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International Journal of Refrigeration 27 (2004) 184–190

REVUE INTERNATIONALE DU FROID
INTERNATIONAL JOURNAL OF
refrigeration

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Three-dimensional measurement of ice crystals in frozen beef with a micro-slicer image processing system

Gab-Soo Do[a,*], Yasuyuki Sagara[a], Mizuho Tabata[a], Ken-ichi Kudoh[b], Toshiro Higuchi[b]

[a]*Department of Global Agricultural Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan*

[b]*Department of Precision Machinery Engineering, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8657, Japan*

Received 7 October 2002; received in revised form 17 February 2003; accepted 11 March 2003

Abstract

A novel technique has been developed for measuring the three-dimensional (3-D) structure and distribution of ice crystals formed in frozen beef by using a micro-slicer image processing system (MSIPS). The system has functions to reconstruct the 3-D image based on the image data of exposed cross-sections obtained by multi-slicing of a frozen sample with the minimum thickness of 1 μm and to display the internal structure as well as an arbitrary cross-section of the sample choosing observation angles. The size and distribution of ice crystals can be determined from the 2-D quantitative information, such as the periphery and area of the crystals. The effects of freezing conditions on the morphology and distribution of the ice crystals were demonstrated quantitatively from the observations of raw beef stained by fluorescent indicator. For the samples frozen at −15 °C, the network structure of ice crystals were observed mainly at intercellular space, having approximately 100 μm in cross-sectional size, while that prepared at −120 °C showed the spherical crystals of 10–20 μm in diameter within the cells. The 3-D image of the sample demonstrated that the growth of ice columns was restricted by the intrinsic structure of muscle fibers. The proposed method provided a new tool to investigate the effects of freezing conditions on the size, morphology and distribution of ice crystals.

Keywords: Meat; Beef; Freezing; Ice; Crystal; Measurement; Process; Equipment

Mesures en trois dimensions des cristaux de glace dans du bœuf surgelé à l'aide d'un système de traitement d'images des micro-tranches

Mots clés : Viande ; Bœuf ; Congéltation ; Glace ; Cristal ; Mesure ; Procédé ; Équipement

* Corresponding author. Tel.: +81-3-5841-7536; fax: +81-3-5841-5335.
E-mail addresses: gabsoo_do@iml.u-tokyo.ac.jp (G.-Soo Do), asagara@mail.ecc.u-tokyo.ac.jp (Y. Sagara).

0140-7007/03/$35.00
doi:10.1016/S0140-7007(03)00042-2

1. Introduction

Biological materials are multicomponent systems of uneven quality containing many substances and water is the most abundant component. Freezing of biological materials usually produces ice crystals. The size of the ice crystals formed in frozen materials is strongly influenced by the freezing rate or heat flux in the temperature zone of maximum crystal formation and the direction of heat flow within the material during freezing.

The ice crystals formed in animal cellular materials induce morphological changes, destruction of cells as well as component degeneration, which may result in textural change and dripping after thawing. An explanation of the growth mechanism of ice crystals and development of optimum freezing method was led to improvement of the quality of frozen foods. The development of a method measuring for ice crystals is required to obtain the 3-D information on the morphology, size and distribution.

Ice crystals in frozen materials are usually difficult to observe because of their dynamic variations in morphology, size, configuration, color, and transparency. Various microscopes [light, scanning electron (SEM) and confocal scanning laser] have been employed to observe ice crystals by staining a specific component, measuring an index of refraction and using the freeze-substitution method. A light microscope is the most common, and many researchers have discussed the observed results for the size and density of ice crystals by direct and freeze-substitution methods [1–5]. Bevilacqua et al. [1,2] measured the histological ice crystals in frozen beef using freeze-substitution methods and expressed the diameter of the intracellular dendrites and extracellular ice crystals in terms of a characteristic freezing time. Payne et al. [4] reported the changes in the cell structure of fish meat during chilling and freezing, evaluating immersion treatment with antifreeze proteins. Evans et al. [6] described how the morphological change in the ice crystals of biological samples during freezing can be directly monitored by the combination of the confocal scanning laser microscope with a heat transfer stage, applying this method to fluorescently labeled egg albumin gel. Kang et al. [7] endeavored to obtain information on the 3-D internal structure of freeze-dried hydrogels based on images obtained by iteractive measurements with the SEM in order to discuss the effects of freezing temperatures on geometrical changes in the structure. However, all of these instruments and procedures provide images at the surface or cross section of the sample, i.e., orthographical images.

