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X-ray microtomography of ice crystal formation and growth in a sponge cake during its freezing and storage

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Abstract:	<p>X-ray tomography has been used to characterize microstructural changes taking place into sponge cake upon freezing and subsequent storage at fluctuating</p> <p>temperatures. The CellDyM cooling cell, initially developed for snow, was used to subject a sponge cake sample to freezing and thermal cycling directly inside the X-ray tomograph. 3D images of the same sample have been scanned at different times during 6 days. An original image processing based on Digital Volumetric Correlation (DVC) has been implemented in order to segment the images and then to quantify porosity changes and ice crystal formation and growth. Overall, the obtained results have shown that (i) all the ice crystals are located at the pore surface and no crystal was found inside the sponge cake crumb matrix and (ii) during the thermal cycling, the size of the ice crystals linearly increases while the number of ice crystals tends towards a plateau. These tendencies can be related to a recrystallization phenomenon occurring due to thermal cycling. More generally, the proposed methodology could be applied to other frozen porous products during freezing and frozen storage.</p>

Amira ZENNOUNE

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Dear Editor,

We wish to submit a research article entitled “**X-ray microtomography of ice crystal formation and growth in a sponge cake during its freezing and storage**” to be considered for publication in Journal of Food Engineering.

In this manuscript, we report the application of an innovative X-ray microtomography-based methodology to analyze the microstructure of frozen sponge cake. The particularity of the studied product is its high porosity and its complexity which makes difficult to study and understand heat and moisture transfer phenomena. A specific cooling stage initially developed for snow was used to subject sponge cake samples to freezing and thermal cycling directly inside a laboratory X-ray microtomography apparatus. This cooling stage allows in situ monitoring of the ice crystals dynamics during freezing and frozen storage of porous foods for the first time. Dynamic X-ray microtomography generates multiple radiographs reconstructed to give the 3D volume of the sample. An original image processing based on Digital Volumetric Correlation (DVC) was implemented in order to segment the images and then quantify porosity changes as well as formation and evolution of the ice crystals.

This study brings new and strong evidences about the impact of dynamic changes of temperature during freezing and frozen storage on ice crystal characteristics (location and size) in sponge cake. Therefore, we believe that our findings could be of interest and useful for the readers of Journal of Food Engineering.

We confirm that this manuscript has not been published and is not under consideration to be submitted for publication elsewhere. All co-authors have seen and agree with the contents of the manuscript and we have no conflicts of interest to disclose.

We hereby suggest the following referees: **Gerard VAN DALEN** Gerard-van.Dalen@unilever.com **Alain Le Bail** alain.lebail@oniris-nantes.fr.

Thank you for your consideration of this manuscript.

Sincerely yours,

Amira ZENNOUNE

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Dear editor,

Thank you for the opportunity to submit a revised version of our manuscript entitled '*X-ray microtomography of ice crystal formation and growth in a sponge cake during its freezing and storage*'. We thank the reviewer for his valuable feedback, which has contributed to the improvement of the manuscript.

Our responses to the comments below are highlighted in [blue](#).

Reviewer #3:

The revised article contains additional supplements that would be helpful for the understanding. Additional information containing the experimental and analysis is also added and the results are compared.

Some minor concerns that the authors may want to pay attention to is:

(1) The resolution after binning is 15 micron/pixel and this is not the real resolution. The real resolution is 30-45 micron depending on conditions. So, the authors may want to modify the description related to resolution.

[The initial pixel size was 7.5 micron and 15 micron after binning. Even if some image treatments such as filtering may probably modify this resolution, their effects are rarely quantified. Thus, we prefer to keep in the manuscript these values, which are commonly used and more reliable.](#)

(2) The scale bar is better to add in the Fig. 5b.

[The Figure 5b has been modified accordingly in the revised version of the manuscript.](#)

(3) the size of scale bar should be noted in fig. 8.

[The Figure 8 has been modified accordingly in the revised version of the manuscript.](#)

Highlights

- First study of ice crystal formation and growth in a sponge cake during freezing and storage.
- In-situ freezing and thermal cycling were performed inside a laboratory X-ray μ CT apparatus.
- Dynamic X-ray μ CT and Digital Volumetric Correlation were implemented to quantify ice crystals.
- Ice crystals were located at the pore surface and their size increased by recrystallization due to the thermal cycling.

1 X-ray microtomography of ice crystal formation and
2 growth in a sponge cake during its freezing and storage

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11 **Abstract**

X-ray tomography has been used to characterize microstructural changes taking place into sponge cake upon freezing and subsequent storage at fluctuating temperatures. The CellDyM cooling cell, initially developed for snow, was used to subject a sponge cake sample to freezing and thermal cycling directly inside the X-ray tomograph. 3D images of the same sample have been scanned at different times during 6 days. An original image processing based on Digital Volumetric Correlation (DVC) has been implemented in order to segment the images and then to quantify porosity changes and ice crystal formation and growth. Overall, the obtained results have shown that (i) all the ice crystals are located at the pore surface and no crystal was found inside the sponge cake crumb matrix and (ii) during the thermal cycling, the size of the ice crystals linearly increases while the number of ice crystals tends towards a plateau. These tendencies can be related to a recrystallization phenomenon occurring due to thermal cycling. More generally, the proposed methodology could be applied to other frozen porous products during freezing and frozen storage.

12 **1. Introduction**

13 Freezing technologies are widely used by the pastry industry in order
14 to preserve the products and to extend their shelf life. This is particularly
15 the case for sponge cake, which is the base of several pastries and one of
16 the most common frozen cake. It is usually produced in large quantities at
17 industrial scale or in pastries following a two stage process: (i) at first, the
18 product is baked and frozen in a factory; (ii) then, after a storage period, it
19 is thawed, prepared and garnished just before the sale in retail stores or in
20 artisanal pastries. This process makes it possible to produce large quantities
21 and to distribute the product over an extended period to distant locations.
22 However, the quality of the thawed sponge cake can be deteriorated by
23 undesirable physico-chemical changes taking place during freezing and frozen
24 storage, resulting in a reduction of consumer acceptance and in economic
25 losses for the industry (Owen and Van Duyn, 1950).

26 It is established that these changes are closely related to ice crystals
27 which are formed during the freezing process and which evolve during the
28 frozen storage due to recrystallization (Le-Bail et al., 2011). In food freezing,
29 heat transfer is always coupled with mass transfer. A temperature gradi-
30 ent appears between the cold surface and the core inducing a conductive
31 heat transfer. Concomitantly, a water vapour pressure gradient between
32 the surrounding air and the food surface occurs leading to a diffusive water
33 transfer from the core to the surface and thus to water evaporation. During
34 the freezing of porous foods at atmospheric pressure, this vapour diffusion is
35 also present in the pores and is the main mass transfer mechanism (Pham,
36 2006). When the freezing point is reached, the liquid water is converted

37 into ice. Inside the pores, the supersaturated vapour also condenses into
38 ice. This mechanism is called evapo-condensation. After freezing, ice re-
39 crystallization occurs during storage. It involves metamorphic changes of
40 ice crystals to minimize the surface free energy of the entire crystal phase.
41 Small ice crystals, which have a large excess of surface free energy and there-
42 fore are thermodynamically unstable tend to lower their energy level in favor
43 of large ice crystals. The net result is the disappearance of small crystals by
44 melting or sublimation and the growing of large crystals leading to the de-
45 crease in crystal number and the increase of the average ice crystal size. Ice
46 recrystallization may occur according to several mechanisms such as isomass
47 rounding, accretion or migratory (also called Ostwald ripening) (Fennema,
48 1973; Cook and Hartel, 2010). Among these mechanisms, Ostwald ripening
49 which involves a melting-diffusion-refreezing process is the most prominent
50 in frozen foods and in case of temperature cycling (Hartel, 1998; Ndoye and
51 Alvarez, 2014; van Westen and Groot, 2018). This phenomenon that in-
52 volves changes in number, size, shape and location of the ice crystals occurs
53 at constant temperatures, but is accelerated by fluctuating temperatures
54 that are often inevitable through the cold chain due to the on/off opera-
55 tion of the refrigerating equipment or to poor practices (temperature abuse)
56 (Zaritzky, 2010).

57 Many efforts have been focused on the relationships between the struc-
58 tural quality of frozen/thawed baked products and the re-arrangement of
59 both the starch granules and the gluten network that may be impaired by
60 ice crystals formation and growth. Most of these studies concerned bread
61 (Bárcenas et al., 2003; Hamdami et al., 2004; Bárcenas and Rosell, 2006;
62 Mandala et al., 2008, 2009; Eckardt et al., 2013). A few were related to
63 cakes (Gómez et al., 2011; Karaoğlu et al., 2008) and to sponge cake (Díaz-

64 Ramírez et al., 2016), the composition of which is different from that of
65 bread and would therefore be likely to react to freezing and frozen storage
66 differently. In opposition, the mechanisms by which ice crystals evolving at
67 local scale interact with the development of the structure of frozen/thawed
68 baked product are scarcely described and thus not fully elucidated. The
69 rare studies that exist also concern bread, especially part-baked bread (Lu-
70 cas et al., 2005a,b; Chen et al., 2012, 2013), but to our knowledge, no study
71 has been carried out about sponge cake. This lack of work can be explained
72 by the complexity of the experimental evaluation of the local dynamics of
73 water and ice during freezing of such porous media. Nuclear magnetic reso-
74 nance imaging (MRI) was used to locally assess physical changes that take
75 place during the pre-chilling and freezing of par-baked bread (Lucas et al.,
76 2005b). The methodology enabled to perform a continuous and non-invasive
77 monitoring of the process, but the MRI signal was not detectable after the
78 beginning of the crystallization. Moreover MRI signal analysis do not pro-
79 vide the ice crystal size and distribution. More recently, Chen et al. (2012)
80 used differential scanning calorimetry (DSC) to investigate the effect of freez-
81 ing rates on the formation and distribution of ice crystals upon the freezing
82 of fresh breadcrumb. The authors have estimated the mean ice crystals
83 sizes in small and large crumb pores (with sizes ranging from nanometre to
84 millimetre) using the endotherm curve obtained by DSC and the modified
85 Gibbs-Thomson equation. They report smaller ice crystals in small crumb
86 pores. But both ice crystals in small and large crumb pores have sizes in
87 the nanometer range (5 - 18 nm), far below the size of bulk ice which should
88 be in the micrometer range or even larger. They argued that the DSC pro-
89 cedure (isothermal stage at -30 °C for 10 min.) used in their work did not
90 afford enough time to make possible water migration and, consequently, the

91 growth of ice crystals. Indeed, the formation and growth of ice crystals are
92 highly correlated with the water distribution and migration that may be
93 constrained by the starch/gluten network and the crumb porosity.

