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Fractal Analysis of Ice Crystals in Frozen Fish Meat

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Fractal analysis of shape of ice crystal particles in frozen fish meat (yellowfin tuna) was carried out by using the technique of image analysis. From a microscopic image of the ice crystal particles, it was confirmed that the perimeter of ice crystal particles could be characterized as a fractal and the fractal dimension of perimeter was evaluated. 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. With increase of the storage time, the d_p value tended to decrease. The higher the storage temperature, the more rapidly the value of d_p decreased. The changes were in agreement with the visual observation of the shape change for the ice crystal particles reported by many researches, indicating that the fractal dimension d_p can be used as a quantitative index reflecting the surface roughness of ice crystal particles.

Key words: fractal, fractal dimension, recrystallization, ice crystal, fish meat

1. Introduction

The natures of ice crystals, such as shape and size, can have great influence on textural and physical properties of many frozen foods. Understanding these natures is important not only for quality control of frozen foods but also for proper design of many freeze-related processes. Many studies have been investigated the natures of ice crystals formed in various kinds of foods. It is well-known that rapid freezing rather than slow freezing results in small size ice crystals in frozen foods [1, 2]. It has been also shown that average size of ice crystals increases during storage by recrystallization, which is dependent upon storage period and storage temperature [3–9].

In most of these studies, however, only the size and the number of ice crystals were investigated. It is reasonable that the shape of the ice crystals is also one of the factors influencing the properties of frozen foods and freeze-related process. One of the examples is the texture and organoleptic characteristics of frozen dessert such as ice cream and popsicle [10]; 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 [10]. Another example is freeze concentration process; the crystals with smooth surface rather than rugged one are required to reduce the loss of solutes adhering to the surface. Therefore, in addition to the size and its distribution, information about the shape of ice crystals should be investigated, which may bring about a new method of improving the quality of foods and freeze-related operation. There have been quite a few literatures that stated the shape of the ice crystals in foods [5, 11, 12], however, most of them carried out only visual observation of the ice crystals and there is little study that investigated quantitatively the form of ice crystals in foods. For systematical understanding of the relationship between the shape of ice crystals and the properties of frozen foods including the freeze-related processes, it is necessary to develop quantitative method for evaluating the shape of ice crystals. The ice crystals in frozen foods were, however, difficult to characterize due to their disordered shape [2–9].

Since Mandelbrot proposed the concept of fractal in 1975 [13], fractal analysis has been recognized as a quantitative analytical method that can characterize many kinds of irregular shape in nature when they have self-similarity [14–16]. According to the fractal concept, the degree of irregularity can be estimated by the non-integer fractal dimension. In general, the higher the fractal dimension, the more rugged the object is. In these days, the fractal concept has been also utilized for characterization of the structure of food materials, such as instant coffee particle [17], food powder products [18, 19], food protein aggregate [20–26] and fat crystal network [27]. In previous study, we have attempted the fractal analysis of ice crystal particles in soy bean curd as a model food and confirmed that the out-

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line of ice crystal particles was fractal [28]. In addition, from the experiment of stored sample, we also have found that the value of fractal dimension decreased with increase of storage period. This means that surface of ice crystal particle became smoother with increasing storage time, which agreed with the result of visual observation of ice crystals reported before [3, 10, 29]. Thus, it is suggested that the concept of fractal is useful tool for quantitative characterization of the shape of ice crystals in foods. However, such approach has not been attempted for real frozen food that is practically utilized. To verify applicability of the fractal analysis for other frozen food, it is necessary to do the same analysis by using other food.

In this study, as a typical frozen food practically in use, frozen fish meat (yellowfin tuna) was chosen and the fractal analysis of the morphology of ice crystal particles was carried out. The change of value of the fractal dimension is also investigated as previous study.

2. Materials and Methods

2.1 Sample preparation

Fresh fillet fish meat of yellowfin tuna was purchased from retail shop. The samples were cut into a disk shape (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 in a heat-sealed polyethylene bag under vacuum and frozen in ethanol brine at –50 ± 0.5°C. During freezing, a core temperature of the sample was 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 crystal particles, other 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.

2.2 Microscopy observation

The frozen samples were processed by the freeze fixation method as the preceding study [5, 8, 28]. After the fixation, the sample was stored at room temperature for 1 day, and washed in water and embedded in melted gelatin [28, 34].

