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1 **Stabilizing highly hydrated wheat flour doughs by adding water in two steps**

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5

6 **Abstract**

7 A major problem in industrial processing of highly hydrated wheat doughs is the stability of
8 the dough over time. Phase separation occurs during storage with a detrimental effect on the
9 viscoelastic properties of the dough. We developed an original production process in which
10 water addition and mixing were done in two steps. The stability of doughs in which water was
11 added in either one step (1S) or two steps (2S) was compared with water contents ranging
12 from 55 to 80% (wb). The 2S process resulted in a significant slowdown of the sedimentation
13 kinetics, hence a higher stability, in doughs with 55 and 70 wt% water. The microstructure
14 and viscoelastic properties of doughs were analyzed by confocal microscopy and rheology.
15 Protein microstructure helped stabilize the dough. The formation of a gluten network during
16 the first step of the 2S process with only 50 wt% of water is a crucial point.

17 **Keywords:**

18 Rheology, microscopy, gluten, network, process, mixing

19

20 **1. Introduction**

21 The wide range of currently consumed cereal products comes from the wide range of
22 ingredients used and of incorporation, mixing and cooking processes. Among the ingredients,
23 water appears to contribute particularly to the texture of the end-product. Hence, to prepare
24 100 g of dough with a T55 wheat flour, about 15 g, 32 g, 43 g and 60 g of water is used to
25 prepare cookie or snack, cake or pasta, bread, crêpes or pancake, respectively (Guibert-Martin
26 et al., 2017; Kiszonas et al., 2015; Monnet et al., 2019).

27 When mixing wheat flour with water (more than 20 g of water for 100 g of dough), the input
28 of mechanical energy caused by mixing destroys the flour grain particles, which are
29 composed of fragments of albumen cells containing starch and proteins, with cell walls
30 mainly composed of pentosans. The proteins hydrate themselves, go through the glass to
31 rubbery transition and soften. The flour grain particles swell and are mechanically broken
32 apart by mixing, but the starch granules remain embedded in the protein matrix (Létang et al.,
33 1999). In contact with water, proteins and the other components of the flour, hydrate and bind
34 a given amount of water depending on their affinity for water. Depending on the study, in
35 non-limited water conditions, native starch binds from 39% to 87% of water per gram of
36 component, damaged starch from 200% and 430%, pentosanes from 500% to 1500% and
37 proteins from 114% to 215% (Berton et al., 2002; Feillet, 2000). Thus, the global maximum
38 capacity of water binding varies depending on the proportion of the different components.
39 Moreover, when the quantity of water is limited, there is competition for water between the
40 ingredients. The resulting distribution of water between them depends on their proportion but
41 also on the way water is added and enters in contact with flour.

42 For bread making, specific kneaders are designed to be able to shear, stretch and compress the
43 dough, in turn. When the water is added to the flour during mixing, this results in the swelling
44 of gliadin and glutenin (the insoluble proteins of the wheat flour). These meet and

45 interconnect through covalent (disulfide bonds) and non-covalent bonds. Connected glutenin
46 form big aggregates. Under alternating stretching, shearing and compression, the bonds are
47 broken and new bonds are formed leading to development of a 3D protein-network, called
48 gluten.

49 The formation of the gluten network depends on mixing time, the water addition kinetics,
50 mixing energy, temperature, flour-to-water ratio and the mechanical stresses applied during
51 mixing (Jongen et al., 2003). The protein network provides the viscoelastic properties
52 required for bread products.

53 A major problem in industrial processing of doughs with high water contents (60% wet basis
54 (wb) and above) concerns the stability of the dough over time. During storage, phase
55 separation can occur leading to the formation of an aqueous upper phase and a sediment
56 bottom phase (Kontogiorgos et al., 2008; Larsson and Eliasson, 1996). In doughs with
57 50 wt% water in the total dough (wt% water) and above, using the usual mixing parameters,
58 the gluten network does not form due to the dilution of proteins and insufficient opportunity
59 for them to meet several times to interact and form bonds. However, as described in the work
60 of Auger et al. (2008), it is possible to form a fully connected gluten network in doughs
61 containing from 50 to 56 wt% water by optimizing the mixing parameters. This is
62 encouraging, but not sufficient for batter containing even more water, i.e. 60 wt% and above
63 because one of the conditions reported by Auger et al. (2008) was a longer mixing time (more
64 than 30 min for a dough containing 56 wt% water) which is not acceptable in terms of input
65 energy but also in industrial processes.

66 As reducing the water content of the dough enables the development of a tridimensional
67 gluten network, one possible way to produce one even at high water content is to add the
68 water in two steps. The first step would allow the formation of the gluten network in a

69 relatively short time. The second step would dilute the gluten network under further water
70 addition and mechanical kneading.
71 Our aim in the present work was to explore the interest of adding water in two steps in doughs
72 containing from 55 to 80 wt% water to improve their stability and prevent phase separation.
73 Doughs were prepared by adding water in two steps and in one step using the same kneader
74 and the same total kneading times. The stability and the rheology of the resulting doughs were
75 evaluated. Results were discussed with the help of the characterization of the protein
76 organization by confocal scanning microscopy.

77 **2. Material and methods**

78 ***2.1. Dough preparation***

79 Wheat flour was provided by Les Fournils du Val de Loire (Agromousquetaires, France). The
80 composition of the flour was determined using normalized ISO methods. The results were
81 14.55 ± 0.01 g moisture per 100 g flour (ISO 712:2009), 11.29 ± 0.04 g protein content per
82 100 g dry flour (ISO 16634-2), and 8.63 ± 0.18 g dry gluten per 100 g flour (21415-4). The
83 flour was stored at -30°C in plastic buckets, then stored at 4°C for one to six days before
84 being brought back to room temperature for an experiment. The calculated water content of
85 the dough, h , also accounted for the water provided by the wheat flour itself, hf (percentage of
86 water in 100g of flour on wet basis). The amount of water to be added (in grams) x , for a
87 weight of wheat flour m , was calculated using equations 1 and 2:

$$88 \quad h = \frac{hf * m + x}{m + x} \quad (1)$$

$$89 \quad x = m * \frac{h - hf}{1 - h} \quad (2)$$

90 The range of water contents, h , was set at 55 wt% to 80 wt% of water of total formula,
91 including the water provided by the flour. Distilled water at a temperature of 25°C was used
92 in all formulations. All the doughs were prepared using a KSS45 EOB CLASSIC, planetary

93 mixer (KitchenAid, USA) fitted with the leaf device. Two dough production processes were
94 tested to produce wheat flour doughs containing 55, 60, 65, 70 and 80 wt% water. In the first
95 process, the water was added to the flour in one step (1S), and the blend was mixed at speed 4
96 (corresponding to about 127 rpm) for 12 minutes. In the second process, water was added in
97 two steps (2S). First, sufficient water was added to the flour to obtain a dough with 50 wt%
98 water then mixed at speed 4 for 3 minutes. Second, the remaining water was added and
99 mixing continued for a further 12 min at speed 4. The quantity of water added in the second
100 step depended on the water content targeted, which were 55, 60, 65, 70 and 80 wt% of water
101 of total formula. Each sample of dough was produced in triplicate.

102 ***2.2. Stability study***

103 The stability of the dough was monitored using a Turbiscan Classic MA 2000V R
104 (Formulacion, France). Dough samples (4 mL) were poured into glass tubes (internal
105 diameter 6.1 mm). Samples were scanned using incident light 880 nm wavelength and a
106 resolution of 40 μm . Light backscattering and light transmission were measured and are
107 expressed as a percentage of incident light. When the transmitted light shows a non-zero
108 value, the sample is translucent and, in that case, the backscattering signal, which is generally
109 about 20%, cannot be interpreted. When the transmission signal is equal to zero, the
110 backscattering scan, whatever the value, can be interpreted and refers to an opaque phase. The
111 average maximum intensity of light backscattering, $\text{MBSm}(\%)$, is defined as the mean of
112 light backscattering values in the plateau formed in the section of backscattering values when
113 the transmission is equal to zero. The top of the sediment phase (TIP) is detected as the
114 height, H , at which the intensity of backscattering equals the $\text{TIP}(t)$ as calculated by equation
115 3.

$$116 \text{TIP}(t) = \text{MBSm}(t) - 2 \% * \text{MBSm}(t) \quad (3)$$

117 Values of $H(t)$ were normalized by $H(0)$ corresponding to the total height of the sample.
118 Dough samples (4 mL) were poured into glass tubes shortly after production. Scans were
119 carried out at one-minute intervals for 15 min at room temperature. The tubes were then
120 stored at 4 °C and scanned 4 hours, 24 hours, 72 hours and 168 hours (seven days) after the
121 first scan. All the doughs were prepared in triplicate.

122 ***2.3. Rheological measurements***

123 Rheological measurements were carried out using a dynamic stress rheometer Carri-Med
124 CLS² 100 (TA Instruments, USA) fitted with a cone-plate geometry of 4 cm of diameter, 4°
125 angle and 106 μm of truncation. Oscillation frequency sweeps were conducted in the range of
126 0.1 to 10 Hz, at 20 °C and at 0.2% of maximum strain amplitude chosen in the linear domain
127 (identified by preliminary strain sweeps). The doughs remained in the geometry for only one
128 minute before the beginning of the test to avoid phase separation of the sample during the test.
129 Values of storage modulus (G') and loss modulus (G'') measured at 1 Hz in the frequency
130 sweep were taken as representative of the G' and G'' levels of the frequency sweep. $\tan \delta$,
131 defined as the ratio of G'' over G' indicates the structure of the dough. For a defined
132 frequency, strain and time, the smaller $\tan \delta$, the more structured the dough.

133 ***2.4. Environmental scanning electron microscopy (ESEM)***

134 An ESEM (FEI Quanta 200) was used to observe the structure of the dough. Samples were
135 observed in the following conditions: secondary electron detector, ESEM mode (GBSED:
136 gaseous BSE detector), pressure 893 Pa, voltage 13 kV, and, spot size 4.5 and work distance
137 approximately 6.5 mm. A temperature of 7 °C was set to prevent solvent evaporation using a
138 Peltier stage. Samples were directly placed in cupped aluminum stubs designed specifically
139 for the Peltier stage.

140 **2.5. Confocal microscopy observations**

141 The organization of the proteins in doughs at different water contents was observed using a
142 confocal laser scattering microscope (CLSM) (Leica TC SP8 Leica, Germany). Using 250- μ m
143 thick spacers, the dough was placed on the lamella and 2 μ L of Dylight 488 was poured on
144 the top of the dough to label the proteins. The stain was excited using a laser with a
145 wavelength of 488 nm and the emitted fluorescence was detected in the range of 496 to 576
146 nm. Photos were taken at magnification: X10.

147

148 **3. Results and discussion**

149 **3.1. Study of dough stability**

150 **3.1.1. Effect of the process on the sedimentation kinetics of doughs at 70 wt% of**
151 **water of total dough**

152 Figure 1 shows the changes in the height of the sediment over time, $H(t)$ in doughs with
153 70 wt% water made using the 1S and 2S processes. $H(t)/H_0$, the normalized height of
154 sediment phase was initially 0.92 in both samples and remained constant for a period of a few
155 minutes. These $H(t)/H_0$ were lower than 1 because of the presence of some little bubbles, a
156 thin layer of water and/or a small meniscus on the top of the sample. The beginning of the
157 destabilization is the time when $H(t)/H_0$ starts to drop from this value: less than 10 min in
158 the 1S process and more than 15 min in the 2S process.

159 The pictures of the tubes containing doughs are given for different times (15 min, 24 h, 1
160 week) after production. At 15 min after production, the doughs made using both processes
161 had a thin layer of aqueous phase on the top of the samples. Their height ratio was 0.85 for 1S
162 dough and 0.92 for 2S dough. After 4 h, the dough made using the 1S process separated into
163 three different phases; a foamy phase at the top, an aqueous phase in the middle and a

164 sediment phase at the bottom of the sample. After 24 h, the phases were easy to distinguish in
165 the picture of the sample. The sediment compacted over time as the height ratio decreased
166 from 0.5 after 4 h to 0.4 after one week. This result is in accordance with the hypothesis of
167 Eliasson and Larsson (1993) who, for a dough with this amount of water, described a
168 suspension of insoluble swollen gluten proteins, starch and fibers in a liquid phase made of
169 water and soluble components. The sedimentation of insoluble components depends on the
170 viscous property of the continuous phase.

171 In the dough containing 70 wt% water made using the 2S process, the sediment height ratio
172 ranged from 0.92 at t_0 to 0.8 after 4 h, pointing to slower phase separation kinetics than in 1S
173 dough (Figure 1). Only two phases were visible: one sediment phase overtopped by an
174 aqueous phase. The distinct kinetics of phase separation and the number of phases obtained
175 using two processes of water addition confirmed the potential interest of adding water in two
176 steps and underlined the probability of a different organization of components in the doughs.
177 The 2S process includes a step during which a gluten network starts to form during mixing of
178 the flour and water at 50 wt% of water (of total dough) for 3 min. This network is probably
179 diluted by the second addition of water and may be broken down by further mixing in the
180 second step.

181 3.1.2. Effect of water content and the process on the dough sedimentation kinetics

182 To investigate the interest of the 2S process in slowing down the phase separation kinetics in
183 more detail, doughs were tested at water contents ranging from 55 to 80% (wb) water. Figure
184 2 compares the differences in $H(t)/H_0$ in 1S and 2S doughs. Based on repeatability equivalent
185 to the dough including 70 wt% water shown in figure 1 and to simplify the reading, only one
186 result per water content and process is reported in figures 2a (1S) and 2b (2S). Two phase
187 separation kinetics were identified in both processes. Doughs containing 55 wt%, 60 wt% and
188 65 wt% water showed almost no sedimentation for at least 4 h after production. Doughs

189 containing 70 wt% and 80 wt% water sedimented rapidly, and a bottom phase formed within
190 a few minutes and compacted between 15 min and 4 h. However, differences in sedimentation
191 kinetics were also observed depending on the water addition process. The dough containing
192 55 wt% water made using the 2S process appeared to be the most stable of all (Figure 2b). Its
193 $H(t)/H_0$ value was equal to 1 at t_0 and remained equal to or greater than 0.97 for at least 1
194 week. The dough containing 55 wt% water but produced using the 1S process had a slightly
195 lower $H(t)/H_0$ ratio (0.93) which started to decrease after only 24h (Figure 2a).

196 According to Auger et al. (2008), more than 30 min is required to form a gluten network when
197 water is added in one step. Thus, considering the mixing time of the present work (1S process,
198 12 min) it is most likely that gliadin and glutenin could only swell and contribute to an
199 increase in the viscosity of the continuous phase. On the other hand, in the 2S process, a gluten
200 network could have formed after 3 min of kneading with 50 wt% of water (of total dough).
201 The addition of only 5 wt% more water and the additional 12 min of mixing only resulted in a
202 small dilution and/or disruption of the gluten network, which could consequently considerably
203 stabilize the dough upon sedimentation over a full week. Yet, the stability of the dough over
204 time was improved possibly thanks to the formation of the gluten network during the first step
205 of the process.

206 The changes in the sediment height in samples produced with the 1S process and containing
207 60 wt% and 65 wt% water were very similar to those that occurred in the samples containing
208 55 wt% water (Figure 2a). A slightly steeper decrease in sediment height after a week was
209 even observed with an increase in water content. This could be due to reduced viscosity of the
210 continuous phase. Indeed, the higher the water content, the lower the probability of collision
211 between swollen flour particles during kneading and formation of gluten aggregates. Using
212 the 2S process, increasing the water content from 55 to 65 % (wb) enhanced the
213 sedimentation kinetics of the doughs (Figure 2b). However, the 60 wt% water and 2S process

214 dough appeared to be at an earlier stage of sedimentation compared to 1S dough with the
215 same water content (Figure 2a and b). Both 1S dough and 2S dough with 65% of water
216 content (wb) showed the same $H(t)/H_0$ after a week despite the fact the two kinetics differed,
217 i.e. an earlier beginning but more progressive decrease in the 2S dough (Figure 2a and b). All
218 these observations are compatible with the hypothesis of the formation of a gluten network
219 stabilizing the dough structure in the 2S process. The addition of the complementary water is
220 hypothesized to dilute and/or disrupt the network. The greater the quantity of water added in
221 the second step, the less efficient the organization of the continuous phase around starch
222 granules to prevent their sedimentation.

223 Interestingly, the sedimentation kinetics observed in the 70 wt% water 2S dough was in good
224 accordance with the kinetics observed in the 55 wt% to 65 wt% water doughs even if it was
225 even more rapid (Figure 2b). The organization of the continuous phase obtained thanks to
226 kneading in the first step with only 50 wt% of water was still in place, even if less efficient.
227 However, in the 80 wt% water 2S dough, sedimentation was drastic and started in the first
228 minutes after production (Figure 2b). The very same evolution was observed in the 80 wt%
229 water 1S dough (Figure 2a). In both cases, it appears that the continuous phases failed to slow
230 down sedimentation. Finally, the dough with 70 wt% water produced with 1S process also
231 involved rapid kinetics and almost complete sedimentation, even if this occurred a little more
232 slowly than in the 80 wt% water doughs. This behavior could be due to a slightly more
233 concentrated continuous phase which could result in higher resulting viscosity than in the
234 doughs containing 80 wt% water.

235 ***3.2. Rheological properties and microstructure of the doughs***

236 To check the hypothesis of differences in continuous phase microstructure, doughs containing
237 55 wt%, 65 wt% and 70 wt% water were analyzed using CLSM. To better understand the
238 changes that take place in the proteins, the doughs were observed at different times in the

239 mixing process. Figure 3 shows the organization of starches and proteins at the end the dough
240 mixing process (1S dough with 55% wt water) and after the end of the first step of the 2S
241 process: 3 min of mixing after addition of only the first part of the water, i.e. 50 wt%.
242 Figure 3.A shows the sizes of the two populations of wheat starch granules (2 and 20 μm in
243 diameter) embedded in a protein matrix which appeared as a homogeneous soft phase
244 connecting starch granules with each other. These observations are in good agreement with
245 those reported by Létang et al. (1999) in doughs with similar water contents but less mixing
246 time. At this scale, the proteins might play a role in the cohesion and friction between starch
247 granules. Depending on water content and mixing parameters, the proteins are hypothesized
248 to form a complex structure, (the gluten network) that is apparent at a larger scale. Figure 3.B
249 shows large green interconnected strands corresponding to a gluten network as observed by
250 Boitte et al. (2013). In the case of dough with 50 wt% of water content, three minutes of
251 mixing is sufficient to enable the formation of a rather well developed protein network. The
252 strands are oriented in the same oblique direction, probably due to slight shearing when the
253 dough was placed between the two microscope slides (Boitte et al., 2013). The small pale
254 green spots on the strands may be starch particles embedded in the gluten strands. The paler
255 green color of the spots could be caused by partial swelling or solvation of the proteins around
256 the starch granules after only three minutes of mixing, leading to a slight overconcentration
257 around the starch granules. Thus, at the end of the first step of the 2S process, the structure of
258 the dough is rather close to that of bread dough (Hackenberg et al., 2018), which is known to
259 remain stable over time with no phase separation.

260 Pictures of doughs containing 55 wt%, 65 wt% and 70 wt% water made using the 1S and 2S
261 processes were taken after 12 min and 15 min of total mixing time (Figure 4). After 12 min of
262 mixing, dough with 55 wt% water made using the 1S process contained large elongated
263 strands of gluten proteins. However, the strands were poorly connected to each other thereby

264 failing to form a real gluten network even if it looked as though one had started to develop.
265 Indeed, three minutes later, after a total mixing time of 15 min, a gluten network is clearly
266 visible. These two images are very similar to those observed by Auger et al. (2008) during the
267 formation of a gluten network in diluted conditions (51 wt% of total dough water) and mixing
268 for a long time. In comparison, the dough made using the 2S process displayed a well-
269 developed gluten network, formed by stretched and interconnected strands of gluten after both
270 12 min and 15 min of mixing. Despite shearing applied for 9 min and even 12 min during the
271 complementary mixing after the second addition of water, the gluten network remained
272 visible. After 9 min of additional mixing (i.e. 12 min total mixing time, 55 wt% water, 2S
273 process, Figure 4), it showed larger and apparently less connected strands compared to those
274 formed after 3 min of mixing with 50 wt% water (Figure 3). However, after 15 min of total
275 mixing time, the strands were again fully interconnected, even if they appeared to be thinner.
276 The two gluten networks obtained after 15 min of total mixing time had different structures,
277 underlining the importance of the order in which the water is incorporated and of the amount.
278 Moreover, after 12 min of mixing in presence of the total amount of water (images squared in
279 red in figure 5 for 55 wt% water), the 1S process appeared to produce no gluten network,
280 whereas the 2S process did produce one. This certainly explains the difference in stability
281 upon sedimentation observed in Figure 2.
282 No gluten network was observed in 65 wt% and 70 wt% water doughs, whatever the 1S or 2S
283 process and the mixing time used (Figure 4). Proteins appeared in the form of particles of
284 different sizes ranging from 10 to 500 μm depending on the process and on the water content.
285 In the dough with 65 wt% water produced using the 1S process, small protein particles were
286 dispersed evenly throughout the matrix after 12 min of total mixing time. These particles may
287 correspond to the only slightly swollen grains in the flours described by Auger et al. (2008).
288 After 15 min of total mixing time, bigger particles were observed probably due to the further

289 swelling and/or the aggregation of the swollen grains (Auger et al., 2008). Larger protein
290 particles were observed in the dough with 70 wt% water using the 1S process, after 12 min of
291 total mixing with no significant change upon further mixing (i.e. 15 min total mixing time). It
292 seemed that after 12 min, flour grains had swollen to a greater extent than in dough with 65
293 wt% water. However, the dilution prevented the occurrence of sufficient collisions between
294 proteins to start formation of a the gluten network during the 15 min of total mixing time both
295 with the dough with 65 wt% and 70 wt% water in the 1S process. The differences in stability
296 apparent in figure 2 were most probably only due to differences in the viscosity of the
297 continuous phase.-Large particles of about 200 μm were observed in dough with 65 wt%
298 water produced with the 2S process. The particles were obtained by adding 15 wt% of water
299 to a dough containing a fully structured gluten network (3 min of mixing with 50 wt% of
300 water, Figure 3.B). These large particles thus corresponded to fragments of the gluten
301 network, which were very similar after 12 or 15 min of total mixing time. Even larger gluten
302 network fragments were observed in dough with 70 wt% water after 12 min of total mixing
303 time using the 2S process. Moreover, small cloudy green particles can also be seen in the
304 bottom part of the image. They probably correspond to thin gluten network afterglows. After
305 15 min of total mixing time, these thin gluten network afterglows seemed to spread
306 throughout the pictures, embedding the big and lighter gluten network fragments. Their
307 presence may explain the improved stability of the dough with 70 wt% water produced using
308 the 2S process compared to the stability produced using the 1S process. The viscoelastic
309 properties of the doughs were measured to quantify the impact of the production processes on
310 structure of the dough. The results obtained for doughs containing 55 to 70 wt% water are
311 shown in figure 5. It was not possible to characterize doughs containing 80 wt% water
312 because they were not sufficiently stable and sedimented during the test.

313 In both processes, G' decreased with an increase in the water content. This result is in
314 agreement with the literature, even if the corresponding results were obtained with doughs
315 containing less water (between 35 to 49 wt% of total formula) (Hibberd, 1970; Navickis et
316 al., 1982). This is logical because of the generally observed softening effect of water. In the
317 range 55% to 65% of water contents (wb), G' values were very similar in the two processes,
318 whereas G' was significantly higher in dough with 70 wt% water doughs produced with the
319 1S process. However, at 55 wt% of water, the dough produced using the 2S process showed a
320 G' slightly higher and a lower $\tan \delta$. Thus, the network observed in Figure 4 provided the
321 dough with increased elastic properties, which is more structured (lower $\tan \delta$) than the dough
322 produced with the 1S process, in which large and poorly connected strands are observed.
323 These results are in agreement with a system that becomes more stable upon sedimentation.
324 Dough with 65 wt% of water produced using the 1S process had a slightly higher G' but also
325 a much lower $\tan \delta$. The many small gluten particles present in the dough produced with the
326 1S process (Figure 4) seemed to provide a more elastic and structured system with a slower
327 sedimentation kinetics than the 2S process (Figure 2). Finally, the G' obtained for the dough
328 with 70 wt% water produced using the 1S process was significantly higher and the $\tan \delta$
329 significantly lower compared to the doughs produced with the 2S process. However,
330 sedimentation occurred very rapidly in the 1S dough while it was much more progressive in
331 the 2S dough (Figures 1 and 2). The large, swollen but irregularly shaped particles shown in
332 Figure 4 (1S process), probably rubbed against each other affording this dough more elasticity
333 than in the 2S process. In the latter case, fragments of the broken gluten network were
334 embedded in the very soft and cloudy gluten network afterglows (Figure 4) that contributed
335 less to the elastic behavior of the dough and increased $\tan \delta$.

336

337 4. Conclusions

338 This study investigated the effect of the addition of water and of the mixing processes on the
339 phase separation and microstructure of highly hydrated doughs (55 wt% to 80 wt%) of total
340 dough water). This range of water range has rarely been studied in the literature although
341 several applications exist. An original production process, in which water was added and the
342 dough mixed in two steps (2S), was developed to produce dough with targeted water contents
343 and was systematically compared with the process in which water was added in only one step
344 (1S). Different protein microstructures were obtained, some of which resulted in different
345 rheological properties. The sedimentation kinetics of the dough was shown to be directly
346 linked to the protein microstructure. The schematic representations of the microstructure and
347 induced doughs behavior at macroscopic scale over time in figure 6 to summarize the main
348 findings of this work. With the 1S process, the dough containing 55 wt% of water was made
349 up of large poorly connected gluten strands, whereas in doughs containing 65 wt% of water,
350 the gluten appeared as dense particles, and in doughs containing 70 wt% of water as swollen
351 diluted particles. Adding water in two steps led to the formation of an even more complex
352 protein structure. The first step consisted in adding only 50 wt% water in order to allow the
353 rapid formation of a well-connected gluten network. This gluten network broke up after the
354 second addition of water and further mixing, resulting in fragments of gluten of different sizes
355 for water contents above 65% (wb). In dough with 55 wt% water, the limited dilution of the
356 dough results in a fully connected gluten network made of thin strands after further mixing.
357 What is more, the same process results in the formation of a diffuse gluten network made of
358 thin connected strands in doughs containing from 55 wt% to 70 wt% water. On average,
359 between 55 wt% and 70 wt% water, stability upon sedimentation was the same or improved
360 with the 2S process. The biggest difference in phase separation kinetics was observed in
361 doughs containing 70 wt% water, when in the 2S process, only two phases separated in 24

362 hours instead of the three phases in the 1S process. The formation of a gluten network at 50
363 wt% water before the addition of any more water and further mixing was found to improve
364 the stability of highly hydrated doughs for more than 24 hours.

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370

371 **6. References**

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417 **7. List of Figure captions**

418 *Figure 1: Changes in sediment height over time in dough with 70 wt% of water of total dough*
419 *obtained using the 1S and 2S processes. The pictures of the dough in Turbiscan tubes were*
420 *taken 15 min, 24 h and one week after production.*

421 *Figure 2: Temporal changes in the height of sediment in refrigerated doughs containing 55*
422 *wt% (triangles), 60 wt% (squares), 65 wt% (diamonds), 70 wt% (empty circles and dotted*
423 *line) and 80 wt% (black dots) water: (a) 1S process, (b) 2S process.*

424 *Figure 3: Dough microstructure observed by MEBE (1S dough with 55 wt% water) and*
425 *CLSM (50 wt% water, 3 min of mixing after the first addition of water during the 2S process).*
426 *Stained proteins are green in panel B.*

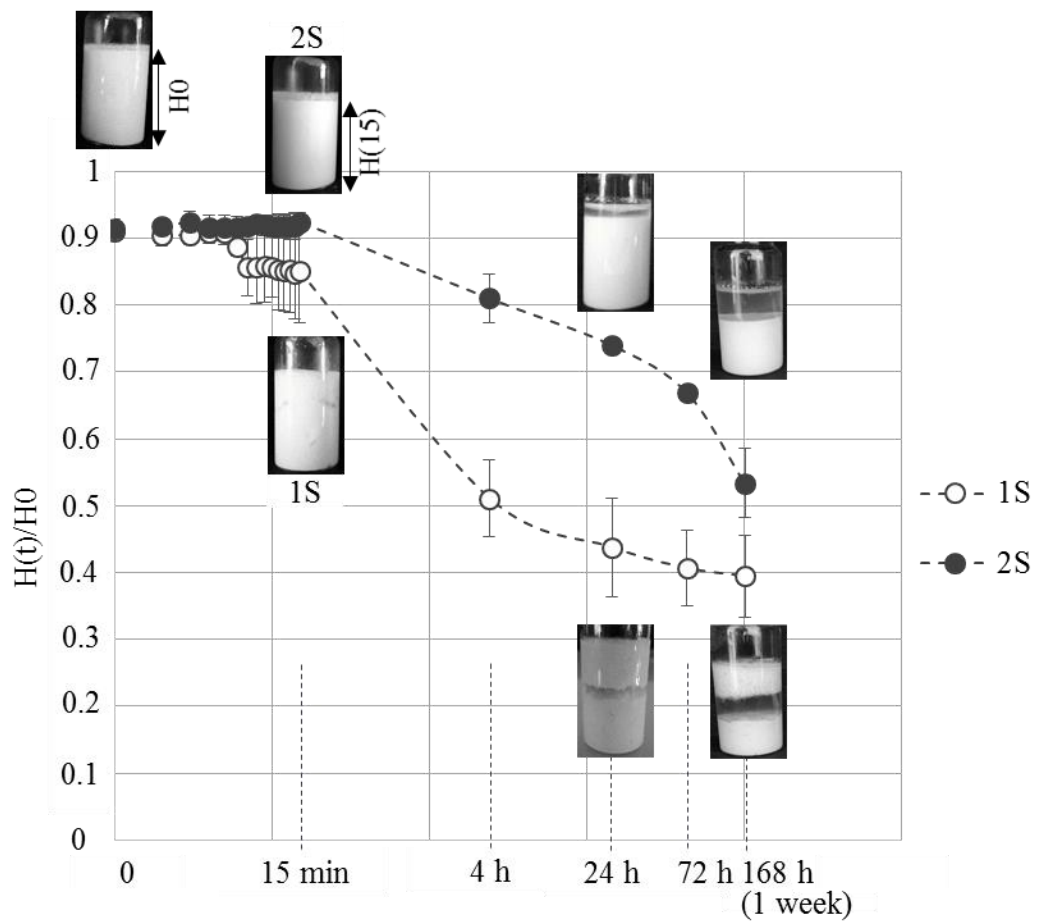
427 *Figure 4: Observations of stained proteins (in green) using CSLM in doughs containing 55*
428 *wt%, 65 wt% and 70 wt% water obtained using 1S and 2S processes with different mixing*
429 *times (the initial time, t_i , indicates the beginning of mixing). The images of doughs*
430 *investigated in the stability study are framed in red. The scale bar is 200 μm .*

431 *Figure 5: Effect of water content on the viscoelastic properties of doughs just after the end of*
432 *mixing. (a) Storage modulus (G'), (b) $\tan \delta (=G''/G')$. Empty symbols show the 1S process,*
433 *Black symbols show the 2S process. 20°C, 1 Hz, 0.2% maximum strain amplitude.*

434 *Figure 6: Macroscopic and microscopic representation of dough structure immediately after*
435 *production (t_0) and after 24 h for doughs containing 55, 65 and 70 wt% of water and*
436 *produced using two different water incorporation processes: one step (1S) and two steps (2S).*

437

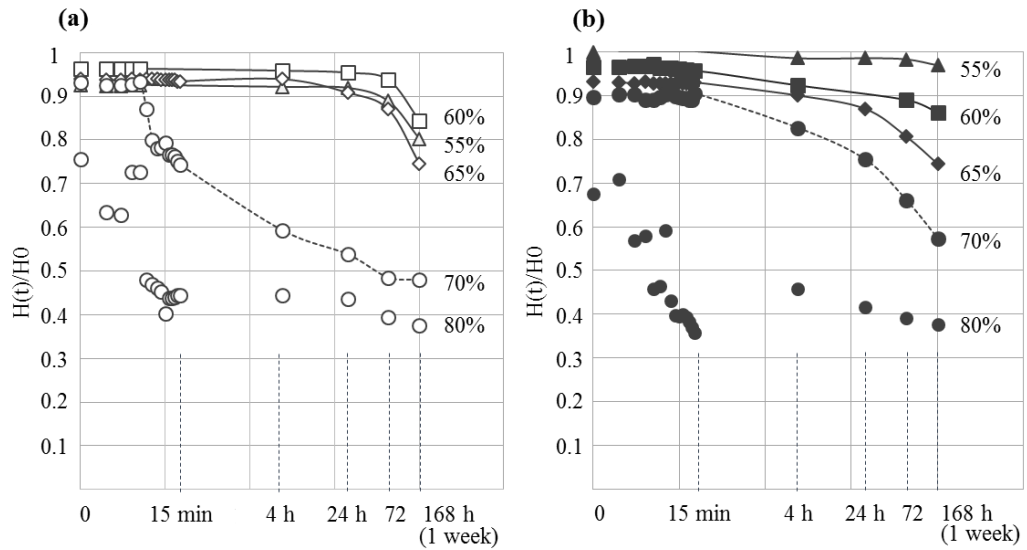
8. Figures



439 9.

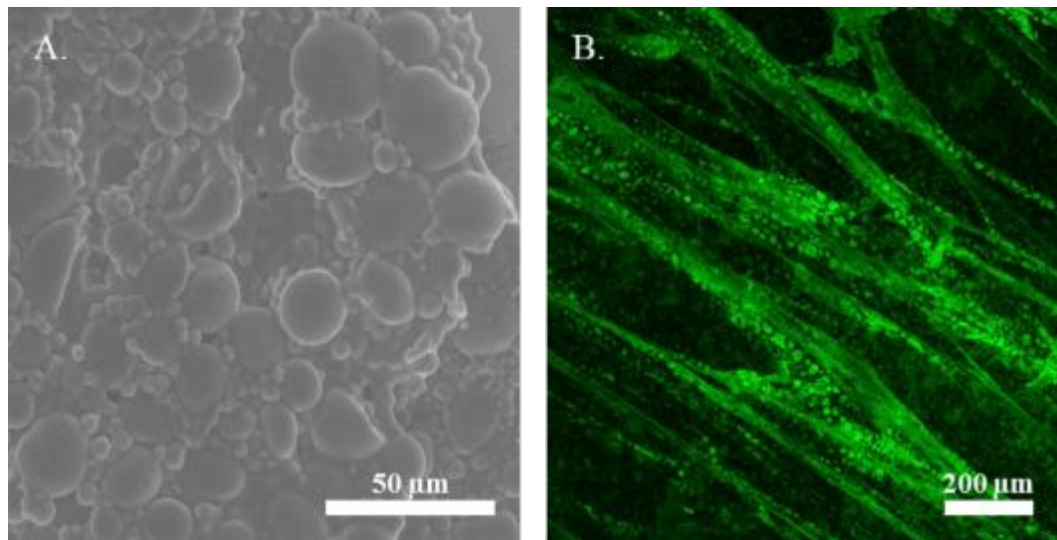
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 442 *Turbiscan tubes were taken 15 min, 24 h and one week after production.*

443



444 11.

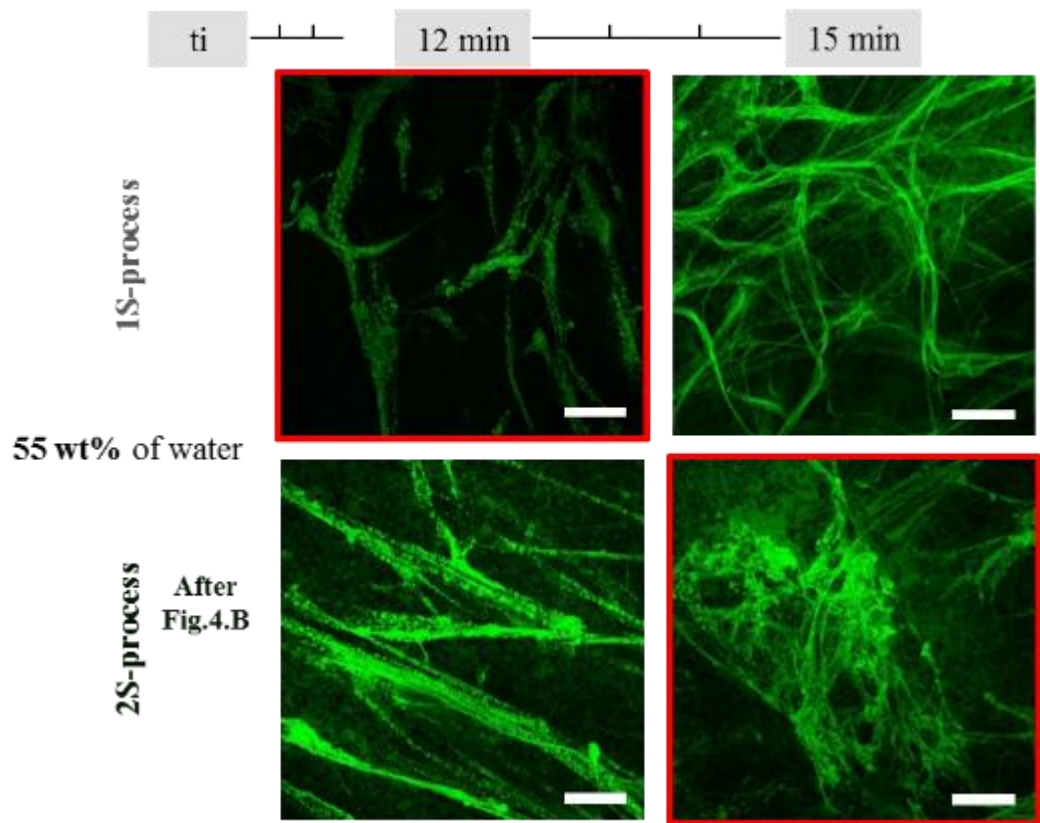
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 447 *(empty circles and dotted line) and 80 wt% (black dots) water: (a) IS process, (b)*
 448 *2S process.*



450 14.

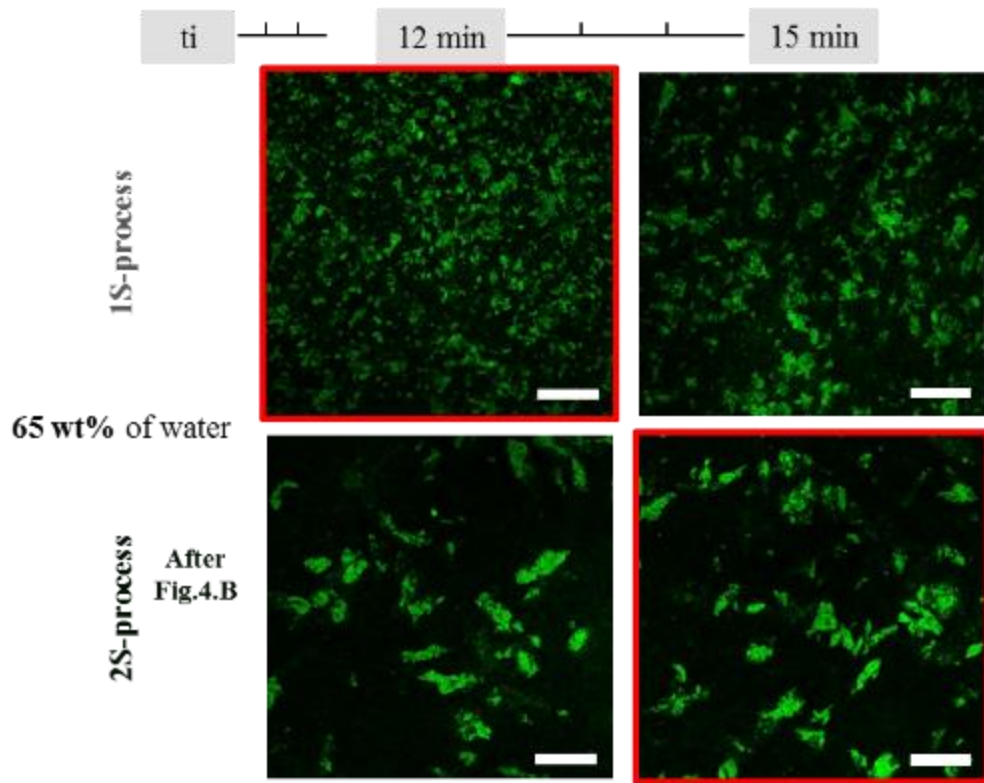
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452 *water) and CLSM (50 wt% water, 3 min of mixing after the first addition of water*
453 *during the 2S process). Stained proteins are green in panel B.*

454 16.

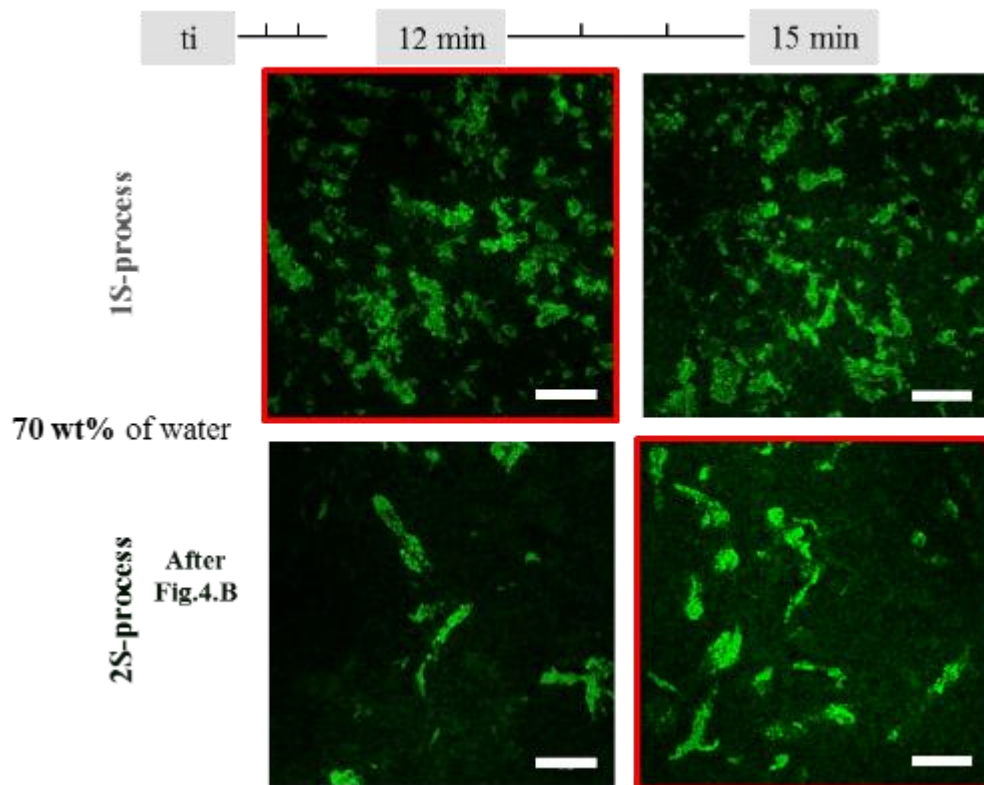


455 17.

