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Amira Zennoune, Hayat Benkhelifa, Frédéric Flin, Fatou-Toutie Ndoye, Jonathan Perrin, et al.. Investigating the influence of freezing rate and frozen storage conditions on a model sponge cake using synchrotron X-rays micro-computed tomography. *Food Research International*, 2022, 162 (Part B), pp.112116. 10.1016/j.foodres.2022.112116 . hal-04152933

**HAL Id: hal-04152933**

**<https://agroparistech.hal.science/hal-04152933>**

Submitted on 5 Jul 2023

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1 **Investigating the influence of freezing rate and frozen storage conditions on a model sponge cake**  
2 **using Synchrotron X-rays micro-computed tomography**

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14  
15 **Abstract**

16 Synchrotron X-rays micro-computed tomography was applied to visualize and quantify 3D ice  
17 crystal changes into a model sponge cake after freezing and subsequent frozen storage. Model  
18 sponge cake samples were submitted to two different freezing rates (fast: 17.2 °C min<sup>-1</sup> and  
19 slow: 0.3 °C min<sup>-1</sup>), then stored at constant and fluctuating temperatures over a two weeks  
20 period. 3D images were acquired at frozen state thanks to a thermostated cell (CellStat) and  
21 processed using a grey level based segmentation method. Image analysis revealed that the ice  
22 volume fraction is conserved during storage but ice crystal size and location change whatever  
23 the freezing rate and the storage conditions. Maximum local thicknesses increase both inside  
24 (from 20 µm to 50 µm) and outside (from 47µm to 70 µm) the matrix during the fourteen days  
25 storage period. Both specific surface areas between starch and ice ( $SSA_{ice/starch}$ ) and between air  
26 and ice ( $SSA_{air/ice}$ ) also evolve with storage duration:  $SSA_{ice/starch}$  decreases up to -30% while  
27  $SSA_{air/ice}$  increases up to +13% depending on the freezing rates and the storage conditions.  
28 These results highlighted that, during storage, ice crystals evolve according to two different  
29 mechanisms depending on the freezing rate: fast freezing leads to a local redistribution of water  
30 both within the starch matrix and within the pores, while slow freezing results in both local  
31 redistribution within the starch matrix and water migration towards the pores. In addition, stable  
32 storage temperatures favor local water redistribution whereas water migration from the starch  
33 matrix towards the pores was greater in the case of fluctuating storage temperatures. This study  
34 shows that freezing and frozen storage conditions have a synergistic effect on the microstructure  
35 evolution of sponge cake due to recrystallization phenomena.

36 **Keywords:** Freezing rate; Frozen storage; Sponge cake; Microstructure; Recrystallization,  
37 Synchrotron X-ray micro-computed tomography.

38 **I. Introduction**

39 Sponge cakes are used as a base product in the manufacture of a wide variety of desserts. To  
40 meet the high demand of the market, large quantities are produced, but sponge cakes have a

41 limited shelf life due to physicochemical changes and microbial deterioration. Therefore,  
42 sponge cakes are frozen just after baking and stored until their distribution in retail stores where  
43 they are thawed and decorated. Freezing and frozen storage have the advantage to preserve the  
44 sponge cake quality and to extend their shelf life. However, the evolution of the microstructure  
45 (pore and ice crystal size and morphology) after the formation and growth of ice crystals could  
46 induce physicochemical changes that could impair the sponge cake organoleptic quality.

47 During freezing, the temperature of the product is lowered, and water is transformed into ice  
48 crystals by crystallization (nucleation and ice crystal growth). The freezing rate controls the  
49 crystallization mechanism, which consequently determines the number of ice crystals as well  
50 as their size and distribution through the matrix. Many authors reported that fast freezing leads  
51 to the formation of a large number of small ice crystals evenly distributed in the matrix. Unlike  
52 fast freezing, slow freezing leads to the formation of bigger ice crystals that can damage the  
53 microstructure of the food (Mousavi et al. 2005, Ban et al. 2016, Mulot et al. 2019). In the case  
54 of porous food, a recent study performed on model sponge cake has shown that fast freezing  
55 leads to the formation of the majority of ice inside the matrix while slow freezing results in ice  
56 mostly formed at the pore surface (Zennoune et al. 2021).

57 In subsequent frozen storage, the microstructure of frozen food continues to evolve, especially  
58 for long term storage. This evolution is due to ice recrystallization: the system tends naturally  
59 to lower the surface free energy of the whole ice phase by ice crystal resizing and redistribution.  
60 Larger and shape-regular ice crystals, which are thermodynamically more stable, are favored at  
61 the expense of small and shape-irregular ice crystals. This leads to an increase of the average  
62 size of ice crystals and to a decrease of their number (Donhowe and Hartel 1996, Hartel 1998,  
63 Hagiwara et al. 2006). Several recrystallization mechanisms were identified. Among them,  
64 migratory recrystallization, also known as Ostwald ripening is recognized as one of the most  
65 prominent in frozen foods. Ostwald ripening is characterized by the gradual detachment of  
66 water molecule from the surface of smaller ice crystals, followed by water molecules diffusion  
67 and redeposition onto large ice crystals. Consequently, small ice crystals tend to disappear while  
68 larger ones become bigger (Pronk et al. 2005). Other recrystallization mechanisms that can take  
69 place in frozen foods are isomass recrystallization (rounding of edges and sharp features of ice  
70 crystals with irregular shape to minimize their surface free energy) and accretive  
71 recrystallization (merging of two crystals in close proximity to form one large crystal). Isomass  
72 recrystallization results in crystals with more regular shape while keeping their number  
73 constant. Accretive and migratory recrystallization lead to an increase of ice crystal size and a  
74 reduction of ice crystal number (Hartel, 2018). Ice recrystallization occurs slowly during long-  
75 term frozen storage at constant temperature but is greatly enhanced by temperature fluctuations  
76 due to the normal on/off operation of the freezer compressor or to poor practices in the cold  
77 chain, as often encountered. Therefore, it is of great importance to properly control the  
78 temperature during storage and distribution in order to maintain the quality of the frozen  
79 products and to minimize product losses.

80 In the specific case of porous foods, the presence of air greatly influences the crystallization  
81 and recrystallization phenomena. Indeed, freezing and frozen storage involve simultaneously

82 heat and water transfer, which are highly related to pore number, size, connectivity and surface.  
83 Pham (2006) reported in his review that water diffusion is accelerated by the presence of pores.  
84 During freezing, conductive heat transfer is driven by the temperature gradient between the cold  
85 surface and the product core. Diffusive water transfer is controlled by the water vapor pressure  
86 gradient between the surrounding air and the product surface. When porous foods are frozen,  
87 these gradients are also created at the pore scale resulting in water diffusion from the matrix  
88 towards the pores where water evaporates. Once the freezing point is reached, the  
89 supersaturated water vapor condenses into ice at the pore surface while liquid water crystallizes  
90 mainly into the matrix. This phenomenon is commonly called evapo-condensation (Pham  
91 2006). This mechanism is in line with our recent findings on ice formation in both pores and  
92 matrix during model sponge cake freezing (Zennoune et al. 2021). Formation of ice crystals is  
93 accompanied by both volumetric expansion and sponge cake dehydration that may alter the  
94 porous microstructure, which is highly related to the product quality.

95 During frozen storage of porous foods, concomitant heat and water transfers still occur. It is  
96 mainly characterized by recrystallization phenomena (Ostwald ripening) where both melting  
97 diffusion-refreezing for the liquid phase and sublimation diffusion-condensation for the vapor  
98 phase take place in the matrix and in the pores, respectively. It results in ice crystal growth and  
99 further microstructural changes that may ultimately deteriorate the product quality. It is obvious  
100 that the final quality of the frozen product is related to the coupling of both freezing and frozen  
101 storage processes. Even though the close relationship between porous/crystalline  
102 microstructural changes and the quality of frozen porous products was established (Bárcenas et  
103 al. 2004, Baier-Schenk et al. 2005, Yi and Kerr 2009, Díaz-Ramírez et al. 2016), a detailed  
104 understanding of the mechanisms implemented at pore and ice crystal scale during a combined  
105 freezing and frozen storage process is still needed. The rare studies reported in the literature  
106 have focused on either freezing (Lucas et al. 2005, Chen et al. 2012) or frozen storage (Chen  
107 et al. 2013, Eckardt et al. 2013). Furthermore, these studies were devoted to bread. Silvas-  
108 García et al. (2014) have investigated the coupled effect of freezing rate and storage time on  
109 bread dough stability and bread quality without characterizing the microstructure. To our  
110 knowledge, the literature does not report any study describing the combined effect of freezing  
111 and frozen storage on the microstructural changes of porous foods and particularly of sponge  
112 cake. Very few works were conducted on frozen sponge cake (Díaz-Ramírez et al. 2016, Van  
113 Bockstaele et al. 2021, Zennoune et al. 2021). Díaz-Ramírez et al. (2016) have shown a  
114 correlation between microstructure changes and quality attributes of sponge cake stored at  $-23$   
115  $^{\circ}\text{C}$  for 6 months. Recently, Van Bockstaele et al. (2021) have rather investigated sensory and  
116 textural properties of frozen sponge cake stored at short-term period. The first work  
117 investigating the effect of freezing rate on porous and crystalline microstructure of frozen  
118 sponge cake was reported by our team (Zennoune et al. 2021). The authors succeeded in  
119 characterizing the evolution of pores and ice crystals in a model sponge cake using synchrotron  
120 X-ray micro-computed tomography (X-ray  $\mu\text{CT}$ ) on products in the frozen state.

121 Imaging methods could be useful and reliable for the assessment and understanding of the  
122 microstructure of frozen products. Microscopy has long been the most commonly used

123 technique to visualize the effect of freezing and frozen storage on the structure of food products  
124 (Bevilacqua et al. 1979, Esselink et al. 2003, Baier-Schenk et al. 2005, Vicent et al. 2019). It  
125 includes light microscopy, which requires a very thin sample of the food product and several  
126 preliminary chemical preparation steps (fixing, coating, staining) in order to enhance the  
127 contrast (Alizadeh et al. 2007, Anese et al. 2013). Transmission and scanning electron  
128 microscopy offer higher resolution than light microscopy but also require intensive sample  
129 preparation such as freeze-drying for contrast enhancement. These preliminary preparation  
130 steps are tedious and likely to alter the microstructure of the product resulting in measurement  
131 artefacts. More reliable results can be obtained using cryo-scanning electron microscopy (cryo-  
132 SEM), which involves less cumbersome and damaging preparation steps (Chassagne-Berces et  
133 al. 2009, Mulot et al. 2019). Cryo-SEM was used to study porous foods like bread dough and  
134 bread (Bárcenas and Rosell 2006) and model sponge cake (Zennoune et al. 2021). However, all  
135 these microscopy methods provide 2D images which depend on the selected cutting plane. In  
136 addition, these 2D images do not afford sufficiently quantitative information that are  
137 representative of the entire volume of the product studied.

