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# 1           **Impacts of the Baking Heating Rate on the Water Mobility, Starch** 2           **Microstructure and Mechanical Properties of Degassed Crumb During** 3           **Staling**

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## 11   **Abstract**

12   In this study, the impacts of different heating rates (HRs) from 6 to 40°C/min were  
13   investigated in terms of the <sup>1</sup>H proton water mobility, starch microstructure, texture, soluble  
14   amylose (AM), amylopectin (AP) retrogradation and AM crystallization during staling. A  
15   degassed breadcrumb baked in a miniaturized baking system was used to ensure better time-  
16   temperature control during baking. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) data  
17   showed that the evolution of the proton *T*<sub>2</sub> relaxation time decreased with increasing HR  
18   during staling. The amount of soluble AM, AP retrogradation degree, AM crystallization  
19   degree and crumb firmness tend to increase at higher HR. Environmental scanning electron  
20   microscopy (ESEM) observations showed that most starch granules in crumbs treated at  
21   higher HR exhibited strong deformation and disruption. These results confirmed that the  
22   increase in crumb firmness during staling with increasing HR was related to a higher level of  
23   starch disruption. This yielded a higher degree of separation between the AM and AP, which  
24   resulted in more pronounced water trapping in the starch crystals. The resulting dehydration

25 of the matrix (gluten network, with AM gel surrounding of starch granules) yielded a loss in  
26 crumb softness, and crumb baked at higher HR tended to have a firmer texture and faster  
27 staling.

28 **Keywords:** Staling, heating rate, AP retrogradation, AM crystallization, <sup>1</sup>H NMR mobility,  
29 firmness.

30 **Abbreviations:** Heating rate (HR), amylose (AM), amylopectin (AP), proton nuclear  
31 magnetic resonance (<sup>1</sup>H NMR), Environmental scanning electron microscopy (ESEM),  
32 conventional baking (CVB), Young's modulus (E), dry flour (d.f.).

### 33 **1. Introduction**

34 Different parameters related to the baking process, such as the baking temperature,  
35 heating rate (HR), baking duration and temperature beyond the gelatinization temperature  
36 affect the texture and staling rate of bread. The HR potentially affects access to water and  
37 therefore the swelling of starch granules and the amount of leached amylose (AM) (Besbes et  
38 al., 2014). Hence, the HR may influence the evolution of the firmness during staling (Besbes  
39 et al., 2014; Palav and Seetharaman, 2007). Besbes et al., (2016) studied the effect of two  
40 HRs on bread staling corresponding to the case of conventional baking (CVB) at 4.67°C/min  
41 and 6.31°C/min for oven temperatures of 220°C and 240°C, respectively. It was observed that  
42 a higher HR resulted in a higher moisture loss and an increased crumb firmness. Furthermore,  
43 Patel et al., (2005) investigated the effect of two HRs in CVB at 16.8°C/min and 6°C/min  
44 with different dough piece sizes. These authors found that staling was more pronounced at  
45 higher HRs (Patel and Seetharaman, 2006). AM crystallization seemed to be affected by the  
46 HR, and this parameter could be considered to monitor crumb staling and the crumb texture,  
47 as reported in previous literature. In addition, the HR affects the availability of water and the  
48 degree of starch granule disruption during baking, which affect the staling parameters.

49 The aim of this study was to assess the impact of the HR on bread crumb staling.  
50 Compared with the existing literature, this study considers a large range of HRs (6°C/min,  
51 20°C/min and 40°C/min) obtained by using a miniaturized baking system to allow the baking  
52 of degassed bread crumb. The properties of the crumb were evaluated to monitor different  
53 parameters related to the progression of staling, including the texture, AP retrogradation and  
54 AM crystallization (Chinachoti et al., 2013; Ribotta and Le Bail, 2007). Water mobility was  
55 assessed by low field NMR (Curti et al., 2017, 2013, 2011; Engelsen et al., 2001), and  
56 environmental scanning electron microscopy was used to examine the extent of starch granule  
57 disruption (Roman-Gutierrez et al., 2002). In addition to the large HR range tested, there is a  
58 novel focus on the amount of AM complexes formed during baking as a function of the HR  
59 with AM crystallization, the amount of soluble AM and water molecular mobility to  
60 investigate the impacts of the HR on starch granule disruption during baking.

## 61 **2. Material and methods:**

### 62 **2.1. Raw materials**

63 The white flour (Giraudineau, Saint-Colomban, France) used for the formulation had the  
64 following properties: 12% water, 10% protein, 1.4% total fat, 64.5% enzymatic starch, and  
65 0.6% ash with a falling number of 450 s. The Alveograph parameters (Chopin Technologies,  
66 Villneuve, France) were as follows: Deformation energy (W: 373.10<sup>4</sup>J), Ratio  
67 Tenacity/Extensibility (P/L: 1.48). The recipe was composed as follows: 100 g of flour, 51.43  
68 g of water, 4.43 g of compressed yeast (l'Hirondelle, Marquette-lez-Lille, France), 1.8 g of  
69 salt (Esco, Levallois-Perret, France), 3.03 g of rapeseed oil, 7.83 g of sugar (Saint Louis,  
70 Paris, France), 1.72 g of improvers (Wheat gluten and alpha-amylases,) purchased from  
71 Puratos, Rungis, France and 0.507 g of emulsifier (Mono and diglycerides of rapeseed fatty  
72 acid) and Xanthan gums (Cargill, Saint Nazaire, France).

## 73 **2.2. Bread-making procedure**

74 A batch of 500 g of ingredients was mixed in an alveograph mixer (Chopin Technologies,  
75 Villeneuve, France) for 7 min at 60 rpm (final temperature of 30°C). The dough was allowed to  
76 rest for 5 min, divided and molded manually to obtain dough pieces that were 30 g each. The  
77 dough pieces were placed in a fermentation cabinet (Hengel, Parigny, France) with a relative  
78 humidity of 80% for 90 min at 38°C. The dough expansion ratio at the end of fermentation  
79 was 3.5, corresponding to a conventional fermentation process. After being fermented, the  
80 dough was placed in a plastic pouch, which was placed in a vacuum sealer machine (Maxima  
81 Kitchen Equipment, Mijdrecht, Holland). Vacuum was applied for 1 min to remove all pores  
82 in the dough before the pouch was sealed. The pouch was flattened and installed in a  
83 miniature baking system composed of two Peltier elements (5 cm x 5 cm) (Le-Bail et al.,  
84 2009). The thickness and the parallelism of the dough sample were controlled by a metallic  
85 spacer (4 cm × 4 cm × 3 mm) located between the two Peltier elements. Three HRs were  
86 applied, namely, 6°C/min, 20°C/min or 40°C/min, until a baking plateau was reached at 98°C,  
87 which was held for 2 min (98°C is the plateau temperature of bread crumb during baking in  
88 conventional conditions), followed by cooling to 20°C in 10 min. At the end of the baking  
89 process, the samples were equilibrated for 40 min in a temperature-controlled cabinet at 20°C  
90 (Memmert, Schwabach, Germany). The degassed crumb samples were detached from the  
91 plastic pouch; disks of degassed baked crumb of 5 mm diameter were obtained using a  
92 cylindrical cutter and stored in Eppendorf tubes at 4°C in a temperature-controlled cabinet  
93 (Memmert, Schwabach, Germany) for 14 days. The baked crumb samples were analyzed for  
94 moisture content, firmness, AP and AM crystallization and soluble AM after 2 h and 1, 3, 7,  
95 and 10 days of storage.

