A comparative study of desktop- and synchrotron radiation-based X-ray microtomography analysing air bubbles and ice crystals in ice cream.

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Introduction

Ice cream is a very popular but also complex food product. Dairy ice cream is made by freezing and aeration of a pasteurised mixture of ingredients including milk, fat, sugars, emulsifiers, stabilisers, flavouring compounds and water¹,²,³. Ice cream contains by volume about 40-50% air bubbles, 30% ice crystals and 5% fat droplets which are held together by a continuous viscous unfrozen aqueous 15% sugar solution¹. Ice cream thus contains all three states of matter simultaneously: solid, liquid and gas.

The size of ice crystals and air bubbles are critical to the ice cream's quality and sensory attributes. Large crystals (> 50 µm) lead to a coarse, grainy and icy texture⁴. Whereas large bubbles will decrease the smoothness and creaminess. The size of ice crystals and air bubbles have to remain below the threshold of perception. Ice cream is inherently unstable. Only at temperatures lower than about -25°C the microstructure will not change. If the temperature increases during shipping, storage at the grocery store, or even during storage (~-18°C) and on exit from the consumer's freezer, the small ice crystals can melt and recrystallize into larger structures with larger interconnected air bubbles. Also pressure fluctuation leads to coalescence of air bubbles. An understanding of the mechanisms behind ice formation and bubble growth would greatly aid manufacturers in predicting the effects of processing and in the development of formulations.

The structure-function relationships in relation to the product properties (stability, mouthfeel, melting behaviour etc.) can be studied and understood using a range of imaging techniques covering length scales from a millimetre down to the nanometre scale. The morphology of ice crystals and air bubbles which are typically ranging from 10 µm to about 100 µm, is generally studied using cryo Scanning Electron Microscopy (SEM)⁵,⁶. Small samples (~ 5 x 5 x 10 mm³) are quench-frozen in liquid nitrogen or nitrogen slush (~-200 °C), transferred to the cryo-SEM, fractured with a knife to create a clean internal surface, sublimated (freeze etching) to allow clear visualisation of the ice crystals and coated with a thin layer of metal to prevent charging effects. A cryo-SEM image of a typical ice cream sample is shown in Figure 1. The ice crystals appear as irregularly shaped light grey objects (smooth flat areas) and the air bubbles as more rounder both light and dark grey objects. The sugar matrix is the continuous phase between air bubbles and ice crystals. The major limiting factor of the cryo-SEM technique is that it is difficult to obtain quantitative data, with respect to air bubble and ice crystal size distributions. Generally, identification of bubbles and ice crystals has to be done manually, drawing around the structures of interest. This is laborious and biased by the influence of the operator. The 2D cryo-SEM images will not represent the true sizes of air bubbles and ice crystals. The apparent size of the object depends on where it has been sliced. Stereological methods are needed to calculate the true size distribution⁷. Cryo-SEM is time consuming and limited to 2D
imaging of small sample areas obtained after destructive sample pretreatment (the maximum useful fractured surface diameter is about 2mm). To obtain a statistical relevant number of air bubbles and ice crystals multiple images have to be made of the fractured surface using manual image acquisition (automatic image acquisition is not possible from the uneven fractured surface due to variability in height, contrast, brightness and focus).

Figure 1  Cryo-SEM image of a fresh ice cream sample (magnification 300x, 1 pixel = 0.37 µm).