138 3D imaging techniques such as X-ray  $\mu$ CT can overcome these limitations. X-ray  $\mu$ CT is a  
139 reliable method based on the measurement, at microscopic spatial resolution, of the intensity  
140 profile of an X-ray beam transmitted through the sample, which depends on the material  
141 characteristics (density, thickness and atomic number). The technique is increasingly used to  
142 visualize and quantify the characteristics of frozen food products (Mousavi et al. 2005, Ullah  
143 et al. 2014, Vicent et al. 2016, Mulot et al. 2019, Masselot et al. 2021). However, most of the  
144 studies so far have focused on the visualization of frozen foods at ambient temperature after  
145 freeze-drying (Mousavi et al. 2007, van Dalen et al. 2013, Kobayashi et al. 2014, Zhao and  
146 Takhar 2017). These studies assumed that voids created after freeze-drying represent ice  
147 crystals. But, freeze-drying may imply shrinkage, which is likely to modify the microstructure,  
148 resulting in inconclusive results (Voda et al. 2012). Freeze-drying can be avoided when the  
149 product is scanned in the frozen state. This is possible when using a cooling cell as illustrated  
150 by several works on frozen food products (van Dalen et al. 2009, Pinzer et al. 2012, Medebacha  
151 et al. 2013, Vicent et al. 2017, Mulot 2019, Vicent et al. 2019, Masselot et al. 2021), and other  
152 porous frozen materials or systems such as snow (Calonne et al. 2014, Calonne et al. 2015) or  
153 ice particles (Haffar et al. 2021, Haffar et al. 2022). In this way, the non-invasive and non-  
154 destructive nature of X-ray  $\mu$ CT can be fully taken advantage of. However, these works were  
155 performed using laboratory scale X-ray  $\mu$ CT, which cannot always offer sufficiently contrasted  
156 images and high resolution for a category of products such as sponge cake. Indeed, sponge cake  
157 has a complex microstructure comprising both macropores and micropores requiring high  
158 resolution for a consistent and complete characterization. Recently, our team studied the effect  
159 of freezing rate on the microstructure of a frozen model-based sponge cake (Zennoune et al.  
160 2021). A thermostated cell (CellStat) (Calonne et al. 2014) was used on the synchrotron  $\mu$ CT  
161 beamline ANATOMIX at Synchrotron SOLEIL, the French national synchrotron light source  
162 in Gif-sur-Yvette, France (Weitkamp et al. 2017). This study demonstrated that the use of  
163 synchrotron can greatly enhance the visibility and discrimination of low contrasted materials in

164 addition to high resolution, allowing to detect better small features (ice crystals and  
165 micropores).

166 The present paper aims to study the combined impact of freezing rate and frozen storage  
167 conditions on the microstructure of a model sponge cake using X-ray  $\mu$ -CT at high spatial  
168 resolution. The samples were frozen at two different freezing rates and each of them were stored  
169 for two weeks at two different conditions (with and without temperature fluctuations). The  
170 model sponge cake samples were scanned at the ANATOMIX beamline using the thermostated  
171 cell CellStat to allow observation in the frozen state. The three phases (air, ice and cake matrix)  
172 of the frozen samples were segmented using an image processing method previously developed  
173 (Zennoune et al. 2021). The coupled effect of freezing rate and frozen storage was then  
174 discussed based on volume fractions of each phase, ice local thickness, ice location and shape  
175 characterizations.

## 176 **II. Materials and methods**

### 177 **II.1. Sponge cake preparation, sampling and freezing**

178 The model sponge cake was prepared using the formulation developed by Bousquières (2017)  
179 as described in Zennoune et al. (2021). The main ingredients are:

- 180 - Native corn starch (Cargill, Minneapolis, MN, USA): 36.55 % (water content about  
181 11%);
- 182 - Methylcellulose (MC) (Dow Chemical, Midland, MI, USA), type SGA7C, 0.46 %;
- 183 - Hydroxypropylmethylcellulose (HPMC) (Dow Chemical, Midland, MI, USA), type  
184 K250M: 0.35 %;
- 185 - Ultrapure water: 62.64 %.

186 Aluminum molds ( $10 \times 6 \times 3 \text{ cm}^3$ ) were filled with 60 g of dough and then baked in an electric  
187 oven (Whirlpool, Benton, IL, USA) for 30 min at 170 °C. After baking, the sponge cakes were  
188 left at room temperature for 45 min before being wrapped in waterproof plastic bags and sealed  
189 to be stored in a refrigerator at 5 °C until the sampling step.

190 For the imaging experiments, cubic samples (of 6 mm side) were cut in the crumb and placed  
191 in sample holders made with polymethyl methacrylate (PMMA) and copper (Figure 3) before  
192 freezing and storage. This sampling was performed in a thermo-hygroscopic chamber at 20 °C  
193 and 80% of relative humidity to avoid sponge cake dehydration during handling.

194 The prepared samples, mounted on their sample-holders, were frozen in the lab (FRISE,  
195 INRAE, Antony, France), before the imaging experiments, at two different freezing rates:

- 196 - Fast freezing ( $17.2 \text{ °C min}^{-1}$ ) using an air blast freezer,
- 197 - Slow freezing ( $0.3 \text{ °C min}^{-1}$ ) using a static chest freezer

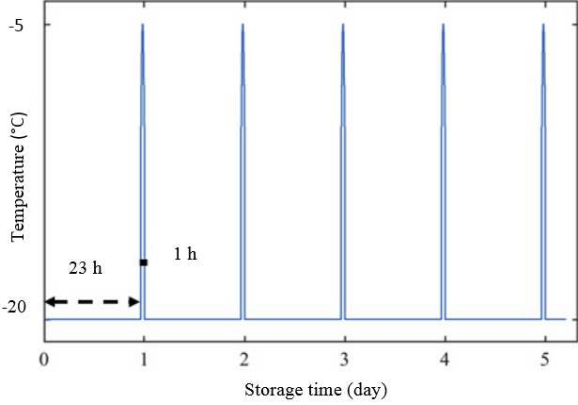
### 198 **II.2. Frozen storage conditions and analysis time points**

199 For both freezing conditions (see Table 1), the frozen samples were stored under two thermal  
200 conditions, before the imaging experiments:

- 201 - Stable storage at  $-20 \pm 0.1 \text{ °C}$ : using a static chest freezer equipped with an electronic

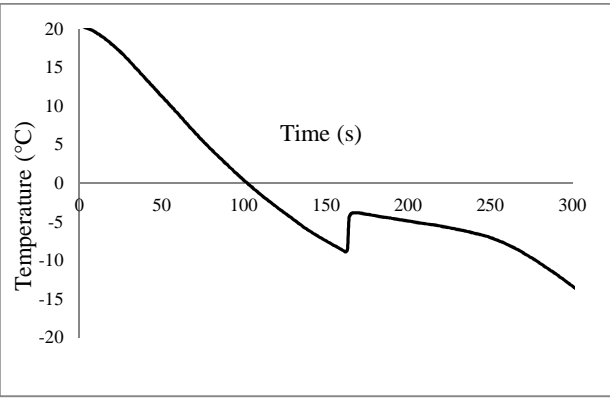
202 temperature controller with one set point (Eliwell WM961, Villeneuve la Garenne,  
 203 France). The samples were placed into a 3 cm thick expanded polystyrene box for  
 204 insulation in order to attenuate the temperature fluctuations related to the on/off  
 205 operation of the refrigeration system.

- 206 - Fluctuating storage between  $-20 \pm 0.1 \text{ }^\circ\text{C}$  and  $-5 \pm 0.01 \text{ }^\circ\text{C}$ : using two different static  
 207 chest freezers. The samples were held during 23 hours in the first freezer, set at  $-20 \pm$   
 208  $0.1 \text{ }^\circ\text{C}$  and then moved to the second freezer set at  $-5 \pm 0.01 \text{ }^\circ\text{C}$  for 1 hour each day  
 209 during a two-week storage period. The fluctuating storage temperature is schematized  
 210 in Figure 1.



211  
 212 **Figure 1:** Schematic representation of the dynamic change of storage temperatures.

213 The stable storage temperature condition intends to reproduce perfectly controlled storage  
 214 conditions of frozen foods in a cold room or in a freezer with no door opening. By contrast, the  
 215 fluctuating storage was designed to make it possible to study the effect of poor practices that  
 216 can be encountered through the cold chain. At  $-5 \text{ }^\circ\text{C}$ , the sponge cake is still in the phase change  
 217 plateau as can be seen in the freezing curve shown in Figure 2 and is likely to melt partially.  
 218 Consequently, the scenario of dynamic change of storage temperature between  $-20 \text{ }^\circ\text{C}$  and  $-5$   
 219  $^\circ\text{C}$  may allow to accelerate moisture migration and ice redistribution (Vicent et al. 2019).



220  
 221 **Figure 2 :** Core temperature kinetic during sponge cake freezing.

222 As summarized in Table 1, combining the two freezing rates and the two storage conditions  
 223 gives rise to four different thermal history scenarios. Three analysis time points were set: just  
 224 after freezing ( $t_0$ ), after 7 days of storage ( $t_7$ ) and after 14 days of storage ( $t_{14}$ ). Analyses at  $t_0$

225 were performed for each freezing rate. Four replicates were performed for each scenario and  
 226 each analysis time point, corresponding to a total of 40 samples as shown in Table 1.