96 **2.3. Moisture content (MC)**

97 The MC was determined according to the AACC method 44-15A (AACC, 1999). Two  
98 grams of bread samples were weighed in aluminum cups using a precision scale with a 1-mg  
99 resolution (SCALETECH, Argenteuil, France) and then dried in an oven (Mettler,  
100 Schwabach, Germany) at 103°C for 24 h. Once it was returned to room temperature using a  
101 desiccator, the dry sample was weighed again. The resulting difference in weight represents  
102 the quantity of water initially present in the bread sample, expressed in g/100 g dm (dry  
103 matter) units. Measurements were conducted in four replicates.

104 **2.4. Mechanical Properties**

$$\text{Stress } (\sigma) = F / S \quad (1)$$

$$\text{Strain } (\varepsilon) = \Delta L / L_0 \quad (2)$$

105           The crumb texture was evaluated using a dynamic mechanical analyzer (DMA Q800-  
106 TA-Waters instruments-78-Guyancourt-France) to perform compression tests on the degassed  
107 bread crumb baked in the Peltier cooker. Two parallel cylinders of 2cm diameter were used to  
108 compress the degassed crumb disks (5mm diameter and 3 mm height) at room temperature  
109 20°C. A static force of 0.02 N was used to start the test and to establish contact between the  
110 parallel cylinders and the sample. The cylinders were slightly lubricated with a piece of tissue  
111 containing rapeseed oil. Each sample of the degassed crumb was exposed to compression at a  
112 rate of 5% per minute up to 30% deformation. The graph of the stress with respect to the  
113 strain was plotted. The stress ( $\sigma$ ) and strain ( $\varepsilon$ ) were calculated using equations (1) and (2),  
114 respectively. Considering the stress as a function of the strain, the corresponding Young's  
115 modulus (E) was calculated based on the slope of the linear part of the curve between -0.5%  
116 and -8% and expressed in N/mm<sup>2</sup> or MPa (Le-Bail et al., 2009). Compression tests were  
117 performed after 2 h and 1, 3, 6 and 10 days of storage to study the evolution of the texture  
118 during staling.

119           Where F is the static force expressed in N, S is the sample surface area in mm<sup>2</sup>,  $\Delta L$  is  
120 the change in length in mm and  $L_0$  is the initial length in mm

## 121 **2.5. Calorimetry Analysis**

122           Two different calorimeters were used to monitor the phase changes of the starch and  
123 starch components after baking. The degree of starch gelatinization and amount of retrograded  
124 AP during storage were studied with micro DSC VII equipment (SETARAM Instrumentation,  
125 Lyon, France), and the degree of AM crystallization was investigated with a TA-Q100  
126 calorimeter (TA Q1000, TA Instruments, New Castle, US-DE). The SETARAM calorimeter  
127 has a very high sensitivity but was unable to reach the high temperature needed to analyze the  
128 AM complexes (done with TA-Q100). A sample of 100 mg to 200 mg of crumb was taken

129 from the degassed crumb slab and placed in a hermetical cell (TA Instruments, New Castle,  
130 US-DE).

131 A Denver balance with 0.01 mg accuracy (Denver Instruments CO., Arvada, US-CO) was  
132 used for weight measurement. The analysis procedure was as follows. After equilibrating the  
133 sample at 20°C for 5 min, a temperature increase was programmed up to 120°C with a  
134 scanning rate of 1.2°C/min. The enthalpies of transition (the melting enthalpy of retrograded  
135 AP starting at approximately 40°C and starch gelatinization at 60-70°C) were obtained by  
136 integrating each peak found in the thermogram and were expressed in units of J/g dry flour.  
137 The degree of starch gelatinization allows for the quantification of nongelatinized starch; this  
138 may occur, particularly in the case of rapid heating and/or a lack of water. The endotherm  
139 linked to starch gelatinization starts at approximately 60°C in excess water (80% water  
140 content, no restriction to water access) (Silverio et al., 1996). The excess water was evaluated  
141 from the dry matter content of the considered product based on the dry matter of the crumb  
142 (dry matter was determined by drying tests performed in a drying cabinet at 103°C for 24 h  
143 according to the AACC method 44-15A, AACC, 1999). The degree of gelatinization was

$$\text{Degree of gelatinization} = \left(1 - \frac{\text{Gelatinization enthalpy of the bread sample}}{\text{Gelatinization enthalpy of dough}}\right) \times 100\% \quad (3)$$

144 estimated according to the equation (3) (Ding et al., 2019):

145 The melting enthalpy of AM-lipid complexes and AM crystallization (from approximately  
146 90/100°C until the end of the endotherm peak) were determined using a Q100 calorimeter  
147 (TA Q100, TA Instruments, New Castle, US-DE). The calorimeter (TA Q100) was  
148 programmed up to 180°C with a scanning rate of 3°C/min; these tests were performed to  
149 monitor and quantify the evolution of the AM crystallized during storage. The associated  
150 enthalpy was determined by integrating the surface of the peak with Universal-ANALYSIS

151 software (TA Instruments, version 4.5A). Measurements were performed in triplicate for  
152 bread baked at different HRs and stored for each storage durations.

## 153 **2.6. Determination of soluble amylose**

154 Determination of soluble AM permits the evaluation of the degree of starch disruption  
155 during baking. The AM leached from the starch granules is dispersed into the matrix and  
156 contributes to the final cohesiveness and texture of the crumb. The AM gel formed during  
157 storage is expected to play a significant role in the mechanical properties of the texture of the  
158 crumb, even though most authors indicated that AP recrystallization is the main driver. The  
159 amount of soluble AM was measured according to the AACC 61-03 method. One hundred  
160 milligrams of baked crumb was stirred in 100 ml of a solution (10% ethanol - 65% v/v, 9%  
161 NaOH 1 N and 81% desalinated water) to evaluate the amount of soluble AM and total AM.  
162 After stirring, the sample was left at room temperature for 18 h (total AM) or 2 h (soluble  
163 AM); 150 µl was sampled after the extraction period and was diluted in a solution containing  
164 60 µl of Lugol, 30 µl of acetic acid (1 N) and 1.5 µl of desalinated water. The solution was  
165 stirred and left for 20 min to rest. The absorbance at 620 nm was determined with a  
166 spectrophotometer (CM 3500-d, Konica Minolta Sensing Inc., Mahwah, US-NJ). Tests were  
167 performed in triplicate for each sample using three 3-ml flasks for each storage duration  
168 because the amount of soluble amylose is known to decrease with increasing storage time due  
169 to the more pronounced organization of the retrograded AM (Patel et al., 2005). Calibration  
170 was performed with a blank and with a sample containing 27% pure AM (Corn starch, Argo,  
171 Best Food, CPC International Inc., Bergen, US-NJ). The percentage of AM was calculated  
172 from the absorbance of the sample multiplied by 27 and divided by the absorbance of the  
173 reference sample (27%). Three repetitions were performed for each data point.

## 174 **2.7. Nuclear magnetic resonance (NMR) measurements**

175 For each HR, NMR tests were performed for samples stored at 4°C for 24 h, 6 days and  
176 14 days after baking. First, 0.5 g of crumb samples (diameter ( $\emptyset$ ) = 8 mm and height (H) = 10  
177 mm) was placed in an NMR tube closed with a cap ( $\emptyset$  = 10 mm). Then, the tube of each  
178 sample was placed in the spectrometer and thermostatically maintained at 277 K. NMR  
179 analysis was carried out using a Minispec mq20 spectrometer (Bruker, Milton, Burlington,  
180 Canada).). The transverse  $T_2$  relaxation time of protons as well as their respective population  
181 in the product was determined using a Carr–Purcell–Meiboom–Gill (CPMG) sequence. The  
182 180° pulse separation was 0.075 ms, 1000 even echoes were collected, and 512 scans were  
183 acquired with a recycle delay of 2 s, resulting in a total acquisition time of approximately 20  
184 min. An inverse Laplace transformation (ILT) was applied to convert the relaxation signal  
185 into a continuous distribution of  $T_2$  relaxation times. The  $T_2$  distributions were then  
186 deconvoluted, and each "peak" was modeled using an asymmetric normal law ( Berman et al.,  
187 2010). The software used was developed (in part) and implemented using MATLAB®R2014a  
188 (MathWorks, Natick, US-MA).

