



HAL
open science

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

Amira Zennoune, Hayat Benkhelifa, Frédéric Flin, Fatou-Toutie Ndoye, Jonathan Perrin, Timm Weitkamp, Mario Scheel, Pierre Latil, Christian Geindreau

► **To cite this version:**

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-04152933v1>

Submitted on 5 Jul 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

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

3 **Amira Zennoune ^{1,2}, Pierre Latil ³, Frederic Flin ³, Jonathan Perrin⁴, Timm Weitkamp ⁴, Mario**
4 **Scheel⁴, Christian Geindreau ⁵, Hayat Benkhelifa ^{1,2} and Fatou-Toutie Ndoye ^{1*}**

5 ¹ Université Paris-Saclay, INRAE, UR FRISE, F-92761 Antony, France; amira.zennoune@inrae.fr; fatou-
6 toutie.ndoye@inrae.fr

7 ² Université Paris-Saclay, INRAE, AgroParisTech, 75005 Paris, France, hayat.benkhelifa@agroparistech.fr

8 ³ Univ. Grenoble Alpes, Université de Toulouse, Météo-France, CNRS, CNRM, Centre d'Etudes de la Neige,
9 Grenoble, France; pierre.latil@meteo.fr; frederic.flin@meteo.fr

10 ⁴ Synchrotron SOLEIL, Gif sur Yvette, France; timm.weitkamp@synchrotron-soleil.fr; mario.scheel@
11 synchrotron-soleil.fr; jonathan.perrin@synchrotron-soleil.fr

12 ⁵ Université Grenoble Alpes, Grenoble INP, 3SR, CNRS, Grenoble, France; christian.geindreau@3sr-grenoble.fr

13 * Correspondence: fatou-toutie.ndoye@inrae.fr

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⁻¹ and
19 slow: 0.3 °C min⁻¹), 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 μCT) 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 μ CT can overcome these limitations. X-ray μ 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 μ CT can be fully taken advantage of. However, these works were
155 performed using laboratory scale X-ray μ 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 μ 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 μ -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 °C min^{-1}) using an air blast freezer,
- 197 - Slow freezing (0.3 °C min^{-1}) using a static chest freezer

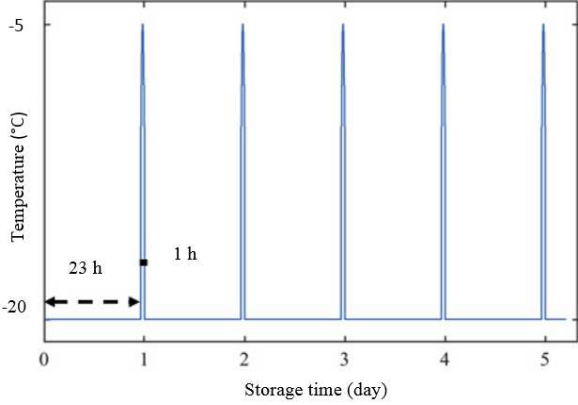
198 **II.2. Frozen storage conditions and analysis time points**

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

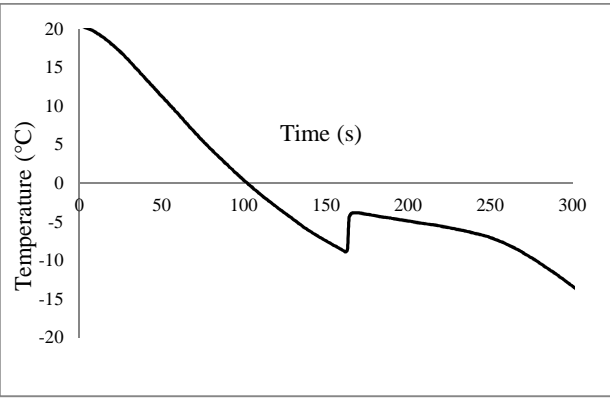
202 temperature controller with one set point (Eliwell WM961, Villeneuve la Garenne,
 203 France). The samples were placed into a 3 cm thick expanded polystyrene box for
 204 insulation in order to attenuate the temperature fluctuations related to the on/off
 205 operation of the refrigeration system.

- 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 ⁻¹		Slow freezing at 0.3 °C min ⁻¹	
	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 ₁₄	4	4	4	4
t ₇	4	4	4	4
t ₀	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.

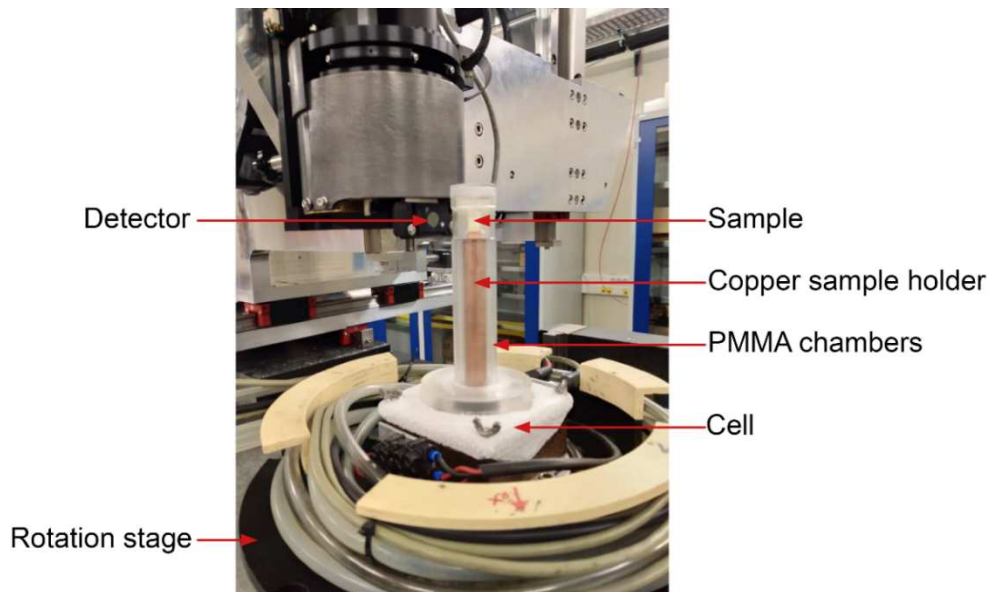
Sponge cake apparent density (kg/m ³)	Porosity (%)	Water content (%)	Freezing point (°C)	Freezable water content (%)
392 ± 15	56 ± 2	60 ± 1.5	-0.52 ± 0.09	44 ± 5.3

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/m³) 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 μ 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.