227

228

229

*Table 1: Number of analyzed samples per condition.*

Time point	Fast freezing at 17.2 °C min <sup>-1</sup>		Slow freezing at 0.3 °C min <sup>-1</sup>	
	Stable storage at -20°C	Fluctuating storage between -20°C and -5°C	Stable storage at -20°C	Fluctuating storage between -20°C and -5°C
t <sub>14</sub>	4	4	4	4
t <sub>7</sub>	4	4	4	4
t <sub>0</sub>	4		4	

230

231 **II.3. Thermo-physical properties of the model sponge cake**

232 The thermo-physical properties of the model sponge cake are summarized in Table 2. All the  
 233 properties were determined as described by Zennoune et al. (2021). The authors have verified  
 234 the reproducibility of the model sponge cake based on its thermo-physical properties (porosity,  
 235 density and water content). They reported that the sponge cake prepared in this way can be  
 236 considered as a model product that represents porous products.

237

*Table 2: Sponge cake thermo-physical properties.*

Sponge cake apparent density (kg/m <sup>3</sup> )	Porosity (%)	Water content (%)	Freezing point (°C)	Freezable water content (%)
392 ± 15	56 ± 2	60 ± 1.5	-0.52 ± 0.09	44 ± 5.3

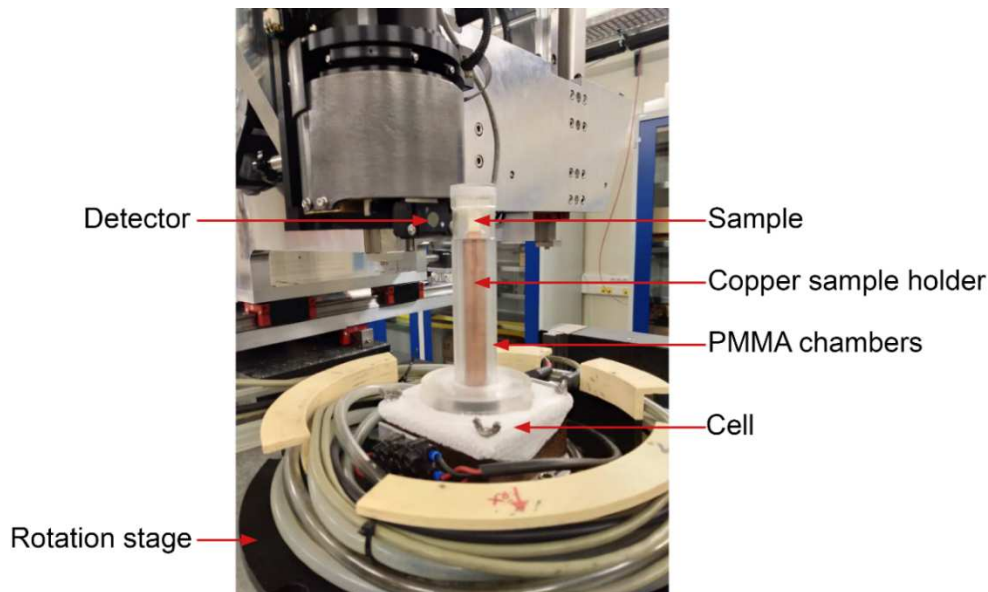
238 The porosity of the sponge cake before freezing was calculated based on the density values  
 239 (apparent density and batter theoretical density without air, which was found to be equal to 899  
 240 kg/m<sup>3</sup>) as explained by Zennoune et al. (2021). The mean value of 56 % is close to that found  
 241 by Roca et al. (2006), where porosities were between 52 % and 86 %. One must keep in mind  
 242 that porosity of a sample is the combination of the bakery process and the randomness of the  
 243 sampling. During baking, sponge cake dough containing initially 75 % of water loses nearly 20  
 244 % of its moisture content. Indeed, the water content measured after baking was about 60%, but  
 245 the freezable water was about 44 % as shown by differential scanning calorimetry  
 246 measurements (Zennoune et al. 2021).



## 247 **II.4. Synchrotron X-ray micro-tomography imaging acquisition and processing**

### 248 *II.4.1. Thermostated Cell*

249 The thermostated cell CellStat, initially developed for snow (Calonne et al. 2014, Haffar et al.  
250 2021), was used to analyze the frozen microstructure of model sponge cake samples under the  
251 synchrotron X-ray  $\mu$ CT environment constraints (Figure 3). It helps to maintain the sample at  
252 the frozen state by means of (i) a Peltier module, set at a temperature of  $-36\text{ }^{\circ}\text{C}$ , on which the  
253 copper sample holder is placed, (ii) a continuous dry and cold air circulation around the sample  
254 and (iii) a heat exchanger for heat dissipation at the warm side of the Peltier module. Additional  
255 details are available in the works of Haffar et al. (2021) and Zennoune et al. (2021). The  
256 samples were stored in a freezer set to  $-30\text{ }^{\circ}\text{C}$  and placed close to the experimental setup, thus  
257 ensuring nearly constant thermal conditions.

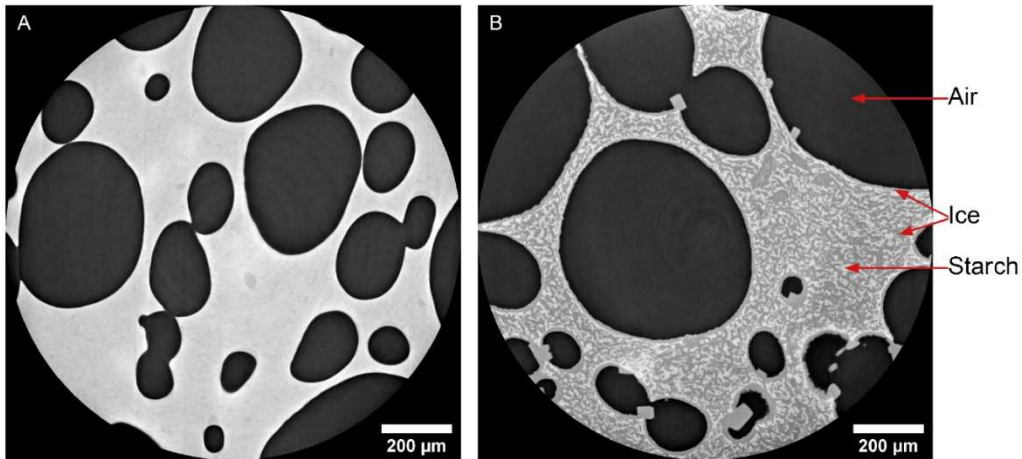


258

259 **Figure 3:** CellStat installed on the ANATOMIX beamline (Synchrotron SOLEIL, Gif-sur-Yvette,  
260 France).

### 261 *II.4.2. 3D Image acquisition*

262 The X-ray micro-computed tomography measurements were made at the beamline  
263 ANATOMIX of Synchrotron SOLEIL in a campaign that lasted three days. The system  
264 operation parameters were optimized as previously described by Zennoune et al. (2021) so as  
265 to yield good contrast between the sponge cake constituents at the voxel size of  $0.65\text{ }\mu\text{m}$ . A  
266 filtered polychromatic “white” beam with a central photon energy around  $30\text{ keV}$  was used; the  
267 distance between sample and detector was  $30\text{ mm}$ . For each volume scan, 2000 projections  
268 were taken over an angular range of  $180^{\circ}$  in continuous rotation. The total time for each scan  
269 was about 5 minutes. For each sample, four scans were acquired at different heights giving a  
270 total height of the reconstructed volume of  $5\text{ mm}$  for a diameter of  $1.3\text{ mm}$ . Figure 4 shows  
271 typical reconstructed slices of an unfrozen sample (Figure 4A) and a frozen sample (Figure 4B).  
272 In that second case, the three components or phases, i.e. air (in black), ice (in grey) and starch  
273 (in light grey), are clearly visible.



274

275

**Figure 4:** X-ray  $\mu$ CT slices of (A) unfrozen and (B) frozen model sponge cake samples.

276

#### II.4.3. Image processing

277

Images were processed according to the methodology described by Zennoune et al. (2021).

278

Firstly, the 3D volumes of size  $2048 \times 2048 \times 2048$  voxels (corresponding to  $1.3 \times 1.3 \times 1.3 \text{ mm}^3$ )

279

were reconstructed with PyHST2 software (Mirone et al. 2013) using a Paganin filter (Paganin

280

et al. 2002). The images were then segmented using the Fiji software according to the procedure

281

resumed in Figure 5. It is based on the global grey level threshold of the 3 phases (air, ice,

282

starch) by applying the Otsu multi-thresholding methodology (Otsu 1979) and taking advantage

283

of the good image contrast between the constituents. This segmentation is followed by a

284

combination of morphological (3D closing) and logical (not/and)

285

operations to distinguish:

286

- The ice volume which is mainly at the air interface (called “ice outside” the matrix)

287

- The ice volume which is mainly blended with the starch phase (called “ice inside” the