## 189 **2.8. Environmental Scanning Electronic Microscopy Images**

190 Environmental Scanning Electronic Microscopy (ESEM) is an electron microscopy  
191 technique (Philips, Leuven, Belgium) capable of producing high-resolution surface images.  
192 ESEM scans the surface of the sample to be analysed with an electron beam to obtain data at  
193 the particle scale. Different detectors that reconstruct a three-dimensional image of the surface  
194 were used to analyze these particles. The crumb samples, stored at 4°C, were fractured into  
195 sizes of approximately 1x1x0.5 cm using a knife and a hammer before the sample was quickly  
196 transferred to the SEM chamber. The interior surface of the samples was coated with an  
197 ultrathin coating of metal (gold) or carbon. A 2D image of the sample was displayed on a  
198 computer monitor. The crumb samples were scanned by a focused electron beam in a Philips  
199 XL30-ESEM system at an accelerating potential of 8 kV at 4°C. Secondary electrons were

200 processed to form enlarged images with a magnification of 500 X. Scans of samples were  
201 realized under the following conditions in the SEM chamber: a vapor pressure of 660 Pa and a  
202 temperature of 4°C.

## 203 **2.9. Staling kinetics**

204 The evolution of the melting enthalpy of AP and AM as a function of the storage time was  
205 modeled according to the Avrami equation (first-order kinetic models) by assuming that the  
206 time exponent was equal to unity (Le-Bail et al., 2012; Le-Bail et al., 2009). The Avrami  
207 equation was used to model the evolution of the melting enthalpy of AP  $\Delta H(t)$  (**equation (4)**)  
208 with  $\Delta H_0$  and  $\Delta H_\infty$  representing the melting enthalpy at initial and final times, respectively,  
209 where  $\tau$  is the time constant and  $t$  is the storage time (in days). A similar equation was used to  
210 model the crumb hardening (from the Young's modulus  $E(t)$ ) of the degassed breadcrumb  
211 (**equation (5)**) with  $E_0$  and  $E_\infty$  corresponding to the Young's modulus at the initial and final  
212 times, respectively.

$$\Delta H(t) = \Delta H_\infty + (\Delta H_0 - \Delta H_\infty) \exp(-t/\tau) \quad (4)$$

$$E(t) = E_\infty + (E_0 - E_\infty) \exp(-t/\tau) \quad (5)$$

213 Fitting the model and the experimental points was performed using Excel Solver  
214 (EXCELSTAT-Microsoft, Las Vegas, US-NV).

## 215 **2.10. Statistical analysis**

216 All experiments were performed in triplicate, and values were expressed as the mean  $\pm$   
217 standard deviation (SD). Statistical analyses were conducted using Statgraphics plus 5.1  
218 software (Statgraphics Technologies, The Plains, US-VA) Fisher's Least Significant  
219 Difference (LSD) test was used to compare the means at the 5% significance level.

### 220 3. Results and discussion

#### 221 3.1. Young's Modulus (E)

222 **Fig. 1A** shows the evolution of the firmness of the baked crumb samples during storage  
223 according to the variation in the E. The results demonstrate that the firmness of the  
224 breadcrumbs increased significantly ( $p < 0.005$ ) for all samples during storage. However, some  
225 differences occurred depending on the baking conditions. It was observed that E was lower  
226 after 2h storage for bread baked at  $6^{\circ}\text{C}/\text{min}$  (long baking and lower heating rate; 0.45 MPa)  
227 than for bread baked rapidly at  $20^{\circ}\text{C}/\text{min}$  (0.65 MPa) and  $40^{\circ}\text{C}/\text{min}$  (0.9 MPa). Indeed, the  
228 lowest value of E obtained at the end of staling (after 14 days storage) was 0.85 MPa for  
229 bread baked at  $6^{\circ}\text{C}/\text{min}$  while the highest Young's modulus at  $\infty$  was 2 MPa and  
230 corresponded to bread baked at  $40^{\circ}\text{C}/\text{min}$ .

231 Bredariol et al., (2019) also reported a significant correlation between heating rate and  
232 crumb hardness (for bread baked without steam). These authors observed that breadcrumb  
233 hardness tended to increase with increasing heating rate. In addition, Shen et al.,  
234 (2018) noticed that the bread samples prepared with different types of sugar (the same sugar  
235 concentration) had the highest crumb firmness when higher baking temperatures for the same  
236 baking time (higher heating rate) were applied. These results could be explained by the fact  
237 that the heating rate during baking affects starch properties, including starch gelatinization  
238 (swelling and dispersion), amylose solubility, and amylopectin recrystallization (Tao et al.,  
239 2020). Therefore, these properties may have an influence on the evolution of crumb firmness  
240 during storage (Patel et al., 2005).

#### 241 3.2. Evolution of AP recrystallization

242 Amylopectin retrogradation is one of the biochemical phenomena most closely associated  
243 with staling. Many authors have correlated this parameter with both bread crumb firmness and  
244 storage time (Ribotta, Cuffini et al., 2004). The degree of AP recrystallization was found to

245 increase during storage and was higher in samples baked at higher HRs (**Fig. 1B**). On 1 of  
246 storage, the AP recrystallization enthalpy was higher in crumb samples baked at higher HRs  
247 (0.38 J/g d.f. for 20°C/min and 0.5 J/g d.f. for 40°C/min) than in those baked at 6°C/min (0.15  
248 J/g d.f.). After 10 days' storage, the AP recrystallization enthalpies increased for all HRs. The  
249 final enthalpy at the end of AP retrogradation was more important for the 40°C/min (1.8 J/g  
250 d.f.) than for the 20°C/min and 6°C/min HRs. Hence, the HR may affect the extent of the  
251 disordering of the AP crystals and the destructuring of starch. It is assumed that the higher  
252 amount of leached AM yields a higher degree of segregation between AM and AP (Tao et al.,  
253 2020).

254 The AP chains were organized in specific domains, resulting in the faster recrystallization  
255 of AP during storage of samples baked with high HRs, even from the first day of storage.  
256 Similar observations were reported by Patel and Seetharaman (2006). They confirmed that the  
257 enthalpy of recrystallized AP was higher in samples baked at higher HRs than in those baked  
258 at lower HRs. According to these results, the higher rigidity of the crumb samples could be  
259 associated with intramolecular dehydration of the crumb cell matrix due to the transfer of water  
260 molecules from gluten to AP crystallites. The capture of water by AP crystals no longer  
261 plasticizes the different networks. This can lead to an increase in crumb firmness and a  
262 decrease in crumb resilience, due to a less flexible gluten network (Goesaert et al., 2009,  
263 Ribotta and Le-Bail, 2007, Gray and Bemiller, 2003). Moreover, bread samples baked at a  
264 higher HR staled faster. This could be related to the higher capability of AP to retrograde at a  
265 higher HR (E. Besbes et al., 2014,).

### 266 **3.3. Soluble amylose and amylose crystallization during storage**

267 The soluble AM contents of the crumb samples were plotted as a function of the HR  
268 during storage (**Fig. 1C**). The amount of soluble AM decreased significantly after two days of  
269 staling for all the samples and continued to decrease slightly throughout the storage period.

