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Swelling Behavior and Structural Characteristics of Wheat Gluten Polypeptide Films

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Wheat gluten films were subjected to controlled thermomechanical treatments to increase the percentage of aggregated sodium dodecyl sulfate (SDS)-insoluble gluten protein, the aggregation reaction being disulfide bonding. The rheological properties of the films were measured under immersion in water, where wheat gluten films are stable and show only slight swelling. The equilibrium swelling of the gluten films in water decreased with the increase of the percentage of SDS-insoluble protein aggregates, and the frequency the independent shear modulus increased sharply with increasing percentage of SDS-insoluble aggregates. Both findings confirm that disulfide bonding between gluten proteins is the predominant cross-linking reaction in the system. A relationship between shear modulus and aggregated protein compatible with a power law (of exponent 3) suggests the existence of a protein network at a molecular scale. However, the classical Flory–Rehner model failed to describe the relationship between the plateau modulus and the gluten volume fraction (a very drastic increase, compatible with a power law of an exponent of about 14). This result shows that gluten cannot be described as an entangled polymer network. The interpretation of both relationships is a network of mesoscale particles which in turn have a fractal inner structure (with a fractal dimension close to 3).

Introduction

Wheat gluten is particularly interesting as a raw material for the development of new biomaterials based on renewable resources, because it is annually renewable and available in large quantities at competitive prices. Additionally, wheat gluten has interesting technological features among which are insolubility in water and viscoelasticity. The unique viscoelastic properties of wheat gluten are the subject of a great research effort. Most studies relate to the field of food chemistry, because gluten plays a key role in the bread-making properties of wheat flour dough. Despite all these efforts, the structure of the gluten polymer system and the mechanisms of its viscoelasticity are still debated.

From a biochemical point of view, gluten is a protein mixture, containing a polymeric protein fraction (glutenin) and a monomeric one (gliadin). The polymeric protein fraction consists of polypeptides linked together with disulfide bonds, forming the so-called glutenin macropolymer with M_r ranging from 150 000 to more than 2 000 000. The monomeric protein fraction is formed by single polypeptide chains (M_r ranging from 15 000 to 150 000), which interact with the glutenin macropolymer “backbone” through secondary bonds.^{1,2}

The rheological investigation of plasticized gluten showed that it behaved like a transient network.^{3–5} Furthermore, the

rheological investigation of wheat flour dough showed that only the glutenin macropolymer contributes to the elastic properties at large deformation, while gliadin contributes to the viscous behavior.^{6–8} Several models of the structural basis of this rheological behavior have been proposed. A widely used approach is to consider the gluten system as a polymer network that is stabilized by various interactions, such as covalent bonds, noncovalent interactions (hydrogen bonds, hydrophobic interactions), and entanglements (to list all of them). According to the “loop and train” model,⁹ the gluten system is mainly stabilized by hydrogen bonds. In the dry state, those bonds are predominately formed between different protein chains. Upon hydration, the protein–protein interactions (trains) become little by little replaced with protein–water interactions, which cause local disaggregating of the chains termed “loops”. When the system is stretched, first the loops are deformed and then the trains are pulled apart.⁹ Another model consists of describing the gluten system as an entangled polymer network.^{8,10,11} In such a network, physical entanglements exist that add resistance to flow. For a long polymer chain, there may be many of such points acting thus as transient cross-links. The extensional properties of gluten would be in consequence determined by two processes; first, the breaking of secondary bonds and, second, slippage of chains through entanglements.¹¹ A quite different structure of the gluten system was suggested very recently. It was proposed that gluten is organized as a transient particle network.^{12,13} With the help of confocal laser scanning microscopy, a particle structure was revealed in wheat gluten gels in 1.5% sodium dodecyl sulfate (SDS).

This structural organization may reflect the structure as it was originally formed in the wheat endosperm.^{14,15}

Technological modifications, such as dough mixing, cooking, or extrusion, imply temperature treatment of the material. It is known that temperature treatments cause gluten proteins to aggregate as a result of disulfide bond interchange reactions.^{16–20} A sound parameter to measure the aggregation reaction is the extractability of wheat gluten in SDS.^{4,19,20} Above a temperature of ~ 40 °C, an increase of the shear modulus of the gluten network can be observed,^{3,5} which is related to the formation of interchain disulfide bonds.⁵

The aim of this study is to examine the quantitative relationship between gluten aggregation, shear modulus, and equilibrium swelling in the light of the two structural models just described (molecular network vs particle network).

The experimental approach consists of the fabrication of gluten film samples with different technological processes resulting in a wide range of protein extractabilities. The samples are subsequently characterized by the measurement of the frequency-independent shear modulus and of the equilibrium swelling.

