Determining the Internal Structure of Ice Cream Using Cryogenic Microtome Imaging and X-ray Computed Tomography

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The size, morphology, and distribution of the internal structure of ice cream, such as ice crystal, bubble, and solid content, were determined in samples prepared at four different overrun levels using a cryogenic microtome spectral imaging system (CMtSIS) and X-ray computed tomography (X-ray CT) at the synchrotron facility SPring–8. Ice cream samples were prepared at four overrun levels and the mean values of the percent overrun by weight (OR) were 11.5%, 22.7%, 44.3%, and 73.8% for very low, low, medium, and high overrun levels, respectively. Percent overrun by volume (ORV for CMtSIS and ORVs for X-ray CT) was also evaluated for the samples. The means of ORV values obtained using CMtSIS were 10.5%, 42.8%, and 77.7% for very low, medium, and high overrun levels, respectively, whereas the means of the measured OR were 11.5%, 44.3%, and 73.8%, respectively. An ORV–S3D of 14.8% was obtained by incorporating the volume of bubbles in the 3-D image using CMtSIS for the ice cream sample with 12.7% OR. For high OR (73.8%) ice cream, the ORVx values ranged from 68.8% to 75.5% with a mean of 71.3%; whereas the value of the corresponding ORVc by CMtSIS was 77.7%. For low OR (22.7%) ice cream, the ORVx values ranged from 17.8% to 25.3% with a mean of 21.7%.

**Keywords:** Ice cream, Ice crystal, Bubble, Internal structure, X-ray computed tomography

1. Introduction

The size and shape of the internal structure of frozen materials strongly influence the freezing rate or heat flux and the direction of heat flow within the material during freezing as well as the final quality. To understand the behavior of heat or mass transfer in frozen food materials and determine the characteristics of freeze–drying, knowledge of the internal structure of frozen food materials is required.

Ice cream usually is consumed in a frozen state, and it has a complex internal structure consisting of bubbles, ice crystals, and milk solids. The final quality of ice cream is influenced by the size, morphology, and distribution of the complex internal structure formed during the manufacturing process.

Attempts have been made to observe bubbles and ice crystals in cross sections of ice cream using optical microscopy [1–4] or by indirect methods such as scanning electron microscopy (SEM) [5–7] or Cryo–SEM [8–10]. The SEM and Cryo–SEM methods allow identification of morphological differences between bubbles and ice crystals by the micro–asperity on a sublimated sample surface, but determining whether a sublimated seam is caused by an ice crystal or by a bubble is difficult. Different cross sections would provide different diameters for the same bubble. Also, the diameter values reported by traditional methods might not correlate well with the final quality of ice cream because the measurements for bubbles, ice crystals, and milk solids are usually obtained from different samples. X-ray computed tomography (CT) has been applied to two– and three–
dimensional (2-D, 3-D) structural elements of food materials [11–19]. The technique is based on the contrast in X-ray images generated by differences in X-ray attenuation due to variations in the density of the material within a sample. Also, food materials containing water are freeze-dried to improve the density contrast for X-ray CT.

Do et al. [20] evaluated bubbles, ice crystals, and milk solids in commercial ice cream samples from microscale to macroscale using CMtSIS images. Sato et al. [11] applied X-ray CT using synchrotron radiation to observe the structure of ice crystals in frozen food. Nakagawa et al. [12] also applied X-ray CT using synchrotron radiation to observe the glassy state relaxation during annealing of frozen sugar solutions.

The present work determined the size, morphology, and distribution of the internal structure of ice cream, including the ice crystal, bubble, and solid content of samples prepared at four different overrun levels using CMtSIS and X-ray CT at the synchrotron facility SPring-8 (BL14B2; JASRI, Hyogo, Japan). The specific objectives were: 1) calculate OR for samples prepared at four overrun levels, 2) calculate ORVc for samples at three OR levels by evaluating the volume of bubbles determined from microscale to macroscale using CMtSIS, 3) construct and evaluate 3-D images of bubbles, ice crystals, and milk solids for a sample with a very low OR level, and calculate ORVc-3D using the total volume of bubbles, and 4) calculate ORVx for ice cream samples with low and high OR levels based on the volume of bubbles using X-ray CT.

