Chapter 11
Principles of Dough Formation

Introduction

The first, basic step in breadmaking is combining water with wheat flour and kneading (imparting mechanical energy to) the mixture to form an elastic dough (Bushuk, 1985; Hoseney, 1985). Flour from wheat, rather than from other cereal grains, is used because wheat storage proteins have unique properties; no other cereal storage protein possesses the ability to form a visco–elastic dough when wetted and kneaded. A full explanation at the molecular level for this uniqueness still eludes researchers.

The events that occur when gluten proteins are hydrated and worked are also elusive. Part of the obscurity is due to the complexity of the system. The basic properties of dough are established by the characteristics of the storage (gluten) proteins in the flour. These characteristics, however, are modified by other flour components, both soluble and insoluble, as well as the additional ingredients added to dough. In studying dough formation we are limited to observing physical events on a macro scale or at the supra-molecular level. Numerous techniques that study molecular properties have been applied to dough including; X-ray analysis, nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), electron spin resonance spectrophotometry (ESR) and scanning electron microscopy (SEM). The interpretation of the results, however, is always complicated by the complexity of the system. X-ray analysis, for example, led to a model of the dough matrix (Grosskreutz, 1961) that included gluten proteins, phospholipids and solid (starch) contributions, but there is no way to confirm independently the accuracy of that model. While these techniques each help us clarify certain aspects of dough structure, the concepts that will be set forth in this chapter remain speculative to a significant degree. This fact must be kept firmly in mind while reading this or any other publication on dough formation.

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The macro-properties of dough change with time. At the end of the mixing process (Chap. 4) the dough has certain visco-elastic characteristics that are considered optimum for subsequent processing. The resting period (floor-time) changes these properties and makes the dough more pliable (relaxed). Dividing and rounding reverses this to some extent and the dough appears more elastic (less relaxed). An intermediate proof period decreases the elasticity, allowing good moulding into the shape of a loaf. During proofing the characteristics are further modified, not only by relaxation but also by changes in matrix composition from the products of fermentation (ethanol, carbon dioxide), by the action of additives (oxidants and enzymes) and possibly by the action of native flour proteases. Again, our understanding of the molecular alterations resulting in these modifications in dough properties is rudimentary at best.

Governing all our discussions about dough formation (and the breadmaking process) is the fact that the ultimate criterion of 'good' or 'poor' structures and processes is the final product—a good loaf of bread. The two main contributors to bread quality are volume (stability in the prover or proof-box and good oven spring) and a fine, silky crumb. These desirable outcomes depend, obviously, on certain optimum properties in the dough matrix. Two characteristics define 'good' dough:

- The ability to retain gas (carbon dioxide), generated during fermentation (proofing), in the form of numerous small gas cells;
- A proper balance of viscous flow and elastic strength so that the loaf can expand adequately during proofing and the early stages of baking, yet retain its rounded form.

Gluten (hydrated wheat storage protein) is the component of dough that determines how well these requirements are met. While other flour components affect gluten functionality, and mechanical energy input during mixing is crucial to developing the proper characteristics, it is still the physicochemical nature of gluten proteins with which we will be mainly concerned in this discussion of dough formation.

Flour and Dough Components

Wheat flour components (dry basis) can be classified into seven groups:

1. Starch;
2. Storage (gluten-forming) proteins;
3. Non-starch polysaccharides (pentosans);
4. Lipids;
5. Water-soluble proteins;
6. Inorganic compounds (ash).
7. Celluloses associated with bran layers, their level is limited in white flour but is higher in wholemeal flours.

Starch is relatively inert during dough mixing, but plays a role as a 'filler' that contributes to increased dough visco-elasticity. (Starch, of course, has a critical influence during the baking process, when it gelatinizes, and during subsequent storage, when retrogradation accounts for the major part of bread staling (see Chap. 10.) Endogenous inorganic materials are relatively unimportant in dough formation, although added salt strongly influences dough properties. The other five component groups listed are actively involved in dough formation during mixing and subsequent processing.

Starch

Starch represents by far the largest portion of flour, making up about 65 % of ordinary flour (14 % moisture basis). Wheat starch comprises about 23 % amylose and 73 % amylopectin (thus the two species represent 15 and 50 % of the flour weight, respectively). Amylose is a linear chain of α-1,4 linked glucose units, with a molecular weight in the range of 100,000 Da, while amylopectin is a highly branched structure, with an estimated molecular weight in the range of 20,000,000 Da (Fig. 11.1). Native starch exists as granules and has a high degree of crystallinity, evidenced by birefringence (the 'Maltese cross' seen when it is examined with a polarizing microscope). These granules are relatively inert during mixing but influence dough elasticity by their presence in the total matrix. In hard wheat flour as much as 15 % of the starch granules (10 % of the flour weight) are 'damaged', that is, they have been deformed during milling and contain cracks and fissures. Damaged starch granules absorb about four times as much water as intact granules, and increase dough water absorption (see below and Chap. 12). Also, damaged starch is much more susceptible to the action of α-amylase than is intact starch, a fact that enters into dough property modification during the proofing stage of processing.

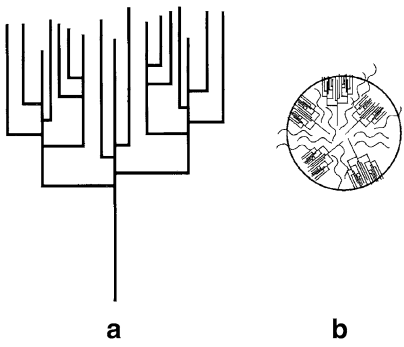

Fig. 11.1 (**a**) Amylopectin, (**b**) a starch granule

Gluten

The storage protein found in flour is not, strictly speaking, gluten; that term designates the hydrated glutelins (glutenins) and prolamines (gliadins) formed when a dough is mixed. However, for convenience in this discussion the anhydrous storage protein of wheat endosperm will be called gluten (as the term is commonly used). Of the total protein in wheat flour, about one-sixth is soluble protein (albumins and globulins), falling into group 5 in the list above. Thus a flour having 12 % protein contains only about 10 % gluten-forming proteins. The molecular characteristics of these gluten proteins will be examined in more detail below.

Pentosans

Non-starch polysaccharides represent only about 2–2.5 % of flour (Michniewicz, Biliaderis, & Bushuk, 1990), but have a disproportionate influence on dough properties. They are sometimes called hemicelluloses because they constitute part of the cell wall materials (formed in conjunction with cellulose) in the seed, made by the plant as the wheat berry is synthesized and ripening. More often the name pentosan is used because approximately 80 % of the sugars present are the pentoses D-xylose and D-arabinose. The pentosans are a heterogeneous group of macromolecules, but the preponderant backbone structure is a xylan, a chain of β-1,4 linked D-xylose units. Various other sugars are attached to this chain by α-1,3 linkages; the major side chain sugar is arabinose, but small amounts of glucose, fructose and mannose are also found. Besides the xylans, a significant amount of arabinogalactan (polygalactose chain with arabinose side chains) is present in the water-soluble portion.

About 65 % of wheat flour pentosans are water-insoluble (WI) pentosans (Michniewicz et al., 1990); these are almost exclusively xylans. The water-soluble (WS) pentosans are approximately half arabinoxylans and half arabinogalactans. Pentosans are gums; they absorb several times their weight in water and form highly viscous solutions. This is especially important in rye flour (Chap. 13), where the pentosan content may be as high as 10 %; the viscosity that allows rye flour to form a dough is due almost exclusively to the pentosans. The water-absorbing property of pentosans is influential in wheat flour doughs (see below) and the viscosity due to the WS pentosans influences the visco-elastic behavior of dough.

Flour pentosans form gels when treated with certain oxidants (Geissman & Neukom, 1973). The mechanism involves ferulic acid, an α,β-unsaturated aromatic carboxylic acid that is esterified to arabinoxylans (Fig. 11.2). Oxidants that generate free radicals, for example hydrogen peroxide, promote cross-linking between ferulic acid residues on adjacent polymer chains and lead to gelation of suspensions of both WI and WS pentosans. The formation of covalent linkages with gluten proteins has been postulated (Neukom & Markwalder, 1978), via reaction between ferulic acid and tyrosine side chains of the protein (Fig. 11.3). Sulfhydryl compounds react with α,β unsaturated aromatic acids. ^{14}C-tagged cysteine binds to WS pentosans

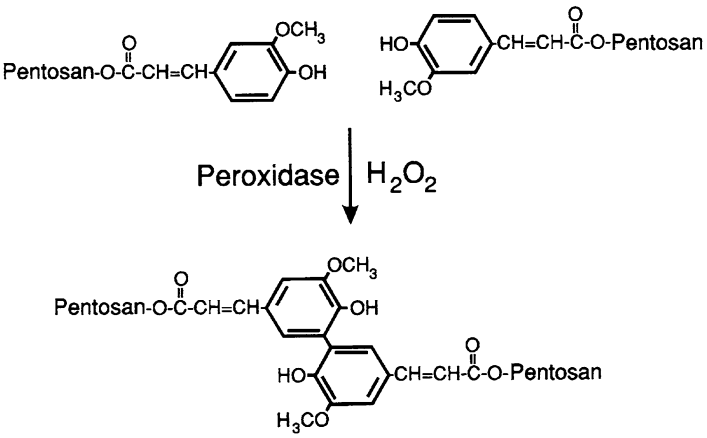

Fig. 11.2 Oxidative crosslinking of ferulic acid in flour pentosans

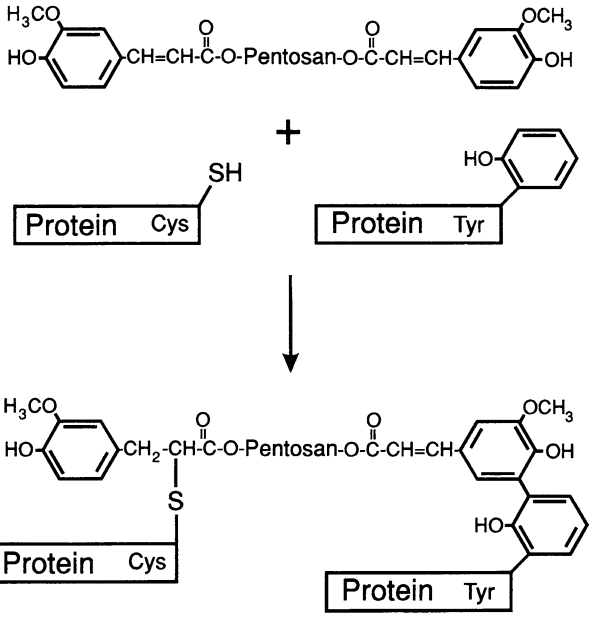

Fig. 11.3 Reactions of water-soluble pentosans with dough proteins. *Left*, addition of –SH to ferulic acid. *Right*, ferulic acid linking to tyrosine

(Sidhu, Hoseney, Faubion, & Nordin, 1980b), presumably through addition of the—SH group across the activated double bond. Cysteine inhibits the oxidative gelation of pentosans (Hoseney & Faubion, 1981). Carboxylic acids with an activated double bond (e.g. fumaric acid and cinnamic acids) drastically reduce the mixing stability of doughs (Hoseney & Faubion, 1981). These facts have been interpreted

to mean that during normal dough mixing feruloyl moieties attached to pentosans are attached to gluten proteins via addition of sulfhydryl groups across the activated double bond, generating cross-links and enhancing dough elasticity (Fig. 11.3).

