Accepted Manuscript

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Please cite this article as: Guo, E., Kazantsev, D., Mo, J., Bent, J., Van Dalen, G., Schuetz, P., Rockett, P., StJohn, D., Lee, P.D., Revealing the microstructural stability of a three-phase soft solid (ice cream) by 4D synchrotron X-ray tomography, *Journal of Food Engineering* (2018), doi: 10.1016/j.jfoodeng.2018.05.027.

1 **Revealing the microstructural stability of a three-phase soft solid**

2 **(ice cream) by 4D synchrotron X-ray tomography**

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4 Enyu Guo [a,c,**], Daniil Kazantsev [c,d], Jingyi Mo [b,c], Julian Bent [e],

5 Gerard Van Dalen [e], Peter Schuetz [e], Peter Rockett [b], David StJohn [f], Peter D Lee [b,c,*]

6

7 [a] Key Laboratory of Solidification Control and Digital Preparation

8 Technology (Liaoning Province), School of Materials Science and Engineering,

9 Dalian University of Technology, Dalian, 116024, China

10

11 [b] Department of Mechanical Engineering, University College London, Torrington

12 Place, London WC1E 7JE, UK

13

14 [c] Research Complex at Harwell, RAL, Didcot, OX11 0FA, UK

15

16 [d] The Manchester X-Ray Imaging Facility, School of Materials, The University of

17 Manchester, Manchester, M13 9PL, UK

18

19 [e] Unilever R&D, Colworth, MK44 1LQ, UK

20

21 [f] School of Mechanical and Mining Engineering, The University of Queensland,

22 St Lucia, Queensland, 4072, Australia

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38 [*, **]Corresponding authors:

39 [*]Peter D Lee: peter.lee@ucl.ac.uk; Tel: +44 (0)1235 567789

40 [**]Enyu Guo: eyguo@dlut.edu.cn; Tel: +86 (0)411 84709500

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44 **Abstract**

45 Understanding the microstructural stability of soft solids is key to optimizing

46 formulations and processing parameters to improve the materials' properties. In this

47 study, *in situ* synchrotron X-ray tomography is used to determine the temperature

48 dependence of ice-cream's microstructural evolution, together with the underlying

49 physical mechanisms that control microstructural stability. A new tomographic data

50 processing method was developed, enabling the features to be segmented and

51 quantified. The time-resolved results revealed that the melting-recrystallization

52 mechanism is responsible for the evolution of ice crystal size and morphology during

53 thermal cycling between -15 and -5 ^{o}C, while coalescence of air cells is the dominant

54 coarsening mechanism controlling air bubble size and interconnectivity. This work

55 also revealed other interesting phenomena, including the role of the unfrozen matrix

56 in maintaining the ice cream's microstructural stability and the complex interactions

57 between ice crystals and air structures, e.g. the melting and recrystallization of ice

58 crystals significantly affect the air cell's morphology and the behavior of the unfrozen

59 matrix. The results provide crucial information enhancing the understanding of

60 microstructural evolution in multi-phase multi-state complex foodstuffs and other soft

61 solids.

62

63 **Keywords:** Ice cream; Microstructure; Tomography; Ice crystals; Coarsening; Soft

64 solid.

65

1. Introduction

Soft solids are important composites that are characterized by complex multi-phase structures and possess inherently complex non-Newtonian rheological properties under external stress [1-3]. Soft solids exist either in nature, e.g. muds, or in many artificially manufactured products such as emulsions, biopolymers, fresh concrete and domestic baking materials. Many soft solids, such as soft foams (e.g. ice cream) and aerated desserts, contain porous phases within a viscous matrix [4-6].

Structural stability is desired for many soft foams and microstructural instability greatly influences the materials' properties and their applications. Take ice cream for example. The microstructure of ice cream, including the size distribution and connectivity of each phase, plays a critical role in determining product quality (e.g. mouthfeel, taste, appearance, etc.) and the product's shelf-life [6-10]. For example, the microstructural change at different storage temperatures has been shown to alter ice cream's viscoelastic properties and hence the oral sensory perception of it [10]. However, irreversible microstructural changes often occur in ice creams (over a range of different timescales), as well as in other similar foam structures that contain air, above a certain temperature (~ -30 °C for ice cream [4]) which may occur during shipping, storage at the grocery store and in domestic freezers (ca. -18 °C), and on the consumer's table.

For ice cream, the structural instability is affected by many factors, including compositions [11, 12] and thermal variations [5, 13]. One of the well-recognized phenomena due to thermal instability is coarsening of microstructure [4, 5, 13-15]. This was initially examined in light microscopy [14, 16-19] and cryo-scanning

3

89 electron microscopy [20, 21], and transmission electron microscopy [22], all of which

90 provide only 2D information of the surface or of cuts through the ice cream sample.

91 These phenomena were recently studied in 3D with X-ray tomography on *ex situ*

92 coarsened samples [4, 5]. Using a synchrotron X-ray tomography technique, prior

93 work by the same authors [5] revealed that after thermal cycling (or thermal 'abuse')

94 between -15 and -5 $^{\circ}$C for a number of cycles/days, both ice crystals and air cells

95 grew in size creating ice dominated structures within a deteriorated ice cream

96 microstructure. More specifically, the size of ice crystals was observed to

97 continuously increase up to 14 cycles; however, the growth rate significantly

98 decreased after 7 thermal cycles. The air cells also increased in size, and they

99 continued to grow into interconnected irregular shapes with long continuous channels

100 after 14 thermal cycles. However, for up to 7 thermal cycles the air cells seemed to

101 remain more or less spherical.

102 From that *ex situ* study, it was observed that the ice crystals within the unfrozen

103 matrix tended to align along the boundary between air cells and the matrix,

104 minimizing surface energy. Those 3D experiments provided valuable insight into the

105 structural changes of ice cream upon thermal cycling. However, the study was

106 performed on *ex situ* thermally cycled samples and thus, the interactions between the

107 microstructural features could not be elucidated. The detailed mechanisms that control

108 the microstructural evolution, which are only available via *in situ* studies, still remain

109 to be explored. Questions regarding the growth mechanisms and relative movement of

110 the phases and their exact interactions during thermal cycling, need to be answered to

111   be able to improve the stability of the ice cream's structure [5, 14, 23]. For example,

112   what are the dynamics of the changes for each phase and how do they impose on each

113   other due to thermal variations, in order to maintain the integrity of ice cream's

114   structure? What are the dominant coarsening mechanisms that control the ice cream's

115   microstructural evolution?

116   This work non-destructively studies the thermal stability of the ice cream

117   microstructure via 4D (3D plus time) synchrotron X-ray tomography to reveal the

118   dynamics of the microstructural changes. This technique has become increasingly

119   used in the study of opaque materials systems to study both coarsening [24] and

120   rheology during deformation [25, 26]. X-ray tomographs were continuously acquired

121   on a sample during a heating and cooling cycle at a well-controlled slow ramp rate of

122   0.05 ^{o}C/min. To analyze the acquired data, an iterative tomographic data

123   reconstruction and image processing method was developed. Through quantitative

124   analysis, the physical mechanisms which dominate the degradation of ice cream's

125   microstructure due to temperature variation, are examined and discussed in detail.

126
127   **2. Materials and methods**

128   **2.1 Sample and experimental methods**

129   Fresh ice cream containing 5% fat was manufactured by Unilever R&D (U. K). A

130   500 ml block of fresh ice cream was initially thermally cycled between -15 and -5 ^{o}C

131   for seven times (1 week) before it was used for the *in situ* synchrotron experiment.

132   The seven cycles created a larger scale of microstructure, enabling easy identification

133   of phases for quantification, and also represent a transition point between the

5

134   observations made in the first seven cycles where change was relatively rapid and the

135   next seven cycles where the size of ice crystals became more stable as reported in the

136   authors' prior *ex situ* studies [5]. Small ice cream samples, each contained in a 3 mm

137   inner diameter kapton tube (67 μm thick, American Durafilm Co. Inc, Holliston, U.S),

138   were cut from the 500 ml block. Details of the sample preparation method are

139   described in [5].