2. Materials & Methods

2.1 Materials

A commercially available vanilla ice cream mix (ZAO; Yamada milk Co., Ltd. Miyagi, Japan) with a formulation of 8% milk fat, 10% milk solids—not-fat, sugar, honey, stabilizer, and emulsifier was used. Ice cream samples at four different overrun levels (very low, low, medium, and high) were produced using an automatic batch freezer (191 P/SP/N; Carpigiani group, Bologna, Italy).

2.2 Overrun by weight

Overrun is the industrial term for the amount of air added to frozen dessert products, and the percentage overrun of ice cream by weight can be calculated using the following equation [21]:

\[
\text{%Overrun by weight (OR)} = \frac{\text{Wt. of mix-Wt. of same vol. of ice cream}}{\text{Wt. of same vol. of ice cream}} \times 100
\]  

2.3 Experimental Equipment

1) Cryogenic microtome spectral imaging system

A CMtSIS technique similar to that used by Do et al. [20] was adapted for this study. The CMtSIS instrument consisted of a microtome unit, an automatic high-precision XY stage, an image acquisition unit (visible, fluorescence, and spectroscopic), and a 3-D image processor. The CMtSIS allows consecutive image acquisition of cross sections of frozen samples exposed by multi-slicing with a minimum thickness of 0.25 μm. The temperature of the heat exchanger is controlled from room temperature to −160°C by regulating the flow rate of liquid nitrogen and the power to the electric heater installed in the heat exchanger. Microscope and macroscale images can be obtained with a high-power objective lens of a microscope installed on the automatic high-precision XY stage (ALD–106–H1P; Chuo Precision Industrial Co., Ltd., Tokyo, Japan) that can be mechanically adjusted with a resolution of 2 μm within a maximum area of 60×60 mm². The image of each cross section is captured with a highly sensitive CCD camera and NIR camera, and is recorded by the 3-D image processor. A 3D image is reconstructed from a series of consecutively acquired 2-D images.

Integrated images using CMtSIS

Each ice cream agglomerate was shaped into a cylindrical sample with a diameter of 10 mm and a height of 10 mm and was shock-frozen in liquid nitrogen. The sample was embedded in an optimal cutting temperature (OCT) compound and a ring of dry ice. The sample, OCT compound, and the ring of dry ice fixed on the heat exchanger were sliced together, and cross-sectional images were then captured by the CCD camera through a microscope. A total of 36 image frames was obtained from a sliced surface using the CCD camera, a 50× magnification lens, and an automatic high-precision XY stage. Each frame covered an area of 194×154 μm². The 36 frames were integrated into a single image by the 3-D image processor.

Identification of the internal structure

Bubbles, ice crystals, and milk solids in the ice cream sample were identified from the CMtSIS images using the microscale to macroscale measurement method previously reported by Do et al. [20]. Bubbles in an ice cream sample were identified as defocused spots in two-dimensional (2D) CMtSIS images due to the difference in the focal distance created by the vacant spaces. The
NIR images of ice crystals in the frozen sample appeared darker than the other components at 1500 nm due to weak reflected light at this peak wavelength. Milk solids were also observed by the flux differences in light reflected from the different interfaces in the ice cream.

Although the bubbles, ice crystals, and milk solids could be recognized from the original 2-D image (Fig. 6a) by visual examination, obtaining binary images for each of these constituents was difficult when applying automatic thresholding techniques. Therefore, the boundaries of individual bubbles, ice crystals, and milk solid were traced manually on a digitizing board (Cintiq 21UXTZ-2100; Wacom Co., Ltd., Saitama, Japan). After extracting the pixels within the traced boundaries and obtaining binary images, the areas of each bubble were calculated using image analysis software (Image-Pro Plus; Media Cybernetics Inc., Maryland, USA).