In recent years, the 3-D images of biological materials have been frequently presented owing to the rapid progress of image processing technologies. Do et al. [8] reported that the MSIPS was useful for measuring the morphology of agricultural products having complex forms, and presented equations to determine both surface area and volume of samples of broccoli from their mass.

The objectives of this work were (a) to develop a technique for measuring the 3-D structure and configuration of ice crystals in frozen beef applying the MSIPS, and (b) to investigate the effects of freezing conditions on the morphology and distribution of ice crystals based on the observed images of a raw beef stained by a fluorescent indicator.

2. Experimental equipment

2.1. Micro-slicer image processing system (MSIPS)

A block diagram of MSIPS is shown in Fig. 1. The MSIPS is composed of a multi-slicing section to expose sample cross-sections, image taking as well as recording devices, personal computer (PC) and 3-D image processor. The PC is used to control the timing for both multi-slicing as well as recording. After the slicing operation, the 3-D sample image is reconstructed based on the image data of exposed cross sections obtained by multi-slicing of a frozen sample with the minimum thickness of 1 μm. Furthermore, the image processor has functions to display the internal structure as well as an arbitrary cross section of the sample by choosing observation angles.

2.2. Configuration and function of MSIPS

Fig. 2 illustrates a multi-slicing section of the MSIPS. The sample was positioned into sample holder (8 mm in diameter × 30 mm in length) of cross-sectioning machine for the slicing operation. The sample was pushed up with a liner actuator using a stepping motor and then sliced by a cutting blade that is installed on the rotating arm with an AC motor and timing belt. The slicing thickness can be mechanically adjusted with the minimum thickness of 1.0 μm [9,10]. Each cross section was captured with a highly sensitive CCD camera (DX930,

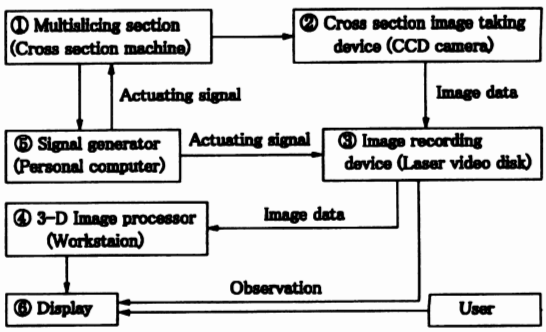

Fig. 1. Block diagram of micro-slicer image processing system.

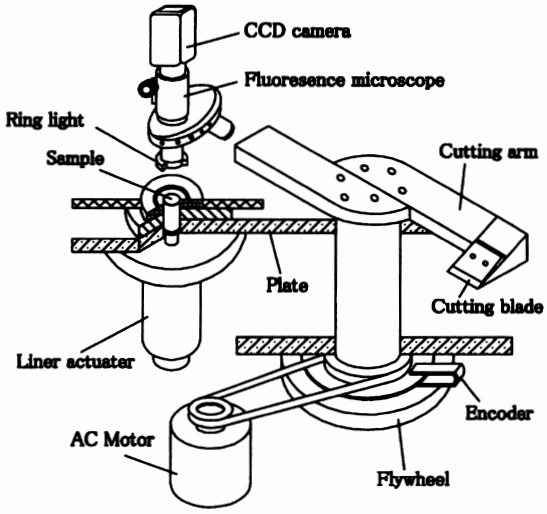

Fig. 2. Multi slicing section.

Sony, Japan) through a fluorescence microscope (BX-FLA, Olympus, Japan), and its images were recorded with a laser videodisc recorder (LVR300AN, Sony, Japan). The micro lens having the ultra-long focal length of 15 mm was used as an objective in order to allow the rotating knife to pass between the lens and sample. To prevent the frozen sample from melting during slicing, the sample holder at multi-slicing section was cooled to about −40 °C.