The embedded sample was sliced into 5–10μm thick specimens with freezing microtome. The direction of slicing was perpendicular to the muscle fiber, which was in same manner as most of preceding researches of recrystallization behavior in myosystem [2, 4, 5, 8]. 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 [8, 28, 34] solution to obtain photographs of good quality for image analysis, and then observed with a light microscope (Se-Ke, 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).

2.3 Image analysis

The same procedures and commercial software as those in a preceding study [28] were used. The obtained bitmap images were converted to binary image. From the binary image, the fractal analysis of ice crystals was done by the area-perimeter method [14, 28, 30–33] as follows.

Figure 1 shows schematic diagram for the image obtained by the microscopic observation of the cross-section of samples. The white part represents the ice crystal particles in the sample. According to the area-perimeter method [14, 28, 30-33], first, the perimeter (outline) lengths L_p (μm) for each ice crystals of different sizes and the areas S_p (μm^2) surrounded by the outline are measured at scale length of 1 pixel unit. When the outline has a fractal property, the fractal dimension of the ice crystal perimeter, d_p (1), is determined using Eq. (1) from the slope of the plot of log S_p *vs.* log L_p:

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

For regular forms such as circles or squares, the value of d_p is 1. When the outline morphology is more complicated and has self-similar characteristics, d_p takes a non-integer value between 1 and 2 [14]. The more rugged the perimeter line is, the higher value d_p takes [14]. To evaluate d_p value adequately by using the area-perimeter method, many ice crystal particles with different size should be analyzed. Numbers of ice crystal particles analyzed per one image were more than 100.

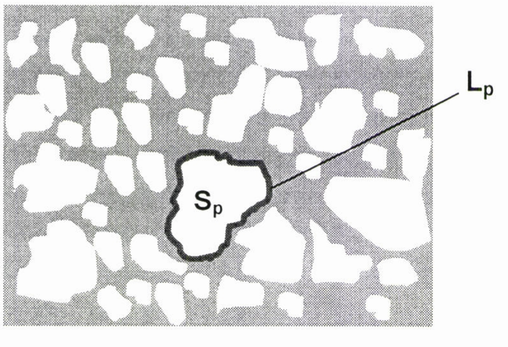

Fig. 1 Schematic diagram for a typical image obtained by microscopy.
L_p: perimeter (outline) length for the ice crystal particle,
S_p: area surrounded by the outline.

And the apparent diameter for each ice crystal, which was defined as a diameter of a circle having the equivalent area S_p was estimated by the same software. From the data set of each of apparent diameter, the number-averaged crystal diameters D_{eq} were calculated.

All analyses were done for the three different replica specimens per condition (storage times and temperatures) and the averaged values were obtained.

3. Results

3.1 Dependences of the average ice crystal size upon storage time and temperature

Figure 2 (a), (c), (e), (g) represent typical, original images of ice crystals stored at −20°C for various storage times. The white part corresponds to the ice crystal particles. The corresponding binary images are shown in Figure 2 (b), (d), (f), (h). The tendency for growth of the ice crystal particle with increase of storage time was observed. In case of other storage temperatures, the same trend of growth in size of ice crystal particle was observed, though the photographic images are not shown here. Fig. 3 shows the plot of the mean diameter D_{eq} vs. storage time, including the results for samples stored at other temperatures. The value of D_{eq} tended to increase with increase of storage time. In addition, at higher storage temperatures, tendency of faster increase in the mean diameters was observed. These were well in agreement with the features of ice crystal particles in foods during storage which were reported by many researchers [3–6, 8], leading to verification of the image analysis method used in this study.