456 18. (Continued)



457 19.



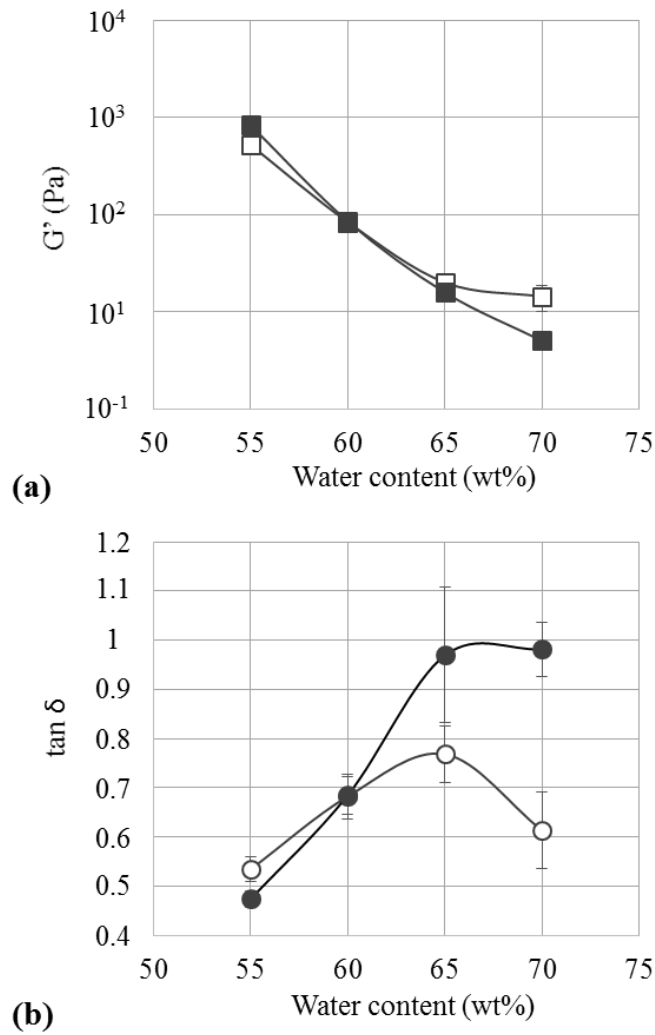
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 461 *with different mixing times (the initial time, t_i , indicates the beginning of mixing).*

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463 *scale bar is 200 μm .*

464 21.

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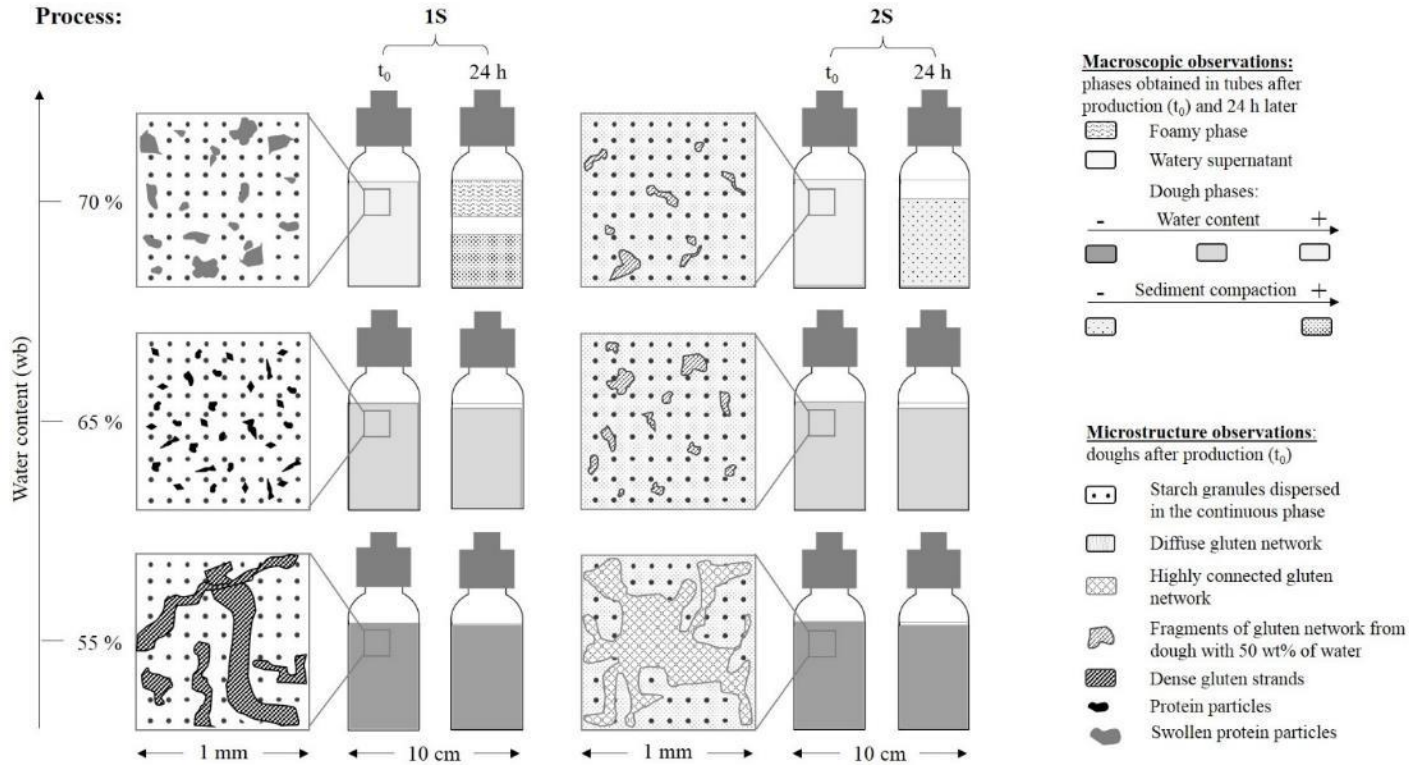


466 22.

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471 24.

472



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