140   The *in situ* synchrotron experiment was conducted on the Diamond Manchester

141   Beamline (I13-2) of the Diamond Light Source (DLS, U.K) using a pink beam. The

142   set-up for running the beamline experiment, together with the cold stage used to

143   provide the sample temperature, is described in [5, 27]. During the *in situ* thermal

144   cycling experiment, the sample was loaded in the cold stage at -15 ^{o}C and stabilized

145   for 10 min. Then, the sample was heated to -5 ^{o}C at a ramp rate of 0.05 K/min and

146   held there for 10 min. After that, the sample was cooled back to -15 ^{o}C at the same

147   ramp rate as the heating stage. A schematic of the thermal cycle history is shown in

148   the inset of Fig. 1. The tomographic scans were acquired using a 2560×2160 pixel

149   PCO Edge 5.5 CMOS camera that was optically coupled to a single crystal $CdWO_4$

150   scintillator during the thermal cycle. For each tomographic scan, 900 projections were

151   recorded with an exposure time of 100 ms (90 s for each scan) and a pixel size of 0.8

152   μm. However, at the end of each tomographic scan, the sample stage was rotated back

153   to the initial position for system re-initiation to start the next tomographic scan,

154   adding an additional delay of ~ 51s, for a cycle time of ~ 141 s. In total, 178

155   tomographic scans were acquired during a thermal cycle.

6

2.2 Image reconstruction and three-phase segmentation approach

Initially, the acquired projection data were reconstructed using the conventional Filtered Back Projection (FBP) algorithm [28], producing extremely noisy reconstructions with low contrast and ring artifacts (see Fig. 2(a)). The poor quality of FBP images was due to angular under-sampling (only 900 projections for a $2k \times 2k \times 2k$ volume), short exposure time and low attenuation contrast between ice and water. In order to improve the image quality suitable for segmentation, a Model-Based Iterative Reconstruction (MBIR) approach was applied.

The MBIR algorithm is based on the Group-Huber data fidelity function to minimize ring artifacts and 3D total variation (TV) regularization penalty [29-31]. . The TV-related regularization sub-problem has been solved using the Split-Bregman method in order to enhance the weak contrast between ice crystals and unfrozen matrix (see Fig. 2(b)).

Although MBIR reconstruction substantially improves the contrast and also removes noise, the reconstructed images suffer from the visible intensity inhomogeneities within various ice-crystals (indicated by arrows in Fig. 2(b)). These artifacts can be a result of the combined effects of strong noise and beam hardening. The latter is possible due to abrupt changes in attenuation coefficients between unfrozen matrix (highly attenuated) and ice crystals (poorly attenuated), therefore introducing non-linearity in a beam. Intensity inhomogeneity within a single crystal restricts successful segmentation by histogram thresholding. One can use more sophisticated segmentation methods (e.g. 3D snake contours can successfully segment features with intensity inhomogeneity using supervised seeding). However, due to the

179   large data size, a computationally efficient approach is required.

180   Here an additional post-processing step was applied to equalize intensity within

181   phases by means of gradient-constrained nonlinear isotropic diffusion [5, 32]. The

182   mask to terminate the diffusion process across selected boundaries was acquired from

183   the image in Fig. 2(b) by thresholding the magnitude of the gradient. Since the

184   gradient magnitude between phases is large and the variations of intensity within

185   regions are gradual, one can run the constrained diffusion until the regions become

186   fully homogeneous. Therefore, 1000 diffusion iterations were run on a GPU to

187   equalize intensities within enclosed regions of the image in Fig. 2(b). The result (Fig.

188   2(c)) is sufficient to implement a simple histogram thresholding operation and

189   generate images with homogeneous contrast within three phases (Fig. 2(d)).

190   Finally, the processed volume was cropped into a smaller volume for 3D analysis.

191   3D rendering of the features, as well as quantification of size/volume, was performed

192   using Avizo® (FEI, Thermo Fisher Scientific, U. S). The thickness of unfrozen matrix

193   was measured using BoneJ in ImageJ [33].

194

195   **3. Results and Discussion**

196   **3.1 General microstructural evolution during thermal cycling**

197   Fig. 3 and supplementary Fig. S1 show the 2D tomographic slices of ice cream's

198   microstructural evolution during thermal cycling between -15 and -5 $^{\circ}$C. A few salient

199   observations can be made based on these images. First, ice crystals were continuously

200   melting, decreasing in size, during the heating stage (Fig. 3(a-c)) and their

8

201 morphologies became more spherical at "high" temperature (e.g. -5 ^{o}C, Fig. 3(c)). It

202 appears that the most significant morphologic change took place in the temperature

203 range -7.5 to -5 ^{o}C. During cooling, the relatively round ice crystals grow into a more

204 irregular morphology again (Fig. 3(d-f)), as expected for ice which has a high

205 anisotropy in interfacial energy. Second, some of the air cells tended to coalesce. One

206 example is indicated by the arrows in Fig. 3(a) and (b), where two neighboring large

207 air cells gradually merged into one.

208   Third, it is observed that during the heating stage the microstructural features

209 moved to the upper right corner of the region in Fig. 3, and observed more clearly in

210 supplementary Fig. S1, because of the volume shrinkage when ice crystals were

211 melted into the unfrozen matrix. An air cell in Fig. 3(b) (indicated by a red arrow)

212 shows the example of this movement. Note that the microstructure seemed to be

213 compressed slightly due to the decreasing volume. It is mentioned that the expansion

214 of volume seemed to be less significant during cooling as compared to the reduction

215 in volume upon heating. The degree of expansion and relative movement during

216 cooling also suggests the unfrozen matrix is quite flexible in response to external

217 thermal change at high temperatures.

218   Microstructures in the longitudinal section were extracted for further examination

219 and the results are shown in supplementary Fig. S1. The microstructural features (e.g.

220 ice crystals and air cells) move less in the sample axis direction as compared to that in

221 the cross section (Fig. 3). In other words, flotation of air cells along the sample axis

222 does not occur, or at least was not observed in this study. This observation suggests

223   that macro-fluid flow was largely inhibited due to the increased viscosity of the

224   unfrozen matrix due to the stabilizers present in the ice cream samples, which would

225   inhibit the mobility of air cells [14]. In addition, it is also difficult to identify if

226   drainage, which can play an important role in the instability of the air structure at high

227   temperature [14], occurred in this work. However, it is also mentioned that

228   microscopic fluid flow in the channels within the unfrozen matrix probably still

229   occurs which cannot be resolved by the technique used in this work.

230

231   **3.2 3D Ice crystal evolution**

232   The volume fraction of ice crystals was examined first (Fig. 1), together with the

233   calculated data based on the thermal properties (i.e. extrapolated melting points at

234   various concentrations) of the ice cream formulation. Generally, the results measured

235   by tomography compare well with the theoretical predictions. Minor errors might be

236   caused by shrinkage and expansion, which would change some of the features

237   measured.

238   Fig. 4 shows the 3D evolution of ice crystals during a thermal cycle. Ice crystals

239   are individually color-rendered according to the equivalent diameter of each ice

240   crystal. In the figure, the blue color corresponds to small size. As expected, ice

241   crystals gradually decreased in size when the sample was heated, reaching the

242   minimum size at -5 $^{\circ}$C. This is consistent with the observation that more small blue

243   ice crystals were present at -5 $^{\circ}$C, as shown in Fig. 4(c). The ice crystals then

244   continuously grew in size when the sample was cooled again to -15 $^{\circ}$C (Fig. 4(d-e)).

245   Detailed examination of the position change for the 3D ice crystals after heating

246   shows that the ice crystals moved upwards slightly, by less than 20 μm. This

247   movement of the ice crystals might be caused by compression due to volume

248   shrinkage, which drives the ice crystals to move upwards towards the center of the

249   sample when the unfrozen matrix is less viscous at warmer temperatures [34].

