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Title

Gas cell opening in bread dough during baking

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Abstract

Background: This literature review describes the evolution during baking of the three main components in dough (starch, proteins, and the aqueous phase) in order to understand what causes gas cells to open. To date, most of the literature has focused on the role played by proteins, gluten having received most attention in the last decades (strain hardening properties, ability to stretch without rupturing etc.). The possible role of a liquid lamella has more recently been proposed. While a number of articles directly evidence its existence, indirect results also provide proof of its presence. The role of starch in the mechanisms of gas cell stabilization/destabilisation has been little considered. The multiple actions of starch described in this review may offer an explanation for this.

Scope and approach: The authors have set out to consider all phases and to understand how they may interact during baking in such a way as to lead eventually to gas cell wall rupture.

Key findings and conclusions: The four most likely situations are presented and discussed:

- gluten with poor ability to stretch: rupture occurs too early during baking.
- gluten with poor ability to stretch but assisted by a liquid lamella: rupture is delayed; extent of delay is dependent on starch's sorption of water.
- gluten with good ability to stretch, starch granules soften early during baking but do not fuse (ideal situation): structure opens late in baking when loaf is able to sustain its own weight.
- too many fusing starch granules: gas cell walls fail to rupture and loaf shrinks during cooling.

1

1. General introduction

2 Dough can be viewed as a dispersion of discrete bubbles in a viscoelastic gluten-starch
3 matrix, hydrated with water and comprising some other minor components (lipids,
4 hydrocolloids). At advanced proofing, the wall that separates adjoining bubbles may become
5 extremely thin, most akin to a film (made of gluten more or less loaded in starch granules or
6 liquid lamella). These bubbles are also described as cells, by reference to the term used for
7 foams. Pore opening which takes place during proofing and/or baking is the process by which
8 the film ruptures and is replaced by a network of inter-connected cells that may coalesce into
9 a single, larger and quite round cell where the Gas Cell Wall (GCW) is still deformable.
10 During baking, hydrothermal reactivity of starch and gluten will much affect the mechanical
11 behavior of the GCW directly, but also indirectly by modifying the water distribution in the
12 GCW.

13 Gas contracts upon cooling, resulting in low pressure in closed bubbles and a decrease
14 in cell volume (Gan, et al., 1995; Mills, et al., 2003). Pore opening is hence required to
15 preserve the inflation obtained during the proofing and baking stages and a normal loaf shape
16 (Fig. 1). In normal conditions, bread crumb tends to display an open foam structure,
17 permeable to gases (Baker and Mize, 1939) i.e. in which local pressure rapidly equilibrates
18 within the connected network (Grenier, Le Ray, et al., 2010). Last, open pores also contribute
19 to the softening of crumb at the chewing step – the relationship between crumb density and
20 elasticity increases two-fold when passing from open to closed pores (Wang, et al., 2011).

21 Gases are retained as long as cells remain closed. The timing of pore opening during
22 the breadmaking process is crucial for optimal loaf rise. Extensive work in the past decades
23 has been dedicated to finding ways of enhancing gas retention during proofing. This has
24 yielded better-performing flours for breadmaking where pore opening is successfully
25 postponed to advanced stages of baking. The exact time of pore opening in the baking process
26 remains key for the control of final crumb texture. Simulations with a numerical model of
27 baking (Lucas, et al., 2015; Nicolas, et al., 2016) showed that the timing of pore opening
28 much affected the spatial distribution of gas fraction at the end of baking, especially near the
29 loaf surface (Fig. 2). While late pore opening during baking favors gas retention, high
30 inflation and uniformity of gas fraction, early pore opening results in collapse of gas cells at

31 the lower part of the loaf, resulting in crumb area of very low density and low overall inflation
32 (Nicolas, et al., 2016).

33 Despite of the impact of pore opening on the final bread characteristics, the
34 mechanisms governing GCW rupture during baking are still poorly documented. Biochemical
35 changes (starch gelatinization, protein denaturation) are often invoked as preliminary to GCW
36 rupture. However, the way these macromolecules are involved in the micromechanics of the
37 GCW has been little considered in the literature. The elucidation of the mechanisms driving
38 the rupture of the GCW requires study on a scale where the gluten-starch matrix can no longer
39 be considered as homogeneous and where interactions between constitutive phases (hydrated
40 gluten, hydrated starch granules and possibly the liquid lamella) predominate (Van Vliet, et
41 al., 1992). However, observations on that scale are not easy when studying opaque material
42 undergoing rapid dynamic changes.

43 The aim of this paper is to review the state of the art related to the rupture of GCWs
44 from advanced proofing to the end of baking, in order to propose a unified vision of
45 governing mechanisms at the microscale together with the compilation of data necessary to
46 achieve this understanding and to identify the gaps to be filled. This review first describes
47 past attempts to monitor pore opening. Most of these measurements were performed at the
48 macroscale (dough scale) and were related to the biochemical events in an indirect manner
49 (through temperature monitoring in fact). The following two sections focus on events at the
50 scale of the GCW and below. First, Section 3 gives a short overview on the size and
51 morphology of GCWs. This section completes the introductory concepts for those new to the
52 field. More expert readers may adjust their perception of dough as a fundamentally
53 heterogeneous foam structure, where thick GCWs are still predominant even at the end of
54 proofing. Section 3 ends with the concept of the “one-layer” state of the GCW, involving only
55 a single layer of oriented starch granules, which precedes the formation of a gluten film at
56 some spots. Then, Section 4 describes the interlinked biochemical and mechanical changes
57 occurring during baking in each constitutive phase of the GCW. It compiles what little data
58 has been reported at the moderate water contents deriving from bread dough and the
59 temperature range applied during baking, but also highlights what is required for
60 investigations in the immediate future. Section 4 ends by questioning the spatial organization
61 of these phases in a GCW as well as their modes of interaction during baking. If the
62 interaction between starch and gluten has quite been studied for crumb i.e. after cooling and
63 starch retrogradation (Guessasma, et al., 2011; Lagrain, et al., 2012; Liu, et al., 2003), there is

64 little information about the nature of the interaction during baking. Last, Section 5 describes
65 the ways each phase favors or postpones the rupture of the GCW. It returns to and refines the
66 mechanisms commonly invoked by the cereal community and proposes some novel ones to
67 complement these. It raises a concern over the stabilization/destabilization role played by the
68 starch granules during baking, which has been far too overlooked in the literature.

69 **2. Estimation of pore opening during baking**

70 In a pioneer investigation, He, et al. (1991b) tentatively linked the timing of pore opening with
71 the initiation of CO₂ release in the oven atmosphere during baking. A precise temperature could be
72 assigned to these observations since the ohmic heating used in this study ensured quite an even
73 temperature throughout the dough. The authors concluded that the “loss in gas-retaining ability”
74 started at 72°C and persisted until 88°C. However, Zhang, et al. (2007) questioned the different
75 mechanisms controlling the major peak of CO₂ release. They assigned the release of CO₂, with the aid
76 of a numerical model of baking, to the development of a pressure gradient consecutive to crust
77 formation. They also indicated that pore opening can occur well before the major peak of CO₂ release
78 and, in such a case, accounts for the smaller levels of CO₂ released. Reports from He and Hoskeny
79 (1991a, 1991b) were re-analyzed in the light of Zhang et al.’s findings, yielding pore opening
80 temperatures of 48-50°C when the dough consistency was optimal. Lower opening temperatures were
81 found for doughs prepared with less water (40-42°C) or with starch or gluten preheated at above 60°C
82 (30°C).

83 Cereal science has also benefited from developments in polymer and metal foam science (e.g.
84 Neff and Macosko (1996) and Zhang, et al. (1998)) allowing indirect investigation of pore opening
85 during bread dough baking (Miś, et al., 2016; Singh and Bhattacharya, 2005). For instance, Neff and
86 Macosko (1996) used rheometers to dynamically load an expanding urea-based polymer in the shear
87 direction, while monitoring its expansion. As for dough, a mixing stage is used to initiate bubbles into
88 the foam. Then, carbon dioxide is chemically produced within the foam and makes the growth of
89 bubbles possible under the plate of the rheometer. During expansion, the load varies with the pressure
90 exerted by the gas onto the GCWs within the foam and is lowered by GCWs opening. The normal
91 force first increased and was related to the stiffening of the polymer matrix. Then, when pore opening
92 occurred, the normal force suddenly dropped. Similar events were observed in the case of bread dough
93 during heating. Unfortunately, temperature uniformity throughout the dough sample was not well
94 controlled or recorded. After re-evaluation of the dough temperature commonly associated with the
95 minimum and maximum dough viscosity levels reported in these studies (55-60°C and 75-80°C

96 respectively, according to (Dreese, et al., 1988; Vanin, et al., 2010)), pressure-peak temperatures were
97 re-estimated at 87°C for Singh, and 85°C for Miś. Note also that Miś, et al. (2016) used a device less
98 subject to auto-tension effects than that of Singh and Bhattacharya (2005). A rheometer was associated
99 with laser displacement sensors to measure the additional lateral expansion of the dough sample
100 compared with its normal displacement.

101 More recently, connectivity between cells consecutive to GCW rupture has been estimated
102 after thresholding of dough/crumb images acquired by 3D X-ray tomography. Connectivity is defined
103 as the ratio of the volume of the largest gas cell to the total volume of gas cells and ranges from zero to
104 the unity. Very high values of connectivity were found in bread crumb, as expected, >98% (Wang, et
105 al., 2011), but also, more surprisingly in fully proofed dough, > 85% (Babin, et al., 2006; Turbin-
106 Orger, et al., 2012). A tiny hole in the GCW is sufficient to connect neighboring cells and the
107 detection of holes will be considerably affected by the spatial resolution and the signal-to-noise ratio
108 in the images to be analysed. In Babin, et al. (2006) and Turbin-Orger, et al. (2012), the thinnest
109 GCWs may not have been detected because of insufficient spatial resolution, leading to an
110 overestimation of connectivity. Zghal, et al. (2002) proposed an alternative method based on 2D
111 images of bread sections; they compared the number of gas cells with a reference obtained using the
112 shortest proving time, and deduced the proportion of “missing” GCWs, which ranged from 0 to 13%
113 depending on flour type.

114 In conclusion to this section, all methods proposed in the literature still require improvements
115 before a definitive estimate can be reached. On the one hand, most of these measurements were
116 performed at the macroscale (dough scale) and were related to the biochemical events in an indirect
117 manner (through temperature monitoring in fact). On the other hand, imaging the GCW in the
118 dynamics of extension still lacks sufficient spatial resolution for confirming evidence of gas cell
119 opening.

120 **3. Size distribution of gas cell walls in relation to the structuring of** 121 **the gaseous phase in dough during the breadmaking process**

122 The mixing step allows for flour hydration and contributes by its dispersive and distributive
123 actions to the development of the gluten network, trapping air in the form of large bubbles that are
124 subsequently broken down into smaller ones (Fig. 3a). During the proofing step, these tiny air bubbles
125 take up the CO₂ and ethanol produced by yeast fermentation and grow. The increase in temperature
126 during baking induces i) the supplementary production of CO₂ by yeast up to its inactivation
127 temperature and the liquid-gas transfer of the dissolved gas into bubbles (Nicolas, et al., 2016),

128 resulting in what is known as oven-rise, and ii) the setting of the structure and the formation of the
129 crust. Gas fraction, defined as the volume of gas per total volume of dough, thus increases across all
130 the breadmaking steps: 8-12% immediately after mixing, 70-75% at the end of proofing and 80-95% at
131 the end of baking (Della Valle, et al., 2014).

132 With increasing gas fraction the thickness of the gluten-starch matrix surrounding gas cells
133 decreases. Yet low values of 300-500 μm in average (Fig. 3b) were already reported for GCW at the
134 end of mixing, which is explained by the already high number of small-sized bubbles, 10^5 - 10^9 per cm^3
135 (Bellido, et al., 2006; Chakrabarti-Bell, et al., 2014). GCWs decrease in thickness down to 240 μm at
136 the end of proofing (Babin, et al., 2006; Besbes, et al., 2013; Turbin-Orger, et al., 2012); this average
137 thickness is associated with a high dispersion of 200 μm which mirrors the broad distribution in
138 bubble diameter (Fig. 3c). Such a foam structure is very different from the first one depicted by
139 Bloksma (1990), which assumed a cubical array of close packed gas spheres of equal size separated by
140 thin walls at multiple contact points. Indeed, GCWs thinner than 20 μm made up less than 0.5% of the
141 GCW material in dough regardless of proofing time (Fig. 3b). Nevertheless, this proportion is
142 indicative only (and might be slightly underestimated) since the spatial resolution of images used for
143 this estimation (Turbin-Orger, et al., 2012) did not capture the thinnest GCWs (this is why the
144 probability density function can be seen to be truncated on the lower side in Fig. 3b). On the other
145 hand, coalescing bubbles during proofing drive local thickening of GCWs (+50 μm over 80 min)
146 (Turbin-Orger, et al., 2012). The thickness of GCWs is not greatly modified on average by baking
147 (Babin, et al., 2006; Turbin-Orger, et al., 2012), but sizes become more dispersed, following further
148 thinning of the GCWs upon extension and local thickening arising from additional coalescence (Fig.
149 3b). In fact, bubble coalescence may continue during baking and was observed until loaf cores reached
150 70°C (Grenier, Le Ray, et al., 2010; Hayman, et al., 1998). Fig. 4 shows microscopic shots of these
151 GCWs at different breadmaking steps. Reports in the literature are of large GCWs with 20 to 50 starch
152 granules embedded from one side to the other, like in Hug-Iten, et al. (1999), see also Fig. 4b. One to
153 two rows of elongated starch granules confined by gluten films are still detectable in the thinnest
154 continuous GCWs available in the literature (Fig. 4c, f). This configuration of a wall composed of only
155 one single particle layer, also called the “one-layer state” in material science, is presented as the
156 preliminary stage to rupture (Bloksma, 1990). In conclusion, thick GCWs are still predominant even at
157 the end of proofing and a minority only conforms to the “one-layer state”. GCW thinning which ends
158 with the rupture must be viewed as a continuous process, taking place at different locations in dough
159 during the whole breadmaking process, from early proving until the end of baking. In this view, the
160 GCWs of intermediate-size (two to three rows of aligned starch granules in the thickness of the GCW)
161 at the end of proving are the ones most likely to rupture in the first part of baking. Next section
162 focuses on the heat-induced changes in the molecular structure leading to the setting of GCWs, as well
163 as the organization of these molecules into GCW phases and the way they interact.

164

165 **4. Constitution, spatial organization and changes in the** 166 **properties of cell walls during the breadmaking process**

167 Once the GCW thins, it can no longer be considered as a continuous homogeneous medium; its
168 characteristic size corresponds to those of its main constituents (gluten film, starch granules). Hence,
169 to address the GCW, it is necessary to consider the phases both individually and as they interact with
170 each other. Three phases are considered: the aqueous granular phase, mostly composed of starch
171 granules, the hydrated gluten and the liquid lamella. Sections 4.1 and 4.2 focus on the first two phases
172 separately. Section 4.3 deals with the organization and the modes of interaction between these two
173 phases in the GCW; this last section also discusses the distribution of water between phases in
174 assembly, with a focus on the resultant liquid lamella (the third phase).

175 **4.1. Wheat starch**

176 The stiffness and size of starch granules affect the minimal size of the thinnest GCWs
177 (Bloksma, 1990) (Fig. 4). Both are themselves impacted by granule moisture, that is, by gelatinization
178 kinetics which are in turn affected by the amount of available water, temperature and also granule
179 composition (amylose/amylopectin ratio, crystallinity). Starch gelatinization is accompanied by
180 amylose leaching which contributes to increased viscosity in the dough water. Transfers into and out
181 of granules together with phase changes during heating are described below and are linked to the size
182 and mechanical properties of starch granules. The focus is on individual granules considered at low to
183 intermediate moisture levels. Since few studies conducted under these conditions are available,
184 frequent reference will be made to studies of water-starch suspensions with excess water and the
185 findings will then be extrapolated by us to lower water contents (WC). All WC values are expressed in
186 wet basis (wb) *i.e.* in g of water per 100g of wetted sample (flour or dough). The analysis spans a large
187 temperature range, extending below and above the heat-induced transition temperatures of starch.

188

189 At or close to ambient temperature

190 The three-dimensional structure of native starch granules is well described in the literature
191 (Gallant, et al., 1997). Starting from the hilum, starch is deposited in alternating amorphous and semi-
192 crystalline concentric growth rings which mainly contain amylose and amylopectin respectively. The
193 distribution in size of wheat starch granules is bimodal. The largest granules (so-called A-type) are
194 lenticular, of 5-15 μm in thickness and 22-36 μm in diameter (Jane, et al., 1994). The smallest

195 granules (B-type) are spherical and their diameter ranges up to 15 μm (Wilson, et al., 2006). They
196 account for 27% of the total starch in weight, but for 97% in number (Soulaka and Morrison, 1985).

197 When in contact with water, starch granules swell due to the strong hydrophilic nature of their
198 constituent macromolecules. At or close to ambient temperature, hydration mainly involves the
199 amorphous parts made up of amylose (French, 1984) and leads to swelling. Reports on swelling of
200 starch granules in excess water are rare for this temperature range. By comparing the density and water
201 content of dry and hydrated wheat starch granules, Dengate, et al. (1978) reported swelling power (SP)
202 of 1.43 in excess water at 20°C (SP being expressed in gram of the hydrated starch granules per gram
203 of the dry original granules). This corresponds to a 1.3-fold increase of the lenticular diameter (34-47
204 μm), assuming constant thickness of the granule (see below). SP varies with the botanical origin of
205 starch and with the proportion of damaged starch in flour which represents between 7-27% of the total
206 starch (w/w db) for wheat (Berton, et al., 2002). Indeed, WC of wheat native starch granules in contact
207 with excess water is between 36 and 49% wb (Rasper and Deman, 1980), while WC in damaged
208 starch is between 67 and 81% wb (Berton, et al., 2002; Bushuk, 1966). There is also a suspicion that
209 swelling will increase with increasing temperature level, even before the onset of starch gelatinization
210 (Kovrljija and Rondeau-Mouro, 2017). However, these changes have been overlooked in studies
211 involving SP at high temperatures which have very often reported values close to unity at temperatures
212 of 40-45°C e.g. (Muñoz, et al., 2015; Tester and Morrison, 1990). Given this evidence, the significant
213 swelling of intact granules before the transition temperature of starch is discarded as a possible
214 mechanism in the following and, consequently, the dimensions of dry starch granules are considered to
215 apply still at the beginning of baking.

216 Additionally, water sorption by granules is accompanied by the release of amylose into the
217 surrounding dough water. There are few studies of leaching at low temperatures. The literature places
218 strong emphasis on the high capacity of damaged starch to leach out at room temperature e.g. (Evers
219 and Stevens, 1984). A more recent study using Time Domain-Nuclear Magnetic Resonance (TD-
220 NMR) showed that 3.34 ± 1.27 g per 100 g of dry wheat starch leached out at 20-40°C and a water
221 content of 35-50% wb (Kovrljija and Rondeau-Mouro, 2017).

222 In the wheat kernel, wheat starch falls within the GPa range of Young's modulus (Chichti, et
223 al., 2013). The literature reports are scarce for wheat starch granules. Starch granules will be
224 considered as rigid particles at the early stages of baking.

225 In the temperature range of baking, where the major phase transitions occur in starch

226 When heated in excess water, wheat starch undergoes an irreversible disruption of its
227 molecular order (crystallite melting) which is part of the gelatinization process. Gelatinization
228 combines several other events, such as absorption of water, change in shape and size of granules,

229 starch solubilization also called leaching (Biliaderis, 2009); these changes will be further described
230 below.

231 SP steeply increases a few degrees below the onset temperature for endothermic transition of
232 gelatinization as measured by DSC (Biliaderis, 2009; Muñoz, et al., 2015) and reaches values up to 20
233 times greater in excess water and temperatures as high as 95°C (Fig. 5a). The thermal dissociation of
234 crystallites permits further water ingress and explains the extent of swelling far beyond that observed
235 at temperatures below starch gelatinization level. Enhanced swelling in turn accelerates the process of
236 disruption of neighboring crystalline parts with rapid propagation within the granule (Bogracheva, et
237 al., 1998). The degree of starch gelatinization and hence swelling depends on both the maximum level
238 of temperature reached and the water available to the starch. Access to water is affected by the nature
239 of the granule surface (Debet and Gidley, 2006) and is improved thanks to constraint relaxation while
240 granules gelatinize, this gelatinization depending on the degree of crystallinity and organization of
241 starch granules (Vermeylen, et al., 2005): A-type versus B-type, crystal defects, any variability due to
242 breeding conditions or cultivars, whether damaged or not at the milling step. The SP of wheat starch
243 granules in water suspensions increased steeply and linearly with temperature (10 to 15% per °C, Fig.
244 5a), and showed a deceleration between 65 and 75-80°C. SP values are scarce at intermediate WC,
245 even for starch-water mixtures. Wang, et al. (2014) reported SP of between 1.8 and 2.2 for wheat
246 starch with a WC of 40-50% wb and 92.5°C (Fig. 5a), corresponding to an increase in granule
247 diameter of 1.4-1.5 (where granule thickness is considered to be constant). Note that swelling is
248 expected to be considerably less at the same WC in dough, because of competition among components
249 for water (see below).

250 Monitoring of the swelling of individual wheat starch granules in excess water e.g. (Cai and
251 Wei, 2013; Patel and Seetharaman, 2006) was consistent with the above picture for starch-water
252 mixtures observed as a whole. Swelling initiates from the hilum and propagates towards the edges of
253 the granule (Cai and Wei, 2013). It was reported to be anisotropic in lenticular granules, no swelling
254 being observed in the thickness direction of the granule (Bowler, et al., 1980). Where SP was higher
255 than 5.8-7.3 as encountered when $T > 70^\circ\text{C}$ in excess water, swelling was accompanied by puckering,
256 with possible impact on the overall dimensions of the swollen starch granules (Bowler, et al., 1980).
257 However, when WC levels are relevant to dough (usually < 0.46) and as reported in Fig. 5a,
258 extrapolation of Bowler's findings indicates that puckering is unlikely for the anticipated SP.

259 The leaching of starch components is fostered at high temperatures, a phenomenon which has
260 so far received little attention at WC levels relevant to dough (Kovrlija and Rondeau-Mouro, 2017;
261 Nivelle, et al., 2019). The main reason for this is the difficulty in accessing the extragranular aqueous
262 phase (Wang, et al., 2014). Leaching in excess water occurs quite late in the heating process, 5.0, 7.5
263 and 35.0 g per 100 g of dry wheat starch being leached at 85, 90 and 96°C respectively after

264 temperature has held for 30 min (Doublier, et al., 1987). Leached amylose and amylopectin form a
265 macromolecular gel at high temperatures with possible cross-linking between themselves and with
266 other constituents (lipids, proteins) at cooling (Biliaderis, 2009). A low heating rate also favors
267 leaching (Doublier, et al., 1987) and, consistently, results in greater firmness of the starch-water
268 mixture after a heating-cooling cycle (Patel, 2006). In general, the heating of wheat bread crumb
269 proceeds at a moderate rate (5-6°C/min) over a short period (10 min) and is succeeded by 10
270 additional minutes where the temperature is held above 95°C while the crust is browning.

271 As has already been mentioned for low temperatures, very few reports exist of direct
272 measurements of the mechanical properties of individual starch granules, especially for wheat starch.
273 In excess water, the shear modulus of swollen potato starch granules was a few hundred Pa (1.5 kPa at
274 most) (Desse, et al., 2010). The work of Carrington, et al. (1998) and Fisher, et al. (1997) was
275 devoted solely to the identification of Young's modulus for swollen potato starch. The granule was
276 gelatinized in excess water at 69°C and then cooled to ambient temperature. Measurements gave
277 Young's moduli of the order of a few hundred Pa. This suggested that swollen granules become easily
278 deformable under the stresses at play in bread baking, with an order of magnitude of 1.7 kPa (Grenier,
279 Lucas, et al., 2010). Transition temperature between rigid and soft particles during gelatinization is
280 still not known exactly. It can only be stated that in excess water granules above 70-80°C are already
281 softened since they lose their integrity under shearing conditions, yielding a steep increase in viscosity
282 e.g. (Debet and Gidley, 2006). Measurements of dough viscosity also showed a slight decrease at
283 temperatures above about 70°C whatever type of mechanical test was used; this transition has been
284 tentatively assigned to granule softening (Vanin, et al., 2013).

285

286 **4.2. Wheat gluten**

287 Gluten is the main protein phase in dough and in the following we focus on its constituents,
288 glutenin and gliadin, in order to shed light on the molecular aspects of the modulation of dough's
289 mechanical properties (MacRitchie, 2016; Orth and Bushuk, 1972). The polymeric and aggregative
290 glutenin and the monomeric gliadin are present in roughly equal proportions. Glutenin polymers, made
291 from disulfide concatenated polypeptides, show molecular weights ranging from 100,000 to several
292 millions; glutenin is completely insoluble in water and the larger polymers cannot be brought into
293 solution whatever the solvent (Shewry, et al., 2002). Gliadin is the water/alcohol soluble component of
294 gluten. It includes monomeric proteins with a molecular weight ranging from 30,000 to 70,000 g /
295 mol. Gliadin and glutenin both contain high levels of glutamine ($\pm 30\%$), a strongly hydrogen-bonding
296 amino-acid (Rhys, et al., 2012). Gluten proteins, even though being insoluble in water, swell up to
297 about 63-64% (wb) in its presence. In mixed dough, glutenin polymers and gliadins, interacting

298 through H-bonds, ionic bonds and hydrophobic bonds, form the basic structure of an elastic protein
299 network.

300 At or close to ambient temperature

301 If the theory of linear polymers is considered to apply to gluten, the elasticity of the dough is
302 connected to the length of glutenin polymers and thereby to their degree of entanglement (Bloksma,
303 1990; Brandner, et al., 2019; Ewart, 1977; Ewart, 1968, 1972; Graveland, et al., 1985; Hosenev and
304 Rogers, 1990; Singh and MacRitchie, 2001). Glutenin chain interactions through H-bonds would
305 proceed along trains interspersed with loops, where hydrogen bonding with water prevails (Belton,
306 1999). As a polymer, the ability of gluten to be extended without rupturing depends on its average
307 molecular weight, on the spacing of entanglements, on the rate at which molecular chains can slip past
308 one another in response to deformation (Singh and MacRitchie, 2001; Termonia, et al., 1988;
309 Termonia and Smith, 1988; Termonia and Smith, 1992). However, the deformation rate during
310 proofing and baking remains moderate, of the order of 10^{-3} s^{-1} , compared to the 10^{-2} s^{-1} beyond which
311 the draw ratio of polymers (ratio of deformed to un-deformed length prior to rupture) is commonly
312 impacted (Termonia and Smith, 1992).

313 Polydispersity and the relative proportions of chains of different lengths is important in
314 controlling the viscoelastic properties of a given polymer (Termonia and Smith, 1992). For a melt of
315 linear monodispersed polymers, the draw ratio prior to rupture increases strongly with the average
316 polymer molecular weight up to a critical molecular weight threshold above which stress-strain curves
317 remain quasi-identical (Termonia and Smith, 1987). Dough maximum resistance to extension was
318 found to be related to the percentage of flour glutenin polymers extracted by sonication and showing a
319 molecular weight over 250,000 g / mol (Bangur, et al., 1997; Sloan, et al., 2009). In reality, the critical
320 molecular weight threshold above which glutenin polymers contribute to gluten elasticity would
321 roughly coincide with their solubility threshold in sodium-dodecyl-sulfate buffers (so above 1 to 2 M g
322 / mol). Accordingly, strong correlations are found between the quantity of flour SDS-insoluble
323 glutenin polymers and flour baking quality (Gupta, et al., 1992).

324 The quantity of glutenin polymers contained in total protein determines extensibility (Sloan, et
325 al., 2009). Indeed, the degree of entanglement determines gluten elastic response, while small
326 polypeptides enable polymer segments to slip between entanglements and enhance the material
327 viscous component. Gliadin, being as prone as glutenin to H-bonding, acts as a perfect diluent
328 (Graveland and Henderson, 1987) when interacting with glutenin polymers. Rheological tests
329 performed on both glutenins and gliadins at 20°C substantiate the viscoelastic and the viscous
330 (slippage) features of glutenins and gliadins respectively (Hernández-Estrada, et al., 2017; Khatkar, et
331 al., 1995). The removal of gliadins causes an increase in stiffness and reduces the extensibility of the
332 residual glutenin-fraction (Song and Zheng, 2008). The successive addition of glutenin fractions of

333 increasing molecular weight to a given flour, at constant protein content, causes loaf volume to
334 increase and, after reaching a maximum, to decrease (Lundh and MacRitchie, 1989; MacRitchie,
335 1987). The occurrence of an optimum volume possibly indicates a balance between strength and
336 extensibility, beyond which the stiffness provided by polymer entanglements decreases the failure
337 strain and might cause lower loaf volumes (Singh and MacRitchie, 2001; Termonia and Smith, 1988;
338 Tsiami, et al., 1997). It is important for there to be a good balance between elasticity and viscosity in
339 the gluten; sufficient elasticity to retain gas but not so much that expansion is lost (Shewry, et al.,
340 1995).

341 The draw ratio at rupture also depends on the number of entanglements in the network
342 (Termonia and Smith, 1988). The steep increase in strain hardening encountered after the yield point is
343 due to increased alignment of monodispersed molecules between entanglements. The higher the
344 number of entanglements in the molecular network, the more quickly molecules align and the less
345 deformable they are. The stress increases more rapidly than strain in greater extent.

346 If the Glutenin Macropolymer (GMP) model is considered to be correct (Graveland, et al.,
347 1985), the mechanical behavior of the gluten would result from the combination of multilevel
348 mechanisms (Van Vliet and Hamer, 2007) and not from molecular-scale mechanisms alone. Among
349 these levels we can distinguish the molecular level –where covalent bonds are essential; the mesoscopic
350 level –where the physical aggregation of glutenin polymers into macropolymers (GMP) is relevant;
351 and the macroscopic level –where processing conditions affect the resulting properties of dough. GMP
352 is believed to resemble a protein particle network (Don, et al., 2003b; Don, et al., 2005). The
353 mesoscopic interactions between glutenin aggregates would govern the macroscopic rheological
354 properties of the dough (Don, et al., 2003a; Don, et al., 2006; Rosell, et al., 2013; Van Vliet and
355 Hamer, 2007).

356 The true nature of gluten and whether the linear polymer model is applicable to it remain
357 bones of contention between specialists (MacRitchie, 2014; Van Vliet, 2008).

358 This makes it quite impossible to be sure whether the above rationale is valid. On the basis of
359 this uncertain microstructure, we cannot infer which gluten feature should be modified in order to
360 control its mechanical behavior. In-depth rheological studies of dough, starch-water mixture, gluten-
361 water mixture and gluten-glass beads-water mixture at room temperature have shown that when gluten
362 is simply considered as a critical gel, it is possible to account for the deformation behavior of gluten
363 within the range of finite strain and strain rate encountered during proofing and baking (Ng and
364 McKinley, 2008; Ng, et al., 2011; Ng, et al., 2006).

365 In the temperature range of baking

366 Beyond 60°C (Dobraszczyk and Morgenstern, 2003; Wang, et al., 2017), the stiffening of the
367 gluten appears to be caused by a heat-induced crosslinking of gluten proteins (Hoseney and Rogers,
368 1990; Millar, et al., 2004) driven by thiol oxidation, thiol/disulfide exchanges and hydrophobic
369 interactions (Gan, et al., 1995; Schofield, et al., 1983). In the range 62-75°C (Fig. 5b), protein
370 unfolding allows exposure of buried intra-molecular disulfide bonds, which can exchange with others
371 forming new inter-chain disulfide bonds (Schofield, et al., 1983). Half of the initial extensibility of the
372 gluten is lost at about 65°C and a proportion of the other half by the end of baking (90°C). Flour of
373 good baking quality displays earlier and steeper increase in elasticity during heating e.g. Dronzek and
374 Butaki (1977); Jeanjean, et al. (1980). According to Stathopoulos, et al. (2006), high gliadin content
375 results in a steeper rise in gluten elasticity above 60°C whereas high glutenin polymer content ensures
376 high elasticity even below 60°C.

377

378 **4.3. The hydrated gluten-starch matrix**

379 At or close to ambient temperatures

380 High-speed centrifugation has indicated that developed doughs include three co-existing
381 phases (Larsson and Eliasson, 1996; Mac Ritchie, 1976; Mauritzen and Stewart, 1966; Mauritzen and
382 Stewart, 1965). The first (Fig.6a, phase 1) is the hydrated gluten. The second (Fig.6a, phase 3) is
383 composed of swollen starch granules. The third (Fig.6a, phase 2) is a water phase which can be more
384 or less viscous and contains albumins, globulins, neutral and charged polysaccharides, and
385 amphiphilic compounds such as fats (Gan, et al., 1995). Electrical conductivity measurements have
386 shown that the water beyond 35% (wb) forms a continuum within the dough (Mac Ritchie, 1976). The
387 aqueous phase suspends starch granules and gluten in the form of filaments or sheets (Eliasson and
388 Larsson, 1993) and is also believed to feed the liquid lamella that lines GCWs (Fig.6a). In fact, there is
389 only indirect evidence of the liquid lamella, the most convincing being the high impact of minor,
390 surface-active components on loaf volume e.g. (Sroan and MacRitchie, 2009). However, little is yet
391 known about its composition which is assumed to be that of the dough liquor extracted by
392 ultracentrifugation by Courtin and Delcour (2002) and Mills, et al. (2003). Water pools were also
393 observed in the protein network, as well as water layers surrounding starch granules (Fretzdorff, et al.,
394 1982) (Fig. 7b,c); likewise, the existence of a mobile aqueous phase has been evidenced by several
395 studies using Thermogravimetry (TGA) (Fessas and Schiraldi, 2001), and NMR (Kim and Cornillon,
396 2001; Kovrlija and Rondeau-Mouro, 2017).

397 The energy transferred to the system through **mixing** makes the formation of a 3D network
398 possible between the co-existing yet interpenetrating phases, even if this is very difficult from a
399 thermodynamic point of view because of the immiscibility of gluten and soluble starch (Tolstoguzov,

400 1997). The continuity of the aqueous phase (see above) leads us to believe that the interaction between
401 starch and gluten (Eliasson and Tjerneld, 1990; Jekle, et al., 2016) involves water trapped between
402 these phases in the form of Van der Waals/hydrogen bonds. However, the way starch and gluten
403 interact is still a matter of debate. In particular, how starch-gluten interactions through Van der
404 Waals/hydrogen bond types would affect mechanical properties when large strain is involved is not
405 very clear (Meerts, et al., 2017). The aqueous phase trapped between the starch granules and gluten
406 makes it possible for gluten chains to slip and align along the main shear direction. During mixing,
407 starch granules behave like ball bearing bodies, actively contributing to the reshaping of the gluten
408 domains which eventually form layers that envelop the starch granules (Tolstoguzov, 1997). Indeed,
409 starch granules are too rigid to lose their shape at the mixing step (Van Vliet, 2008). The gluten is
410 assumed to be greatly stretched between starch granules when the proportion of gluten to starch is low
411 (Ahmed and Jones, 1990) and, consistent with this view, stiffer blends have been observed.
412 Interactions between starch and gluten are crucial for predicting the mechanical behavior of the gluten-
413 starch matrix and further investigation is required that will build on the theoretical work carried out by
414 Mohammed, et al. (2013).

415 The shearing that occurs during mixing is subsequently relayed by the biaxial extension in
416 GCWs **during proofing** (Dobraszczyk and Morgenstern, 2003). At advanced stages of GCW thinning,
417 the gluten network will line the gas cell interface, at least partially (Eliasson and Larsson, 1993;
418 Sandstedt, et al., 1954); also see Fig. 4c,f. In the meantime, the remaining pools of water (Fig. 7b,c)
419 are likely to be expelled and spread across the gas-matrix interface, locally feeding the liquid lamella.
420 The liquid lamella also stretches as the cell inflates. The liquid lamella may be stabilized by lipids and
421 in such cases its stretching is governed by the Gibbs-Marangoni mechanism. Deformation of a lamella
422 will cause local thinning and deplete the local surfactant concentration. The amphiphilic/water
423 molecules will naturally migrate to the depleted area to reduce the concentration gradient, restoring the
424 lamella to its original concentration. Proteins also stabilize the liquid lamella. By unfolding at the
425 interface and interacting strongly with each other, they provide greater elastic properties to the surface.
426 As a consequence, the mechanical properties of the liquid lamella will depend heavily on its
427 composition. In fact, both lipids and protein are likely to co-exist at the gas-lamella interface, the
428 mixture being notoriously unstable (Primo-Martín, et al., 2006). However, it is not known whether the
429 gas-cell interfacial film is first formed from a lipid layer into which proteins may insert themselves
430 later, or vice versa (Eliasson and Larsson, 1993). This composition also plausibly changes at the
431 advanced stages of proofing where the distance between two interfaces increasingly narrows (Gan, et
432 al., 1995). Last, while the gluten is extended, the largest wheat starch granules orient themselves
433 within the protein veil in the direction of extension, with their smaller dimension (5-15 μm) making up
434 the thickness of the GCW (Fig. 4 a versus b). As the gluten mass develops from 3D to 2D objects
435 (sheets or filaments), starch granules join together, increasing their surface of contact (Eliasson and

436 Larsson, 1993). The bimodal distribution in size of wheat starch granules also helps them to be closely
437 packed, increasing contact between the granules (Eliasson and Larsson, 1993). These authors
438 suggested that this positioning of the starch is key for the continuity of the aqueous granular phase in
439 dough.

440 In the temperature range of baking

441 Upon heating, the strain hardening index of the gluten-starch matrix gradually decreases. The
442 higher the baking performance of the flour used for the preparation of dough, the higher the
443 temperature at which this decrease will occur (Dobraszczyk and Morgenstern, 2003); even in the case
444 of excellent baking performance, the GCW becomes likely to rupture beyond 60°C (Dobraszczyk and
445 Morgenstern, 2003).

446 The descriptions of heat-induced modifications in gluten and starch in Sections 4.1 and 4.2
447 also apply to the gluten-starch matrix. During bread baking, starch swelling remains low due to the
448 limited WC of the dough. Another limiting factor is the competition among dough components for
449 water, which is little documented – data on chemical potentials are particularly scarce at different
450 temperatures, e.g. (Viollaz and Rovedo, 1999). In Fig 5 a, we assumed that the water trapped within
451 gluten is not available for starch gelatinization in dough, and is only partially available once the gluten
452 has been denatured by heat (62-63°C and beyond). Under these hypotheses, starch swelling during
453 baking would range between 1.36 and 1.57, depending on the dough's initial WC. Values reported by
454 Schirmer, et al. (2014) – to our knowledge, the only study of starch swelling of individual starch
455 granules in a dough environment and with increasing temperature – was consistent with these
456 hypotheses, i.e. significant swelling was observed from 60°C, reaching a final swelling factor of 1.15
457 at 70°C (the maximal temperature tested in this study). After absorbing water and swelling upon
458 heating, the still quite rigid starch granules come into closer contact with each other and the viscosity
459 of the gluten-starch matrix increases, as has been observed, at temperatures above approx. 60°C
460 (Bloksma and Nieman, 1978; Dreese, et al., 1988). The matrix is said to stiffen. Then, as the gluten is
461 stretched further and temperature of about 70°C is reached, starch granules which have become soft
462 upon heating are also squeezed and become elongated in the direction of GCW stretching; a slight
463 decrease in dough viscosity is then observed. We have performed a morphological analysis of starch
464 granules in crumb compared with starch granules at the end of proofing on the basis of the images
465 provided by Hug-Iten, et al. (1999) and Sandstedt, et al. (1954). This analysis showed an increase in
466 starch granule width by a factor greater than 1.4 (up to 2.2) and a decrease in thickness by a factor of
467 0.6-0.9. In contradiction to the common opinion, the elongated shape of starch granules in crumb has
468 more to do with granules being flattened after they are squeezed between the two gluten films upon
469 stretching, than with swelling. This flattening process further increases the contact surface area
470 between neighboring granules. Last, the heat-induced cross-linking of gluten proteins also contributes

471 to the stiffening of the matrix (Section 4.2) but remains difficult to separate from the contribution of
472 starch modifications which occur in the same temperature frame.

473 **4.4. Conclusion to this section**

474 In the following, we adopted the GCW organization depicted by Eliasson and Larsson (1993)
475 at advanced stages of thinning, namely a core wall constituted of close-packed starch granules
476 suspended in an aqueous solution (“aqueous granular phase”), sandwiched between the continuous
477 films of hydrated gluten, behind the liquid lamella (Fig. 6 c). The occurrence of the latter is
478 conditioned to the water amount in the recipe and also the affinity of flour constituents for water and
479 we will consider both options in Section 5. The granular phase is assumed to be rather continuous up
480 to the “one-layer” state (introduced in Section 2); further stretching beyond the “one-layer” state will
481 separate starch granules from each other, making appear a gluten film in between (Fig. 6c). The longer
482 sides of the wheat starch granules (A-type) are oriented in the plane of the GCW.

483 The above suggests that the changes in the cell wall’s mechanical properties as a result of the
484 hydrothermal reactivity of starch and protein are poorly documented. The hydrothermal reactivity of
485 starch and gluten has also been little studied in the conditions of baking, in restricted amount of water
486 or large deformations. Given this overall lack of knowledge, the biochemical/mechanical transitions in
487 the GCW were assumed for the discussion of the rupture in Section 5; these assumptions are
488 summarized below. We are aware that our choices are arguable, but we believe that this disputability
489 precisely will foster further investigation in these areas. This together with the variability of properties
490 made possible in reconstituted flours made us consider different timings of transition in Section 5.6.

491 Starch granules do not swell much (1.36-1.57 at most) and late during the dough baking
492 process because of low amount of water and its distribution between the GCW phases. Swelling
493 proceeds most probably in the GCW stretching direction for the largest, lenticular wheat starch
494 granules. Rheological measurements on dough together with morphological analysis applied to
495 microscopic observations of GCWs suggested that wheat starch granules soften during baking despite
496 of the low amount of water available in dough. 70°C was retained as the transition temperature for
497 granule softening in wheat bread dough. The leaching of granule material is completely neglected in
498 the literature because of the low level of hydration; however the stickiness of starch granules might be
499 a property crucial for explaining some unexpected results obtained with some specific starches
500 involved in the dough recipe (see Section 5). Due to heat-induced denaturation, gluten is hypothesized
501 to lose half of the initial extensibility at about 65°C and a proportion of the other half by the end of
502 baking (90°C).

503 If hydration of each constituent is recognized as essential in the mastering of dough aeration,
504 we dispose of little information on the exact distribution of this water during baking, which will drive

505 the mechanical properties of the phases, directly or indirectly (through the hydrothermal reactivity
506 mentioned above). The plausible feeding of the liquid lamella with water expelled by the gluten under
507 extension has been discarded and its persistence while starch granules swell during baking has not
508 been questioned. This is an additional reason for considering the liquid lamella as optional in Section
509 5. We considered in the above subsections that each granule has the same environment, which is
510 doubtful (Jekle, et al., 2016). Because the dynamics of heating are faster than those of water migration,
511 the above picture is complicated by spatial heterogeneities. These will be discussed again at the end of
512 Section 5.

513 Since starch occupies most of the volume of the dough, we will take the view in Section 5 that
514 dough structure sets mostly as the result of starch gelatinization upon heating and that the gluten
515 mainly controls the extent of gas cell inflation at the early stages of baking while starch granules are
516 considered to act simply as a granular charge. The draw ratio of the hydrated gluten will much depend
517 on the distribution in molecular weight of its constitutive protein, glutenin and gliadin, but also on the
518 way the hydrated network is formed at the mixing step. Given the controversy about starch-gluten
519 interactions, and the lack of data in the baking conditions, the hypothesis of low interaction, allowing
520 the slippage of gluten on the starch granules, has been retained in the following; this hypothesis
521 minimized speculations.

522 **5. Mechanisms governing thinning and opening of gas cell walls during** 523 **baking**

524 The spatial organization of the GCW, mechanical transitions due to biochemical reactions and
525 mode of interactions between GCW phases given in Section 4 are used for the discussion of the
526 rupture below.

527 **5.1. Revisiting and completing characterization of these mechanisms for the thinnest** 528 **gas cell walls**

529 The driving force for the deformation of the GCW is the gas pressure resulting from the
530 equilibration between the gas intake within gas cells and the mechanical resistance and/or tensional
531 forces that issue from the bi-extension of the GCW. Rupture of the GCW will begin at the spot where
532 the stress concentrates until it exceeds the yield stress. Before, stress increases with strain rate and
533 increase of strain within the GCW. As strain is greatest in the middle of the GCW, the triggering of the
534 rupture might occur very close to that point.

535 The formation of significant pressure differences between gas cells during baking is very
536 unlikely. Where surface tension is relevant, pressure differs up to 3 kPa between adjoining gas cells of
537 widely differing sizes. This estimate of gas pressure was calculated for cells of 50 μ m and 1mm in
538 diameter using Laplace's law and assuming a surface tension of 0.04 N.m⁻¹ (Kokelaar and Prins, 1995;
539 Van Vliet, et al., 1992). This is of the order of magnitude of about 1 to 30% of the modulus of
540 elasticity of dough. The GCW may bend slightly between the two gas cells. The contribution of
541 surface forces is expected to decrease during baking as the cell size increases. Further, pressure
542 measurements during baking have shown no relevant difference in pressure (a few kPa at most)
543 between the core of the dough and the region close to the crust (Baker and Mize, 1939; Grenier, Le
544 Ray, et al., 2010; Miś, et al., 2016; Singh and Bhattacharya, 2005; Sommier, et al., 2005).

545 In the following, we adopted the alternative view that rupture is initiated by excessive GCW
546 bi-extension and thinning. In Section 2 and 3, we saw that thinning has already reached a great degree
547 at the end of proofing, and that part of the gas cells was already connected. In Section 5, we assumed
548 that most of the gas cells are still sealed at the beginning of baking so that gas cannot transfer from one
549 cell to another according to Darcy's law (Sections 2 and 3). Most GCWs are still thick (Fig. 3 b). At
550 various locations within the dough (< 0.5 % of the dough volume, see Section 2), GCW thickness
551 approximates the size of the smallest starch granules (Section 2, Fig. 4 including both cases c and e).
552 Mechanisms favoring or postponing the rupture of these GCWs are described below for each
553 constitutive phase defined in Section 4.

554

555 **5.2. Thinning of a "thick" GCW down to the size of starch granules**

556 Although the mechanical behavior of molten metals and bread dough differ (the former is
557 purely viscous and the latter visco-elastic), it can be inferred from studies on metal foam (Korner,
558 2008) that solid particles such as starch granules first act as a stabilizing filler in the GCWs until they
559 reach the one-layer state as depicted in Fig. 6c. The one-layer state refers to a wall composed of only
560 one single particle layer (also see Section 3). The rheological behavior of the dough depends on the
561 relative proportions of gluten and starch (Jekle, et al., 2016). In the bakery industry, the addition of
562 gluten is a common practice to improve baking performance. There is also a particular proportion of
563 small to large starch granules (75/25 kg/kg) that combines the greatest loaf volume with optimal
564 crumb grain (Lelievre, et al., 1987; Roman, et al., 2018; Soulaka and Morrison, 1985). The underlying
565 mechanical interactions between the two phases mean that there is an optimal surface area for starch to
566 be exposed to gluten. Beyond this optimum, the stability of the dough decreases with the increase in
567 the proportion of small starch granules (Park, et al., 2005). It is worth noting that these major trends do
568 not account for a number of contradictory results obtained on bread as reported in the literature. These
569 contradictory results might result from the fact that the proportion of small to large granules affects

570 other starch properties (for instance the hydration capacity of starch) that are also involved in the
571 stabilization/destabilization of GCWs during baking. These effects were not thoroughly characterized
572 and disentangled in these previous studies. These starch properties will be further described in the
573 following subsection.

574

575 **5.3. Rupture of gluten in mechanical interaction with solid particles**

576 Where the thickness of the GCW becomes less than the thickness of the largest solid particles
577 (starch granules, bran inclusions etc.), stress in the gluten increases to a greater extent than that in the
578 surrounding area: solid particles act as stress concentrators (Bloksma, 1990; Van Vliet, et al., 1992).
579 The rupture of the gluten film happens at the spot where stress is the greatest, most probably at the
580 edge of the starch granule (Fig. 6b (c,d)). Strain hardening in the gluten film acts as a stabilizing factor
581 against rupture for as long as gluten is still likely to feed the region where tensional stress has
582 increased; this stabilizing phenomenon works well when the strain-hardening factor (as defined by
583 VanVliet and co-workers) remains greater than 2 (Dobraszczyk, 2017; Turbin-Orger, et al., 2015; Van
584 Vliet, 2008). The gluten then quickly thins down (Van Vliet, et al., 1992) and ruptures (Fig. 6b (a,b)).
585 Note that some authors found that gluten exhibited strain softening at low strain rates (10^{-3} s^{-1}) relevant
586 to baking (Ng and McKinley, 2008). Once the gluten has ruptured, a hole is created in place of the
587 GCW, allowing connection between adjoining gas cells. In crumb, Stokes and Donald (2000) noticed
588 the existence of gluten strings devoid of starch granules across some of these holes (Fig 6b (b,c)).
589 These strings may counteract the increase in the size of the holes. In holes devoid of strings, the holes
590 slowly become round in shape (Fig. 6b (B-C-D)) at a rate that mainly depends on the viscosity of the
591 dough, as evidenced in the case of metal foams (Korner, 2008). Image sequences taken during dough
592 proofing have shown that the movement of the GCWs occurs on a time scale of the order of several
593 tens of minutes where dough viscosity is low (Babin, et al., 2006) (Fig. 8). From the hole shapes
594 observed in crumb by Stokes and Donald (2000), it can be inferred that the holes had time to widen
595 before the dough stiffened upon heating and hence that they formed well before the end of starch
596 gelatinization and protein denaturation (Section 4).

597 Wheat starch granules in the middle of the gluten were assumed to remain stiff up to about
598 70°C (Section 4.1.) and the pattern of stress concentration within the gluten described above applies
599 up to this temperature. When heated towards 70°C , starch granules also swell (Section 4.1). Given that
600 the swelling predominantly follows the gluten film's direction of tension (Section 4.1), the thickness
601 of the GCW is little modified. We take the view that, despite the disappearance of the extra-granular
602 aqueous solution, there is little friction between starch granules and gluten because of their
603 immiscibility and because the gluten slips on the surface of the swollen starch granule. For all these
604 reasons, the shear stress within gluten might thus not be greatly affected by starch swelling.

605 When the temperature reaches 62-63°C the gluten begins to stiffen and its ability to be
606 stretched progressively decreases upon further heating (Section 4.2). From the onset of this gluten
607 stiffening, an already greatly stretched and stressed gluten film will possibly break, even without
608 further stretching. The stress might increase more than the yield stress. This rupture without further
609 stretching could even be greater if the heat-induced denaturation of gluten and water loss from the
610 gluten were to be accompanied by shrinkage, but we found nothing in the literature to substantiate this.
611 Again, such a rupture would look like cracks located along the rims of the solid particles.

612

613 **5.4. The liquid lamellae may provide an additional delay in the complete opening of** 614 **the GCWs**

615 Relative to the mechanical strength of the gluten network, the contribution of the liquid
616 lamella to GCW integrity has been shown to be negligible or low (Van Vliet, 2008). However, once
617 the gluten film has broken, at prolonged proving times or early baking, the liquid lamella may patch
618 the hole left by gluten withdrawal (Fig. 6c) and act as a gas barrier (Gan, et al., 1995; Sroan and
619 MacRitchie, 2009), keeping cells closed. Then, as the GCW extends, the surface tension within the
620 liquid lamella is not sufficient to sustain the strong increase in stress and the liquid lamella ruptures.
621 The mechanical resistance of the liquid lamella is highly dependent on its composition (Section 4.3),
622 which has not yet been totally elucidated and it is plausible that it evolves during baking. The rupture
623 of the liquid lamella is much more sudden than that of the gluten film (previous sub-section), due to
624 the sudden release of stored surface energy. Even if the broken edges of the gluten film have not
625 completely relaxed at that time, the holes being contained within the GCW will grow faster from this
626 point, resulting in a much bigger and rounder hole (Fig. 6c).

627 Simultaneously with the further extension of the GCW upon heating, starch granules begin to
628 absorb any additional water available in their environment, not only from the extra-granular aqueous
629 phase but also, it seems likely, from the liquid lamellae. This process starts quite early in the baking
630 process, from 45-53°C and is soon limited by the amount of water available (Section 4.1, Fig. 5a). The
631 liquid lamella will thin after water has been sucked from it and, where it is acting as a patch to contain
632 an emerging hole in the GCW (Fig. 10c), this will allow the GCW to open. For this reason, the liquid
633 lamella is not believed to persist long once starch gelatinization has started. Consistently with this
634 overall view, loaf volume decreased linearly with increasing water absorption in reconstituted flours
635 (with equal gluten levels and water solubility, but with different starch origins) measured at 20°C (Fig.
636 9). Surprisingly, with some exceptions, e.g. Park, et al. (2005), GCW destabilization by starch through
637 the process of water redistribution is neglected in the literature.

638

639 **5.5. Starch granules act sequentially to destabilize then stabilize the gas cell wall, the**
640 **tipping point being granule softening upon starch gelatinization**

641 Starch granules start by destabilizing the GCW at the one-layer state, either by stress
642 concentration in gluten films under extension for as long as these particles remain more rigid than
643 gluten, or by sorption of water leading to the disappearance of the liquid lamellae. These modes of
644 action have been described in the above sub-sections. It has been highlighted in Section 4 that
645 transition temperatures for granule softening and water sorption by starch granules in the complex
646 environment of dough remain ill-defined and were hence not available to past studies seeking to
647 compare the effects of different starches on bread baking performances. Indeed, the use of
648 gelatinization endotherm or pasting temperatures in these past studies may not have been appropriate
649 to describe softening and sorption dynamics and this may explain the failure to pinpoint the effects of
650 starch.

651 As starch granules soften, stress is no longer concentrated in the gluten but is redistributed
652 across the entire section of the GCW including that occupied by starch granules. Assuming that the
653 gluten still remains slightly extensible at that time, both starch and gluten thin down and the thickness
654 critical to rupture is attained much later in the baking process (Fig. 10d as compared to Fig. 10a).
655 Before being dispersed in the well-extended gluten film, starch granules are packed together forming a
656 continuous granular suspension phase trapped inside the GCW. For certain starches, granule swelling
657 and flattening combined with amylose/amylopectin leakage, even if the latter is confined to the very
658 granule surface, will increase the cohesiveness and the viscosity of the GCW (Section 4.3). It is worth
659 underlying that at these baking stages, the granular phase may act for the GCW stabilization.

660 Wheat starch granules probably keep their stiffness until late in the heating process (Section
661 4.1), limiting the above-mentioned effects of stabilization on GCWs to advanced stages of baking.
662 Their leaching levels are also lower compared to other starches. By contrast, some starches with a low
663 amylose fraction (tapioca starch, used in reconstituted flours or waxy wheat flours) can soften and lose
664 their integrity early in the heating process (Fig. 10d). The early increase in viscosity of GCWs does
665 not appear to be detrimental to the extension of the GCWs, which proceeds in a normal way during
666 baking (Kusunose, et al., 1999). And, consistent with the increased cohesiveness in the granular phase
667 and its positive impact on the GCW stabilization proposed above, greater loaf expansion has even
668 been reported where the proportion of such starches remained low (Blake, et al., 2015; Kusunose, et
669 al., 1999) (Fig. 1). However, where the proportion is too high, individual loaf volumes will decrease.
670 The stabilization effect is so efficient that rupture of GCWs becomes rare. Within predominantly
671 sealed gas cells, pressure drop occurs at the very end of baking (since air cannot enter a closed gas cell
672 and compensate for the amount of carbon dioxide that escapes by diffusion through the GCW) and
673 after baking (because of temperature decrease), causing the loaves to contract and explaining the lower

674 individual loaf volumes. Wheat starch can perform like these particular starches when it is attacked by
675 enzymes, being able to sorb more water and, plausibly, soften earlier during baking. It appears
676 however that the cohesiveness between wheat starch granules after enzymatic attack is not sufficiently
677 increased to impede the rupture of GCWs since increased loaf volumes have often been reported
678 (Sandstedt, 1961).

679

680 **5.6. Effects of relative timing of the softening of starch granules and the loss of gluten** 681 **stretchability on final alveolar structure**

682 Fig. 10 presents different hypothetical scenarios of GCW opening which might happen at
683 different temperatures depending on the occurrence of the mechanisms involved in stress increase and
684 thinning of GCWs. The different mechanisms described in previous sub-sections occur over finite
685 ranges of temperature (defined in Section 4) which are not well-characterized and vary between wheat
686 genotypes, crop conditions and, even more widely, between starches from different botanical origins
687 (found in reconstituted flours). The temperature range for heat-induced denaturation of gluten has been
688 assumed to be 62-75°C but lower values can be found in some reports (Fig. 5b), the origin of such
689 variability being unclear. The temperature of starch granule softening in wheat has been assumed to be
690 70°C, but could also occur at lower temperature in doughs prepared with reconstituted flours
691 containing other starches (see scenario in Fig. 10a versus Fig. 10d).

692 If the temperature associated with the maximal ability of the gluten to stretch is much lower
693 than that associated with the softening of starch granules, stress stores within the gluten until it reaches
694 the yield stress without ever taking advantage of the softening of the starch granules (Section 5.1, Fig.
695 10b). Gas cells coalesce repeatedly, and gas escapes to the loaf outside, leading to a coarse crumb and
696 a non-expanded loaf. This is typical of dough made with flour of poor breadmaking performance. In
697 such a configuration, liquid lamellae play a crucial role in postponing the opening of the GCWs (Fig.
698 10c compared to Fig. 10a or b) until the starch eventually absorbs the liquid lamellae. In this role, the
699 liquid lamellae both bridge the gaps where the gluten films have broken and reinforce the mechanical
700 strength of the thin GCW before gluten rupture.

701 By contrast, if the temperature associated with the softening of starch granules is much lower
702 than that of the gluten's maximal ability to stretch, the stress stored within the gluten relaxes and
703 redistributes early throughout the GCW which tends to equalize in thickness (Section 5.1). It delays
704 rupture and is favorable to cell inflation (Section 5.1); indeed rupture may never happen as evoked in
705 Section 5.5 and depicted in Fig. 10d.

706 The ideal configuration is when the softening of starch granules occurs just before the gluten
707 reaches its maximum ability to stretch (Fig. 10a). The gluten partly relaxes and becomes less likely to

708 rupture. Upon further stretching the stress increases again and eventually reaches the yield stress at
709 some locations. Most importantly, most gas cells open when the structure has stiffened sufficiently to
710 support its own weight in the still-thick GCWs close to the GCW that has just ruptured. Collapse is
711 minimal. Nor does a sufficiently open structure of this type shrink under the contraction of gases upon
712 cooling. This configuration is ideal because both collapse and shrinkage are avoided, or at least
713 minimized.

714

715 **5.7. Opening mechanisms also depend on the position of the GCW in the loaf**

716 Mechanisms of GCW opening have been discussed at the scale of thin GCWs. The mechanical
717 behavior of gluten, starch and dough lamellae were considered separately and in interaction. The
718 mechanical property of each phase is variable during baking and different scenarios of time-course
719 change were envisaged. Up to this point, the variability of these mechanisms and mechanical
720 properties across the dough has not been discussed.

721 Importantly, the mechanical properties of each phase also greatly depend on their water
722 content and temperature. The water content of each phase during baking greatly depends on the
723 availability of water to feed the phases. Water availability depends on local temperature but also on
724 gradient in temperature throughout the dough as well as variability in the local proportion or
725 arrangement of hydrocolloids.

726 First, flour components that are present in low proportions (a few %) but that strongly affect
727 water redistribution such as damaged starch or arabinoxylans may not be present in all GCWs at the
728 final stages of thinning. Likewise, the enclosure of starch granules by a gluten barrier (Jekle, et al.,
729 2016) has a high impact on the amount of water available to the granule and, again, this barrier may
730 vary from one granule to another.

731 Second, there may be differences in the redistribution of water between the core and peripheral
732 layers of dough. In deck and convective ovens, temperature first increases in the outer layers. With the
733 increase in temperature and the subsequent increase in partial water vapor pressure, the evapo-
734 condensation-diffusion (ECD) phenomenon feeds the colder core of the dough with water while some
735 water is withdrawn from the outer layers (Ureta, et al., 2019). It is possible that ECD feeds the internal
736 lining water of gas cells located in the loaf core. The influence of ECD on the formation of liquid
737 lamellae has never been studied. The transport of water vapor to the core is greater in regions
738 containing many open pores than in those with predominantly sealed pores. In the latter case, the
739 diffusion of condensed water through the GCWs slows down the overall transport of water vapor to
740 the core; diffusion of liquid water being slower than diffusion of water vapor. These different rates in
741 water transport to the core will lead to local variations in water content and water availability. These

742 spatial variations in water content and temperature will undoubtedly lead to differences in starch
743 softening, gelatinization, amylose leaching, mechanical properties, stress, and strain softening or
744 hardening. Similarly, because of the drying process within the crust, GCWs stiffen before gas
745 formation has had a chance to inflate gas cells (Vanin, et al., 2009) and opening of GCWs does not
746 happen at the same rate and for the same size of gas cells as in the loaf's core. Permeability may be
747 lower in the crust than it is in the loaf core (Jefferson, et al., 2007; Zhang, et al., 2005).

748 All this means that the mechanisms by which a given GCW opens may be very different from
749 those of its neighbors and that several of the mechanisms described in the above subsections may co-
750 occur in different locations during baking, making mastery of dough inflation and GCW opening a
751 delicate matter, as cereal engineers and chemists have discovered in the past decades.

752 6. Conclusion

753

754 This paper has described the mechanisms leading to gas cell wall rupture during bread baking.
755 It takes into account the contributions of the different phases that make up the GCW: hydrated gluten,
756 granular aqueous phase, liquid lamella. Following Gan, et al. (1995), we believe that the different
757 phases, including the aqueous granular phase, play a role in stabilizing the GCW and could even
758 substitute for each other if the duration of their active involvement during baking were adjusted.
759 Focusing our vision on the GCW scale, we highlighted the complexity and interdependency of the
760 mechanisms controlling GCW opening; this complexity was enlarged to the scale of dough at the end
761 of our analysis. By bringing together all phases present, their interactions and their spatial variability,
762 the overview provided a better understanding of the reasons for the difficulties encountered by cereal
763 engineers and chemists in mastering dough inflation over the past decades.

764 The micromechanical approach has been very little developed for agrifoods. It has so far been
765 applied only to stable materials, as opposed to thermo-reactive ones such as dough during baking. Yet
766 ongoing advances in micro-imaging and computational multiphysics are now making it possible to
767 model the deformation of a dough wall as a multiphase and reactive process, and to provide
768 experimental validation at the same scale. Micromechanical approaches in other domains of
769 application could also benefit research on bread dough.

770 The present paper showed that there is little data available on the mechanical properties of
771 starch granules and hydrated gluten in the dough environment, at temperatures, gaseous environment
772 and strain rates relevant to bread baking. Similarly, the nature of the interaction between starch and
773 gluten is still being debated in the literature; here, the impact of baking on the interplay between these
774 two dough phases as they undergo rapid physicochemical changes is also totally disregarded. This
775 topic would benefit from theoretical approaches and from experimental approaches focused on

776 interfacial design (Jekle, et al., 2016). Last, the spatial organisation of the GCW, as well as its
777 reorganisation in response to the redistribution of water following starch gelatinisation, also require
778 more investigation. Combined microscopy and NMR can provide relevant information in this respect.
779 Acquisition of new insights on these topics is needed to supply the micromechanical models proposed
780 above. In their absence, the processes of GCW opening during baking will remain very hard to fathom
781 and, when dealing with the setting of crumb structure, our ignorance must be acknowledged. In this
782 context, the present review does not pretend to offer a definitive account, but to provide a mechanistic
783 vision of a more systemic nature that has yet to be tested, completed and corrected by further
784 investigation.

785

786

Journal Pre-proof

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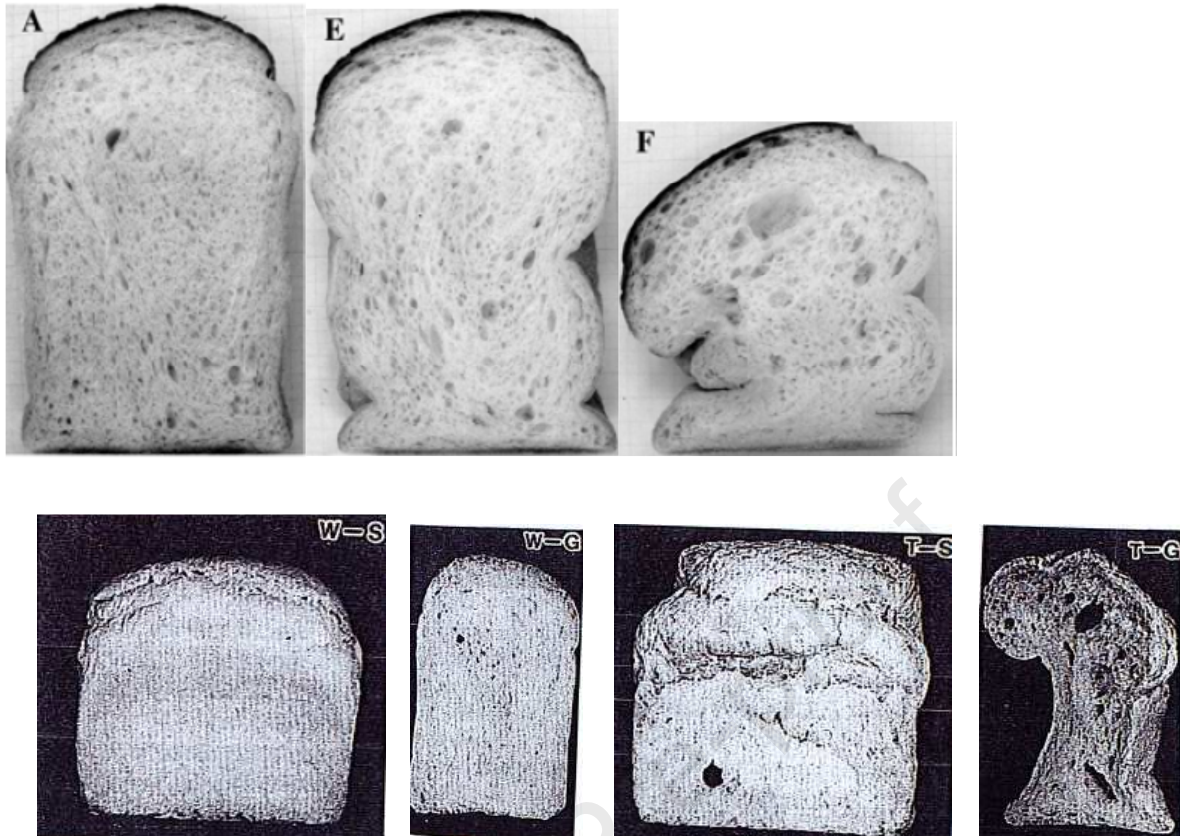


Fig. 1. Images of loaves after cooling. Top row: doughs prepared by Hayakawa, et al. (2004) using different proportions of normal and waxy wheat flours: 100-0 (A), 50-50 (E), 0-100 (F). Bottom row: doughs prepared by Kusunose, et al. (1999) with reconstituted flours using wheat (W-S, W-G) or tapioca (T-S, T-G) starch; side views (W-S, T-S) show the greater gas retention of the “tapioca” loaf while the cross section in T-G shows the results of the contraction of sealed gas cells during cooling, suggesting low levels of pore opening during baking.

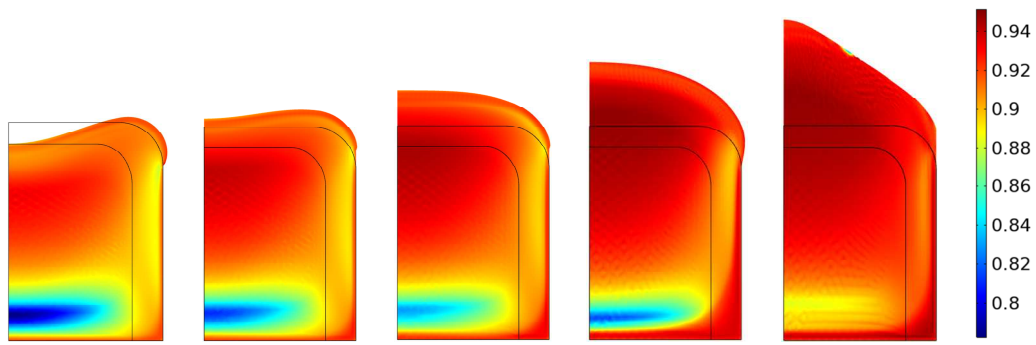


Fig. 2. Distribution of the gas fraction in a finished pan-loaf calculated by the baking model described in (Nicolas, et al., 2016) for different pore-opening temperatures: 47, 52, 57, 62, 67°C (left to right). Dough stiffening begins at ~65°C. Color bar in m^3 of gas/ m^3 of crumb.

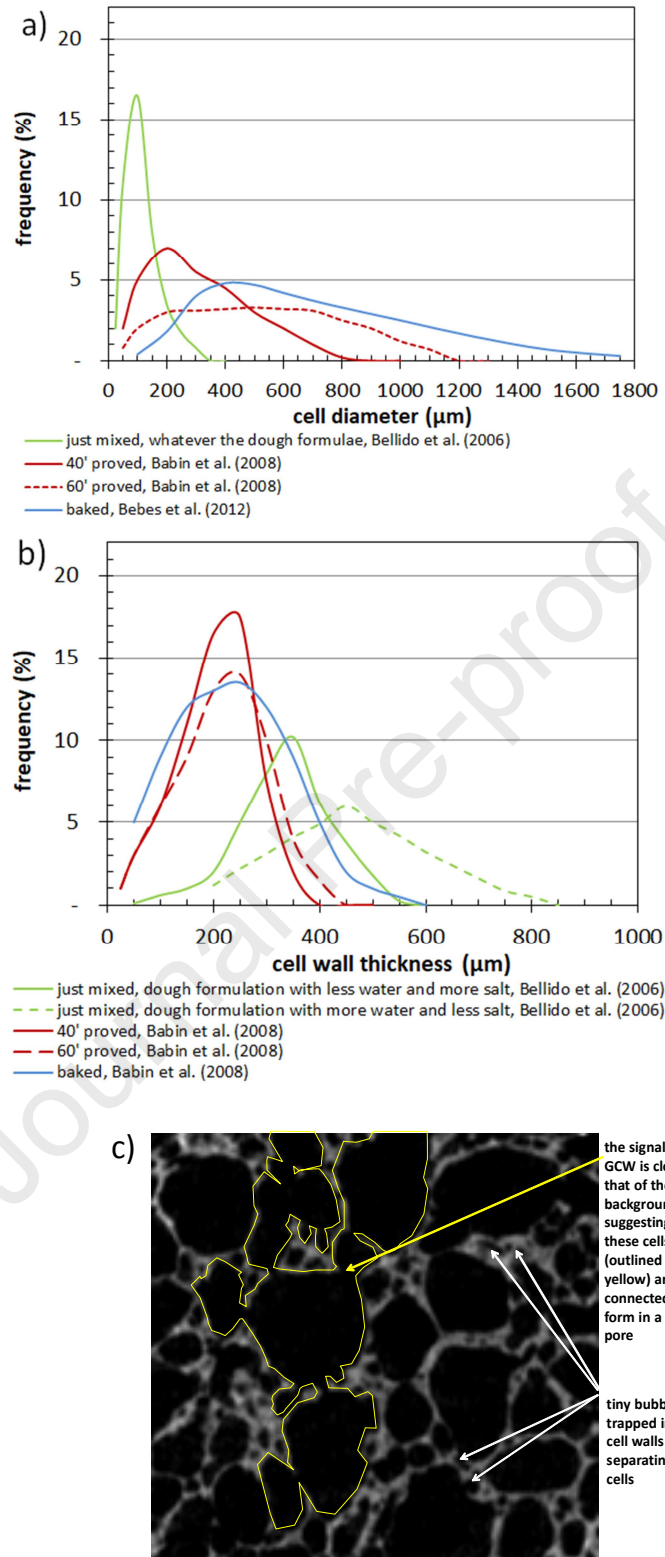


Fig. 3. Probability density function of a) gas cell diameter and b) gas cell wall thickness at the different steps of breadmaking obtained from a compilation of literature data (Babin, et al., 2006). These distributions are depicted in c) for crumb (Besbes, et al., 2013). Voids in the gas cell wall were assumed to exist where signal intensity was close to that of the image background; given the spatial resolution of this image obtained by X-ray μ tomography (22 μm), very thin cell walls might not be visible (known as the partial volume effect).

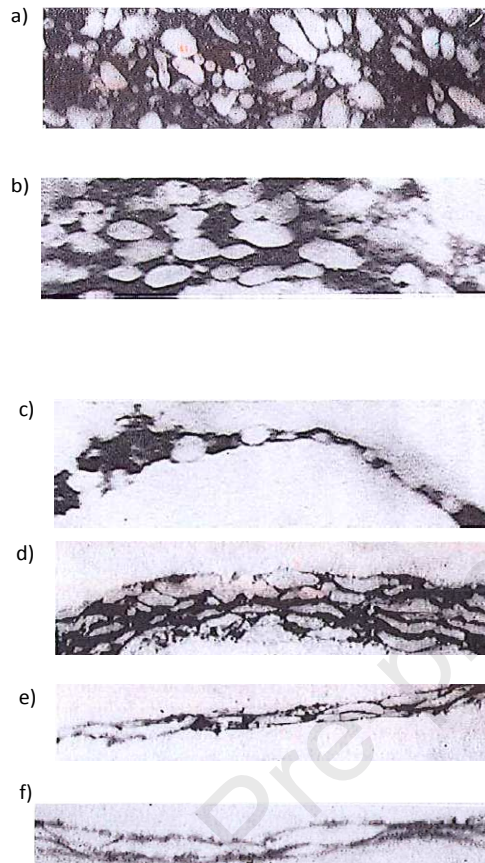


Fig. 4. Observations of gas cell walls using a light microscope. Dark-colored proteins were previously stained with Cotton C₄ Blue, leaving the starch granule section white (Sandstedt, et al., 1954): a) freshly mixed dough, with non-oriented starch granules; b) and c) cell walls in dough of various thicknesses at the end of proofing (b—50 μm, c—3 to 10 μm, varying locally according to the size of the starch granule embedded in the gluten film); d)-f) cell walls in bread crumb, of various thicknesses ranging from d) about 50 μm down to e) and f) 10-20 μm. Magnification ×400 for all except d) and e) (×200) and f) (×380).

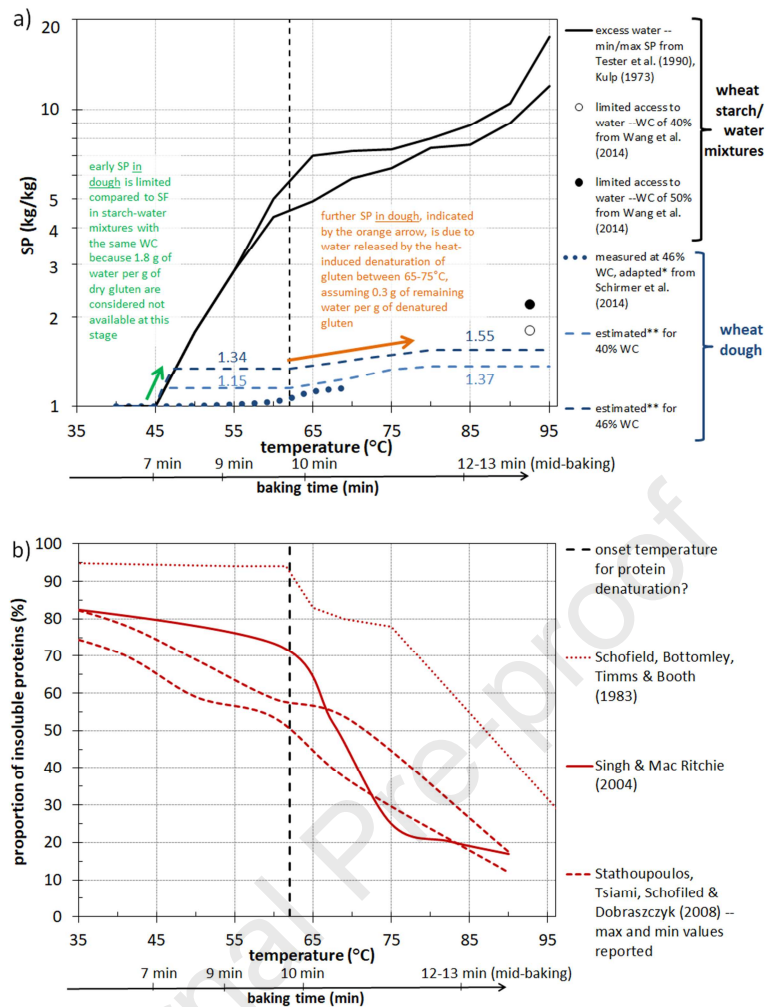


Fig. 5. Biochemical changes in flour components studied separately or in dough during heating: a) SP in wheat starch granules, expressed in kg of hydrated granules per kg of dry starch granules, plotted as a function of temperature for different amounts of available water; b) loss in gluten solubility as a function of temperature (compilation of literature data). *data converted from m^2/m^2 to g/g assuming that the thickness of the lenticular granules does not change during swelling, and densities of water and original starch at 40°C of 998 and 1358 kg/m^3 respectively; **calculation for a flour containing 12% gluten and 70% starch (dry basis) and gluten and starch hydration levels in the dough (before baking) of 1.80 and 0.43 g of water per g of dry matter respectively.

