



MINI REVIEW

Application of Polymer Science to Properties of Gluten

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ABSTRACT

Much of the knowledge from polymer science studies can be usefully applied to increase understanding of the properties of gluten and how they are related to composition. Low solubility of gluten proteins compared to many other proteins that have been studied evidently arises from the low entropy of mixing of the largest-sized glutenins and a relatively high value for the Flory-Huggins interaction parameter mainly due to a low concentration of ionisable groups. Grain hardness is a property that appears to depend on the proteins that are concentrated at the starch/matrix interface and how they control adhesion. Dough mixing properties, dough strength and extensibility can be understood in terms of the extension of large glutenin molecules giving rise to rubber elasticity and to the presence of molecular entanglements which contribute strength and extensibility to dough systems.

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INTRODUCTION

Suitability of wheat flours for different products is largely based on physical properties such as grain hardness and dough behaviour. The main components of wheat flour, starch and protein, are polymers. Since a large amount of knowledge about the physical properties of polymers and their relation to chemical composition has been acquired, this can be applied to increase our understanding of flour properties. When a dough is developed by mixing or sheeting, the gluten protein forms a continuous viscoelastic network throughout the dough. The properties of dough are therefore the properties of hydrated gluten, modified by a high loading of filler, the starch present as granules. In this article, we will explore some areas of polymer science that can be usefully applied to aspects of gluten protein properties. Although some mathematical equations will be introduced, the aim will be to facilitate their understanding by emphasising physical interpretations.

SOME UNIQUE PROPERTIES OF POLYMERS

There are a number of properties that are unique for polymers.

Segmental motion

The activation energy for flow of large molecules is found to depend, not only on the whole molecule, but on a portion or segment of the molecule¹. Segments of polymer molecules can move, to some extent, independently as kinetic units. The size of a segment, in the case of proteins, can be in the order of 6–10 amino acid residues².

Molecular weight distribution (MWD)

Synthetic polymers normally do not have a discrete MW but are a mixture of different sized molecules. Gluten comprises two main groups of proteins, gliadins and glutenins. Gliadins are single chain

polypeptides with similar MWs but glutenins are formed from subunits by a post-translational polymerisation, thus giving rise to a wide molecular weight distribution.

Entanglements

Rheological properties (particularly of linear polymers) such as viscosity usually increase with increasing molecular weight but, at a critical molecular weight, characteristic for each polymer, the molecular weight increment of viscosity increases sharply.³ Above this value, in addition to normal frictional forces between molecules, there is believed to be an additional resistance to flow associated with widely spaced points along the chain, referred to as entanglements. These act as transient cross links. For very large molecules, there may be many of these points, thus contributing strength through entanglement coupling.

Glass transition temperature (T_g)

As the temperature of a polymer is lowered, kinetic motion of the backbone decreases. At a critical temperature, all segmental motion practically ceases. The polymer behaves as a glass. The glass transition temperature⁴ can be lowered by incorporation of a plasticiser such as water.

SOLUBILITY PROPERTIES

One of the problems in studying gluten proteins is that they are difficult to solubilise, usually a requisite for their characterisation. Solubility in a given solvent requires that the solute disperse molecularly with a decrease in free energy of the system. For amorphous polymers such as gluten proteins,

$$\Delta G_m = \Delta H_m - T\Delta S_m \quad (1)$$

where ΔG_m is the change in free energy of the system upon mixing (joules/mole); ΔH_m is the change in enthalpy (heat content) of the system upon mixing (joules/mole); ΔS_m is the change in entropy of the system upon mixing (joules/mole/kelvin); T is the temperature in kelvins

ΔH_m is concerned with the energies of interaction between molecules and is measured by changes in heat content. When molecules of different species interact strongly, there is a decrease in energy of the system with an

accompanying emission of heat. ΔS_m relates to changes in order/disorder of molecules.

A useful description of the free energy (G_m) for a polymer-solvent system is given by the equation⁵

$$G_m = RT[(n_1 \ln v_1 + n_2 \ln v_2) + \xi v_1 v_2 (n_1 + m n_2)] \quad (2)$$

where R is the universal gas constant; T is the absolute temperature (K); n_1 and n_2 are the numbers of moles of solvent and polymer respectively; $m = V_2/V_1$ is the ratio of the molar volume of polymer to solvent; v_1 and v_2 are the volume fractions of solvent and polymer respectively; ξ is the Flory-Huggins interaction parameter, a measure of the interaction between a given solvent and a given polymer. It is a free energy parameter that contains both entropy and enthalpy terms. A low value (<0.3) indicates considerable interaction; i.e., the liquid is a relatively good solvent for the polymer. A high value (>0.8) indicates little interaction; i.e., the liquid is a relatively poor solvent for the polymer. The first two terms in Equation 2 represent the entropy of mixing. This is always negative and results from the increase in disorder of the system when solute and solvent molecules are mixed up. The degree of mixing is related to the number of ways of arranging solvent and solute molecules. The third term in Equation 2, containing the Flory-Huggins interaction parameter should be a small positive number or negative in order to obtain the negative change in ΔG_m required for solubility.