250   The ice crystals in Fig. 4 were quantified in terms of equivalent diameter and size

251   distribution (Fig. 5). The average equivalent diameter of ice crystals decreases upon

252   heating from 101 μm at -15 ^{o}C to 87 μm at -5 ^{o}C, and then increases again to ~103 μm

253   at -15 ^{o}C after cooling (Fig. 5(a)). Interestingly, the average equivalent diameter of the

254   ice crystals is increased by ~ 2 μm at -15 ^{o}C after a thermal cycle (Fig. 5(a)),

255   suggesting a mild coarsening of ice crystals after thermal cycling. This coarsening of

256   ice crystals at a storage temperature (e.g. -5 ~ -18 ^{o}C) with imposed oscillation

257   temperatures (e.g. ±2.5 ^{o}C) have been previously observed [10, 23, 35, 36]. It is

258   pointed out that the performed thermal cycling in this study leads to much higher

259   coarsening rates than isothermal storage at a certain temperature due to the

260   temperature oscillations which, through observed melting-recrystallization process,

261   increases the rate of the coarsening process [23].

262   The size distribution of the ice crystals at different temperatures was analyzed

263   and the results are plotted in Fig. 5(b-d). In general, the size distribution curves reflect

264   the temperature change, where the curves shift to the left upon heating (Fig. 5(c)),

265   while they shift to the right during the cooling stage (Fig. 5(d)). It is also shown in Fig.

266   5(b) that the distribution of the two curves at -15 ^{o}C, before and after the thermal

267 cycle, is similar indicating only a minor change in the overall size after the thermal

268 cycle was completed. This observation confirms that the ice crystals follow mainly a

269 "melting-recrystallization" mechanism and that other proposed mechanisms [37] play

270 a small or negligible role.

271   Fig. 6 shows the morphological evolution of five individually separated ice

272 crystals extracted for detailed examination. The morphology of the ice crystals

273 changed during thermal cycling being more irregular at low temperature, e.g. -15 ^{o}C

274 (Fig. 6(a) and (f)), while they became more spherical at "high" temperature, e.g. -5 ^{o}C

275 (Fig. 6(c) and (d)).

276   The five ice crystals shown in Fig. 6 are quantified in terms of volume, volume

277 change, specific surface area and sphericity, and the results are presented in Fig. 7. It

278 is seen that the volume of each ice crystal keeps decreasing during heating and then

279 increasing during cooling. The volume change (as compared to -15 ^{o}C before thermal

280 cycling) shows that the volume of four of the ice crystals increased by 5.5-11%,

281 indicating an increase in size of ice crystals, which is consistent with the observation

282 of the overall increased equivalent diameter of ice crystals due to coarsening (Fig.

283 5(a)). However, the volume of ice crystal 3 decreased slightly, by ~4%. This ice

284 crystal is one of the smallest of the 5, and most likely the other four ice crystals grew

285 at the expense of ice crystal 3 via an Ostwald Ripening mechanism [37].

286   In this study, complete melting of ice crystals was not observed when the sample

287 was heated to -5 ^{o}C since the smallest crystals will have dissolved in the seven

288 thermal cycles prior to the *in situ* experiment. After seven cycles the ice crystals have

289   grown to a large enough size such that they don't completely melt during heating to -5

290    $^{\circ}$C. This critical size can be extracted from Fig. 5(b). Note that the smallest crystals

291   measured are about 60 μm diameter at -15 $^{\circ}$C, and reduce in size by ~25 μm when

292   heated to -5 $^{\circ}$C. This observation suggests that ice crystals with an equivalent

293   diameter less than 25 μm have a high probability of being completely melted during

294   the applied thermal cycling. This is further supported by the observation that there is

295   no significant number of crystals less than 30 μm diameter at -5 $^{\circ}$C. This finding

296   confirms the measurements made in a previous study by the same authors (i.e. Fig.

297   9(d) in ref. [5]) showing a significant reduction in the number of ice crystals during the

298   first 7 cycles.

299   As mentioned, the morphology of ice crystals also changes during thermal

300   cycling (Figs. 3 and 6). This change is quantified using measures of specific surface

301   area and sphericity in Fig. 7 (c) and (d). For example, both the values of specific

302   surface area and sphericity increased continuously as the ice crystals melt, decreasing

303   as the ice crystals recrystallized. These changes support the observation that ice

304   crystals become more spherical during melting (to minimize interfacial energy) and

305   then became more facetted (irregular) during the recrystallization stage as they strive

306   to reach the Wulff shape driven by anisotropy in interfacial energy [38, 39]. However,

307   the ice crystals show only minor morphological changes after a thermal cycle, as

308   indicated by the average sphericity of more than 300 ice crystals where the value

309   increased from ~0.80 at -15 $^{\circ}$C to 0.84 at -5 $^{\circ}$C during the heating stage, and then

310   decreased to ~0.80 when cooled back to -15 $^{\circ}$C. A similar trend was observed for the

311 specific surface area. The minor changes to the size and morphology of ice crystals

312 after one thermal cycle support the previous findings that only small differences in

313 size and morphology of ice crystals were observed between the sample thermally

314 cycled for 7 days and the sample cycled for 14 days [5].

315 **3.3 Unfrozen matrix evolution**

316 Fig. 8 and supplementary Fig. S2 (larger volume) show the 3D morphological

317 evolution of the unfrozen matrix during a thermal cycle. The unfrozen matrix forms a

318 very complex 3D network-like shape with ice crystals and air bubbles dispersed

319 within the matrix. The 3D images in Fig. 8(a-f) show that the unfrozen matrix

320 appeared thicker between air cells upon heating, while they became thinner as the

321 sample cooled down presumably due to the melting of the ice crystals during heating

322 and recrystallization during cooling. This is reflected by the quantified thickness of

323 the unfrozen matrix analyzed in a $721 \times 721 \times 504$ μm^3 volume (supplementary Fig. S2),

324 as plotted in Fig. 8(g) where the thickness monotonically increased from ~12.6 μm at

325 -15 ^{o}C to 27.0 μm at -5 ^{o}C, and then decreased to ~19.6 μm at -15 ^{o}C at the end of the

326 thermal cycle.

327 Figure 8 shows that the thickness of the unfrozen matrix is greater during the

328 cooling stage than during the heating stage, such that the thickness increases by ~ 6

329 μm after the thermal cycle was completed. After detailed examination, it is

330 hypothesised that the formation of a local region of the matrix (e.g. upper right corner

331 in Fig. 3) that is concentrated with more water molecules, is responsible for this

332 change. The shrinkage of the sample, and the associated macro-flow induced by the

14

333   compression effect, might have accelerated the formation of a larger region of low

334   viscosity matrix. During the cooling stage, no additional new ice crystals were

335   nucleated under the cooling rate studied. Thus, the measured thickness of the unfrozen

336   matrix in this region is higher compared to that before thermal cycling.

337   In addition, the 'strength' (or viscosity) of the unfrozen matrix would decrease at

338   the higher temperatures during heating due to a reduction in viscosity allowing more

339   movement of the matrix to accommodate the overall shrinkage of the sample (see

340   above). Thus, a shift of the structure was observed during the heating stage,

341   suggesting a significant negative impact of the ice melting process on structural

342   stability.

343

344   **3.4 3D air cell evolution**

345   A few 3D air cells were extracted to examine the coarsening mechanism. Fig. 9

346   shows one example where two separate air cells gradually merge into one. It is

347   observed that the air cells at -15 $^{\circ}$C are not necessarily round, instead, they have many

348   concave regions (indicated by an arrow in Fig. 9(a)), or even an elongated shape for

349   some cases as seen in Fig. 3. Upon heating, the two air cells merged by creating a

350   bridge between them (Fig. 9(b)), and then the bridge (or neck) continued to thicken

351   with increasing temperature during the heating stage. Meanwhile, some of the

352   concave regions on the air cell surface gradually disappear forming a smooth or

353   spherical surface. The gradual rounding was driven by the reduction in surface energy

354   of the air/unfrozen matrix interface [40].

355 Decreased viscosity of the unfrozen matrix at "warmer" temperatures upon

356 heating increases the diffusion rate of gas between air cells and promotes coalescence

357 of air cells. It is mentioned that adding stabilizers and emulsifier to ice cream helps

358 reduce air cell coarsening, due to the increased extent of fat destabilization and the

359 increased viscosity of the matrix phase, respectively [14]. It is also noticed that the

360 surrounding ice crystals significantly affect the shape of the area around the neck

361 between merging cells. One example is shown in Fig. 9(d-2). It is likely that those ice

362 crystals in the vicinity of the neck limit further coalescence of the two air cells due to

363 the constraint imposed by the ice crystal imbedded unfrozen matrix (also see

364 supplementary Fig. S3).