The analysis of the experimental data in light of the structural model considering gluten to be a cross-linked polymer network will be done with the help of the Flory–Rehner theory.²¹ In this treatment, the swollen polymer is considered an elastic solution. The extent of swelling represents a competition between two forces. The free energy of mixing will cause the solvent to penetrate and try to dilute the polymer phase. As the polymer chains in the cross-linked polymer network begin to elongate under the swelling action of the solvent, they generate an elastic retracting force in opposition to this deformation. The volumetric swelling reaches steady state (equilibrium swelling) when both forces balance each other. To account for the change of the chemical potential of the solvent accompanying the formation of the real solution, the interaction parameter χ has to be introduced, which in turn depends on the temperature and composition of the system. Following these assumptions, the equilibrium swelling of a cross-linked polymer network is a direct function of the cross-linking density in the network, which is linearly related to the frequency-independent shear modulus.²² In consequence, swelling measurements are a simple, low-cost method for the determination of the network cross-linking degree.

The analysis of the experimental data in light of the structural model considering the presence of a particle network will be done with the help of a weak-link fractal scaling theory.²³ According to the theory, the elastic modulus of a colloidal aggregate network is influenced by the amount and spatial distribution of network mass and by particle properties, including size, shape, rigidity, and particle–particle interactions. In the weak-link rheological regime, flocs are mechanically stronger than the links between them. The Young modulus scales with the change of internal energy per floc–floc bond as a result of deformation multiplied by the volume fraction of the polymer in the fractal aggregate network. The scaling relation allows, thus, conclusions to be drawn on the spatial distribution of the aggregates within the network.

Materials and Methods

Vital wheat gluten was provided by Amylum (Aalst, Belgium). Protein, starch, lipid, and ash contents were 76.5, 11.8, 5.0, and 0.8% of dry mass, respectively. The moisture content was 7.2% (wet mass basis). Chemicals were obtained from Sigma in p.a. quality unless stated differently.

Gluten Film Preparation. To produce pressed gluten–water films, vital wheat gluten was equilibrated for 4 days at 100% relative humidity. Thereafter, the average water content, which was determined by drying the samples overnight at 105 °C, was found to be $19.40 \pm 0.11\%$ (wet mass basis). The wet gluten powder (3 g) was hot-molded under a heating press (Techmo, Nazelles-Negrin, France) between two Teflon sheets for 10 min at 150 bar and different press temperatures (70, 80, 100, 120, and 150 °C). The resulting films were immediately cooled to 0 °C and stored at -28 °C in a commercial freezer. Samples fabricated by this process are labeled with “eq” (equilibrated) and the corresponding press temperature.

For the fabrication of mixed and pressed gluten–water films, vital wheat gluten (39.89 g) was mixed with water (10.11 g) in a two-blade counter-rotating batch mixer, type Plasticorder 50 W (Brabender, Duisburg, Germany) turning at 3:2 differential speed. The mixing chamber (50 cm³) was thermoregulated at 20 °C with a cryostat. Samples (50 g) were mixed for 5 min at 30 rpm. The water content of the resulting blend was $22.49 \pm 2.20\%$. The blend was subsequently pressed for 10 min at different temperatures (70, 80, 100, 120, and 150 °C, respectively). The film samples were cooled and stored as described previously. Samples fabricated by this process are labeled with “mi” (mixed) and the corresponding press temperature.

To produce pressed gluten–glycerol blends, vital wheat gluten was hand-mixed with glycerol (35% glycerol, Fluka Chemie, Buchs, Switzerland) in a mortar for 3 min and subsequently rested for 30 min. The blend (3 g) was pressed under the heating press at 150 bar for various times at different press temperatures (70, 80, 100, and 120 °C). The resulting films were immediately cooled and stored as described previously. The film samples’ labels contain the plasticizer name, the fabrication temperature, and the press time. Therefore, the label “gly70-120” stands for at gluten–glycerol film pressed at 70 °C for 120 min.

Gluten Protein Solubility. The gluten samples were analyzed according to Domenek et al.²⁰ The ground gluten–starch blend (160 mg) was agitated for 80 min at 60 °C in 20 mL of 0.1 mol/L sodium phosphate buffer (pH 6.9) containing 1% SDS and subsequently centrifuged (Beckmann Avanti J30-I, 37 000 g, 30 min). The supernatant contained the SDS-soluble protein fraction (F_s). The SDS-insoluble protein fraction (F_i) was extracted with 5 mL of SDS–phosphate buffer containing 20 mmol/L dithioerythriol (DTE) for 60 min at 60 °C, and the tip was sonicated for 3 min to bring the gluten proteins into solution. The supernatant (centrifugation 37 000 g, 30 min) was mixed 1:1 with SDS–phosphate buffer containing 40 mmol/L iodoacetamide (IAM). IAM alkylated the thiol groups, which prevented their subsequent oxidation. Both extracts (20 μ L), F_s and F_i , were submitted to size-exclusion HPLC (SE-HPLC) fractionation.

SE-HPLC analysis was carried out on a TSK-G 4000 SWXL (TosoHaas) size exclusion analytical column (7.5 × 300 mm) preceded by a TSK 3000-SW XL (TosoHaas) guard column (7.5 × 75 mm). The columns were eluted at room temperature with a 0.1 mol/L sodium phosphate buffer (pH 6.9) containing 0.1% SDS. The flow rate was 0.7 mL/min, and proteins were detected at 214 nm. The column was calibrated with protein standards according to Dachkevitch and Autran.²⁴ In the following, F_i is expressed in percent of the total protein quantity, which was estimated from the sum of the F_s and F_i areas (once corrected for their different solid-to-solvent ratios).

Swelling Measurement. For the determination of the swelling of the gluten films, dry samples were immersed in deionized water (containing 0.1% NaN₃ to avoid microbial growth) at a constant temperature (25 °C) for 24 h (according to standard method ASTM D2765-95, method C). To weight the swollen samples, excess water was carefully removed with a paper towel. The samples were subsequently dried over P₂O₅ at room temperature until constant weight. For the calculation of the sample swelling, the dry weight measured *after* swelling and drying was used to take into account the sample weight loss due to the solubility of glycerol in water. The swell ratio q is defined as

$$q = \frac{1}{v_2} = \frac{W_d + |W_s - W_d|K}{W_d} \quad (1)$$

where v_2 denotes the polymer volume fraction, W_d is the sample dry weight (g) *after* swelling, W_s denotes the swollen sample weight (g), and K is the ratio of the densities (g/mL) of the polymer to the solvent = 1.2/0.997; density values from Redl et al.).¹⁰ Each determination was repeated at least four times.

Rheological Investigation of the Cross-Link Density. Tensile experiments on gluten films were conducted with a Dynamic Mechanical Thermal Analyzer MK III (DMTA, Rheometric Scientific, Piscataway, NJ). To standardize the plasticizer content, measurements were performed on samples immersed in deionized water, whereby the plateau modulus can be obtained at ambient temperature using a medium frequency range. The maximum deformation applied was 0.05% (within the domain of a linear elastic response) under a constant static force of 2 g, and the frequency ranged from 1.59 to 0.0159 Hz. Samples were pre-equilibrated in water prior to measurement. The pre-equilibration times had no significant influence on the measured moduli for all the types of films, a behavior which is most likely due to the film geometry (thickness between 150 and 300 μm, length 20 mm, width 5 mm). Therefore, the pre-equilibration of gluten–water films was shortened from 24 h (equals swelling time) to 2 h. The pre-equilibration time of gluten–glycerol films was between 10 min and 2 h. The investigation of the glycerol loss during the pre-equilibration phase revealed that glycerol was completely dissolved after 15 min. Each experiment (irrespective of the equilibration time) was repeated at least four times. In Figure 1 are shown typical results for the average storage modulus of the three types of film samples (eq80, mi70, and gly120-35). The storage and

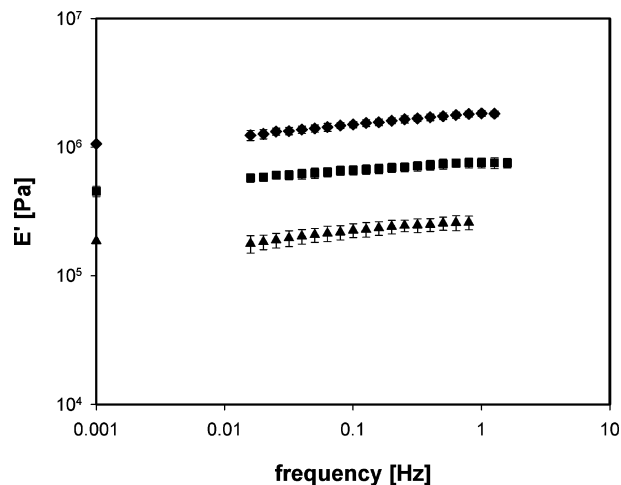


Figure 1. Mechanical spectrum of the storage modulus E' for gluten film samples prepared with varying methods. The value at 0.001 Hz corresponds to the measure of the Young modulus in simple extension. The measurement was conducted on water-immersed samples. Preparation conditions: Gluten–glycerol film pressed at 120 °C for 35 min (◆), gluten–water film mixed for 5 min and subsequently pressed at 70 °C for 10 min (■), and gluten–water film pressed from wet gluten powder at 80 °C for 10 min (▲).

loss moduli were independent of the frequency at 22.5 °C (Figure 1), demonstrating the occurrence of the rubbery plateau.²² As a result of the profound weight loss (35%), the gluten–glycerol films were extremely fragile, a problem that was amplified for lightly cross-linked gluten–glycerol films. As a consequence, either such samples were torn apart during the frequency sweep experiment or the force, which is measured in response to the deformation, was close to the detector limit, which caused nonreproducible and scattered data. Because all the samples that allowed the frequency sweep experiment to be carried out showed the plateau zone in the same frequency range (Figure 1), the value of the modulus was approximated by the Young modulus (E) measured at a given frequency within the plateau zone. E was determined with the DMTA (Rheometric Scientific, Piscataway, NJ) by unidirectional elongation experiments (strain rate 0.001 s^{−1}) on immersed samples at room temperature (22.5 °C). Experiments were repeated at least five times. The Young modulus was computed from the slope of the initial linear increase in engineering stress caused by the strain increase. The points at 0.001 Hz shown in Figure 1 correspond to the so-measured Young modulus. In the following, we will only use the shear modulus (G) of the film samples, which was computed from the modulus measured in tensile experiments (E) by virtue of the relation

$$E = 3G \quad (2)$$

which applies to isotropic incompressible solids.²²

Results and Discussion

Relationship between Gluten Solubility, Shear Modulus, and Film Swelling. Wheat gluten films show a good stability under immersion in water. The experimental results of the sample series, prepared according to three different protocols, are given in Table 1. The percentage of SDS-insoluble protein (F_i) rises with the severity of the heat

Table 1. Biochemical and Rheological Properties of Gluten Films Fabricated under Different Conditions

sample ^a	F_i (%)	E^c (kPa)	v_2^d
vital	13.63 (0.52)	n.d.	0.315
eq70	27.48 (0.41)	$2.60 (0.10) \times 10^1$	0.436 (0.012)
eq80	38.94 (0.58)	$1.81 (0.16) \times 10^2$	0.485 (0.008)
eq90	46.79 (0.70)	$4.30 (0.74) \times 10^2$	0.521 (0.009)
eq100	68.32 (1.02)	$1.90 (0.21) \times 10^3$	0.572 (0.004)
eq120	87.22 (1.31)	$2.99 (0.23) \times 10^3$	0.612 (0.002)
eq150	75.58 (1.13)	$3.69 (0.24) \times 10^3$	0.589 (0.004)
mi70	37.29 (0.56)	$4.53 (0.42) \times 10^2$	0.520 (0.003)
mi80	53.53 (0.80)	$1.02 (0.26) \times 10^3$	0.546 (0.004)
mi90	61.72 (0.93)	$1.15 (0.011) \times 10^3$	0.564 (0.002)
mi100	80.89 (1.21)	$2.83 (0.46) \times 10^3$	0.579 (0.004)
mi120	86.97 (1.30)	$2.55 (0.22) \times 10^3$	0.582 (0.027)
mi150	69.66 (1.04)	$1.72 (0.24) \times 10^3$	0.532 (0.029)
gly100-2	21.52 (0.33)	$7.56 (2.09) \times 10^1$	0.454 (0.004)
gly70-300	27.94 (0.07)	$8.69 (0.61) \times 10^1$	0.389 (0.010)
gly80-80	29.66 (0.33)	$9.67 (0.54) \times 10^1$	0.465 (0.010)
gly80-240	32.50 (1.00)	$1.51 (0.19) \times 10^2$	0.468 (0.015)
gly80-140	33.22 (0.51)	$1.35 (0.32) \times 10^2$	0.461 (0.010)
gly100-10	34.86 (1.61)	$9.95 (2.23) \times 10^1$	0.481 (0.010)
gly100-25	42.75 (0.06)	$1.64 (0.30) \times 10^2$	0.484 (0.021)
gly120-2	45.75 (0.34)	$2.74 (0.38) \times 10^2$	0.485 (0.022)
gly100-60	53.91 (0.69)	$2.39 (0.32) \times 10^2$	0.499 (0.020)
gly120-20	78.61 (2.60)	$7.53 (0.063) \times 10^2$	0.544 (0.20)
gly120-35	83.31 (2.40)	$9.91 (0.50) \times 10^2$	0.553 (0.005)

^a Preparation conditions: pressed gluten–water films “eq”, mixed and pressed gluten–water films “mi”, and pressed gluten–glycerol films “gly”.

^b F_i : biochemically determined content in aggregated protein. ^c E : equilibrium Young modulus at 0.001 Hz. ^d v_2 : gluten volume fraction; the values inside the parenthesis denote the standard deviation of the measurement.

treatment from 13.63 to 87.22%. We observe that the value for the native gluten is quite high, which is explained by the industrial provenance of the gluten. Interestingly, the highest F_i value is obtained for the sample treated at 120 °C (eq120, 87.22%) and not for the sample treated at 150 °C (eq150, 75.58%). Obviously, above a certain severity degree of heat treatment the material begins to degrade. The hypothesis is supported by the observation of an important browning reaction. It had already been shown with kinetic studies that the maximum F_i , attainable with the present experimental setup, is about 90%.²⁰ The Young moduli are similar to the moduli measured on hydrated elastin (at 28–45% H₂O), a well-studied elastomeric protein,²⁵ and at low F_i (<35%), similar to the Young modulus reported for chemically cross-linked glutenin subunits measured in water ($\sim 1.1 \times 10^2$ kPa; strain rate not given).²⁶ The range of the swelling values is quite restricted. The maximum swelling of vital gluten (polymer volume fraction $v_2 = 0.315$) corresponds to the water holding capacity of native gluten (ca. 2.8 g of water for 1 g of gluten). The minimum swelling value obtained was observed for sample eq120 ($v_2 = 0.612$) and corresponded to a water uptake of 0.83 g/g gluten. Gluten materials show, thus, a quite satisfying form stability when brought into contact with water.