288

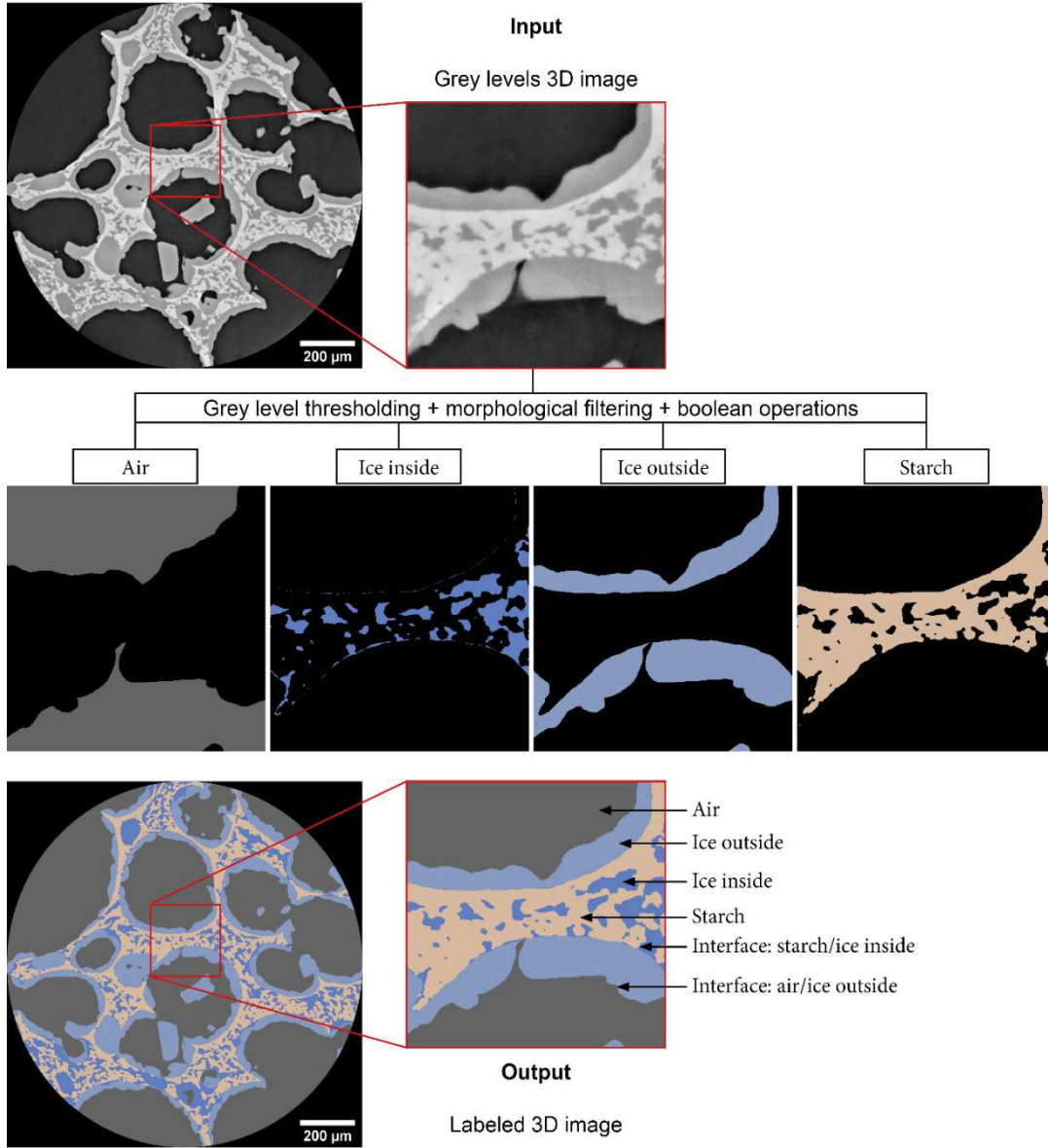
matrix).

289

The result of the procedure is then a labeled 3D image as depicted in Figure 5, which can be

290

used to compute microstructural descriptors.



291

292

**Figure 5: Image segmentation procedure.**

293 *II.4.4. Microstructural description*

294 To describe the microstructure of the different samples, the following parameters were  
 295 computed from 3D labeled images:

- 296 - The air volume fraction or porosity :

$$\varphi_{\text{air}} = \frac{V_{\text{air}}}{V_{\text{total}}} \quad (1)$$

297 where  $V_{\text{total}} = V_{\text{air}} + V_{\text{ice}} + V_{\text{starch}}$  is the total analyzed volume,  $V_{\text{air}}$ ,  $V_{\text{ice}} =$   
 298  $V_{\text{ice inside}} + V_{\text{ice outside}}$  and  $V_{\text{starch}}$  are the volumes of air, ice, ice inside and outside the  
 299 matrix, and starch, respectively. They are calculated using a classical voxel counting  
 300 algorithm implemented in Fiji software (Schindelin et al., 2012).

- 301 - The ice fraction in the solid phase (without the air),

$$\varphi_{ice} = \frac{V_{ice}}{V_{ice} + V_{starch}} \quad (2)$$

302 - The volume fraction of the ice inside ,

$$\varphi_{ice\ inside} = \frac{V_{ice\ inside}}{V_{ice} + V_{starch}} \quad (3)$$

303 - The specific surface area (*SSA*) of the ice/air interface, defined as

$$SSA_{ice/air} = \frac{S_{ice/air}}{V_{total}} \quad (4)$$

304 where  $S_{ice/air}$  is the surface area of the air/ice interface (Figure 5) and is computed using  
305 the dedicated *air/ice* tool in (Legland et al. 2016).

306 - The specific surface area (*SSA*) of the ice/starch interface, defined as

$$SSA_{ice/starch} = \frac{S_{ice/starch}}{V_{total}} \quad (5)$$

307

308 where  $S_{ice/starch}$  is the surface area of the air/starch interface (Figure 5).

309 - The local thickness distributions of ice inside and outside the matrix (Schindelin et al.  
310 2012): it represents the diameter of the largest sphere at a given point that can fit inside  
311 the object and containing the given point. It depicts the characteristic dimensions of the  
312 ice phases (inside and outside).

313

#### 314 *II.4.5. Statistical analysis*

315 A statistical analysis based on the analysis of variance (ANOVA) method (Brownlee 1965) was  
316 performed to study the significance of the combined effect of freezing rate and frozen storage  
317 conditions on model sponge cake microstructure evolution during freezing and storage. The  
318 Tukey Kramer test for multiple range comparisons ( $p < 0.05$ ) was used to identify the difference  
319 between the measured mean values for volume fractions and *SSA*.

### 320 **III. Results**

#### 321 **III.1. Qualitative analysis**

322 Figure 6 presents synchrotron X-ray micro-computed tomography images (horizontal slices) of  
323 the model sponge cake (air in grey, ice outside the matrix in light blue, ice inside the matrix in  
324 dark blue and starch in brown) obtained after fast freezing (Figures 6A1 to 6A5) and slow  
325 freezing (Figures 6B1 to 6B5). It shows typical microstructure evolution of model sponge cake  
326 with storage duration for the four storage scenarios applied (fast freezing/stable storage, fast  
327 freezing/fluctuation storage, slow freezing/stable storage; slow freezing/fluctuating storage).

328 *III.1.1. Initial state  $t_0$*

329 Figures 6A1 and 6B1 describes microstructures obtained just after freezing ( $t_0$ ) at fast and slow  
330 freezing, respectively. The corresponding 3D visualizations are shown in Figures 7A1 and 7B1  
331 It can be seen that:

- 332 • fast freezing leads to small ice objects homogeneously distributed inside the matrix and  
333 to a thin layer of ice that is formed at the air interface;
- 334 • slow freezing leads to a thick ice layer at the air interface and larger ice objects  
335 heterogeneously distributed inside the matrix.

336 The physical phenomena that led to such microstructure were explained in our previous  
337 research article (Zennoune et al. 2021) and are reported in section IV.

338 *III.1.2. Effect of storage conditions*

339 For the case of fast freezing, Figures 6A1 to 6A3 show the typical microstructures of samples  
340 after 0, 7 and 14 days of stable storage, respectively. Figures 6A4 and 6A5 show typical  
341 microstructures obtained in the fluctuating storage conditions for 7 and 14 days of storage  
342 duration, respectively. Figures 7A1 and 7A5 represents the 3D visualizations of images shown  
343 in Figures 6A1 (fast freezing,  $t_0$ ) and 6A5 (fast freezing, fluctuating storage temperature  $t_{14}$ ),  
344 respectively. The comparison between these figures shows that:

- 345 - The ice and starch structures are coarser at  $t_7$  and  $t_{14}$  than at  $t_0$  for both stable and  
346 fluctuating storage conditions
- 347 - The ice layer at the air/starch interface is thicker at  $t_7$  and  $t_{14}$  than at  $t_0$ ,
- 348 - There are no visible significant differences of the microstructure between stable and  
349 fluctuating storage.

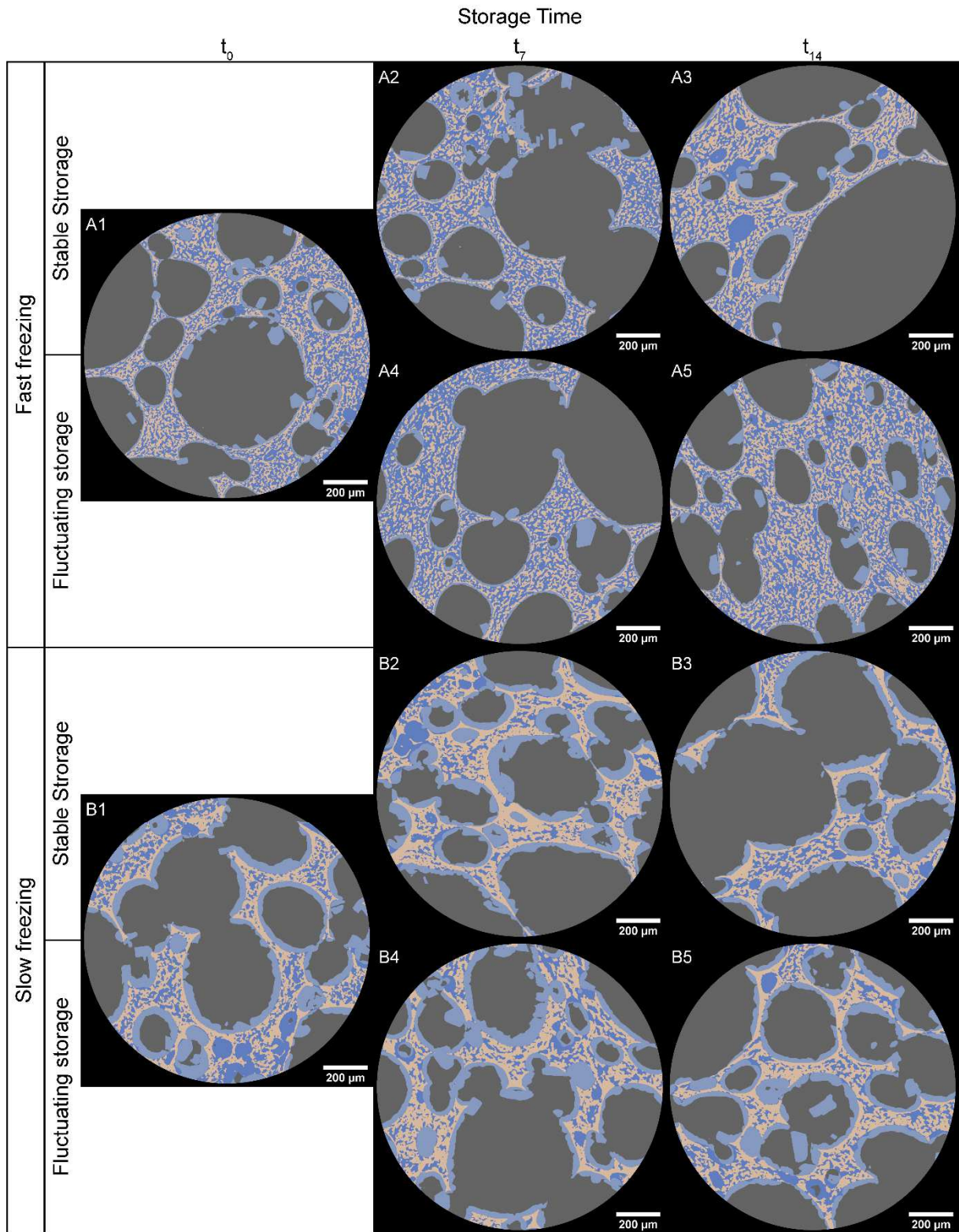
350 In the case of slow freezing, 2D images (Figures 6B1 to 6B5) as well as 3D visualizations,  
351 (Figures 7B1 and 7B5) bring the following comments:

- 352 - The ice and starch structures are coarser at  $t_7$  and  $t_{14}$  than at  $t_0$  for both stable and  
353 fluctuating storage conditions
- 354 - The ice layer at the air/starch interface is slightly thicker at  $t_7$  than at  $t_0$  and  $t_{14}$ ,
- 355 - Visually, there are no significant differences of the microstructure between stable and  
356 fluctuating storage.