270 Breads baked at higher HRs had higher soluble AM contents than bread crumb samples baked  
271 at lower HRs. After 2 h of storage, the crumb baked at 40°C/min had 8% soluble AM, crumbs  
272 baked at 20°C/min had 5.2% soluble AM and crumbs baked at 6°C/min had 4.7% soluble  
273 AM.

274 These results are in agreement with the hypothesis on AM leaching reported by  
275 Seyhun et al., 2005. They mentioned “the Higo effect” as a rapid change of AM when bread  
276 is baked at higher HR. This effect was explained by a considerable leaching of AM out of  
277 starch granules when bread is heated at a higher HR (40°C/min), as in microwave heating.  
278 The leached AM was found to be more disoriented during storage and more water molecules  
279 were bound to AM crystals (associations) than conventionally heated bread (6°C/min)  
280 (Seyhun et al., 2005). Moreover, during storage, the soluble AM decreased more in crumb  
281 samples baked at higher HRs than in those baked at lower HRs. After 10 days’ storage, the  
282 soluble AM content decreased by 6.3%, 3.5% and 3.4% for bread baked at 40°C/min,  
283 20°C/min and 6°C/min, respectively. The decrease in the soluble AM content during storage  
284 can be explained by the increase in AM molecule associations in their crystalline form. The  
285 latter increases during storage, as shown in **Fig. 1D**.

286 **Fig. 1D** shows the evolution of the AM associations (crystallized) as a function of the  
287 storage time. Such approaches appear novel, and so far there are no similar studies have been  
288 found in the literature. The amount of crystallized AM was found to increase with increasing  
289 HR. This was attributed to the increase in the concentration of soluble AM released from  
290 starch granules caused by a higher HR. After 10 days’ storage, the crystallized AM enthalpies  
291 reached 4.3 J/g d.f. for an HR of 40°C/min, and 3.7 J/g d.f. and 3.2 J/g d.f. for bread baked at  
292 20°C/min and 6°C/min, respectively. When baking at higher HR, the leached AM seemed to  
293 be more concentrated at the surface of starch granules, where the latter were richer in AP.  
294 Upon cooling and during storage, the surrounding AM molecules aligned into a crystalline

295 structure and formed a gel. This gelation induced the formation of ordered structures such as  
296 double helices during storage and the hardening of the crumb (Seyhun et al., 2003,Uzzan et  
297 al., 2007).Patel and Seetharaman (2006) reported that at higher HRs, AM leaching out of  
298 starch granules is rapidly associated with the surface of starch granules and/or phase  
299 separation from AP within the granules. These results confirm findings on the impact of the  
300 HR on the bread texture related to starch modification and concentrations.

### 301 **3.4.Staling kinetics**

302 The Avrami equation was developed to analyze the staling kinetics of starchy food such as  
303 bread (E. Besbes et al., 2014) and cake (Lee et al., 2020). The Avrami equation was used to  
304 model the evolution of crumb firmness and starch retrogradation during staling (Sang et al.,  
305 2015). Several authors have stated that for starch crystallization, the time exponent (n) is  
306 related to the crystal nucleation mechanism and growth with n varying between 1 and 4  
307 (Mouneim et al., 2012; Rojas et al., 2001; Gujral et al., 2003). On the other hand, according  
308 to Le-Bail et al., (2009), the time exponent was equal to one for bread firming. For n=1, the  
309 time constant  $\tau$  can be determined from the slope of the linear regression of the difference  
310 between the final value (long storage) and current value of the parameters evaluated  
311 (Young's modulus and starch crystallization enthalpies) as a function of time. The slower the  
312  $\tau$  was, the higher the staling kinetics was.(E. Besbes et al., 2014; Lee et al., 2020). To fit the  
313 evolution of Young's modulus and the melting enthalpy of AP and AM crystallization (staling  
314 parameters) during storage a simple first order model (n=1) was used for this study (Equation  
315 5 and 6). This model can fit very well with the experimental results for samples baked in a  
316 miniaturized baking system according to Mouneim et al., 2012 and Le-Bail et al., (2009).

317 **Table 1** presents the parameters of the first-order model obtained from the model  
318 calculated by Excel-Solver (Excel-Solver, Microsoft USA) using an Avrami model. For the  
319 AP retrogradation model and AM crystallization, the value of the origin (time zero = day of

320 baking) was set to zero, which corresponds to the observed results in **Fig. 2**. At time zero,  
321 Young's Modulus was significantly higher at 40°C/min and 20°C/min and lower at 6°C/min.

322 At the end of storage, the enthalpy linked to AP retrogradation and AM crystallization  
323 increases with increasing HR. This trend was also observed for E (2 MPa at 40°C/min versus  
324 0.85 MPa at 6°C/min), which refers to the evolution of crumb firmness. The time constant  $\tau$  is  
325 presented in Table 1; as  $\tau$  decreases, faster staling is observed. It was shown that the time  
326 constants of all the parameters (AM, AP and E) decreased with increasing HR. Using the first  
327 order kinetic model (Besbes et al 2014), this confirms that faster baking leads to faster staling.  
328 First, this trend explained by the reorganization and crystallization of AM and retrogradation  
329 of AP. These phenomena take more time with increasing HR due to the greater amount of  
330 crystallized material (as shown in **Fig. 1**). Second, the smaller values of the time constants  
331 obtained for E are followed by AM crystallization and AP retrogradation; the average values  
332 of the time constant were approximately 0.6, 1.05 and 3.48 days at 40°C/min.

333 In previous studies, most researchers found a direct relationship between the  
334 retrogradation of AP and the evolution of crumb firmness during staling. Kang et al., (2021)  
335 reported that the recrystallization of amylopectin is the main reason for samples staling during  
336 storage and bread firmness. Gray and Bemiller, (2003) reported similar observations. It was  
337 noted that starch retrogradation/recrystallisation, plays a major role in bread firming, after the  
338 initial cooling process. This was not the case in this study, as the increase in bread firmness  
339 appeared roughly at the same time as the increase in AM crystallization. Therefore, in this  
340 study using a specific recipe with low water content and improvers induced the formation of  
341 a large amount of AM complexes which can affect (restrict) the evolution of crumb firmness  
342 during storage, as discussed by Kang et al., (2021).

### 343 3.5. Starch swelling and microstructure

344 Starch granules morphology and the degree of gelatinization, is directly dependent on  
345 baking temperature, time and the resulting crumb firmness. **Fig. 2** illustrates ESEM images at  
346 550 x magnification for the starch granules in bread crumbs baked in the Peltier system at  
347 different HRs. **Fig. 2A** shows that the starch granules in crumbs baked at 6°C/min had a  
348 regular, swollen elliptical shape with smooth surfaces in which the starch granules seemed to  
349 be completely gelatinized (with a degree of gelatinization of 100%, as indicated by a  
350 calorimetry study). Similar observations were reported by Ozkoc et al., (2009) for bread  
351 baked by conventional baking where the heating rate corresponded to 6°C/min. It was seen  
352 that starch granules observed by SEM were completely gelatinized and had a continuous  
353 starch granule structure.