**Measurement of ORV using CMtSIS**

The CMtSIS could capture cross-sections exposed by multi-slicing of a frozen sample with a minimum thickness of 1 μm and reconstruct 3-D images utilizing a series of consecutively acquired 2-D images. The volume rendering method was employed as optimal for measurement of internal structures. Using this method, the volume could be determined from the surface areas of quantitative 2-D information and the slice thickness of CMtSIS. The volume of each bubble and ice crystal was calculated from the 3-D images using image processing software (TRI/3D VOL; Ratoc System Engineering Co., Ltd., Tokyo, Japan). The ORV could be determined as a percent increase in volume of the mix occurring as a result of air addition, and could be calculated using the following equation [21]:

\[
\%\text{Overrun by volume (ORV)} = \frac{\text{Vol. of ice cream} - \text{vol. of mix used}}{\text{Vol. of mix used}} \times 100
\]  

(2)

**2) Experimental procedure using synchrotron X-ray CT**

An X-ray CT technique performed at the synchrotron facility SPring-8, similar to that used by Sato et al. [11], was used for this study. The X-ray energy was adjusted to 12.4 keV. Cylindrical ice cream samples of 5×15 mm (diameter×height) were placed on a block of dry ice to keep them frozen. The temperature of the sample was maintained at about −30°C by blowing liquid nitrogen over the sample. The distance from the sample to the X-ray camera was set to 100 mm so that the resulting image pixel size was 2.9×2.9 μm².

The principle of X-ray tomography is based on contrast in the X-ray image by X-ray attenuation due to density and compositional differences within a sample; therefore, dark regions correspond to air bubbles and the brightest regions represent ice crystals and milk solids. The raw X-ray tomography image of ice cream was binarized after sequential application of noise removal and particle segregation processes using ImageJ software (ImageJ; U. S. National Institutes of Health, Bethesda, Maryland, USA).

**3. Results and Discussion**

**3.1 Overrun by weight**

The OR of commercial ice cream ranges from 25% (super premium) to 120% (standard). The mean values of the OR measurements in this study were 11.5%, 22.7%, 44.3%, and 73.8% for very low, low, medium, and high OR levels, respectively (Table 1). The OR for commercial ice cream is 100–120% of that for standard (milk fat: 10–12%), 60–90% of that for premium (milk fat: 12–14%), and 25–50% of that for super premium (milk fat: 14–18%). The mean values were 11.5%, 22.7%, 44.3%, and 73.8% for very low, low, medium, and high OR levels, respectively (Table 1). These results confirmed that the OR was controlled in the 11.5% to 73.8% range by an automatic batch freezer.

**3.2 Measurement of internal structure by CMtSIS**

**1) Measurement of bubbles**

Figure 1 shows an integrated raw image and a binarized image representing the boundaries of identified bubbles covering a measurement area of 1164×924 μm² by a total of 7800×6180 pixels. Figure 2 shows the distribution of equivalent bubble circle diameters in the sample with 11.5% OR (very low overrun). A total of 480 bubbles was recognized from the integrated image: the equivalent circle diameters ranged from 1.1 to 101.9 μm.

| Statistic | Very low | Low | Medium | High |
|-----------|----------|-----|--------|------|
| OR        | 12.7     | 25.8| 46.7   | 75.7 |
| Minimum   | 10.3     | 19.3| 40.9   | 72.0 |
| Mean      | 11.5     | 22.7| 44.3   | 73.8 |
| SD        | 1.2      | 2.3 | 1.4    | 1.9  |