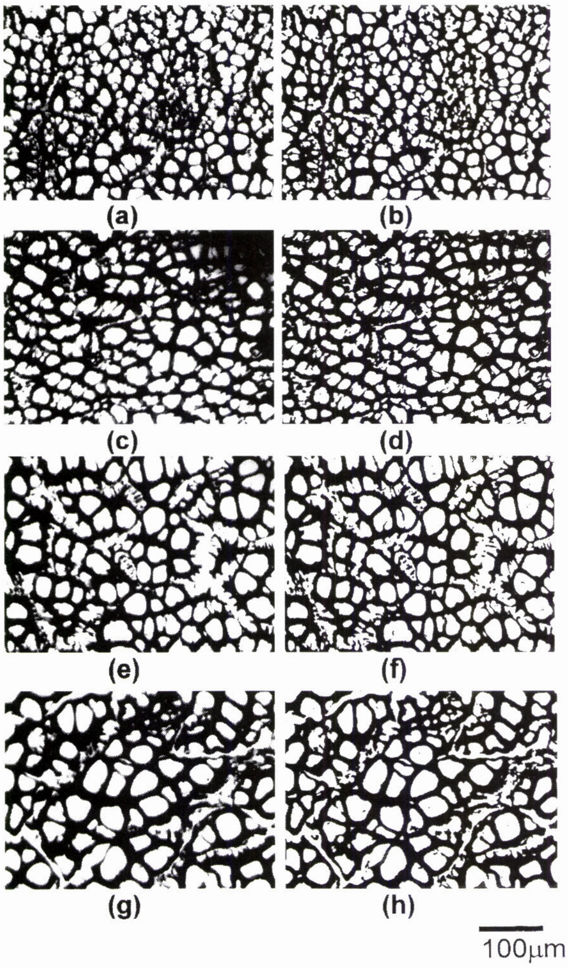

Fig. 2 Microscopic images for yellowfin tuna samples stored at −20°C.
(top) non-storage; (a) original image, (b) binary image.
(second) 30 days; (c) original image, (d) binary image.
(third) 60 days; (e) original image, (f) binary image.
(bottom) 80 days; (g) original image, (h) binary image.

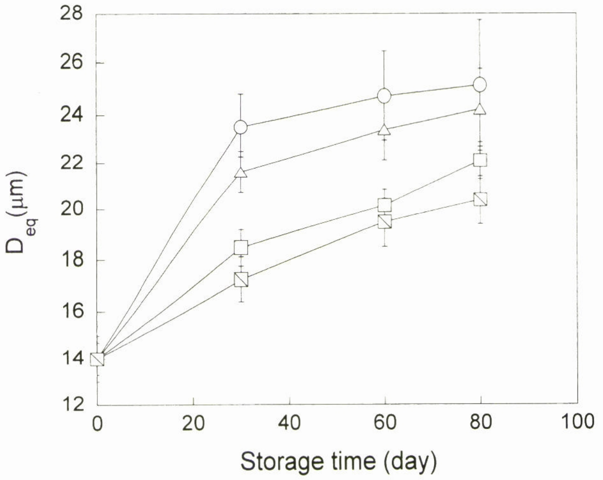

Fig. 3 Dependences of the mean diameter D_{eq} on storage time at various temperatures.
○ −5°C; △ −20°C; □ −30°C; ⧅ −50°C. Each symbol is represented as the mean ± S.E.

3.2 Fractal Analysis

Many ice crystal particles with various shapes were observed in the photographs. Without classification the ice crystals by apparent shape, we carried out the fractal analysis because it was difficult to classify them objectively due to uncertainty from individual variation. Figures 4 (a)–(d) are typical plots of log S_p vs. log L_p for the corresponding images in Fig. 2. The plots showed good liner relationship with different slopes, and the values of fractal dimension d_p could be evaluated from the slopes, according to the Equation (1). This indicates that in spite of the diversity of apparent shape, an average shape quality of the ice crystal particles could be characterized as a single d_p value. In other words, the d_p value obtained here is considered as a reflection of average quality of the shape of ice crystal perimeters [31]. As for the samples stored at −5°C,

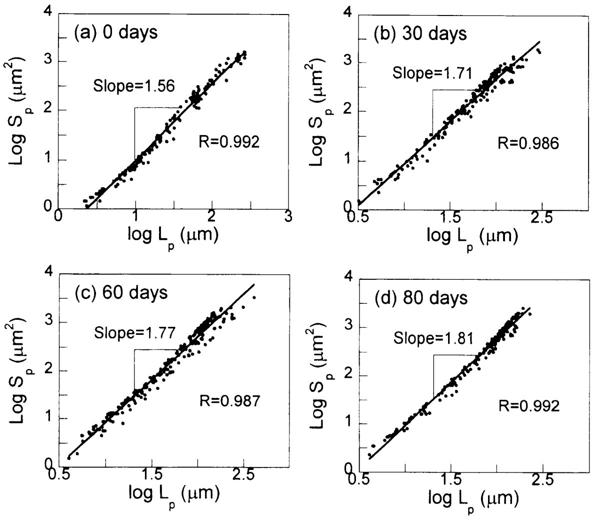

Fig. 4 Plots of log S_p vs. log L_p for the samples stored at −20°C. (a) 0 days; (b) 30 days; (c) 60 days; (d) 80 days.