354 In **Fig. 2B**, a HR of 20°C/min led to an increase in granule shape deformation with  
355 complete disruption and some superficial cracks. A large increase in HR (40°C/min) results in  
356 incomplete folding of starch granules, with the complete destruction and rupture of most of  
357 them (**Fig. 2C**). Additionally, the starch granules appeared to be nonhomogeneous and  
358 irregular, with many granular remnants. The starch underwent significantly less gelatinization  
359 at 40°C/min. The enthalpy of gelatinization associated with the nongelatinized starch in  
360 crumbs baked at 40°C/min was 0.65 J/g of dry flour, representing 80% of the gelatinized  
361 starch. Consequently, a higher HR will potentially alter the swelling kinetics and the  
362 disorganization of AP crystals and AM leaching. These changes can subsequently have an  
363 impact on the bread texture during storage (Kinner M. et al., 2021). The results obtained are in  
364 agreement with Bredariol et al., (2019) and Bilbao-Sáinz et al., (2007). These authors found  
365 that high heating rates lead to an increase in the enthalpy of gelatinization above the levels of  
366 native starch granules with a decrease in the gelatinization temperature. This is explained by  
367 the fact that starch granules are severely damaged when exposed to very high temperatures for

368 a short baking time (higher heating rate) and do not have enough time to complete its  
369 gelatinization.

### 370 **3.6. Effect of the HR on the molecular water mobility and distribution of stored crumb**

371 Measurement of the moisture content of degassed crumb (baked in a sealed pouch and  
372 stored in hermetically plastic Eppendorf tube) showed a nonsignificant difference for each HR  
373 and during staling. The limited changes in degassed crumb MC meant that changes in the  
374 proton distributions of the  $T_2$  relaxation time during storage were exclusively attributed to  
375 changes in the starch and gluten networks (Bosmans et al., 2013; Curti et al., 2017, 2011). In  
376 our study, the  $T_2$  relaxation time was measured by the CPMG sequence, and a continuous  
377 distribution is shown in **Fig. 3**. The continuous distribution permits us to characterize up to  
378 five proton populations and name them according to the length of their relaxation times A  
379 (0.3-0.5 ms), B (0.9-2.2 ms), C (3-10 ms), D (12-20 ms), and E (40-60 ms). The population  
380 abundance distribution was calculated and is represented in **Table 2**.  $P_{2A}$  and  $P_{2B}$  were the  
381 smallest populations, representing 3% of the total protons in baked degassed dough.  
382 Population C represents more than 80% of the total proton population, and D and E  
383 encompassed 7% of the total proton population. Moreover, our values of the  $T_2$  relaxation  
384 time of different populations were reported in some studies where each population  
385 corresponded to a specific proton domain.

386 According to Engelsen et al. (2001), a relaxation time peaking at 0,5 ms ( $T_{2A}$ )  
387 corresponds to protons associated with water-gluten. Population B with a peak time of 2 ms is  
388 attributed to protons of intragranular water retained by starch (Assifaoui et al., 2006).  
389 Population C observed at 5.0 ms could probably be assigned to mobile protons of gluten and  
390 water associated with these molecules (Engelsen et al., 2001). Population D, ranging between  
391 10 ms and 20 ms, is related to protons associated with gelatinized starch (and pentosane)  
392 (Curti et al., 2011; Engelsen et al., 2001) and corresponds to intergranular water, especially in  
393 interactions and competition with sucrose and starch (Assifaoui et al., 2006). Bosmans et al.,

394 (2014) reported that a relaxation time peak at 10 ms is associated with the mobile protons of  
395 water exchanging in the formed gel network (Bosmans et al., 2014). The  $T_{2E}$  of the last  
396 population, ranging from 50–60 ms, is related to the most mobile protons of water (less  
397 interacting with macromolecules) available in the aqueous phase of bread (Assifaoui et al.,  
398 2006; Rondeau-Mouro et al., 2019). The  $T_2$  relaxation time and population abundance were  
399 affected by the HR and varied during storage.  $T_{2A}$  slightly decreased for the two higher HRs  
400 of 20°C/min and 40°C/min from 0.52 ms to 0.4 ms and increased for the lowest HR of  
401 6°C/min from 0.44 ms to 0.53 ms following storage. Similarly,  $T_{2B}$  significantly decreased for  
402 the 2 higher HRs of 20°C/min and 40°C/min from 1.8 ms to 1.3 ms and 1.8 ms to 1.5 ms,  
403 respectively, and a slight but nonsignificant decrease was observed for 6°C/min. The relative  
404 amount of protons in populations A and B varied significantly at the higher HR after 14 days  
405 of storage, decreasing to 2.8% and increasing to 4.4% and varied insignificantly for samples  
406 baked at lower HRs.

407 Populations A and B might reflect the hydration state of the gluten network or the  
408 degree of gluten-water association. The decrease in their relaxation time is explained by the  
409 loss of water from the gluten network in bread and the migration of water from gluten to  
410 starch at higher HR. The slight increase in  $T_{2A}$  and the unchanged  $T_{2B}$  values upon storage for  
411 the 6°C/min rate might be attributed to the higher degree of gluten network hydration and the  
412 more plasticization that occurred during storage and resulted in crumb firmness limitations  
413 (Bosmans et al., 2013; Curti et al., 2011). This decrease was also observed in the starch water  
414 model with 47% MC stored for up to 7 days, as reported by Bosmans et al. (2013). These  
415 authors suggested that in the gluten-starch model, dehydration of gluten occurs due to water  
416 migration from gluten during storage. The majority of detectable protons in population C  
417 decreased in abundance after 14 days of storage from 80% to 77% for the higher HR of  
418 40°C/min, decreased from 84% to 80% for the 20°C/min HR and remained stable for the

419 6°C/min HR. The relaxation time slightly decreased for all HRs. In addition, the  $T_{2D}$   
420 component decreased significantly after 14 days of storage from 15 ms to 13 ms for the  
421 40°C/min HR and slightly decreased for the 20°C/min and 6°C/min HRs. The decreases in the  
422 relaxation time for components C and D upon storage could be due to the retrogradation of  
423 AP and the incorporation of water molecules in starch crystallites, which become less mobile  
424 at a higher HR. In contrast, at lower HRs, the crystallization phenomena are slower, which  
425 explains the slight decrease in  $T_{2C}$  and  $T_{2D}$ .

426 During storage, starch crystallization phenomena such as AP retrogradation and AM  
427 crystallization occur (particularly at a higher HR). Those crystalline structures require water,  
428 which explains the migration of water from gluten to starch. The crystallized rigid starch  
429 network formed during staling traps the bound water in its structure (Hemdane et al., 2017).  
430 These mechanisms lead to an increase in crumb firmness, as observed in **Fig. 1A**. The  
431 population abundance ( $P_{2E}$ ) and relaxation time ( $T_{2E}$ ) of the longest component (population E)  
432 that characterized the extragranular water fraction decreased significantly ( $p < 0.05$ ) for the two  
433 higher HRs whereas it did not vary for the lowest. This could be due to complete starch  
434 granule gelatinization at lower HRs, which was not achieved at higher HRs according to a  
435 calorimetric study.

#### 436 **4. Conclusion:**

437 In this study, the impacts of different HRs on crumb was investigated using a  
438 miniaturized baking process (Peltier-baking system) that allows for good control of the HR  
439 and the time-temperature profile during baking. The staling kinetics were assessed based on  
440 the water mobility, starch microstructure, thermodynamic properties and mechanical  
441 properties of crumb. The  $^1\text{H}$  NMR relaxation time of the five identified populations decreased  
442 with increasing HR and during staling. This could be explained by the loss of water from the  
443 gluten network, which undergoes dehydration, from the migration of water from gluten to

444 starch, and its incorporation in starch crystallites. In addition, baking at higher HR (40°C/min)  
445 yielded an incomplete starch gelatinization (80%) of crumb samples. In morphological ESEM  
446 observations, severe deformation and disruption were observed for most starch granules in  
447 crumbs treated at a higher HR (40°C/min). The results confirm that the firmness of bread  
448 crumb is related to the hydration of starch granules and the water trapped by AP crystals as  
449 well as the presence of AM associations that strongly affect the crumb texture. These results  
450 add useful findings to the literature and provide an important insight at industrial scale when  
451 non-CVB processes such as microwave baking are considered.