357 All these results could be explained by ice redistribution and water migration during storage as  
358 will be discussed in section IV. However, it is worth noting that the qualitative analysis requires  
359 a complementary quantitative characterization to establish reliable conclusions.

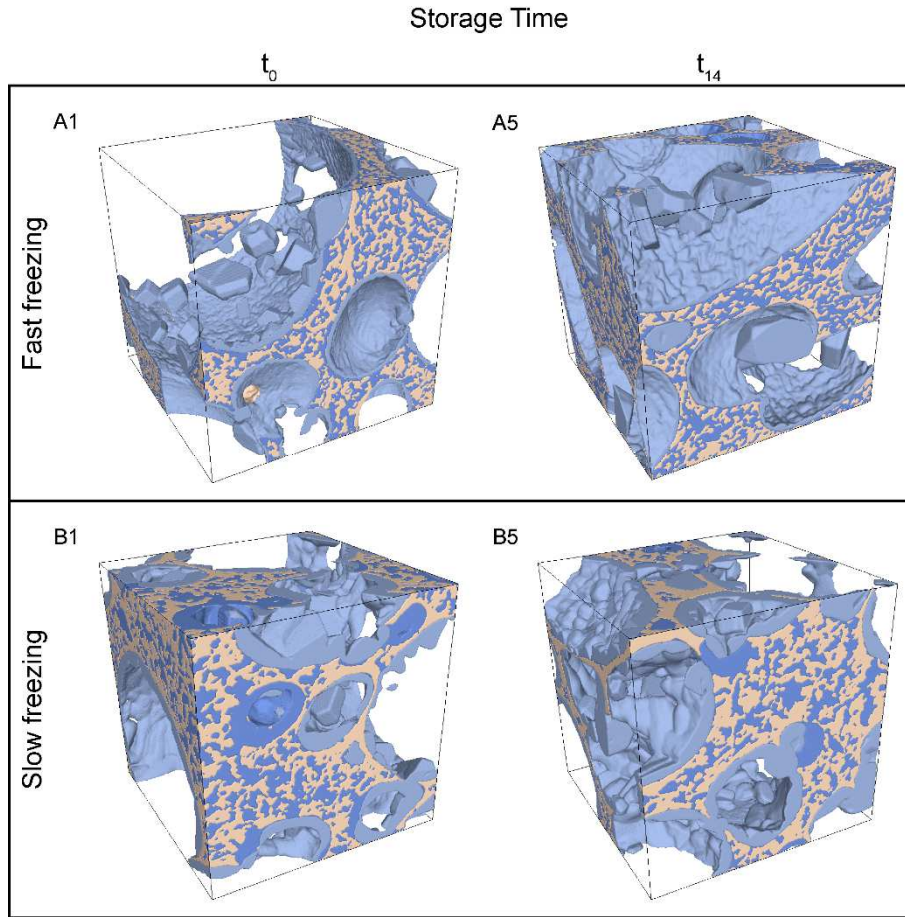
360





361

362 **Figure 6:** Synchrotron X-ray  $\mu$ CT slices of the labeled 3D images for the sponge cake samples frozen  
 363 at (A) fast rate and at (B) slow rate (Air in grey, ice outside the matrix in light blue, ice inside the  
 364 matrix in dark blue and starch in brown).



365

366 **Figure 7:** 3D visualization of the segmented Synchrotron X-ray  $\mu$ CT images for the sponge cake  
 367 samples frozen at (A) fast rate and at (B) slow rate. (Ice outside the matrix in light blue, ice inside the  
 368 matrix in dark blue and starch in brown). A1, A5, B1 and B5 are the corresponding 3D visualizations  
 369 of the slices with the same caption in Figure 6. Each cube is 500  $\mu$ m a side.

### 370 III.2. Quantitative analysis

#### 371 III.2.1 Volume fractions

372 Tables 3 summarized the volume fractions computed from the 3D images for the different  
 373 conditions. These results are also plotted in the Figure 8.

374 **Table 3:** Mean volume fraction of the different phases (air, ice and ice inside) for different conditions.

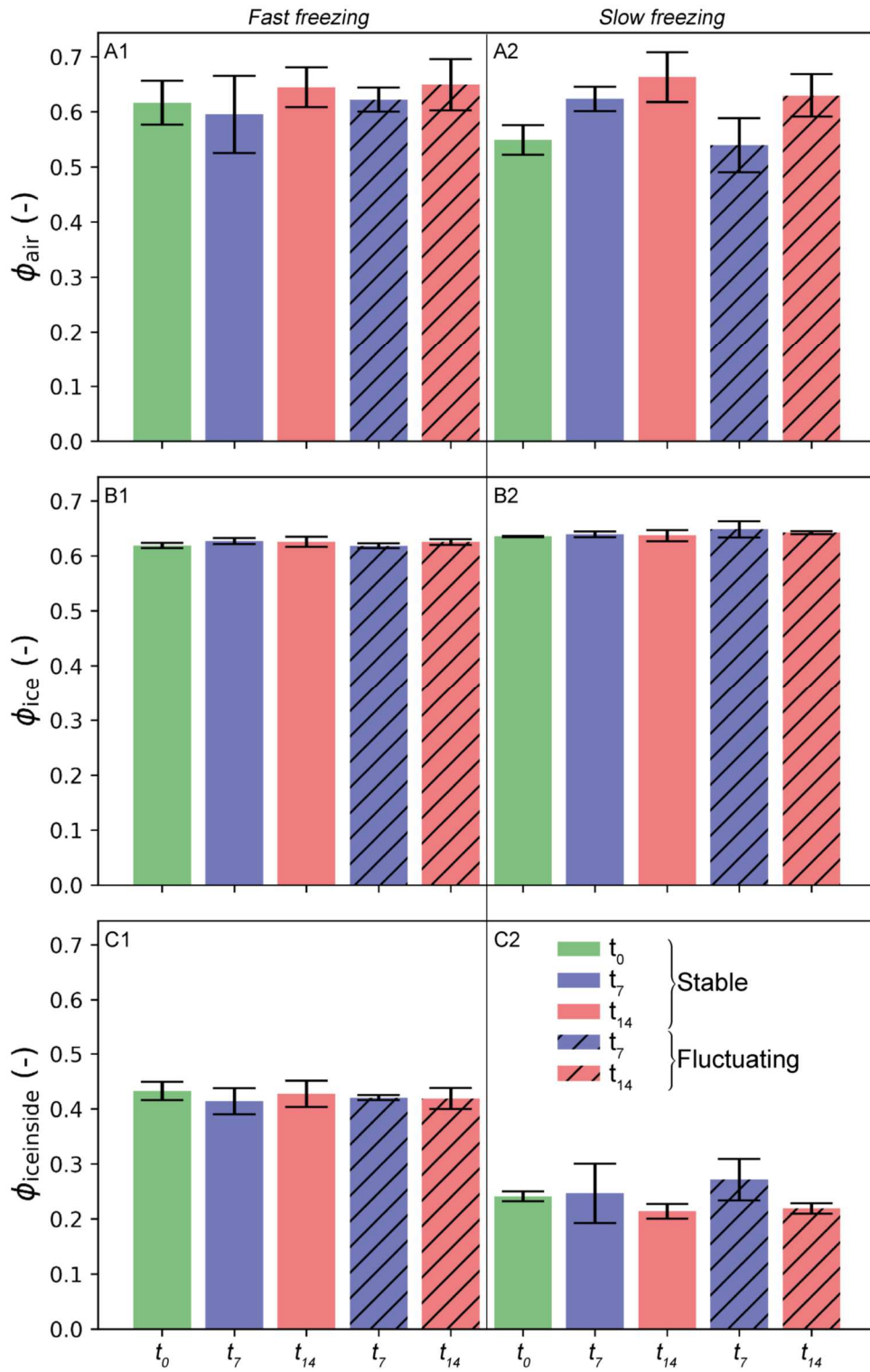
Storage conditions	Storage duration (days)	Fast freezing			Slow freezing		
		Air	Ice	Ice inside	Air	Ice	Ice inside
	0	61.6 $\pm$ 4.6 <sup>a</sup>	61.9 $\pm$ 0.6 <sup>a</sup>	43.3 $\pm$ 2.0 <sup>a</sup>	54.9 $\pm$ 2.7 <sup>a</sup>	63.6 $\pm$ 0.1 <sup>a</sup>	24.1 $\pm$ 1.0 <sup>a</sup>
Stable storage	7	59.5 $\pm$ 8.1 <sup>a</sup>	62.6 $\pm$ 1.2 <sup>a</sup>	41.2 $\pm$ 2.7 <sup>a</sup>	62.3 $\pm$ 2.2 <sup>b</sup>	63.9 $\pm$ 0.5 <sup>a</sup>	24.5 $\pm$ 6.3 <sup>a</sup>
	14	64.4 $\pm$ 4.1 <sup>a</sup>	62.6 $\pm$ 1.0 <sup>a</sup>	42.7 $\pm$ 2.8 <sup>a</sup>	66.3 $\pm$ 4.5 <sup>b</sup>	63.7 $\pm$ 1.2 <sup>a</sup>	21.4 $\pm$ 1.5 <sup>b</sup>
Fluctuating storage	7	62.2 $\pm$ 2.2 <sup>a</sup>	61.9 $\pm$ 0.3 <sup>a</sup>	42.0 $\pm$ 0.8 <sup>a</sup>	53.5 $\pm$ 4.9 <sup>a</sup>	64.6 $\pm$ 1.6 <sup>a</sup>	27.2 $\pm$ 4.3 <sup>c</sup>
	14	64.9 $\pm$ 4.7 <sup>a</sup>	63.1 $\pm$ 1.1 <sup>a</sup>	42.0 $\pm$ 2.6 <sup>a</sup>	63.0 $\pm$ 3.9 <sup>b</sup>	64.1 $\pm$ 0.3 <sup>a</sup>	22.0 $\pm$ 1.0 <sup>b</sup>

375 Comparisons were made between data in the same column. A same index letter indicate that there is no significant  
 376 differences between values within a single column,  $p > 0.05$ .

