QUANTITATIVE CHARACTERIZATION OF MORPHOLOGY OF ICE CRYSTALS IN FROZEN FOODS BY USING THE CONCEPT OF FRACTAL

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ABSTRACT

A quantitative characterization of ice crystal morphology in frozen foods was attempted for soybean curd and tuna fish meat by using the concept of fractal. From a microscopic image analysis of the ice crystals it was found that the perimeter of the ice crystal could be recognized as a fractal and the shape of ice crystal was estimated quantitatively by a fractal dimension of the perimeter, d_p. Effects of storage time and storage temperature on d_p value 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 crystals during storage reported by many researches, suggesting that the fractal dimension d_p could be used as a quantitative indicator reflecting the surface roughness of ice crystals

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 (Hartel, 1998). 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 (Reeve, 1970; Bevilacqua *et al.*, 1979). Also, it has been reported that ice crystals grow in size during storage by recrystallization, depending on storage time and temperature (Fennema, 1973; Bevilacqua and Zaritzky, 1982; Martino and Zaritzky, 1988; Sutton *et al.*, 1996; Takai *et al.*, 1997).

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. One of the examples is the texture and organoleptic characteristics of frozen dessert such as ice cream and popsicle (Hartel, 2001); it is pointed out that smooth, rounded crystals flow across each other easily and give a smooth texture, whereas crystals with jagged edges do not flow nearly as well and may result in a stiffer, more brittle texture (Hartel, 2001). Another example is freeze concentration process; the crystals with smooth surfaces rather than rugged ones are required to reduce the loss of solutes adhering to the surface. Therefore, the information about the shape of the ice crystals should be investigated, since it could offer another way of improving the quality of foods and freeze-related operations. There have been several studies that focused on the shape of ice crystals in food until now (Hartel, 1998; Fennema, 1973; Kanda *et al.*,, 1992; Fuchigami and Teramoto, 1997). But, most of these used only visual observation of the ice crystals and 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 since the ice crystals often make complicated, and eventually irregular shapes.

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 (Mandelbrot, 1982; Viscek, 1989). Using this concept, the degree of irregularity can be estimated by a non-integer fractal dimension. In general, the higher the value of the fractal dimension, the more rugged the object is. Previously, the fractal concept has been also utilized for characterization of the structure of food materials, such as instant coffee particle (Peleg and Normand, 1985), food powder products (Suzuki and Yano, 1990), food protein aggregate (Bremer *et al.*, 1993; Hagiwara *et al.*, 1997; Hagiwara *et al.*, 1998; Ikeda *et al.*, 1998) and fat crystal network (Narine and Marangoni, 1999). However, there are no studies that applied the fractal analysis to the characterization of ice crystal morphology in frozen food.

In this study, frozen soybean curd and frozen tuna meat were selected as samples and the fractal analysis of morphology of ice crystals in frozen foods was carried out. Additionally, the change of fractal dimension during storage at subzero temperatures was also investigated.

1 MATERIALS AND METHODS

1.1 Theory for Fractal Analysis of Ice Crystals

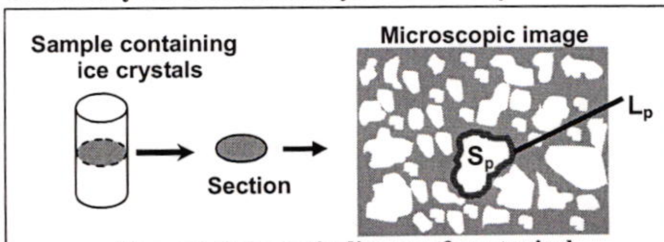

Figure 1. Schematic diagram for a typical image obtained by microscopy.

Examination of ice crystals in frozen food is often carried out by microscopic observation of the cross-section of samples (Bevilacqua *et al.*, 1979; Bevilacqua and Zaritzky, 1982; Martino and Zaritzky, 1988; Takai *et al.*, 1997). A schematic diagram of a typical microscopic image is shown in Fig. 1; many crystal particles can be seen appearing like islands. We carried out fractal analysis of the images of ice crystal by the area-perimeter method (Mandelbrot, 1982; Gladden *et al.*, 1995; Suzuki *et al.*, 1997). 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 holds from the fractal measure relations (Mandelbrot, 1982, Suzuki *et al.*, 1997; Takayasu, 1990; Lovejoy, 1982):

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

where d_p is a fractal dimension for an outline of ice crystals on the image. For regular forms such as circles or squares, d_p=1. When the outline morphology is more complicated, d_p takes a non-integer value between 1 and 2 (Mandelbrot, 1982); the more rugged the perimeter line is, the higher value d_p takes (Mandelbrot, 1982).