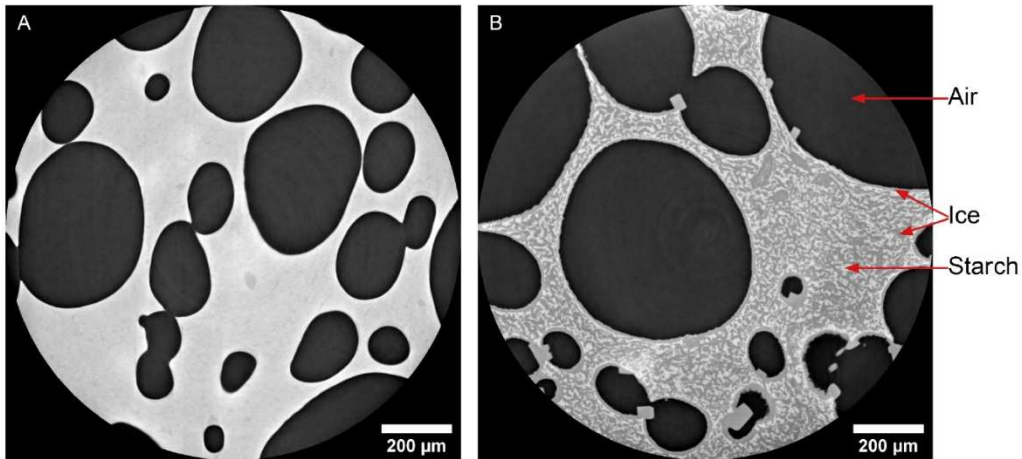


258

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 keV was used; the
267 distance between sample and detector was 30 mm . For each volume scan, 2000 projections
268 were taken over an angular range of 180° 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 mm for a diameter of 1.3 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

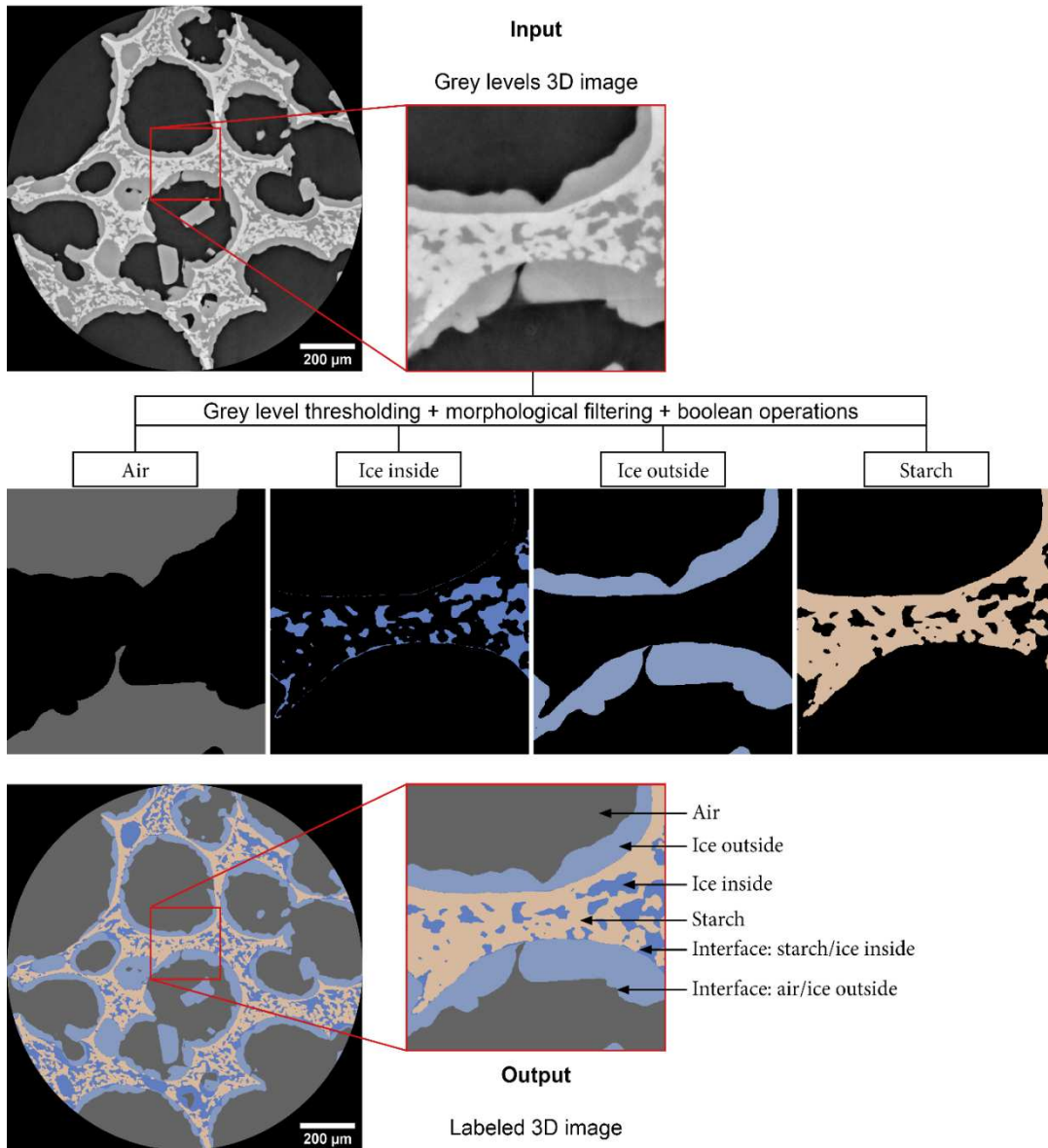
Figure 4: X-ray μ CT slices of (A) unfrozen and (B) frozen model sponge cake samples.

