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Review

New approaches to study the molecular basis of the mechanical properties of gluten

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Abstract

Recent work contributing to the understanding of the molecular basis of the mechanical properties of gluten and dough are critically reviewed. Rheological, chemical and spectroscopic methods are considered and the results compared. It is concluded that, despite some inferences to the contrary, the behaviour of gluten and dough on mixing is characterised by the build-up of a polymeric species and that particulate theories of dough rheology are not tenable.

Keywords: Rheology; Infrared; Near infrared; NMR

1. Introduction

Understanding the mechanical properties of wheat gluten is at the heart of understanding the processing behaviour of wheat products. As such the mechanical behaviour of gluten and doughs has been the subject of intense study over many years. A great deal of the work reported has been based on the use of industrial mixers or the apparatus that is thought in some way to measure parameters that are indicative of actual processing behaviour. In most cases, therefore, true rheological parameters that can be related to physical models have not been obtained, although much useful empirical knowledge has resulted (for a recent review of these matters see (Dobraszczyk (2003)). In addition rheological measurements yielding more tractable results have been carried out (Dobraszczyk, 2003) but much of this has been criticised on the basis that it has not been carried out in a region of extension or at a rate that is appropriate to the actual mixing processes that are going on (Dobraszczyk and Morgenstern, 2003).

Another approach has been to extract material from dough or gluten during the mixing process and attempt to relate its properties to the mechanical changes occurring during mixing. This is essentially a molecular approach to the problem. The least perturbing method of exploring molecular changes during the input mechanical energy is to combine spectroscopic and rheological measurements to concurrently measure molecular and mechanical changes.

In this review these various approaches will be critically considered and the consequences for understanding the molecular basis of dough rheology discussed.

2. Rheological measurements

2.1. General background

Rheology measures the deformation of a material in response to the application of mechanical force The force is usually defined in terms of stress, the amount of force applied per unit area, with strain being the resulting deformation. Both of these terms need to be considered with respect to the instantaneous state of the material during deformation. If a sample is stretched by uniaxial extension then its cross sectional area will reduce; thus the stress applied will be time dependant and cannot be treated as

Abbreviations: FTIR, Fourier transform infrared; HMW, high molecular weight; NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulphate.

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a constant, even though the applied force may be constant. Similarly as the length changes the strain, which is defined in the change in length per unit length, needs to be recalculated.

Two responses to the application of stress are possible for a material such as dough. The first is a simple elastic deformation; this is what might be observed in a perfectly elastic rubber band. On application of force immediate deformation occurs and when the force is removed the rubber band recovers to its original dimensions. The energy absorbed by the band is thus recovered fully by the restoration process. The second process is flow; this occurs in dough and gluten with the elastic response being accompanied by permanent displacement of the molecules. This process dissipates energy since the energy used to displace the molecules is not recovered on the removal of the stress. In many practical measurements the stress is applied sinusoidally using a rheometer in which the base of the sample is held still and a suitably shaped plate is used to apply force to the top of the sample. The sample is thus in shear, and shear moduli can de defined. The modulus corresponding to the energy storage is a called the storage modulus and is represented by G' and the dissipative part associated with flow is called the loss modulus, G''.

In general, the response of materials to mechanical perturbation will depend on the rate at which the perturbation is applied. If the perturbation is applied very rapidly compared to the time scale on which the molecules can respond then the system will behave as a hard solid. This is because there will be insufficient time for large deformation and/or flow. For a simple molecular liquid the time scale for molecular response can be characterised by a single time constant. For more complex systems such as a synthetic polymer melt, a range or distribution of time constants is involved. This distribution gives rise to a distribution of time scales over which different rheological responses are observed. Thus the values of G' and G'' are dependent on the rate at which force is applied. This is illustrated in Fig. 1 for the changes in G'. When the rate of application of stress is very slow the system has time to internally rearrange itself and accommodate the stress by flow. In this case the value of the storage modulus, G', is small. Typically, in a polymeric system, this time scale would be that in which the polymers can disentangle themselves from one another and respond to the stress by relocation. As the time scale for stress application is decreased the response of the system becomes more limited; there is not enough time for rearrangement and the displacement of the material becomes more difficult. As there is less time for relocation there is less dissipative response and more elastic response, thus G' increases. A plateau region is therefore reached in this region, relocation is very limited and the internal rearrangements of the system are limited to displacements, which are not accompanied by relocation. Thus, when the stress is released the original arrangement of internal elements is regained and the value