94 From the above literature review, it is obvious that the methodologies
95 used to characterize the dynamics of ice crystals formation and growth dur-
96 ing freezing of porous food such as bread do not provide a satisfactory quan-
97 titative assessment. More reliable techniques are still needed to improve
98 the understanding of the complex mechanisms taking place at this micro-
99 scopic scale, particularly into such porous frozen foods. The potential of
100 X-ray micro-computed tomography (X-ray μ CT) as a reliable methodology
101 to visualize and quantify the 3D microstructure of frozen foods with a non-
102 destructive approach was recently showed (Vicent et al., 2017). X-ray μ CT
103 generates 3D images reconstructed from multiple radiographs acquired from
104 different angles around a sample. X-ray μ CT images reflect the sample with
105 different grey level depending on the X-ray attenuation properties of the dif-
106 ferent components. Since commercial μ CT devices are adapted for ambient
107 temperature imaging and not equipped with thermostated stage, specific
108 tools have been developed to maintain the samples at frozen state during
109 imaging. Vicent et al. (2017) developed a new X-ray μ CT based methodol-
110 ogy that enables to image frozen foods at the frozen state ($-20\text{ }^{\circ}\text{C}$), thanks
111 to a cooling stage made with phase change material. This methodology pro-
112 vided valuable insights into the microstructural changes in frozen minced
113 beef as affected by the freezing rate (Mulot et al., 2019), in frozen carrots
114 upon storage with fluctuating temperature (Vicent et al., 2019) and in fresh
115 ice sorbet after freezing (Masselot et al., 2021). However, the cooling stage
116 used in these works does not allow analysing the frozen sample more than
117 20 minutes, thus making in-situ monitoring of the water/ice crystals dy-

118 namics during an extended period impossible. Moreover, it does not allow
119 dynamic monitoring of temperature in order to perform thermal cycling that
120 may mimic temperature fluctuations occurring through the cold chain. Guo
121 et al. (2018) successfully applied in situ synchrotron X-ray μ CT to inves-
122 tigate dynamic microstructural changes of ice cream subjected to thermal
123 cycling, using a cooling stage specially designed to operate between ambient
124 temperatures to -40 °C. A similar cooling stage was used by Calonne et al.
125 (2015) in a laboratory scale X-ray μ CT apparatus to dynamically monitor
126 the metamorphism of snow. The advantage of this cooling stage, named Cell
127 for Dynamic Monitoring (CellDyM), is to allow precise setting of the tem-
128 perature at the top and the bottom of the sample using Peltier coolers. An
129 aluminium sample holder was used allowing a vertical heat conduction and a
130 subsequent uniform temperature within the sample. But all overmentioned
131 studies concerned non porous foods or systems containing a high water frac-
132 tion and with ice crystals already formed. In the specific case of sponge cake,
133 the water content is low and is trapped in a porous starch/gluten matrix.
134 Following the formation and growth of ice crystals, as well as the evolution
135 of pore structure into such a complex microstructure is really challenging.

136 The principal purpose of the present study was to apply a dynamic X-
137 ray μ CT based methodology to assess the local physical changes taking
138 place into sponge cake upon freezing and subsequent storage at fluctuating
139 temperatures. To this end, the CellDyM cooling stage developed by Calonne
140 et al. (2015) for snow was used to subject sponge cake samples to freezing
141 and thermal cycling directly inside the X-ray μ CT apparatus. Scans were
142 performed at different times during 6 days-period of analysis. An image
143 analysis strategy based on Digital Volumetric Correlation (DVC) (Buljac
144 et al., 2018) was implemented to quantify porosity changes and ice crystals

145 formation and evolution. Cryo-SEM analysis was also performed to obtain
146 high resolution imaging and to qualitatively compare the microstructure
147 obtained with cryo-SEM and μ CT image visualization.

148 **2. Material and methods**

149 A specific sponge cake formulation and preparation protocol was defined
150 and optimized (see section 2.1) to ensure reproducible cakes in terms of
151 thermophysical properties (density, water content and freezing point) (see
152 section 2.2).

153 *2.1. Sponge cake preparation and sampling*

154 Sponge cakes were home made using the following formulation: 25 %
155 (w/w) wheat flour (farine de blé, Tarwebloem T55, Germany) , 25 % (w/w)
156 sucrose (powdered sugar, Daddy, France), and eggs (Carrefour Bio, France):
157 30 % (w/w) egg white, 15 % (w/w) egg yolk, and 5% (w/w) of pure water.
158 These ingredients were purchased from local supermarket.

159 The batter was prepared using a kitchen food processor (Masterchef
160 Gourmet QA510110, Moulinex, France) into two steps:

- 161 1. First, egg yolk was homogenized for 10 s, then sugar was incorporated
162 and blended for 10 min 12 s , afterwards sifted white flour was gradu-
163 ally poured over 2 min 12 s and finally water was added and blended
164 for 1 min. All these blending operations were performed at maximal
165 speed rotation of the kitchen food processor. The mixture is then left;
- 166 2. Egg whites got beaten separately using the same food processor at high
167 speed. Finally, the beaten egg whites were gradually incorporated into
168 the first mixture using a flat beater at low speed. Next, a batch of nine
169 pieces of sponge cake was prepared by pouring 60 g of batter in each

170 rectangular aluminium pan (10×6×3 cm) and baked in an electrical
171 oven (Arianna LED Bakerlux, Unox) for 20 minutes at 170 °C.

172 After baking, the nine sponge cakes were allowed to cool at ambient temper-
173 ature for 45 minutes (ambient relative humidity RH=50-60%). They were
174 then individually packaged in impermeable plastic bags and sealed before
175 storage in a fridge at 5 °C (for about 7-10 days). In order to make sure
176 that all samples have similar thermal history during baking and cooling,
177 preliminary tests were performed using temperature sensors to record cakes
178 temperatures. Among the nine sponge cakes prepared for this study, eight
179 were used for DSC, density and water content measurements. According
180 to the reproducibility of these measurements on the sponge cakes (see sec-
181 tion 2.2), it was reasonably assumed that the latter one, which was used for
182 X-ray tomography analysis, presents similar properties.

183 For X-ray μ CT analysis, a sample (diameter = 9.8 mm and height = 10
184 mm) was extracted from the cake crumb in a cold room (-5 °C, RH=20 %)
185 using a core drill mounted on a milling machine. The sample was transported
186 in a sealed container at -5 °C before its installation in the cooling stage
187 CellDyM, which ensures the needed thermo-hygroscopic stability (Figure
188 2).

189 *2.2. Characterization of thermo-physical properties of the sponge cake*

190 *2.2.1. DSC measurements*

191 The freezing point and the freezable water content were calculated using
192 differential scanning calorimeter (DSC 1 STARe System, Mettler-Toledo,
193 Columbus, USA). The heat flows and temperature were measured on three
194 replicates with an accuracy of 0.02 μ W and 0.02 °C respectively. For each
195 analysis, a sponge cake sample of approximately 9 mg was placed in an

196 aluminium pan and hermetically sealed. For each scan, the sample was
197 first equilibrated at 25 °C for 10 min, then cooled to -40 °C at 10 °C/min,
198 then after an isothermal holding stage at -40 °C for 10 min, the sample was
199 heated back to 25 °C at 5 °C/min. The onset temperature (T_o) and the peak
200 temperature (T_p) were determined from the endothermic ice melting peak
201 using the STARe Evaluation software (Mettler-Toledo, Columbus, USA). T_o
202 represents the freezing point. It is defined as the intersection point between
203 the baseline and the inflexion tangent at the beginning of the melting peak.
204 A typical thermogram of melting is shown in Figure 1). It shows that ice
205 melting started when the temperature reached -30 °C ; the endothermic ice
206 melting peak was obtained at around -10 °C.

207 T_o measured from the ice melting endotherm was about -21 °C for sponge
208 cake samples. Based on these DSC results, the thermal cycling during the
209 microtomographic analysis was set between -30 °C and -20 °C , correspond-
210 ing to the temperature range of the phase change into the sponge cake.
211 This temperature range mimics the thermal fluctuations encountered dur-
212 ing frozen storage.

213 2.2.2. Density

214 The theoretical density of batter (in kg/m³) was first determined by
215 considering the proportions and densities of each ingredient. The density
216 of wheat flour, sucrose, egg white and egg yolk were measured by weigh-
217 ing (Mettler AE 163, precision 10⁻⁴ g) 10 mL of each ingredient using a
218 measuring cylinder (three replicates). The theoretical density of batter ob-
219 tained was then 941 ±3 kg/m³. Then, the density of batter was measured
220 by weighing 100 mL of batter (two replicates) in a measuring cylinder. The
221 measured average density of batter was equal to 700 ± 2 kg/m³. In fact, air

222 was incorporated in the batter during whipping explaining this lower value
223 of density.

224 The apparent density of the sponge cake was calculated by weighing
225 (about 19 g) a parallelepiped-shaped ($5.5 \times 5.1 \times 2.5 \text{ cm}^3$) sample previously
226 cut in the sponge cake crumb. Four replicates were measured. In order to
227 determine the density of the sponge cake without air (real density), the same
228 samples were compacted using a vacuum sealing system (Foodsaver, V2860)
229 to remove the air from the pores, before measuring the new dimensions
230 for calculating the new volume. The mean apparent density and the real
231 density of sponge cake were equal to $405 \pm 18 \text{ kg/m}^3$ and $684 \pm 29 \text{ kg/m}^3$
232 respectively. The real density is close to the density of batter (700 ± 2
233 kg/m^3) but lower than the theoretical density of batter ($941 \pm 3 \text{ kg/m}^3$).
234 Even after compaction, a fraction of air seems to be still present and trapped
235 in the cake. The amount of air present inside the sponge cake was then
236 calculated considering the ratio between the apparent density (405 ± 18
237 kg/m^3) and the batter theoretical density ($941 \pm 3 \text{ kg/m}^3$). The value of
238 the mean porosity of the sponge cake equals $57 \pm 2 \%$.

239 2.2.3. Water content

240 Five samples ($6 \times 5.8 \times 2.2 \text{ cm}^3$) from different sponge cakes were cut and
241 weighed (about 24 g). Then the water content of the samples was determined
242 by weighing (Mettler AE 163, precision 10–4 g) before and after complete
243 dehydration in an oven (Themarks TS4057) set at 105 °C for 24 hours.

244 Water content is calculated as follow:

$$X_w = \frac{m_0 - m_f}{m_0} * 100 \quad (1)$$

245 where X_w is the water content of the sponge cake sample, m_0 is the mass
246 (kg) of the wet sample and m_f is the mass (kg) of the dried sample. The

247 average water content was calculated from five replicates and was equal to
248 $30.50 \pm 1.7 \%$.

249 *2.3. Cryo-SEM Analysis*

250 Cryo-SEM measurements were carried out in an external laboratory
251 equipped with scanning electron microscopy (Electron Microscopy Facility,
252 IBPS, Paris, France), in order to compare imaging results to those obtained
253 with the microtomographic method. Both previously frozen and unfrozen
254 samples were analyzed. Fracture was performed in liquid nitrogen. Frac-
255 tured surfaces were observed using cryo-SEM (GeminiSEM 500, Zeiss) at
256 $-120 \text{ }^\circ\text{C}$, the pressure in the equipment was $1.6 \times 10^{-4} \text{ Pa}$. The accelerating
257 voltage was 3.00 kV or 0.790 kV and the accessible magnification range was
258 from x13 to x500 000. Frozen samples were first observed at $-120 \text{ }^\circ\text{C}$ then
259 after partial sublimation at $-90 \text{ }^\circ\text{C}$ for 1 min.