1.1 Freezing and Storage Procedure

The Soybean curd was cut into disk shapes (7 mm height×30mm diameter) and set into a polystyrene support to keep their shape during experiment. Then the samples were packed under vacuum in a heat-sealed polyethylene

bag and frozen in ethanol brine at -50±0.5°C.
Fresh fillet tuna meat (*yellowfin tuna*) was cut into disk shapes (8 mm height×40 mm diameter) so that the center axis of the disk was parallel to the muscle fibers. Then, the samples were packed and frozen at -50±0.5°C in the same way as the soybean curd.
During freezing, a core temperature of the samples were measured. After the temperature reached -50°C, some samples were used for preparation of microscopic observation. To investigate the effects of storage upon the morphology of ice crystals, other samples were stored at subzero temperatures. In case of the soybean curd, storage temperatures were -5±0.5°C, -20±0.5°C, -30±0.5°C, and -50±0.5°C, respectively. The tuna meat samples were stored at -5±0.5°C, -20±0.5°C, -40±0.5°C, and -50±0.5°C, respectively. The storage durations were set to 30, 60 and 80 days for both samples.

1.2 Microscopic observation.

The frozen samples were processed by the freeze fixation method (Martino and Zaritzky, 1988; Takai *et al.*, 1997). After the fixation, the samples were embedded in melted gelatin (Kageyama and Watanabe, 1978). The embedded sample was sliced into 5-10μm thick specimens with freezing microtome. The direction of slicing was perpendicular to the heat transfer direction during the freezing process as preceding studies (Martino and Zaritzky, 1988; Takai *et al.*, 1997). Only the specimen located at the same distance from the initial surface was used for observation. The sliced specimens were stained with a 1% Eosin Y (Takai *et al.*, 1997) solution and then observed with a light microscope (Se-Ke, Nikon corp., Japan) equipped with a camera (Nikon corp., Japan). The photographic images were scanned by image scanner (GT-7000, Seiko Epson Corp., Japan) as bitmap images for consequent image analysis. Typical image size was about 1400 pixels×1000 pixels (1 pixel=0.056μm).

1.3 Image Analysis

The obtained bitmap images were converted to binary image. Then, the lengths of the perimeters, L_p, and the areas S_p of the ice crystals were evaluated at 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 the equation (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 averaged values were obtained.

2 RESULTS

2.1 Dependences of the average ice crystal size upon storage time and temperature (Hagiwara *et al.*, 2002; Hagiwara *et al.*, 2003)

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 would be shown.
Fig. 2 shows typical original microscopic images (left) and binary ones (right) for the soybean curd stored at -5°C for various storage times. In Fig. 3, typical original images (left) and binary ones (right) for the tuna meat stored at -20°C for various storage times are presented. The white part corresponds to the ice crystals in the samples. From the original images of both samples, trend for growth of the ice crystals with increase in storage time was observed. The same trend of growth in size was also observed at other storage temperatures (the photographic images are not shown here). Fig. 4 shows the dependence of the mean diameter, D_{eq}, on storage time for the soybean

curd (A) and the tuna meat, which were estimated from the binary images, including the results for samples stored at other temperatures. The value of D_{eq} of both samples tended to increase with increasing storage time which agreed with a visual observation of the original images. Additionally, at higher storage temperatures, tendency of larger D_{eq} was observed. These behaviors mentioned above were well in agreement with the features for ice crystals in foods during storage previously reported (Bevilacqua and Zaritzky, 1982; Martino and Zaritzky, 1988; Sutton *et al.*, 1996; Takai *et al.*, 1997), leading to verification of the experimental and analytical methods used in this study.

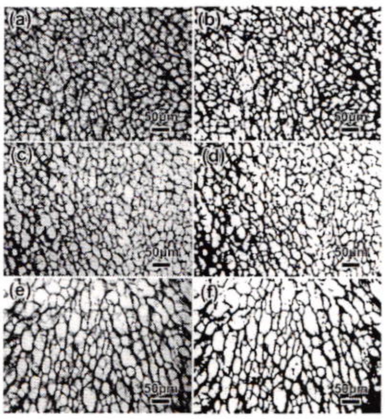

Figure 2. Typical original microscopic images (left) and binary ones (right) for the soybean curd stored at -5°C. Top row: non-storage. Second row: 30days. Bottom row: 60days.

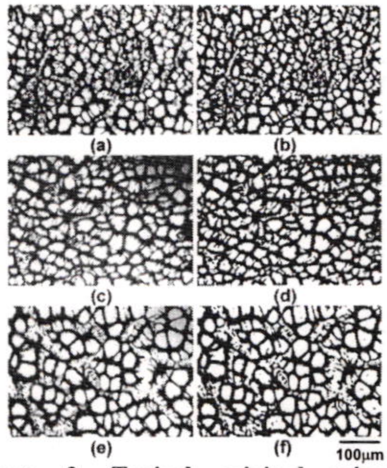

Figure 3. Typical original microscopic images (left) and binary ones (right) for the tuna meat stored at -20°C. Top row: non-storage. Second row: 30days. Bottom row: 60days.