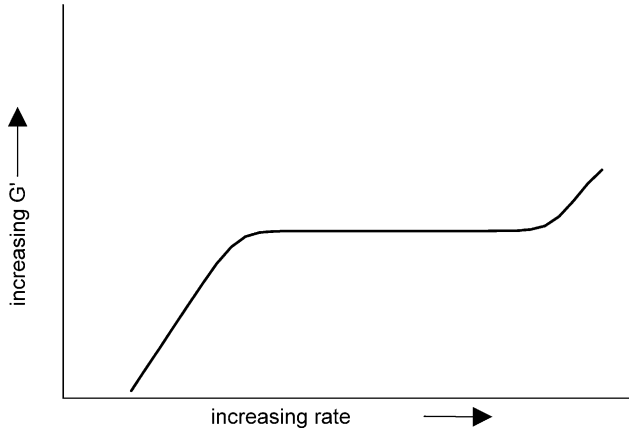

Fig. 1. An illustration of the variation of storage modulus of polymeric system with increasing rate of shear.

of the storage modulus is high. Since the time scale of the stress rate is small compared to the time scale of the displacements and recoveries, the modulus remains independent of the stress rate until it reaches the same time scale as the displacements. When polymeric systems are subjected to this regime the chains may be distorted by the application of stress but are held in place by entanglements, whose time scale for motion is much longer. Finally, the stress rate becomes so fast that it is faster even than the rates of distortion and recovery in the plateau region and the modulus rises again.

2.2. Applications to gluten and dough

Dobraszczyk and Morgenstern (2003) have pointed out that the frequency ranges used in conventional measurements are often limited to the plateau region and that these are not relevant to the rates observed during fermentation and baking. Furthermore, they argue that the important differences between the behaviour of polymers in the dough will only become apparent at frequencies where the disentanglement processes are observable. The term 'polypolymers' in this instance must be taken to be some generalised form of entangled species, it is not possible on the basis of this rheological evidence alone to specify the chemical nature of the polymers. The plateau region is essentially independent of molecular weight over a large range and has no significant correlation with parameters such as bread volume. Another complication (Dobraszczyk and Morgenstern, 2003; and Kokelaar et al., 1996) is that both gluten and dough are strain hardening. This implies that as a sample is elongated its resistance to further extension increases. This effect is shown diagrammatically in Fig. 2. The measurement of stress–strain relationships in these materials therefore needs care.

In addition to these complications yet another phenomenon is exhibited. This is shear thinning (Lefebvre et al., 2003). If a stress is applied to a gluten sample for an extended period creep is observed. This is the continued

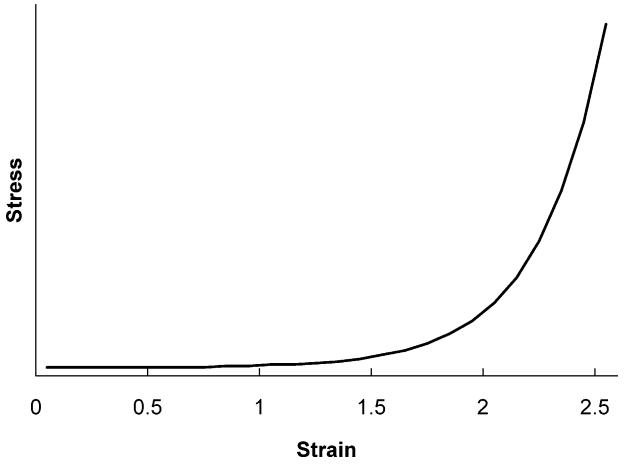

Fig. 2. An illustration of the changes in stress with increasing strain of a strain hardening system.

deformation of the sample due to viscous flow. Lefebvre et al. (2003) showed that when the stress was below about 150 Pa the effective viscosity for the flow was independent of the applied stress, however, above this level of stress there was a sharp decrease in effective viscosity. In effect, as the stress was increased the viscosity fell.

The final factor, which complicates studies of gluten and dough, is the change in rheological properties as the total amount of mechanical energy applied to the system increases. This is a very well-known phenomenon in dough mixing and is often observed as a maximum in the torque required during mixing. A particularly careful study of this and related phenomena was carried out by Gras and co-workers (2000) using a Mixograph. They showed that the mixing action was predominantly elongational and drew the following conclusions:

1. Dough development can be considered as being in two stages. The first is a hydration stage and the second is the input of energy through deformation.
2. Dough deformation results in the storage of energy in the dough through the modification of molecular structures. In effect, mixing results in the storage of elastic energy in the dough.
3. Dough resistance reaches a maximum in a Mixograph during mixing. The height of the maximum increases with decreasing water content.
4. On resting for 1 h all mixed doughs show a decrease in resistance to extension with mixing time.
5. On resting for 1 h all mixed doughs show a decreased degree of extension to break with mixing time.
6. On resting for 1 h the resistance to extension of the mixed dough decreases with water content.
7. On resting for 1 h the degree of extension to break of the mixed dough increases with water content.