260 *2.4. In situ X-ray micro-tomography*

261 *2.4.1. Cryogenic cell*

262 CellDyM is a cryogenic cell that allows the sample to be monitored
263 by time-lapse X-ray μCT with a scanner operating at room temperature
264 (Calonne et al., 2015). The geometrical arrangement of the cell enables a
265 360° rotation during a tomographic acquisition (Figure 2). CellDyM gives
266 the possibility to precisely impose the temperature (about $+40$ to $-40 \text{ }^\circ\text{C}$)
267 at the top and bottom of the sample (minimal thermal gradient lower than
268 $3 \text{ }^\circ\text{C}/\text{m}$) with copper cylinders while its lateral sides are thermally insulated
269 from the outside using a vacuum chamber. The sample holder mechanically
270 links the two copper cylinders and is in contact with the lateral sides of the
271 sample. This key part can be made of poly methyl methacrylate (PMMA),
272 whose advantage is to have low X-ray linear absorption and then maximise

273 the images contrast of low X-rays absorption samples. However, it has the
274 drawbacks not to fully prevent from thermal radiative exchange with the
275 outside and it is not a good thermal conductor. Alternatively, it can be made
276 of aluminium, which allows a rather good thermal conductivity with the top
277 and bottom copper cylinders and prevents thermal radiative transfers. In
278 this case, the higher linear X-ray attenuation impinges the contrast within
279 the sponge cake sample. After some trials, the aluminium sample holder
280 appeared as the best compromise for this experiment.

281 *2.4.2. Freezing and thermal cycling*

282 Figure 3 shows the temperature applied to the sample with respect to the
283 time, where the dots represent when the X-ray μ CT scans (noted Si) were
284 performed. The scans were acquired at least 30 minutes after the thermal
285 loading to prevent the blurring of the 3D images due to the induced sample
286 movement during acquisition. Then, after two scans (S0 and S1) in the
287 initial state at 5 °C , the sample was frozen at -30 °C with a cooling rate of
288 about -5 °C min⁻¹ (S2). It was then warmed up at -20 °C and cooled at -30
289 °C 7 times at the same temperature rate (+ or - 5 °C min⁻¹). These values
290 were measured at the cooling modules and not directly within the sample,
291 consequently the temperatures and the cooling rate may be slightly lower.
292 These temperature oscillations were simulating the possible temperature
293 fluctuation during frozen food handling and storage. The whole experiment
294 took about 6 days and all the 37 scans, hereafter noted Si with $i \in [0, 37]$,
295 were not equally spaced in time (Figure 3) due to experimental and practical
296 reasons.

297 *2.4.3. 3D image acquisition*

298 The sample was placed in CellDyM cell, which was previously installed
299 in the tomograph cabin (RX Solutions, France) and the temperature was
300 set to 5 °C. The system was operating at a X-ray tube voltage of 65 kV
301 and a current intensity of 115 μA . A voxel size of 7.5 μm was set according
302 to the dimensions of the cryogenic cell (Figure 2). The total scanning time
303 was set at 2 hours 10 min (1440 radiographies on a 360° rotation) for one
304 acquisition. These parameters were chosen as the best compromise between
305 image quality (air/ice/cake contrast) and scan duration that prevents possi-
306 ble sample evolution during the acquisition. XAct 2 software (RX Solutions,
307 France) was used to reconstruct the 3D volumes using the classical filtered
308 back-projection algorithm and applying ring artefact filter.

309 *2.4.4. Image processing*

310 The 3D image processing involved several operations summarised in Fig-
311 ure 4:

312 *Registration.* All the 3D images (i.e. 37 in total), were registered in order to
313 define and follow the same volume of analysis inside the aluminium sample
314 holder between the two copper cylinders (Figure 5 (a)). The registration
315 was done by setting vertical the axis of the aluminium cylinder.

316 *Crop.* All the 3D images (i.e. 37 in total), were cropped to exclude the cop-
317 per cylinders absorption artefacts (Figure 5 (a)). Consequently, the height
318 and the volume of the analyzed sample were $H = 7.9$ mm and $V_0 = 593$
319 mm^3 .

320 *Binning.* A $2 \times 2 \times 2$ binning was applied to all the 3D images leading to a
321 voxel size of 15 μm and reducing the overall number of voxels by 8 to optimize

322 subsequent images processing, such as the digital volume correlation step.

323 *Air segmentation.* The air phase was segmented using a global grey level
324 threshold. As shown by the grey levels histogram (Figure 5 (c)), the seg-
325 mentation of air, matrix and ice was not trivial. However, the grey levels
326 of the air phase are fairly well described by a Gaussian distribution and are
327 expected to be constant during the experiment (no density change). The
328 grey level threshold was chosen such that, statistically, the major part of
329 the voxels belonging to the air phase are selected. It corresponds to the
330 right part of the Gaussian curve fitted on the air grey levels (Figure 5 (c)).
331 If the cake matrix grey levels are described by a Gaussian distribution, the
332 total grey level histogram still exhibits a non negligible number of voxels
333 with intermediate grey levels. These voxels can at least be related to both
334 the ice crystals (Figure 5 (b)) and the partial volume effects of the matrix,
335 *i.e.* the voxel that are at the interface of the cake matrix and the air have
336 intermediate grey levels which is a well known artefact (Glover and Pelc,
337 1980). Even if it was possible to visually distinguish the ice crystals, an ac-
338 ceptable segmentation was not achievable for two reasons: (i) the poor grey
339 level contrast between the matrix and ice, and (ii) some similar morpholog-
340 ical characteristics of the ice and matrix (small and narrow). Subsequently,
341 Digital Volumetric Correlation (DVC) has been implemented to detect the
342 ice crystals in the 3D images.

343 *Digital Volume Correlation (DVC).* This technique is widely used in me-
344 chanics of materials to measure the deformations of a sample that has been
345 mechanically loaded (Lenoir et al., 2007; Roux et al., 2008; Hild et al., 2011;
346 Pierron et al., 2013), but also to detect cracks (Limodin et al., 2011; Lep-
347 lay et al., 2013; Wang et al., 2016). According to Buljac et al. (2018), the

348 principle of DVC is the following: suppose two 3D images denoted as $f(\mathbf{x})$
 349 (assumed to be the *reference* image) and $g(\mathbf{x})$ (the *deformed* image), where
 350 f and g are the grey levels at each voxel location \mathbf{x} . For the two 3D images to
 351 be correlated, DVC rests on the basic assumption that upon a mere change
 352 in the position of voxels, defined by a displacement field $\mathbf{u}(\mathbf{x})$, the two im-
 353 ages can be brought to perfect coincidence such that $g(\mathbf{x} + \mathbf{u}(\mathbf{x})) = f(\mathbf{x})$.
 354 Although this equation appears trivial at first glance, its resolution is not,
 355 and presents a number of common issues discussed in Buljac et al. (2018).
 356 In the present work, the YaDICs software (Seghir et al., 2014) with a Fi-
 357 nite Element Regularized DVC method was used to correlate the 3D images
 358 acquired during the experiment.

359 In the following, the S3 image (at -30°C) was used as the reference 3D
 360 image and was then correlated to the other 3D images (S4 to S36). It was
 361 not possible to use S0, S1, and S2 as reference images for two reasons: (i)
 362 the sample deformation between S1 (or S0) and S2, *i.e.* when the sample
 363 was frozen (Figure 3), was too important and thus DVC did not work, and
 364 (ii) the S2 image was a bit blurred due to sample evolution during the
 365 acquisition.

366 *Ice segmentation.* Besides the three components of the displacement vector
 367 $\mathbf{u}(\mathbf{x})$, the software provides the residual map of the correlation $r(\mathbf{x}) = g(\mathbf{x}) -$
 368 $f(\mathbf{x})$ (Figure 5 (d)). On this residual map, a grey level $r(\mathbf{x}) = 0$ indicates
 369 that the correlation is perfect, a grey level $r(\mathbf{x}) > 0$ represents a part of the
 370 image that has disappeared, *e.g.* bright voxels that became darker like when
 371 a crack occurs. Lastly, a grey level $r(\mathbf{x}) < 0$ represents a part of the image
 372 that has appeared, *i.e.* dark voxels that became brighter, or in the present
 373 case, when ice crystals appear in pores of the matrix. The obtained residual

374 maps mainly exhibits a good average correlation between the images with

$$\langle r(\mathbf{x}) \rangle = 1/V \int r(\mathbf{x}) dV \approx 0 \quad (2)$$

375 as shown by the grey level histogram plotted in Figure 5 (f). Ice crystals
376 are clearly visible on the 3D residual maps (in black in Figure 5 (d) and
377 (e)) and in the histogram (Figure 5 (f)) represented with grey values below
378 -10.0. Indeed, the offset between the Gaussian fit that models the correlation
379 residue of the random noise (which is function of the acquisition, the image's
380 reconstruction and the data processing), and the $r(\mathbf{x})$ data highlights that
381 objects have appeared during the experiment. Consequently, the obtained
382 3D residue images have been used to segment the ice phase using a global
383 grey level threshold. The threshold level was set such that no voxel belonging
384 to the correlation error is taken into the ice phase. The ice phase was thus
385 segmented as all the voxels with grey values lower than -10.0 (Figure 5 (f))
386 and errors were estimated using two more threshold values (-12.0 and -9.0).

387 *Matrix segmentation.* The matrix (cake) phase was segmented as the re-
388 maining volume.

389 This image processing leads to 3D images where the 3 phases of the
390 sample are labelled, *i.e.* 3D images with artificial grey levels that represent
391 each phase, here $f(\mathbf{x}) = 0$ outside the region of interest (ROI), $f(\mathbf{x}) = 1$ for
392 air, $f(\mathbf{x}) = 2$ for ice and $f(\mathbf{x}) = 3$ for the remaining voxels that belong to
393 the matrix phase (respectively in white for air, yellow for matrix and blue
394 for ice in Figure 5 (g) and (h)).

395 2.4.5. Microstructural parameters

396 Several microstructural parameters have been computed from the 3D
397 labelled images in order to characterize the evolution of the microstructure
398 induced by the thermal loading:

400 *Porosity / ice volume fraction / matrix volume fraction.* These three pa-
 401 rameters were computed using a standard voxel counting algorithm. The
 402 porosity, the ice volume fraction, the matrix volume fraction are defined as
 403 V_{air}/V_0 , V_{ice}/V_0 and V_{matrix}/V_0 respectively, where V_{air} , V_{ice} and V_{matrix} are
 404 the volume of each phase and V_0 is the initial volume of the sample (Figure
 405 6). All these quantities are computed using the initial volume of the sample
 406 V_0 as reference (Lagrangian quantities). As will be shown later, the sample
 407 volumes change during the experiment due to the matrix shrinkage (Section
 408 3.4), so the current volume of the sample is smaller than the initial one.

409 *Specific surface areas.* The surface areas of the interfaces, $S_{\text{matrix/air}}$, $S_{\text{ice/matrix}}$,
 410 $S_{\text{ice/air}}$ have been computed using the MorphoLibJ tools (Legland et al.,
 411 2016) on the labelled 3D images. It was then possible to estimate the cor-
 412 responding Specific Surface Areas (SSA) defined as $\text{SSA} = S(\text{interface})/V_0$
 413 (Figure 6) and expressed in mm^{-1} .