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_{air} , $V_{\text{ice}} =$
 298 $V_{\text{ice inside}} + V_{\text{ice outside}}$ and V_{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:

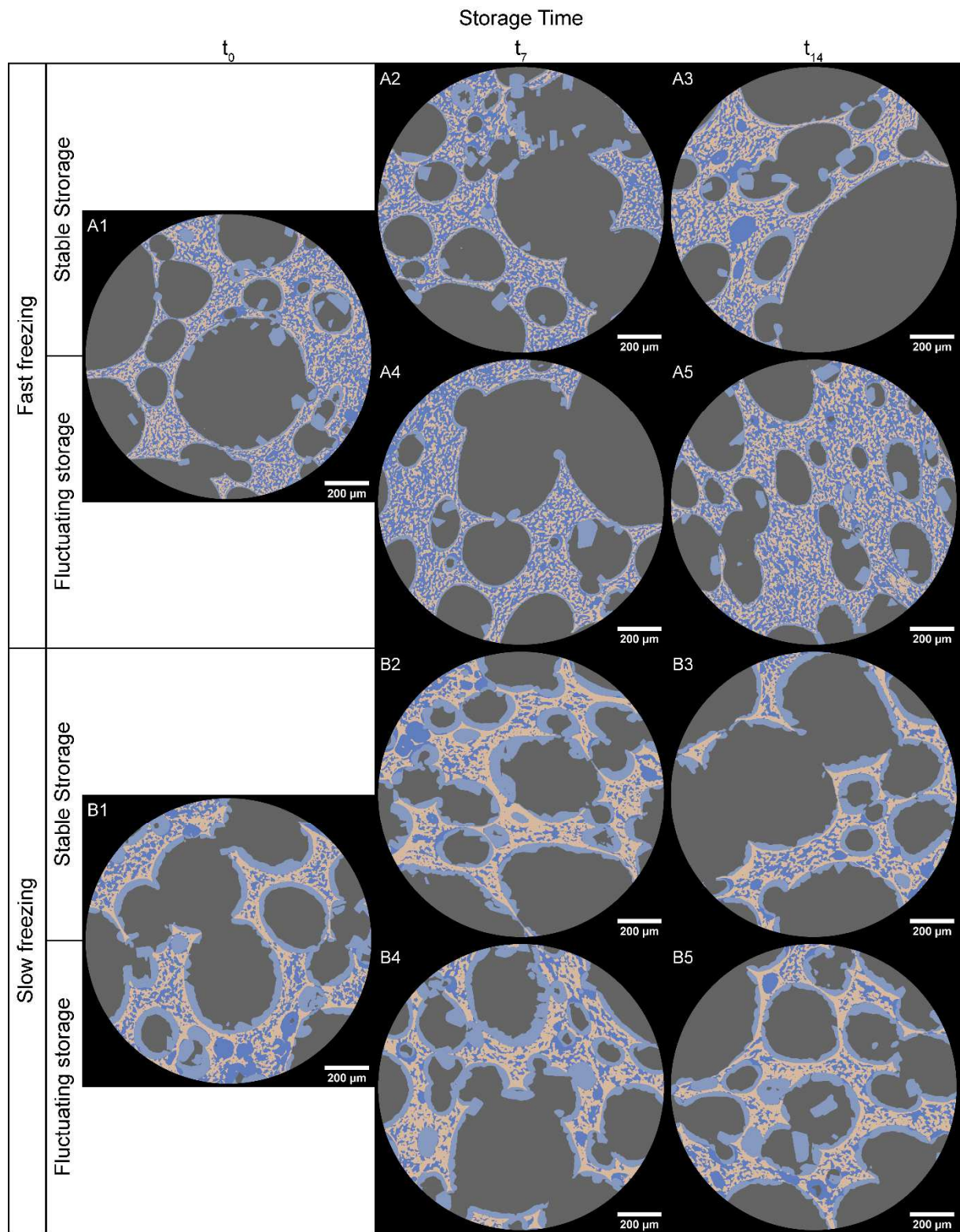
- 345 - The ice and starch structures are coarser at t_7 and t_{14} than at t_0 for both stable and
346 fluctuating storage conditions
- 347 - The ice layer at the air/starch interface is thicker at t_7 and t_{14} than at t_0 ,
- 348 - There are no visible significant differences of the microstructure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

