Rheological Investigation of Swollen Gluten Polymer Networks: Effects of Process Parameters on Cross-link Density

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Summary: Wheat gluten samples were subjected to different thermo-mechanical treatments. Kinetics of protein aggregation and changes in network structure were investigated through biochemical and rheological measurements. Temperature induced protein aggregation through disulphide cross-links. Shear treatment alters the aggregation mechanism since a lower energy of activation was observed. Accumulation of aggregated protein enhances the elastic behaviour of the material. The strong correlation found between the extent of protein aggregation and the molecular weight between cross-links reveals the important role of covalent bonds in the network connectivity.

Keywords: activation energy; biopolymer; cross-linking; kinetic; wheat gluten

Introduction

The rheology of wheat flour dough has been a topic of interest for several decades, because the rheological properties of dough play a key role in the successful manufacturing of bakery products. The gluten protein governs the unique viscoelastic properties of wheat flour dough. In addition to the study of its functionality in baked products, wheat gluten has been widely investigated as a raw material for the development of biodegradable materials, because it is a low-cost material derived from agricultural resources, that is annually renewable and readily available.

A large variety of texturization processes, such as batch mixing or extrusion, has been applied to wheat gluten protein, mainly for food uses. Process parameters like thermal and mechanical energy inputs, or plasticizer chemical structure and content, determine the structure and mechanical properties of the end product. However, the effects of those parameters on the final

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protein network structure are still not well understood to date.

Gluten proteins, in contrast to homopolymers, have a very diverse structure. The amino acids offer a large variety of possible chemical interactions and reactions. Vital wheat gluten is a mixture of mainly two types of protein, i.e., glutenin and gliadin. Gliadin consists of monomers of globular protein up to 65% of the total protein mass, whereas glutenin is a polymeric protein. The glutenin macropolymer derives from the post-translational polymerization of glutenin subunits which gives rise to a wide molecular weight distribution. The precise structure and size of the glutenin macropolymer are still under discussion. It is, however, widely accepted that it might exceed several millions.[1] Owing to its low content of charged amino acids and to its huge size, glutenin is insoluble in water. Denaturing agents such as sodium dodecyl sulphate are needed to bring it into solution. Wheat proteins are furthermore characterized by their high glutamine content,[2] which generates a great capacity to form intra- and inter-molecular hydrogen bonds.

In order to use wheat gluten in technological processes to produce biodegradable materials, we need a clear understanding of the gluten aggregation mechanism. Temperature has a polymerizing effect on wheat gluten in that it induces the formation of covalent bonds between proteins.[3,4] The decrease of gluten extractability due to technological treatments is often taken as a measure of the protein aggregation. Shear, on the other hand, is supposed to have a depolymerizing effect on gluten, because of the resulting chain scission due to mechanical stress.[5,6]

The purpose of this study is to investigate the effects of shear and temperature on the structure of the wheat gluten network. In order to meet this objective gluten was processed under various conditions. The formation of the gluten network was monitored with solubility tests and its structure was assessed by rheological measurements.

Material and Methods

The Amylum Group (Aalst, Belgium) graciously provided vital wheat gluten. Protein, starch, lipid, and ash quantities were 76.5, 11.8, 5.0, and 0.8 % of dry mass, respectively. Moisture content was 7.2 % (wet mass basis).

Sample Preparation

In order to produce mixed gluten samples, the gluten powder was mixed with glycerol (35 %) in a two-blade counter-rotating measuring mixer (Plasticorder PL 2000, Brabender, Germany) at 100 rpm. The mixing chamber was preheated at T_{reg} = 40, 60, and 80 °C with a cryostat (Lauda RC 20). The effective sample temperature recorded in the middle of the mixing chamber stabilized after a sharp initial increase at 77, 88, and 100 °C, respectively. In order to follow the changes in gluten structure five samples were taken at different times throughout the mixing process. The materials were then shaped into 4 mm sheets by using the hot-moulding press at 70°C. Small disks were cut out from these sheets for the rheological determinations.

For the fabrication of hot-moulded gluten samples, the gluten-glycerol blend (35 % glycerol) was hand-mixed in a mortar and then pressed (150 bar) in a heating press (Techmo PL 10T, France). The reaction was stopped by cooling the samples immediately to –28 °C by placing them in a domestic refrigerator. The resulting film samples had an average thickness of 120 µm. The press temperature and press time were varied in order to get an insight into the protein aggregation kinetics (T_p = 71, 82, 104, 124, and 150 °C).