X-ray microtomography (micro-CT) has proven to be a very useful technique for the 3D non-invasive imaging of relative large volumes of food microstructures at µm resolution and suitable for 3D quantitative analysis. The technique requires minimum sample preparation and is widely used for imaging food products at ambient temperatures. A few studies concerning the application of micro-CT to investigate the ice cream microstructure have been performed up to now. Imaging of ice cream requires special cooling facilities. A laboratory micro-CT instrument with a two stage solid-state Peltier cooling stage was used to image fresh and temperature abused (cycling between -20°C and -10°C) ice cream (Figure 2). The images show clearly the effect of temperature cycling (formation of larger air bubbles and ice crystals). However, the contrast between the ice crystals and the continuous sugar matrix is too low for quantitative analysis. The used cooling stage should reach 35-40°C below ambient temperature. However the cooling capacity is insufficient for low stability ice creams and doesn’t allow accurate temperature settings. The laboratory micro-CT instrument can also be placed in a cold room. The instrument should be slightly modified to withstand the cold temperature. The contrast between ice crystals and the continuous sugar matrix can be increased using iodine, making automatic quantitative analysis of the different phases possible. However, contrast agents are not preferred because they may induce structural changes and can only be used for model samples produced on lab scale and not for samples manufactured under food grade pilot plant or factory conditions (not for commercial samples).
A better contrast and resolution can be obtained using Synchrotron Radiation (SR) micro-CT. The high photon flux results not only in high signal-to-noise ratios at high spatial resolution but also in a reduction in measuring times (few min.) which is needed to study in situ thermal cycling. Beside absorption contrast also phase contrast can be observed due to X-rays with a high degree of coherence. Phase contrast is generated in regions of a highly-localized change in the refractive index of the sample, such as its borders and interfaces between the sample matrix and inclusions\(^\text{13}\). A feasibility study using SR micro-CT was performed in 2009 at the ID19 beamline at the ESRF (European Radiation Synchrotron Facility) in Grenoble using a continuous nitrogen-flow cooling device (Oxford cryostream cooler 700) to blow a cold nitrogen gas stream (~170°C) on the sample during its rotation\(^\text{14,15}\). Using fast scanning (1 min) 700 projections (1024x512 pixels 0.020s) were acquired over 180° resulting in a reconstructed stack of 1024 x 1024 x 512 pixels with a pixel size of 0.56 µm. Figure 3 represents a comparison between fresh and temperature abused ice creams (temperature cycled). These images show different contributions of phase and absorption contrast. The phase contrast can be seen in the characteristic black/white fringes at the edges between air and solid. The edges between ice crystals and the continuous sugar matrix of the fresh ice cream are faintly visible. After temperature abuse the ice crystals are clearly distinguishable. These images show the formation of large ice crystals and air bubbles after temperature abuse. However quantitative analysis of phase contrast images will be difficult\(^\text{13}\). Recently more sophisticated reconstruction procedures have become available\(^\text{16}\) to retrieve the different phases which could improve the segmentation and quantification. The cooling can further be...
optimised to reduce frost formation (e.g. using a dry shielding nitrogen flow and an enclosure). For in-line temperature cycling a cryostream or cryojet is less suitable because the temperature of the sample is difficult to control. More advanced cold-stages with accurate temperature control have been developed which can be used within a synchrotron set-up. Experiments performed at the TOMCAT beamline at the Swiss Light Source and at the Diamond Manchester Branchline (DMB) of the Diamond Light Source in the UK showed that these systems can be used for in-situ temperature cycling of ice cream to follow the bubble and ice crystal growth in time. This paper will show the potential of the SR micro-CT imaging of ice cream at the DMB using a cold-stage providing temperature variation from ambient conditions to -40°C with a controlled accuracy approaching 0.1°C.

Figure 3  SR Micro-CT images of cross sections of fresh (A) ice-cream and temperature-abused (B and C) ice cream obtained using the ESRF in Grenoble using a cryojet. (1 pixel = 0.56 µm). C shows the 3D visualisation of air and ice crystals (clipped at 50% of the volume).
Method
Samples and sampling
500 ml blocks of 5% fat ice cream were prepared using a scraped surface heat exchanger. Sample kapton tubes (American Durafilm Co. Inc, Holliston U.S) with an inner diameter of 3 mm, wall thickness of ~0.06 mm and length of 10.5 mm were inserted into the ice cream before blast freezing at -35°C and subsequent storage at -25°C. The ice cream filled kapton tubes were cut from the block of ice cream on a bed of dry ice shortly before X-ray analysis. The ice cream was also investigated after deliberate temperature abuse by cycling the blocks of ice cream between -15°C and -5°C every twelve hours for 1 and 2 weeks (started after 24 hours at -25°C).