365 Upon cooling, some of the phenomena observed during the heating stage act in

366 reverse. That is, the surface of air cells became rough or even distorted again at

367 "cooler" temperatures, e.g. -12 $^{\circ}$C in Fig. 9(g). This is most obvious at the lowest

368 temperature of -15 $^{\circ}$C (Fig. 9(h) and (h-2)). The changes are likely to be caused by

369 two main factors. One is that the growing ice crystals continue to push towards the air

370 cells through the unfrozen matrix. This is realized more easily when the unfrozen

371 matrix becomes thinner and thinner as more water molecules are attached to the

372 recrystallizing ice crystals. In total, twenty ice crystals were observed to grow around

373 the air cells shown in Fig. 9 (see supplementary Fig. S3). The second factor is that the

374 pressure within the air cell decreases with the decreasing temperature according to the

375 ideal gas law (PV=nRT), releasing some of the force on the surface that resists

376 morphological change. It is mentioned that the final morphological change is a result

16

377   of competition between the force imposed on the air cell surface by the growing ice

378   crystals and the surface tension of the air cell/matrix interface. It seems that for the

379   case in Fig. 9 the force imposed by the growing ice crystals through the matrix was

380   greater than the surface tension at temperatures lower than ~ -12 $^{\circ}$C, under which the

381   surface started to deform significantly. In addition, the coalescence process seemed to

382   be inhibited by the increased viscosity at low temperatures during the cooling stage,

383   indicating a significantly reduced rate of morphological change of the air cells than

384   during the heating stage.

385   Pelan et al. [41] and Rohenkohl and Kohlus [42] both suggested that the

386   coalescence of air cells to create large coarsened air pockets was the major

387   destabilizing mechanism in the ice cream they studied. A previous study revealed that

388   the storage of ice cream without emulsifier or stabilizer at -15 $^{\circ}$C for 16 days lead to

389   interconnected channels [14]. The recent observations by Guo et al. [5] also showed

390   that thermal cycling of ice cream between -15 and -5 $^{\circ}$C for 14 days resulted in a very

391   complex interconnected air structure [5]. Although Ostwald ripening was observed in

392   the aerated emulsions [43], the *in situ* observations in this study strongly suggest that

393   for ice cream that was cycled for seven times coalescence is the dominant mechanism

394   responsible for the creation of complex interconnected air structures. It should be

395   noted that gas formation can occur due to radiation damage, resulting in molecular

396   bond cleavage (H-H and O-O) or water photolysis, as reported in water under high

397   pressures [44]. If this is occurring, it could explain the increase in bubble volume

398   fraction and the coarsening of bubbles. However, the increase in bubble volume will

399 not have a significant impact on the coarsening of the ice crystals. Gas formation due

400 to irradiation is an open question, as is how this might affect bubble coarsening.

401 Apart from the coarsening of air cells, another interesting phenomenon was

402 observed, i.e. the reduced volume of some of the air cells after thermal cycling. Fig.

403 10 shows the evolution of three individually extracted air cells during thermal cycling,

404 and their corresponding quantified volume changes are plotted in Fig. 11. The overall

405 volume fraction of air cells was also analyzed and the result was observed to decrease

406 monotonically during the heating stage, and continued to decrease until 0.256 at ~ -7

407 ^{o}C during cooling before it started to rise upon further freezing (Fig. 11(a)). The

408 volume fraction after the thermal cycle (~ 0.285) was lower compared to that before

409 thermal cycling began (~0.330). This corresponds to a reduction of volume fraction

410 by ~ 13.8%. The trend of the volume change of the three individual air cells is

411 consistent with that for the overall volume change. It is also noticed that the change of

412 volume is even more than 50% for Air 2 and Air 3, and that those two air cells did not

413 grow in size during the cooling stage. Detailed mechanisms here are still unknown. It

414 is unlikely that the hydrostatic pressure causes such a large change, as the sample

415 height is quite small. The shift of the sample during the thermal cycle might

416 contribute to some measurement errors; however, the volume (thus size) change of the

417 air cells is proposed to be the main contribution, which is supported by the volume

418 change of all three air cell cases (Fig. 11(c-d)). The diffusion of gas into the matrix

419 and the surrounding air cells, as well as out of the whole sample, at the warm

420 temperatures might have contributed to this change. The detailed mechanisms will be

421 investigated in a future study.

422

423 **3.5 Summary of microstructural evolution mechanisms**

424 Here, the mechanisms that control the microstructural evolution of ice cream as

425 observed in this *in situ* study are summarised (Table 1). Generally, the microstructural

426 evolution of ice cream during thermal cycling is controlled by the interaction of three

427 phases.

428 Regarding ice crystals, nucleation of new ice crystals does not occur under the

429 cooling condition studied in this work. The melting-recrystallization mechanism

430 hypothesized as an important mechanism in the previous study by Guo et al. [5] was

431 quantified by analyzing the 4-D tomographs during the thermal cycle. The melting

432 and recrystallization of ice crystals also affects the air cell's morphology, as well as

433 its coarsening process, through the unfrozen matrix layer between the ice crystals and

434 the air structures. For the air phase, coalescence of air cells is clearly observed to be

435 responsible for the coarsening mechanism. For the sample that was initially thermally

436 cycled for seven times, Ostwald ripening takes a less important role in the coarsening

437 of both ice crystals and air cells during thermal cycling. The continuous reduction of

438 air cell volume needs further investigation. The third phase, the unfrozen matrix, is a

439 crucial component controlling the microstructural stability of ice cream. It acts as the

440 reservoir for the water from dissolving ice during heating and releases water for

441 recrystallization of the ice crystals during the cooling cycle. The network of unfrozen

442 matrix, reinforced by the distributed ice crystals (and dissolved hydrocolloids), holds

443 the whole structure together and greatly influences the structural stability of ice cream

444 when subjected to external temperature variations.

445

446 **4. Conclusions**

447 Using 4D synchrotron X-ray tomography, the effect of thermal variation on the

448 microstructural stability of ice cream was investigated during a heating and cooling

449 cycle between -15 ^{o}C and -5 ^{o}C, at a ramp rate of 0.05 ^{o}C/min. A new data

450 reconstruction and image processing method was developed, enabling the large 4D

451 data sets to be segmented and quantified. The experimental set-up, as well as the

452 image processing routine developed, can be applied to a wide range of soft materials.

453 The dynamic evolution of individual microstructural features, i.e. an ice crystal,

454 air cell, and unfrozen matrix, was quantitatively analyzed. The findings integrate the

455 *ex situ* observations made in Guo et al. [5] enhancing the understanding of the

456 mechanisms controlling ice cream's microstructural evolution. The experimental

457 results in this study reveal important physical mechanisms that influence

458 microstructural instability: that is, the coarsening of air cells takes place mainly

459 through the coalescence of neighboring air cells, while ice crystal growth results from

460 the melting-recrystallization mechanism during thermal cycling, both of which lead to

461 degradation of ice cream's microstructure. The unfrozen matrix plays an important

462 role in maintaining the integrity of the structure of ice cream while being flexible

463 enough at the higher temperatures to reduce the stresses imposed during heating and

464 then cooling by the melting and recrystallization of the ice crystals.

20

Data statement

Representative samples of the research data are shown in the figures. Other datasets generated during and/or analysed during this study are not publicly available due to their large size but are available from the corresponding author on reasonable request.

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597

598

Figure captions

599

600 Fig. 1 Change in ice volume fraction as a function of temperature. The inset shows the

601 thermal cycling history of the ice cream sample for the *in situ* synchrotron

602 experiment. The measured phase diagram (i.e. extrapolated melting points at various

603 concentrations) of the ice cream formulation is presented in [5].

604

605 Fig. 2 Reconstructed images using (a) conventional FBP reconstruction method and (b)

606 MBIR reconstruction method; (c) post-processed image of reconstructed MBIR image

607 of (b), note more homogeneous (equalized) intensities compared to (b) within

608 ice-crystals; (d) 3-phases segmentation using (c). A, I and M in (b) stand for air cell,

609 ice crystal and unfrozen matrix, respectively. Scale bar =150 μm.