FACTORS INFLUENCING SOLUBILITY OF GLUTEN PROTEINS

Proteins contain a mixture of polar and non-polar amino acid side chains. It is the non-polar side chains that contribute to lack of solubility in aqueous solution. The failure of non-polar moieties such as hydrocarbon chains to dissolve in water is referred to as the hydrophobic effect. Contrary to what might be expected, the insolubility is not due to an unfavourable enthalpic interaction but to a negative entropy change. When a hydrocarbon molecule is introduced into water, the hydrogen bonds of water molecules adjacent to the hydrocarbon are broken. This causes surrounding water molecules to reorientate and form a more ordered hydrogen bonding arrangement. Non-polar side chain frequency (NPS) is a simple parameter that provides a measure of the hydrophobic

Table I Solubility—related parameters for proteins

Protein	NPS	CHF	Ref
Glutenin	0.34	0.13	6
Gliadin	0.39	0.08	6
Myoglobin	0.32	0.34	7
Haemoglobin	0.35	0.27	7
Ovalbumin	0.34	0.24	7

effect. It is defined as the number of tryptophan, isoleucine, tyrosine, phenylalanine, proline, leucine and valine residues divided by the total number of residues. A comparison of the NPS of gluten proteins with some standard proteins shows that they are of a similar order (Table I). Another important parameter is the charged group frequency (CHF) which is defined as the number of aspartic and glutamic acids, histidine, lysine and arginine expressed as a fraction of the total number of residues. Among the polar amino acid side chains these ionisable ones are those that interact most strongly with water. In this case, the CHF is appreciably smaller for gluten proteins than the standard proteins (Table I). The frequency of charged groups is therefore a limiting factor in the solubility of gluten proteins.

As a macromolecule increases in size, it becomes less soluble. This is because the number of arrangements with solvent molecules decreases leading to a lower entropy of mixing. Glutenins have been hypothesized to be the largest protein molecules in nature⁸. The reduction in solubility with increasing molecular weight has been used to estimate the molecular weight of polymers. A plot of the critical precipitation temperature versus $v M^{1/2}$ where v is the specific volume of polymer, is usually linear. In the case of gluten proteins, the percentage of polymeric protein (mainly glutenin) not extractable by a given solvent has been used as a relative measure of the MWD⁹⁻¹¹. In summary, the difficulty in solubilising gluten proteins arises predominantly from a lack of ionisable groups and the very high molecular weight of the glutenins.

GRAIN HARDNESS

Grain hardness is an important property that determines the suitability of wheat for different products. It is known to be associated with a gene (s) on the short arm of chromosome 5D but the physical basis of differences in hardness is still

being investigated. Work by Simmonds and co-workers^{12,13} focused attention on the starch granule/protein interface as the key to explaining hardness and work since then has pursued this concept. The continuity of the grain texture was emphasised by Stenvert and Kingswood¹⁴. In hard kernels, starch granules are firmly embedded in the surrounding protein matrix whereas, with soft kernels, strong adhesion between starch granules and matrix is not evident and discontinuities appear. Thus, when hard kernels are subjected to shear forces as in milling, the continuity of the matrix can cause fracture through the starch granules. In the case of softer kernels, the matrix is more friable as a result of the discontinuities and less starch damage results. Since the work by Simmonds and co-workers and Stenvert and Kingswood, research has focused on a group of proteins that appear to concentrate at the starch granule surface of soft wheats¹⁵. This group of proteins, called friabilin, contain puroindolines a and b (pin A and pin B), hydrophobic proteins with molecular weights of about 15 000^{16,17}. Giroux and Morris^{18,19} have shown that grain hardness was linked to either non expression of pin A or a glycine to serine mutation in pin B.

Wheat endosperm resembles polymer composite systems which have been studied extensively in recent times. In these systems, a filler is incorporated in the polymer to modify its properties. In wheat endosperm, this filler is the starch, dispersed as granules. A large number of investigations into the effects of fillers on the mechanical properties of polymeric materials, has found that different behaviour of systems is mainly due to the adhesion between filler and polymer. A concentration of hydrophobic proteins at the starch surface of soft kernels suggests that the starch granule surface may itself be hydrophobic. Lipids are known to be present and would confer hydrophobicity. Prior to maturation (water concentration high), there will be competition for the starch surface between the different surface active proteins and lipids. Stability at the surface depends on the hydrophobic/hydrophilic nature of the protein which in turn depends on a delicate balance between polar and non-polar amino acid side chains. Thus, substitution of a highly polar side chain, serine, for glycine could conceivably reduce the free energy of adsorption sufficiently to prevent the molecule from being strongly adsorbed. It may be that, in hard kernels, a greater concentration of gluten proteins is present at the starch surface.