377 Overall, it is noteworthy that the air volume fractions obtained by image analysis and presented  
378 in Table 3 and Figure 8 are in line with the mean porosity value ( $56 \pm 2$  %) determined by  
379 experimental measurements (see Table 2). However, significant differences ( $p < 0.05$ ) were  
380 obtained for air volume fractions at slow freezing rate whatever storage condition and duration,  
381 while there was no significant difference ( $p > 0.05$ ) at fast freezing rate. These differences may  
382 be related to the local heterogeneity of the initial porosity of the sample as shown in our previous  
383 work (Zennoune et al. 2021). Indeed, it was reported that the model sponge cake has a multiple  
384 scale porosity that is heterogeneously distributed, making the sample porosity greatly  
385 dependent on the sampling location.

386 Table 3 and Figure 8 also show that the total ice volume fractions are similar (about 60%)  
387 whatever the freezing rate, the storage condition and storage duration. However, the ice location  
388 is different according to the freezing rate. The mean volume fraction of ice inside the matrix  
389 obtained for fast freezing is higher (quite twice) than the one found for slow freezing,  
390 corresponding to almost two thirds of the total ice formed. Inversely, for slow freezing, two  
391 thirds of the total ice are mainly formed at the air-starch interface. These quantitative results  
392 are consistent with the observations described in Figures 6A1, 6B1, 7A1 and 7B1 and with the  
393 physical phenomena occurring at the initial state, that was also described in our previous work  
394 (Zennoune et al. 2021).





395

396 **Figure 8:** Volume fractions of (A) air, (B) ice and (C) ice inside the matrix. (A1,B1,C1) for samples  
 397 obtained in the fast freezing conditions; (A2,B2,C2) for samples obtained in the slow freezing  
 398 conditions.

399

400

401 *III.2.2 Specific surface area and local thickness*

402 Considering the SSA values shown in Table 4 and Figure 9, significant differences were  
 403 obtained for both air/ice and ice/starch interfaces. The SSA values can be related to the local  
 404 thickness distributions of ice as shown in Figure 10. The standard deviations of the distributions  
 405 are represented by the shaded areas in Figure 10.

406 **Table 4:** Specific Surface Area ( $mm^{-1}$ ).

Storage conditions	Storage duration (days)	Fast freezing		Slow freezing	
		$SSA_{air/ice}$	$SSA_{ice/starch}$	$SSA_{air/ice}$	$SSA_{ice/starch}$
	0	$8.0 \pm 0.9^a$	$50.0 \pm 9.7^a$	$7.7 \pm 1.0^a$	$27.0 \pm 3.0^a$
<b>Stable storage</b>	7	$8.6 \pm 2.4^a$	$45.2 \pm 14.9^a$	$8.5 \pm 3.4^a$	$23.4 \pm 7.1^a$
	14	$7.4 \pm 1.7^a$	$38.6 \pm 9.5^b$	$8.0 \pm 2.0^a$	$18.6 \pm 5.4^b$
<b>Fluctuating storage</b>	7	$7.9 \pm 0.4^a$	$48.5 \pm 5.3^a$	$7.7 \pm 0.7^a$	$26.1 \pm 2.9^a$
	14	$8.7 \pm 1.0^b$	$46.3 \pm 4.9^c$	$8.7 \pm 0.4^b$	$21.0 \pm 4.7^c$

407 Comparisons were made between data in the same column. A same index letter indicate that there is no significant  
 408 differences between values within a single column,  $p > 0.05$ .

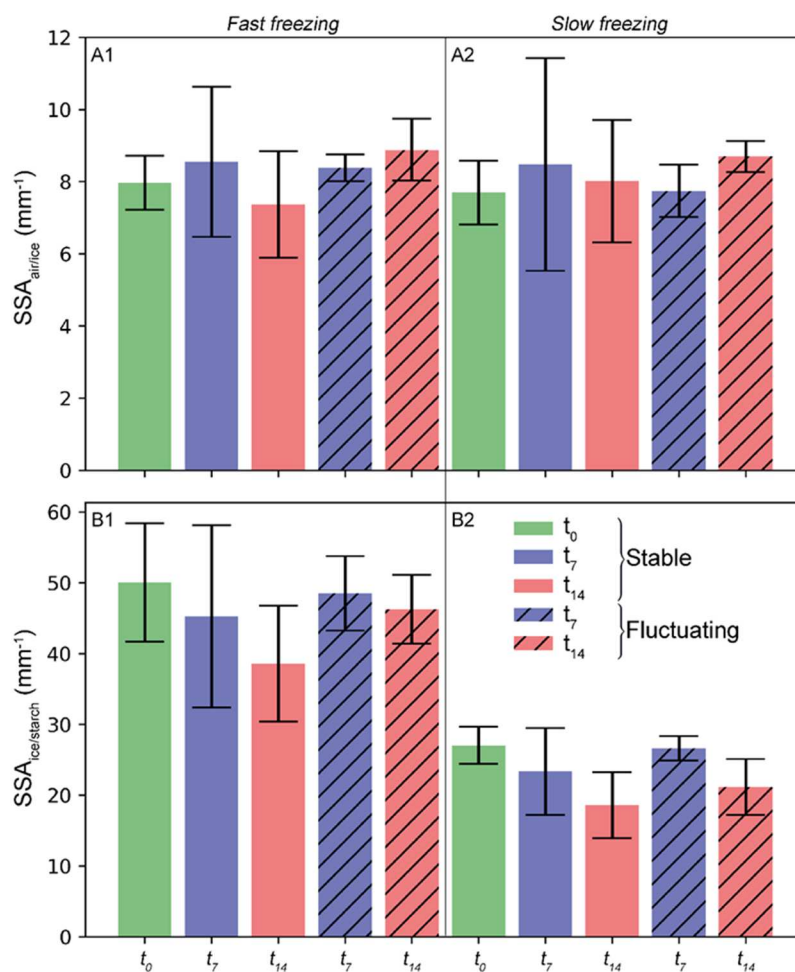
409 For fast frozen samples:

410 - Table 4 and Figure 9A1 show that the  $SSA_{air/ice}$  exhibit values between  $7.4 \text{ mm}^{-1}$  (stable  
 411 storage -  $t_{14}$ ) and  $8.7 \text{ mm}^{-1}$  (fluctuating storage -  $t_{14}$ ). In the case of a fluctuating storage,  
 412 the  $SSA_{air/ice}$  increases significantly ( $p < 0.05$ ) with the storage duration. This can be due  
 413 to an increase of the roughness of the ice layer or by the growth of individual crystals at  
 414 the interface. This seems to be confirmed by the evolution of the local thickness of the  
 415 ice outside presented in Figure 10. Indeed, it can be observed on Figures 10A2, B2 and  
 416 C2, in the case of a fluctuating storage, that the local thickness of the ice outside  
 417 increases with the storage duration: at  $t_0$ , the maximum local thickness (for 99% of ice)  
 418 is nearly  $47 \mu\text{m}$  whereas it is  $63 \mu\text{m}$  and  $70 \mu\text{m}$  at time  $t_7$  and  $t_{14}$ , respectively. It means  
 419 that, locally, a part of the ice layer has grown leading to a coarser and heterogeneous ice  
 420 surface. By contrast, in the case of a stable storage, there is no evident trend for the  
 421  $SSA_{air/ice}$  mostly because of the high standard deviations (Table 4 and Figure 9A1). This  
 422 may represent an increasing heterogeneity between and within the samples with  
 423 increasing storage duration.