Lipids

Wheat flour contains about 2.5 % lipids. Of this, about 1.0 % is non-polar lipids (triglycerides, diglycerides, free fatty acids and sterol esters). The two main groups of polar lipids are galactosyl glycerides (0.6 %) and phospholipids (0.9 %). During mixing, both classes of lipids are complexed with gluten and become relatively unextractable with any of the usual solvents (Daniels, Wendy-Richmond, Russell-Eggitt, & Coppock, 1976; Wootton, 1976; DeStefanis, Ponte, Chung, & Ruzza, 1977). Grosskreutz (1961) interpreted his X-ray spacings to indicate the presence of bimolecular layers of polar lipids in the gluten complex, and polar lipids have been proposed as adhesive agents between starch granules and gluten protein in several other models (Chung, 1986).

Flour lipids appear to have little effect on mixing requirements; the mixogram is identical for control and defatted flours (Schroeder & Hoseney, 1978). On the other hand, the addition of anionic surfactants such as sodium dodecyl sulfate strengthens the dough and increases mixing time (Danno & Hoseney, 1982). While these are not flour components, the effect offers further insight into the nature of the gluten complex (and how mechanical mixing modifies it) and will be discussed in more detail below. Lipids have a major influence on baking performance of bread (Wilde, 2012), especially with respect to oven spring (loaf volume) and the keeping quality of the finished product. Protein-lipid interactions are formed in bread dough though their role in the stability of bread dough is not always clear with both beneficial and negative effects being observed depending on the concentration and nature of the lipid itself. With the increased use of lipase enzymes (Kornbrust, Forman, & Matveeva, 2012) the contribution of flour lipids to bread quality is once again attracting attention.

Water-Soluble Proteins

The water-soluble fraction of flour (approximately 2–3 % of total flour weight) contains albumins and globulins as well as WS pentosans. The proteins include enzymes, enzyme inhibitors, lipoproteins, lectins, and globulins of unknown function. It is reported that two-dimensional electrophoresis shows over 300 components. These undoubtedly have some biological function in the seed, relating to its primary role as the progenitor of the next wheat plant. The only clearly identified role played by any of these compounds in baking is the action of β-amylase on starch, generating maltose which serves as a fermentable sugar for yeast during proofing of lean doughs.

Reconstitution studies with fractionated flour components show that the water-soluble fraction plays a role in the breakdown of over-mixed dough (Schroeder & Hoseney, 1978). A mixogram of only the gluten and starch fractions showed a long mixing time with extremely long mixing tolerance; when the water-soluble fraction was also included the mixogram resembled that of the control (un-fractionated) flour. The fraction was dialyzed (removing low molecular weight materials) and then heated (denaturing proteins). The remaining soluble material (presumably mainly WS pentosans), when added back to the gluten plus starch fractions, gave a mixogram similar to that of the control, i.e. mixing to a peak followed by relatively rapid breakdown.

Ash

White wheat flour contains about 0.5 % ash which is a measure of the level of bran that is present (see Chap. 12), in wholemeal flour the levels are significantly higher. The inorganic material which comprises ash no significant influence on dough formation or subsequent bread quality. The inorganic materials which comprise ash are closely associated with the bran layers in wheat which is why their measurement is so important to the miller (Cauvain, 2009) and baker. In the context of dough formation and stability, the presence of bran particles should be seen as a negative in that the presence of the bran particles disrupts the formation of a cohesive network and contributes to instability during the gas bubble coalescence processes which take place in the later stages of proof and the early stages on baking. The particle size of the bran can have a significant impact on the gas retention ability of the dough with smaller bran particles having a large negative impact than large. In part this account for the fact that stoneground wholemeal flours often deliver lower bread volume than those based on the reconstitution from roller-ground fractions (Cauvain & Young, 2001). The negative impact on bread particles on dough gas retention can often be overcome by the addition of other functional ingredients which deliver improved gas retention, e.g. fat (Chap. 3).

Flour Components and Water Absorption

An important factor in commercial bread dough production is the proper water to flour ratio (Cauvain & Young, 2008). In common usage this ratio is called ‘absorption’, and expressed as a percentage of the flour mass. The adjective ‘proper’ differs depending upon the kind of dough being made (absorption is much lower for a bagel dough than for a white pan bread dough) and the method used in its measurement (Farinograph absorption can be 2–4 % lower than operational absorption). Operational (or baking) absorption, of course, means the water to flour ratio that results in a dough having the handling (machinability), proofing and baking

(loaf volume), and finished product (appearance, eating quality) characteristics necessary to give the desired baked food (bread). As stated above and discussed in Chap. 1, the criterion of 'good' or 'poor' is determined by the final product and the consumer. The contribution of various flour components to absorption has usually been made using some sort of instrumental measurement, with the instrument defining 'correct' absorption. This is most often the Brabender Farinograph (Chap. 12), which records mechanical resistance as a simple mixture of flour and water is kneaded. Farinograph absorption is the water to flour ratio that results in a recorder trace which, at its maximum, is centred on the 500 (or 600 in the UK) Brabender units line. This is generally lower than baking absorption—the water to flour ratio, determined by an experienced mixer operator that gives optimum dough handling and final product qualities. The absorption numbers that are presented here must be understood as representing the relative water uptake by the various components, and not an attempt to allow precise calculation of baking absorption for a given flour based upon analytical data.

Four white flour components absorb water; protein, native starch, damaged starch and pentosans. The relative absorptions (in grams of water per gram of component) are given in Table 11.1. Using analytical data (typical for a hard red spring wheat flour) shown in column 3, an absorption of 68.4 % is calculated (total of column 4), which is reasonable for such a flour.

Both soluble and insoluble (gluten) proteins absorb water (Greer & Steward, 1959; Bushuk, 1966). We might expect that absorption by the insoluble gluten proteins would have more effect on dough rheology than solution of the soluble proteins, but the specific evidence for such a conclusion is somewhat indirect. The greater influence of gluten proteins on baking absorption is highlighted in a study by Tipples et al. (1978). They measured total protein, wet gluten, gluten 'quality' (wet gluten quantity divided by total protein), damaged starch and pentosan contents of flours from a number of milling streams of Canadian hard red spring wheat. They also measured Farinograph absorption and baking absorption (for several different baking protocols) of the flours. The most important predictor of Farinograph absorption was damaged starch content; inclusion of total protein improved the prediction equation significantly, but including the other factors produced no further improvement (i.e. no increase in r^2). Of the five baking tests they used, the one most like commercial (North American) production methods was the remix test that included 0.3 % malt. This test is similar to the standard American Association of Cereal Chemists International (AACCI) baking test, with a long (nearly 3 h) bulk

Table 11.1 Influence of flour components on absorption

Component	Water per g component (g)	Amount per 100 g flour (g)	Absorption per 100 g flour
Protein	1.3	12	15.6
Intact starch	0.4	57	22.8
Damaged starch	2.0	8	16.0
Pentosans	7	2	14.0

fermentation, but the dough is then remixed before being moulded and panned. The most important single factor in predicting baking absorption was gluten quality. Adding simple protein level as a prediction factor significantly increased reliability (r^2), but the other factors (damaged starch and pentosan content) were not statistically significant.

Native starch granules are relatively impermeable to water. This may be due in part to the lipids and protein found on the surface of the granules, probably derived from the cell wall of the amyloplasts present in the ripening wheat berry (Greenwood, 1976). While native starch is the largest single contributor to absorption, this is due to its preponderance in flour. During baking, of course, when these granules swell and gelatinize, the contents become readily hydratable and are probably the main water-binding species in baked bread.

Most damaged starch is formed during milling (though amylase action in sprouted grain can also cause starch damage). During the process of reducing chunks of endosperm from the break rolls to flour on the reduction rolls, the particles are subjected to extreme pressure. The granules are somewhat elastic and return to their original shape after the pressure is relieved, but some granules are left with cracks and fissures. These represent spots where water can readily penetrate to the interior of the granule and interact with the amorphous regions found there. More pressure at the reduction rolls is needed to break up hard wheat endosperm than for soft wheat; hence hard wheat flours typically have a higher damaged starch content (6–12 %) than soft wheat flours (2–4 %). These cracks also represent points of susceptibility to amylase action, in contrast to intact starch granules which are resistant to amylolytic attack under ordinary conditions. Digestion by amylases in dough releases maltose, which can be fermented by yeast, as discussed in Chap. 3. Also, during proofing digestion of damaged starch decreases its water-holding capability, releasing more water into the dough matrix and increasing pan flow. Significant amylolytic activity requires some period of time, and is not a factor during the relatively short time involved in dough formation.

Studies on the water-absorbing capabilities of pentosans give rather varied results. Kim and D'Appolonia (1977) added isolated pentosans to flour and measured the change in Farinograph absorption. The addition of 1 % WS pentosan increased absorption by 4.4 %, while 1 % WI pentosan increased absorption by 9.9 %. Michniewicz et al. (1990) added WI pentosan to various hard wheat flours at different levels, and measured the changes in Farinograph absorption. They found increases in absorption ranging from 3.2 to 5.6 g of water per gram of pentosan; the increment was smaller when the intrinsic baking quality of the test flour was better. Patil et al. (1976) used flour fractionation and reconstitution studies to explore the effect of flour water solubles and WS pentosan fractions on absorption, mixing time and loaf volume. They found essentially no effect of WS pentosan on baking absorption (and a small, variable effect on mixing times). Based on published reports, a median value of 7 g of water absorbed per gram of flour pentosans was chosen for inclusion in Table 11.1.

Bran is of course a mixture of many different wheat polysaccharides, some of which are discussed above. In practical terms it is important to recognise that the

level of bran also impacts the water absorption capacity of flours not just in absolute terms but also with respect to the rate at which water is taken up during doughmaking. The physical structure of bran particles commonly means that they are slow to hydrate. This has practical implications for dough processing in that high-bran doughs (e.g. based on wholemeal) may appear to have a satisfactory consistency on leaving the mixer but as the water is slowly absorbed into the bran the doughs become firmer and this may have an adverse behaviour on the moulding and shaping processes which follow dough mixing. In some cases a short pre-hydration phase may be incorporated at the start of the mixing process typically this comprises mixing the ingredients for a short length of time at a slower speed prior to full development of the dough.

Wheat Gluten Proteins

Wheat proteins have occupied a central position in protein studies since early times. Gluten was first recognized as the rubbery component of wheat flour in 1729 (Bailey, 1941), although at that early stage it was not called protein (the term had not yet been coined). The common method of characterizing proteins based on their solubility was developed using wheat proteins (Osborne, 1907). According to Osborne's scheme, proteins were divided into four groups:

- Albumins, soluble in distilled water;
- Globulins, soluble in dilute salt solutions;
- Prolamines, soluble in 70 % aqueous ethanol;
- Glutelins, soluble in dilute acid.

Gluten proteins are members of the latter two groups.

Amino Acid Composition

Wheat gluten proteins are anomalous, even compared to other cereal storage proteins, in their amino acid make-up (Kasarda, 1989). About one-third of the residues are glutamyl residues, which are almost entirely in the form of glutamine (the amide of the side chain carboxyl group). The amide, a non-ionizing group, readily forms hydrogen bonds with electron donors (other amides and water molecules). The content of basic amino acid residues (arginine, lysine and histidine) is relatively low, and the amount of carboxylic acid residues (aspartic and glutamic acid) is even lower. As a result the proteins have a rather low surface charge density, even at pH values somewhat removed from the isoelectric point. Since the charge repulsion between molecules is low the protein chains can approach each other and interact (form hydrogen bonds) in the aqueous dough matrix. The addition of sodium chloride further suppresses charge repulsion, increasing molecular interaction.

Gluten also contains a higher level (about 14 % of the residues) of proline than is usual in proteins. This amino acid favours the formation of β-sheets (Belton, 2012) and similar structures that are thought to be responsible for some of the elastic characteristics of gluten (see discussion below). While the content of hydrophobic amino acids is not unusual, the lack of ionic character makes hydrophobic interactions between protein chains possible. The hydrophobicity of gluten proteins has been demonstrated experimentally by chromatography of acid-solubilized gluten on hydrophobic gel media such as phenyl-sepharose (Chung & Pomeranz, 1979). These authors examined gluten from two flours having different baking properties, and found that glutenin from the good-baking flour was more strongly absorbed to the gel than the glutenin from the poor-baking flour. Surprisingly, the relationship was reversed for gliadin; that from the poor-baking flour was bound somewhat more strongly than gliadin from the good-baking flour. Kaczkowski et al. (1990), on the other hand, found gliadin from good-baking wheat to be slightly more hydrophobic than gliadin from wheat of medium-baking quality. They used binding capacity for sodium dodecyl sulphate as their criterion of hydrophobicity, a difference in technique that might account for the discordant results.

Gliadin

Actually a heterogeneous group of prolamines, more than 70 different gliadin species have been identified, using chromatography and electrophoresis. They are rather hydrophobic, hence their insolubility in water or salt solutions, but can be divided into groups based upon their degree of hydrophobicity. More hydrophobic gliadins (the γ-gliadins) increase bread loaf volume, while gliadins from the more hydrophilic end of the spectrum (θ-gliadins) decrease loaf volume (van Lonkhuijsen, Hamer, & Schreuder, 1992; Weegels, Marseille, de Jasger, & Hamer, 1990). Gliadin proteins are relatively small, with molecular weights in the range 30,000–100,000 Da. They are single-chain proteins (i.e. no cross-links between chains) and disulphide bonds as occur are all intra-molecular (Fig. 11.4a). Concentrated solutions of isolated gliadin are highly viscous, with little measurable elasticity.

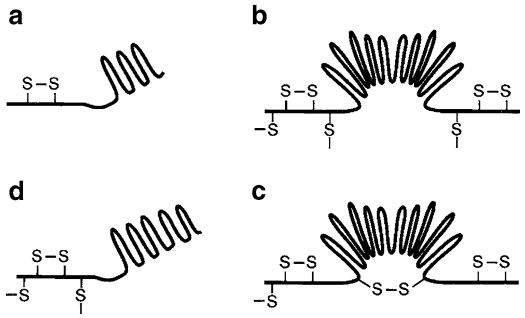

Fig. 11.4 Schematic depiction of gluten proteins. (**a**) Gliadin, (**b**) HMW glutenin subunit, showing possible action of the β spirals as 'molecular spring', (**c**) HMW glutenin subunit, showing disulphide bond preventing extension of the β spirals, (**d**) LMW glutenin subunit

Glutenin

Glutenin is the type example of Osborne's glutelins. Like gliadin it is quite hydrophobic (its amino acid composition is similar to that of gliadin) but it has a very different molecular structure; glutenin is a polymeric protein. The average molecular weight of native glutenin is stated to be about 3×10^6 Da, a number that is highly approximate and serves only to characterize the wide molecular weight distribution of glutenin (Kasarda, 1989). Polymerization takes place via intermolecular disulphide bonds. Reduction of these bonds with a reagent such as dithiothreitol (DTT) frees the basic glutenin subunits, which can be separated using SDS-PAGE (electrophoresis in a polyacrylamide gel in the presence of a high concentration of sodium dodecyl sulphate, a technique that separates proteins on the basis of their molecular weights). Two groups of subunits are identified. High molecular weight glutenin subunits (HMW-GS) have apparent molecular weights in the range 80,000–120,000 Da, while the molecular weights of low molecular weight glutenin subunits (LMW-GS) are about 40,000–55,000 Da. The molar ratio of LMW-GS to HMW-GS is 2:1 or higher; the amounts of the two kinds of subunits are roughly equal on a weight basis.

The molecular architecture of glutenin subunits is unusual (Fig. 11.4). In HMW subunits cysteine is concentrated in the regions near each end of the chain, with a long stretch of other amino acids between these two ends. The cysteine residues are involved in both intra- and inter-molecular disulphide bond formations. LMW subunits have a similar concentration of cysteine residue, but at only one end of the chain (Fig. 11.4d). Thus, both ends of the HMW protein can enter into polymerization reactions, while only one end of the LMW protein can react this way. The interior regions of both species, but the HMW subunits in particular, are postulated to form β-turn spirals, which in turn can fold into a helical sheet structure that can possibly be likened to a coil spring (Fig. 11.4b). This is only a hypothesis, but it is an attractive one that could account for the elastic nature of glutenin. An intramolecular disulphide bond can restrain this 'spring' (Fig. 11.4c); this bond can be broken during mixing (see below) to 'develop' the gluten structure. Isolated glutenin, when re-hydrated, forms an elastic, rubbery mass that has almost no viscous flow characteristics.

Numerous proposals have been put forward for the structure of glutenin polymers in dough (Fig. 11.5). Unfortunately, because glutenin is such an intractable protein to examine, these must of necessity be considered speculative. Graveland et al. (1985) postulated a basic 'building block' of three glutenin subunits linked through disulphide bonds (glutenin IIIa) and a tetramer of this basic structure (glutenin IIIb). These react with linear proteins having two or more reactive sulphydryl sites, to form a larger molecule called glutenin II. Glutenin I is a highly polymerized, insoluble protein which is thought to be the glutenin protein present in wheat flour. It is partially depolymerized during mixing and reforms during the resting stage of dough processing. Gao et al. (1992) examined the effects of small amounts of DTT on dough consistency in the Farinograph, and arrived at a slightly different model. They also postulate a subunit structure similar to Graveland's, but specify both

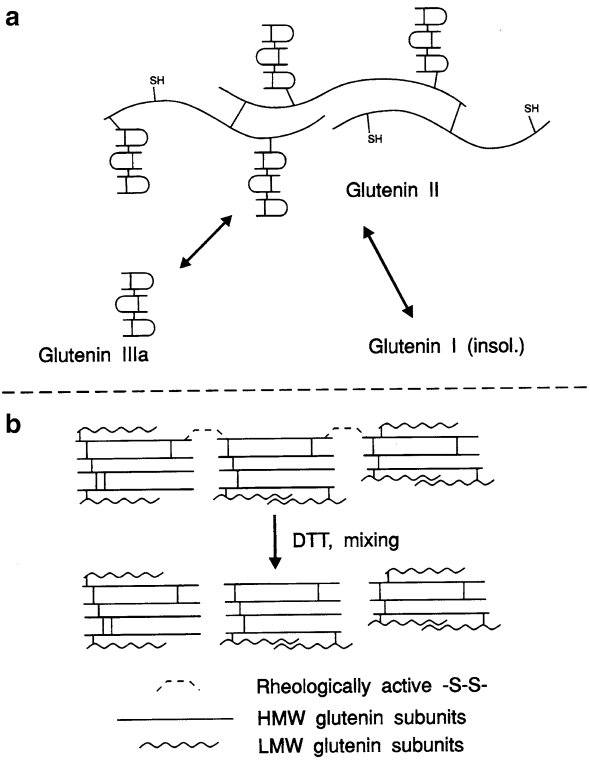

Fig. 11.5 Two models of glutenin structure in dough. (**a**) Adapted from Graveland, Bosveld, Lichtendonk et al. (1985), (**b**) adapted from Gao, Ng, and Bushuk (1992)

HMW and LMW subunits in their 'building block'. In their work DTT reduction of a small portion of disulphide bonds produced the maximum decrease in consistency; they called these 'rheologically effective —S—S—'. Jones et al. (1974) used a similar approach (a change in Farinograph consistency in the presence of small amounts of DTT) to conclude that only about 3 % of the disulphide groups in flour affect the rate of dough development, and about 12 % of the disulphide groups are involved in mixing resistance. The best current picture of glutenin is that it is a large linear polymer, and interactions between glutenin chains in dough is through non-covalent forces, namely hydrogen and hydrophobic bonds (Ewart, 1977).