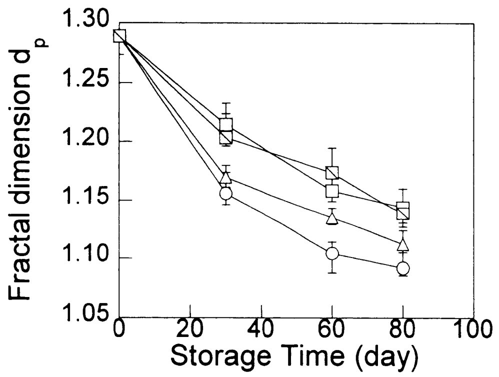

Fig. 5 Dependences of the fractal dimension d_p on storage time at different storage temperatures.
○ −5°C; △ −20°C; □ −30°C; ⧅ −50°C. Each symbol is represented as the mean ± S.E.

−30°C, and −50°C, the plots of log S_p vs. log L_p also showed the linear behavior, and the values of d_p could be also calculated (data plots are not shown here). Figure 5 shows the dependence of storage time on the fractal dimension d_p for the samples stored at the different temperatures. Increasing in storage time, the values of d_p tended to decrease from initial value of 1.29. At higher the storage temperature, more rapidly the value of d_p decreased; at −5°C, the value of d_p decreased to 1.09 after 80 days storage.

4. Discussion

The fractal dimensions d_p for all of the samples were greater than 1. This indicates that the outlines of the ice crystal particle in yellowfin tuna have self-similar geometric, i. e. fractal [14]. In previous study [28], we have already shown that the outlines of ice crystal particles in soybean curd are also fractal. From these aspects, it is suggested that the fractal nature of ice crystal particles may be common property for many kinds of frozen foods. It has been observed that the ice crystal particles take various shapes, depending on freezing condition [3, 35]. Under different conditions, ice crystal particles are expected to have different fractal dimension from those in this study.

It has been observed that the shape of ice crystals in frozen food becomes rounder during storage by the recrystallization process [3, 10]. Bevilacqua and Zaritzky 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 [4]. According to the fractal concept, decreasing the value of d_p in Fig. 5 indicates that the outline of ice crystals become smoother with increase of 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 crystal particles by the recrystallization [3, 10]. 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 crystal particles. As a parameter for estimating surface texture of particle, shape index, such as the degree of circularity or the surface shape factor, are known [36–38]. In the field of powder technology, these parameters are considered as a standard index of surface roughness of powder particle. There have been little studies that applied them for ice crystal particles, though. The fractal dimension may be a potential standard parameter for estimating surface roughness of particle.

We observed here the section of yellowfin tuna meat, of which the direction of sectioning was perpendicular to the muscle fiber in same manner of preceding studies [2, 4, 5, 8]. It was reported that the existence of the parallel, elongated, cylindrical structure like muscle frequently affected the direction of ice crystal growth; sometimes ice crystals were seen like long parallel spears when a longitudinal section of the parallel structure was observed [3]. Thus, if we observe the longitudinal section slide of the yellowfin tuna muscle meat, the ice crystal will show different form from that in this study and may take different dp value. Observing the ice crystals from various directions will lead to better understanding of the shape of ice crystals.