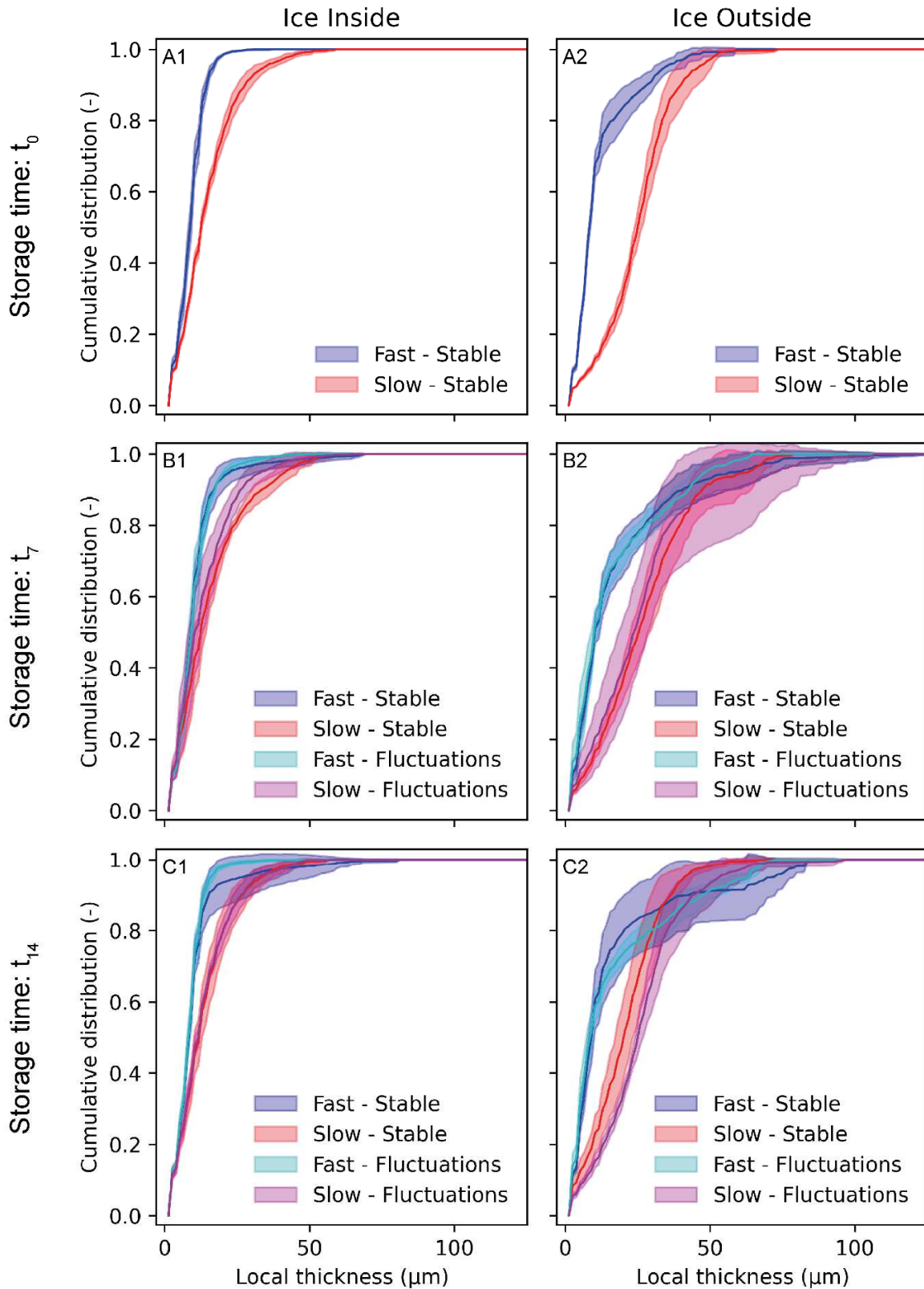
424 - The  $SSA_{ice/starch}$  ranges between  $50.0 \text{ mm}^{-1}$  (for  $t_0$ ) and  $38.6 \text{ mm}^{-1}$  (stable storage -  $t_{14}$ )  
 425 (Table 4 and Figure 9B1). The  $SSA_{ice/starch}$  values tend to decrease with the storage  
 426 duration regardless the storage conditions (stable or fluctuating). This decrease of the  
 427 surface between the ice inside and the starch, which is more pronounced in the case of  
 428 the stable condition, reflects probably a decrease in the number of small crystals and a  
 429 growth of the larger ice crystals. Indeed, in the case of a stable storage Figures 10A1,  
 430 B1 and C1 show that at  $t_0$ , the maximum local thickness of the ice inside is  $20 \mu\text{m}$  for  
 431 more than 99% of ice volume whereas it is  $50 \mu\text{m}$  at  $t_{14}$ .

432 For slow frozen samples:

- 433 - The  $SSA_{air/ice}$  ranges between  $7.7 \text{ mm}^{-1}$  (at  $t_0$ ) and  $8.7 \text{ mm}^{-1}$  (at  $t_{14}$  - fluctuating storage)  
 434 as can be seen in Table 4 and Figure 9A2. As for the fast frozen samples, the  $SSA_{air/ice}$   
 435 increases slightly with the storage duration in the case of temperature fluctuations.  
 436 Similarly, there is no clear trend in the case of a stable temperature storage, which shows  
 437 large standard deviations.
- 438 - The  $SSA_{ice/starch}$  values vary between  $27 \text{ mm}^{-1}$  (at  $t_0$ ) and  $18.6 \text{ mm}^{-1}$  (at  $t_{14}$  - stable storage)  
 439 as shown in Table 4 and Figure 9B2. It can be seen a clear decrease the  $SSA_{ice/starch}$  values  
 440 with the storage duration whatever the storage conditions. Once again, this decrease is  
 441 more pronounced in the case of stable storage temperatures. It can also correspond to a  
 442 growth in the size of ice crystals, even if this increase is not well pronounced on the  
 443 local thickness values in Figure 10A1, B1 and C1.



444  
 445 **Figure 9:** (A) Specific Surface Area of the air/ice interface  $SSA_{air/ice}$  (B) Specific Surface Area of the  
 446 ice/starch interface  $SSA_{ice/starch}$ . (A1, B1) for samples obtained in the fast freezing conditions and (A2,  
 447 B2) for samples obtained in the slow freezing conditions.



448

449 **Figure 10:** Cumulative distribution of local thickness of. (1) ice inside the matrix and (2) ice outside  
 450 the matrix after (A) freezing, (B) 7 days of storage and (C) 14 days of storage, both for stable and  
 451 fluctuating storage temperatures. The standard deviations of the distributions are represented by the  
 452 shaded areas.

## 453 **IV. Discussion**

### 454 **IV.1. Water migration and redistribution**

455 Sponge cake samples were visualized using synchrotron X-ray  $\mu$ CT. The samples were first  
456 frozen at two different freezing rates (fast and slow freezing). Figures 6A1, 6B1, 7A1 and 7B1  
457 clearly evidenced that the freezing rate has an impact on ice formation and location. Fast  
458 freezing leads to the formation of numerous small ice crystals homogeneously distributed inside  
459 the matrix and to a thin ice layer at the pore walls while slow freezing leads to the formation of  
460 bigger ice crystals heterogeneously distributed and to a thick ice layer at the pore surface. In  
461 addition, the volume fractions of ice inside the matrix ( $43.3 \pm 2.0$  % for fast freezing and  $24.1$   
462  $\pm 1.0$  % for slow freezing) and  $SSA_{ice/starch}$  ( $50.0 \pm 9.7$   $\text{mm}^{-1}$  for fast freezing and  $27.0 \pm 3.0$   
463  $\text{mm}^{-1}$  for slow freezing) displayed in Table 3 and Table 4, respectively, highlights a second  
464 finding. They show that ice was mainly formed inside the matrix when the product was frozen  
465 quickly whereas it was largely located inside the pores for slow freezing. This corroborates our  
466 earlier reports (Zennoune et al. 2021) in which we argued that both nucleation and ice crystal  
467 growth are controlled by the freezing rate through heat transfer (thermal resistance) and  
468 moisture transfer (freezable water diffusion). In addition, the high porosity of the model sponge  
469 cake affects water mobility and ice distribution during freezing. On the one hand, the fast  
470 freezing rate favors the creation of ice nuclei with low critical size but limits the water diffusion  
471 for ice crystal growth and towards the pores, leading thus to the formation of numerous small  
472 ice crystals, especially inside the matrix. On the other hand, during slow freezing, the nucleation  
473 phenomenon is limited due to the high critical size of nuclei, but water has enough time to  
474 diffuse through the matrix and towards the pores, resulting in fewer but large ice crystals inside  
475 the matrix and a thick ice layer at the pores walls.

476 After freezing, both quickly and slowly frozen samples were stored at two different conditions  
477 (stable and fluctuating temperatures) for two weeks.

478 In the case of fast freezing, comparison of images of Figures 6A1 to A5 and Figures 7A1 and  
479 A2 reveals that both ice morphology and size seem to evolve with storage time, even though  
480 ice distribution inside and outside the matrix seem to be the same. Moreover, this evolution is  
481 more noticeable for stable storage temperature than for fluctuating temperature. These  
482 observations are in line with the evolutions of both the volume fractions of the ice inside the  
483 matrix (Table 3) and the  $SSA_{ice/starch}$  (Table 4). As a matter of fact, volume fractions of the ice  
484 inside did not vary significantly with storage time and storage temperature conditions (Table 3,  
485  $p > 0.05$ ) while the  $SSA_{ice/starch}$  significantly decreases with storage time whatever the storage  
486 condition (Table 4,  $p < 0.05$ ). These results show that ice migration from the starch matrix  
487 towards the pores is limited or inexistent under these conditions. Instead, a local redistribution  
488 of water within the starch matrix seems to happen, resulting in crystal enlargement and crystal  
489 number decrease accompanied by mass conservation. This result may be explained by  
490 recrystallization phenomena driven by differences in the stability of ice crystals as a function  
491 of their size. In the case of fast freezing, the crystalline system is mainly constituted of small  
492 crystals, which have high free surface energy and are energetically unstable. Therefore, the  
493 system will tend towards a lower energy state by forming larger ice crystals that are more stable.

494 Given the evolution of the ice inside the starch matrix, several recrystallization mechanisms  
495 may occur. These include, at first, recrystallization by accretion when two crystals are close  
496 enough to form a bridge and then grow into a single crystal. Then, Ostwald ripening may happen  
497 involving water molecules of the small ice crystals to detach gradually from the ice crystal  
498 surface and diffuse through the matrix before deposition on larger ice crystals, leading to a  
499 decrease in the number of small crystals and a growth of the larger ones.