Each n=9

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with a mean of 24.8 μm, and the measured areas ranged from 0.9 to 8144.9 μm^2 with a mean of 637.8 μm^2. Figure 3 shows the distribution of equivalent circle diameters of the bubbles found in the sample with 44.3% OR (medium overrun). A total of 1898 bubbles was recognized from the integrated image: equivalent circle diameters ranged from 1.1 to 140.4 μm with a mean of 25.8 μm, and the areas ranged from 0.9 to 15479.8 μm^2 with a mean of 749.0 μm^2. Figure 4 shows the distribution of the equivalent circle diameters of bubbles in the high overrun sample (73.8% OR). A total of 692 bubbles was recognized from the integrated image: equivalent circle diameters ranged from 1.1 to 248.2 μm with a mean of 40.3 μm, and the areas ranged from 0.9 to 48362.0 μm^2 with a mean of 2035.9 μm^2. The extended area of the integrated image (1,075,536 μm^2) allowed the recognition of bubbles from a microscale of 0.9 μm^2 to a macroscale of 48362.0 μm^2, where the area of the largest bubble surpassed that of a single frame. The number of bubbles of medium OR was greater than that of very low and high OR. For medium OR samples, bubbles less than 20 μm in equivalent circle diameter were 64.0% for 29.8% of high OR, and bubbles greater than 50 μm were 2.5% for 20% of high OR. The total amount of ORvc was significantly affected by the size of the bubble.

2) Overrun by volume

The means of ORvc values obtained were 10.5%, 42.8%,
and 77.7% for very low, medium, and high OR levels, respectively, with corresponding OR measurements of 11.5%, 44.3%, and 73.8%. Figure 5 compares the OR values calculated from the weight measurements and the ORVc values obtained by identifying the area of bubbles on integrated images analysis using CMtSIS (slice thickness: 2 μm) for ice cream samples prepared at three OR levels.

3) Three-dimensional measurements of the internal structure of ice cream

An ice cream sample of 12.7% OR (maximum of the very low overrun level) was prepared for 3-D internal structure measurements. All of the 3-D images for bubbles, ice crystals, and milk solids were reconstructed using separate sets of 100 binarized cross-sectional images (slicing thickness: 4 μm) as shown in the lower row of Fig. 6. Each 3-D image was 260 μm long, 206 μm wide, and 400 μm high.

The 3-D images (Figs. 6a, b) showed that the bubbles were fairly evenly distributed in the ice cream sample, but the ice crystals were not. Milk solids formed a retinal structure circumscribing bubbles and ice crystals as shown in Figs. 6a, d.

A total of 1178 bubbles was recognized by reconstructing the circles in the 2-D images into 3-D spheres, with volumes that ranged from 0.125 mm³ to 490.421 mm³ with a mean of 2.342 μm³, as shown in Fig. 7. Furthermore, an ORVc-3D of 14.8% was obtained by incorporating the volume of bubbles in the 3-D image for the ice cream sample of 12.7% OR (Fig. 8). A total of 186 ice crystals was reconstructed from a set of 100 2-D images and had volumes that ranged from 0.125 mm³ to 5493.684 mm³ with a mean of 34.483 mm³, as shown in Figure 9.

Fig. 5 OR and ORVc values for different overrun levels using CMtSIS.

Fig. 7 Distribution of bubble volume in the sample with 12.7% OR using CMtSIS.

Fig. 6 Morphology and distribution of the internal structure of the ice cream sample with 12.7% OR (upper: 2-D, lower: 3-D).
Bubbles were observed within ice crystals and milk solids. Do et al. [20] reported microscale to macroscale measurement of bubbles, ice crystals, and milk solids in commercial ice cream using CmTIS images; however, the relation between OR and ORvc was not clear. In the present study, a comparison between OR and ORvc was possible from the CmTIS images. Measurement of bubbles is difficult using SEM or Cryo-SEM because of sublimation at the sample surface. Ice crystals and bubbles within a sample can be resolved by the vacant spaces left after freeze–drying. Since all vacant spaces are treated as ice crystals and bubbles after freeze–drying, distinguishing between ice crystals and bubbles may be difficult. Therefore, this 3-D method for determining the size, morphology, and distribution of internal structures such as ice crystals, bubbles, and solid content within an ice cream sample using CmTIS is an advancement.