414 *Ice crystals and pore granulometry.* Using a typical connected component
 415 analysis, performed with the MorphoLibJ library (Legland et al., 2016), it
 416 was possible to compute the granulometry of the ice crystals, *i.e.* to count
 417 the ice crystals N^{ice} in the 3D image and to compute their characteristic
 418 dimension defined here as the equivalent diameter of an ice crystal (the
 419 diameter of a sphere with the same volume as the particle):

$$d_{\text{eq}}^{\text{ice}} = 2\sqrt[3]{(3/4\pi)V^{\text{ice}}}, \quad (3)$$

420 with V^{ice} its volume. According to the resolution after the binning opera-
 421 tion, the critical size of the ice crystals which can be measured is 15 μm . It

422 was also possible to compute the pore granulometry. Even if the pores are
423 connected, the porous network was artificially divided into individual pores
424 using the 3D watershed algorithm of the MorphoLibJ library (Legland et al.,
425 2016). An additional borderkill algorithm (Legland et al., 2016) was applied
426 to remove the pores in contact with the aluminium sample holder. Then, as
427 for the ice crystals, it was possible to count the number of pores N^{pore} and
428 to compute their equivalent diameter $d_{\text{eq}}^{\text{pore}}$.

429 **3. Results and discussion**

430 *3.1. Microstructural observations*

431 Cryo-SEM was used to visualize the microstructure of model sponge
432 cake after freezing. Figure 7 shows a micrographs of a frozen sample at
433 magnification of x500. The formation of ice crystals can be observed; they
434 are partially embedded into the wall and protrude into the pores. The
435 typical size of such crystals is about 20 μm . Even if there are few crystals
436 which are a little bit smaller, the used resolution for the X-Ray 3D images
437 after binning (15 μm) is sufficient to capture most of the crystals.

438 Figure 8 presents vertical slices of some 3D images acquired during the
439 experiment. The global evolution of the sample can be observed, as well
440 as the local one through the insets in the surroundings of a pore of char-
441 acteristic dimensions of $1.2 \times 0.8 \text{ mm}^2$. The sponge cake exhibits a highly
442 porous microstructure: (i) the air volume fraction is about 79 % and (ii) the
443 connected pore network presents a large variety of pore sizes. This mean
444 porosity is higher than the one initially determined by density measurements
445 (57%) and described in section 2.2.2. These differences can be mainly ex-
446 plained by the heterogeneity of the sponge cake, the presence of large pores

447 and the volume of the sample that was nearly 80 times higher in the case of
448 density measurements.

449 Overall, the sample seems not to undergo many modifications during the
450 experiment, except at the beginning during the freezing stage. Indeed, from
451 S0 (T=5 °C, t=0 h) and S3 (T=-30 °C, t=8.8 h), an important shrinkage
452 of the sample can be observed as shown by the black arrows on the S3
453 slice (Figure 8). In the initial state S0, the sample is in contact with the
454 aluminium sample holder (Figure 8) while in S3 there is an average air gap
455 of 0.25 mm between the aluminium holder and the sample. Then, this gap
456 slightly decreases during each warming-up. At the end of the experiment
457 (S36), this gap is at an average of around 0.3 mm.

458 At the pore scale, small crystals (of about 50 μm or less) mainly ap-
459 pear and grow at the matrix-air interface (red arrows in Figure 8), which is
460 consistent with Cryo-SEM observations (Figure 7). These particles have a
461 grey level intensity, *i.e.* a linear X-ray attenuation coefficient below the one
462 of the matrix (about 10% less). The number and the size of these crystals
463 depend on the freezing time and the temperature. During the warming-up
464 stage, some ice crystals can totally or partially disappear (see red arrows in
465 S18-S22 and S27-S31, Figure 8).

466 3.2. Porosity and pore sizes

467 As already mentionned, the sponge cake sample exhibit a highly porous
468 microstructure and about 99% of the air volume was found to be connected,
469 *i.e.* the volume of pores trapped in the matrix was less than 1% of the total
470 air volume in the sample.

471 The pores size and distribution computed from the 3D images are pre-
472 sented in Figure 9. Figure 9 (a) shows that most of the pores are within

473 the range 0.1 to 0.9 mm. The number of pores with $d_{\text{eq}}^{\text{pore}} < 0.4$ mm slightly
 474 decreases during the experiment. No pore with $d_{\text{eq}}^{\text{pore}} < 0.1$ mm is detected
 475 for the S36 scan. This can be explained by the shrinkage of the sample
 476 that decreases the pores size and makes the smallest ones not detectable
 477 by the experimental set-up. This is not an image processing artefact since
 478 $d_{\text{eq}}^{\text{pore}} = 0.1$ mm is much larger than the image voxel size (0.015 mm). On
 479 the other hand, the characterization of the largest pores (typically with
 480 $d_{\text{eq}}^{\text{pore}} > 1.0$ mm) may undergo some image processing issues. Some of the
 481 biggest pores might be over-splitting while others are under-splitting by the
 482 3D watershed algorithm. Despite such image processing issues, the 3D im-
 483 ages clearly show that the sponge cake matrix presents some large pores
 484 whose diameters are within the range 1 – 5 mm.

485 The number of pores is initially about 5000 (Figure 9 (b)), and their
 486 mean equivalent diameter is about 0.4 mm (Figure 9 (c)). During the ex-
 487 periment, the number of pores decreases while the mean equivalent diameter
 488 increase. These evolutions are mainly related to the shrinkage of the matrix
 489 during the experiment. Indeed, as already underlined, due to the shrinkage,
 490 the smallest pores with a diameter below 0.1 mm may disappear or become
 491 too small to be detectable. Consequently, the number of pores decreases
 492 and the mean equivalent pore size increases.

493 *3.3. Sample shrinkage*

494 Figure 10 presents the evolution of sample volume during the experiment
 495 divided by its initial volume. The shaded area represents the estimated
 496 errors obtained with different grey level thresholds (see Section 2.4.4). This
 497 figure shows that during the first freezing stage (from S0 (T=5 °C, t=0
 498 h) to S3 (T=-30 °C, t=8.8 h), the shrinkage of the sample is about 4%.

499 After 32 hours at $-30\text{ }^{\circ}\text{C}$ (S11), this shrinkage attains nearly 6%. During
500 the seven warming-up/cooling cycles, this shrinkage is almost stable and
501 oscillates between 5% and 8%. The shrinkage phenomena is discussed in the
502 section 3.7.3.

503 *3.4. Evolution of the volume fraction of each phase*

504 The vertical profiles of the 3 phases' volume fractions are plotted in
505 Figure 11. These profiles show that the vertical distribution of the three
506 phases is not perfectly homogeneous. The variations are typically around
507 10% for the matrix and air volume fractions (Figure 11 (a) and (b)). De-
508 spite such heterogeneities, Figure 11 shows that the shape of these profiles
509 are quite similar during the experiment, indicating an homogeneous phe-
510 nomenon along the height of the sample. On the other hand, the ice volume
511 fraction profiles plotted in Figure 11 (c) show that the ice content increases
512 during the experiment and that this phenomenon is also rather homogeneous
513 in the sample.

514 Figure 12 shows the evolution of the volume fractions of the 3 phases
515 during the whole experiment. Both matrix and air volume fraction are
516 mainly decreasing, respectively at a rate of $-8.5\ 10^{-5}\ \text{h}^{-1}$ and $-8.6\ 10^{-5}\ \text{h}^{-1}$,
517 as shown by the linear regressions plotted on Figure 12 (a) and (b). By
518 contrast, the volume fraction of ice increases and reaches a final value of
519 about 0.024 ± 0.006 at the end of the experiment. All these evolutions are
520 mainly related to the shrinkage process and the crystals growth. During the
521 freezing stage, the water initially present in small pores of the matrix has
522 migrated in the larger pores network and then has been transformed in ice
523 crystals. This leads to a decrease of the volume of the matrix, but also of
524 the volume of the pores due to both the disappearance of the smallest pores

525 within the matrix and the crystals growth within the large pores.

526 Finally, the ice volume fraction was computed and presented in Figure
527 12 (c). Using S3 scan as a reference, this measurement does not take into
528 account the ice initially present in S3 due to the freezing. However, the
529 Figure 12 (c) shows that after 8 hours (S4), the ice volume fraction is very
530 low and is about 0.02%. As a consequence, it seems reasonable to suppose
531 that the ice volume fraction that might be present in the reference scan S3,
532 made 2 hours after freezing might be as low as 0.005%. Consequently, the
533 error that was made by considering the S3 scan as a reference is negligible.

534 3.5. Ice crystals properties

535 Figure 13 presents 3D rendering of the sponge cake after segmentation
536 at different time of the experiment. Within the chosen 3D images, the first
537 ice crystals (in blue) are visible for S11. They cover the matrix surface fairly
538 evenly with maybe a preference for narrow regions (small pores), and are
539 small (characteristic dimensions of 20 – 25 μm) with round shapes.

540 During the thermal cycle, the number of ice crystals increases. They
541 present different shapes and dimensions, ranging from small rounded parti-
542 cles to angular shaped crystals with a length of 0.1 mm and an aspect-ratio
543 between 3 and 4. During the warm-ups, the ice crystals size decreases glob-
544 ally. Consequently, some of the smallest crystals can disappear and the
545 number of crystals thus decreases. However it seems that some large crys-
546 tals are not affected, their size remain almost constant and even grow (see
547 S27-S31 in Figure 13).

548 The distribution of the ice crystals equivalent diameter $d_{\text{eq}}^{\text{ice}}$ is plotted in
549 Figure 14 (a). This figure shows that in the early stage of the experiment,
550 about 80% of the total ice volume is composed of ice crystals such as $d_{\text{eq}}^{\text{ice}} < 30$

551 μm . By contrast, in the last steps of the experiment, more than 95% of the
552 total ice volume is composed of particles with size higher than $30 \mu\text{m}$. The
553 maximum equivalent diameter measured is about $150 \mu\text{m}$.

554 Figures 14 (b) and (c) show that the total number of ice crystals N^{ice}
555 strongly increased up to 350,000 and that the mean ice crystal volume $\langle v^{\text{ice}} \rangle$
556 increased linearly up to $40,000 \mu\text{m}^3$. It must be mentioned that during
557 the first steps after the freezing, the granulometry of the ice crystals is less
558 accurate due to the resolution of images and the precision of the image
559 processing, *i.e.*, the smallest particles may not be detected or with a low
560 precision. Finally, as already mentioned, the number of ice crystals decreases
561 of about 15 – 20% after each warm-up cycle and the size of the crystals also
562 decreases.

563 3.6. Specific surface areas

564 Figure 15 presents the evolution of the three SSAs: $\text{SSA}_{\text{matrix/air}}$, $\text{SSA}_{\text{ice/air}}$
565 and $\text{SSA}_{\text{ice/matrix}}$. We can observe that $\text{SSA}_{\text{matrix/air}}$ is about 10 mm^{-1} at
566 the beginning and slightly decreases during the experiment. This decrease is
567 linked to the apparition of the ice crystals mainly at the interface air-matrix.
568 Consequently, the $\text{SSA}_{\text{ice/matrix}}$ increases and tends towards 1 mm^{-1} at the
569 end of the experiment, *i.e.* around 10% of the air-matrix interface is covered
570 by ice crystals.