Solubility Tests

Analysis of gluten protein was performed by size-exclusion high performance liquid chromatography (SEC) according to Redl *et al.*[5] The ground gluten samples were first extracted in a sodium phosphate buffer (0.1 M, pH 6.9) containing 1% sodium dodecyl sulphate (SDS) in order to get the soluble protein fraction (F_s). The pellet was extracted using a sonication device after reduction of disulphide bonds in the same SDS-buffer containing dithioerythriol. The insoluble protein fraction (F_i) and F_s were analysed by SEC in order to check the recovery of total protein mass.

Rheological Measurements

The rheological investigation of mixed gluten samples was carried out using a Rheometrics Dynamic Spectrometer (MkIII torsion head, Rheometrics, USA) equipped with two coaxial circular parallel plates. A time sweep was run for 60 min in a frequency range from 0.03 to 30 Hz. The amplitude of oscillations was 1.59 µrad, which corresponds to a strain amplitude of

γ_0 = 0.39 %. Samples were measured at 80 °C in constant force mode, applying a force of 5 N. The obtained spectra were quantified using Cole-Cole distributions.[7]

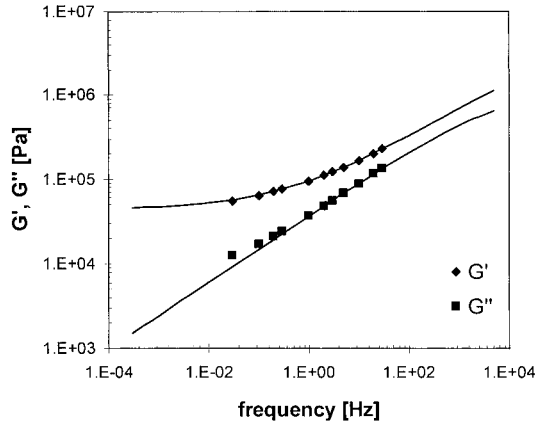

Fig. 1. Cole-Cole fit to the mechanical spectrum of a gluten sample mixed at 88 °C for 8 min.

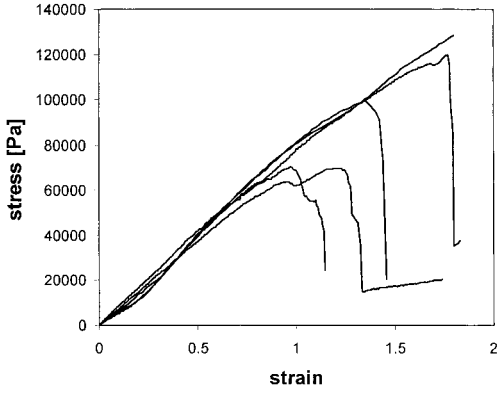

Fig. 2. Reproducibility of tensile experiments with gluten films. The samples were hot-moulded at 100 °C for 10 min.

The analysis of hot-moulded film samples was carried out with a Dynamic Mechanical Thermal Analyzer MK III (Rheometrics, USA). Samples were immersed in de-ionized water at room temperature (22.5 °C) in order to standardize their plasticizer content. A tensile deformation (strain) was applied to the sample at a rate of 0.001 s^{-1}. The Young modulus (E) was calculated

from the slope of the linear stress increase. The Young Modulus was converted into shear modulus (G) with the approximation E(t) = 3 G(t) for isotropic incompressible solids.[8]

According to the theory of rubber elasticity, the density of cross-linked network strands can be estimated from the equilibrium modulus G_e[8] from the equation:

$$G_e = \frac{\rho}{M_e} RT, \qquad (1)$$

where ρ is the density of the material ($\rho = 1.2 \cdot 10^6$ g m^{-3}), M_e is the molecular weight of network strands terminated by cross-links or entanglements (g mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is the temperature (K).

Results and Discussion

Kinetics of Gluten Aggregation

Initially, approximately 85 % (w/w) of the wheat gluten protein is soluble in SDS-buffer. Mixing and hot-moulding of gluten-glycerol blends cause a solubility loss of the proteins, which accelerates with increasing temperature (Figure 3). The drop in extractability is related to the gluten protein aggregation. The formation of covalent bonds between proteins is likely to be involved in the aggregation phenomenon, since SDS disrupted only non-covalent interactions. The fact that gluten solubility can be fully restored by the reduction of disulphide bonds reveals the substantial contribution of new disulphide cross-links in the aggregation mechanism.[3,4,6]

The samples pressed at 150 °C showed a maximum in F_i after 2 min (Figure 3). This finding might be attributed to the beginning of a degradation mechanism.

The increase of F_i during hot-moulding can be described by an apparent first order kinetic law, involving two active sites,

$$F_i = F_{i,Start} + S_{H,1} \cdot \left[1 - \exp\left(-k_{H,1} t\right)\right] + S_{H,2} \cdot \left[1 - \exp\left(-k_{H,2} t\right)\right], \qquad (2)$$

where $F_{i,Start}$ [%] is the SDS-insoluble protein fraction of native gluten [%], t the reaction time, $S_{H,1}$ and $S_{H,2}$ the two reactive sites [%], and $k_{H,1}$ and $k_{H,2}$ are the respective rate constants [s^{-1}]. It was observed that both rate constants follow an Arrhenius law with the same energy of activation, so that all data points can be reduced to a single master curve (Figure 4). We suggest

that the decrease of the reaction rate as the cross-linking reaction proceeded was due to increasing hindrance of the diffusive motion of the molecules in the system.

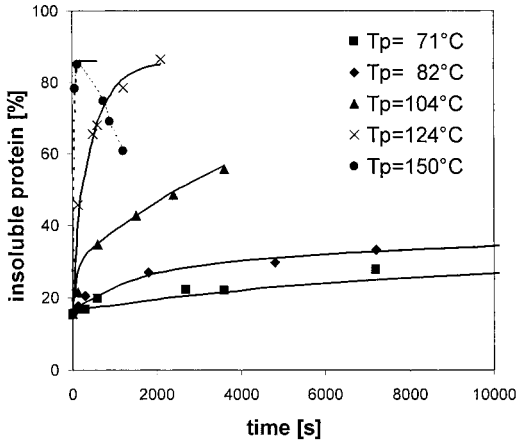

Fig. 3. Increase of the SDS-insoluble gluten protein fraction during hot-moulding at different temperatures.

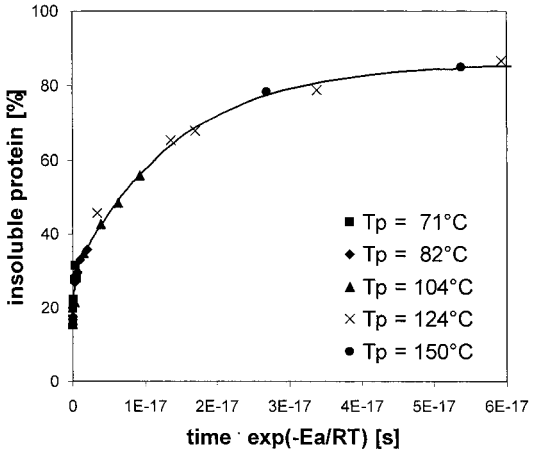

Fig. 4. Master curve of the increase of the SDS-insoluble protein fraction during the hot-moulding process.

In the case of mixing, the kinetic of gluten aggregation can be described by a simple apparent first order law. Diffusive hindrance was not observed in the mixed system (Figure 5). Here, the

optimization of the kinetic parameters with the help of a master curve is advantageous, because it allows the simultaneous fit of all data, irrespective of the mixing temperature.

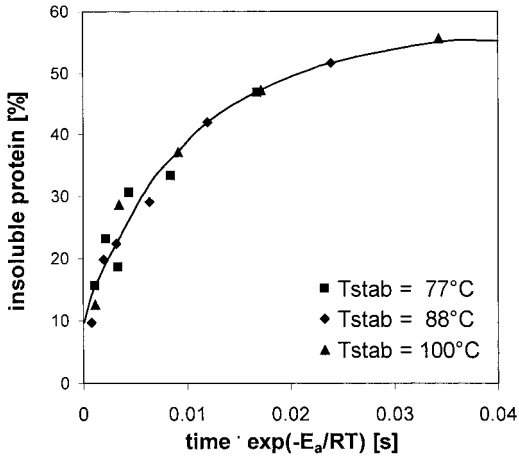

Fig. 5. Master curve of the increase of the SDS-insoluble protein fraction during the mixing process.