### 3.3 Measurement of internal structures by X-ray CT

Figure 10 shows an X-ray tomography image and binarized image of the ice cream sample with a high OR (73.9%) obtained through X-ray CT at the synchrotron facility SPring–8 (BL14B2). Figure 10b shows a binary image of a 1000×1000 μm$^2$ region within the white square in Fig. 10a. The volume of each bubble was calculated as a multiple of surface area and pixel height of 2.9 μm for each layer. Figure 11 shows the volume distribution for the bubbles on a typical binary image for the sample with 22.7% OR. A total of 8636 bubbles was recognized from the binary image with a volume from 26.8 to 4498.8 μm$^3$ with a mean of 464.1 μm$^3$. Figure 12 shows the volume distribution for the bubbles with 73.8% OR. A total of 7515 bubbles was recognized from the binary image with volumes from 26.3 to 15427.5 μm$^3$ with a mean of 1438.6 μm$^3$. Figure 13 and Table 2 show OR,
ORVc, ORVc–3D, and ORVx with values of OR and ORVc from Table 1, Fig. 5, and 8. For high OR (73.8%) ice cream samples, the ORVx value ranged from 68.8% to 75.5% with a mean of 71.3%; whereas the corresponding mean ORVc by CMtSIS showed a value of 77.7%. For low OR (22.7%) ice cream, the ORVx value ranged from 17.8% to 25.3% with a mean of 21.7%.

A recent study applied X-ray CT to obtain information on food microstructures [13, 15, 16, 17]. X-ray CT image contrast acquired in X-ray tomograms is determined by the absorption coefficient of the sample. Thus, frozen food materials containing moisture require pretreatment, such as freeze–drying, to obtain vacant spaces for a high absorption coefficient and to clarify density contrast. In this study, the internal structure of ice cream was determined in the frozen state without pretreatment using X-ray CT at the synchrotron facility SPring–8.

### 4. Conclusions

A new technique to investigate the internal structure of ice cream samples produced at different overrun levels was developed and its high accuracy was verified using the CMtSIS and X-ray CT at the synchrotron facility SPring–8. Results showed:

1. The actual size of integrated images by CMtSIS was $1164 \times 924 \mu m^2$. These images demonstrated that the areas of recognized bubbles ranged from a microscale of 0.9 to a macroscale of $48362.0 \mu m^2$.

2. The means of ORVc obtained using CMtSIS were 10.5%, 42.8%, and 77.7% for very low, medium, and high overrun levels, respectively, whereas the means of the

| Table 2. Percent overrun by weight and volume (ORVc–3D was calculated using the 3D image) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Statistics                      | Overrun levels (%) |                |                |                |                |                |                |
|                                | Very low         | Low            | Medium         | High           |                |                |                |
|                                | OR              | ORVc           | ORVc–3D        | OR              | ORVx           | OR              | ORVc           |
| Maximum                        | 12.7            | 12.2           | 25.8           | 25.3           | 46.7           | 43.9           | 75.7           |
| Minimum                        | 10.3            | 8.8            | 19.3           | 17.8           | 40.9           | 40.7           | 72.0           |
| Mean                            | 11.5            | 10.5           | 14.8           | 22.7           | 21.7           | 44.3           | 42.8           |
| SD                              | 1.2             | 1.7            | 2.3            | 3.8            | 1.4            | 1.7            | 1.9            |
| n                               | 9               | 3              | 9              | 3              | 9              | 3              | 3              |

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measured OR were 11.5%, 44.3%, and 73.8%, respectively.

3. For high OR (73.8%) ice cream, the ORx values ranged from 68.8% to 75.5% with a mean of 71.3%; whereas the corresponding ORv by CMTsis showed a value of 77.7%. For low OR (22.7%) ice cream, the ORx values ranged from 17.8% to 25.3% with a mean of 21.7%. These results indicate that CMTsis and X-ray CT were useful for determining OR and ORx values and to examine the internal structure of commercial ice cream within the OR range of 73.8% to 11.5%.