500 Still in the case of fast freezing, it is noteworthy that the growth of ice crystal size inside the  
501 matrix with storage time is more pronounced for stable storage than for fluctuating storage  
502 condition. Indeed, Table 4 shows a high decrease of the  $SSA_{ice/starch}$  for stable storage (about  
503  $-23\%$  from  $t_0$  to  $t_{14}$ ) compared to that for fluctuating storage (about  $-7\%$  from  $t_0$  to  $t_{14}$ ). This  
504 result is confirmed by the local thickness analysis. In fact, measured local thicknesses of ice  
505 inside the matrix show a larger increase of ice crystal size for stable storage than for fluctuating  
506 storage condition. This result is not intuitive, given that it is well recognized that temperature  
507 fluctuations promote recrystallization and therefore crystal growth (Donhowe and Hartel 1996a,  
508 Donhowe and Hartel 1996b, Ndoye and Alvarez 2015). An explanation might be related to the  
509 heat transfers inside the starch matrix. Indeed, the fluctuation duration (1h at  $-5^\circ\text{C}$ ) may not be  
510 sufficient to significantly affect the microstructure inside the starch matrix. On the other hand,  
511 the fluctuating storage temperature seems to affect the evolution of ice outside the starch matrix  
512 (ice inside the pores). The local thickness of ice outside the matrix exhibits a noticeable increase  
513 with storage duration for fluctuating storage condition, while the evolution is less pronounced  
514 for stable storage condition (Figure 10B2 and C2). This trend is supported by the evolution of  
515 the  $SSA_{air/ice}$ , which is larger for fluctuating storage, compared to stable storage conditions after  
516 14 days of storage (Table 4). These results reveal an increase of the ice layer at the pore  
517 interface. Local water redistribution and ice resizing rather than ice migration, keeping in mind  
518 that no variation of ice volume fraction was observed (Table 3), can plausibly explain this ice  
519 size increase. Unlike the matrix, the pore phase is affected by temperature fluctuations which  
520 may favor migratory recrystallization (Ostwald ripening) at the pore interface by a sublimation-  
521 diffusion-condensation mechanism leading to larger ice crystal size and to a more stable state.

522 In the case of slow freezing, visual observations in Figures 6B1 to B5 and Figures 7B1 and B5  
523 show no discernible difference in the microstructure evolution with storage time for both stable  
524 and fluctuating storage scenarios. These observations are not in line with the changes of the  
525 volume fractions of ice crystals inside the matrix (Table 3), of the  $SSA_{ice/starch}$  (Table 4) and of  
526 the local thicknesses (Figure 10) during storage, for both storage conditions. Volume fractions  
527 of ice inside the matrix decreased significantly after 14 days ( $p < 0.05$ ) when the storage  
528 duration increase for stable as well as for fluctuating storage temperatures. Moreover, the  
529  $SSA_{ice/starch}$  exhibit large decreases with storage time both for stable (nearly  $-30\%$  from  $t_0$  to  
530  $t_{14}$ ) and fluctuating (around  $-20\%$  from  $t_0$  to  $t_{14}$ ) conditions. In addition, the increase of  $SSA_{air/ice}$   
531 values both for stable (about  $+4\%$  from  $t_0$  to  $t_{14}$ ) and for fluctuating (around  $+13\%$  from  $t_0$  to  
532  $t_{14}$ ) temperatures reveals ice size increase at the pore interfaces. These results reflect, at the  
533 same time, the decrease in the number of small ice crystals and the growth of large ice crystals  
534 inside the starch matrix and locally ice layer increase at the pore surface. On another note, these  
535 results are underpinned by the increase of local thickness with storage time both inside and

536 outside the matrix. Two hypotheses can be put forward to explain these findings: (i) a migration  
537 of water from the matrix to the pores where it is deposited on the starch surface or on the existing  
538 ice layer by sublimation-diffusion-condensation phenomenon (Ostwald ripening); (ii) a local  
539 redistribution of water in the matrix through recrystallization phenomena (mainly Ostwald  
540 ripening) leading to an enlargement of the ice crystals. The first hypothesis may explain both  
541 the decrease of ice volume fractions inside the matrix and the increase of the ice characteristic  
542 size in the pores after 14 days. The second hypothesis can plausibly explain the ice crystal  
543 growth inside the starch matrix. Local redistribution inside the starch matrix seems to be more  
544 important at stable temperature storage while water migration from the matrix towards the pores  
545 appears to be predominant in the case of fluctuating storage conditions. Chen et al. (2013)  
546 reported that during long-term storage significant water redistribution was noticed. This  
547 redistribution was accompanied by ice recrystallization and growth. Similar results were found  
548 by Eckardt et al. (2013) who studied bread and dough and by Kontogiorgos and Goff (2006)  
549 who studied flour-water mixture. In both cases, the authors explained ice crystal growth by  
550 recrystallization resulting in the destruction of the microstructure.

#### 551 **IV.2. Coupled effect of freezing rates and frozen storage conditions**

552 As mentioned previously, fast and slow freezing of the model sponge cake give rise to,  
553 respectively, small and coarse ice crystals inside the starch matrix. At the same time, more or  
554 less ice layer is formed at the pore interfaces depending on the freezing rate. In the case of slow  
555 freezing, the majority of the ice is formed at these pore interfaces reflecting a greater water  
556 migration from the matrix towards the pores. During frozen storage, the crystalline  
557 microstructure evolves regardless of the initial freezing rate and the storage conditions.  
558 However, the changes in microstructure are more significant when the products are slowly-  
559 frozen as evidenced by the larger decrease of the  $SSA_{ice/starch}$  compared to fast freezing (Table  
560 4). This means that when the same storage conditions are applied, ice crystals grow more in  
561 sponge cake frozen at slow freezing rate. This result is confirmed by the local thickness values  
562 depicted in Figure 10. For both ice locations (inside and outside the matrix), local thickness  
563 values are always higher for slow freezing than for fast freezing and for the three time points  
564 ( $t_0$ ,  $t_7$  and  $t_{14}$ ). As mentioned above, local water redistribution and ice resizing by  
565 recrystallization mechanisms are favored in fast frozen samples while water migration is added  
566 to the local redistribution in slowly frozen samples, explaining the greater changes observed in  
567 the latter. It is noteworthy that coupling fast freezing with poor storage conditions (fluctuating  
568 temperatures) leads to smaller ice crystals and less damage to the microstructure than  
569 combining slow freezing with good storage conditions (stable temperatures). This result can be  
570 explained by the fact that fast freezing initially leads to a more homogeneous ice crystal  
571 population than slow freezing. It is well known that homogeneous population is energetically  
572 more stable than heterogeneous population and therefore is less likely to undergo ice  
573 recrystallization (Hartel 1998, Kobayashi et al. 2018).

574 Moreover, it is important to point out that microstructural changes affect the texture of the  
575 sponge cake and consequently its quality. This impact is more pronounced as the size of the ice  
576 crystals is bigger. Under the conditions used in this study, the benefits of fast freezing are

577 reduced at the end of the two-week storage period, but the products still have a better quality  
578 than slowly-frozen samples. To our knowledge, such results about the combined effect of  
579 freezing and frozen storage conditions are reported for the first time. The combined effects of  
580 freezing rate and storage time and temperature on the quality of frozen porous foods was studied  
581 for bread dough and bread (Yi and Kerr 2009, Eckardt et al. 2013) and for sponge cake (Díaz-  
582 Ramírez et al. 2016, Van Bockstaele et al. 2021). However, none of these works has  
583 investigated pore and ice crystal evolution as impacted by the coupled freezing and frozen  
584 storage processes.

## 585 **V. Conclusion**

586 Freezing and frozen storage of sponge cake have an impact on ice formation and growth which  
587 can cause undesirable changes to the product. In this article, the impact of freezing rate and  
588 storage conditions on ice formation and location was studied. The analysis of synchrotron  $\mu$ CT  
589 images leads to the conclusion that the ice volume fraction (about 60%) obtained after freezing  
590 is conserved during frozen storage, whatever the storage conditions. However, ice redistribution  
591 and relocation occur during storage depending on both initial freezing and frozen storage  
592 conditions, as shown by the evolutions of volume fraction of ice inside the matrix, and the SSA  
593 values. During the whole storage period, slow freezing gives rise to a decrease of the volume  
594 fraction of ice inside the matrix (up to  $-13\%$ ) combined to the  $SSA_{ice/starch}$  values drop (up to  
595  $-30\%$ ) and the  $SSA_{air/ice}$  values increase (up to  $+13\%$ ) meaning ice local redistribution and ice  
596 migration from the matrix to pores. For fast freezing, no change in ice inside volume fraction  
597 was noticed, but the  $SSA_{ice/starch}$  values decreased (up to  $-23\%$ ) and the  $SSA_{air/ice}$  values  
598 increased (up to  $+11\%$ ) evidencing a local redistribution of ice both inside the matrix and in the  
599 pores without migration towards the pores. It was shown that fast freezing causes less  
600 microstructural changes, regardless of the storage conditions, even though the microstructure  
601 continues to evolve during frozen storage, due to ice migration and recrystallization.

602 Before this study, the mechanism of ice formation in porous foods according to the freezing  
603 process, as well as the mechanism of pore and ice crystal evolution during frozen storage were  
604 poorly understood. This work is an important step forward, but further investigations are still  
605 needed. For example, the impact of temperature fluctuations was not clearly evidenced,  
606 especially inside the starch matrix. It would be of interest to study the impact of more aggressive  
607 fluctuations to bring a better knowledge of the influence of temperature fluctuation. Another  
608 limitation of this study is related to the fact that each sample is different, even if their mean  
609 porosity and water content was similar. Following the microstructural evolution of the same  
610 sample during freezing and storage should limit the effect of heterogeneity arising from the  
611 sampling location in the sponge cake. In addition, it would be useful to formalize the link  
612 between the evolution of the microstructure and the classical quality criteria such as texture or  
613 drip loss.

614 The freezing process and the frozen storage are the main components of the frozen cold chain.  
615 A better understanding of the mechanisms involved during these two steps would be very useful



616 for a better control of microstructure and induced quality changes of frozen products throughout  
617 the cold chain.

## 618 **Acknowledgments**

619 We would like to thank Alain Denis from UR FRISE INRAE for his technical help. We  
620 acknowledge SOLEIL for provision of synchrotron radiation facilities on ANATOMIX and  
621 financial support concerning the proposal study number 20200227. ANATOMIX is an  
622 Equipment of Excellence (EQUIPEX) funded by the *Investments for the Future* program of the  
623 French National Research Agency (ANR), project *NanoimagesX*, grant no. ANR-11-EQPX-  
624 0031. The authors would like to thank Guillaume Daniel of the ANATOMIX team for technical  
625 support at the beamline. We acknowledge also CNRM/CEN which is part of the LabEx  
626 OSUG@2020 (*Investments for the Future* program, grant agreement ANR10 LABX56) and  
627 3SR Laboratory which is part of the LabEx Tec21 (*Investments for the Future* program, grant  
628 agreement ANR11 LABX0030).

629 **Conflict of Interest:** The authors declare no conflict of interest. The funders had no role in the  
630 design of the study; in the collection, analyses, or interpretation of data; in the writing of the  
631 manuscript, or in the decision to publish the results.

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