Swelling of polymers involves a balance between the interactions of solvent with the polymer causing a reduction in free energy and the opposing elastic forces of the polymer network. During desiccation of the kernel, water is removed from the endosperm setting up contracting stresses within it. Whether these stresses lead to discontinuities in the matrix depends on the adhesion between starch and matrix. There appear to be two factors that could influence the force of adhesion between starch and matrix. The first is the strength of attachment of protein molecules, measured by the free energy for desorption of the adsorbed protein. Polymers adsorb as trains (molecular segments attached at the surface), loops (segments that protrude into the adjacent phase) and tails (ends of molecules that also tend to be in the adjacent phase)^{20,21}. The free energy of desorption per molecule is equal to the free energy of desorption per segment multiplied by the number of attached segments. This will be relatively low for smaller molecules; e.g. those in the order of 15 000 MW. If very large molecules such as glutenins are adsorbed, there will be many attached segments and the total free energy for desorption could attain high values.

The other factor is the role of the loops and tails. These interact with other molecules in the matrix. In the case of glutenins, long sequences of segments could form entanglements with neighbouring molecules or even form bridges between starch granules. Whether the stresses set up in the desiccating kernel cause the matrix and starch granules to separate thus would depend on the strength of attachment of the adsorbed protein to the starch and to the strength of interaction with the matrix. Based on this model, hard grained wheats might be expected to have a greater proportion of glutenins at the starch surface whereas soft grained wheats would have smaller proteins such as the puroindolines. Reconstitution studies following up on the work of Malouf and Hosney²² could throw more light on the role of the starch/matrix interface in grain hardness.

DOUGH DEVELOPMENT AND BREAKDOWN

Three factors are required to form a dough—flour, water and energy. Doughs are concentrated systems which are different from those discussed in the section on solubility where we are dealing with relatively dilute solutions. As a result, their treatment is necessarily different and extrapolation

of results from dilute systems may not be appropriate. Shear and tensile forces imparted by mixing or sheeting cause discrete masses of gluten protein to coalesce and form a continuous network throughout the dough. During development, the dough acquires viscoelastic properties which become optimum at peak consistency. If only gliadin is present in admixture with starch, a viscous liquid is formed with no elastic strength and no significant development stage. When only glutenin is mixed with starch, a rubbery material is formed which is difficult to develop, at least at normal mixing intensities. At a molecular level, it appears that dough development involves the use of shear and tensile forces to extend the large glutenin molecules from their equilibrium conformations. Extended molecules give rise to elastic restoring forces similar to what occurs in rubber elasticity. The high viscosity arising mainly from entanglement coupling of glutenin molecules retards the molecular retraction and maintains the elasticity during resting. A threshold intensity of mixing is required to develop a dough to optimum²³. As the dough strength of a flour (measured by parameters such as dough development time and Extensograph maximum resistance) increases, this threshold intensity is raised. The threshold intensity is related to the strain rate required to extend the largest glutenins. When a dough is developed by mixing at an intensity above its threshold value and subsequently mixed at a speed below the threshold, the dough undergoes changes corresponding to a reversal of the development process. The dough loses elasticity and resembles an undeveloped dough. This process has been termed 'unmixing'²⁴. At a molecular level, it appears that the glutenins retract from their extended conformations and, on a macroscopic scale, the continuity of the gluten matrix is lost. The faster retraction of the glutenins during low intensity mixing compared to resting is probably facilitated by the greater opportunity for segmental diffusion resulting from the mixing process.

As a dough is mixed past its peak development time, the torque decreases and it progressively loses its elasticity and acquires plastic and sticky properties. Polymer molecules respond to the application of stress by three main processes, disentanglement, chain orientation and bond rupture. All three probably occur during dough mixing. Scission of the largest glutenin molecules results from chains not being able to slip free at entanglement points quickly enough in response to

the stresses²⁵. The covalent bonds that are broken are the disulphide bonds between glutenin sub-units. The energy of a disulphide bond is about half that of a peptide bond. Chain scission is an activated process²⁶. Because the rate equation involves an exponential of the bond energy, the probability that a disulphide bond breaks is many times that for a peptide bond. Another important result is that the highest stresses occur at the centres of molecules so that this is where the probability of chain scission is greatest.

Mixing stresses produce orientation of molecules. After the largest glutenins are broken, the entanglement network diminishes in strength and chains are able to slip through entanglement points more easily. The effect of disentanglement leads to a weaker, more plastic dough with sticky properties.

DOUGH STRENGTH

The force required to stretch a dough is measured in cereal laboratories by the Extensograph using uniaxial extension and the Alveograph which uses biaxial extension. Here we will consider only the former which operates similarly to a tensile stress test. Tensile stress tests for polymers involve stretching a polymer sample until it ruptures. The maximum stress attained is the ultimate tensile strength and elongation to break is the length that the sample can be stretched before breaking. Tensile strength (σ) of polymers has been described by an equation introduced by Flory²⁷

$$\sigma = \sigma_0(1 - M_T/M_n) \quad (3)$$

where σ_0 = the limiting tensile strength at high MW; M_T = a threshold MW; M_n = the number average MW.

Bersted and Anderson tested this equation and found good agreement for monodispersed polymers but not for polydispersed polymers²⁸. They introduced a modification of the Flory equation based on the concept that only molecules with molecular weights above the effective molecular weight for entanglements contributed to tensile strength. The tensile strength was given by

$$\sigma = \sigma_0(1 - M_T/M_n^*) \mathcal{O} \quad (4)$$

where M_T is the threshold molecular weight for effective entanglements; \mathcal{O} is the fraction of polymers with $M > M_T$; M_n^* is the number average molecular weight of this fraction. Equation 4 was

found to predict the tensile strength of poly-dispersed polymers reasonably well.