610

611 Fig. 3 Reconstructed tomographic slices (prior to the equalization step) showing the

612 overall microstructural evolution of ice cream during a thermal cycle: (a) -15 $^{\circ}$C, (b)

613 -7.6 $^{\circ}$C, and (c) -5 $^{\circ}$C during heating, (d) after holding at -5 $^{\circ}$C for 10 min, (e) -7.5 $^{\circ}$C,

614 and (f) -15 $^{\circ}$C during refreezing. A, I and M in (f) stand for air cell, ice crystal and

615 unfrozen matrix, respectively. Scale bar equals 150 μm.

616

617 Fig. 4 3D ice crystal evolution in a 1416×1416×504 μm^3 volume during a thermal

618 cycle: (a) -15 $^{\circ}$C, (b) -7.6 $^{\circ}$C, (c)-5 $^{\circ}$C, (d) -7.5 $^{\circ}$C, and (e) -15 $^{\circ}$C. Ice crystals are

619 size-colored using the equivalent diameter. Scale bar equals 500 μm.

620

621 Fig. 5 Quantified ice crystal size during a thermal cycle: (a) change of average

622 equivalent diameter of ice crystals during a thermal cycle; (b-d) size distribution of

623 ice crystals during (b) a complete thermal cycle, (c) heating stage and (d) cooling

624 stage. The arrow in (b) indicates the size shift of the curves. Note, more than 300 ice

625 crystals were analyzed.

626

627 Fig. 6 3D morphological evolution of five ice crystals during a thermal cycle: (a) -15

628 ^{o}C, (b) -7.6 ^{o}C, (c) -5 ^{o}C, (d) after holding at -5 ^{o}C for 10 min, (e) -7.5 ^{o}C, and (f) -15

629 ^{o}C. The time is indicated in each figure during the thermal cycle. Scale bar 150 μm for

630 all images. Numbers in (c) match the ice crystals analyzed in Fig. 7.

631

632 Fig. 7 Quantified results of five ice crystals during a thermal cycle: (a) volume, (b)

633 volume change, (c) specific surface area, and (d) sphericity. Note the colours of the

634 plots in each figure are identical to the colour-rendered ice crystals in (b).

635

636 Fig. 8 3D morphological evolution of unfrozen matrix within a 259×243×243 μm^3

637 volume during a thermal cycle: (a) -15 ^{o}C, (b) -7.6 ^{o}C, (c) -5 ^{o}C, (d) after holding at -5

638 ^{o}C for 10 min, (e) -7.5 ^{o}C, and (f) -15 ^{o}C; (g) Average thickness of the unfrozen matrix

639 as a function of temperature. Note, the thickness is measured within a 721×721×504

640 μm^3 volume, identical to the domain as shown in supplementary Fig. S2. Figures (a-f)

641 share the same scale bar. Scale bar equals 100 μm.

642

643 Fig. 9 Coalescence of two air cells during the heating stage (a-d) and cooling stage

644 (e-h) of a thermal cycle. (d-2) and (h-2) show the morphological relationship between

645 the surrounding ice crystals and the air cell at -5 ^{o}C and -15 ^{o}C, respectively. Scale bar

646 100 μm for all images.

647

648 Fig. 10 Morphological evolution of three individual air cell cases during a thermal

649 cycle. Scale bar 100 μm for all images.

650

651 Fig. 11 Volume change of air cells as a function of temperature during a thermal cycle:

652 (a) overall volume change of air cells in a 1416×1416×504 μm^3 volume, (b) volume

653 change of three individual air cells.