571 Finally, the differences between the SSA values of the two first scans
572 S0 and S1 are very small. While their acquisition time was separated by 3
573 hours, these results show: (i) the stability of the sample in the experimental
574 set-up, and (ii) the ability of the image analysis to provide repeatable data.

575 *3.7. Discussion - Physical processes*

576 *3.7.1. Ice crystals location*

577 One specificity of applying X-ray μ CT to follow ice crystals formation
578 and growth is the possibility to get a mapping of ice crystals into the frozen
579 sponge cake crumb. Figures 5 (g) and (h) give a 2D representation of this ice
580 crystals mapping. A 3D rendering is shown in Figure 13. In both renderings,
581 ice crystals labelled in blue seem to be located essentially at the matrix-air
582 interface. This observation is more precisely demonstrated in a schematic
583 representation at local scale (Figure 6). All ice crystals are found to surround
584 partially the pores surface and no crystal was located inside the sponge cake
585 crumb matrix. Furthermore, the SSA evolution of ice shown in Figure 15,
586 points out that the ice-air interface is always larger than the ice-matrix
587 interface. Moreover, the SSA of matrix with air decreases inversely with
588 the increase of SSA of ice with air. But SSA of the matrix with air and
589 ice remains constant over time. This result also supports the hypothesis
590 that ice crystals are mainly formed at the periphery of the pores and grow
591 inside the pores. Similar conclusion were reported by Chen et al. (2012)
592 who studied ice crystals formation and distribution in wheat bread, using
593 DSC measurements. The DSC thermograms obtained in their study show
594 a minor ice melting endotherm that precedes a major one. The authors
595 attributed the minor endotherm to the melting of ice crystals located in
596 tiny pores and capillaries within the gluten-starch matrix. In contrast, the
597 major endotherm would be due to the melting of large ice crystals housed
598 in gas cells that form large pores. It is worth noting that the smallest pore
599 and ice crystal size observable in our study corresponds to the image voxel
600 size (15 μ m). Nevertheless, it is quite possible that even smaller pores are
601 embedded inside the matrix and that ice crystals have formed and grown

602 inside without reaching a size detectable with the method used in this study.
603 Other authors also argued that ice crystals formed upon freezing of porous
604 food materials are mainly originated from pores (Baier-Schenk et al., 2005;
605 Kontogiorgos and Goff, 2006; Kontogiorgos et al., 2008). Baier-Schenk et al.
606 (2005) imaged ice formation during freezing of bread dough and observed
607 first ice crystals formation at the pore interfaces. They concluded that gas
608 pore interfaces are preferential sites for ice nucleation thus favouring the
609 growth of ice crystals into the pores. They also admitted that small ice
610 crystals formed inside the dough matrix but are of smaller size than the ice
611 crystals inside the gas pores. The random nature of nucleation makes it
612 difficult to know the exact mechanisms involved on the surface of the pores.
613 But, the structured aspect of pores surface may promote the nucleation
614 because of irregularities that can constitute nucleation sites (Baier-Schenk
615 et al., 2005). There is no consensus about the mechanism by which ice
616 nucleation occurs at the pores surface. The most plausible mechanism would
617 be related to the coupled heat and moisture transfers that take place during
618 freezing of porous foods and known as evapo-condensation phenomena. This
619 theoretical model has been found to correctly predict the large variations
620 of thermophysical properties with temperature in porous foods (Hamdami
621 et al., 2004). In the case of sponge cake, when the product temperature is
622 cooled down, the matrix phase becomes warmer than the pores. Indeed, the
623 thermal diffusivity of the air inside the pores is much higher (nearly 10^{-5}
624 m^2/s) than the sponge cake matrix thermal diffusivity (around 10^{-8} m^2/s).
625 Water thus diffuses through the matrix and evaporates inside the pores.
626 Once the pores surface reaches the freezing point, the water vapour inside
627 the pores condenses at the pores surface, thus initiating ice formation.

628 *3.7.2. Ice crystals growth and recrystallization*

629 In-situ X-ray μ CT allows us to follow the ice crystals profile into the
630 same frozen sponge cake crumb during thermal cycling (-30 °C to -20 °C)
631 over a period of 6 days. Ice crystals size distributions over time are depicted
632 on Figure 14(a). It can be seen that the distributions are shifted to the right
633 side when aging time rises, showing an increase of ice crystals size with time.
634 First observable ice crystals were detected for S4 (about 18h at -30 °C) with
635 sizes ranging from 15 to 50 μm and 80% of the particles with sizes smaller
636 than 30 μm . For S11 (about 41h at -30 °C) which is the last scan before the
637 beginning of the thermal cycling, the ice crystals size distribution slightly
638 enlarged to a maximum size of 60 μm , but 70% of particles are still very
639 small with sizes lower than 30 μm . This result shows a very low growth
640 of ice crystals between S4 and S11. On the other hand, the number of ice
641 crystals increased 10 times during this period at -30°C as can be seen on
642 Figure 14(b). The present result may be related to the fast freezing rate
643 ($15\text{ }^\circ\text{C min}^{-1}$) that implies a high super-cooling leading to the formation of
644 numerous ice nuclei with small critical size for growth. Furthermore, the fast
645 freezing rate combined to the low temperature slows down water diffusion
646 resulting on limited growth of nuclei and small ice crystals. The increase in
647 ice crystals number could be explained assuming two mechanisms that hap-
648 pen concomitantly (Figure 16): (i) nucleation continues and new ice crystals
649 are formed, (ii) existing ice crystals grow from local freezable water in the
650 surrounding space to reach an observable size. From S12 to S36 (the last
651 scan), ice crystals size distributions continue to expand steadily reaching a
652 maximum size of 150 μm as shown in Figure 14(a). During thermal cycling,
653 the mean ice crystal volume exhibits a quasi-linear increase (Figure 14(c))

654 while the increase of the number of ice crystals is strongly slowed down
655 and seems to tend towards a plateau (Figure 14(b)). Temperature cycling
656 near the melting point of ice in the sponge cake crumb (-21 °C) increased
657 water mobility and promotes further growth of ice crystals by recrystalliza-
658 tion phenomenon based on Ostwald ripening. In the case of porous foods,
659 recrystallization by Ostwald ripening may involve both melting-diffusion-
660 refreezing or sublimation-diffusion-condensation processes (Zaritzky, 2008).
661 Indeed, the DSC thermogram displayed in Figure 1 shows the co-existence
662 of ice and liquid water (from melted ice) within the thermal cycling temper-
663 ature range (-30 °C / -20 °C). Depending on the temperature and pressure
664 local conditions within the product, melting or sublimation of ice can occur.
665 When the sponge cake is warmed up, small ice crystals, which have a low
666 energy stability, tend to melt/sublime and can even completely disappear.
667 Water molecules thus released are redistributed through the sample and are
668 redeposited onto large ice crystals, which are thermodynamically favoured.
669 As mentioned earlier, in the case of porous materials, water redistribution oc-
670 curs preferentially towards the pores where large ice crystals get bigger. Both
671 melting-diffusion-refreezing and sublimation-diffusion-condensation mecha-
672 nisms yield to a decrease of ice crystals number and to larger ice crystals size
673 in the sponge cake. The fact that the number of ice crystals does not effec-
674 tively diminish in this study might be explained by the continuous growth of
675 small ice crystals, which become more and more detectable as the thermal
676 cycling progresses. These results are in line with the findings of Chen et al.
677 (2013) who reported ice crystals growth by recrystallization during aging
678 of frozen wheat bread, eventhough they do not measure ice crystals size
679 directly. Bárcenas and Rosell (2006) also reported similar results for long-
680 term storage of frozen par-baked bread without evidencing the ice crystals

681 growth by direct measurements. Recrystallization was also found to be re-
682 sponsible for ice crystals growth and consequent microstructure destruction
683 during aging of frozen flour-water mixture using nuclear magnetic resonance
684 and transmission electron microscopy (Kontogiorgos and Goff, 2006). But,
685 to our knowledge, there is no published work describing *in situ* ice crystals
686 formation and growth while quantifying ice crystals size in frozen sponge
687 cake.

688 3.7.3. *Shrinking behaviour*

689 Sponge cake shrinkage of about 4-6 % was observed at the early stage of
690 the freezing process when the product temperature passes from 5 °C to -30
691 °C (Figure 10). During the thermal cycling, this volume retraction fluctuates
692 between 5% (at -20 °C) and 8% (at -30 °C). The volume change that occurs
693 at the cooling-freezing step could be related to the migration of the water
694 initially inside the sponge cake matrix towards the macro-pores, where ice
695 crystals are preferentially formed. When the sponge cake is warmed up to
696 -20 °C, ice crystals sublimation can lead to dilatation particularly inside
697 micropores where small crystals are more likely to sublimate. This results
698 in a slight reduction of the volume retraction as can be seen in Figure 10.
699 When the product is cooled down again to -30 °C, water diffuses through
700 the matrix and evaporates inside the macropores but at a slower rate than
701 during the first freezing step. Moreover gas entrapped in the closed pores
702 may contract. Consequently, micropores may become smaller or disappear
703 leading to the sample volume retraction. This mechanism is consistent with
704 the results depicted in Figure 9 (b) that shows a decrease of the total number
705 of pores during the whole experiment and an increase of the number of pores
706 when the sponge cake is warmed up to -20 °C. This pore number increase is

707 accompanied by a decrease of the mean equivalent diameter of pores (Figure
708 9 (c)) meaning that these new pores are of small size (see section 3.2). To
709 our knowledge, such results were not reported in the literature about sponge
710 cake. Nevertheless, the shrinking phenomenon was extensively studied in
711 bread crumb. Ribotta and Le Bail (2007) reported similar trend during
712 freezing of bread. The authors argued that the retraction of bread crumb
713 could be related to the dehydration of the matrix during freezing due to the
714 removal of water from the protein-starch-water system in the form of ice. A
715 bit later, Jiang et al. (2008) showed that cellular bread crumb (with pores)
716 shrinks during freezing whereas degassed bread crumb expands. They also
717 explained their results by the matrix dehydration following the formation of
718 crystals inside the pores during freezing in the case of cellular bread crumb,
719 while these crystals are formed inside the matrix in the case of degassed
720 bread. More recently, this phenomenon was also noticed by Ben Aissa et al.
721 (2010) while comparing cellular and degassed bread crumbs. Besides the
722 hypothesis of the dehydration of the matrix, these authors assumed the
723 compression of the gas inside the pores to explain the shrinking behaviour
724 of the cellular bread crumb. It is noteworthy that the levels of shrinkage
725 reported in these works on bread are about 3% and therefore well below the
726 results obtained in the case of this study with sponge cake. The difference
727 could be related to the product composition, porosity or to freezing and
728 frozen storage conditions. This remains to be investigated.