Conclusions 2–5 indicate the strong dependence of the mechanical behaviour of dough on its history and on water content.

2.3. Interpretations of results

The explanation of these phenomena in terms of the internal structure of gluten is challenging. However, careful rheological measurements do allow some insight. Lefebvre et al. (2000) measured the effects of heating and changes in High Molecular Weight (HMW) glutenin subunit composition on the viscoelastic properties of gluten. The work was carried out with strain amplitudes of 3%, which is in the low strain region and corresponds to a situation where the ratio stress/strain remains approximately independent of strain. Strain rates were varied between 10^{-3} and 10^{2} s^{-1}, which allow at least some insight into the long time behaviour of the system. They observed that when the gluten was heated between 20 and 40 °C no irreversible changes took place but when the gluten was heated above 40 °C changes were observed in the absence of a sulphydryl (SH) blocking agent. They concluded that the reversible changes were due to hydrogen bonding effects and the higher temperature effects arose because of aggregation caused by rearrangement of disulphide bonds. At low temperatures the effect of heating was to reduce both G' and G''. Maintaining the sample at 70 °C resulted in an increase in G' and very little change in G''.

The authors note that after heating to 70 °C attempts to extract proteins from the gluten resulted in a decrease in the recovered amounts of monomeric gliadins as well as a decrease in recovery of higher molecular weight species. This may suggest that a major change in the conformations of all the protein components took place after heating and that a network containing both low and high molecular weight proteins was formed. This new network would be expected to have properties, which are significantly different from those of parent network.

In a second paper (Lefebvre et al., 2003) reported results obtained with gluten from a single cultivar. Both dynamic and creep measurements were carried out which allowed the construction of a mechanical spectrum covering the range 10^{-6}–10^{2} ras s^{-1}. As noted above, these experiments demonstrated the existence of shear thinning and also showed that after long periods of imposed stress aging phenomena were observed. Both the height of the viscoelastic plateau and the storage modulus measured at 1% strain and a sheer rate of 1 rad s^{-1} decreased with time. This is not unexpected as one would expect that, given enough time, any system would rearrange itself to accommodate, even small, imposed stresses and strains. It does not seem likely that, under these very mild conditions, the phenomena observed are the same as in the Mixograph experiment. A detailed analysis of the frequency spectrum indicated a bimodal distribution of responses corresponding to time scales of about 10 s and 10^{4} s. It was concluded that the type of behaviour observed was consistent with a colloidal particle gel in which the particles were aggregated by hydrogen and hydrophobic bonds. The authors suggested that disulphide bonds were probably not directly involved in

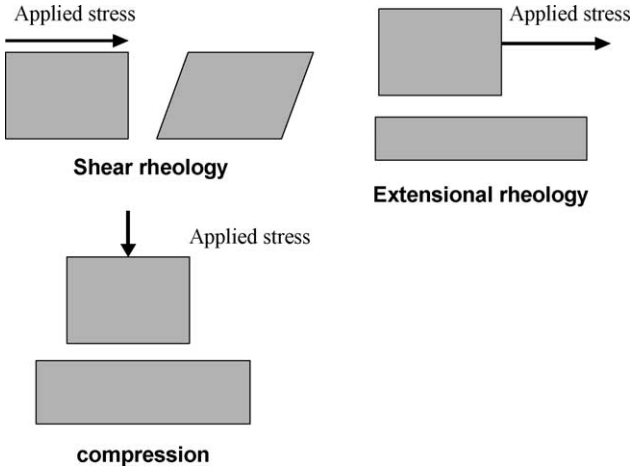

Fig. 3. Methods of measuring the response of a material to stress.

the macroscopic network. However, since they used an agent to block SH groups and hence preclude disulphide bond formation, the basis for this statement is not clear.

In contrast to the approach of Lefebvre and co-workers (2000, 2003a,b) and Dobraszczyk and co-workers (Dobraszczyk, 2003; Dobraszczyk and Morgenstern, 2003) have explored the regions of high strain and used a stress relaxation approach to measure slow processes. An important distinction is also made between the effects of shear and the effects of extension (Dobraszczyk and Morgenstern, 2003). In shear rheology the sample is fixed at the base and a stress is applied to the top surface, in extension rheology one end of the sample is fixed and an extending force is applied to the opposite end. A further form of extension rheology is compression, which results in biaxial extension. These ways of applying stress are shown diagrammatically in Fig. 3. The authors point out that the responses of materials to extension or shear may be substantially different. Typically, the effective viscosity is similar in shear and extension at short times, but diverges at long times with extensional viscosity becoming much larger.