2.3. Accuracy of sample feed and 3-D image

The slicing thickness is one of the parameters most important in reconstructing a 3-D image. To confirm both accuracy of sample feed and slicing stability, the variations in the upper surface displacement of a brass cylindrical test piece, which was processed with a precision lathe, were continuously measured with a non-contact micrometer of eddy current type during pushing-up motion with a stepping motor. As shown in Fig. 3 the sample feed could be stably controlled in thickness ranging from 1.0 to 50 μm per rotation. Although many image processing methods such as wire frame and surface have been proposed to reconstruct the 3-D image, the volume rendering method was employed as an optimum one for the system to measure internal structures. Using this method the surface area and volume can be determined from the 2-D quantitative information, such as periphery and area of the sample as shown in Fig. 4.

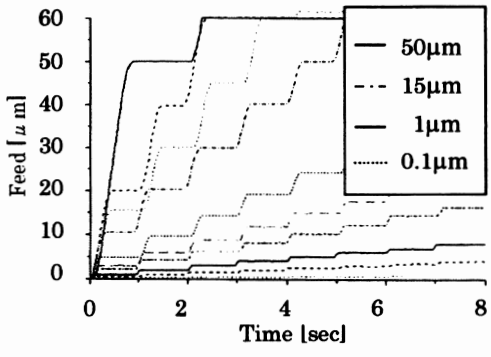

Fig. 3. Accuracy of sample feeding and multi-slicing stability.

3. Materials and methods

3.1. Staining method

To investigate the possibility for visualizing ice crystals in frozen foods, the raw beef was chosen as a typical protein sample. A cylindrical sample of raw beef of approximately 4 mm in diameter and 20 mm in length was prepared by cutting out along the principal axis perpendicular to the muscle fibers. Then it was soaked for 3 h in a 10 μm diluted solution of fluorescent indicator (CellTracker™ Blue CMF_2HC, Molecular Probes). After freezing the stained sample under an air temperature of −30 °C for 3 h, samples having a thickness of 20 μm were obtained using a microtome. Images of exposed surfaces were taken with the CCD camera through a fluorescence microscope. Fig. 5 shows a cross sectional image of muscle fiber cells stained by a fluorescent indicator. The image demonstrates that muscle fibers are fluoresced with the chioromethyl derivative bonded mainly with the protein of muscle fibers. This enables the fibers to be distinguished from ice crystals.

3.2. Sample fixation method

Before slicing, the sample was embedded and fixed with paraffin or optima! cutting temperature (OCT) compound. Fig. 6 is a sketch of the sample embedded

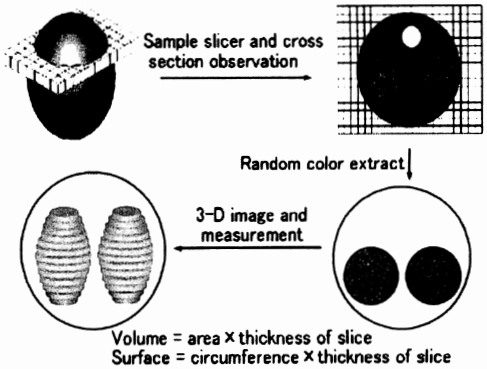

Fig. 4. Construction of 3-D internal structure and the measurement of surface area and volume.

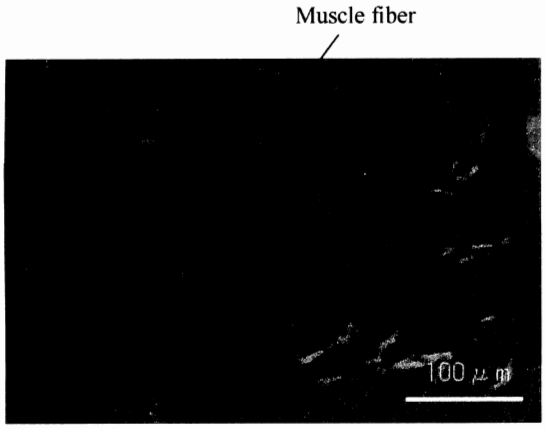

Fig. 5. Muscle fibers of raw beef stained by fluorescent indicator.

and fixed on a copper column with OCT compound in a sample holder, which is a cylindrical paraffin dish of 4 mm in diameter and 20 mm in height. For accomplishing one-dimensional freezing of the sample, glass fiber insulation was placed around the side of the sample holder. The copper column located at the lower part of the holder was used to determine the heat flux across the bottom of the sample from its thermal conductivity and the temperature distribution measured during freezing. The temperature of copper cooling plate can be controlled at a constant temperature ranging from 0 to −120 °C. The samples were frozen from the bottom surface at copper cooling plate of −120 °C (quick freezing) and −15 °C (slow freezing) for about 2 h, to compare freezing rate and ice crystal size, respectively. The frozen sample was sliced together with its holder at the revolution rate of 60 rpm with a thickness of 1.0 μm. The 3-D image of internal structure was reconstructed utilizing the series of 2-D images. The size and distribution of ice crystals were determined based on the 2-D quantitative information such as periphery and area of the crystals.