SR micro-CT
The ice cream samples were imaged using the DMB of the Diamond Light Source in Oxfordshire, UK. The sample tube was inserted inside a custom-made cold stage developed by the Manchester X-ray Imaging Facility (Figure 4). The cold-stage provides temperature variation from ambient conditions to -40°C with a controlled accuracy approaching 0.1°C. Details are reported by P. Rockett et al.19. The DMB was operated using a pink beam with a 2mm Al filter. For optimum image quality the distance between the sample and the scintillator was ~ 3.5 cm. A 2560 × 2160 pixel PCO Edge 5.5 CMOS camera was optically coupled to a single crystal CdWO4 scintillator. For the tomography, a total of 3600 projections were obtained over 180° rotation with an exposure time of 0.1 s. The reconstructed volumes were 1980 × 1980 × 1830 voxels with a pixels size of 1.6 µm (2x objective) and 2560 × 2560 × 2128 voxels with a pixels size of 0.81 µm (4x objective). For tomographic reconstruction a single-distance non-iterative phase retrieval algorithm described by David Paganin16 was used. After phase retrieval air and solid can clearly be identified based on a difference in grey level (2 distinct peaks in the histogram, see Figure 5). The grey level of the sugar matrix is slightly higher than the grey level of the ice crystals. However no separate peaks can be observed in the histogram. A drawback of the phase retrieval is the possible smoothing of edges which can hamper the identification of ice crystals which are separated by a thin layer of the continuous sugar phase.

![Figure 4](image4.png)

Figure 4 Cold-stage assembly used for SR micro-CT imaging of ice cream at the DMB of the Diamond Light Source.
For 3D visualisation and quantitative image analysis the AvizoFire software (version 9.01) from the FEI - Visualization Sciences Group was used.

Figure 5  Standard (left) versus Paganin phase retrieval (right) reconstruction of a SR μCT image of fresh ice cream with inserts showing the histograms of each image.

Results
Initial micro-CT experiments were performed on DMB of the Diamond Light Source with fresh and temperature abused ice cream using the cold stage at a constant temperature of -20°C. The used temperature abuse procedure simulates the behaviour in the cold supply chain (from production, storage, transport, point of sale to the consumer home) by cycling the temperature of blocks of ice cream (500ml) between -15°C and -5°C during 1 and 2 weeks. Horizontal cross sections through SR micro-CT images of the fresh and ex-situ temperature abused ice cream are compared in Figure 6. The grey levels in the images range from dark grey (air) to light grey - white (sugar matrix). The ice crystals are visible by their intermediate grey level (light grey). These images show a clear effect of extended temperature cycling on the microstructure of ice cream. Both bubbles and ice crystals are coarsened over time. Many small ice crystals recrystallize to fewer bigger crystals and air bubbles increase in size and finally become irregular shaped.
Figure 6 SR-μCT images of horizontal cross sections through ice cream after production and after storage during which temperature fluctuation occur (ex-situ temperature abuse: cycling between -15°C and -5°C) with enlarged view (magnification =4x, 1 pixel = 0.81 µm).

Quantitative analysis of the volume fraction of air bubbles and their size distribution from these images can be done straightforward by thresholding using a clear minimum in the grey level histogram (Figure 5). Surface renderings of the segmented images are shown in Figure 7. Ice crystals are much more difficult to identify in these images. After median filtering a local thresholding method was used to identify the ice crystals. However this method leads to a lot of misclassified pixels which have to be corrected using laborious manual correction methods and is therefore not suitable for automatic image analysis (especially for the image of the fresh sample which is more difficult to segment). To obtain a statistically accurate size distribution, many particles (~500) must be measured which makes manual analysis very laborious and time consuming.
**Conclusion**

Initial synchrotron micro-CT experiments on the Diamond Light Source in the UK give insight in the 3-dimensional development of air bubbles and ice crystals in ice cream during thermal abuse. In the context of these transformations, this information will allow better assessment of the mechanisms involved that lead to structural failure during temperature oscillations. These insights will allow us to design and test novel and better intervention strategies to increase product stability.

The results have shown successful imaging of fresh and temperature abused ice cream using a custom build cold stage at a constant temperature of -20°C. The major bottleneck is that automatic quantitative analysis of ice crystals is not possible. This can be obtained by improving the contrast and resolution (optimisation of acquisition and phase retrieval) or by developing more sophisticated image analysis methods. The technique can be further used for in-situ temperature cycling\(^9\). This is of particular interest to study the 3D development of ice crystals as a function of changing temperature.
References: