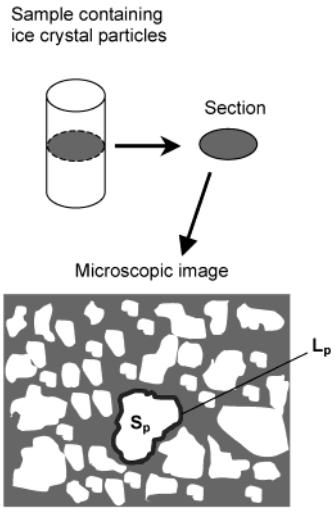

Figure 1. Schematic diagram for a typical image obtained by microscopy. The white part represents the ice crystal particles. L_p, perimeter (outline) length for the ice crystal particle; S_p, area surrounded by the outline.

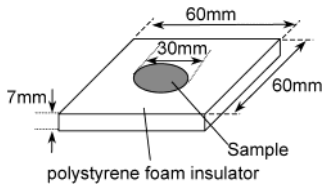

Figure 2. Schematic diagram of the sample insulator for unidirectional heat transfer from top and bottom sides.

diagram of a typical image obtained by microscopy is shown in **Figure 1**; many crystal particles can be seen appearing like islands. We carried out fractal analysis of the images of ice crystal particles by the area−perimeter method, which has been used to measure the fractal dimensions of the coastlines of islands (*13*), the perimeters of porous catalyst support pellets (*27*), and powder particle silhouettes (*28*). According to this method, first, the perimeter (outline) length L_p for ice crystals of different sizes and the area S_p surrounded by the outline of the image are evaluated by a measure with same unit length. Between L_p and S_p, the following relationship exists (*13*, *28*−*30*):

$$S_p \propto L_p^{2/d_p} \quad (1)$$

where d_p is a fractal dimension for an outline of ice crystal particle on the image. For regular forms such as circles or squares, $d_p = 1$. However, when the outline morphology is more complicated and has self-similar characteristics, d_p takes a noninteger value between 1 and 2 (*13*); the more complicated the perimeter line is, the higher the value of d_p (*13*).

Freezing and Storage Procedures. The soybean curd (tofu) was bought from a retail store. Because the soybean curd has a relatively homogeneous structure, it was selected as a simple sample for the analysis of the shape of ice crystal particles. The soybean curds were cut into disk shapes (7 mm height × 30 mm diameter) and set into a polystyrene foam insulator as shown in **Figure 2**. Then the samples were packed under vacuum in a heat-sealed polyethylene bag and immersed in ethanol brine thermostated at −50 ± 0.5 °C. Lateral insulation of the specimens, provided by the polystyrene foam, brought about unidirectional heat transfer from top and bottom only. The temperature of the center of samples was monitored during freezing. After the core temperature reached −50 °C, some samples were subsequently prepared for microscopic observation.

To examine the effects of storage on ice crystal structure, other samples were stored at −5, −20, −30, and −50 °C after freezing. The storage periods were set to 30, 60, and 80 days.

Microscopic Observation. In this study, a fixation method similar to that of Martino and Zaritzky (*6*, *9*) was carried out. The frozen samples were cut into a cylindrical shape (7 mm height × 7 mm diameter) in a chamber at −20 °C and were then immersed in a mixture of formalin and ethanol (*31*) for 12−14 days at −50 °C to fix the protein body and replace the ice with the solution. For fixation of the stored samples, the operation was done at the same temperature as storage (*3*, *6*, *9*). After fixation, the samples were stored at room temperature for 1 day, washed in water, and embedded in melted gelatin (*9*, *31*).

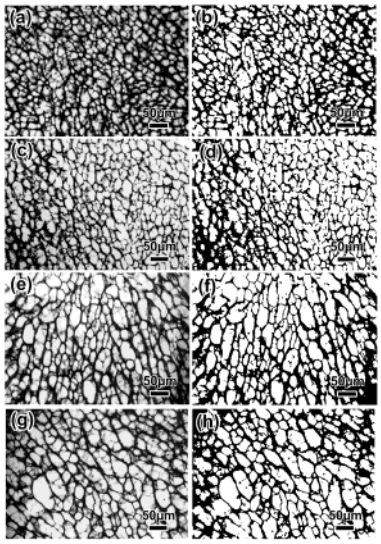

Figure 3. Microscopic images for tofu samples stored for different times at –5 °C. Top row: nonstorage; (a) original image, (b) binary image. Second row: –5 °C, 30 days; (c) original image, (d) binary image. Third row: –5 °C, 60 days; (e) original image, (f) binary image. Bottom row: –5 °C, 80 days; (g) original image, (h) binary image.

The embedded samples were sliced into 5−10-μm thick specimens with a freezing microtome, with the direction of slicing perpendicular to the heat transfer direction. Only the specimens which were the same distance from the freezing surface (presenting same freezing rate) were used for observation. The sliced specimens were stained with a 1% Eosin Y (*31*) solution to obtain photographs of good quality for image analysis, and then observed with a Se−Ke type light microscope (Nikon Corp., Japan), equipped with a camera (Nikon).

Image Analysis. The obtained photographs were scanned by an image scanner (GT-7000, Seiko Epson Corp., Japan) as bitmap images, which were finally converted to binary images. The size of the images was about 1400 pixels × 1000 pixels (1 pixel = 0.056 μm). Then, the lengths of the perimeters, L_p, and the areas, S_p, of the ice crystal particles were evaluated using a scale of 1 pixel unit length. For these procedures, commercial image analysis software WinROOF (Mitani Corp., Japan) was used. Using these data, the fractal dimension d_p was determined from the relationship to eq 1 as explained before.

Also, the apparent diameter for each ice crystal, defined as a diameter of a circle having the equivalent area S_p, was estimated, using the same software. From the data set of each apparent diameter, the mean crystal diameter, D_{eq} was calculated.

All analyses were done for the three different specimens per condition (storage times and temperatures) and the average values were obtained. Numbers of ice crystal particles per one image were more than 100.

RESULTS

First, the results about the size of ice crystals were described, then by confirming that these were not contradictory to past research results, the validity of the experimental and analytical methods in this study could be shown. In **Figure 3a**, a typical original microscopic image for the nonstorage samples after freezing at −50 °C is presented. The white part corresponds to the ice crystal particles in the samples. Many ice crystal particles, like islands, were observed as shown in **Figure 1**. The corresponding binary image is presented in **Figure 3b**. The

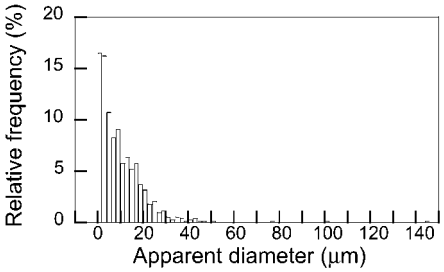

Figure 4. Example of relative frequency distributions of the apparent crystal diameter.

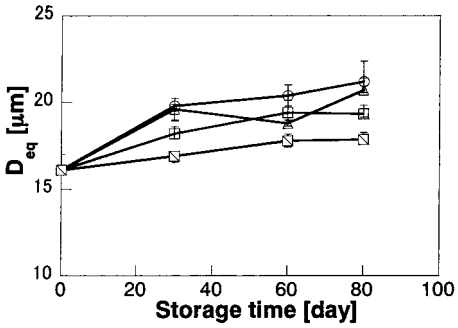

Figure 5. Dependences of the mean diameter D_{eq} on storage time at various temperatures. Each symbol is represented as the mean ± S. E.: (○) –5 °C; (△) –20 °C; (□) –30 °C; (⊠) –50 °C.