Stages in Dough Formation

The word 'dough' connotes a semi-solid mass that resists mixing. In a recording mixer such as the Mixograph, a dough in the mixing bowl gives enhanced (and variable) resistance as the mixing head rotates, while a batter (a semi-liquid mass) causes low resistance with little variability (narrow band width). The progression from a simple mixture of water and flour to a dough correlates with an increased

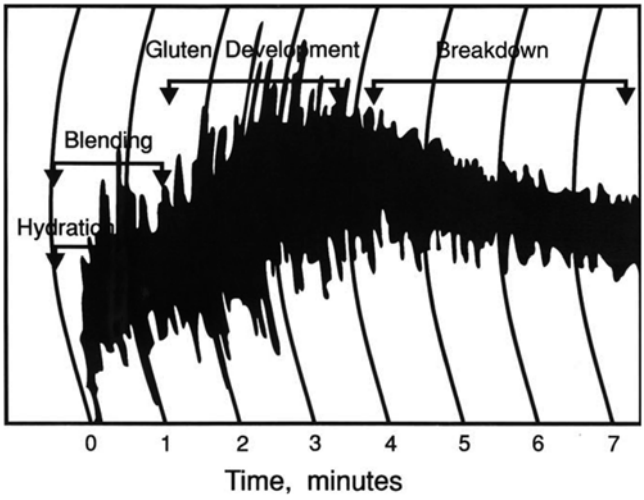

Fig. 11.6 (**a**) Mixograph trace, indicating the four main stages of dough formation

resistance to mixing, however that may be recorded. A Mixograph is just as much a mixer as any large commercial dough mixing machine, and the trace can be easily translated into consistency changes during dough mixing. In Fig. 11.6 a typical Mixograph trace shows the various stages of dough formation: hydration, blending, gluten development and breakdown. These same stages can be observed in a commercial horizontal mixer (Pyler, 1988). Bakers in the USA refer to them as the 'pickup stage' (Pyler, 1988, Fig. 14.3), 'cleanup stage' (Pyler Fig. 14.4), 'development' (Pyler Figs. 14.5–14.7) and 'letdown' and 'breakdown' (Pyler Figs. 14.8 and 14.9), respectively. Similar changes can be observed in most dough mixing and development systems in use around the world. An example of a mixing curve obtained from a Tweedy mixer use in CBP dough production is illustrated in Fig. 7.1 and shows how similar the shape of that curve is to that from a Mixograph or Farinograph despite the very different action of this type of mixer.

Hydration

In flour most of the protein exists as a flinty material. An analogy for the hydration of this protein is hydration of a bar of soap. If the soap is simply immersed in a bowl of water, the water slowly penetrates the outer layer of the bar. Rubbing the soap wipes off this soft, hydrated layer, and water proceeds to penetrate further into the soap. In the same way, the initial action of the mixer hastens the conversion of the flinty protein bodies into a soft, hydrated (but not truly dissolved) protein dispersion that is further modified during gluten development. Simultaneously the WI pentosans and damaged starch granules are absorbing water, and the water-soluble flour components (and added water-soluble ingredients such as salt and sugar) are dissolving.

The soap analogy is not strictly accurate. When water is brought into contact with flour particles and the process is observed under a microscope, the particles seem to explode; strands of protein are rapidly expelled into the aqueous phase (Bernardin & Kasarda, 1973). Movement of the cover glass stretches the protein strands, indicating their extensibility (Amend & Belitz, 1990). The rapid extrusion of protein fibres appears in part to be due to surface tension at the air–water–protein interface. Amend and Belitz (1990) submerged flour particles in acetone, which was then replaced by water. The particles swelled but no fibre formation was evident.

The input of mechanical energy is crucial to dough formation. A simple exercise demonstrates this fact. Blend cold wheat flour with powdered ice (in a 100:65 ratio) and then allow it to warm to room temperature. The result is a thick slurry that has no dough-like properties. When this slurry is stirred it rapidly increases in consistency, forming a soft (undeveloped) dough. Hydration alone is not sufficient to make a dough. Tkachuk and Hlynka (1968) substituted D_2O for water to show the importance of the formation of hydrogen bonds in dough. The mixing energy required to develop a dough using D_2O was much greater than that when water was used indicating that the hydrogen bonds formed with D_2O were significantly stronger.

Blending

Flour particles are agglomerates of starch granules embedded in a network of protein (Fig. 11.7). As the protein network is softened by hydration and agitated by mixing, the starch granules become less firmly attached to the protein, but nevertheless remain associated with the protein fibres (Fig. 11.8). Most of the starch can be removed by washing and kneading the dough (the basis for isolating wet gluten) but it cannot be totally removed. SEM photos of optimally mixed dough indicate that most of the starch is readily removable, but a small number of granules appear to have protein fibrils strongly attached to them (Amend & Belitz, 1990, Fig. 20). The actual strength of the starch–protein interactions has not, of course, been measured, but only inferred from observations such as those described. During this early stage of mixing all the ingredients of dough are being blended, to give a dough mass that

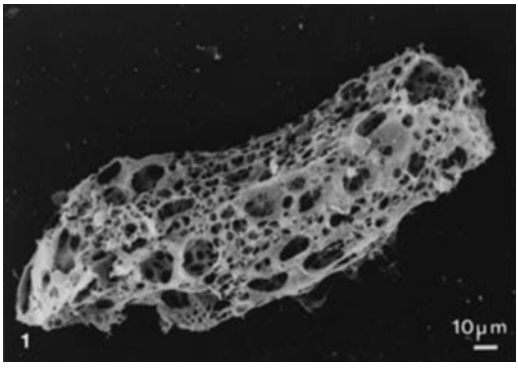

Fig. 11.7 Hydrated flour particle after starch is removed by enzymatic digestion, showing the protein framework (from Amend, 1995, by permission)

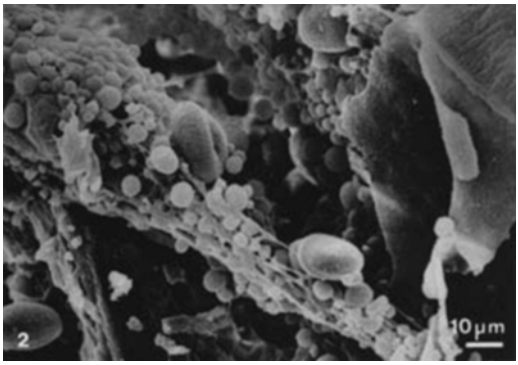

Fig. 11.8 Protein film and associated starch granules in a hydrated, stretched flour particle (from Amend, 1995, by permission)

is, at least at the millimetre scale, homogeneous. Lipids (flour and added lipids) are uniformly distributed and brought into contact with the protein fibres, and soluble materials are fully dissolved and distributed in the aqueous matrix.

Gluten Development

The pivotal step in forming a wheat-flour dough is the increase in consistency (increased resistance to mixing) that is generally called 'dough development'. During this stage of mixing, the flour–water mixture is converted from a thick, viscous slurry to a smooth visco-elastic mass, characterized by a dry, silky appearance (and feel) and the ability to be extended into a thin continuous membrane. The most important practical parameter is mixing time (which can be equated to total energy input, however, in this context the impact of mixing speed on the degree of dough development discussed in Chap. 2 should be noted), the time required to reach the peak consistency (maximum resistance) of the dough. Dough mixed to this point gives the maximum loaf volume, as compared with dough that is under-mixed or significantly over-mixed. It should be noted that in commercial practice mixing is usually extended slightly beyond the peak, giving a dough with better machinability in the subsequent moulding step, and one less likely to exhibit a 'wild shred' during baking. Data published by Millar and Tucker (2012) has also shown that maximum bread volume is achieved after mixing beyond an 'NIR optimum' for dough and that the finest cell structure that could be achieved in bread made by the CBP occurred sometime before the volume optimum. Such observations support the practical experience of bakers for a need to mix beyond peak dough resistance to deliver optimum bread quality.