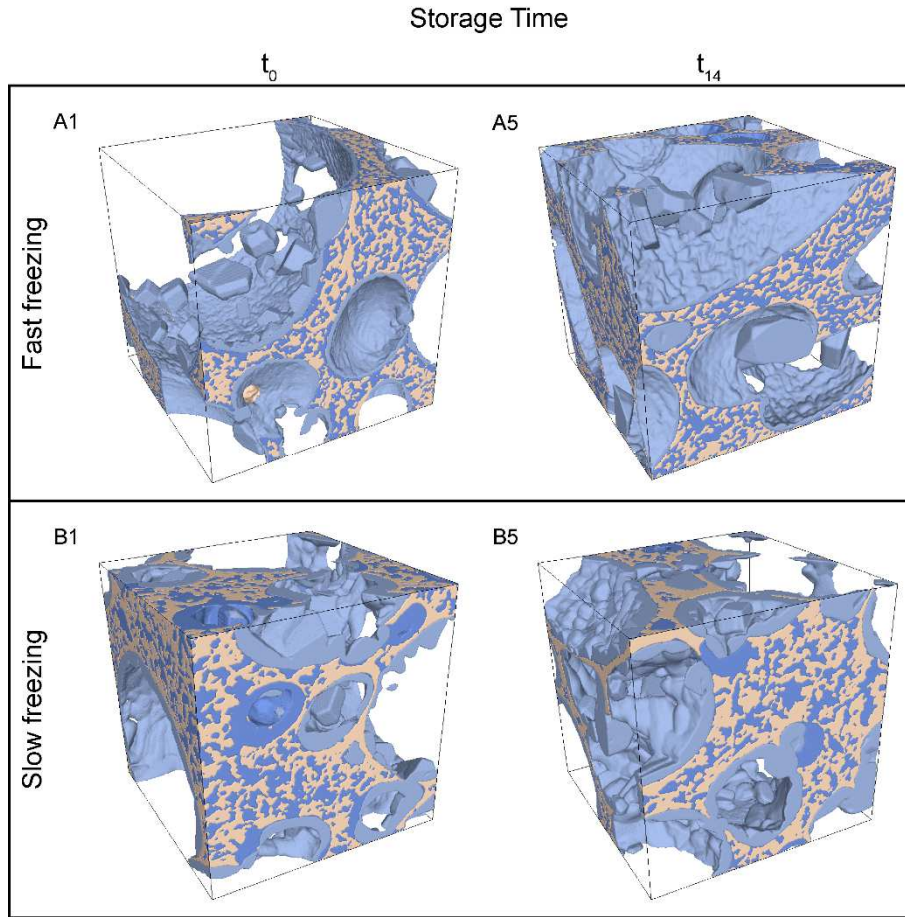
357 All these results could be explained by ice redistribution and water migration during storage as
358 will be discussed in section IV. However, it is worth noting that the qualitative analysis requires
359 a complementary quantitative characterization to establish reliable conclusions.

360



361

362 **Figure 6:** Synchrotron X-ray μ 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 μ 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 μ 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.

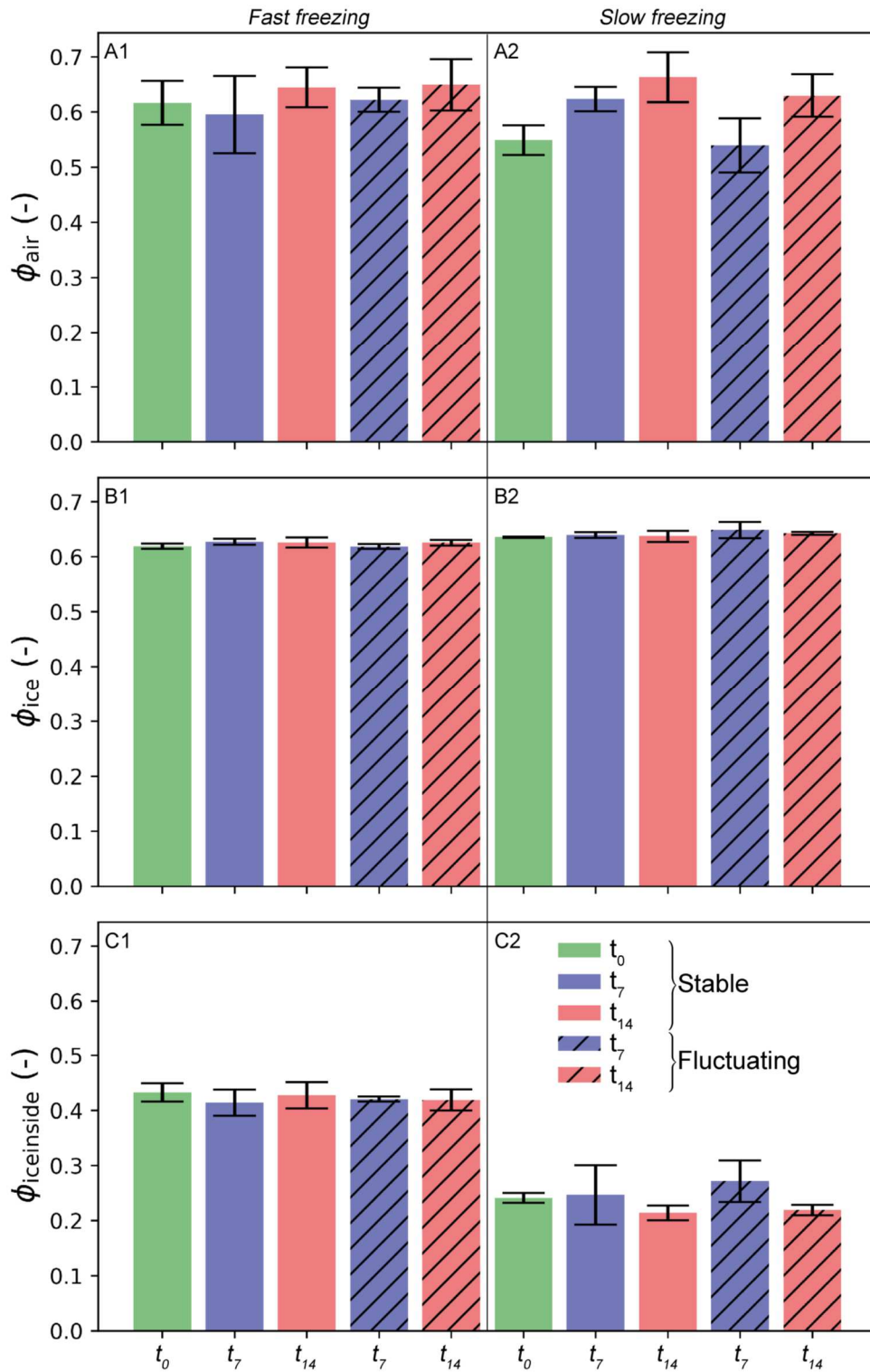
374 **Table 3:** Mean volume fraction of the different phases (air, ice and ice inside) for different conditions.

