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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. **General introduction**

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

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

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

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

The aim of this paper is to review the state of the art related to the rupture of GCWs from advanced proofing to the end of baking, in order to propose a unified vision of governing mechanisms at the microscale together with the compilation of data necessary to achieve this understanding and to identify the gaps to be filled. This review first describes past attempts to monitor pore opening. Most of these measurements were performed at the macroscale (dough scale) and were related to the biochemical events in an indirect manner (through temperature monitoring in fact). The following two sections focus on events at the scale of the GCW and below. First, Section 3 gives a short overview on the size and morphology of GCWs. This section completes the introductory concepts for those new to the field. More expert readers may adjust their perception of dough as a fundamentally heterogeneous foam structure, where thick GCWs are still predominant even at the end of proofing. Section 3 ends with the concept of the “one-layer” state of the GCW, involving only a single layer of oriented starch granules, which precedes the formation of a gluten film at some spots. Then, Section 4 describes the interlinked biochemical and mechanical changes occurring during baking in each constitutive phase of the GCW. It compiles what little data has been reported at the moderate water contents deriving from bread dough and the temperature range applied during baking, but also highlights what is required for investigations in the immediate future. Section 4 ends by questioning the spatial organization of these phases in a GCW as well as their modes of interaction during baking. If the interaction between starch and gluten has quite been studied for crumb i.e. after cooling and starch retrogradation (Guessasma, et al., 2011; Lagrain, et al., 2012; Liu, et al., 2003), there is
little information about the nature of the interaction during baking. Last, Section 5 describes
the ways each phase favors or postpones the rupture of the GCW. It returns to and refines the
mechanisms commonly invoked by the cereal community and proposes some novel ones to
complement these. It raises a concern over the stabilization/destabilization role played by the
starch granules during baking, which has been far too overlooked in the literature.

2. Estimation of pore opening during baking

In a pioneer investigation, He, et al. (1991b) tentatively linked the timing of pore opening with
the initiation of CO$_2$ release in the oven atmosphere during baking. A precise temperature could be
assigned to these observations since the ohmic heating used in this study ensured quite an even
temperature throughout the dough. The authors concluded that the “loss in gas-retaining ability”
started at 72°C and persisted until 88°C. However, Zhang, et al. (2007) questioned the different
mechanisms controlling the major peak of CO$_2$ release. They assigned the release of CO$_2$, with the aid
of a numerical model of baking, to the development of a pressure gradient consecutive to crust
formation. They also indicated that pore opening can occur well before the major peak of CO$_2$ release
and, in such a case, accounts for the smaller levels of CO$_2$ released. Reports from He and Hoseney
(1991a, 1991b) were re-analyzed in the light of Zhang et al.’s findings, yielding pore opening
temperatures of 48-50°C when the dough consistency was optimal. Lower opening temperatures were
found for doughs prepared with less water (40-42°C) or with starch or gluten preheated at above 60°C
(30°C).

Cereal science has also benefited from developments in polymer and metal foam science (e.g.
Neff and Macosko (1996) and Zhang, et al. (1998)) allowing indirect investigation of pore opening
during bread dough baking (Miś, et al., 2016; Singh and Bhattacharya, 2005). For instance, Neff and
Macosko (1996) used rheometers to dynamically load an expanding urea-based polymer in the shear
direction, while monitoring its expansion. As for dough, a mixing stage is used to initiate bubbles into
the foam. Then, carbon dioxide is chemically produced within the foam and makes the growth of
bubbles possible under the plate of the rheometer. During expansion, the load varies with the pressure
exerted by the gas onto the GCWs within the foam and is lowered by GCWs opening. The normal
force first increased and was related to the stiffening of the polymer matrix. Then, when pore opening
occurred, the normal force suddenly dropped. Similar events were observed in the case of bread dough
during heating. Unfortunately, temperature uniformity throughout the dough sample was not well
controlled or recorded. After re-evaluation of the dough temperature commonly associated with the
minimum and maximum dough viscosity levels reported in these studies (55-60°C and 75-80°C
respectively, according to (Dreese, et al., 1988; Vanin, et al., 2010)), pressure-peak temperatures were re-estimated at 87°C for Singh, and 85°C for Miś. Note also that Miś, et al. (2016) used a device less subject to auto-tension effects than that of Singh and Bhattacharya (2005). A rheometer was associated with laser displacement sensors to measure the additional lateral expansion of the dough sample compared with its normal displacement.

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

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

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

The mixing step allows for flour hydration and contributes by its dispersive and distributive actions to the development of the gluten network, trapping air in the form of large bubbles that are subsequently broken down into smaller ones (Fig. 3a). During the proofing step, these tiny air bubbles take up the CO₂ and ethanol produced by yeast fermentation and grow. The increase in temperature during baking induces i) the supplementary production of CO₂ by yeast up to its inactivation temperature and the liquid-gas transfer of the dissolved gas into bubbles (Nicolas, et al., 2016),
resulting in what is known as oven-rise, and ii) the setting of the structure and the formation of the crust. Gas fraction, defined as the volume of gas per total volume of dough, thus increases across all the breadmaking steps: 8-12% immediately after mixing, 70-75% at the end of proofing and 80-95% at the end of baking (Della Valle, et al., 2014).

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

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

4.1. Wheat starch

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

At or close to ambient temperature

The three-dimensional structure of native starch granules is well described in the literature (Gallant, et al., 1997). Starting from the hilum, starch is deposited in alternating amorphous and semi-crystalline concentric growth rings which mainly contain amylose and amylopectin respectively. The distribution in size of wheat starch granules is bimodal. The largest granules (so-called A-type) are lenticular, of 5-15 µm in thickness and 22-36 µm in diameter (Jane, et al., 1994). The smallest
granules (B-type) are spherical and their diameter ranges up to 15 µm (Wilson, et al., 2006). They account for 27% of the total starch in weight, but for 97% in number (Soulaka and Morrison, 1985).

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

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

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

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

When heated in excess water, wheat starch undergoes an irreversible disruption of its molecular order (crystallite melting) which is part of the gelatinization process. Gelatinization combines several other events, such as absorption of water, change in shape and size of granules,
starch solubilization also called leaching (Biliaderis, 2009); these changes will be further described below.

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

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

The leaching of starch components is fostered at high temperatures, a phenomenon which has so far received little attention at WC levels relevant to dough (Kovrlija and Rondeau-Mouro, 2017; Nivelle, et al., 2019). The main reason for this is the difficulty in accessing the extragranular aqueous phase (Wang, et al., 2014). Leaching in excess water occurs quite late in the heating process, 5.0, 7.5 and 35.0 g per 100 g of dry wheat starch being leached at 85, 90 and 96°C respectively after
temperature has held for 30 min (Doublier, et al., 1987). Leached amyllose and amylopectin form a macromolecular gel at high temperatures with possible cross-linking between themselves and with other constituents (lipids, proteins) at cooling (Biliaderis, 2009). A low heating rate also favors leaching (Doublier, et al., 1987) and, consistently, results in greater firmness of the starch-water mixture after a heating-cooling cycle (Patel, 2006). In general, the heating of wheat bread crust proceeds at a moderate rate (5-6°C/min) over a short period (10 min) and is succeeded by 10 additional minutes where the temperature is held above 95°C while the crust is browning.

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

### 4.2. Wheat gluten

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

At or close to ambient temperature

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

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

The quantity of glutenin polymers contained in total protein determines extensibility (Sroan, et al., 2009). Indeed, the degree of entanglement determines gluten elastic response, while small polypeptides enable polymer segments to slip between entanglements and enhance the material viscous component. Gliaidan, being as prone as glutenin to H-bonding, acts as a perfect diluent (Graveland and Henderson, 1987) when interacting with glutenin polymers. Rheological tests performed on both glutenins and gliadins at 20°C substantiate the viscoelastic and the viscous (slippage) features of glutenins and gliadins respectively (Hernández- Estrada, et al., 2017; Khatkar, et al., 1995). The removal of gliadins causes an increase in stiffness and reduces the extensibility of the residual glutenin-fraction (Song and Zheng, 2008). The successive addition of glutenin fractions of
increasing molecular weight to a given flour, at constant protein content, causes loaf volume to increase and, after reaching a maximum, to decrease (Lundh and MacRitchie, 1989; MacRitchie, 1987). The occurrence of an optimum volume possibly indicates a balance between strength and extensibility, beyond which the stiffness provided by polymer entanglements decreases the failure strain and might cause lower loaf volumes (Singh and MacRitchie, 2001; Termonia and Smith, 1988; Tsiami, et al., 1997). It is important for there to be a good balance between elasticity and viscosity in the gluten; sufficient elasticity to retain gas but not so much that expansion is lost (Shewry, et al., 1995).

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

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

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

This makes it quite impossible to be sure whether the above rationale is valid. On the basis of this uncertain microstructure, we cannot infer which gluten feature should be modified in order to control its mechanical behavior. In-depth rheological studies of dough, starch-water mixture, gluten-water mixture and gluten-glass beads-water mixture at room temperature have shown that when gluten is simply considered as a critical gel, it is possible to account for the deformation behavior of gluten within the range of finite strain and strain rate encountered during proofing and baking (Ng and McKinley, 2008; Ng, et al., 2011; Ng, et al., 2006).
Beyond 60°C (Dobraszczyk and Morgenstern, 2003; Wang, et al., 2017), the stiffening of the gluten appears to be caused by a heat-induced crosslinking of gluten proteins (Hoseney and Rogers, 1990; Millar, et al., 2004) driven by thiol oxidation, thiol/disulfide exchanges and hydrophobic interactions (Gan, et al., 1995; Schofield, et al., 1983). In the range 62-75°C (Fig. 5b), protein unfolding allows exposure of buried intra-molecular disulfide bonds, which can exchange with others forming new inter-chain disulfide bonds (Schofield, et al., 1983). Half of the initial extensibility of the gluten is lost at about 65°C and a proportion of the other half by the end of baking (90°C). Flour of good baking quality displays earlier and steeper increase in elasticity during heating e.g. Dronzek and Butaki (1977); Jeanjean, et al. (1980). According to Stathopoulos, et al. (2006), high gliadin content results in a steeper rise in gluten elasticity above 60°C whereas high glutenin polymer content ensures high elasticity even below 60°C.

### 4.3. The hydrated gluten-starch matrix

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

The energy transferred to the system through mixing makes the formation of a 3D network possible between the co-existing yet interpenetrating phases, even if this is very difficult from a thermodynamic point of view because of the immiscibility of gluten and soluble starch (Tolstoguzov,
The continuity of the aqueous phase (see above) leads us to believe that the interaction between starch and gluten (Eliasson and Tjerneld, 1990; Jekle, et al., 2016) involves water trapped between these phases in the form of Van der Waals/hydrogen bonds. However, the way starch and gluten interact is still a matter of debate. In particular, how starch-gluten interactions through Van der Waals/hydrogen bond types would affect mechanical properties when large strain is involved is not very clear (Meerts, et al., 2017). The aqueous phase trapped between the starch granules and gluten makes it possible for gluten chains to slip and align along the main shear direction. During mixing, starch granules behave like ball bearing bodies, actively contributing to the reshaping of the gluten domains which eventually form layers that envelop the starch granules (Tolstoguzov, 1997). Indeed, starch granules are too rigid to lose their shape at the mixing step (Van Vliet, 2008). The gluten is assumed to be greatly stretched between starch granules when the proportion of gluten to starch is low (Ahmed and Jones, 1990) and, consistent with this view, stiffer blends have been observed. Interactions between starch and gluten are crucial for predicting the mechanical behavior of the gluten-starch matrix and further investigation is required that will build on the theoretical work carried out by Mohammed, et al. (2013).

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

**In the temperature range of baking**

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

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

4.4. Conclusion to this section

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

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

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

If hydration of each constituent is recognized as essential in the mastering of dough aeration, we dispose of little information on the exact distribution of this water during baking, which will drive
the mechanical properties of the phases, directly or indirectly (through the hydrothermal reactivity mentioned above). The plausible feeding of the liquid lamella with water expelled by the gluten under extension has been discarded and its persistence while starch granules swell during baking has not been questioned. This is an additional reason for considering the liquid lamella as optional in Section 5. We considered in the above subsections that each granule has the same environment, which is doubtful (Jekle, et al., 2016). Because the dynamics of heating are faster than those of water migration, the above picture is complicated by spatial heterogeneities. These will be discussed again at the end of Section 5.

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

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

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

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

The driving force for the deformation of the GCW is the gas pressure resulting from the equilibration between the gas intake within gas cells and the mechanical resistance and/or tensional forces that issue from the bi-extension of the GCW. Rupture of the GCW will begin at the spot where the stress concentrates until it exceeds the yield stress. Before, stress increases with strain rate and increase of strain within the GCW. As strain is greatest in the middle of the GCW, the triggering of the rupture might occur very close to that point.
The formation of significant pressure differences between gas cells during baking is very unlikely. Where surface tension is relevant, pressure differs up to 3 kPa between adjoining gas cells of widely differing sizes. This estimate of gas pressure was calculated for cells of 50µm and 1mm in diameter using Laplace’s law and assuming a surface tension of 0.04 N.m⁻¹ (Kokelaar and Prins, 1995; Van Vliet, et al., 1992). This is of the order of magnitude of about 1 to 30% of the modulus of elasticity of dough. The GCW may bend slightly between the two gas cells. The contribution of surface forces is expected to decrease during baking as the cell size increases. Further, pressure measurements during baking have shown no relevant difference in pressure (a few kPa at most) between the core of the dough and the region close to the crust (Baker and Mize, 1939; Grenier, Lé Ray, et al., 2010; Miś, et al., 2016; Singh and Bhattacharya, 2005; Sommier, et al., 2005).

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

### 5.2. Thinning of a “thick” GCW down to the size of starch granules

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

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

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

Wheat starch granules in the middle of the gluten were assumed to remain stiff up to about
70°C (Section 4.1.) and the pattern of stress concentration within the gluten described above applies
up to this temperature. When heated towards 70°C, starch granules also swell (Section 4.1). Given that
the swelling predominantly follows the gluten film’s direction of tension (Section 4.1), the thickness
of the GCW is little modified. We take the view that, despite the disappearance of the extra-granular
aqueous solution, there is little friction between starch granules and gluten because of their
immiscibility and because the gluten slips on the surface of the swollen starch granule. For all these
reasons, the shear stress within gluten might thus not be greatly affected by starch swelling.
When the temperature reaches 62-63°C the gluten begins to stiffen and its ability to be stretched progressively decreases upon further heating (Section 4.2). From the onset of this gluten stiffening, an already greatly stretched and stressed gluten film will possibly break, even without further stretching. The stress might increase more than the yield stress. This rupture without further stretching could even be greater if the heat-induced denaturation of gluten and water loss from the gluten were to be accompanied by shrinkage, but we found nothing in the literature to substantiate this. Again, such a rupture would look like cracks located along the rims of the solid particles.