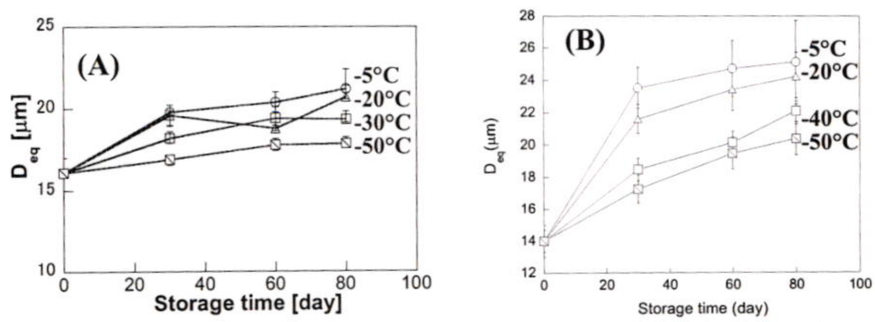

Figure 4. Dependences of the mean diameter D_{eq} on storage time. Sample: (A) soybean curd, (B) tuna meat.

2.2 Fractal Analysis (Hagiwara *et al.*, 2002; Hagiwara *et al.*, 2003)

Typical plots of log S_p vs. log L_p for the corresponding image in Fig. 2 and Fig.3 are shown in Fig. 5 and Fig. 6, respectively. The plots showed good linear relationship (correlation coefficient R > 0.99, in most cases) and, from the slope of the plot, the value of d_p was evaluated according to Equation (1). At other storage temperatures, the plots of log S_p vs. log L_p also showed a linear behavior, and values of d_p were also evaluated for both samples (data plots are not shown). Figure 7 shows the dependence of storage time on the fractal dimension d_p for the soybean curd (A) and the tuna meat (B), which were stored at the different temperatures. With an increase of storage time, the values of d_p tended to decrease for both samples. The higher the storage temperature, the more rapidly the value of d_p decreased.

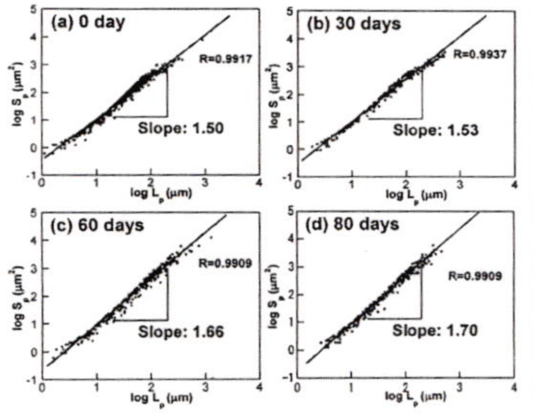

Figure 5. Plots of logL_p for the soybean curd stored at -5°C.

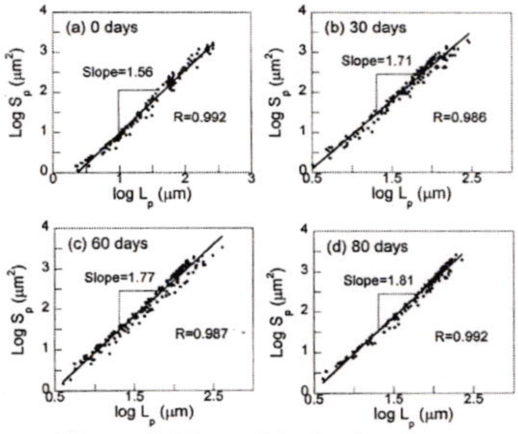

Figure 6. Plots of logL_p for the tuna meat stored at -20°C.

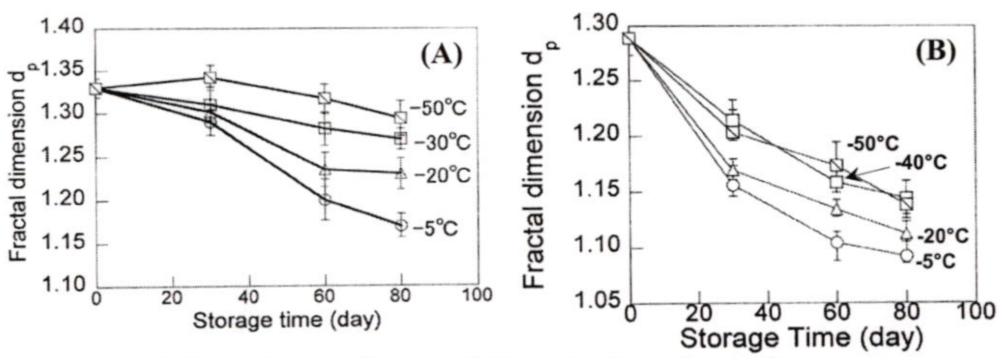

Figure 7. Dependences of the fractal dimension D_p on storage time. Sample: (A) soybean curd, (B) tuna meat.