It is clear that if rheological measurements are to be related directly to breadmaking behaviour these factors must be taken into account. A more detailed discussion of this has been given by Dobraszczyk and Morgenstern (2003).

3. Chemical measurements

The role of certain groups of proteins in determining gluten and dough rheology has been long recognised, in particular the HMW subunits have been identified as being of primary importance (Shewry et al., 2003). There is considerable evidence that the HMW subunits are aggregated in some way to form large polymeric species (Carceller and Aussenac, 2001; Lindsay and Skerritt, 1999). Given that the subunits can readily interact by hydrogen bonds, disulphide bonds and, probably, hydrophobic interactions (Shewry et al., 2003) this seems perfectly reasonable. However, the characterisation of this polymer is problematic. If it is formed by a combination of relatively weak non-covalent bonds and labile disulphide bonds any extraction technique is liable to result in damage to the structure and, given the lability of the system, may result in the formation of new structures which do not reflect the structure in the dough. Nevertheless, a number of studies (Li et al., 2003; Weegels et al., 1996a,b) have shown that treatment of dough or gluten with a suitable solvent system (sodium dodecyl sulphate (SDS) or acetic acid) gives an unextractable residue whose properties are correlated to the loaf quality or the dough rheology. The amount of material that remains unextractable by SDS decreases as the mixing process proceeds (Weegels et al., 1996a). This is a curious observation; the unextractable material (often referred to as the macropolymer) clearly has properties that relate to breadmaking quality and the rheology of the dough and is assumed to represent a large polymeric species formed in the dough, which is similar in nature to the one described above. The decrease in the amount of this material on mixing would seem to imply depolymerisation (Weegels et al., 1996a), however, as the mixing process goes on, the resistance of the dough to extension increases. It seems very unlikely that a depolymerisation event would result in an increase in the resistance to extension. To explain the increase one would expect that at least the state of polymerization would be unchanged or increased.

The explanation for this apparent contradiction may lie in the methods used for extraction. Typically, the dough or gluten is treated with 1.5% sodium dodecyl sulphate (SDS) solution and then centrifuged for 30 min at 80,000*g* (Don et al., 2003a). This process would be unlikely to preserve the structure of a very labile polymer and it does not seem reasonable to suppose that this extraction reflects the equilibrium solubility of the material. The apparent solubility may thus be a reflection of kinetic rather than thermodynamic processes. A more detailed discussion of the general solubility problem is given in Shewry et al. (2003). During the mixing process the kinetics may become more favourable to dissolution since mixing is likely to fold air into the system and change its effective surface area. This alone may account for the apparent changes in extractability.

Confocal laser scanning micrographs (Don et al., 2003b) of the insoluble protein fraction have indicated that it is particulate in nature, typical sizes are of the order of 5–30 μm. This has led to the hypothesis (Don et al., 2003b) that these particles are related to the protein bodies in wheat and that gluten is a particulate system. This hypothesis is consistent with the conclusions of Lefebvre et al. (2003). No length scales are suggested in that paper so a direct comparison of the proposed nature of the particles cannot be made. However, by implication these particles may be identified with those of Don and co-workers as a publication

by Lefebvre and van Vliet (2003) specifically alludes to the size of the particles as measured by Don and co-workers.

The paper by Lefebvre and van Vliet (2003) is also interesting in that it makes the assumption that the insolubility of gluten is due to the fact that water is a 'bad solvent' for glutenins and thus the 'glutenin 'polymers' should not be viewed as coiled chains (and gluten as an entangled polymer system) but as colloidal particles formed by collapsed concatenations'. This presumably implies that water is a 'bad solvent' in the Flory sense that insolubility implies weak protein–solvent interactions. As has been pointed out elsewhere (Shewry et al., 2003) this is erroneous as the low solubility of gluten arises from very strong protein–protein interactions not from weak water–protein interactions. The notion that insolubility denotes weak solvation is equivalent to concluding that the actomyosin complex in muscle must be a coacervate because it is not in solution. The suggestion that the proteins must be present as particles is thus weakened.

A number of other considerations also suggest that the particle hypothesis is weak. To observe the particles the samples were dispersed in 1.5% SDS and placed in tubes on a roller bank for 3 h. SDS is a powerful detergent; its main commercial use is to suspend material as a particulate in washing. It is not surprising therefore that the material appears to be a particulate suspension after such treatment. Indeed, it would perhaps be surprising if it did not. Further evidence against the particle hypothesis comes from microscopy studies (see for example Lindsay and Skerritt, 1999; Roman-Gutierrez et al., 2002) which give no evidence of a particulate structure.

In spite of the reservations concerning the representative nature of the unextractable material the results of Li et al. (2003) provide some insights into the role of the various protein types in the gluten. The experimental procedure was to extract the gluten from mixed dough samples, and after freeze drying and defatting, re-extract this sequentially with ethanol and acetic acid. Experiments were then carried out on the whole gluten and the residual, unextractable material. Measurements were made of the stress relaxation over 1800 s and the results plotted as a relaxation spectrum of intensity of relaxation versus relaxation time. This in effect gives a measure of the distribution of relaxation times. The results showed that both gluten and the unextractable material had two common characteristic relaxation processes: one at short times and one in the region of 10–1000 s. From the graphs shown in Li et al. (2003) typical values for these peaks can be estimated as around 1 and 500 s. Although the actual time scales are shorter, the appearance of two processes at long and short times is consistent with the results of Lefebvre and co workers. It was concluded that the behaviour of the gluten and the unextractable protein was characteristic of an entangled and physically cross-linked network. The short-time peak, which was also observed in soluble gliadins and glutenins, was assigned to proteins not in a network. This interpretation contrasts with that of Lefebvre et al. (2003) who attributed the fast relaxation to changes in the internal structure of the particles and the long time process to the network connectivity of the particles. The analysis of Li et al. (2003) also suggests that the model in which there is a breakdown of polymeric structure with mixing is incorrect. If the rheology of gluten depends on the existence of polymeric structures, the breakdown of the structure would result in reduced entanglement and interaction and, thus, reduction in resistance to extension as mixing proceeds. This is not what was observed.

4. Spectroscopic methods

In principle, spectroscopy offers a non-invasive approach to observing changes in the molecular structure of gluten whilst under mechanical perturbation. Near infrared studies have been carried on dough during mixing (Alava et al., 2001, Wesley et al., 1998), showing changes in the intensities of bands at 1160 and 1200 nm which were attributed to changes in water (1160 nm) and possibly protein (1200 nm). The changes in both peaks appeared to show minima, which were related to the peak power consumption point in the mixer. Alava et al. (2001) used a multivariate analysis of their data to determine the most affected wavelengths. They found, in agreement with the results of Wesley et al. (1998), that changes in the region 1125–1180 nm were a good indicator of the progress of mixing and showed minima associated with, but not at, the peak torque during mixing. The indications of this work are that mixing in some way changes the behaviour of water in the system. This is not an unreasonable conclusion. Christopher et al. (1992) showed that in a reverse micelle system undergoing stepwise hydration changes in water band frequency continued up to about 12 water molecules per head group. Since there may be as little as six water molecules per amino acid residue present in gluten (Belton, 2003) changes might occur on mixing due to the hydration of the proteins. However, it is suggested that the 1160 nm band is due to a combination of an OH stretch and bend (Wesley et al., 1998). The results of Christopher et al. (1992) clearly demonstrate that there is no change in the extinction coefficient of the OH stretch even at ratios of water to head group of 1:1 and there seems no reason to suppose that the OH bend will be significantly different in its changes in extinction coefficient. It is possible that the assignment of the 1160 nm peak solely to water is incorrect. A peak at this position certainly appears in the spectrum of water and it disappears in dough when water is replaced by D_2O (Wesley et al., 1998). However, this does not prove that it is only derived from water; any exchangeable groups contributing to the intensity would also be affected. It is possible that changes in these groups contribute to the changes in intensity. Other possible causes also need to be eliminated:

during mixing air is entrapped in the dough; since a diffuse reflectance technique is being used it needs to be shown that the changes in optical properties due to this entrapment are not having an effect. Similarly the temperature of the dough rises during mixing and this may have an effect directly on the intensity or, if indeed the peak is due to water, indirectly through disruption of the water hydrogen bonding.

The possible role of hydrogen bonding is also highlighted by the work of Callaghan and Gil (1999) who observed changes in the NMR spectra when samples of gluten were exposed to shear or biaxial extension. In both cases, changes in the intensities of amide protons assigned to glutamine side chains were observed which were consistent with the breaking of hydrogen bonds during the application of shear or extension and the recovery of bonding during relaxation. The time scale for the recovery process after shear was in the order of hundreds of seconds.