Table 1. Arrhenius parameters for the gluten protein aggregation reaction during the hot-moulding and the mixing process.

Hot-moulding process		Mixing process	
$E_{a,H}$ [kJ · mol^{-1}]	149	$E_{a,M}$ [kJ · mol^{-1}]	33.7
$A_{H,1}$ [s^{-1}]	$7.02 \cdot 10^{16}$	A_M [s^{-1}]	$9.56 \cdot 10^{1}$
$A_{H,2}$ [s^{-1}]	$4.91 \cdot 10^{18}$		
R^2	0.9953	R^2	0.9807

$E_{a,H}$	activation energy of the hot-moulding process
A_H	Arrhenius frequency factor of the hot-moulding process
$E_{a,M}$	activation energy of the mixing process
A_M	Arrhenius frequency factor of the mixing process
R^2	correlation coefficient for fitted values versus raw data

The fitted Arrhenius constants are given in Table 1. The activation energy of 34 kJ ·mol^{-1} for mixing is significantly lower than that calculated for the hot-moulding process (149 kJ ·mol^{-1}). The latter is similar to the value 172 kJ·mol^{-1} found for a simple heat-treatment of gluten-

glycerol blends.[3] The considerably decreased temperature dependence of the protein aggregation upon mixing indicates that the shear treatment of gluten changed the protein aggregation mechanism. We suggest that mechanical scission of disulphide bonds may contribute to the formation of free radicals in the system, which could be responsible for the increased reactivity of gluten.[6]

Network Structure

The gluten protein network is supposed to be stabilized by covalent (disulphide) bonds as well as by non-covalent interactions; the latter being hydrogen bonds and hydrophobic interactions. In the conceptual scheme of entanglement coupling or cross-linking of polymer chains, the average molecular weight between coupling loci (M_e) is considered as a measure of the spacing between topological restraints of various chemical/physical origins.

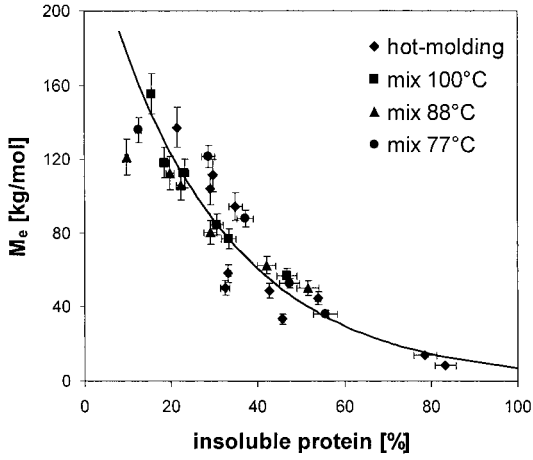

Fig. 6. Relationship between the percentage of SDS-insoluble protein and the average molecular weight between cross-links M_e.

Gluten protein aggregation, which is assessed by an increase in F_i, was found to be strongly related to M_e. Figure 6 shows that M_e decreases while F_i increases, where smaller M_e values correspond to an increase of the protein network connectivity. Since the rise of F_i was found to be caused by the formation of disulfide bonds, covalent bonds seem to have a preponderant

influence on the network structure. The main role of disulphide bonds is further supported by the unique F_i - M_e relationship obtained, whatever the sample preparation for the rheological measurement. Actually, for the submerged samples, higher M_e values might have been expected because of the weakening of the hydrophilic bonds in this measurement mode.

Conclusion

The kinetic investigation of gluten aggregation during both types of processing (i.e. mixing and hot-moulding) reveals the dominant influence of temperature. Shear seems to alter the aggregation mechanism, because it significantly lowers the temperature dependence of the aggregation reaction, although in both cases the main cross-links formed are disulphide bonds. Processing of wheat gluten increases the network cross-link density and concomitantly its elastic property. Rheological measurements in combination with biochemical investigations revealed the important role of the formed disulphide bonds for the connectivity of the gluten protein network.

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