In this study, to obtain the value of d_p, we used the area-perimeter method. Another method is sometimes used, which is often called the divider method based on the principle of changing coarse-graining level [14, 32]. According to the method, the perimeter is converted to a polygonal approximation using different slide lengths r and the total number of slides $N(r)$ is counted. Then, the value of d_p is evaluated using following equation.

$$N(r) \propto r^{-d_p} \quad (2)$$

A merit using this method is that information about scale length at which fractal relation holds is also available by checking the range of r where the equation (2) holds. However, it is pointed out that this method requires the specific procedure mentioned above, which many of image analysis softwares usually do not support [31]. On the contrary, the area-perimeter method used in this study requires only the data set of length of perimeter L_p and the area surrounded by the perimeter S_p and is easier to check the fractal properties and evaluate the value of d_p than the method of changing coarse-graining level [31]. From the point of easiness to evaluate d_p, the area-perimeter method used in this study has an advantage over the divider method, although the information about scale length is difficult to obtain.

5. Conclusion

By using the fractal analysis, the shape of ice crystal particles in fish meat (yellowfin tuna) was characterized quantitatively. The shape change during storage could be described by the fractal dimension, which agreed well with the visual observation of ice crystal particles as reported by past researches. It is suggested that the fractal nature of ice crystal particles are common property for many kinds of frozen foods and that the fractal analysis is a useful tool to evaluate the shape of ice crystals for frozen food. 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.

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***** 和文要約 *****

凍結魚肉中の氷結晶のフラクタル解析

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氷結晶の形状，大きさ，その分布は，凍結食品の性状や物性に影響を及ぼすのみならず凍結操作を利用した製造・貯蔵工程の設計および制御とも深い関係がある．これまで，凍結速度と生成する氷結晶の大きさの関係，貯蔵中の再結晶化に伴う氷結晶の成長挙動など，食品中の氷結晶に関する様々な事柄が多数の研究者によって調べられてきた．しかしながら，その多くは氷結晶の大きさ，数についてのみ言及しており，氷結晶の形状について詳細に調べた研究例は，あまり見当たらない．その理由としては食品中に生成した氷結晶は一見すると複雑かつ不規則であり，こうした構造を科学的に把握することが困難であったためと思われる．我々は，先の研究で，近年発達したフラクタル解析の手法を用いることにより，モデル食品系（大豆カード）に生成した氷結晶の形状を定量的に把握することが可能であることを示した．本研究では，実際の凍結食品に対しても，フラクタル解析が適用可能であるか検証するため，キハダマグロの凍結魚肉を用いて，同様の検討を行なった．

実験試料は−50 ℃でブライン凍結させ，凍結置換法により観察用のプレパラートを作製し，氷結晶形状の顕微鏡観察を行なった．観察画像の撮影プリントをスキャナーでビットマップ形式のデジタル画像として読み込み，市販の画像処理ソフトウェアを用いて画像処理を行い，area-perimeter 法により，氷結晶の輪郭のフラクタル次元 d_p の算出を試みた．貯蔵温度，貯蔵時間がフラクタル次元に及ぼす影響についても検討するため，−5 ℃，−20 ℃，−40 ℃，−50 ℃でそれぞれ30日，60日，80日貯蔵した試料中の氷結晶に関してもフラクタル次元の算出を行なった．

その結果，キハダマグロの凍結魚肉氷結晶の輪郭はフラクタルとして認識でき，輪郭のフラクタル次元 d_p を求めることが可能であった．また，貯蔵時間が増すほど，フラクタル次元の値は減少する傾向にあり，貯蔵温度が高いほど，減少の速度は大きかった．これは貯蔵時間が長くなるにつれ，氷結晶の表面構造が平滑化していくこと，そしてその進行は貯蔵温度が高いほど速いことを意味している．これらの結果は貯蔵中の氷結晶構造変化を視覚的に観察した既往の研究結果と一致した． 以上の結果から，フラクタル次元 d_p は凍結魚肉中の氷結晶の表面構造の粗さを反映する定量的な指標として用いることが可能であることが判った．前述したように，我々は既に大豆カードを試料に用いた場合でも，フラクタル次元は，氷結晶形状を反映した指標として有用であることを示している．複数の食品素材で，同様な結果が得られたことは，多くの凍結食品において，氷結晶のフラクタル性は共通に見られる性質であり，氷結晶の形状把握のための手法として，フラクタル解析は有用であることを示唆している．また，既往の研究により，凍結条件に応じて，氷結晶は様々な形状を取りうることが観察されており，本研究と別の条件では，d_p は異なる値をとることが十分考えられ，この点については今後検討すべき課題であると考えられた．

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