Application to gluten proteins in dough

In cereal laboratories, the Brabender Extensograph is used to measure tensile properties of doughs. The main parameters that are measured are the maximum resistance to extension, R_{\max} (corresponding to ultimate tensile strength) and extensibility (corresponding to elongation to break). In a study of 74 recombinant inbred wheat lines grown at one location, R_{\max} was plotted against the percentage of total polymeric protein (PPP) and the percentage of unextractable polymeric protein (UPP) evaluated by SE-HPLC^{9,29}. No significant correlation was found with PPP but a very high correlation ($r^2 = 0.86$) was found for UPP. It is deduced from this result that only the least soluble polymeric proteins and therefore the fraction with highest molecular weight is contributing to R_{\max} . This is consistent with the results of a study of 158 wheat lines in which the correlation coefficients for R_{\max} against the cumulative percentage of the polymeric protein eluting up to varying elution times in SE-HPLC was evaluated³⁰. The maximum correlation coefficient was obtained for the percentage of protein that had eluted up to a time corresponding to elution of about 60% of the polymeric protein. This fraction was estimated to have MWs greater than 250 000 although this value is subject to some uncertainty because of the calibration with standard proteins. Nevertheless, the concept that not all of the glutenin but only glutenin of MW above a certain value contributes to R_{\max} seems to be valid. This is therefore consistent with the Bersted-Anderson theory and points to the important role that molecular entanglements play in dough properties.

DOUGH EXTENSIBILITY

Doughs suitable for leavened products such as breads need to have properties that enable them to stretch in response to gas expansion. The dough films must therefore have sufficient strength to prevent collapse but, at the same time, be capable of stretching without rupture. In the theory of Termonia and Smith,³¹ the extensional properties of polymers are determined by two main kinetic processes; firstly, the breaking of secondary valence bonds and, secondly, slippage of chains through

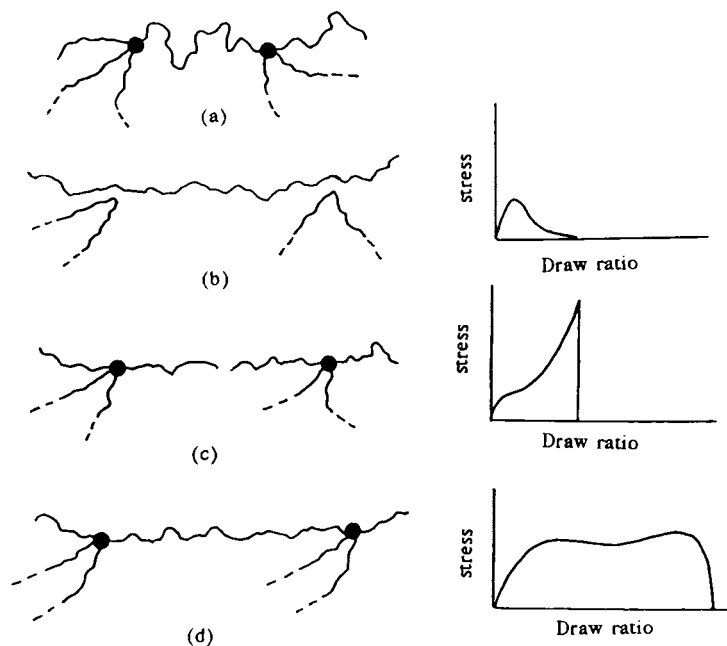


Figure 1 Schematic illustration of the effect on the draw ratio (extensibility) of the rate of chain slippage through entanglements relative to the rate of elongation of the sample. (a) Initial state; (b) slippage rate \gg elongation rate; (c) elongation rate \gg slippage rate; (d) slippage rate optimum for elongation rate.

entanglements. Each process has its own activation energy. Once non-covalent bonds between entanglement nodes are broken and chains are stretched, the only way in which chains can move relative to one another is if they slip through entanglements. The extensional behaviour is then determined by the relative rates of sample elongation and of chain slippage. The model is illustrated schematically in Figure 1 in a simplified way. Initially, the chain strands are in a relaxed state [Fig. 1(a)]. On application of a tensile stress, non-covalent bonds between chain strands are broken and the chains are extended. If the rate of chain slippage is much greater than the rate of elongation of the sample, chain slippage occurs rapidly, providing no points of resistance. Tensile strength and draw ratio (length of sample at break/original length) will both be low [Fig. 1(b)]. If the rate of chain slippage is very low compared to the rate of elongation, chains will not slip through entanglements sufficiently rapidly in response to the applied stress and will break. Although a high strength may be attained, the draw ratio will be very low [Fig. 1(c)]. When the rate of slippage is optimum, as in Figure 1(d), the draw ratio will be maximised; i.e. entanglement points will contribute resistance but chains will slip free

sufficiently rapidly to prevent breakage of covalent bonds.

The theory of Termonia and Smith has been presented in a simplified form. For a more detailed and mathematical treatment, the reader is referred to the original papers. In these papers, the effects of a number of variables have been studied.

Entanglement spacing

For a pure polymer, there is a characteristic value for M_T . In the presence of solvent, the value of M_T increases with increasing volume fraction of solvent. The stress-strain behaviour changes, first because of the increase in M_T through dilution of the polymer and secondly through a plasticising effect of the solvent or diluent. The entanglement spacing factor (Θ) was defined by:

$$\Theta = (M_T)^{\text{melt}} / (M_T)^{\text{soln}} \quad (5)$$

where $(M_T)^{\text{melt}}$ is the MW between entanglements in pure polymer; $(M_T)^{\text{soln}}$ is the MW between entanglements in presence of solvent. The spacing factor thus corresponds to the volume fraction of polymer. Termonia and Smith³² varied Θ for a monodispersed polymer by using solutions of different concentrations and then removing the

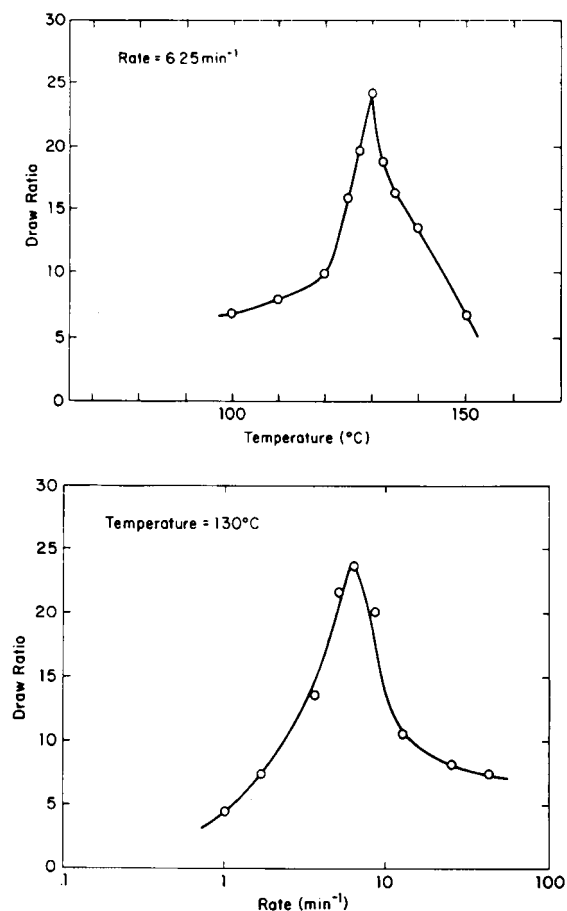


Figure 2 Calculated dependence of the maximum draw ratio on temperature at constant elongation rate (top) and on elongation rate at constant temperature (bottom) for a monodisperse polyethylene of $M = 143\,000$ according to the theory of Termonia *et al.*³³

solvent so as to reveal only the effect of the initial volume fraction and eliminate possible plasticising effects of the solvent. Calculated and experimental stress-strain curves showed good agreement. As the spacing factor decreased from 1 (pure polymer) down to 0.01, tensile strength decreased while strain at break increased spectacularly. When the MW between entanglements reached the MW of the polymer, the plastic deformation leading to high values of the strain at break no longer occurred and ductile failure was observed.

Deformation rate and temperature

Studies, again with monodisperse polymers showed that, for each MW, there exists a narrow temperature or elongation rate window within which maximum drawability occurs³³. This is illustrated in Figure 2 showing the dependence of

polyethylene (MW 143 000). Increasing the elongation rate has an influence similar to increasing the temperature. This is consistent with the time/temperature superposition principle. In essence, this means that a particular value of the draw ratio may be obtained either by varying the elongation rate at a given temperature or by varying the temperature at a given elongation rate. A comparison of the draw ratio as a function of elongation rate at a temperature of 130 °C is shown in Figure 3 for two samples of polyethylene of MW 6800 and 143 000. It is seen that increasing the MW leads to a reduction of the optimum elongation rate and of the width of the window. Furthermore, there is an appreciable increase in the maximum attainable draw ratio for the higher MW sample. This is because the maximum draw ratio is ultimately determined by the largest number of statistical segments between entanglements that can be reached through slippage before disentanglement occurs.

Polydispersity

Most of the results so far have been based on monodisperse polymer samples. This has given insight into the molecular processes governing drawability and thus provides basic concepts that can be used to understand the behaviour of the more commonly encountered and more complex polydispersed systems. Termonia and Smith³⁴ have found two parameters to be useful in characterising polydisperse polymer systems for interpreting their stress-strain behaviour. The first is an average MW (M_t) first introduced by Graessley³⁵ that has been found to be useful for describing the entanglement friction factor of polydisperse systems. The value of M_t lies between the weight-average MW (M_w) and the z-average MW (M_z). The second is a measure of polydispersity given by the ratio of M_z to M_w (M_z/M_w). The calculated dependence of the maximum draw ratio on the polydispersity (M_z/M_w) of polymer samples all having the same value of M_t based on the Termonia-Smith theory is shown in Figure 4. As the polydispersity is increased from 1.19 to 3.45, there is a strong decrease in the draw ratio and an accompanying widening of the optimum rate window. This behaviour is consistent with an optimum elongation rate window for every chain length. Calculations of the maximum draw ratio for a blend of two different MW samples of polyethylene were also made, thus

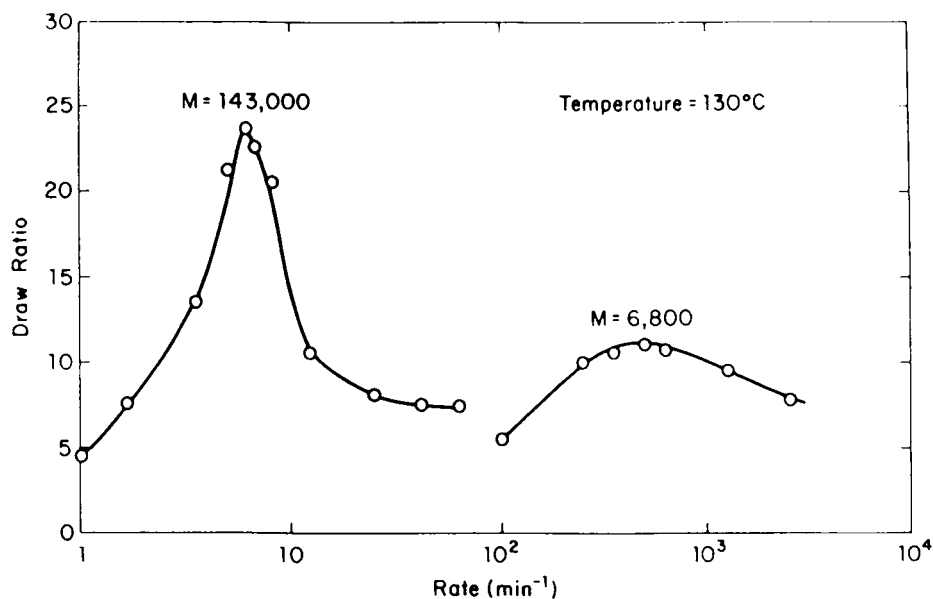


Figure 3 Calculated dependence of the maximum draw ratio on elongation rate for monodispersed polyethylene samples of $M = 143\,000$ and 6800 according to the theory of Termonia *et al.*³³

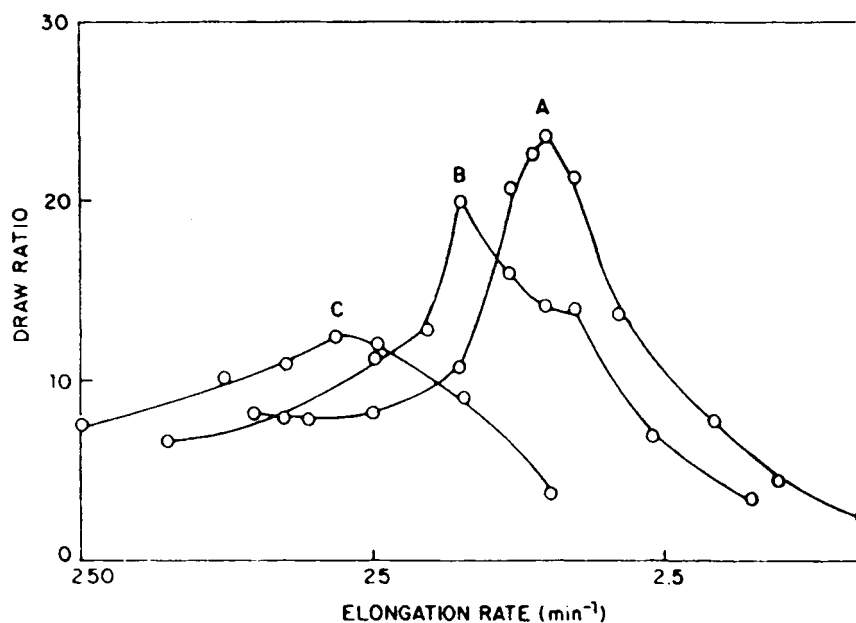


Figure 4 Calculated dependence of the maximum draw ratio on polydispersity (M_z/M_w) at constant average MW ($M_t = 160\,000$) for characterised polyethylene samples.³⁴ A, $M_z/M_w = 1.19$; B, $M_z/M_w = 1.62$; C, $M_z/M_w = 3.45$.

giving an insight into the effects of a bimodal MW distribution. The behaviour was unusual in that a linear relationship was not obtained. A maximum

in the draw ratio at an intermediate weight fraction was observed for a mixture of equal weights of the two MW fractions ($M = 6840$ and $148\,000$).

Conclusions pertinent to dough properties

Instruments such as the Extensograph measure dough properties at a fixed rate of elongation and fixed temperature. As a result, the ranking of dough samples for extensibility will depend on how close these fixed conditions are to optimum for the different samples. This is acceptable providing the elongation rate that is used corresponds closely to that which occurs in the dough during processing assuming that high extensibility is required; or, that extensibility measured in this test correlates with processing quality. The effect of which elongation rate is chosen can be appreciated by reference to Figure 3. If we compare the two polymer samples at a rate of 10^{10} min^{-1} , the higher MW sample will be superior. Its maximum draw ratio (i.e. its value at the optimum rate) is also appreciably higher. In wheat flour doughs, of course, we are dealing with a distribution of molecular sizes. As mentioned, extensibility will be determined by the number of statistical segments between entanglements that can be reached through slippage before disentanglement occurs. It can therefore be surmised that, as the size distribution of polymeric proteins is shifted to higher MW, extensibility should initially increase. However, because a constant elongation rate applies, a point is reached when further increase in molecular size causes extensibility to decrease because chains will not be able to slip through entanglements sufficiently rapidly. Extensibility, as measured routinely in cereal laboratories, is not a fundamental parameter although it is valuable as a relative measure. It needs to be interpreted in terms of how entanglement slippage is affected by the molecular size distribution of the protein in relation to variables such as elongation rate and temperature. Low dough extensibility can result either because the rate of entanglement slippage is too rapid [cf. Fig. 1(b)] or because it is too slow [Fig. 1(c)]. In the latter case, extensibility could be enhanced by increasing the proportion of gliadin to act as a plasticiser and increase the entanglement spacing.

Extensograph extensibility

Extensograph extensibility has been found to correlate highly with flour protein content. This, however, likely occurs because flour protein content correlates with flour glutenin content and the latter is what appears to govern extensibility.

Correlations of extensibility with flour glutenin content are usually found to be higher than with flour protein content. The framework in a dough that is responsible for extensibility is therefore the hydrated glutenin. Surveys have shown that, when there is a wide range of protein contents in wheat lines, some 70% of the variation in extensibility can be ascribed to differences in the flour glutenin content³⁶. When flour glutenin content is similar between lines, other factors affecting extensibility can be studied. In a pair of lines having the same flour glutenin contents but differing appreciably in extensibility, it was found that the UPP was greater in the line having the lower extensibility³⁷. This suggests that, when the MW distribution of the glutenin is shifted to high values, extensibility can be reduced. Based on the considerations of polymers discussed above, this can arise when the rate of slippage of chains through entanglements becomes lower than optimum.

MODIFICATION OF GLUTEN PROTEINS

Physical and chemical modification of gluten can be used to alter the functional properties in desirable ways. For example, no procedure has been found for solubilising gluten proteins completely. Since characterisation requires complete molecular dispersion, modification is a way to achieve this. Other modifications of interest commercially are those that enhance the foaming, emulsification, gelling and textural properties.

Sonication

Exposure of polymers to ultrasound is well known to cause degradation which can be followed by reduction in viscosity. This has been utilised to increase the solubility of glutenin. The mechanism of degradation is not well understood. Cavitation is known to occur under certain conditions but polymer degradation has been observed under conditions which exclude cavitation. One explanation of the effect is that it results from frictional forces between the ultrasonically accelerated faster moving solvent molecules and the larger less mobile macromolecules^{38,39}. The effects of sonication on gluten proteins is similar in nature to those of dough mixing in which shear forces have been shown to cause scission of the larger glutenins and thus increase the solubility⁴⁰⁻⁴³.

One of the earliest theories of mechanical

degradation of polymers was that of Bueche who derived an equation to predict the kinetics. This was a standard rate equation which included a pre-exponential term and an exponential term containing the energy to break a bond. The basis of the process is that long chain molecules cannot disentangle sufficiently rapidly and therefore break. Several results are predicted from the theory: (1) The weaker covalent bonds will be broken preferentially. In the case of glutenin, these are the disulfide bonds linking subunits; (2) The probability of scission is greatest near the centres of molecules where the stresses are highest; (3) Only molecules above a critical MW (M_{\max}) are broken down and the degradation products fall in the size range $M_{\max} > M > M_{\max}/3$. The value of M_{\max} depends on the bond energy and the strain rate.

In the study of gluten proteins, the aim has been to apply conditions just sufficient to break down the largest glutenins and induce complete solubility without altering the size range of the glutenin. This requirement has been monitored using size-exclusion HPLC⁴⁴.

Apart from its application in the controlled degradation of polymers, sonication can induce chemical reactions as a result of production of free radicals. This is an area that has not been investigated for gluten proteins. It offers the potential for incorporating different chemical groups which could alter the properties. For example, the incorporation of highly polar moieties might be used to increase the solubility in aqueous solution or the water binding capacity. It has the attraction of being an environmentally friendly procedure.

Other modifications

There is an increasing trend to produce modified proteins for use in foods (e.g. texturised proteins for meat substitutes)⁴⁵ as well as the non-food industry where biodegradability is an important factor^{46,47}. The main types of protein modifications have been physical (e.g. extrusion, heat), enzymatic (e.g. proteolysis) and chemical. As mentioned earlier, one reason for the low solubility of gluten proteins is the dearth of ionisable groups. Deamidation has been used to convert the large number of amide groups on glutamine and asparagine residues to carboxylic acid groups⁴⁸. In theory, this does not affect the MW appreciably. Proteolysis, on the other hand, increases solubility

by reducing the molecular size. Transglutaminase is an enzyme that modifies proteins by catalysing crosslink reactions, deamidation and incorporation of amines, the reactions being dependent on the source of the enzyme⁴⁹. The possible uses of gluten are numerous and this has stimulated research on different modification procedures to target specific end-uses. Apart from deamidation and proteolysis already mentioned, others have included alkaline treatment and succinylation to improve solubility⁵⁰ and crosslinking such as through dityrosine formation⁵¹.

The glass transition temperature (T_g) is becoming recognised as an important parameter in defining the properties of proteins and this is very sensitive to modifications and to the presence of plasticisers^{52,53}. Water is one of the best plasticisers for gluten⁵⁴ and glutenin⁵⁵ and causes a dramatic reduction in T_g . In order to obtain good viscoelastic properties, protein needs to be well above its T_g . This may be a consideration when it comes to explaining why most cereals other than wheat do not form viscoelastic doughs. Maize proteins have been found to exhibit viscoelasticity when heated well above room temperature⁵⁶ and this may be related to the T_g ⁵⁷.

Heat is another way to modify proteins^{58,59}. Weegels *et al.*^{60,61} have studied the changes in properties when gluten is heated (up to 80 °C) at different moisture contents. At low moisture contents (below 20%), there was a decrease in extractability of larger glutenins and in hydrophobicity of acetic acid unextractable protein. At higher moisture contents, disulphide bonding and irreversible conformation changes were reported. At temperatures above 90 °C, gliadins polymerise⁶². The alpha, beta and gamma gliadins have numerous cysteine residues which form only intra-chain disulphide bonds. Evidently, on heating to temperatures above 90 °C, these bonds participate in disulphide-sulphydryl interchange. SE-HPLC has shown that the gliadin peak is reduced and the glutenin peak is increased for gluten samples that had been heated to temperatures above 130 °C (Singh & MacRitchie, unpubl. results). This indicated that the gliadins had bonded with glutenins.

This review has emphasised the application of polymer concepts to help explain some properties of gluten proteins, particularly in regard to the rheological behaviour. A linear amorphous polymer can exist as a hard solid (glassy state), a viscoelastic material or a liquid, depending on

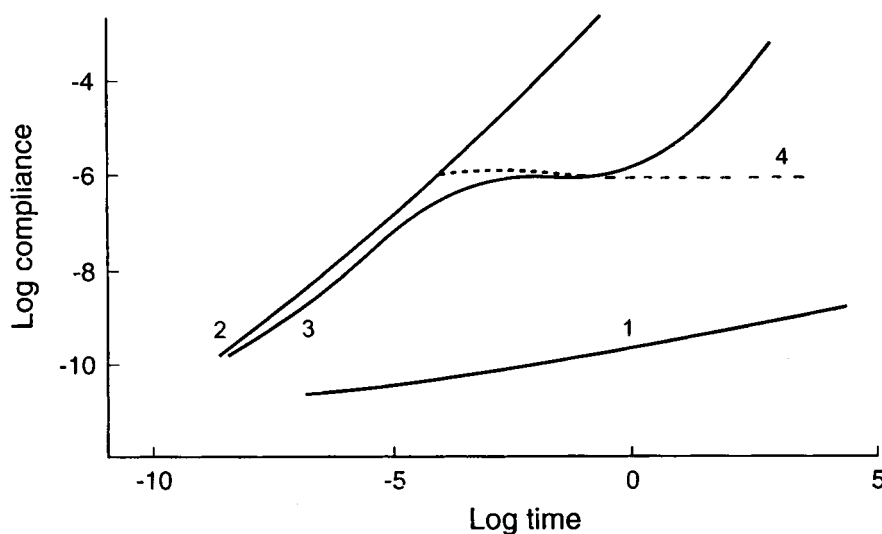


Figure 5 Log compliance vs. log time for creep of various polymer samples. 1, polymer at a temperature below its T_g ; 2, polymer of low MW; 3, polymer of high MW; 4, cross-linked polymer.

the temperature and MWD of the polymer. The different behaviours are illustrated by the relationships between creep compliance (the ratio of strain to stress) and time as shown in Figure 5. In a creep measurement, a constant stress is applied and the strain (deformation) is monitored as a function of time. Curve 1 shows the behaviour for a polymer below its T_g . Below this temperature, backbone configurations are immobilised throughout the whole time scale. Curves 2 and 3 show behaviour for polymers of low and high molecular weights respectively, above their T_g . At long times for these polymers, the creep compliance increases without limit because of the contribution from viscous flow. Curve 2 corresponds to gliadins. Curve 3 corresponds to glutenins. The delayed strain (curve 3) results from slippage of chains through entanglements, acting as transient cross links. Curve 4 is for a true cross linked polymer. In this case, once chains between cross links become fully stretched, no further deformation is possible unless the stress is sufficiently high to break bonds. This type of behaviour may occur with gluten as a result of heating to high temperatures.

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