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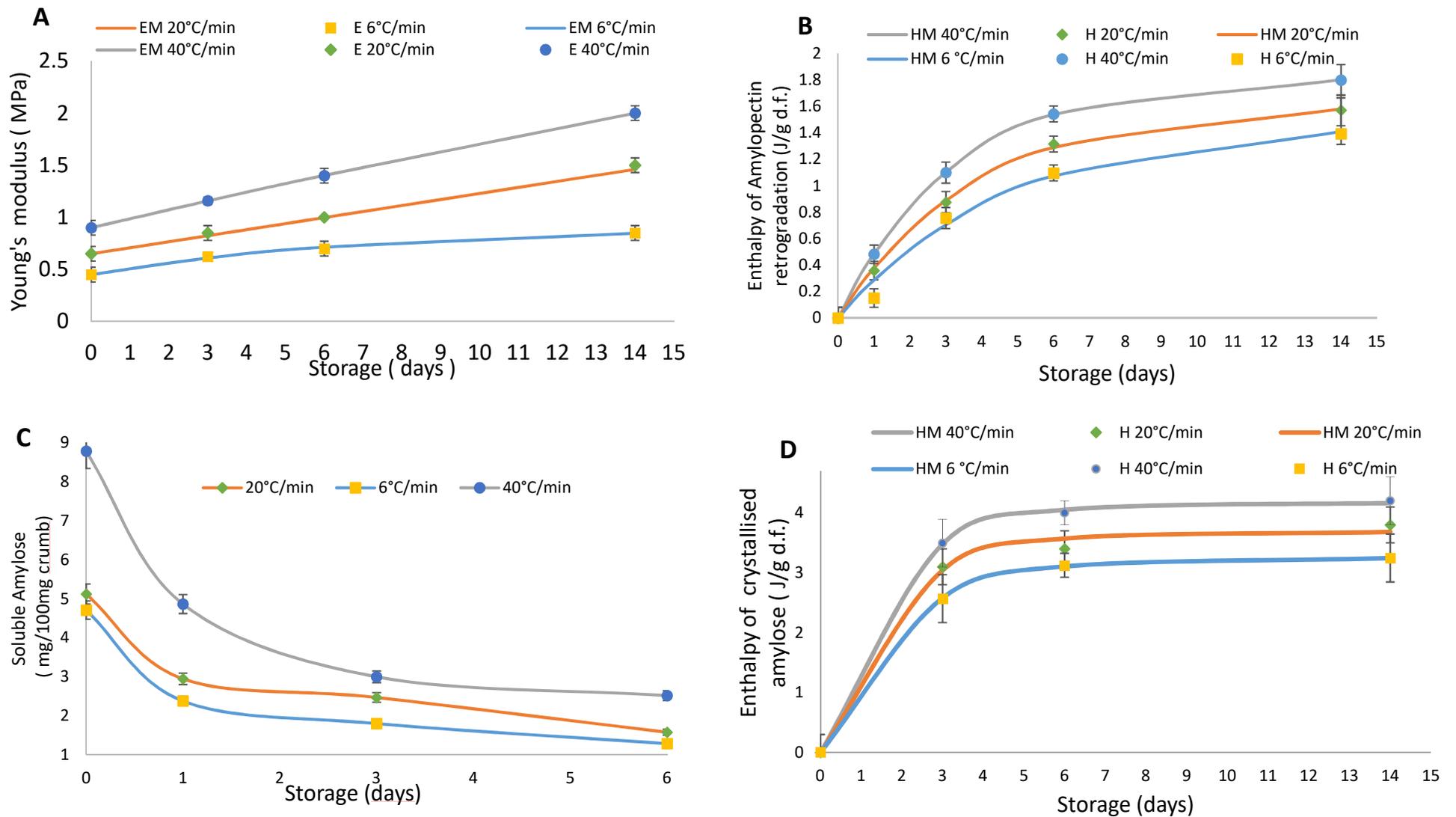
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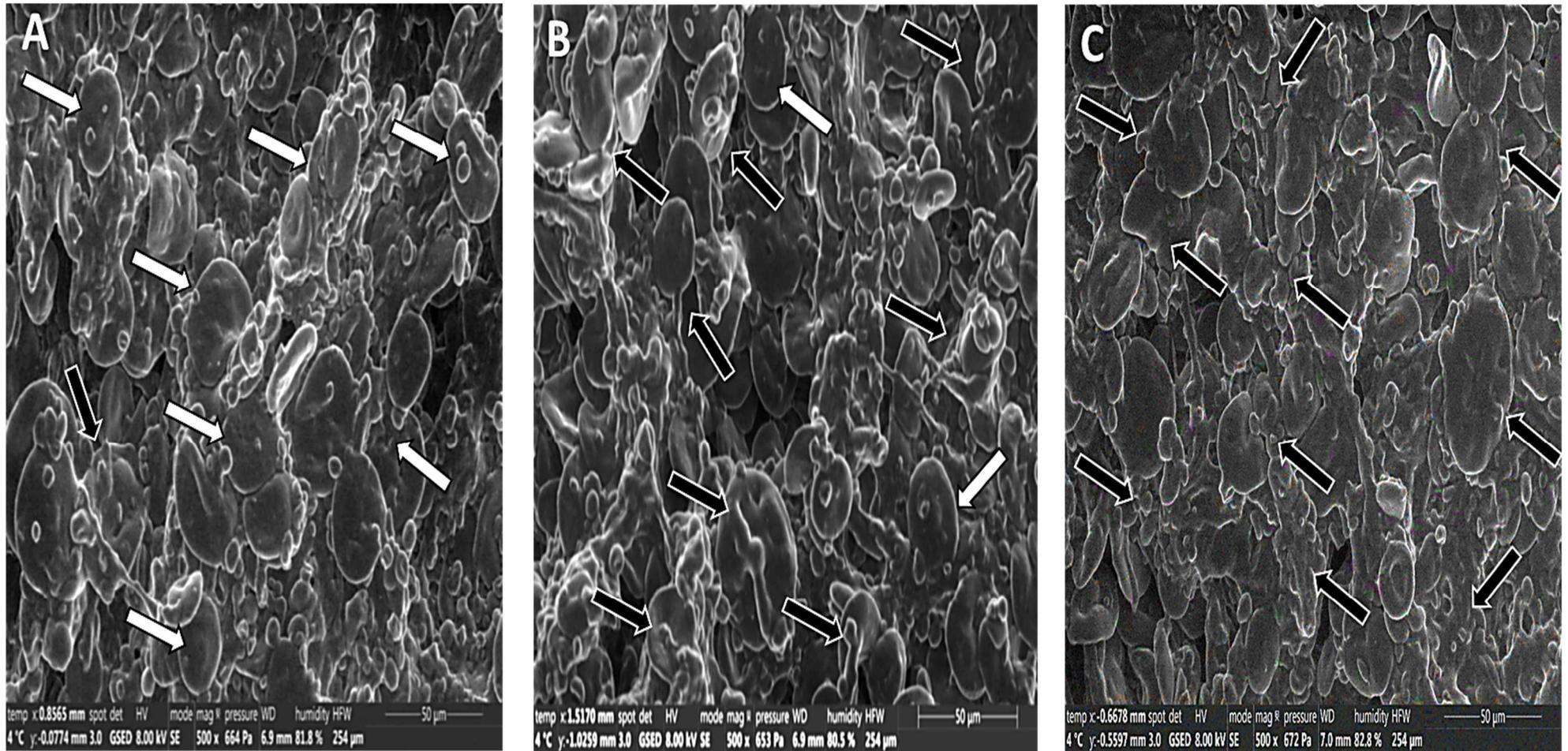
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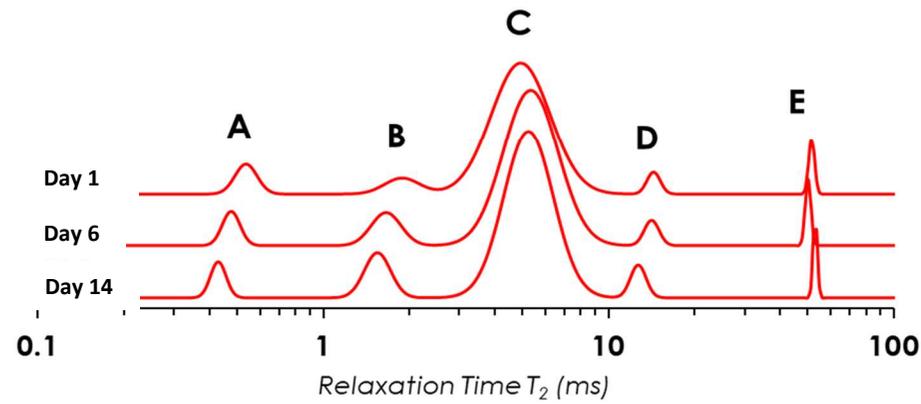