729 **4. Conclusion**

730 An original set-up, initially developed for snow, was used in order to
731 characterize microstructural changes of a sponge cake sample during freez-
732 ing and subsequent storage at fluctuating temperatures using X-ray tomog-

733 raphy. 3D images of the same sample were performed at different times
734 during 6 days. An image processing based on Digital Volumetric Correla-
735 tion (DVC) was implemented in order to segment the images and then to
736 quantify porosity changes and ice crystal formation and growth. Several
737 microstructural parameters (volume fraction of ice, air and matrix, number
738 and equivalent diameter of pores and ice crystals, specific surface areas) were
739 then computed from the 3D images. Overall, the obtained results of this
740 first experiment have shown that:

- 741 1. during the freezing step, the sample was submitted to an important
742 shrinkage of about 4-6%. During the seven warming-up/cooling cycles,
743 this shrinkage was almost stable and oscillated between 5% and 8%.
744 The macro-pores network of the sample is slightly affected by this
745 shrinkage, whereas the small pores inside the matrix have disappeared.
- 746 2. during the thermal cycles, ice crystals mainly formed and enlarged at
747 the macro-pore interface, whereas no crystal was found in the porous
748 matrix. Thermal cycling was found to promote ice crystals growth
749 due to the recrystallization phenomenon. Ice crystals size increased
750 linearly with thermal cycling duration whereas their number tends
751 towards a plateau.

752 In a near future, this experiment, which is long and complex, must be re-
753 peated in order to confirm the findings and to take into account the variabil-
754 ity of food products. The obtained results open the way for future modeling
755 to characterize the impact of freezing and frozen storage on the microstruc-
756 ture and quality of sponge cake. Moreover, the proposed methodology, which
757 allows to study the microstructural changes of the same sample induced by
758 complex thermal loadings, could be applied on other frozen porous products.

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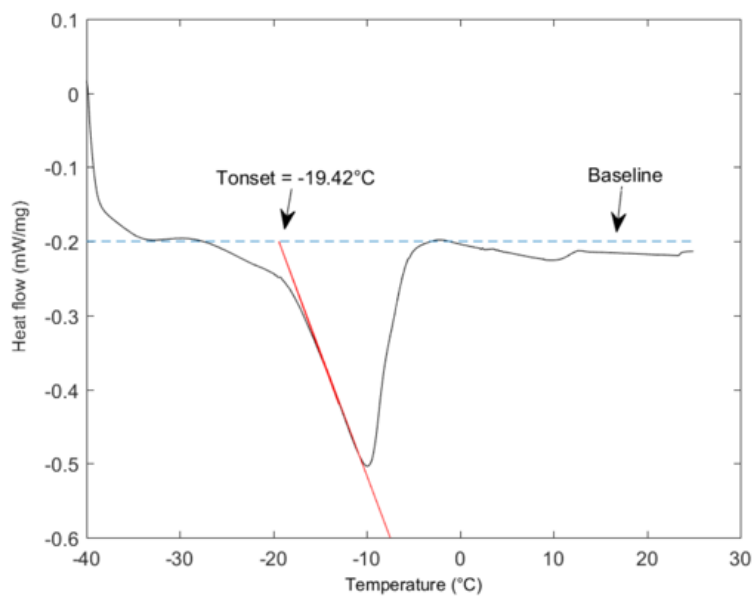


Figure 1: Typical ice melting endotherm obtained upon the thawing of frozen sponge cake with a heating rate of 5 °C/min.

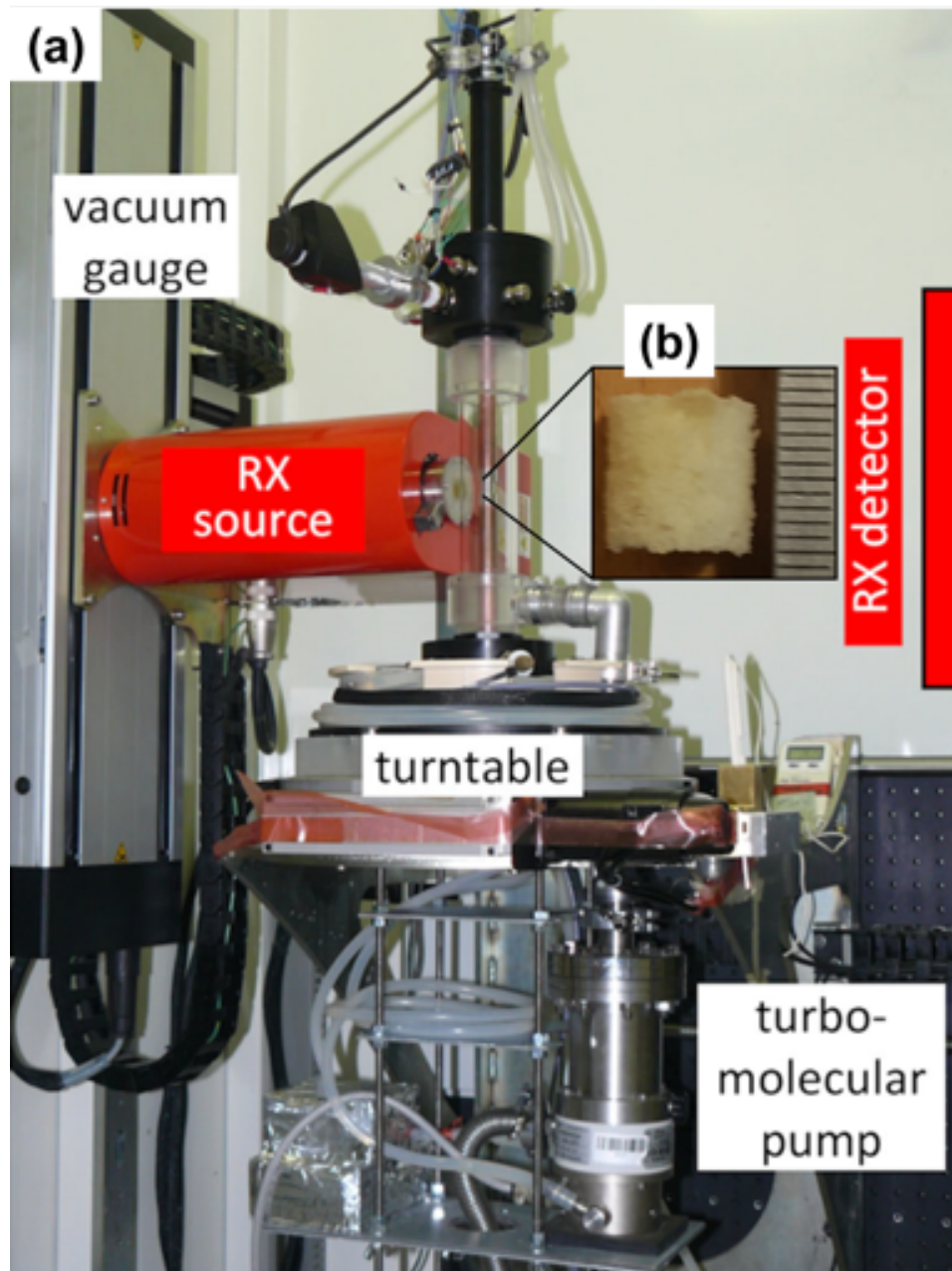


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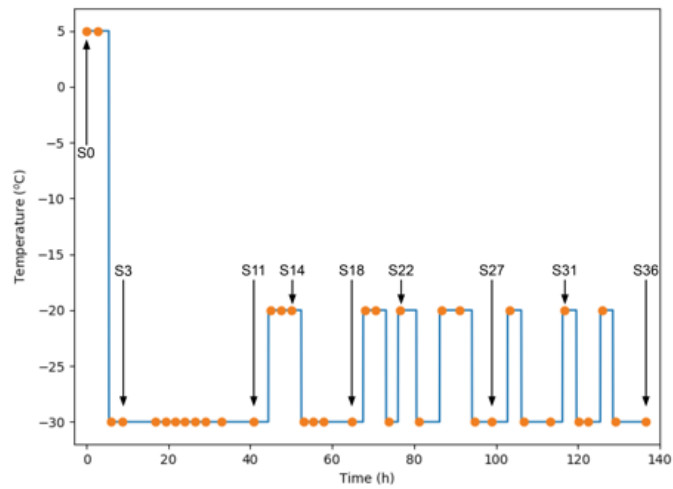


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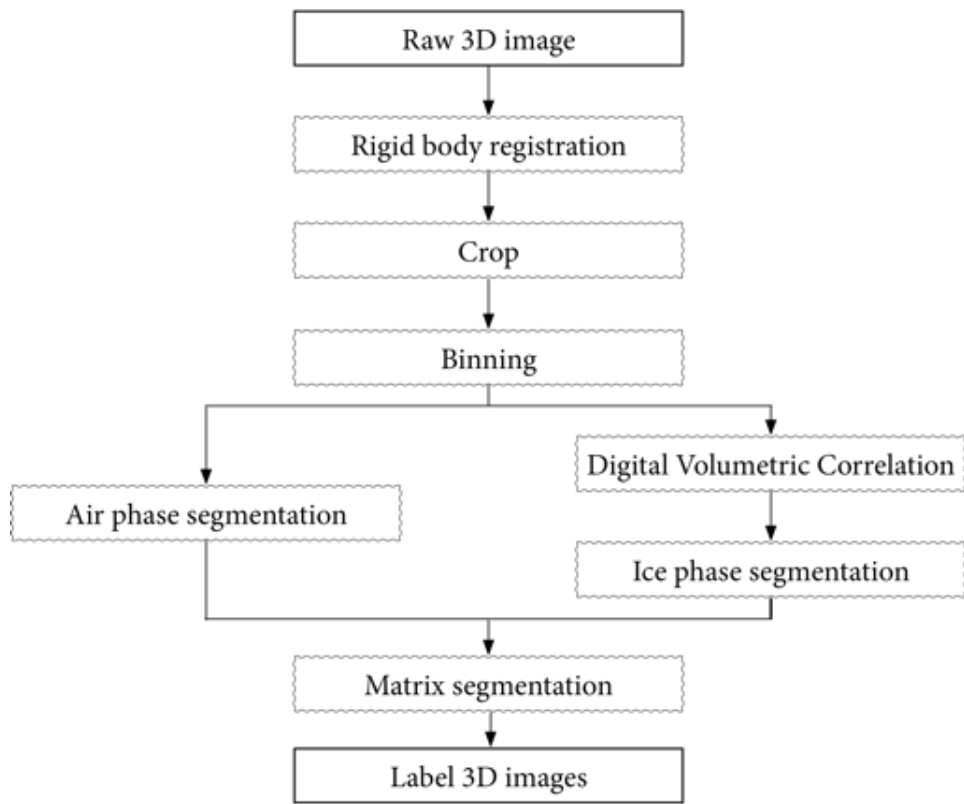