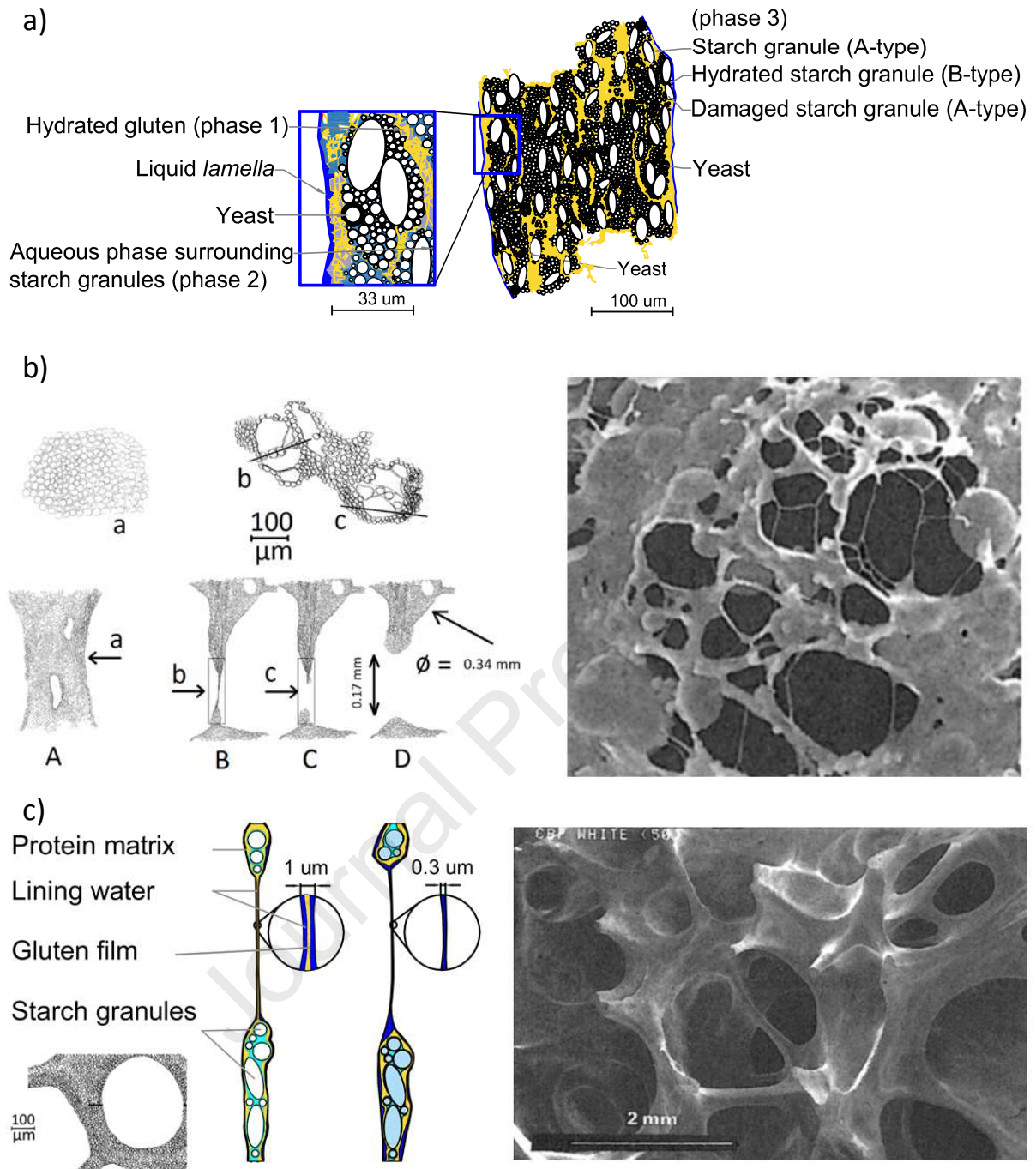


Fig. 6. a) Schematic GCW showing the spatial arrangement of dough components (250 μm thick); b) cross-section (A) of a GCW without liquid lamella and (B, C, and D) evolving cross-section of a rupturing GCW, along with what can be seen from the corresponding lower-case letters (a, b, c). The image of the complex structure of holes with strings crossing them was captured by (Gan, et al., 1990); c) cross-section of a GCW with liquid lamella. The water already lining the GCW and any water that may be expelled from the gluten matrix reinforce the wall until it ruptures. Starch granules hydrate during baking; the darker the blue, the more hydrated the starch granule. The hole left after the second rupture (that of the liquid lamella) is rounder in shape than that observed when no liquid lamella is present. The round holes visible in the right-hand image (Gan, et al., 1990) had previously contained water that had been sublimated at the sample-preparation steps preceding SEM.

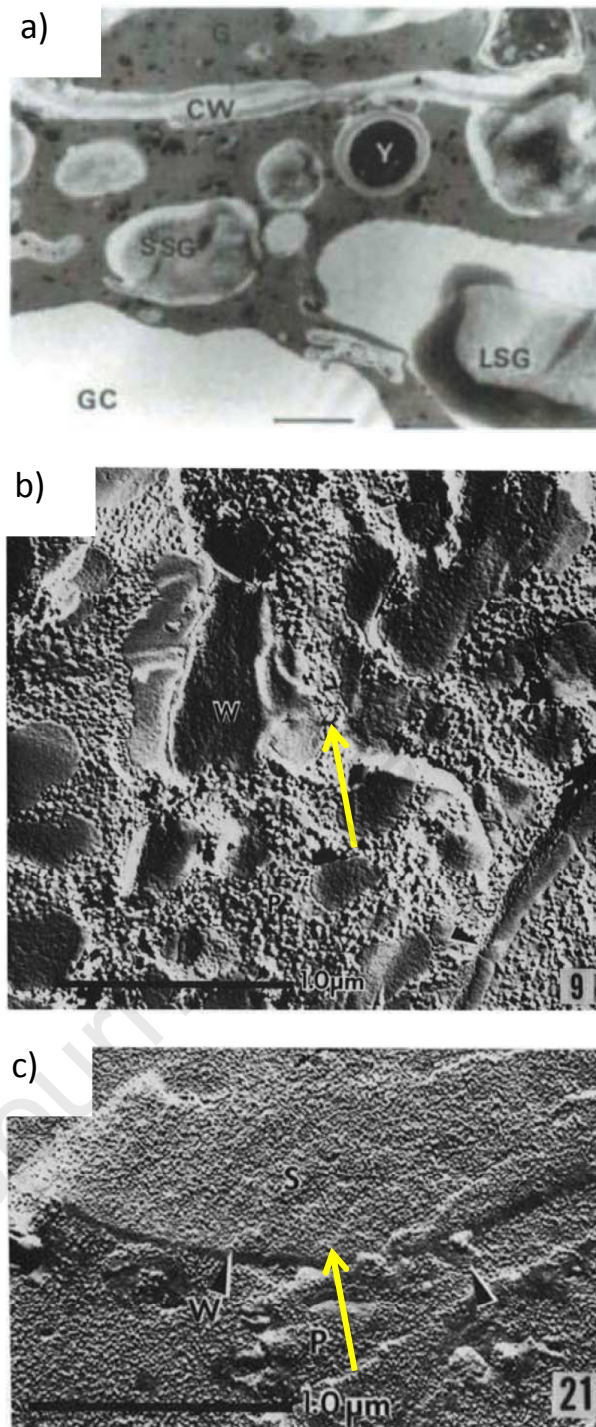


Fig. 7. Transmission electron microscopy images of bread dough constituents taken from a) Shewry, et al. (1995) and b), c) Fretzdorff, et al. (1982). Y = yeast; SSG = small starch granule; LSG = large starch granule; CW = fragment of cell wall from a wheat seed; GC = gas cell; (W) water pool in the protein phase (P).

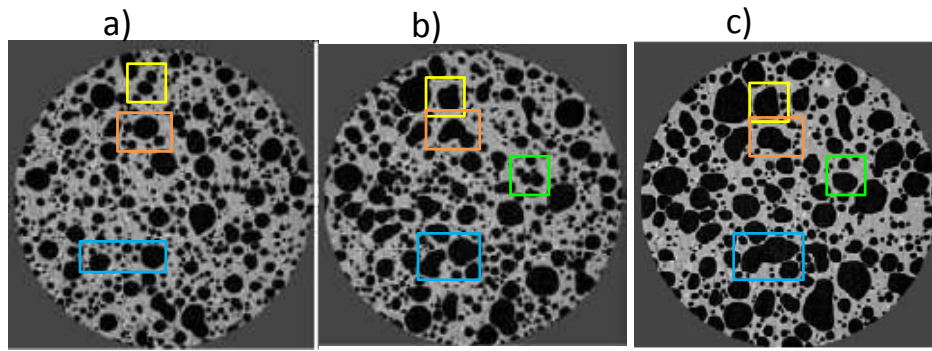


Fig. 8. Cross sections of dough during proofing (Babin, et al., 2006): a) 80 min, b) 90 min, c) 110 min; NB under the conditions of this study, the minimum value in mean GCW thickness (and hence the onset of coalescence) occurred at 80 min of proofing.

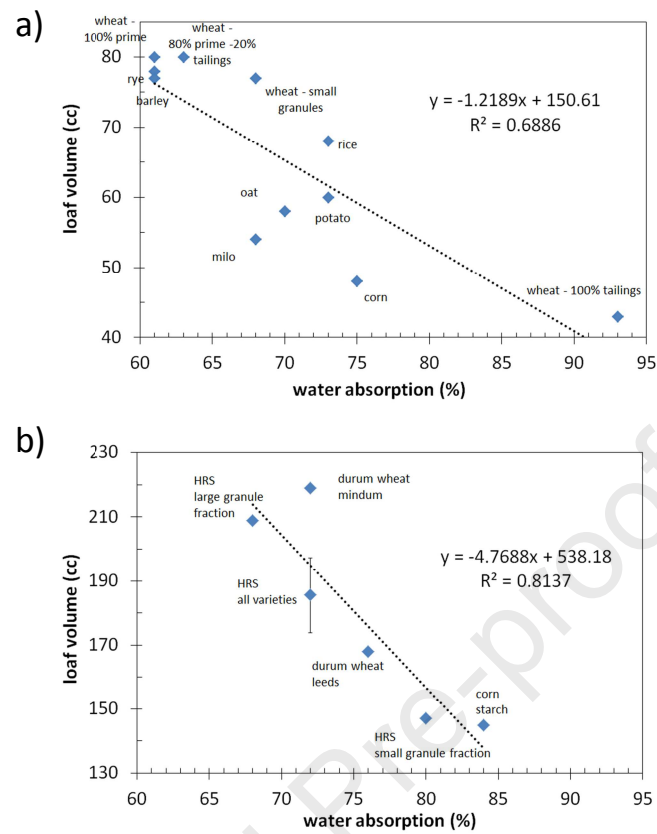


Fig. 9. Loaf volume as a function of the water absorption of the different gluten-starch blends at ambient temperature; adaptation of uncollated data found in a) Hoseney, et al. (1971) and b) D'appolonia, et al. (1971).

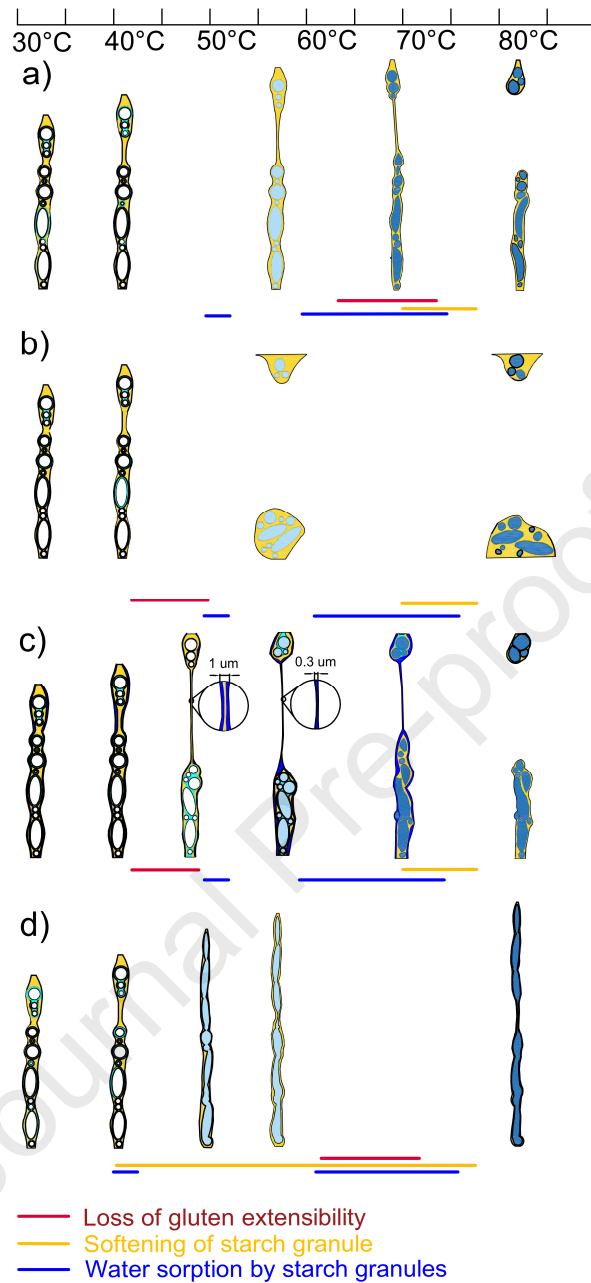


Fig. 10. Gas cell opening involving dough lamellae in the case of a) consistent dough and b) non-consistent dough where the gluten film ruptures early in the growth bubbles, without or with liquid lamella (a) and c) respectively), or d) is impeded by merging starch as a cohesive core forms in the gas cell wall. For the liquid lamella the scale has not been respected since it would not be visible in the figure. For the same reason the chosen void size is among the smallest voids observable in bread crumb.

Highlights

- Gas cell wall (GCW) rupture in bread dough during baking results from multiple physics.
- Changes in dough phase interactions modify GCW rupture mechanisms.
- The scale of dough constituents lacks knowledge of mechanical properties.
- Each GCW phase plays a role in the GCW stabilization.
- The role of starch is antagonistic, first destabilizing GCWs and then stabilizing them.