The previous paragraphs describe (dough gluten) development on the macroscopic scale. Scanning electron microphotographs of gluten at various stages of development have been published. One such series is shown in Fig. 11.9 (Amend, 1995). At early stages (corresponding to the 'hydration' segment of Fig. 11.6) the

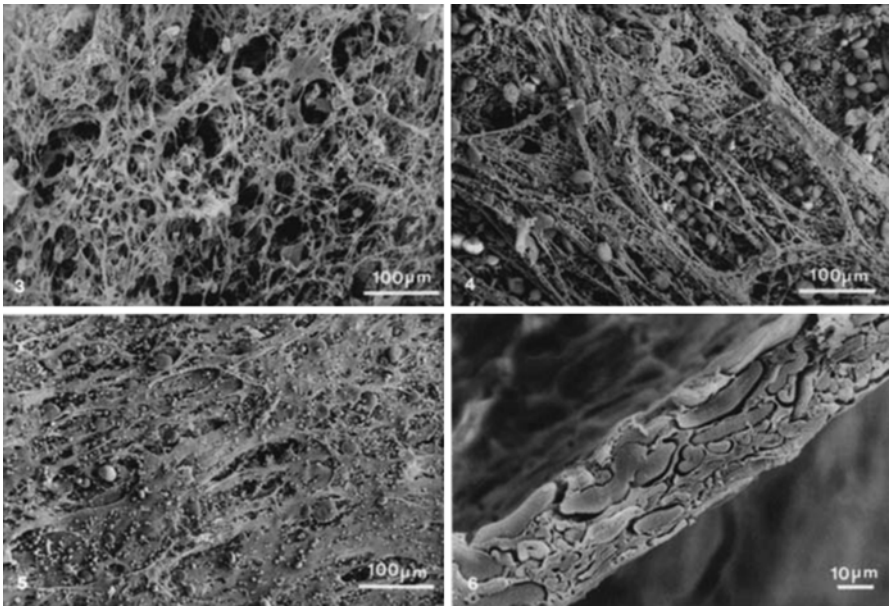

Fig. 11.9 Gluten network in dough at various stages of mixing. (**a**) Early in the process (at about the middle of the hydration stage), (**b**) partially mixed dough, (**c**) dough at maximum development stage (from Amend, 1995, by permission)

fibrils of hydrated protein adhere to each other, forming a rather coarse, random network of large strands (Fig. 11.9a). The action of the mixer stretches these strands, thinning them but also orienting them along the direction of the stretching action, allowing them to interact with each other (Fig. 11.9b). At the peak of consistency (Fig. 11.9c) the protein fibrils have been significantly reduced in diameter, and they appear to interact two-dimensionally, rather than just along the individual strand axes. In other words, at this stage the gluten appears able to form the continuous film, or gluten sheet, that is used by a baker (by hand-stretching a piece of dough) to evaluate completeness of mixing.

The crucial question, and one that continues to generate much research, is 'What happens on the molecular level during dough mixing?' The research is complicated by the complexity of the dough system, and the fact that the main species involved (glutenin) is a high molecular weight polymeric protein that is, to a large extent, insoluble. Nevertheless, progress towards the answer is being made.

It seems clear that mixing breaks the high molecular weight glutenin into smaller units, which then reform to some extent. Graveland et al. (1985 and references therein), for example, found that during short high-energy mixing the amount of glutenin having a lower molecular weight (less than 1 MDa) increased sharply, but then decreased again when the dough was allowed to rest. This is the basis for the model shown in Fig. 11.4a; the insoluble glutenin I (assumed to be the form in the dry endosperm) is depolymerized, perhaps down as far as the glutenin III subunits,

but also repolymerizes to glutenin II during a subsequent resting stage. Ewart (1977) came to a similar conclusion in his consideration of mixing action, that glutenin macromolecules are broken during mixing. The points of scission are thought to be at the disulphide bonds, forming thiol radicals (—S—S— → 2—S*). The presence of free radicals in flour was shown by Redman et al. (1966) and Dronzek & Bushuk (1968). Sidhu et al. (1980a) showed that fumaric acid formed an adduct with cysteine residues of glutenin, a reaction that probably proceeds via addition of the thiol radical to the α,β double bond of fumaric acid.

The importance of stress-mediated scission of disulphide bonds in developing gluten is consonant with several lines of evidence. It is well known that increasing the absorption in a dough in the bakery increases the mixing time. Tipples and Kilborn (1977) found that the critical speed of mixing (the minimum mixing rpm necessary to achieve good loaf volume) increased as absorption increased. They could make a well-developed dough at 100 % absorption (using a high-quality Canadian wheat flour) by running the mixer at a high speed. As the dough consistency decreased, a higher rate of energy input was required to achieve the necessary stress to break disulphide bonds. The mixing time (at fixed rpm) required to develop doughs is highly correlated with the amount of glutenin in the flour (Orth & Bushuk, 1972; Singh, Donovan, & MacRitchie, 1990); more glutenin requires more energy input to be broken down and rearranged.

As disulphide bonds are broken, they reform between adjacent molecules that have been aligned along the lines of stress in the dough. Several different combinations can be envisioned:

$$2\text{—}S^* \rightarrow \text{—}S\text{—}S\text{—}$$
$$\text{—}S^* + \text{—}SH \rightarrow \text{—}S\text{—}S\text{—} + H^*$$
$$\text{—}S^* + \text{—}S_1\text{—}S_2\text{—} \rightarrow \text{—}S\text{—}S_1\text{—} + \text{—}S_2{}^*$$

The end result of these rearrangements is the linear glutenin polymers envisioned in Fig. 11.5.

A common picture of this process is simple thiol–disulphide interchange, as proposed by Goldstein (1957). This is unlikely, however, because such a reaction proceeds via nucleophilic attack of the thiolate anion on the disulphide. At dough pH (approximately five) less than 0.1 % of the thiol groups would be ionized (pK_a of —SH is approximately 8.5). These interchanges are more likely to involve a free radical mechanism, as described here.

While rearrangement of glutenin is the major consumer of mixing energy, it is not the only process occurring. The protein also incorporates lipids from the flour and any added emulsifiers and shortening. Grosskreutz (1961) used X-ray studies to conclude that developed gluten has a lamellar structure, with lipid bi-layers interleaved with protein layers. Other researchers (e.g. Chung, 1986) have proposed different models for the protein–lipid interaction. All that can be confidently stated is that most polar lipids and a significant fraction of non-polar lipids become tightly associated with the gluten protein (Chung, Pomeranz, & Finney, 1978; DeStefanis et al., 1977). The precise role played by these included lipids in dough properties (and final loaf volume) is still not fully clarified (Pomeranz, 1985).

The final result of development is thought to be an alignment of extended, nearly linear polypeptide chains, interacting through ionic and hydrophobic forces (Ewart, 1977). This will be discussed more fully below.

The Formation of Other Bonds

The dominance of the di-sulphide bond in dough formation is undisputed but other bonds are formed during mixing which contribute to dough development. The formation of hydrogen bonds has already been introduced. Disruption of the hydrogen bonds, e.g. with urea (Wrigley et al., 1998) weakens the dough while for metal chloride ions (e.g. sodium chloride) gluten strength is increased (Eliasson & Larsson, 1993) because higher charge densities result in more hydrogen bonding in the structure.

The recent application of spectroscopic techniques led Belton (1999) to develop the so called 'loop and train' model for the interaction of glutenin subunits in dough. In his model Belton postulates that individual glutenin subunits interact with one another by disulphide bonds at the ends of the subunits and hydrogen bonds along repeat regions. The 'loops' formed at repeat regions are where the water is bound and when extension is applied to the system, such as during mixing, the loops disappear and the 'trains' are formed. If the extension force is removed and the polymer relaxes then loops may be re-formed.

More recently a hypothesis has been developed (Tilley et al., 2001) for the formation of dityrosine cross-links in dough as a contribution to dough development. Tilley et al. postulated that the addition of a free tyrosine source prevents the over-formation of tyrosine cross-links and enhances dough stability. Miller et al. (2005) examined the effect of adding free tyrosine and concluded that the effect of tyrosine addition varied with flour type and in one case a soft milling variety with weak gluten characteristics recorded an improvement in dough rheological properties as assessed with the DoCorder. The role of enzymic activity in the modification of tyrosine cross-links has also been reported (Tilley & Tilley, 2005).

Breakdown

If mixing continues after peak development is reached the dough becomes softer, less resistant to mixing action, and loses its ability to retain gases during proofing. SEM photographs indicate that the protein strands become shorter and thicker compared with those in optimally mixed dough (Amend & Belitz, 1990). The viscosity of dough proteins extracted into 1 % sodium dodecyl sulphate solutions were lower in over-mixed doughs compared to optimally mixed doughs, indicating a smaller average molecular weight (Danno & Hoseney, 1982).

Several α,β-unsaturated carbonyl compounds, such as fumaric acid, maleic acid, sorbic acid, ferulic acid and *N*-ethylmaleimide all increase the rate of dough breakdown during mixing (Schroeder & Hoseney, 1978). ^{14}C-Fumaric acid reacts with cysteinyl groups in gluten proteins during mixing; forming *S*-succinyl adducts (Sidhu, Nordin, & Hoseney, 1980a). It did not react with cysteine in soluble proteins or with added —SH compounds, leading the authors to conclude that it was combining with thiol radicals on the gluten proteins. Flour water solubles also contribute to the breakdown phenomenon (Schroeder & Hoseney, 1978; see above). Presumably it is the ferulic acid present in the WS pentosans which causes this effect. Fumaric acid and sorbic acid have been suggested as agents for reducing mixing time; at normal levels of use, and in practical situations, their effect may be too powerful, and the practice has not been widely accepted.

To summarize, dough breakdown appears to be simply a continuation of the process by which flour glutenin I is converted to (relatively) medium weight protein polymers that impart the desired rheological properties to dough.

Unmixing

Tipples and Kilborn (1975) reported an unusual phenomenon, the reversible decrease of resistance of a fully developed dough when it is mixed at a much lower rpm. When mixer speed is returned to that used for original development, dough consistency (and loaf volume potential) rapidly returns to that originally achieved. They termed this 'unmixing'. It is not the same thing as allowing a dough to rest (no mixing action). If a nearly developed dough is allowed to rest, when the mixer is restarted the consistency first drops to the level that would be the case if it were mixed at low speed, then rises to full consistency. An explanation that has been made (Ewart, 1977) is that with low-speed mixing the gluten molecules are no longer being constrained to extended parallel alignment by shear forces. They tend towards more random configurations, and the low-shear mixing allows these molecules (presumably somewhat more globular in shape) to form interactions that stabilize the less extended configurations.

Air Incorporation

More than 60 years ago Baker and Mize (1941) showed that achieving a fine crumb grain depended, in part, on incorporating air into the dough and subdividing the air bubbles into small cells. These serve as nuclei for expansion of the gases formed during fermentation and baking. Junge et al. (1981) determined the course of air incorporation during mixing in a Mixograph (Fig. 11.10). Little air is incorporated during the hydration and blending stages of mixing. Entrapment begins only after the dough begins to develop resistance to mixing and some internal structure that

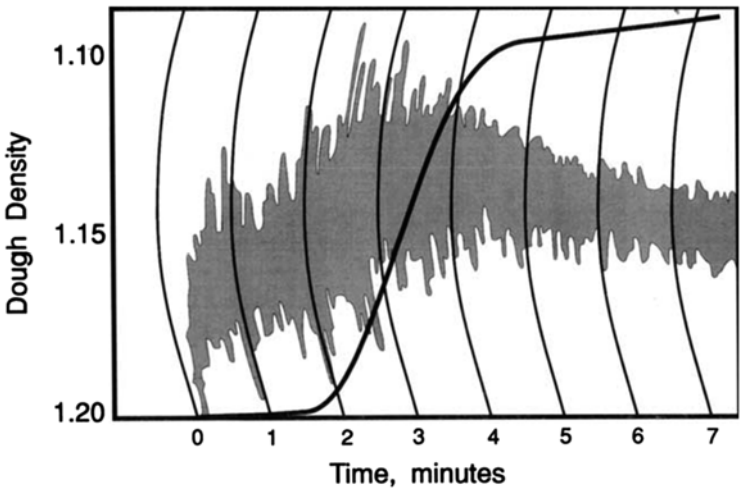

Fig. 11.10 Incorporation of air in dough mixed in a Mixograph (adapted from Junge, Hoseney, & Varriano-Marston, 1981)

Fig. 11.11 Structure of bread mixed using the CBP in 100 % oxygen atmosphere (courtesy *BakeTran*)

can envelop the air bubbles. An interesting point is that incorporation continues well past the mixing peak, into the breakdown portion of the mixogram. Thus it is not simply the elasticity of the dough that is responsible for entraining air; viscosity also seems to play a role (and perhaps also the ability of dough proteins to stabilize foams). Chamberlain and Collins (1979) found an interesting corollary to the observation of Baker and Mize; the entrapped gas must contain some nitrogen. They mixed doughs under a pure oxygen atmosphere. The final bread had an extremely coarse grain, with only a few large cells (Fig. 11.11). Their conclusion was that yeast

consumed all the oxygen during early stages of fermentation, leaving relatively few gas bubble nuclei for expansion of fermentation gases during proofing and baking, resulting in large voids in the bread (see also Chaps. 2 and 4).

The Gluten Matrix

The final product of dough mixing is a visco-elastic mass that, after appropriate proofing and baking, produces an aerated solid called bread. Bread has a sponge-like structure (the voids are interconnected) with the structural elements being primarily gelatinized starch and denatured protein. The rheological characteristics of dough are primarily responsible for achieving the desired result. Dough rheology, however, is (or should be) traceable to the nature of the matrix elements which are, in this case, gluten-forming proteins. A great deal of dough research has to do with measuring its rheological characteristics, correlating them with bread characteristics (the effects of additives such as oxidants, reductants and surfactants, proofing behaviour, loaf volume and crumb grain), and attempting to connect those measurements with such physical characteristics of gluten as can be determined. Much of this research has been presented and reviewed. Some excellent sources are Bloksma and Bushuk (1988); Bloksma (1990a, b) for Cauvain (2012a) and Eliasson and Larsson (1993); Faridi and Faubion (1990) and Hoseney and Rogers (1990).

While much more is known about dough now than, say, 70 years ago, the current situation might be summarized as follows:

- Dough is an extremely complicated system that cannot be fully described in simple rheological terms (springs and dashpots);
- Many practical instruments make measurements that are difficult to interpret in fundamental rheological terms, and may or may not be applicable to events during proofing and baking;
- Statements about the structure of gluten protein polymers are still largely hypothetical;
- There is great scope for further fundamental research in this area.

Dough Rheology

Numerous discussions of dough rheology are available. Menjivar (1993) presents basic rheological concepts, while Bloksma and Bushuk (1988) apply them more specifically to dough. An important point is that two types of stress are involved: shear and extensional (Fig. 11.12). In shear stress opposing forces are applied parallel to each other, in opposite directions to the matrix element. A strain is set up at right angles to the two surfaces. If it remains constant (and the element returns to its original shape when stress is relieved) the deformation is elastic, and elastic modulus is defined as: E = stress/strain. Intuitively, E is larger for more 'solid' materials;

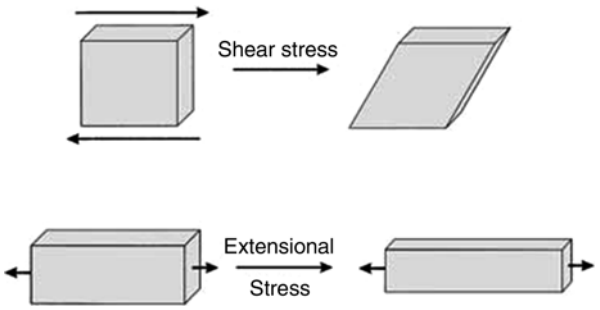

Fig. 11.12 Diagram of shear and extensional deformation

a cube of hard rubber has much higher value of *E* than a cube of sponge rubber. If the strain decreases as a function of time, then when stress is relieved the element does not return to its original shape, and the deformation is viscous. For a simple (Newtonian) fluid viscosity is defined as μ = stress/strain rate. In a dough mixer, Mixograph or Farinograph shear stress is the dominant mode. In extensional stress the opposing forces are applied in opposite directions, but at the opposite faces of the matrix element (Fig. 11.12). The definitions of elastic modulus and viscosity are the same as in shear stress, but the dimensional effects on the element are different. Whereas in shear the element maintains the same cross-section, in extension the cross-section decreases as the element lengthens (the volume remains the same in both cases). Extensional stress is applied to a dough by the Extensograph or Alveograph, and also during fermentation (proofing) and baking (oven spring).

Dough is visco-elastic, that is, it has both viscous and elastic characteristics. The simplest mechanical model that can be used to interpret rheological studies on dough is the Burgers body (Fig. 11.13). When stress is applied to dough the immediate response is elastic deformation (element A), followed by a delayed elastic response due to stretching of element B as element C undergoes viscous flow. Viscous flow by element D relaxes the instantaneous elastic strain on A. When the stress is relieved, any remaining elastic deformation of A is immediately removed. The removal of strain on element B is relieved only as C undergoes viscous flow (in the opposite direction). There is no force to reverse the flow that has occurred in D, so that amount of dough deformation remains when final equilibrium is reached.

A typical creep and recovery curve is shown in Fig. 11.14. The strain (deformation) continues as long as stress is maintained on the dough piece. The contributions of the various elements of the Burgers body can be identified on the curves, based on the previous discussion. However, it should be noted that each element is a composite of many elastic and viscous elements in the dough, so that element A (for example; Fig. 11.14) actually represents a spectrum of elastic moduli and D comprises a range of viscosities. By collating the results of many such creep and recovery studies an equation relating apparent dough viscosity (element D) to shear stress (Bloksma & Bushuk, 1988, Figs 6 and 7) was developed. Dough is a shear-thinning material, and its viscosity was calculated as 1.6×10^5 Pa s at a shear rate of 10^{-3}/s and 1.1×10^2 Pa s at a shear rate of 10^2/s.

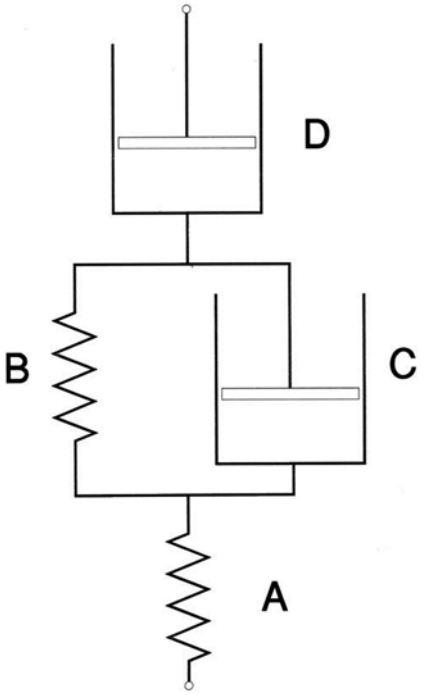

Fig. 11.13 The Burgers body mechanical model of dough rheology

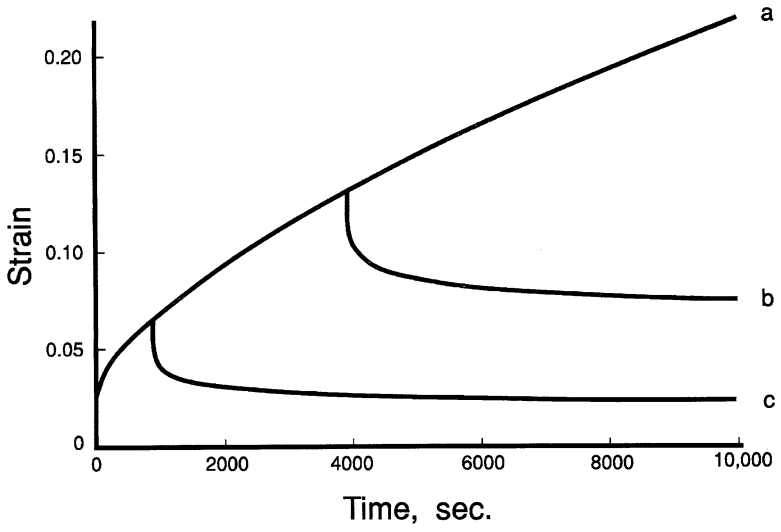

Fig. 11.14 A typical creep and recovery curve for dough under extensional stress. (**a**) Stress maintained throughout, (**b**) stress removed after 4,000 s, (**c**) stress removed after 1,000 s (adapted from Hibbered & Parker, 1979

Bloksma (1990a) presents some figures relating shear rates in various laboratory instruments to the situation in dough. They are:

- Dough mixers, 10–100/s;
- Farinograph, Mixograph, 10/s;
- Extensograph, Alveograph, 0.1–1/s;
- Proofing, 10^{-4} to 10^{-3}/s;
- Baking (oven spring), 10^{-3}/s;

The problem with relating test results to actual dough function thus becomes apparent. Extensograph and Alveograph testing involves dough with a viscosity of $(2–8)\times10^3$ Pa s (calculated according to Eq. 1 of Bloksma & Bushuk, 1988), some two orders of magnitude lower than viscosity in proofing dough. While results from such testing may correlate with dough properties (and qualities such as loaf volume), these should not be taken as 'explanations' of what is actually occurring in the dough.

A typical extensogram is shown in Fig. 11.15. The parameters of interest are *R* (the height of the curve at 5 cm extension), *E* (the length of curve until the dough piece breaks) and *A* (the area under the curve). A dough having large values of *R* but small *E* is extremely 'bucky', while one with small values of *R* and large *E* is very soft and pliable. Extensogram curves have been transformed into stress–strain diagrams (Rasper, 1975) but little use has been made of this work. More often, one or more of these measurements is correlated with dough properties. One example is the report by Singh et al. (1990), where they found that *E*, *R* and *A* for a series of 15 flours were all highly correlated with final loaf volume (the three parameters were strongly inter-correlated, as might be expected, so there was really only one Extensograph test of loaf volume potential).

Bloksma (1990b) expresses the opinion that:

> The only rheological properties required for good breadmaking performance appear to be extensibility and a sufficiently large viscosity. Extensibility can be translated into structure; a large quantity of high-molecular-mass glutenins enhances extensibility. The latter of these two conditions, a large viscosity, is met by virtually all doughs; it has no discriminating power.

Before considering the meaning of the term extensibility, we must think about the structure to which Bloksma refers.

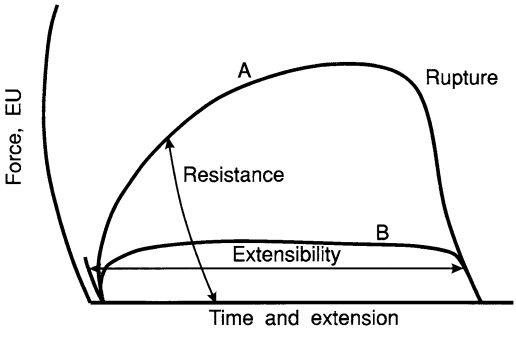

Fig. 11.15 An extensogram. (**a**), Dough having good elasticity and good breadmaking properties; (**b**), dough with poor elasticity and poor breadmaking properties

Gluten Structure

Meredith (1964) proposed that in developed dough the gluten consists virtually of one giant molecule, comprising glutenin extensively cross-linked by disulphide and other bonds. The comparison was made (by other authors) to vulcanized rubber. Bloksma (1990a and references therein) pointed out that this comparison was invalid; temperature changes had opposite effects on the elastic and viscous moduli for dough and for rubber. Ewart (1968, 1977) pointed out that there were several other lines of evidence that substantiated rejection of the 'giant molecule' hypothesis, and proposed that gluten structure was due to interactions between long, linear glutenin polymers. He made the analogy between dough 'strength' and the strength of a rope; while rope fibres are not physically cross-linked; the longitudinal forces between fibres give it a high elastic modulus (resistance to deformation in extensional shear). The glutenin 'fibres' impart elasticity to dough by virtue of the (non-covalent) bonds between them. The linear glutenin hypothesis of Ewart envisions numerous long glutenin molecules, aligned somewhat as shown in Fig. 11.16a. The contribution of gliadin to dough properties cannot be ignored (van Lonkhuijsen et al., 1992; Weegels et al., 1990). The scheme in Fig. 11.16b includes gliadin molecules, which contribute to the interactions between glutenin chains. Some of the possible consequences for dough rheology due to these models are discussed below.

Bonding Between Protein Chains

There are three possible types of non-covalent bonds in dough: ionic, hydrogen and hydrophobic bonds (Wehrli & Pomeranz, 1969). Glutenin has a low density of ionizable (acidic and basic) amino acids, so that such bonds would appear to be relatively unimportant in dough. At low pH (for example, in a sponge subjected to long

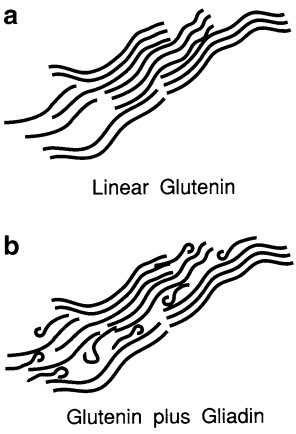

Fig. 11.16 Models of the structure of gluten. (**a**) The linear glutenin hypothesis (Ewart, 1977), (**b**) inclusion of gliadin in the structure

fermentation with consequent formation of much acetic and lactic acid) protonation of the few carboxylic side chains present leads to a significant net positive charge on gluten proteins, weakening inter-chain interactions by ionic repulsion.

The high percentage of amide (glutamine) side chains contributes to extensive hydrogen bonding between chains. The importance of this interaction to gluten elasticity was clearly demonstrated by Beckwith et al. (1963). They treated gluten with methanolic hydrochloric acid, converting amide groups to esters. Conversion increased solubility, decreased intrinsic viscosity of protein solutions and decreased cohesion of the hydrated gluten. Individual hydrogen bonds are relatively weak (about 4.2–6.3 J/mol or 1–1.5 kcal/mol) but the presence of large numbers of them lends overall strength to the inter-chain interactions. The fact that the resistance of dough to elastic deformation decreases with increasing temperature (Bloksma & Nieman, 1975) emphasizes the importance of hydrogen bonds in the proteins. Besides inter-chain bonding, hydrogen bonds also stabilize the β-turn spirals in the central portions of glutenin molecules. These play a role in the interpretation of elasticity presented below.

The relative importance of hydrophobic bonding in dough is difficult to assess accurately. When a dough is mixed in deuterium oxide (D_2O) rather than ordinary water (H_2O) it is much more elastic (Hoseney, 1976). Both hydrogen (deuterium) bonds and hydrophobic bonds are stronger in the presence of D_2O, so this test is not decisive. The addition of various salts, however, does discriminate between the two types of bonds. The Hofmeister (lyotropic) series arranges ions according to their ability to 'salt in' (increase hydration of) proteins as well as other hydrophobic materials. This is interpreted as being primarily an effect on water structure. Lyotropic salts (e.g. magnesium thiocyanate) decrease water structure and increase solubility of ('salt in') hydrophobic chains. Non-lyotropic salts (e.g. sodium chloride and sodium phosphate) enhance water structure and decrease solubility of ('salt out') hydrophobic chains (Tanford, 1973). (The term 'chaotropic' is used synonymously with lyotropic. The advantage is its mnemonic nature; a chaotrope is an ion or molecule that increases the 'chaos' in water structure.) The effect of salts on dough elasticity, absorption and mixing tolerance has been studied by numerous authors (Holmes & Hoseney, 1987; Kinsella & Hale, 1984; Salovaara, 1982). Lyotropic salts (e.g. sodium thiocyanate) increased water absorption by the protein (enhanced its solubility in water), while non-lyotropic salts (e.g. sodium fluoride) decreased absorption. The reported results have been interpreted in terms of protein hydration (Stauffer, 1990), but they can equally well point to the role of hydrophobic bonds in gluten structure.

Glutenin and gliadin are rather hydrophobic proteins, as shown by numerous studies using gel chromatography on hydrophobic media such as phenylsepharose (Chung & Pomeranz, 1979; Weegels et al., 1990). Chung and Pomeranz (1979) found that acid-soluble glutenin extracted from a good-quality flour was more hydrophobic than that from a poor-quality flour. Hydrophobic gliadins increase bread loaf volume, while hydrophilic gliadins decrease loaf volume (van Lonkhuijsen et al., 1992; Weegels et al., 1990). Flour lipids (Daniels et al., 1976; Wootton, 1976) and added emulsifiers (DeStefanis et al., 1977) are bound to gluten during dough

mixing, which must be in large part due to hydrophobic interactions. Hydrophobic interactions are weaker than hydrogen bonds (approximately 2,500 J or 600 cal per CH_2 group) but again, because of the rather large number of available interaction sites, the overall contribution to gluten structure is significant.

The relative contributions of ionic, hydrogen and hydrophobic bonds to aggregation of glutenin proteins were estimated to be 17.3, 56.3 and 26.4 %, respectively, in a good-quality gluten, and 12.8, 80.1 and 7.1 %, respectively, in a poor-quality gluten. In discussing chain interactions in gluten, then, hydrogen bonding (glutamine side chains) is of primary importance, and hydrophobic interactions play a lesser, but not negligible, role, particularly when lipids are involved.

Gluten Elasticity

What is the source of gluten elasticity? A reasonable hypothesis (Tatham, Miflin, & Shewry, 1985) is that β-turn spirals, and the hydrogen bonding between them (which connects them into β sheet structures), can be slightly extended and act as springs (Fig. 11.17a). Under stress, hydrogen bonds can be slightly extended. While each such extension might amount to only a fraction of a nanometre, summed over many thousands (or even millions) of such deformations, the total dough deformation can amount to several percent, as indicated in Fig. 11.13. While Tatham et al. (1985) proposed an analogy with elastin, Bloksma (1990b) pointed out that elasticity in the two proteins has opposite temperature dependence.

A second source of elasticity could be entropic. Ewart (1977) considered the individual glutenin molecules to be roughly spherical in shape; several such spheres are concatenated to form the 'linear glutenin' of his hypothesis. Under stress each glutenin molecule could be extended (Fig. 11.17b) into a less-favourable configuration. Relieving stress allows the protein molecule to recoil to its preferred (lower-energy) state. A similar picture has been suggested by Amend (1995), based upon SEM pictures of extended (stretched) gluten membranes.