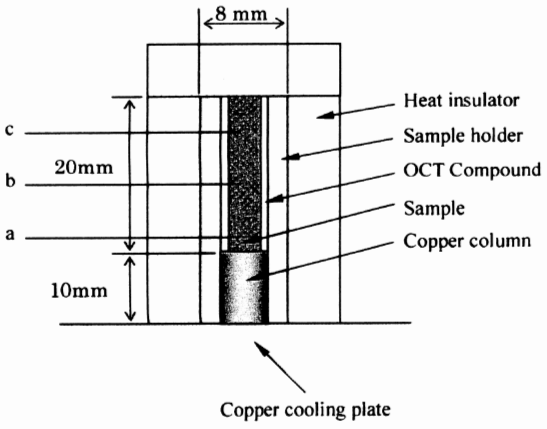

Fig. 6. Sample fixed in sample holder.

4. Results and discussion

4.1. Observation of ice crystals

Fig. 7 shows freezing curves (Fig. 6a–c) at copper cooling plate temperature of −15 °C. Fig. 8 shows the typical cross-sectional images obtained from the upper, center and lower parts of the sample frozen at copper cooling plate of −15 °C (Slow freezing: S-①–②) and −120 °C (Quick freezing: Q-①–②), respectively. These images clearly showed both ice crystals and muscle fibers; the former was distinguished by dark color, while is paler due to fluorescence produced by the protein of muscle fibers. The morphology of ice crystals is indeterminate volume with typical sizes ranging from 10 to 150 μm. The marked effects of freezing rates on the morphology, size and configuration of ice crystals were observed as shown in Fig. 8. In the upper portion of the sample (Q-②), the matrix of ice crystals were mainly observed at intercellular locations, enclosing and destroying muscle fibers. Their maximum size were assessed to be approximately 50 μm, and the similar network structure was also found at central portion showing the relatively narrow width of approximately 30 μm (Q-③). In this portion, globular ice crystals were also developed in intracellular spaces, indicating almost the same diameter with intercellular ice. In the lower portion, the relatively small ice crystals were observed in intra- and extra-cellular spaces as shown in Fig. 8 (Q-①), and the diameter of intra-cellular ice crystals was in the range of 10–20 μm, indicating their increased population. These cross-sectional images demonstrated the variation in morphology as well as configuration of ice crystals. Also, the tissues in frozen beef reflected the difference in heat transfer rates during freezing processes. These images were also found to support the well-known hypothesis [11] that the site of ice crystal growth was transferred gradually from extra- to intracellular location as the freezing rate increased, forming

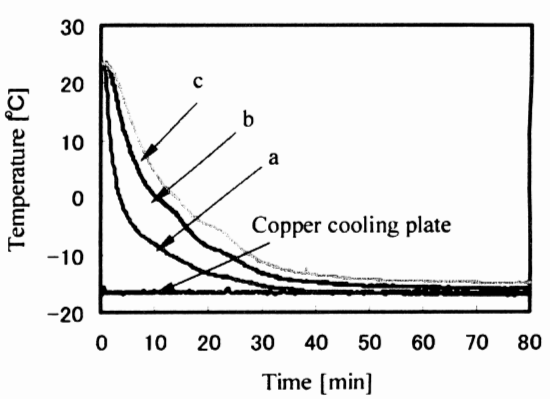

Fig. 7. Freezing curves at copper cooling plate temperature of 15 °C (a, b and c are shown in Fig. 6).

smaller spherical ice crystals and increasing their population within the cells.