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

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

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

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

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

Wheat starch granules probably keep their stiffness until late in the heating process (Section 4.1), limiting the above-mentioned effects of stabilization on GCWs to advanced stages of baking. Their leaching levels are also lower compared to other starches. By contrast, some starches with a low amylose fraction (tapioca starch, used in reconstituted flours or waxy wheat flours) can soften and lose their integrity early in the heating process (Fig. 10d). The early increase in viscosity of GCWs does not appear to be detrimental to the extension of the GCWs, which proceeds in a normal way during baking (Kusunose, et al., 1999). And, consistent with the increased cohesiveness in the granular phase and its positive impact on the GCW stabilization proposed above, greater loaf expansion has even been reported where the proportion of such starches remained low (Blake, et al., 2015; Kusunose, et al., 1999) (Fig. 1). However, where the proportion is too high, individual loaf volumes will decrease. The stabilization effect is so efficient that rupture of GCWs becomes rare. Within predominantly sealed gas cells, pressure drop occurs at the very end of baking (since air cannot enter a closed gas cell and compensate for the amount of carbon dioxide that escapes by diffusion through the GCW) and after baking (because of temperature decrease), causing the loaves to contract and explaining the lower
individual loaf volumes. Wheat starch can perform like these particular starches when it is attacked by enzymes, being able to sorb more water and, plausibly, soften earlier during baking. It appears however that the cohesiveness between wheat starch granules after enzymatic attack is not sufficiently increased to impede the rupture of GCWs since increased loaf volumes have often been reported (Sandstedt, 1961).

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

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

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

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

The ideal configuration is when the softening of starch granules occurs just before the gluten reaches its maximum ability to stretch (Fig. 10a). The gluten partly relaxes and becomes less likely to
rupture. Upon further stretching the stress increases again and eventually reaches the yield stress at some locations. Most importantly, most gas cells open when the structure has stiffened sufficiently to support its own weight in the still-thick GCWs close to the GCW that has just ruptured. Collapse is minimal. Nor does a sufficiently open structure of this type shrink under the contraction of gases upon cooling. This configuration is ideal because both collapse and shrinkage are avoided, or at least minimized.

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

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

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

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

Second, there may be differences in the redistribution of water between the core and peripheral layers of dough. In deck and convective ovens, temperature first increases in the outer layers. With the increase in temperature and the subsequent increase in partial water vapor pressure, the evapo-condensation-diffusion (ECD) phenomenon feeds the colder core of the dough with water while some water is withdrawn from the outer layers (Ureta, et al., 2019). It is possible that ECD feeds the internal lining water of gas cells located in the loaf core. The influence of ECD on the formation of liquid lamellae has never been studied. The transport of water vapor to the core is greater in regions containing many open pores than in those with predominantly sealed pores. In the latter case, the diffusion of condensed water through the GCWs slows down the overall transport of water vapor to the core; diffusion of liquid water being slower than diffusion of water vapor. These different rates in water transport to the core will lead to local variations in water content and water availability. These
spatial variations in water content and temperature will undoubtedly lead to differences in starch softening, gelatinization, amylase leaching, mechanical properties, stress, and strain softening or hardening. Similarly, because of the drying process within the crust, GCWs stiffen before gas formation has had a chance to inflate gas cells (Vanin, et al., 2009) and opening of GCWs does not happen at the same rate and for the same size of gas cells as in the loaf’s core. Permeability may be lower in the crust than it is in the loaf core (Jefferson, et al., 2007; Zhang, et al., 2005).

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

6. Conclusion

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

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

The present paper showed that there is little data available on the mechanical properties of starch granules and hydrated gluten in the dough environment, at temperatures, gaseous environment and strain rates relevant to bread baking. Similarly, the nature of the interaction between starch and gluten is still being debated in the literature; here, the impact of baking on the interplay between these two dough phases as they undergo rapid physicochemical changes is also totally disregarded. This topic would benefit from theoretical approaches and from experimental approaches focused on...
interfacial design (Jekle, et al., 2016). Last, the spatial organisation of the GCW, as well as its reorganisation in response to the redistribution of water following starch gelatinisation, also require more investigation. Combined microscopy and NMR can provide relevant information in this respect. Acquisition of new insights on these topics is needed to supply the micromechanical models proposed above. In their absence, the processes of GCW opening during baking will remain very hard to fathom and, when dealing with the setting of crumb structure, our ignorance must be acknowledged. In this context, the present review does not pretend to offer a definitive account, but to provide a mechanistic vision of a more systemic nature that has yet to be tested, completed and corrected by further investigation.


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 μtomography (22μm), 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 m²/m² 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.