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

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 ± 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 ± 0.9^a	50.0 ± 9.7^a	7.7 ± 1.0^a	27.0 ± 3.0^a
Stable storage	7	8.6 ± 2.4^a	45.2 ± 14.9^a	8.5 ± 3.4^a	23.4 ± 7.1^a
	14	7.4 ± 1.7^a	38.6 ± 9.5^b	8.0 ± 2.0^a	18.6 ± 5.4^b
Fluctuating storage	7	7.9 ± 0.4^a	48.5 ± 5.3^a	7.7 ± 0.7^a	26.1 ± 2.9^a
	14	8.7 ± 1.0^b	46.3 ± 4.9^c	8.7 ± 0.4^b	21.0 ± 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, $p > 0.05$.

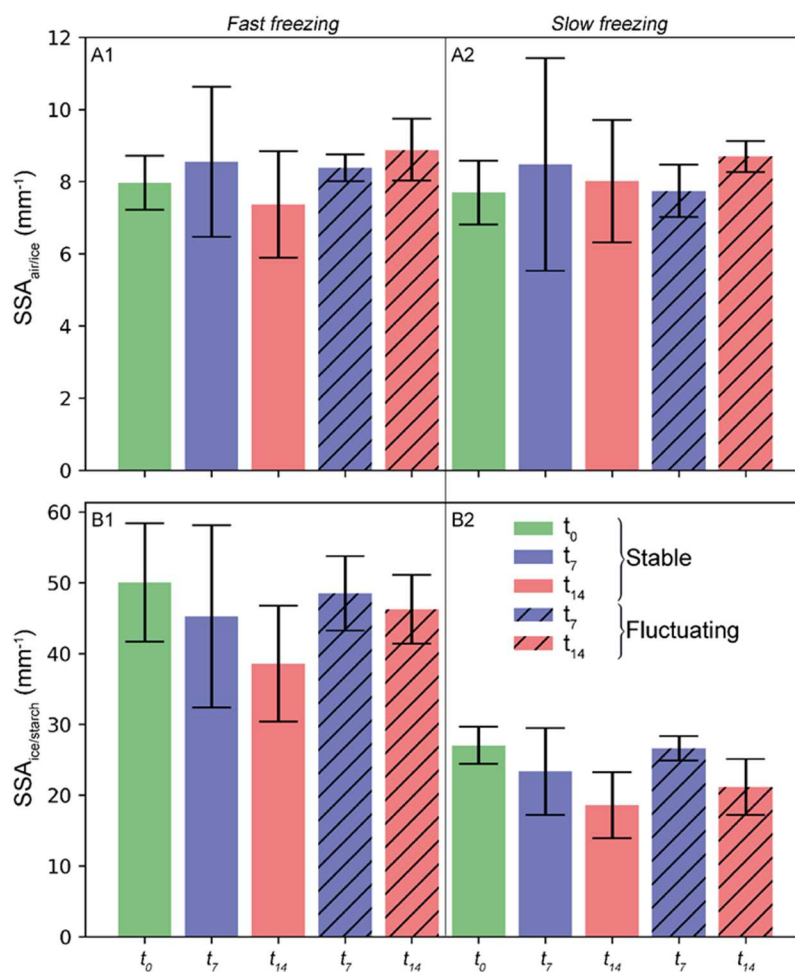
409 For fast frozen samples:

410 - Table 4 and Figure 9A1 show that the $SSA_{air/ice}$ exhibit values between 7.4 mm^{-1} (stable
 411 storage - t_{14}) and 8.7 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.