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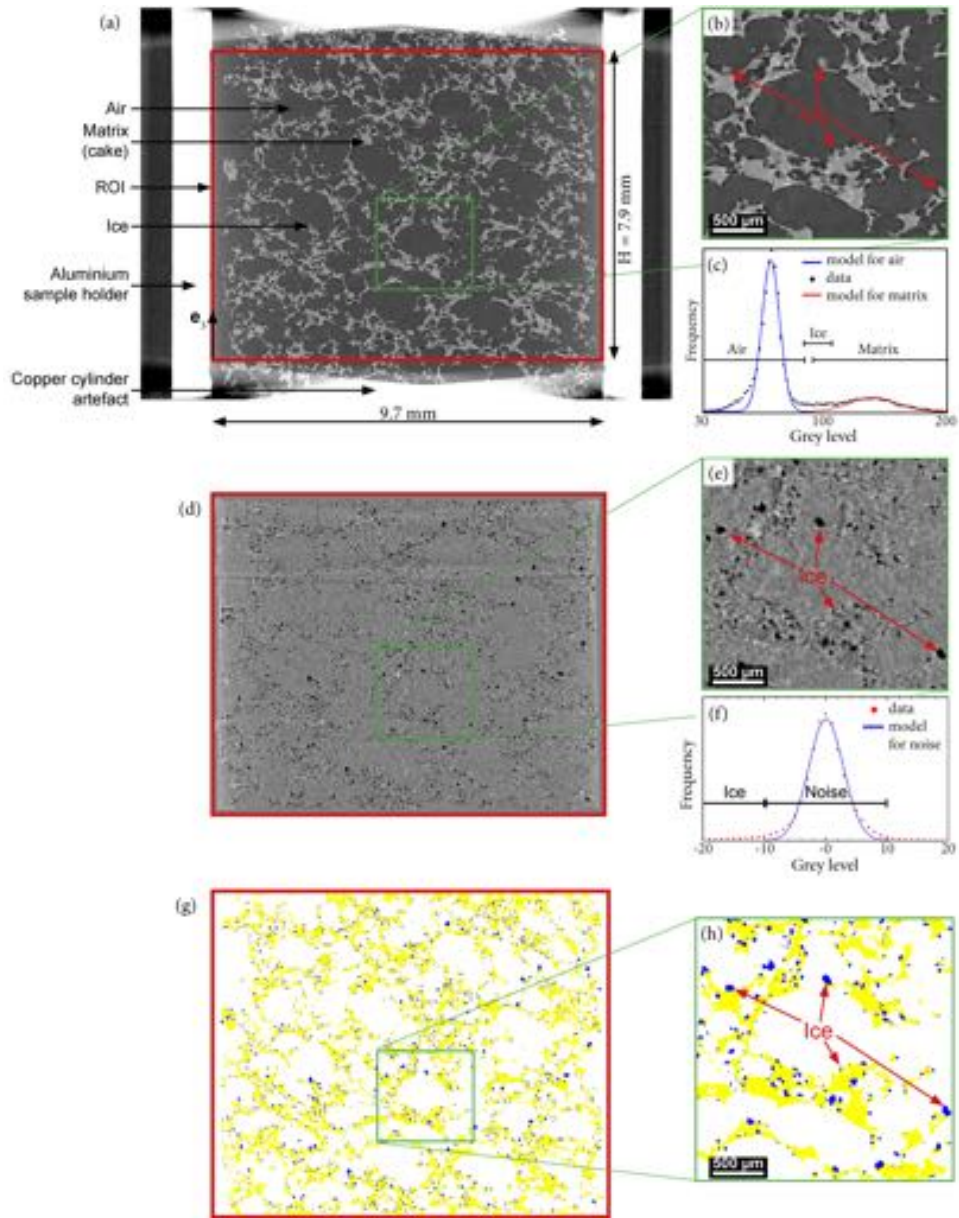


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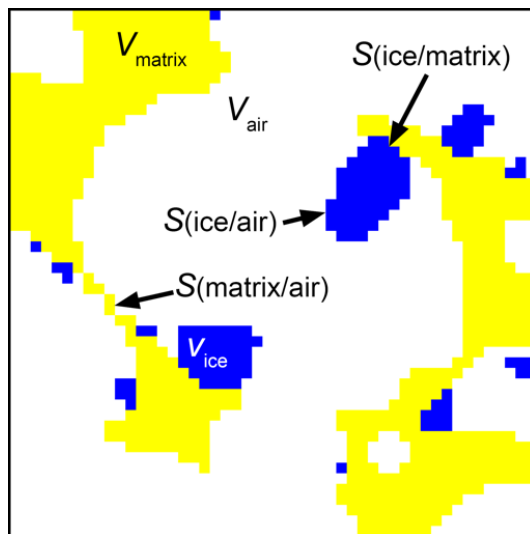


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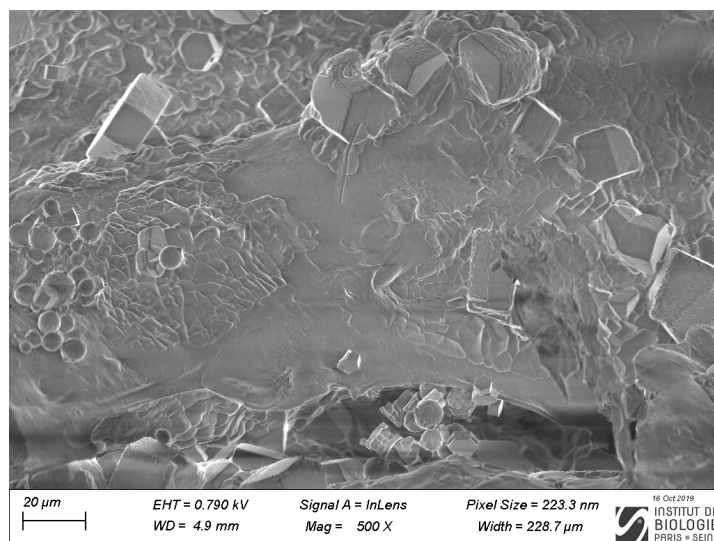


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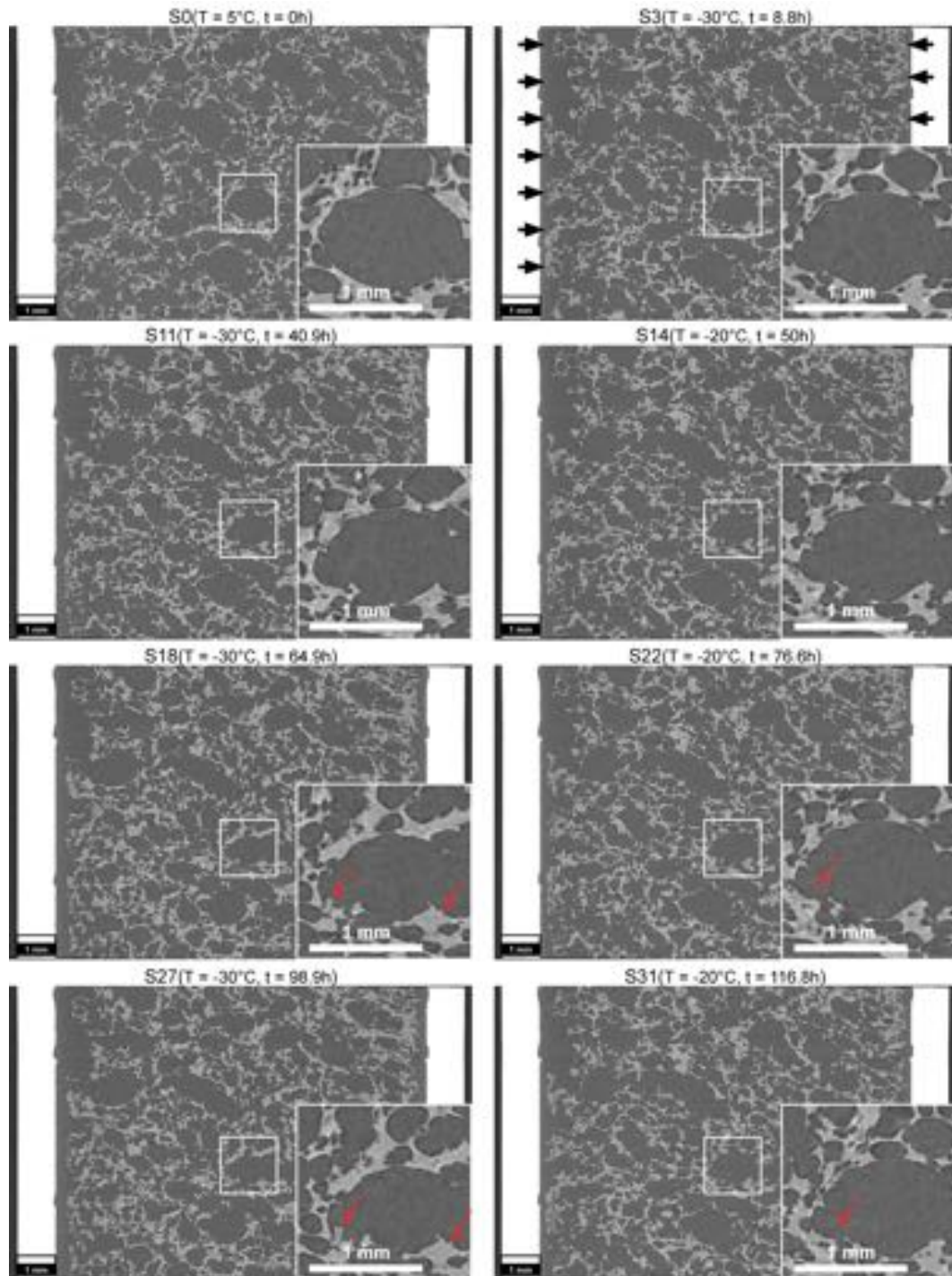


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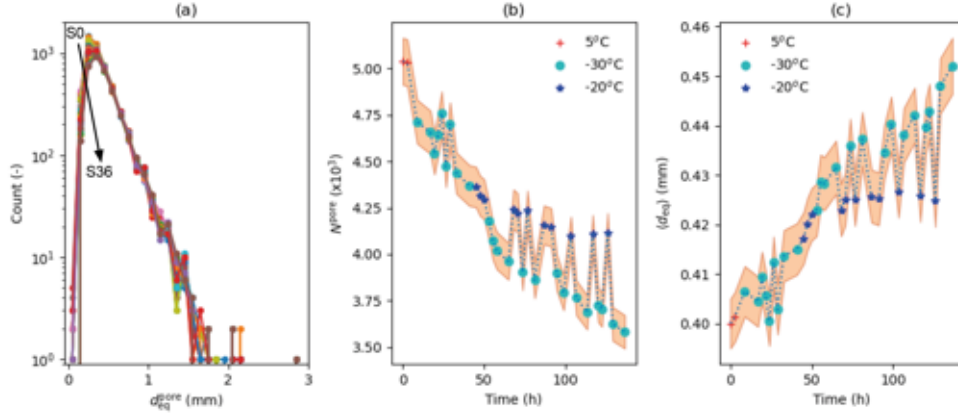