3 DISCUSSION

The values of d_p for the soybean curd and the tuna meat in this study were greater than 1. This indicates that the outlines of the ice crystals do not have the same geometric nature as circles or squares (regular shape) (Mandelbrot, 1982). Therefore, this confirmed that, from the point of view of the fractal measure relations (Mandelbrot, 1982), the outline of the ice crystals was recognized as fractal. Furthermore, considering that the ice crystals in both the foods showed the fractal property, it is suggested that the fractal nature of ice crystals is a common property for many kind of frozen foods.

It has been observed that the shape of ice crystals in frozen food becomes rounder during storage by the recrystallization process (Fennema, 1973; Hartel, 2001). Bevilacqua and Zaritky (1982) 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. According to the fractal concept, decreasing the value of d_p in Fig. 7 indicates that the outline of ice crystals become smoother with increase in storage time and that rate of the smoothing process is larger at higher storage temperature, which is in agreement with the reported results of a smoothing of ice crystals by the recrystallization (Fennema, 1973; Hartel, 2001). Therefore, the decrease in value of d_p during storage would be a reflection of this rounding process, indicating that the fractal dimension d_p can be used as a quantitative index reflecting the surface roughness of ice crystals.

The fractal dimensions d_p in this study took various values, depending on the storage time, storage temperature and the samples, as shown in Figure 7. It has been empirically shown that the morphology of ice crystals varies with the freezing situations (e.g., cooling rate and temperature; Fennema, 1973; Franks, 1985) and a type of sample (Fennema, 1973). Therefore, under the different conditions from those in this study, ice crystals may have different fractal dimension from those in this study. Further study should be done to perform the fractal analysis for ice crystals formed under various conditions. This kind of research would be useful for prediction and control of the structural properties of ice crystals in frozen food.

In this study, the outlines of ice crystals 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 crystal surface, d_s, can be obtained from the value of d_p by using the following equation (Mandelbrot, 1982; Viscek, 1989; Takayasu, 1990):

$$d_s = d_p + 1 \tag{2}$$

This equation has been used to evaluate d_s of super water-repellent surfaces by Shibuichi *et al.* (1996). The d_s value may bring about a new hint for dealing a growth behavior of ice crystals because several researchers have suggested that that information about the surface of ice crystals is one of key factors determined recrystallization properties (Bevilacqua and Zaritky, 1982, Hartel, 2001). To use Eq. (2) for evaluating the fractal dimension of the surface of ice crystals, it is necessary to verify whether the surface structure of the ice crystals has isotropy.

CONCLUSION

By using the concept of fractal analysis, the shape of ice crystals in soybean curd and tuna meat was described more accurately. The changes in shape during storage could be described by the fractal dimension, which agreed well with the visual observation of ice crystals as reported by past researchers. These suggest that the fractal analysis is a useful tool to determine the quantitative parameter for the geometric shape of crystals which grow during storage and that the fractal nature of ice crystals is a common property for many kinds of frozen foods. By recognizing the fractal nature of ice crystals, physical properties of frozen foods may be better understood in future. Further study to reveal the relationship between the fractal dimension of ice crystal and physical properties of frozen food should be done.

ACKNOWLEDGEMENT

The authors thank Ms. Y. Yamada and Mr. Y. Sato for their preparation of the microscopic samples.

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Caractérisation quantitative de la morphologie de cristal de glace dans les produits congélés en employant le concept de fractal

RESUME : Une caractérisation quantitative de la morphologie du cristal de glace dans les produits congélés etait essayée pour la pâte de soja et pour la viande de thon en employant le concept de fractal. Par l'analyse d'image micrographique sur le cristal de glace, il était trouvé que le périmètre de cristal de glace puisse être reconnu comme un fractal et la forme de cristal de glace etait estimée quantitativement par la dimension fractale du pétrimètre d_p. Les effets du temps de dépôt et la température de stockage sur la valeur de d_p étaient aussi examinées. Quand le temps de dépôt augmentait, la valeur d_p avait un penchant à diminuer. Quand la température avait augmentée, la valeur de d_p était diminuée plus rapidement. Les changements correspondaient à l'observation visuelle du changement de la forme pour les cristaux de glace pendant le stockage comme rapportés par un grand nombre de chercheurs, et ce fait suggère que la dimension fractal d_p puisse être utilisée comme un indicateur quantitatif, qui se reflète la rugosité d'une surface du cristal de glace.