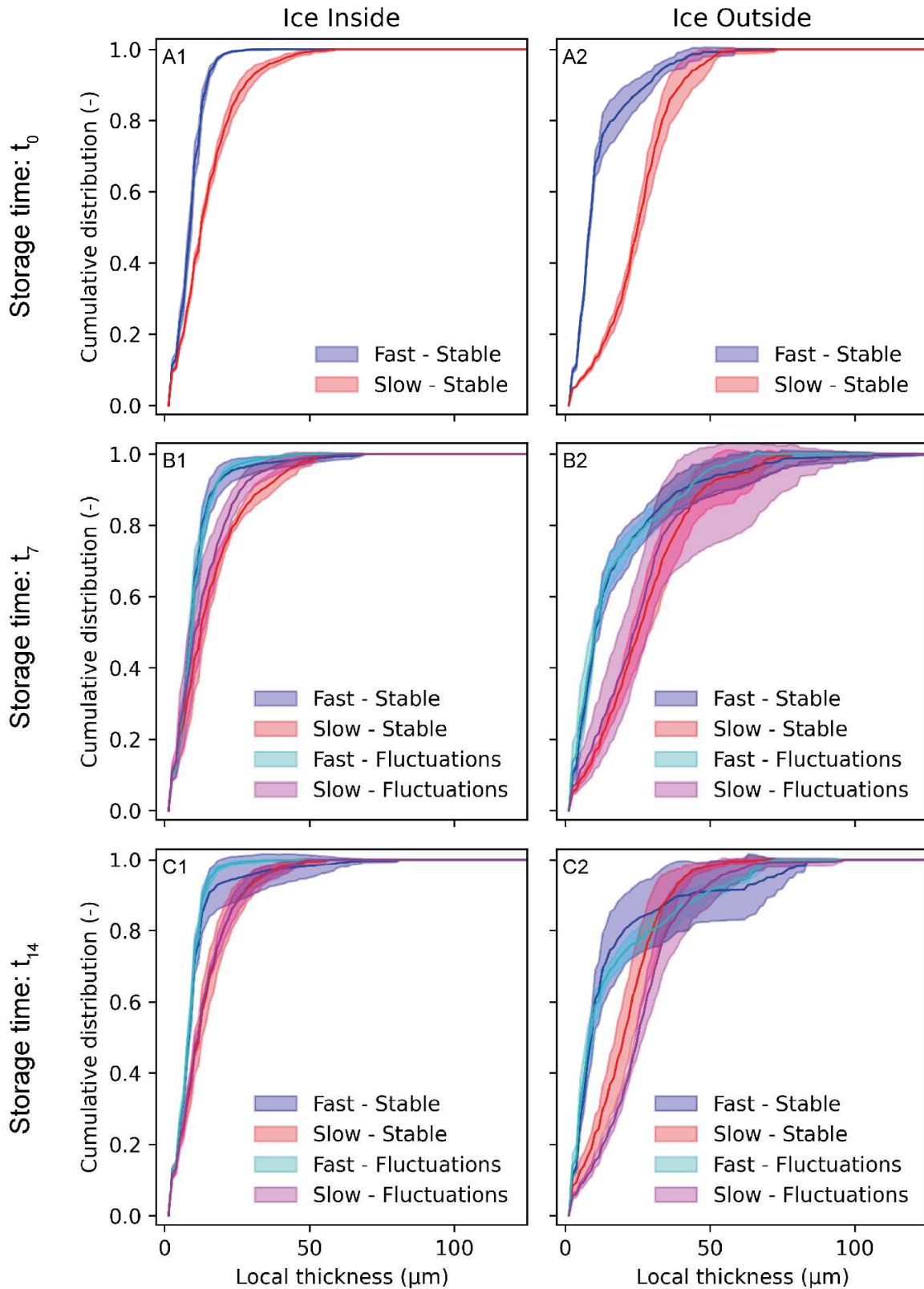
424 - The $SSA_{ice/starch}$ ranges between 50.0 mm^{-1} (for t_0) and 38.6 mm^{-1} (stable storage - t_{14})
 425 (Table 4 and Figure 9B1). The $SSA_{ice/starch}$ values tend to decrease with the storage
 426 duration regardless the storage conditions (stable or fluctuating). This decrease of the
 427 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 mm^{-1} (at t_0) and 8.7 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 mm^{-1} (at t_0) and 18.6 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 μ 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 ± 2.0 % for fast freezing and 24.1
462 ± 1.0 % for slow freezing) and $SSA_{ice/starch}$ (50.0 ± 9.7 mm^{-1} for fast freezing and 27.0 ± 3.0
463 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°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 μ 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.

References

- Alizadeh, E., Chapleau, N., de Lamballerie, M. and Le-Bail, A. (2007). *Effect of different freezing processes on the microstructure of Atlantic salmon (Salmo salar) fillets*. *Innovative Food Science & Emerging Technologies*, 8(4): 493-499.
- Anese, M., Mirolo, G., Beraldo, P. and Lippe, G. (2013). *Effect of ultrasound treatments of tomato pulp on microstructure and lycopene in vitro bioaccessibility*. *Food chemistry*, 136(2): 458-463.
- Baier-Schenk, A., Handschin, S. and Conde-Petit, B. (2005). *Ice in prefermented frozen bread dough—an investigation based on calorimetry and microscopy*. *Cereal Chemistry*, 82(3): 251-255.
- Ban, C., Yoon, S., Han, J., Kim, S. O., Han, J. S., Lim, S. and Choi, Y. J. (2016). *Effects of freezing rate and terminal freezing temperature on frozen croissant dough quality*. *Lwt-Food Science and Technology*, 73: 219-225.
- Bárceñas, M. a. E., Benedito, C. and Rosell, C. M. (2004). *Use of hydrocolloids as bread improvers in interrupted baking process with frozen storage*. *Food Hydrocolloids*, 18(5): 769-774.
- Bárceñas, M. E. and Rosell, C. M. (2006). *Effect of frozen storage time on the bread crumb and aging of par-baked bread*. *Food Chemistry*, 95(3): 438-445.
- Bevilacqua, A., Zaritzky, N. and Calvelo, A. (1979). *Histological measurements of ice in frozen beef*. *International Journal of Food Science & Technology*, 14(3): 237-251.
- Bousquière, J. (2017). *Impact de la composition et des procédés sur la réactivité d'un produit modèle alvéolé de type cake*, Université Paris-Saclay.
- Brownlee, K. A. (1965). *Statistical theory and methodology in science and engineering*. New York, John Wiley and Sons.
- Calonne, N., Flin, F., Geindreau, C., Lesaffre, B. and Rolland du Roscoat, S. (2014). *Study of a temperature gradient metamorphism of snow from 3-D images: time evolution of microstructures, physical properties and their associated anisotropy*. *The Cryosphere*, 8(6): 2255-2274.
- Calonne, N., Flin, F., Lesaffre, B., Dufour, A., Roulle, J., Puglièse, P., Philip, A., Lahoucine, F., Geindreau, C., Panel, J.-M., du Roscoat, S. R. and Charrier, P. (2015). *CellDyM: A room temperature operating cryogenic cell for the dynamic monitoring of snow metamorphism by time-lapse X-ray microtomography*. *Geophysical Research Letters*, 42(10): 3911-3918.
- Chassagne-Berces, S., Poirier, C., Devaux, M. F., Fonseca, F., Lahaye, M., Pigorini, G., Girault, C., Marin, M. and Guillon, F. (2009). *Changes in texture, cellular structure and cell wall composition in apple tissue as a result of freezing*. *Food Research International*, 42(7): 788-797.
- Chen, G., Jansson, H., Lustrup, K. F. and Swenson, J. (2012). *Formation and distribution of ice upon freezing of different formulations of wheat bread*. *Journal of Cereal Science*, 55(3): 279-284.
- Chen, G., Öhgren, C., Langton, M., Lustrup, K. F., Nydén, M. and Swenson, J. (2013). *Impact of long-term frozen storage on the dynamics of water and ice in wheat bread*. *Journal of cereal science*, 57(1): 120-124.
- Díaz-Ramírez, M., Calderón-Domínguez, G., Salgado-Cruz, M. d. I. P., Chanona-Pérez, J. J., Andraca-Adame, J. A. and Ribotta, P. D. (2016). *Sponge cake microstructure, starch retrogradation and quality changes during frozen storage*. *International Journal of Food Science & Technology*, 51(8): 1744-1753.
- Donhowe, D. P. and Hartel, R. W. (1996). *Recrystallization of ice during bulk storage of ice cream*. *International Dairy Journal*, 6(11-12): 1209-1221.
- Donhowe, D. P. and Hartel, R. W. (1996a). *Recrystallization of ice in ice cream during controlled accelerated storage*. *International Dairy Journal*, 6(11): 1191-1208.
- Donhowe, D. P. and Hartel, R. W. (1996b). *Recrystallization of ice during bulk storage of ice cream*. *International Dairy Journal*, 6(11): 1209-1221.
- Eckardt, J., Öhgren, C., Alp, A., Ekman, S., Åström, A., Chen, G., Swenson, J., Johansson, D. and Langton, M. (2013). *Long-term frozen storage of wheat bread and dough—Effect of time, temperature and fibre on sensory quality, microstructure and state of water*. *Journal of Cereal Science*, 57(1): 125-133.