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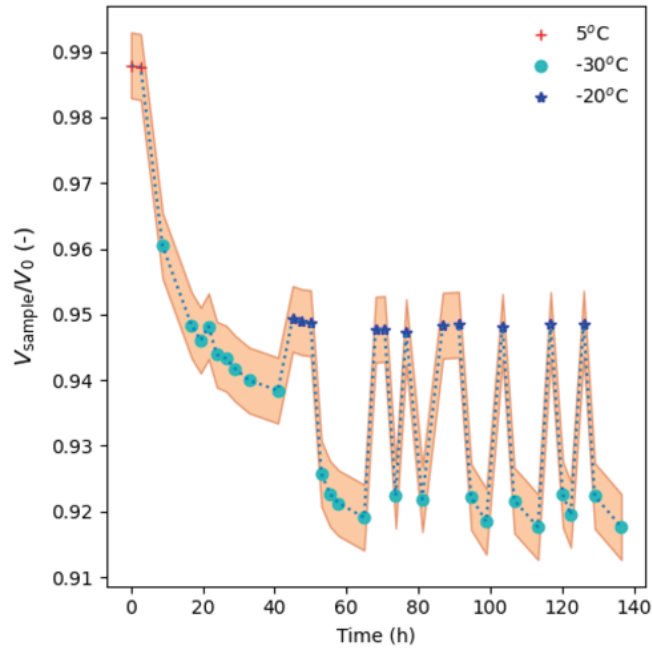


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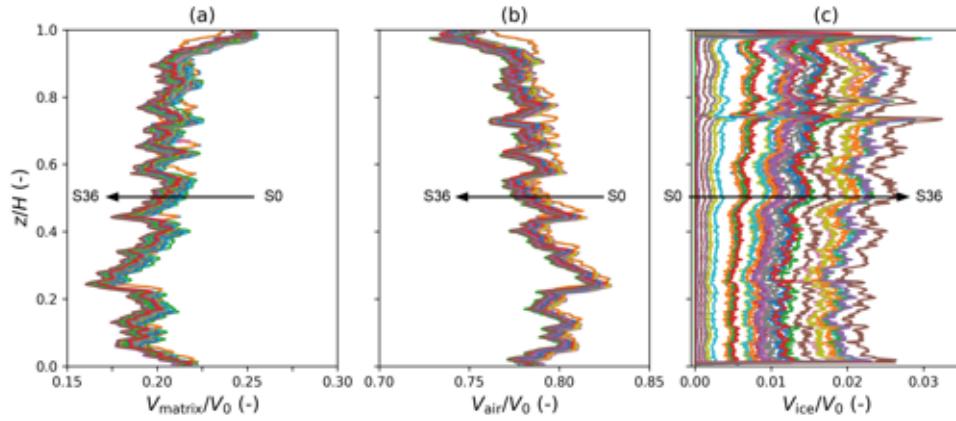


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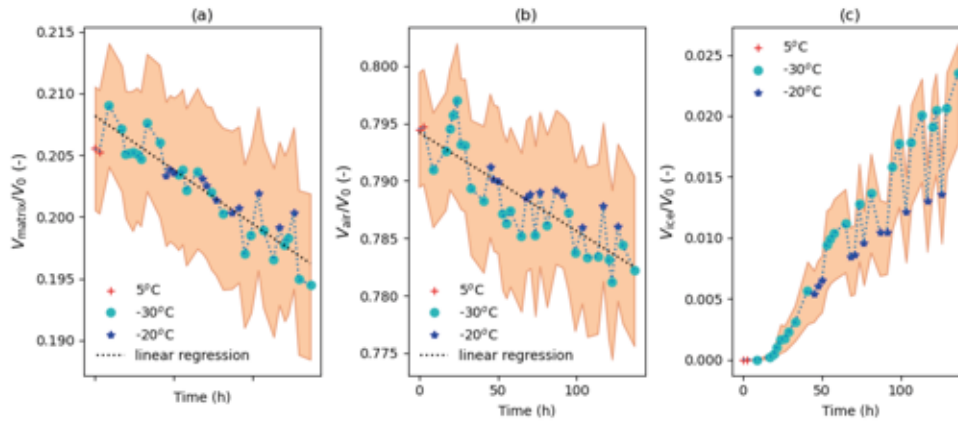


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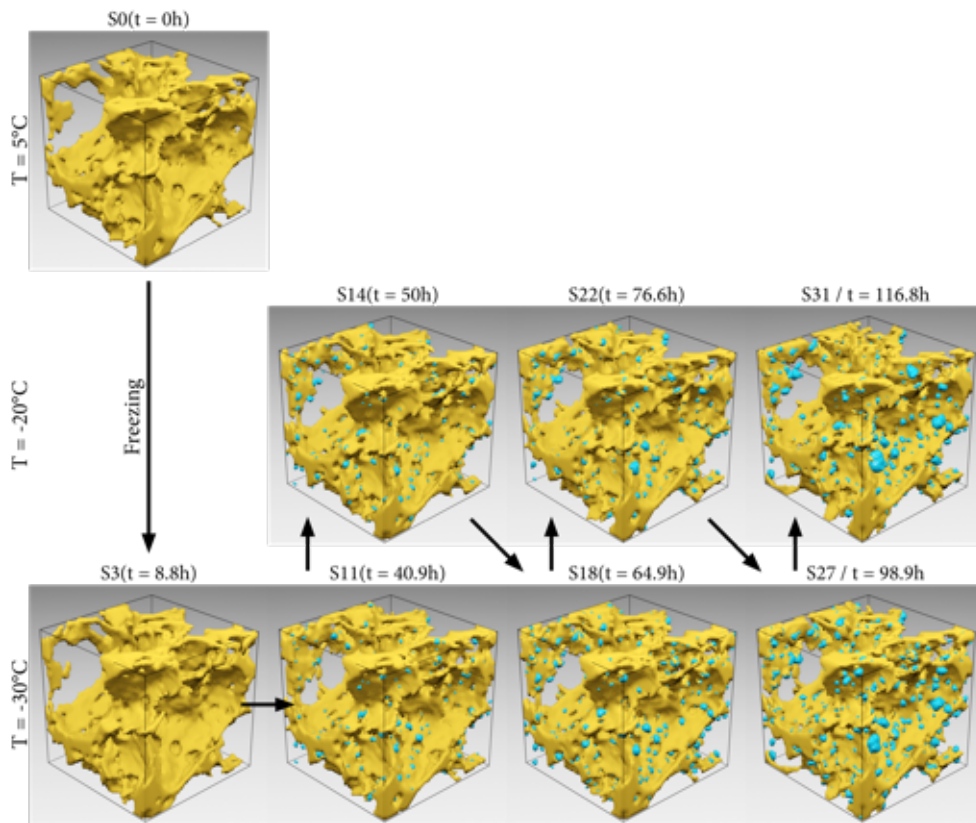


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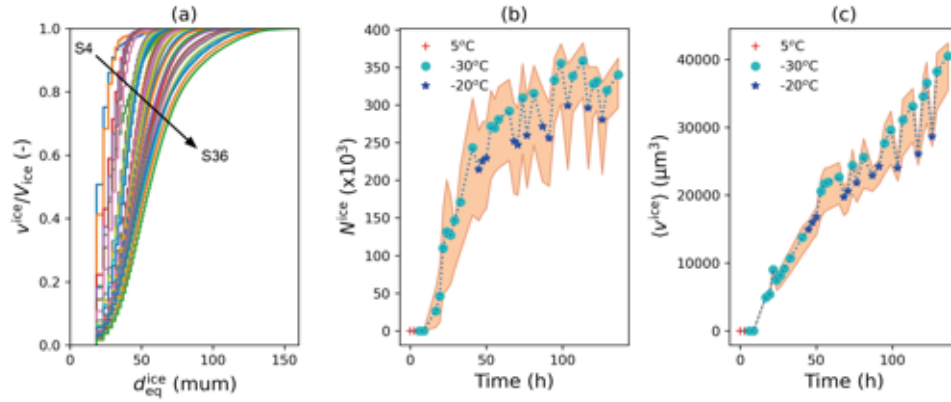


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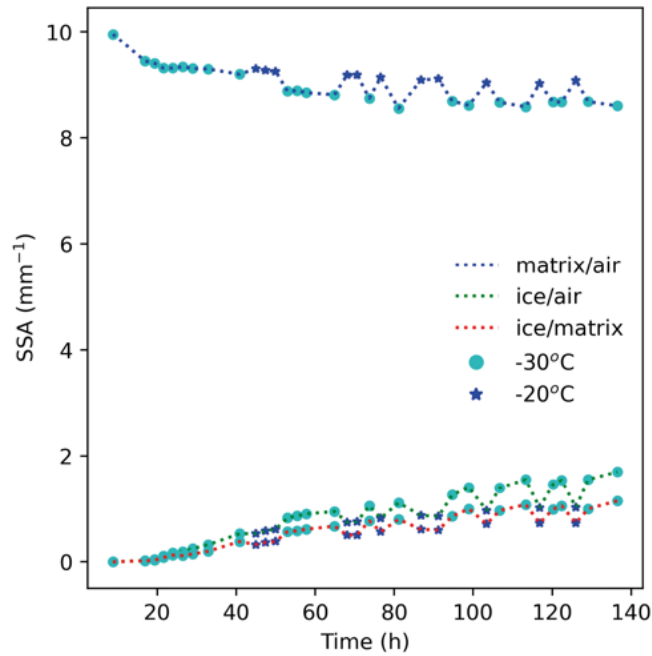


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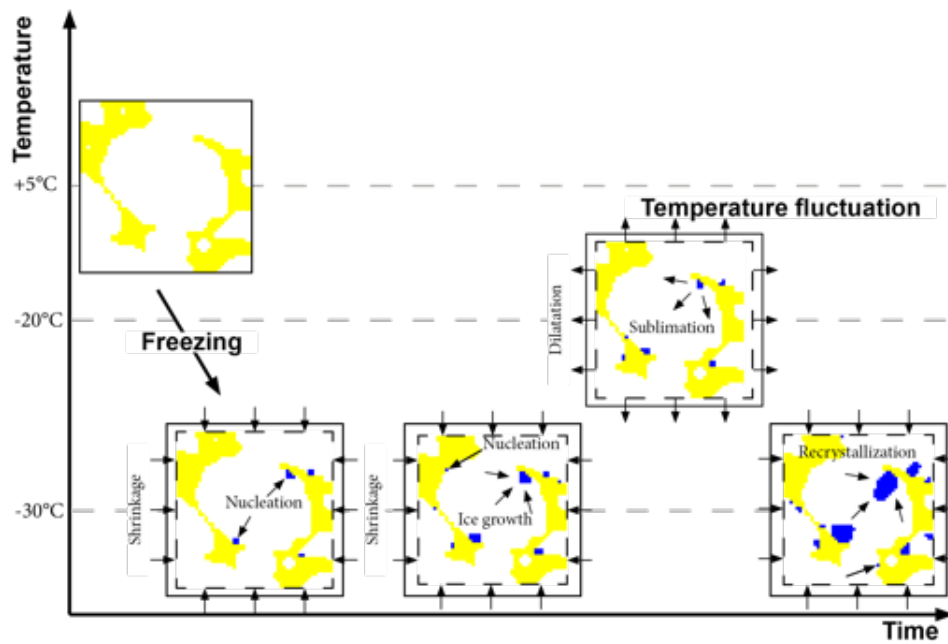


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CRedit author statement

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Conflict of Interest and Authorship Conformation Statement

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Dear Editor,

We confirm that this manuscript has not been published and is not under consideration for publication elsewhere. All co-authors have seen and agree with the contents of the manuscript, and we have no conflicts of interest to disclose.

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