Nomenclature

CMTsis: Cryogenic microtome spectral imaging system
OR: Overrun by weight, %
ORv: Overrun by volume using CMTsis (bubble area slice thickness), %
ORv-3D: Overrun by volume using CMTsis (reconstructed 3-D image), %
ORx: Overrun by volume using X-ray CT with synchrotron radiation, %

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アイスクリームの内部構造は、主に気泡、氷結晶、乳製品で構成され、これらは、アイクリームの最終品質に大きな影響を与える。アイスクリームの内部構造計測は、光学顕微鏡、電子顕微鏡、X線CTなどがあるが、これらの計測には、凍結置換法、凍結固定法、凍結乾燥法などによる試料の前処理が必要で、とくに、電子顕微鏡、X線CT計測には、前処理として試料の凍結乾燥が行われる。このため、アイスクリーム内の氷結晶が昇華し、空隙として残り、元来の気泡との区別が困難である。

本研究の目的は、極低温ミクロトームイメージングシステム（Cryogenic Microtome Spectral Imaging System, CmSIS）とX線CTを用い、アイスクリームの内部構造を計測することにある。具体的には、1) アイスクリームの6種類オーバーラン（Overrun: OR）をコントロール可能なフリーザーを用い、4種類（Very Low, Low, Medium, High）のオーバーランを製造し、重量を基準にしたオーバーラン（OR）を測定した。2) CmSISにより自動位置きめにより得られた結合画像から気泡の表面積を求め、CmSISの切削厚を掛け体積を求めた。この気泡の体積を基準にした3種類（Very Low, Medium, High）のオーバーラン（ORv）を算出した。3) CmSISにより得られた連続2次元断面画像を用い、気泡、氷結晶、乳製品の3次元像を構築し、気泡と氷結晶の体積を算出し、さらに、気泡の3次元像の体積を基準にしたVery Lowのオーバーラン（ORv-3D）を算出した。4) X線CTにより得られた画像から気泡の表面積を求め、X線CTの3次元画像構築に伴う画素の厚さを掛け気泡の体積を求めた。この気泡の体積を基準にした2種類（Low, High）のオーバーラン（ORv）を算出した。

CmSISは、試料を切削するミクロトーム部、自動XYステージ、分光観察部で構成される。ミクロトーム部の熱交換器は液体窒素を冷媒とし、室温から-100℃まで、切削刃は、熱交換器を通った液体窒素により-50℃で制御される。X線CTは、大型放射光施設SPRING-8のビームライン（BL14B2）で、X線エネルギーは12.4keVである。試料周辺は、液体窒素を吹き付け、約-30℃で制御される。結果は以下である。1) アイスクリームの重量を基準にしたORの平均値は、Very Lowが11.5%, Lowが22.7%, Mediumが43.3%, Highが73.8%であった。2) CmSISの自動位置きめにより得られた36枚の結合画像から、気泡、氷結晶、乳製品を識別した。また、結合画像の実寸法は、1164×924μmで、気泡のミクロ（相当円直径：0.9μm）からマクロ面積（相当円直径：48362.0μm）までの計測が可能になった。3) CmSISによるORvの平均値は、Very Lowが10.5%, Mediumが42.8%, Highが73.7%で、この際、ORはそれぞれ11.5%, 44.3%, 73.8%であった。4) CmSISによるORv-3Dの平均値は、14.8%で、この際、ORは12.7%であった。5) X線CTによるORvの平均値は、Lowが21.7%, Highが71.3%で、この際、ORはそれぞれ22.7%, 73.8%であった。本研究の手法は、従来のような凍結乾燥、凍結置換、材料構成分の染色などの試料の前処理が不要となり、かつ、アイスクリームの内部構造を直接に計測できる大きな特色がある。