**Fig. 1.** Evolution of (A) crumb firmness, (B) AP recrystallization, (C) soluble AM, (D) AM crystallization in crumb baked at 6°C/min, 20°C/min and 40°C/min during storage (days) E : Experimental enthalpy, E: Experimental Young's Modulus, and HM : modeled enthalpy and EM : Modeled Young's Modulus at ■ 6°C/min, ◆ 20°C/min and ● 40°C/min.

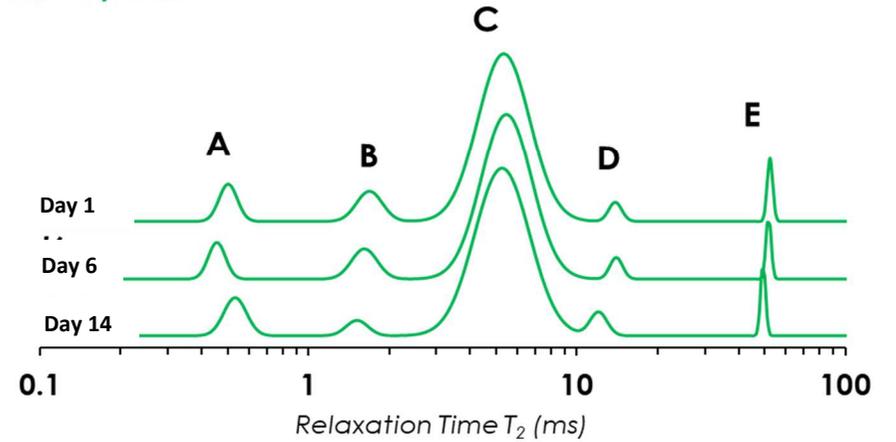


**Fig.2. ESEM images for the microstructure of breadcrumbs taken at 500 × magnification baked in A) at 6°C/min, B) 20°C/min and C) 40°C/min. \*\*The black arrows represent the destructured and deformed starch granules whilst the white arrows indicate swollen and non-deformed starch granules.**

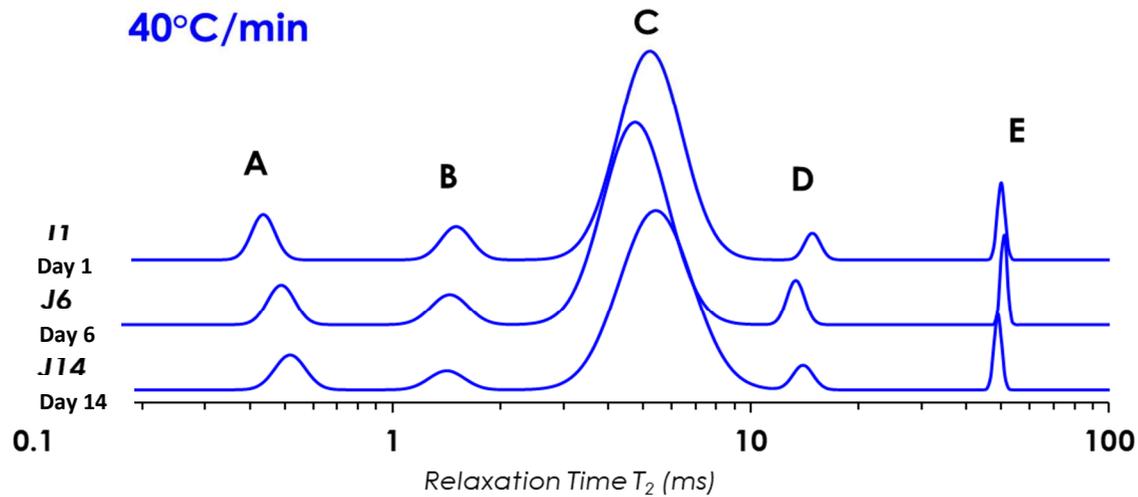
6°C/min



20°C/min



40°C/min



**Fig. 3.** Proton population distributions as a function of the T<sub>2</sub> relaxation time of the crumb sample baked at 6°C/min, 20°C/min and 40°C/min during 1, 6, and 14 days of storage.

\*The different proton population are indicated with capital letters according to their increasing mobility. Analyses were performed at 4°C.

1 **Table 1**

2 Effect of HR on the evolution of the melting enthalpy (E) of the retrograded amylopectin, amylose Crystallization  
 3 and Young's Modulus (H) after baking and at the end of storage and on the time constant for the three-studied  
 4 parameters  
 5

HR (°C/min)	Staling parameters	E0 (MPa)/ΔH0 (J/g d.f.)	E <sub>∞</sub> (MPa)/ ΔH <sub>∞</sub> (J/g d.f.)	τ(days)
6°C/ min	Young's Modulus	0.45 ± 0.05 <sup>c</sup>	0.85 ± 0.80 <sup>c</sup>	1.14 ± 0.07 <sup>a</sup>
	Amylopectin retrogradation	0.00 ± 0.00 <sup>a</sup>	1.40 ± 0.02 <sup>b</sup>	5.75 ± 0.02 <sup>a</sup>
	Amylose crystallization	0.00 ± 0.002 <sup>a</sup>	3.24 ± 0.03 <sup>c</sup>	2.00 ± 0.06 <sup>a</sup>
20°C/min	Young's modulus	0.65 ± 0.07 <sup>b</sup>	1.50 ± 0.07 <sup>b</sup>	0.76 ± 0.05 <sup>b</sup>
	Amylopectin retrogradation	0.00 ± 0.00 <sup>a</sup>	1.60 ± 0.06 <sup>b</sup>	4.28 ± 0.41 <sup>b</sup>
	Amylose crystallization	0.00 ± 0.004 <sup>a</sup>	3.69 ± 0.02 <sup>b</sup>	1.54 ± 0.01
40°C/min	Young's modulus	0.90 ± 0.07 <sup>a</sup>	2.00 ± 0.14 <sup>a</sup>	0.60 ± 0.02 <sup>c</sup>
	Amylopectin retrogradation	0.004 ± 0.001 <sup>a</sup>	1.90 ± 0.15 <sup>a</sup>	3.48 ± 0.29 <sup>c</sup>
	Amylose crystallization	0.002 ± 0.005 <sup>a</sup>	4.17 ± 0.05 <sup>a</sup>	1.05 ± 0.05 <sup>c</sup>