Let's first investigate the relationship between the biochemical parameter F_i and the structural parameters equilibrium modulus (G) and polymer volume fraction (v_2). Figure 2 shows v_2 as a function of F_i , where we observe the previously described large change of F_i and the restricted

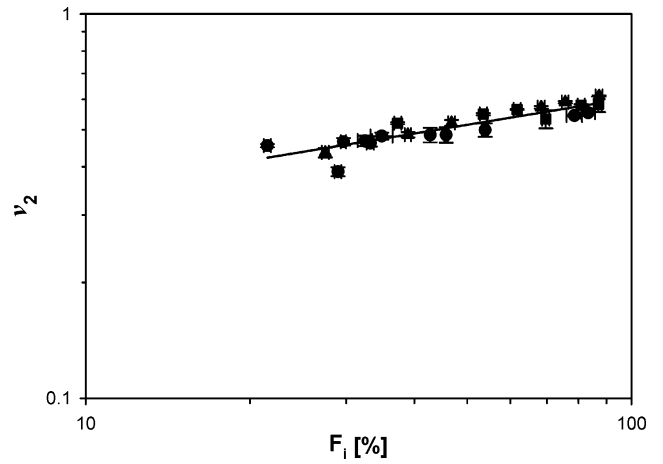


Figure 2. Polymer volume fraction (v_2) as a function of the percentage of aggregated proteins (F_i). Data show a good agreement with a power law of exponent 0.23 (solid line). Symbols signify the different sample preparation methods: eq (●), mi (■), and gly (◆).

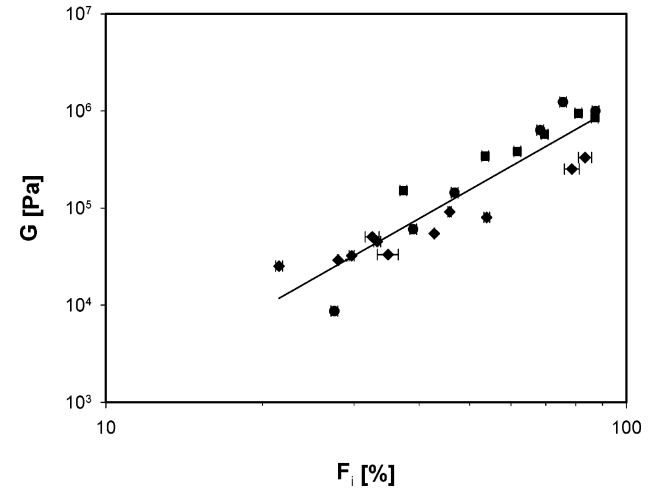


Figure 3. Shear modulus (G) as a function of the percentage of aggregated proteins (F_i). Data are aligned on a power law of exponent 3 (solid line). Sample preparation methods: eq (●), mi (■), and gly (◆).

range of v_2 . Furthermore, we see that the film swelling decreases significantly with more severe heat-treatments and that there is a unique and quite satisfying correlation between both parameters irrespective of the film preparation procedure. Figure 3 shows the relationship between the Young modulus E (and thus G) and F_i , where we observe that G rises over 3 orders of magnitude with increasing F_i . This proves increasing network connectivity within the film samples. In our case, the cross-links may correspond to different types of interactions, because the gluten protein network is supposed to be stabilized by covalent (disulfide) bonds as well as by noncovalent interactions; the latter being hydrogen bonds and hydrophobic interactions. It is known that the F_i rise is caused by the formation of disulfide bonds between different gluten polypeptides,^{2,6,7,16,27–29} which is indirectly proven by the analytical method used. Actually, a reducing agent of disulfide bonds (DTE) is required to bring the SDS-insoluble proteins (F_i) into solution. SDS alone is insufficient because it has a strong disrupting effect only on noncovalent interactions. A single continuous relationship was found, between the parameters F_i and G , indicating the predominant influence of covalent bonds on the rheological