654
655

Table

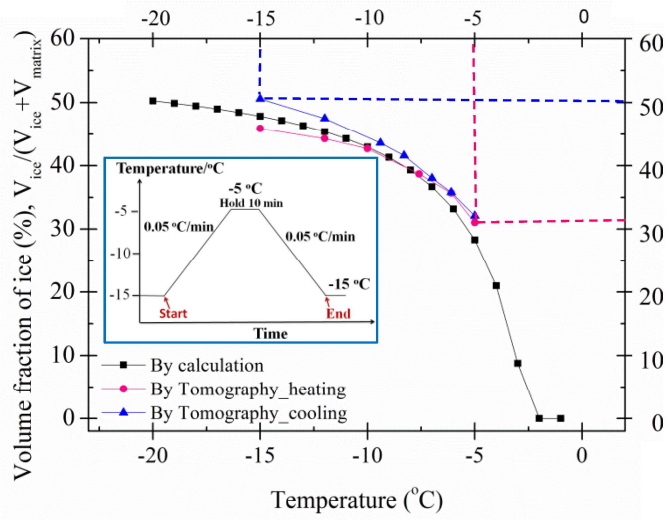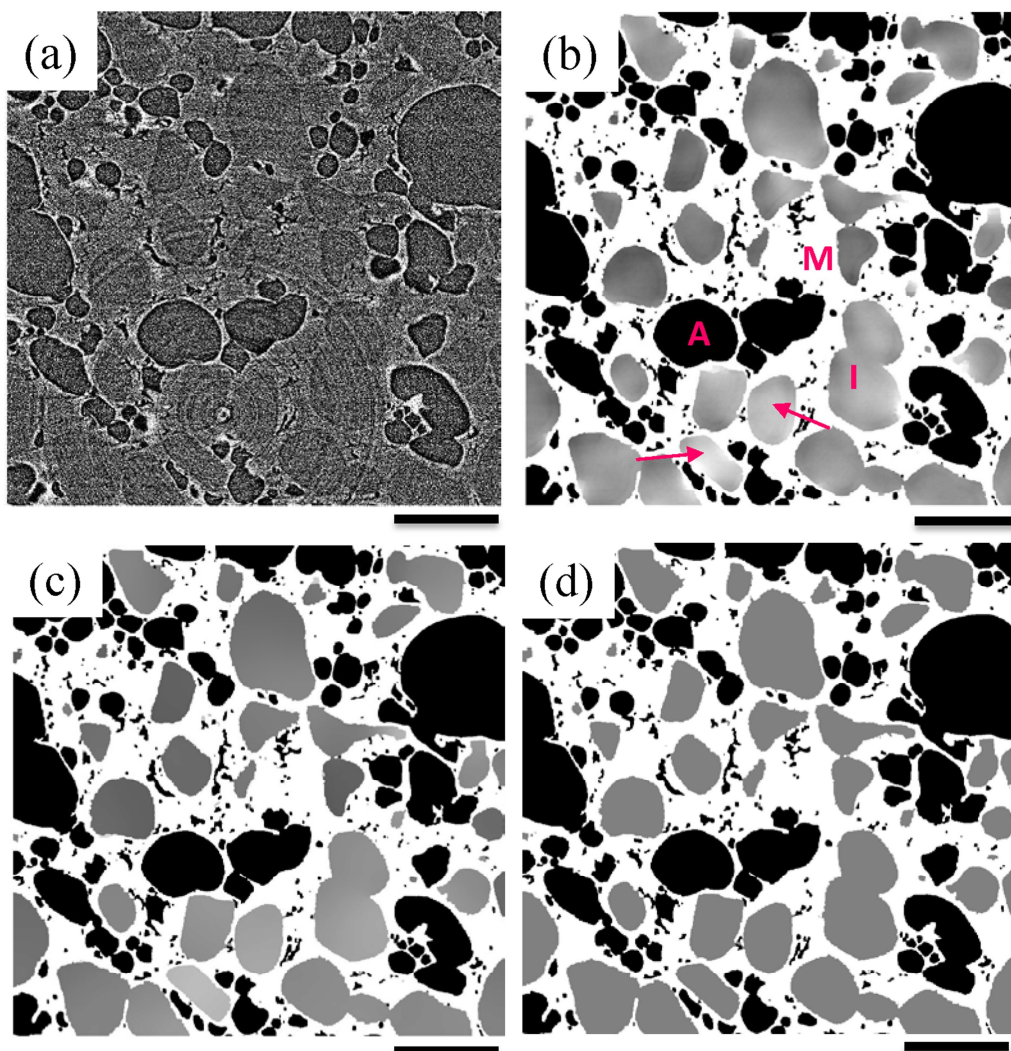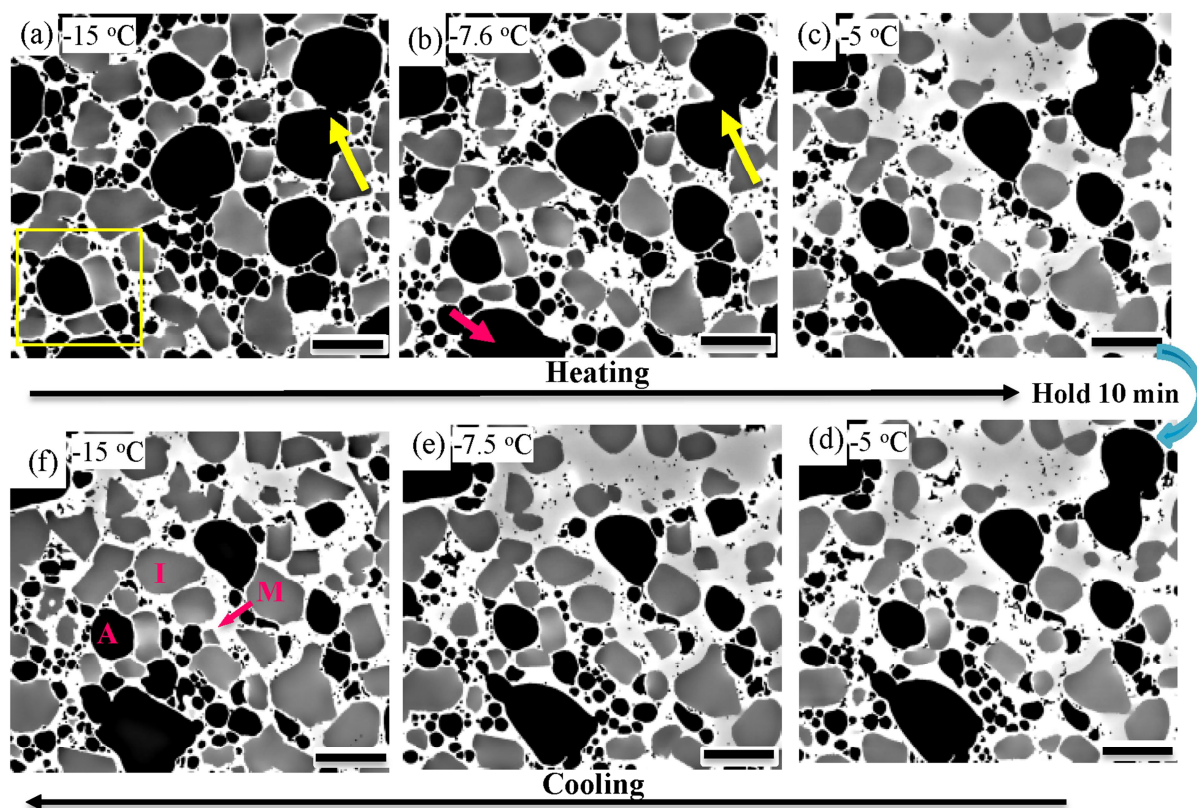Table 1 A summary of the microstructural changes that occur during a thermal cycling from -5 to -15 ^{o}C highlighting the differences between what occurs during the first seven cycles and the following seven cycles. The arrows indicate that part of the cycle over which most change occurs.
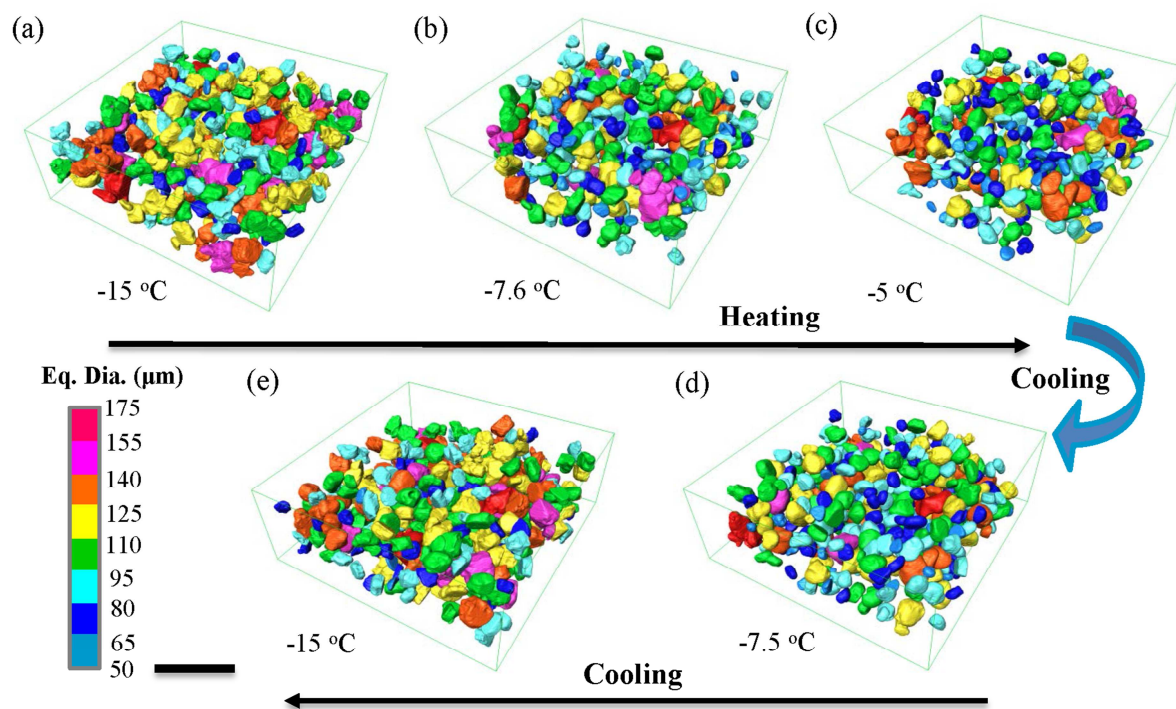
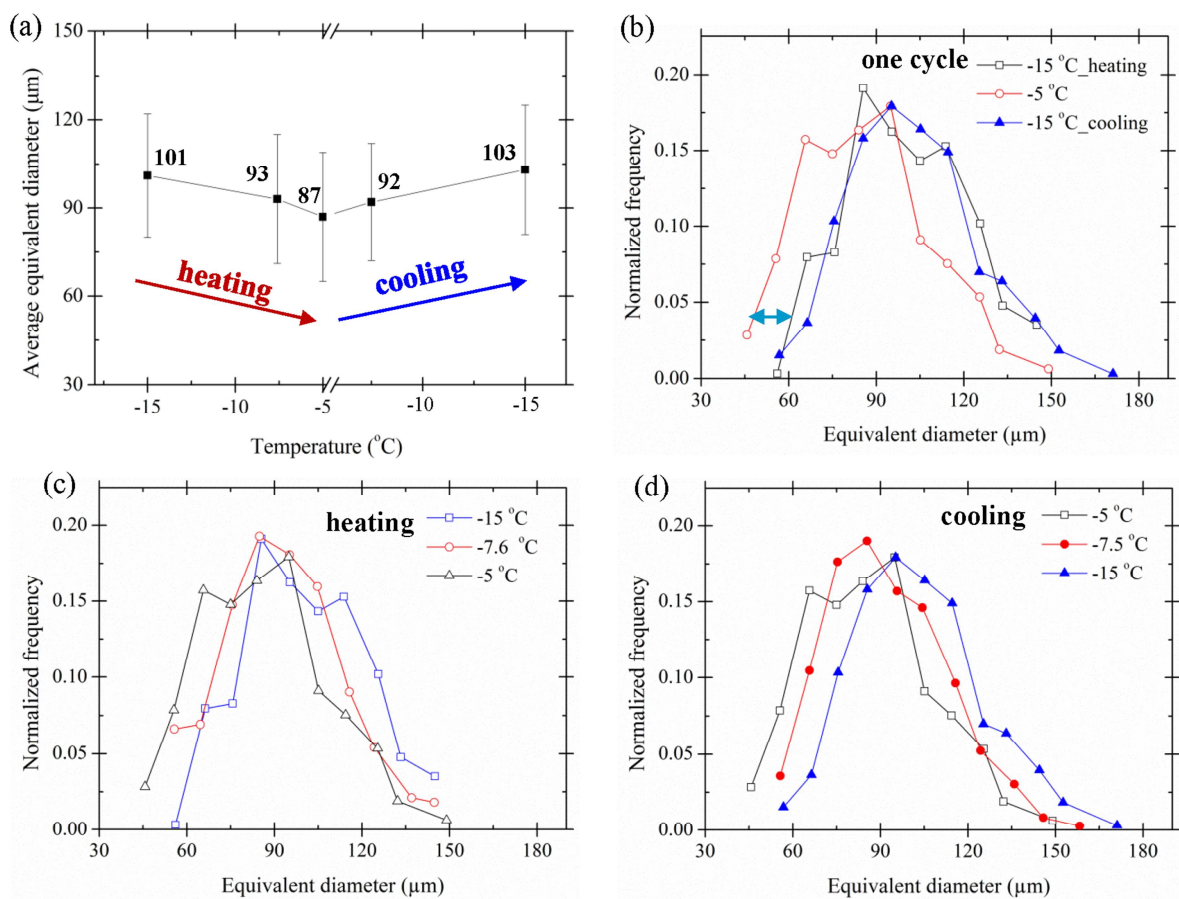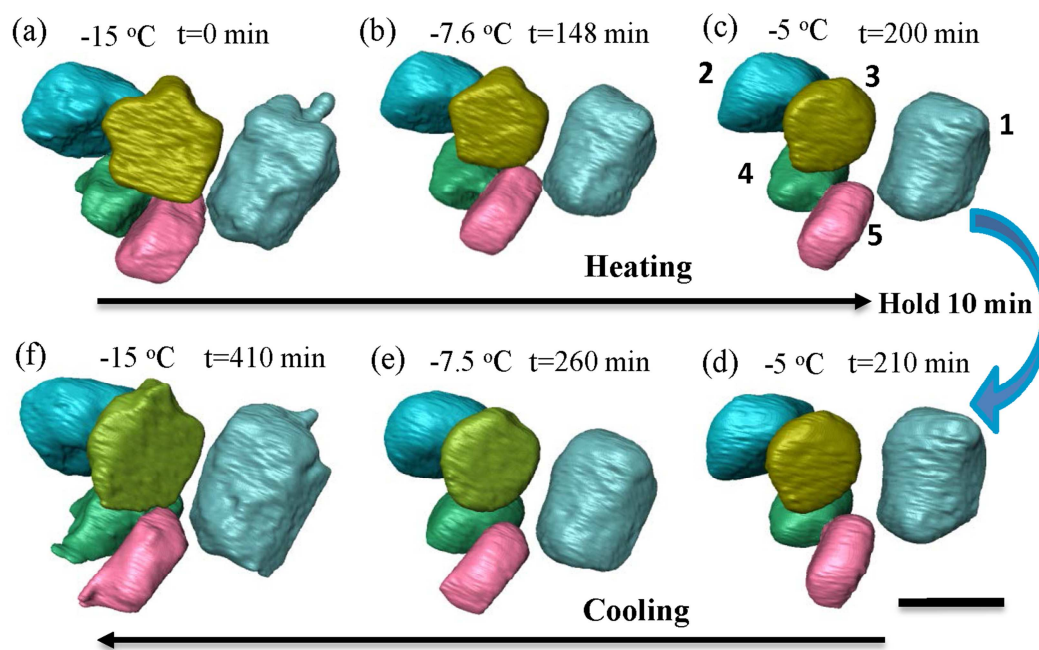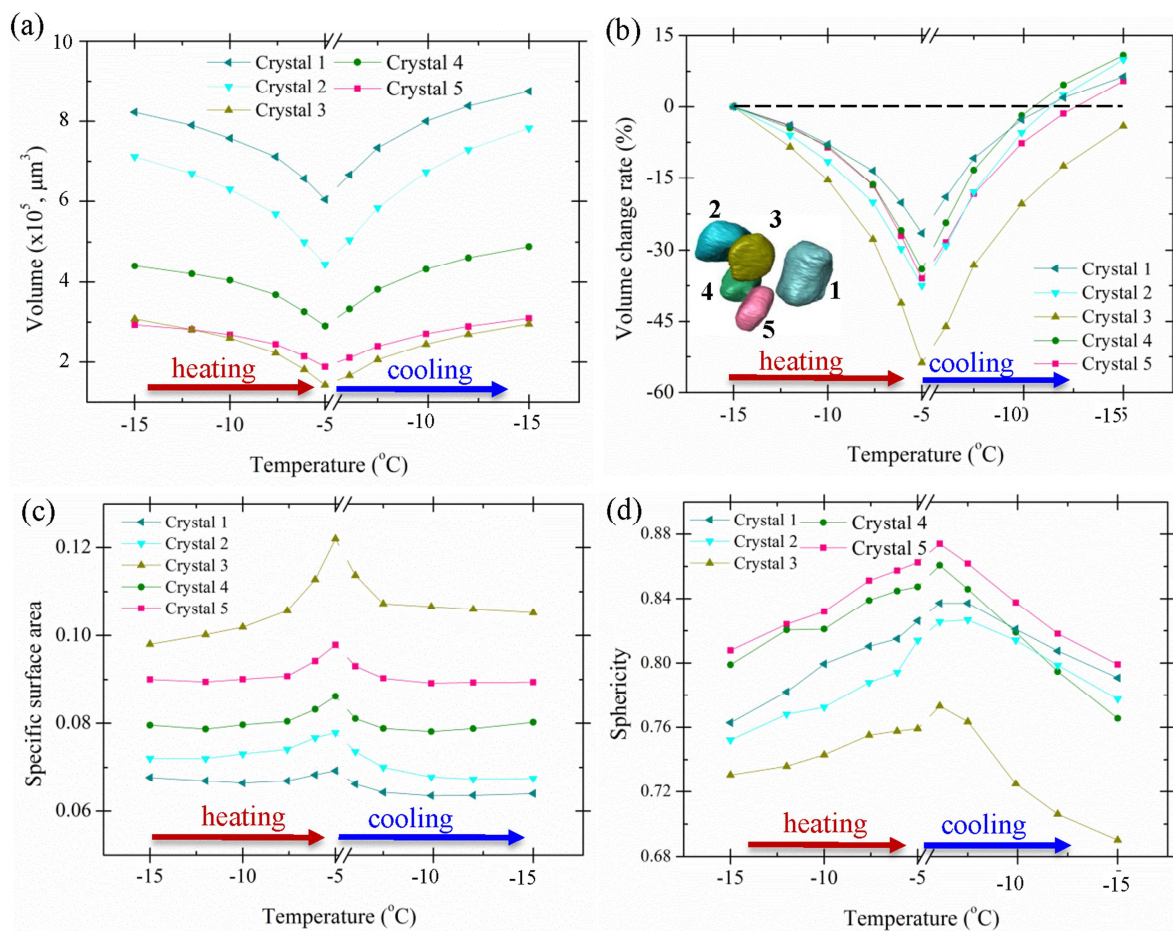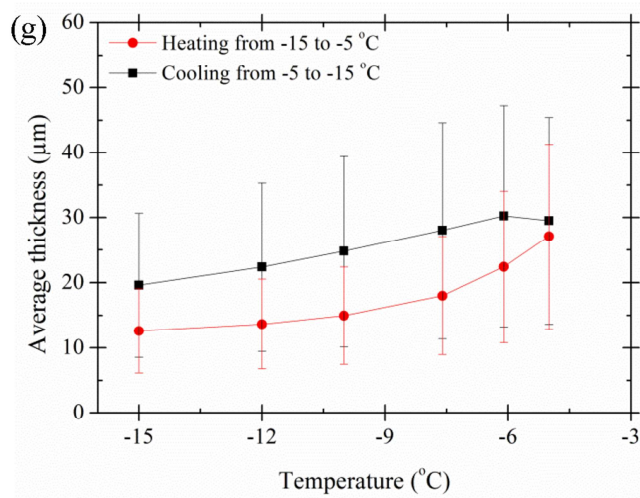|	Heat to -5 ^{o}C	Hold at -5 ^{o}C	Cool to -15 ^{o}C	Hold at -15 ^{o}C
Air cells				
Coalescence of neighbouring air cells.	→→→→→→→→→→→→→→→			
1 to 7 cycles	Size increases although some small air cells remain leading to a bimodal distribution and remain equiaxed.		The air cells shrink.	Cells remain relatively spherical.
8 to 14 cycles	Coalescence of neighbouring air cells.		The air cells become irregular due to constraint by matrix and ice crystals.	Air cells become interconnected and form channels within the matrix network.
Air cells continue to grow into a large interconnected network of irregular shapes as the number of thermal cycles increase. The morphology is constrained by the network of unfrozen matrix and ice crystals.				
Ice crystals				
	Melt by ~40%		Grow by ~66%	
	Dissolution →→→→→→→→→		Recrystallization	