relative frequency distribution of apparent crystal diameter for the nonstorage samples, which was obtained from the binary image, is shown in **Figure 4**. The distribution ranged from a few to 50 μm, and the mean crystal diameter D_{eq} was estimated to be 15.4 ± 1.6 μm. Miyawaki et al. (*26*) reported the mean sizes (shortest diameters (chord); length of the shortest aspect of the particle (*32*)) of ice crystals in frozen soy protein curd were from about 50 to 500 μm, much larger than found in this study. The freezing temperature used in this study (−50 °C) was much lower than those in the Miyawaki et al. study (>−15 °C). Furthermore, the size of soybean curd used for this study was much smaller than theirs. Therefore, it is reasonable to estimate that these differences brought about a faster freezing rate for this study, resulting in a smaller size of ice crystals. **Figure 3c**, **e**, and **g** represent typical, original images of ice crystals stored at −5 °C for various storage times. The corresponding binary images are shown in **Figure 3d**, **f**, and **h**. From the original images, the trend for growth of the ice crystal particles corresponded to increase in storage time, which agreed with the features of ice crystal particles in food during such storage reported previously (*6*−*11*). **Figure 5** shows the dependence of the mean diameter, D_{eq}, on storage time, which was estimated from the binary images, including the results for samples stored at other temperatures. It should be noted here that a conversion to a binary image from an original image sometimes causes bias of image data (*33*), which may bring about an unrealistic result. In **Figure 5**, the value of D_{eq} tended to increase with increasing storage time which agreed with a visual observation of the original images. In addition, for the samples of same storage time, the tendency of larger D_{eq} value at higher storage temperature was observed. This phenomenon was well in agreement with the features for ice crystal particles in foods during storage previously reported (*6*−*11*). Therefore, the bias caused by converting to a binary image is minimal for this study.

Typical plots of log S_p vs log L_p for the corresponding image in **Figure 3** are shown in **Figure 6**. For most of the range of L_p (about 10^0 to 10^3 μm), the plots showed good linear relationship (correlation coefficient $R > 0.99$) and, from the slope of the plot, the value of d_p could be evaluated according to eq 1. At −20, −30, and −50 °C, the plots of log S_p vs log L_p also showed a linear behavior, and values of d_p were also evaluated (data plots are not shown). **Figure 7** shows the dependence of storage time on the fractal dimension d_p for the samples stored at the different temperatures. With an increase of storage time, the values of d_p tended to decrease. And the higher the storage temperature, the more rapidly the value of d_p decreased.

DISCUSSION

The values of d_p for all of the samples in this study were greater than 1. This indicates that the outlines of the ice crystal particles do not have the same geometric nature as a circle or a square (regular shape) (*13*). Therefore, this confirmed that,

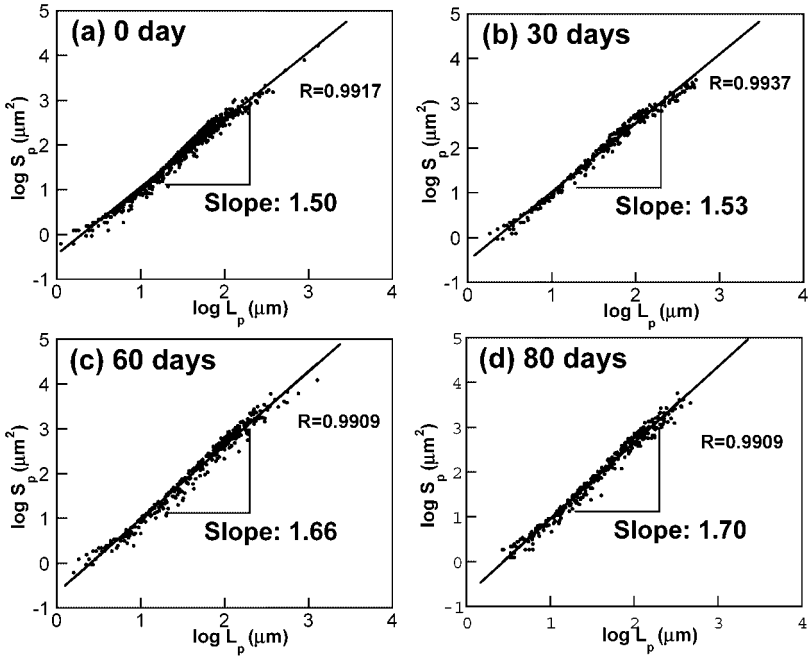

Figure 6. Plots of log S_p vs log L_p for the samples stored at –5 °C: (a) 0 days; (b) 30 days; (c) 60 days; (d) 80 days.

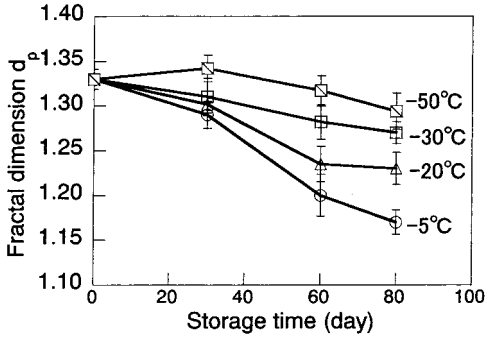

Figure 7. Dependence of the fractal dimension d_p on storage time at different storage temperatures. Each symbol is represented as the mean ± S. E.: (○) −5 °C; (△) −20 °C; (□) −30 °C; (⧄) −50 °C.

from the point of view of the fractal measure relations (*13*), the outlines of the ice crystal particle could be recognized as fractal.

The plots of log S_p vs log L_p were generally regarded as a straight line as shown in **Figure 6**. Strictly speaking, however, there were slight deviations from a linear relationship: for example, in **Figure 6a**, a broad shoulder appeared at $L_p \approx 10^2$; for the plot in **Figure 6c**, the data points at $L_p \approx 10^{2.5}$ were not linear. There are no clear explanations for these deviations; however, from many research studies on the growth behavior of ice crystal particles in foods, it has been shown that there are several mechanisms for the growth of ice crystals during storage, such as migratory recrystallization or accretive recrystallization (*1*, *4*). Furthermore, for aqueous fructose solutions, it has been reported previously that, depending on the ice crystal size, the mechanism of development for these ice crystals was different; accretive recrystallization dominated when crystals were small and close together, whereas migratory recrystallization occurred mainly as crystals grew and separated (*7*). These results suggest that the difference in the growth mechanism may bring about a morphological change in ice crystals, resulting in a slight departure from a linear behavior. To prove the hypothesis, it will be necessary to perform the same analysis as this study, using a sample such as fructose.

In this study, the outlines of ice crystal particles correspond to the cross sections of the surface. Thus, assuming that the surface of the ice crystal has an isotropic geometry, the fractal dimension of ice crystals surface, d_s, can be obtained from the value of d_p by using the following equation (*13*, *14*, *29*):

$$d_s = d_p + 1 \qquad (2)$$

This equation has been used practically to evaluate d_s of super water-repellent surfaces by Shibuichi et al. (*34*). To use eq 2 for evaluating the fractal dimension of the surface of ice crystal particles, it is necessary to verify whether the surface structure of the ice crystal particle has isotropy.

It is known that the shape of ice crystals in frozen food becomes rounder during storage through the recrystallization process (*1*, *4*). Bevilacqua and Zaritky explained that this phenomenon was caused by the movement of water molecules from the more convex surface region, where free energy of the molecules is greater, and by simultaneous deposition of the molecules on the concave or less convex region (*5*). According to the fractal concept, the tendency of decreasing d_p in **Figure 7** means that increasing storage time results in the outline of ice crystals becoming smoother and that this smoothing process proceeded more rapidly by a higher storage temperature, which corresponds well with the reported result of a smoothing of ice crystal particles through the recrystallization process (*1*, *4*). Therefore, the decrease in value of d_p during storage would be a reflection of this rounding process, suggesting that the fractal dimension d_p could be used as a quantitative indicator reflecting the surface roughness of ice crystal particles.

The fractal dimensions d_p for the outline of ice crystal particles in this study were 1.17 to 1.34, depending on the storage time and storage temperature, as shown in **Figure 7**. It has been empirically shown that the morphology of ice crystal particles varies with the condition of freezing (e.g., cooling rate and temperature; *4*, *35*). Therefore, under conditions different from those in this study, ice crystal particles may have different fractal dimension from those found here. Further study should be done to perform a fractal analysis for ice crystal particles formed under various freezing conditions. This kind of research would be useful for prediction and control of the structural properties of ice crystal particles in frozen food.

Finally, by using the concept of fractal analysis, the shape of ice crystal particles can be described more accurately. The changes in shape during storage could be described by fractal dimension, which agreed well with the visual observation of ice crystal particles during storage as reported by many researchers previously. These results could be significant for determining the quantitative parameter for the geometric shape of crystals which grow during storage.

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