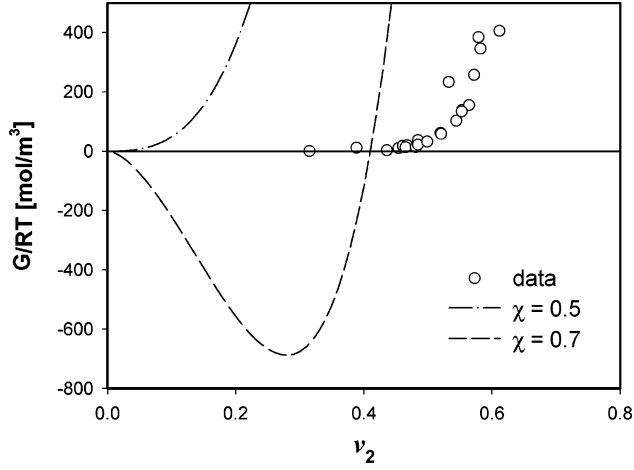


Figure 4. Comparison between the Flory–Rehner theory for two constant interaction parameters (using the water’s $\varphi_1 = 18.0182$ mL/mol) and the experimental data.

behavior. The single relationship notwithstanding, the film fabrication method shows furthermore that differences in the physical treatment or in the type of the plasticizer do apparently not change the structure of the protein system. Furthermore, the strong variation of G when changing F_i shows that either the contribution of the noncovalent bonds is negligible or increases in parallel with the covalent cross-links. The power law relationship between F_i and G was found to result in

$$G \propto F_i^3 \quad (3)$$

Such behavior is known from the field of gelation, which in turn is a percolation problem. Above a certain critical concentration of activated cross-links (N_c), a macroscopic cluster appears. Its mass fraction, which is essentially our quantity F_i , is found to vary linearly with the distance from N_c , which is the normalized cross-links number above the critical value $(N - N_c)/N_c$.³⁰ The system’s shear modulus G , on the other hand, varies like the cube of this distance.³⁰ Taken together, this predicts G to vary as F_i^3 , which is in good agreement with eq 3. Note that the critical cross-link number will be reached at $F_i = 0$.

Gluten Network Structure. Both structural parameters, G and v_2 , were well-correlated to F_i , which raises the question of the theoretical basis of the relationship between G and v_2 . The relationship in the case of our data is given in Figure 4, which shows a steep increase of the modulus with increasing polymer volume fraction. In the field of rubber elasticity, widely accepted and commonly used for entangled (and cross-linked) polymers, a classical model exists to formalize this relationship, the Flory–Rehner theory of rubber elasticity.²¹ According to this theory, the density of cross-links (G/RT) and, thus, the equilibrium shear modulus G ,^{21,31} is related to the polymer volume fraction v_2 by

$$\frac{G}{RT} = \frac{1}{\varphi_1} \frac{\ln(1 - v_2) + v_2 + \chi v_2^2}{v_2/2 - v_2^{1/3}} \quad (4)$$

where φ_1 is the solvent’s molar volume (here water with $\varphi_1 = 18.0182$ mL/mol) and the interaction parameter χ describes the energy gain ($\chi < 0$) or penalty ($\chi > 0$) of a

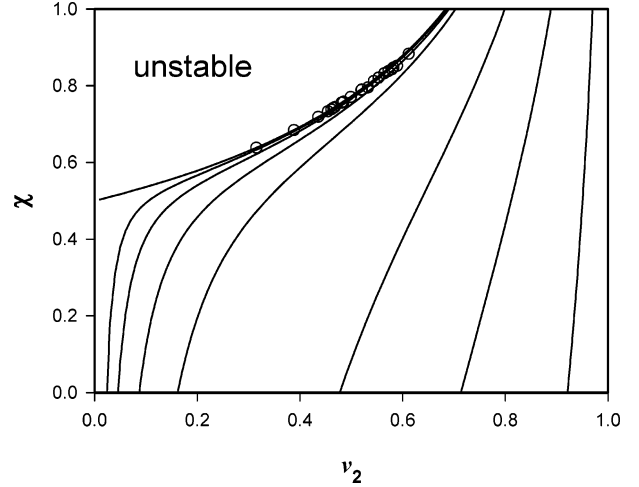


Figure 5. Logarithmic contour plot of $g \equiv \varphi_1 G/(RT)$ for 10 different values: $\log(g) = -\infty, -3, -2.5, -2, -1.5, -1, -0.5, 0, 0.5$, and 1 (from left to right). The region above $g = 0$, marking a thermodynamically unstable system, defines a border of the theory’s applicability. Our data (eq 4 solved for χ , open circles) are found in the immediate vicinity of the limiting curve $G = 0$, because of the small shear modulus, that is, $G/(RT) \ll 1/\varphi_1$.

monomer surrounded by the solvent. However, as shown in Figure 4, no correspondence is achieved by implementing eq 5 with a constant χ .