- Eckardt, J., Öhgren, C., Alp, A., Ekman, S., Åström, A., Chen, G., Swenson, J., Johansson, D. and Langton, M. (2013). *Long-term frozen storage of wheat bread and dough – Effect of time, temperature and fibre on sensory quality, microstructure and state of water*. *Journal of Cereal Science*, 57(1): 125-133.
- Esselink, E. F., van Aalst, H., Maliepaard, M. and van Duynhoven, J. P. (2003). *Long-term storage effect in frozen dough by spectroscopy and microscopy*. *Cereal chemistry*, 80(4): 396-403.
- Haffar, I., Flin, F., Geindreau, C., Petillon, N., Gervais, P.-C. and Edery, V. (2021). *X-ray tomography for 3D analysis of ice particles in jet A-1 fuel*. *Powder Technology*, 384: 200-210.
- Haffar, I., Flin, F., Geindreau, C., Petillon, N., Gervais, P.-C. and Edery, V. (2022). *3D microstructure evolution of ice in jet A-1 fuel as a function of applied temperature over time*. *International Journal of Heat and Mass Transfer*, 183: 122158.
- Hagiwara, T., Hartel, R. W. and Matsukawa, S. (2006). *Relationship between recrystallization rate of ice crystals in sugar solutions and water mobility in freeze-concentrated matrix*. *Food Biophysics*, 1(2): 74-82.
- Hartel, R. W. (1998). *Mechanisms and kinetics of recrystallization in ice cream. The properties of water in foods ISOPOW 6*, Springer: 287-319.
- Kobayashi, R., Kimizuka, N., Suzuki, T., Watanabe, M. and Int Inst, R. (2014). *Effect of supercooled freezing methods on ice structure observed by X-ray CT*. 3rd IIR International Conference on Sustainability and the Cold Chain. Paris, Int Inst Refrigeration. **2014**: 392-396.
- Kobayashi, R., Kimizuka, N., Watanabe, M., Takenaga, F. and Suzuki, T. (2018). *Property Changes during Frozen Storage in Frozen Soy Bean Curds Prepared by Freezing Accompanied with Supercooling*. *Transactions of the Japan Society of Refrigerating and Air Conditioning Engineers*, 35(3): 269.
- Kontogiorgos, V. and Goff, H. D. (2006). *Calorimetric and microstructural investigation of frozen hydrated gluten*. *Food Biophysics*, 1(4): 202-215.
- Legland, D., Arganda-Carreras, I. and Andrey, P. (2016). *MorphoLibJ: integrated library and plugins for mathematical morphology with ImageJ*. *Bioinformatics*, 32(22): 3532-3534.
- Lucas, T., Le Ray, D. and Davenel, A. (2005). *Chilling and freezing of part-baked bread. Part I: An MRI signal analysis*. *Journal of food engineering*, 70(2): 139-149.
- Masselot, V., Bosc, V. and Benkhelifa, H. (2021). *Analyzing the microstructure of a fresh sorbet with X-ray micro-computed tomography: Sampling, acquisition, and image processing*. *Journal of Food Engineering*, 292: 110347.
- Medebacha, A., Pinzera, B. R., Dubois, C., Limbach, H. J., Schneebelic, M. and Stampanonia, M. (2013). *X-ray phase contrast tomography of ice cream : A time-lapse study*. *Food Symposium*. Leuven, Belgium.
- Mirone, A., Gouillart, E., Brun, E., Tafforeau, P. and Kieffer, J. (2013). *The PyHST2 hybrid distributed code for high speed tomographic reconstruction with iterative reconstruction and a priori knowledge capabilities*. *Nuclear Instruments and Methods in Physics Research Section B Beam Interactions with Materials and Atoms*, 324.
- Mousavi, R., Miri, T., Cox, P. W. and Fryer, P. J. (2005). *A novel technique for ice crystal visualization in frozen solids using X-ray micro-computed tomography*. *Journal of Food Science*, 70(7): E437-E442.
- Mousavi, R., Miri, T., Cox, P. W. and Fryer, P. J. (2007). *Imaging food freezing using X-ray microtomography*. *International Journal of Food Science and Technology*, 42(6): 714-727.
- Mulot, V. (2019). *Caractérisation expérimentale et modélisation multi-échelles des transferts thermiques et d'eau lors de la congélation des produits alimentaires*, Institut agronomique, vétérinaire et forestier de France.
- Mulot, V., Fatou-Toutie, N., Benkhelifa, H., Pathier, D. and Flick, D. (2019). *Investigating the effect of freezing operating conditions on microstructure of frozen minced beef using an innovative X-ray micro-computed tomography method*. *Journal of Food Engineering*, 262: 13-21.
- Ndoye, F. T. and Alvarez, G. (2015). *Characterization of ice recrystallization in ice cream during storage using the focused beam reflectance measurement*. *Journal of Food Engineering*, 148: 24-34.