Another approach to the problem of observing changes in protein structure during biaxial extension is to use mid-infrared spectroscopy. van Velzen et al. (2003) have reported changes in the amide I and amide II bands of the mid infrared spectra of dough which had been extended. A more detailed study by Wellner et al. (2004) measured the changes in secondary structure which occurred in gluten during deformation by biaxial extension. It was observed that the biaxial extension caused an increase in beta-sheet conformers in the gluten. The degree of change on initial compression was variable, but subsequent changes proved to be reproducible. In order to observe the cumulative effect of compression, a cycle of five compressions was used. The changes observed are shown in Fig. 4 which shows the ratio of intensities at 1620 cm^{-1} (corresponding to the absorption of beta-sheet) and the intensity at 1650 cm^{-1}, which arises from a combination of random structure, a small amount of alpha helix and absorbance from glutamine amide group. The results are consistent with the predictions of the loop and train model (Belton, 1999) in that the extension of gluten should result in changes in protein conformation and that these should take the form of an increase in the beta-sheet content. Analysis of the whole spectrum shows that there is a corresponding decrease in beta-turn content as predicted. The effects seen are small which is to be expected if, as postulated, changes are due mainly to high molecular weight subunits. The HMW subunits comprise only 10–12% of the gluten proteins and therefore a 10% change in conformation would therefore only produce a 1% change in the spectrum overall. Fig. 4 shows that the amount of beta-sheet increases sharply on each extension and then decays away on a time scale of minutes. This result is consistent with both rheological and NMR observations. However, a build up of beta-sheet structure occurs on repeated extensions. On the basis of simple extension of a protein network it would be expected that repeated extension in the same direction would result in the same degree of overall structural change. This is because, even if there is incomplete relaxation of the protein between extensions, no new proteins will become available on each new extension. There must therefore be a build up of some kind of system that has a high beta-sheet content on each new extension. A scheme that illustrates this process is given in Fig. 5.

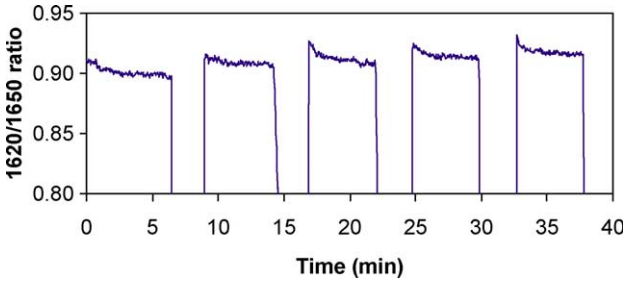

Fig. 4. The changes in beta-sheet content, represented as the ratio of intensities at 1620/1650 cm^{-1}, of sample of gluten subjected to repeated biaxial extension.

When the system is extended all available HMW subunits respond and in some cases individual subunits or clusters of subunits will be extended as envisaged in the original loop and train model. However, in some cases the extension will give rise to alignments of HMW subunits that make the formation of polymeric complexes possible. It is envisaged that the polymers will be stabilised by a combination of intermolecular disulphide linkages and beta-sheet formation, which act synergistically. Both beta-sheets and disulphide bridges are assumed to be involved since both hydrogen bonding and the presence of disulphide linkages have been shown to be important factors in gluten rheology (see Belton, 2003 for a discussion). The polymer will thus be stable with respect to other stretched proteins or protein complexes and will not relax rapidly back to the unstretched state when stress is removed. This model explains the build up of beta-sheet structure that occurs on biaxial extension and is consistent with a number of pieces of evidence that indicate that a polymeric network is formed during dough development.

Possible arrangements are shown in Fig. 6. In Fig. 6a, the relaxed protein network is shown, for simplicity only high molecular weight subunits are indicated schematically. These interact with each other in random fashion by the formation of beta-sheet trains and some disulphide bonds. In the real system there will be other proteins present which may be interacting with the HMW subunits by covalent or non-covalent interactions: these may assist

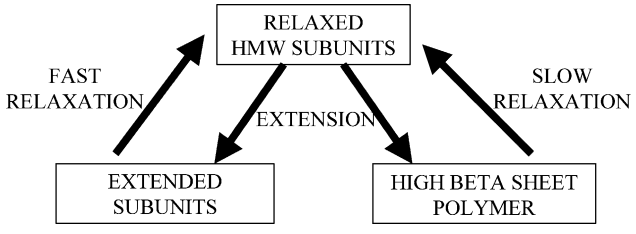

Fig. 5. The two processes occurring when a sample of gluten is extended.