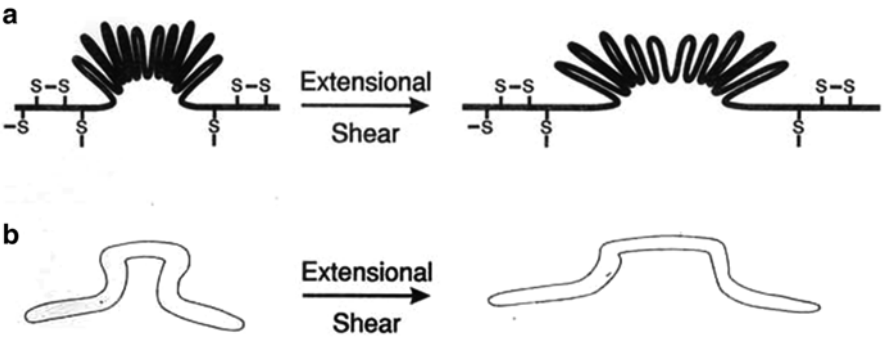

Fig. 11.17 A proposal for the source of glutenin elasticity. (**a**) Extension of the β-turn spirals and sheets, (**b**) deformation of compact glutenin molecules into a more linear configuration

Gluten Viscosity

For dough to undergo viscous flow the glutenin molecules must move relative to each other. Several mechanisms have been proposed by which this might occur. At the time Goldstein (1957) suggested sulfhydryl–disulphide interchange as such a mechanism, disulphide cross-links were considered to be the most important feature of gluten structure. Today that picture of the 'giant molecule' seems unlikely, for reasons given above. In freshly mixed dough, however, thiol free radicals appear to be present. These disappear during a 10 min resting period (Graveland et al., 1985), probably through interaction with disulphide bonds. Thus during this relaxation period (bulk fermentation or floor-time in common parlance) the dough undergoes viscous flow (releasing elastic stress) via thiol–disulphide interchange.

Movement of glutenin molecules is more likely to occur via hydrogen bond and hydrophobic bond interchange. Some of this may happen as a result of molecular motion and Brownian movement. A certain fraction of the hydrogen bonds between chains can be disrupted, and one chain move relative to another, before re-establishing hydrogen bonds. This happens more readily at higher temperatures, and dough viscosity decreases by a factor of five over the range 26–60 °C (79–140 °F) (Bloksma, 1990a).

Gliadin may also play a role as a mobile, small-molecule intermediary of these interchanges. While gliadin can certainly interact via hydrogen bonding because it contains a higher percentage of glutamine than glutenin, the fact that its hydrophobicity contributes to bread quality indicates involvement of this aspect of its nature, as emphasised in Figure 11.18. By facilitating the movement of adjacent glutenin molecules, gliadin may be characterized as 'molecular ball bearings'.

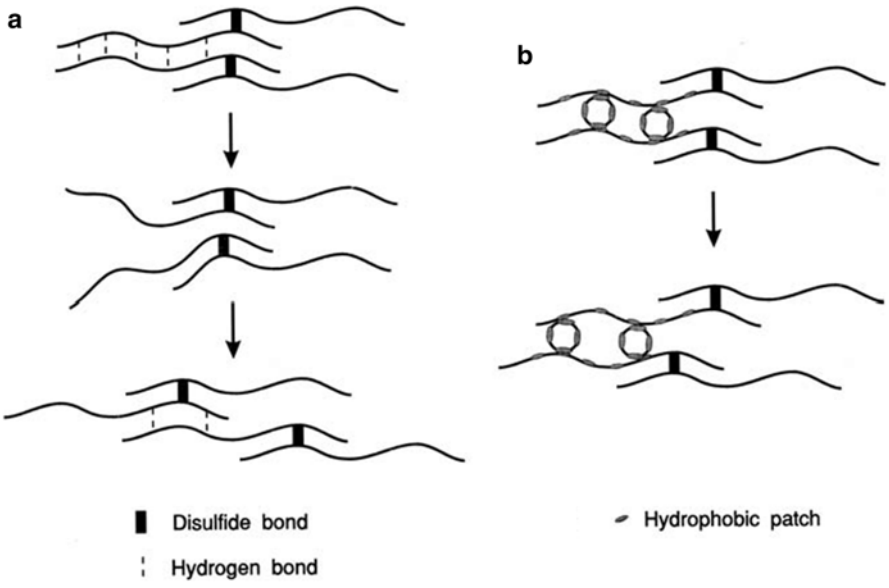

Fig. 11.18 Viscous flow and dough relaxation. (**a**) Brownian motion and chain realignments; (**b**) involvement of gliadin in glutenin realignment

A portion of gluten viscosity may be simply due to a high concentration of macromolecules in the aqueous phase. The viscosity of gum solutions increases tenfold for each 1 % increase in concentration. The concentration of glutenin can be estimated at 15 % as a lower boundary. Solutions of non-gelling gums at this concentration show a viscosity of the order of 10^6 Pa s or more. Viscosity is also strongly dependent on the average molecular weight of the protein, and glutenin molecular weights are of the order of 10^6 Da. Even if there were no interactions between glutenin chains, one would intuitively predict a high viscosity for a suspension such as that found in dough.

The postulated formation of links between gluten proteins and WS pentosans (Hoseney & Faubion, 1981; Fig. 11.2) must not be overlooked. To the extent that this happens in dough, the glycoprotein (pentosan–glutenin) would have an even higher molecular weight than the glutenin complex alone, thus increasing viscosity. It would also hinder relaxation, increasing dough elasticity.

Extensibility

Extensibility is difficult to define in precise rheological terms, although it is easy to find familiar instances; bubble gum and bread dough are common examples. Under extensional stress, the material thins to form a membrane. At the limit of extensibility, holes appear in the membrane and expand as extension continues. In the Extensograph this corresponds to the distance the centre of the dough piece (of defined initial dimensions) can be stretched before the dough ruptures. To some degree this distance depends on the rate of extension; at a lower rate the dough will extend further before rupturing. Thus, viscous flow is involved to some degree. Elasticity is involved in defining the amount of stress that can be applied before the dough piece breaks (tensile strength). With a less elastic dough the amount of extension may be the same as for a more elastic one, but the actual stress at rupture will be higher for the more elastic dough (Fig. 11.15).

Slade et al. (1989) show polarized light photomicrographs of stretched films of a synthetic chewing gum base (polyisobutylene elastomer) and of gluten. The similarities between the two photographs are striking. These films were stretched in one direction, and the authors point out that film strength is maximum along that axis, and minimum at right angles. Holes begin to form when the fibrils separate laterally, and the holes expand perpendicularly to the direction of stretching. In bread dough during proofing and expansion of gas cells, and in the Alveograph, extension of dough is biaxial with the gluten film being stretched along both dimensions. (In the third dimension, perpendicular to the film surface, the membrane grows thinner.) This results in the maximum strength for gluten membranes.

Bloksma (1990a) reviews at some length the various components of extensibility (viscosity, elasticity and tensile strength) that influence the overall performance of dough during proofing and baking. His discussion emphasizes that all those factors

of importance to the baker are developed during dough mixing. In other words, he confirms the experience of every bakery technical service person: if a bakery is having trouble producing good bread, one of the first places to look is at the mixer. If the mixing is right, the rest of the process should be relatively trouble-free.

Stickiness and the Behaviour of Dough During Processing

One dough property which has major implications for dough processing but is still not understood is stickiness. In the commercial bakery, especially where mechanical handling of dough is practised, stickiness is of major importance as equipment surfaces may become smeared with dough. This is especially true with rounder and final moulders (Chap. 4) and the progressive build-up of traces of dough on metal surfaces can be significant enough to bring processing halt. Plant stoppages clearly need to be avoided because they increase waste, disrupt production and reduce production capacity. A particular problem in commercial is that the practical reaction to 'sticky' doughs is to reduce the recipe water level. To some extent this water reduction does have an ameliorative effect but the relationship between dough softness (consistency) is not an absolute one. Indeed lowering recipe water levels too far can result in other quality defects arising from the interaction of the stiffer dough with moulding equipment (Cauvain & Young, 2008). The alternative to reducing recipe water levels to combat dough stickiness is to use a liberal dusting of the dough with flour.

Observations carried out on dough processing readily reveal that dough stickiness is associated with the manner in which the dough is processed. In particular it can be readily seen that subjecting the dough to shear (Menjivar, 1993) increases the property of dough which bakers interpret as stickiness. In commercial practice shearing of dough is most commonly seen during the dividing and moulding processes. A further practical observation is that stickiness is to some extent, transitory in that the rheological properties of dough which rests after being sheared change and there is a noticeable decrease in dough stickiness. Even the gentle manipulation of a sticky dough by hand results in the loss of dough stickiness.

The reasons behind dough stickiness are not well understood, in part because of the problems of measuring this particular dough property. A number of methods are available for measuring dough stickiness (Cauvain & Young, 2009) but often manipulation and passage of time associated with testing the dough reduces the ability of a test to measure the property concerned. Recently a test has been developed which attempts to mimic high shear dough processing (Cauvain, 2012b). In the test a knife blade is driven downwards into a dough piece held in a box of fixed dimensions. A slot in the lid facilitates the movement of the blade down through the dough piece (Fig. 2.1a) and upon withdrawal (Fig. 2.1b) the stickiness of the dough is measured using the negative curve so obtained (Fig. 2.2). In its action the test is similar to that of a dough divider and as the cut surface of the dough is not exposed to the air or manipulation (other than the cutting blade) some of the transitory nature of stickiness is avoided.

Table 11.2 Effect of salt on dough stickiness (as measured with the Warburtons dough stickiness test)

Salt level (% flour weight)	Stickiness (kg) (adhesion peak)	Work of adhesion (kg.s) (area)
2 (standard)	0.82	0.94
1 (50 % reduction)	2.75	3.71

Higher values indicate stickier dough

The open question is then what fundamental changes causes dough to become sticky? Perhaps one clue is provided by the observations that when salt levels in dough are reduced, stickiness increases (Table 11.2). Sodium chloride is of course, very good at binding water (Cauvain & Young, 2008), probably more so than wheat protein and pentosans. It would be possible to consider that some of the water bound into the dough protein structure may be temporarily released when the dough is exposed to significant shear forces during processing. The temporarily free water may well be a significant contributor to dough smearing and stickiness. Upon resting or under gentle manipulation (by hand or sheeting) it appears that the attractive forces in the dough are able regain control of the temporarily free water. If this is the case then it is likely that hydrogen bonding is involved. Another observation worthy of investigation is the addition of sugars such as sucrose which cause the dough to become both softer and stickier.

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