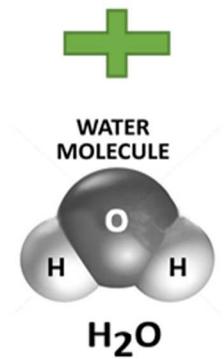
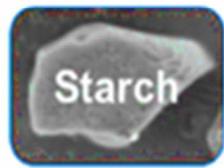
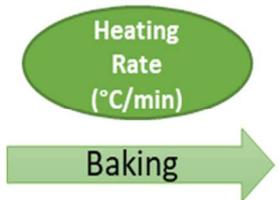
6 \*Within a column, Values with the same small letter for different HR and same staling parameter are not  
 7 statistically significant (p<0.05), ±SD  
 8  
 9

10 **Table 2**

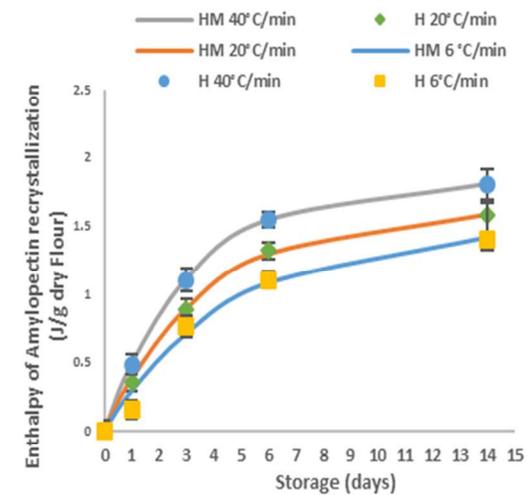
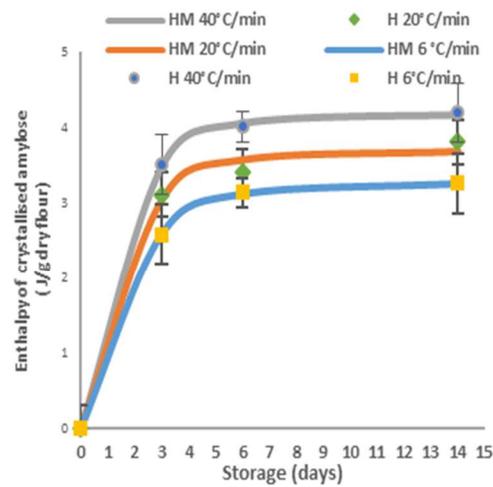
11 NMR population abundance for each crumb samples baked at different HR in function of storage time (days)  
 12

HR (°C/min)	Storage time (days)	Population abundance (%)				
		P2a	P2b	P2c	P2d	P2e
6°C/min	1	3.5 ± 0.02 <sup>aB</sup>	2.8 ± 0.04 <sup>aA</sup>	81.2 ± 0.01 <sup>aB</sup>	6.8 ± 0.02 <sup>aA</sup>	7.9 ± 0.008 <sup>aA</sup>
	6	3.3 ± 0.04 <sup>aA</sup>	3.0 ± 0.01 <sup>aB</sup>	81.0 ± 0.03 <sup>aA</sup>	5.8 ± 0.01 <sup>bB</sup>	7.1 ± 0.012 <sup>bA</sup>
	14	3.2 ± 0.01 <sup>aA</sup>	3.0 ± 0.05 <sup>aB</sup>	80.0 ± 0.02 <sup>bA</sup>	4.4 ± 0.02 <sup>cB</sup>	6.9 ± 0.004 <sup>bA</sup>
20°C/min	1	3.4 ± 0.02 <sup>aB</sup>	2.5 ± 0.01 <sup>bB</sup>	84.4 ± 0.03 <sup>aA</sup>	7.0 ± 0.05 <sup>aA</sup>	7.4 ± 0.01 <sup>aA</sup>
	6	3.1 ± 0.01 <sup>aA</sup>	3.0 ± 0.01 <sup>aB</sup>	80.3 ± 0.01 <sup>bA</sup>	4.2 ± 0.01 <sup>bc</sup>	6.0 ± 0.003 <sup>bB</sup>
	14	2.9 ± 0.03 <sup>bB</sup>	3.0 ± 0.02 <sup>aB</sup>	80.2 ± 0.02 <sup>bA</sup>	1.9 ± 0.02 <sup>cc</sup>	5.5 ± 0.006 <sup>cB</sup>
40°C/min	1	4.3 ± 0.04 <sup>aA</sup>	3.0 ± 0.01 <sup>cA</sup>	80.1 ± 0.01 <sup>aB</sup>	6.8 ± 0.01 <sup>cA</sup>	7.3 ± 0.008 <sup>aA</sup>
	6	3.1 ± 0.01 <sup>bA</sup>	3.9 ± 0.01 <sup>bA</sup>	81.5 ± 0.03 <sup>bA</sup>	9.3 ± 0.01 <sup>bA</sup>	5.8 ± 0.012 <sup>bB</sup>
	14	2.8 ± 0.01 <sup>cB</sup>	4.4 ± 0.02 <sup>aA</sup>	77.6 ± 0.04 <sup>cB</sup>	10.0 ± 0.03 <sup>aA</sup>	5.2 ± 0.04 <sup>cc</sup>

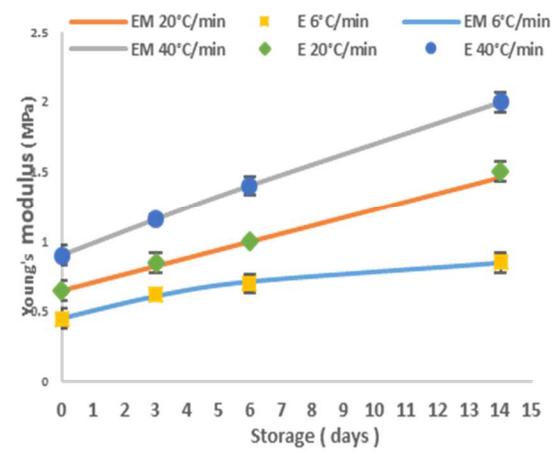
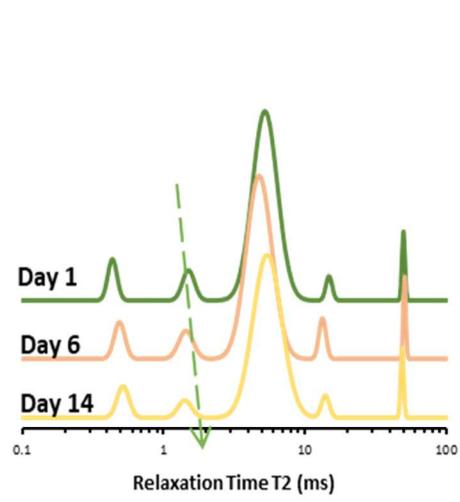
13 \*Within a column, Values with the same small letter at different storage times of one HR and with the same  
 14 capital letters at the same storage time for different HR are not statistically significant (p<0.05), ± SD  
 15  
 16



**Crumb Staling (14 days Storage)**



**Increase of starch gelification ( Amylose crystallization + Amylopectine retrogradation)**



**Gluten dehydration, more Bound water in starch crystallites → Increase of crumb firmness**