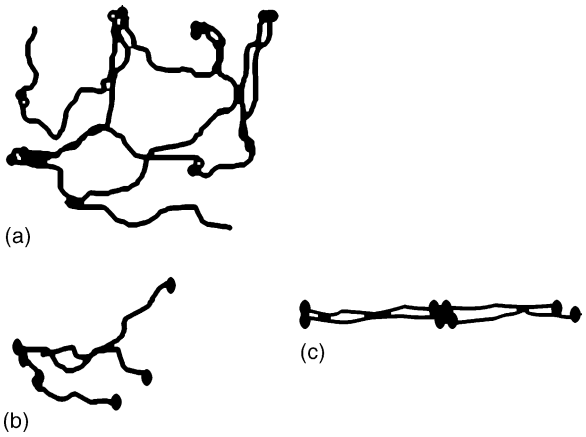

Fig. 6. The two possible changes that can occur on a molecular scale when gluten is extended. See text for further details.

or resist network formation. Other proteins may simply be present and not be interacting and the role of these in the network would be to act as a diluent to the interactions. In Fig. 6b the HMW subunits are extended due to the imposition of mechanical perturbation but are not in a favourable configuration for forming protein/protein interactions by intermolecular beta-sheets or disulphide bonds, thus the beta-sheet content is low. In Fig. 6c the alignment favours both beta-sheet formation and the formation of disulphide bonds, by disulphide interchange, between the C- and N-termini of the subunits (shown as elliptical regions). The relaxation time of this configuration to the initial state will be very slow, whereas the relaxation time of the less aligned system will be more rapid. On the next extension some of the relaxed protein resulting from the less aligned state will be converted to the highly aligned state and because of its slow relaxation this configuration will build up with time.

A model based on these assumptions can be developed to illustrate the expected behaviour. The model is as follows: suppose at the start of the first cycle that there are N_r relaxed HMW subunits. On extension these form N_q rapidly relaxing assemblies and N_s slowly relaxing assemblies. The amounts of N_q and N_r formed are given by

$$N_q = P_q N_r \text{ and } N_s = P_s N_r \quad (1)$$

The time dependence of N_s and N_q is given by

$$N_q(t) = N_q(t=0)\exp(-k_q t) \text{ and}$$
$$N_s(t) = N_s(t=0)\exp(-k_s t) \quad (2)$$

This assumes a first order rate law; this is clearly unlikely to be the actual law but nevertheless can represent functions of different decay rates.

Assuming that

$$P_s < P_q \text{ and } k_s < k_q \quad (3)$$

The lower probability of the formation of the aligned state and its slower rate of decay can be modelled using appropriate values of P and k

The beta-sheet contents B_i of the two forms is given by

$$B_q = C_q N_q \text{ and } B_s = C_s N_s \quad (4)$$

With

$$C_s > C_q. \quad (5)$$

If it is assumed that $P_s = 0$, this is equivalent to assuming that no slowly relaxing components are formed and that the only mechanism is the relaxation of the single faster relaxing process. This corresponds to the formation of assemblies of type b (Fig. 6) only. If the relaxation time constant, k_q, is chosen so that relaxation is slow compared to the time between the applications of biaxial extension, then the results show no build up of beta-sheet structure. This is shown in Fig. 7.

In Fig. 8, a different set of parameters have been used. The ratio P_s/P_q has been set to 0.3/0.7 to reflect the notion that the formation of the aligned species is less likely than that of the unaligned species. The ratio $k_q/k_s = 0.7/0.003$ accounts for the relatively high stability of the aligned species and ensures that most of the relaxation of the unaligned species takes place during the interval between extensions. The results of the calculation clearly show that the model is consistent with the observed behaviour.

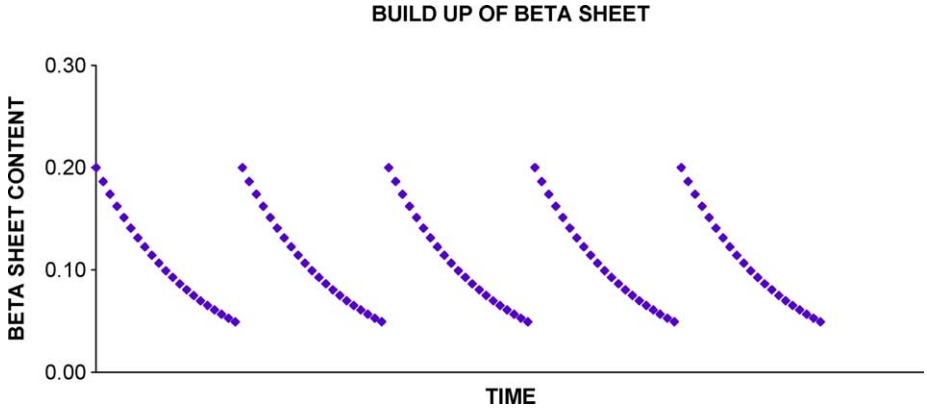

Fig. 7. The build up of beta-sheet content predicted when no slow relaxing high beta-sheet polymer is formed.