The possibility of a nonconstant χ has already been considered in the original work,²¹ and it is not uncommon to fit the function $\chi(v_2)$ (obtained by solving eq 4 for χ) by a polynomial (typically of degree 2).^{31–33} The corresponding fit to our data yields

$$\chi = 0.530 + 0.0848v_2 + 0.797v_2^2 \quad (5)$$

with an, at first sight stunningly, excellent correlation coefficient of 0.9992. However, as already indicated by Figure 4, we deal with a discrepancy of a shear modulus G that is several orders of magnitude lower (for a given value of φ_1 and range for v_2) than typical values given by the Flory–Rehner theory, and a comparison with Figure 5 shows that the latter permits these very low values only in a very narrow region in the vicinity of the line $G = 0$, beyond which the system becomes thermodynamically unstable. As shown in Figure 5, our data practically coincide with this line; that is, the obtained result $\chi(v_2)$ bears little physical meaning.

An alternative approach would be the introduction of an effective molar volume $\bar{\varphi}_1$ and an effective relative solvent volume \bar{v}_2 , which corresponds to rescaling the axes in the plot G versus v_2 . Figure 6 shows this approach in a double logarithmic plot, where rescaling reduces to shifting the curve. Obviously, no agreement between data and modeled curves can be obtained, whatever the rescaling of the axes. Irrespective of the actual v_2 dependence of G , it can be clearly seen from Figure 6 that the variation of G over 3 orders of magnitude cannot be a generic result of the Flory–Rehner theory [which is able to produce such variations only at its very limits, and for that it needs an extremely well-tuned interaction “parameter-function” $\chi(v_2)$]. Therefore, something other than a gradual change of rubber elasticity must be going on.

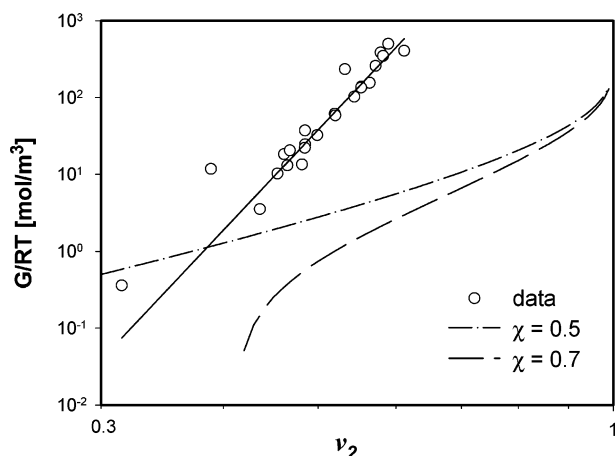


Figure 6. System's shear modulus in a double logarithmic plot compared to (rescaled by an unphysical factor of 1800 with respect to the molar volume of water) results of the Flory–Rehner theory for $\chi = 0.5$ and $\chi = 0.7$. The exponent of the indicated power law amounts to 14.

Actually, the data are fitted rather well by a power law, albeit with an unusually large exponent of about 14, that is,

$$G \propto \nu_2^{14} \quad (6)$$

Another answer is that of a particle network. Marangoni²³ derived a relationship between the shear modulus and the volume fraction in a particle network where the mesoscopic particles are formed by microscopic entities (i.e., proteins in our case) in a fractal manner:

$$G \propto \nu_2^{1/(3-D)} \quad (7)$$

where D is the fractal dimension of the packing within the particles and 3 is the Euclidian dimension in which the particles themselves pack. Accordingly, an exponent of about 14 would correspond to a fractal dimension of the gluten particles of $D \approx 2.93$, which is close to the range of dimensions observed for fractal flocs in different types of foodstuff (2.37–2.88).²³

There are other hints that support this interpretation: Hamer and van Vliet¹² proposed that the gluten network is organized as a particle network, and recently their research team showed such a structure for the gluten macropolymer in solution in SDS¹⁴ using confocal scanning laser microscopy. The authors supposed this structure to reflect the initial structure of gluten particles deposited in the wheat endosperm. Although Don et al.¹⁵ showed that the particle structure was quite stable even during the mixing process, the observation of an artifact due to the use of SDS cannot be ruled out completely. On the other hand, a hint for a fractal inner structure of particles was found by means of small angle neutron scattering and light scattering by Popineau (Yves Popineau, personal communication) and at least the existence of a particle network is discussed by Lefebvre et al.¹³

The rather high value of the fractal dimension might indicate a quite “dense packing” inside the particles, but we have also to keep in mind that the cause of the higher volume fraction (i.e., the greater number of cross-links) can have a simultaneous effect on the structures inside the particles, something that is not contained in the theory.²³ A quantitative

hint to this complicated interdependence is given by the relationship between F_i and ν_2 (Figure 2), obtained from combining eqs 3 and 6 as

$$\nu_2 \propto F_i^{3/14} \quad (8)$$

On the other hand, it should be admitted that the quality and the span of the data is not sufficient to actually *prove* a power law exponent of 14 for the algebraic dependence of G on ν_2 (already due to their scattering, a lower exponent of about 11 cannot be ruled out), but it indicates the possibility of the suggested scenario, resulting in such a strong variation of G .