4.2. Three-dimensional structure of ice crystal

The 3-D images of the samples frozen at copper cooling plate of −15 °C and −120 °C were presented in Fig. 9. The MSIPS demonstrated to be useful to observe the growth of inter- and extra-cellular ice columns. These images showed that the ice crystals formed the columns that grew towards the interior of the meat in the opposite direction to heat flux, starting at the bottom or cooling surface of sample. The ice column was developed mainly in intercellular spaces when the sample was frozen at copper cooling plate of −15 °C, causing a large distortion of cells as shown in cross sectional images in Fig. 9(a). The growth of ice columns appeared to be restricted by the intrinsic structure of muscle fibers.

Furthermore, Fig. 9(b) showed that the continuity of columns had a tendency to be lost when the sample was frozen at copper cooling plate of −120 °C, because of smaller crystal size formed. The continuity of ice columns appeared to be an important factor, which affects not only the texture of frozen beef after thawing and cooking but also the permeability of water vapor flow-

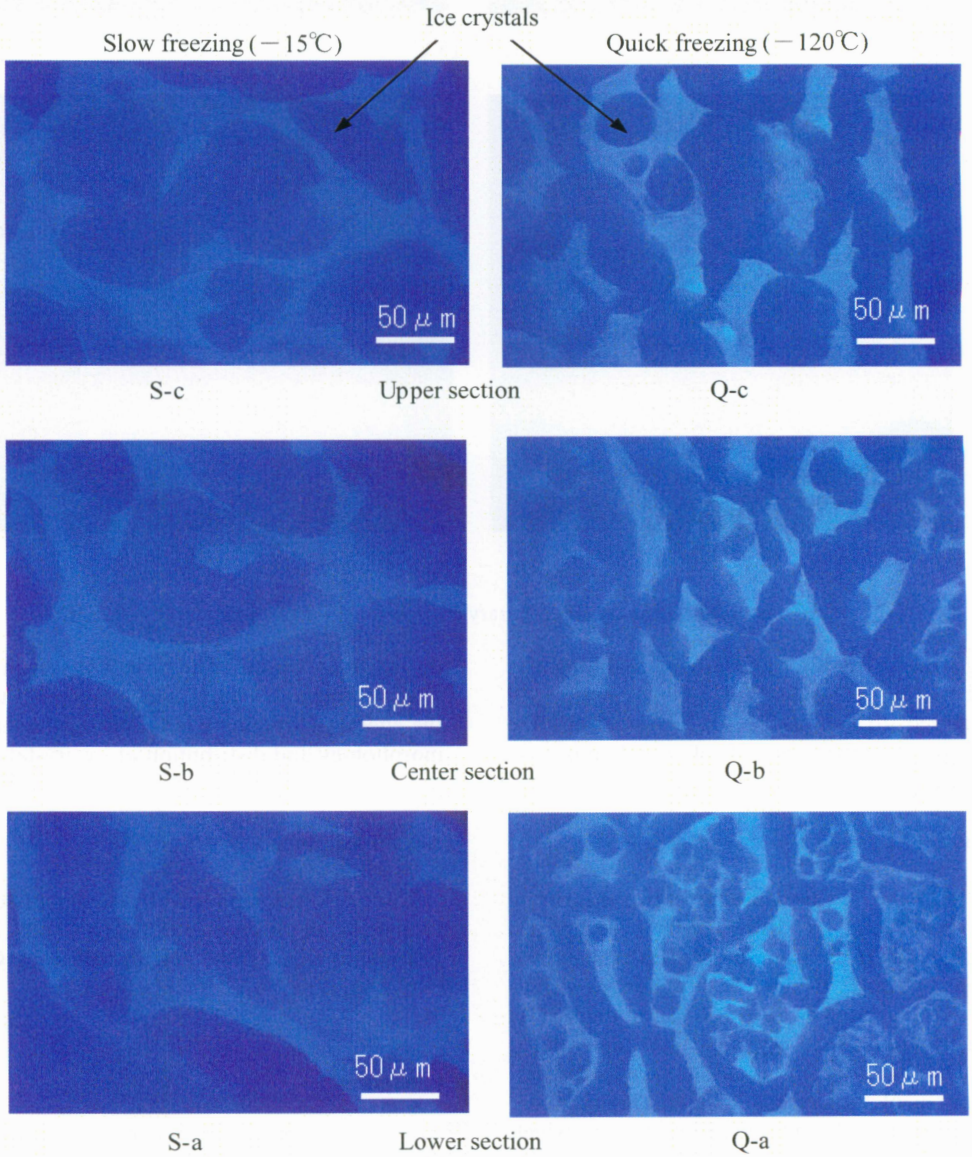

Fig. 8. Images of three cross sections.