1 to 7 cycles	Size of ice crystals decrease and those < 25 μm melt completely. The morphology becomes rounded.	The size and morphology of crystals change little.	Size of ice crystals increase and no nucleation of new crystals occurs. The morphology becomes irregular during recrystallization.	Over the cycle, the size increases significantly and the number decrease significantly.
8 to 14 cycles	Size decreases by ~ 25 μm dissolvingand the number changes little.	The size of crystals change little.	Size of ice crystals increase by about 25 μm. The number remains unchanged.	Over the cycle, the size increases by a small amount and the number do not increase.
After 7 thermal cycles, the number changes little and the size of ice crystals increase slowly. The ice crystals form networks within the unfrozen matrix network.				
Unfrozen matrix				
Water content	Increases	High	Decreases	Low
Viscosity	Viscosity decreases	Low	Viscosity increases	High
Mechanical response	Matrix becomes and remains flexible, reducing residual stresses		Matrix becomes less flexible	Matrix is effectively rigid
Total volume	Ice cream expands	May shrink somewhat	Ice cream shrinks	Relatively constant
Alignment of ice crystals with the unfrozen matrix network occurs to minimise surface energy and reduce local stress with each additional thermal cycle. At the warmer temperatures the matrix becomes flexible also reducing stresses developed by the constraint of ice crystal and air cells.				