Otsu, N. (1979). *A Threshold Selection Method from Gray-Level Histograms*. IEEE Transactions on Systems, Man and Cybernetics, SMC-9(1): 62-66.

Paganin, D., Mayo, S. C., Gureyev, T. E., Miller, P. R. and Wilkins, S. W. (2002). *Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object*. Journal of microscopy, 206(1): 33-40.

Pham, Q. T. (2006). *Modelling heat and mass transfer in frozen foods: a review*. International Journal of Refrigeration, 29(6): 876-888.

Pinzer, B. R., Medebach, A., Limbach, H. J., Dubois, C., Stampanoni, M. and Schneebeli, M. (2012). *3D-characterization of three-phase systems using X-ray tomography: tracking the microstructural evolution in ice cream*. Soft Matter, 8(17): 4584-4594.

Pronk, P., Ferreira, C. I. and Witkamp, G.-J. (2005). *A dynamic model of Ostwald ripening in ice suspensions*. Journal of Crystal Growth, 275(1-2): e1355-e1361.

Roca, E., Guillard, V., Guilbert, S. and Gontard, N. (2006). *Moisture migration in a cereal composite food at high water activity: Effects of initial porosity and fat content*. Journal of Cereal Science, 43(2): 144-151.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S. and Schmid, B. (2012). *Fiji: an open-source platform for biological-image analysis*. Nature methods, 9(7): 676-682.

Silvas-García, M., Ramírez-Wong, B., Torres-Chávez, P., Carvajal-Millan, E., Barrón-Hoyos, J., Bello-Pérez, L. A. and Quintero-Ramos, A. (2014). *Effect of freezing rate and storage time on gluten protein solubility, and dough and bread properties*. Journal of Food Process Engineering, 37(3): 237-247.

Ullah, J., Takhar, P. S. and Sablani, S. S. (2014). *Effect of temperature fluctuations on ice-crystal growth in frozen potatoes during storage*. LWT - Food Science and Technology, 59(2, Part 1): 1186-1190.

Van Bockstaele, F., Debonne, E., De Leyn, I., Wagemans, K. and Eeckhout, M. (2021). *Impact of temporary frozen storage on the safety and quality of four typical Belgian bakery products*. LWT, 137: 110454.

van Dalen, G., Don, A., Nootenboom, P. and C G Blonk, J. (2009). *Determination of bubbles in foods by X-ray microtomography and image analysis*. SkyScan Micro-CT user meeting. Ghent, Belgium: 15-23.

van Dalen, G., Koster, M., Nijse, J., Boller, E. and van Duynhoven, J. (2013). *3D imaging of freeze-dried vegetables using X-ray microtomography*. Micro-CT user meeting SkyScan, Hasselt, Belgium.

Vicent, V., Ndoye, F.-T., Verboven, P., Nicolai, B. and Alvarez, G. (2019). *Effect of dynamic storage temperatures on the microstructure of frozen carrot imaged using X-ray micro-CT*. Journal of Food Engineering, 246: 232-241.

Vicent, V., Ndoye, F. T., Verboven, P., Nicolai, B. and Alvarez, G. (2016). *Applicability of X-ray Microtomography for Characterizing the microstructure of Frozen Apple during Storage*. 4th IIR International Conference on Sustainability and the Cold Chain. A. East, J. Carson, D. Cleland and R. Love. Paris, Int Inst Refrigeration: 414-420.

Vicent, V., Verboven, P., Ndoye, F.-T., Alvarez, G. and Nicolai, B. (2017). *A new method developed to characterize the 3D microstructure of frozen apple using X-ray micro-CT*. Journal of Food Engineering, 212(Supplement C): 154-164.

Voda, A., Homan, N., Witek, M., Duijster, A., van Dalen, G., van der Sman, R., Nijse, J., van Vliet, L., Van As, H. and van Duynhoven, J. (2012). *The impact of freeze-drying on microstructure and rehydration properties of carrot*. Food Research International, 49(2): 687-693.

Weitkamp, T., Scheel, M., Giorgetta, J., Joyet, V., Le Roux, V., Cauchon, G., Moreno, T., Polack, F., Thompson, A. and Samama, J. (2017). *The tomography beamline ANATOMIX at Synchrotron SOLEIL*. Journal of Physics: Conference Series, IOP Publishing.

Yi, J. and Kerr, W. L. (2009). *Combined effects of freezing rate, storage temperature and time on bread dough and baking properties*. LWT-Food Science and Technology, 42(9): 1474-1483.

Zennoune, A., Latil, P., Ndoye, F.-T., Flin, F., Perrin, J., Geindreau, C. and Benkhelifa, H. (2021). *3D Characterization of Sponge Cake as Affected by Freezing Conditions Using Synchrotron X-ray Microtomography at Negative Temperature*. *Foods*, 10(12): 2915.

Zhao, Y. and Takhar, P. S. (2017). *Micro X-ray computed tomography and image analysis of frozen potatoes subjected to freeze-thaw cycles*. *LWT - Food Science and Technology*, 79: 278-286.