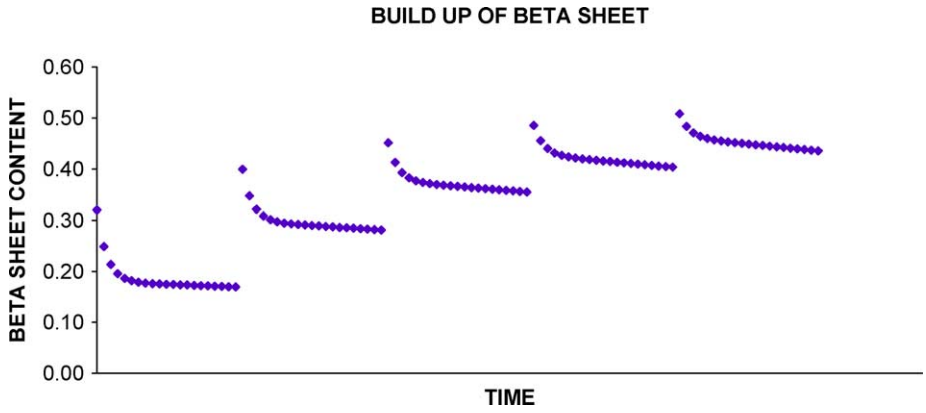

Fig. 8. The build up of beta-sheet content predicted when a slow relaxing high beta-sheet polymer is formed.

5. Discussion

Despite the considerable effort that has been put into characterising the rheology of dough, even the appropriate conditions under which to carry out the experiments remain controversial. Interpretation may be in terms of a particulate system or of an entangled polymer network. Nevertheless, despite these differences in views as to the correct measurement conditions and interpretation, there does seem to be agreement that relaxation processes are bimodal with long and short time components. Any successful theory of dough or gluten rheology must explain these observations as well as the results of the mixing experiments reported by Gras et al. (2000). It is not obvious how a particulate theory of rheology would explain the mixing experiment results. If the particulate model is taken further, as in the work of Don et al. (2003a,b) and Weegels et al. (1996a,b) the problems get greater. As discussed above, this model is based on observations of the insoluble fraction of dough, which, it is claimed, is particulate. As the dough is mixed to peak resistance this fraction becomes near to zero and its relationship to the internal structure of the dough is problematic. The proposed model (Don et al., 2003a) seems to suggest that the initial particle size determines the energy requirement for mixing to peak. At this point, however, the particles are no longer apparent. Presumably, therefore, the rheological properties at the peak are not determined by the particles but by their decomposition products. This seems to present a logical difficulty in the explanation of the observed relationship between the insoluble fraction and dough rheology.

This logical difficulty may be resolved by assuming that that the amount of insoluble material is more an artefact of the kinetics of extraction than an indicator of internal processes in the dough. The process does, however, tend to fractionate the aggregated high molecular weight polymers and it is these that are the indicators of rheological behaviour. Some support for this view comes from the results of FTIR spectroscopy. These imply that the original loop and train hypothesis (Belton, 1999) needs modifying. It has been shown (Belton, 2003; Shewry et al., 2003) that this model adequately explains the observations of Gras et al. (2000) but as discussed above it needs extending to include an explanation of how an aligned polymer network builds up in the system. This polymer network may be identified with the large polymer systems that a number of workers have proposed. The implication of the extended loop and train model is that the input of work to the dough causes the build up rather than break down of a polymeric structure. Maximum resistance occurs when the build up of the polymer is a maximum while excess input of work will result in break down of the polymer. If this is the case then the amount of unextractable material is not indicative of any significant changes taking place in the dough.

Within this model the observations of strain hardening and shear thinning may be accommodated: at low levels of work input strain hardening will be observed when the loops of the system have been stretched and further work is needed to stretch the trains formed by inter- protein interactions. At high work level inputs the system will be intrinsically stiffer due to the formation of larger polymers with lower levels of turns. Under these circumstances strain hardening is likely to occur at lower degrees of extension. In the case of shear thinning disruption of the beta-sheet structure of the trains will result in less resistance to extension and hence thinning; it would be expected that after high levels of work input the thinning effects would be less due to the formation of stronger polymeric structures.

Intrinsic to the loop and train model is a bimodal distribution of mechanical relaxation times. It would be expected that fairly rapid creep behaviour would be observed due to rearrangements of loops and slower process would be observed due to trains. The theory is thus consistent with the rheological observations.

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