(a) -15 °C

(b) -7.6 °C

(c) -5 °C

Heating

Cooling

Eq. Dia. (μm)

175
155
140
125
110
95
80
65
50

(e) -15 °C

(d) -7.5 °C

Cooling

(a) -15 °C t=0 min

(b) -7.6 °C t=148 min

(c) -5 °C t=200 min

2 **3**

1

4

5

Heating

Hold 10 min

(f) -15 °C t=410 min

(e) -7.5 °C t=260 min

(d) -5 °C t=210 min

Cooling

(a) -15 ºC

(b) -7.6 ºC

(c) -5 ºC

Heating

Hold 10 min

(f) -15 ºC

(e) -7.5 ºC

(d) -5 ºC

Cooling

(g)

(a) -15 °C (b) -10 °C (c) -7.6 °C (d) -5 °C (d-2) -5 °C

Heating

Hold 10 min

(h-2) -15 °C (h) -15 °C (g) -12 °C (f) -9.4 °C (e) -5 °C

Cooling

Air1 (a-1)   (a-2)   (a-3)   (a-4)   (a-5)

-15 °C   -7.6 °C   -5 °C   -9.9 °C   -15 °C

Heating   **Cooling**

Air 2 (b-1)   (b-2)   (b-3)   (b-4)   (b-5)

-15 °C   -7.6 °C   -5 °C   -9.9 °C   -15 °C

Heating   **Cooling**

Air 3 (c-1)   (c-2)   (c-3)   (c-4)   (c-5)

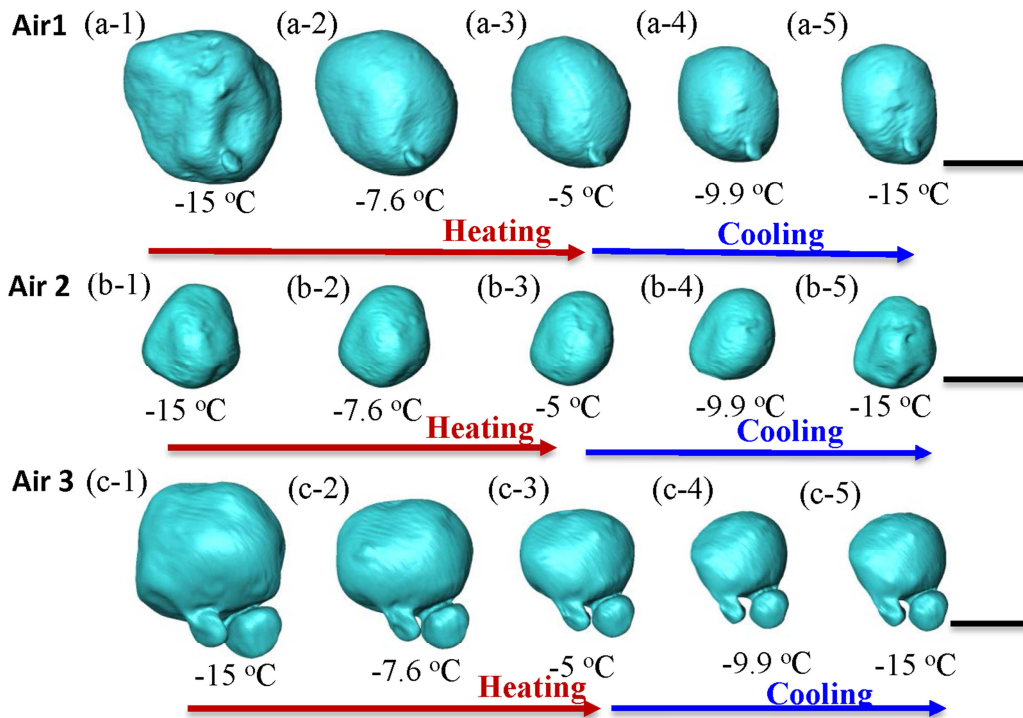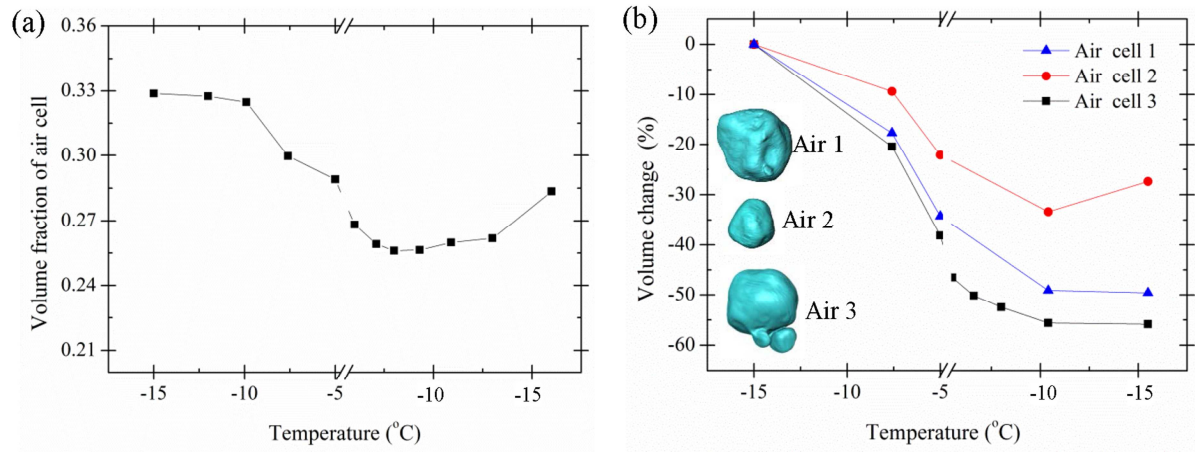-15 °C   -7.6 °C   -5 °C   -9.9 °C   -15 °C

Heating   **Cooling**

Highlights

- In situ synchrotron tomography reveals the dynamics of ice cream emulsion's thermal stability

- A new tomographic data processing method was developed, enabling the features to be quantified

- The melting-recrystallization mechanism is responsible for ice crystal evolution

- Coalescence of air cells is the dominant coarsening mechanism controlling air bubble's evolution

- The unfrozen matrix is important in maintaining the ice cream's microstructural stability