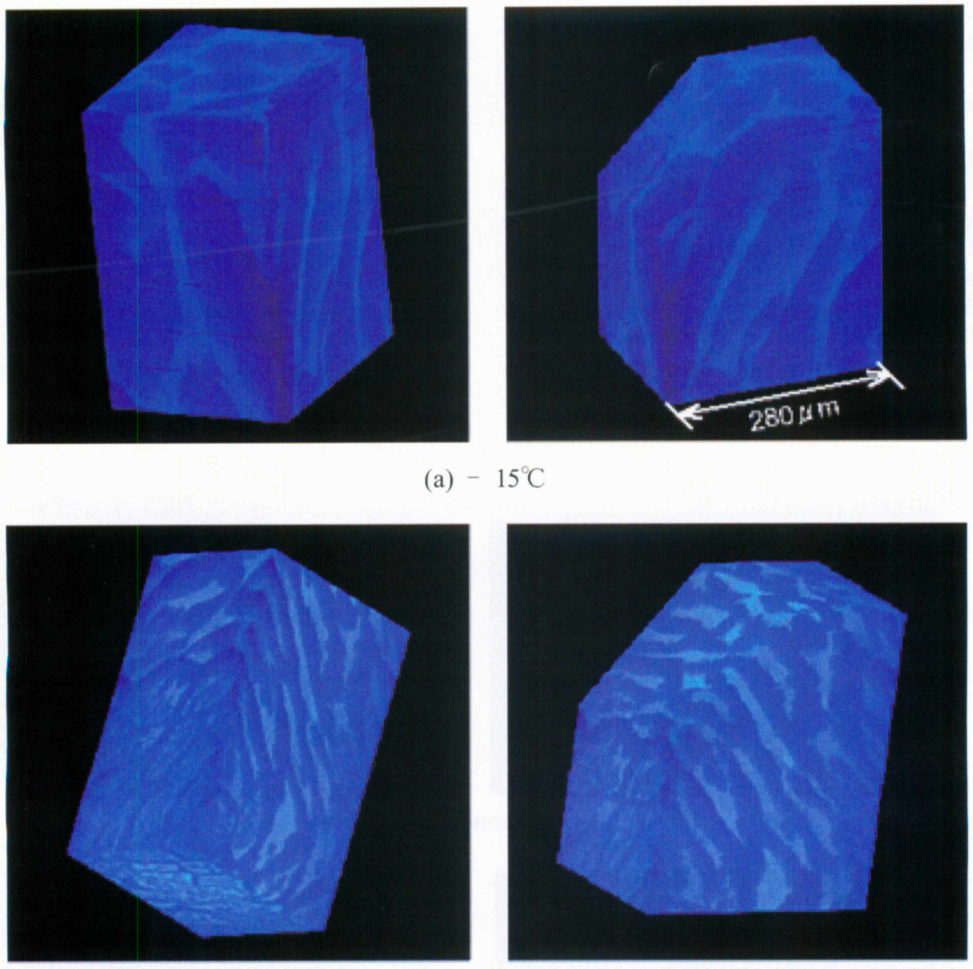

Fig. 9. Reconstituted 3-D image and arbitrary cross section at (a) −15 °C and (b) −120 °C.

ing through the dried layer during freeze-drying. As described here, the 3-D images of a frozen beef tissue were recognized to be useful for investigating the mechanisms in the growing process of ice columns.

5. Conclusions

A novel technique has been developed for measuring the 3-D structure and the distribution of ice crystals formed in frozen beef by using the MSIPS. The system has functions to display the internal structure as well as its arbitrary cross section at any observation angle. The size and distribution of ice crystals can be determined from the 2-D quantitative information, such as the periphery and area of the crystals. The continuity of ice columns appeared to be an important factor, which affects not only the texture of foods but also the permeability of water vapor flowing through the dried layer during freeze-drying. The proposed system and method were demonstrated to provide a new tool to investigate the effects of freezing conditions on the structure, size, morphology and distribution of ice crystals.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science (JSPS). The JSPS Postdoctoral Fellowship is gratefully acknowledged by Gab-Soo Do (No. P00199).

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