Conclusion

We have investigated the wheat gluten network structure upon immersion in water. Both the equilibrium shear modulus and the equilibrium swelling were well-correlated to the biochemically determined percentage of aggregated proteins in gluten, showing the high importance of disulfide bonds for the gluten protein network structure. The single relationship between aggregated proteins and the modulus or swelling notwithstanding the sample fabrication conditions shows that the network formed is independent from the thermomechanical treatment of gluten. The failure of the Flory–Rehner model to describe the swelling properties of gluten shows clearly that the protein network, although elastic, cannot be modeled with the classical theories of rubber elasticity. Entropy as the primary driving force for the elasticity of gluten is, therefore, quite questionable. In terms of the Flory–Rehner model, the network strands are not completely floppy (i.e., free from free energy contributions) as assumed by this meanfield model, but rather rigid, not being able to give rise to a significant swelling, despite their comparatively low moduli. This implies further that wheat gluten cannot be considered as an entangled network. Instead, the very strong rise of the modulus with increasing polymer volume fraction indicates a structure being organized as a dense network of mesoscale particles with an inner irregular, fractal structure. This mesoscale image strengthens the suggestion that the network organization reflects the gluten structure as deposited during the growth of the wheat endosperm.¹⁴ How the cross-link structure acts in detail as confining “laces” to yield the gluten’s high volume fraction and in how far it influences the particles’ inner structure directly, giving rise to the observed scaling exponents, remain to be investigated.

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References and Notes

- (1) Wrigley, C. *Nature* **1996**, *381* (June), 738–739.
- (2) Shewry, P.; Tatham, A. *J. Cereal Sci.* **1997**, *25*, 207–227.
- (3) Tsiami, A.; Bot, A.; Agterof, W. *J. Cereal Sci.* **1997**, *26*, 279–287.
- (4) Redl, A.; Morel, M.; Bonicel, J.; Guilbert, S.; Vergnes, B. *Rheol. Acta* **1999**, *38* (4), 311–320.
- (5) Lefebvre, J.; Popineau, Y.; Deshayes, G.; Lavenant, L. *Cereal Chem.* **2000**, *77* (2), 193–201.

- (6) Weegels, P.; van de Pijpkamp, A.; Graveland, A.; Hamer, R.; Schofield, J. *J. Cereal Sci.* **1996**, 23, 103–111.
- (7) Weegels, P.; Hamer, R.; Schofield, J. *J. Cereal Sci.* **1996**, 23, 1–18.
- (8) Macritchie, F. *Cereal Foods World* **1999**, 44 (4), 188–193.
- (9) Belton, P. *J. Cereal Sci.* **1999**, 29, 103–107.
- (10) Redl, A.; Morel, M.; Bonicel, J.; Vergnes, B.; Guilbert, S. *Cereal Chem.* **1999**, 76 (3), 361–370.
- (11) Singh, H.; MacRitchie, F. *Cereal Chem.* **2001**, 78 (5), 526–529.
- (12) Hamer, R.; van Vliet, T. In *Wheat Gluten – Proceedings of the 7th International Workshop Gluten 2000*; Shewry, P., Tatham, A., Eds.; Royal Society of Chemistry: London, 2000; pp 125–131.
- (13) Lefebvre, J.; Pruska-Kedzior, A.; Kedzior, Z.; Lavenant, L. *J. Cereal Sci.* **2003**, 38 (3), 257–267.
- (14) Don, C.; Lichtendonk, W.; Plijter, J.; Hamer, R. *J. Cereal Sci.* **2003**, 37, 1–7.
- (15) Don, C.; Lichtendonk, W.; Plijter, J.; Hamer, R. *J. Cereal Sci.* **2003**, 38, 157–165.
- (16) Schofield, J.; Bottomley, R.; Timms, M.; Booth, M. *J. Cereal Sci.* **1983**, 1, 241–253.
- (17) Weegels, P.; Verhoek, J.; deGroot, A.; Hamer, R. *J. Cereal Sci.* **1994**, 19, 31–38.
- (18) Weegels, P.; Verhoek, J.; deGroot, A.; Hamer, R. *J. Cereal Sci.* **1994**, 19, 39–47.
- (19) Morel, M.; Redl, A.; Guilbert, S. *Biomacromolecules* **2002**, 3 (3), 488–497.
- (20) Dömenek, S.; Morel, M.; Bonicel, J.; Guilbert, S. *J. Agric. Food Chem.* **2002**, 50 (21), 5947–5954.
- (21) Flory, P. *Principles of polymer chemistry*; Cornell University Press: London, 1953.
- (22) Ferry, J. *Viscoelastic properties of polymers*, 3rd ed.; John Wiley and Sons: New York, 1980.
- (23) Marangoni, A. *Phys. Rev. B* **2000**, 21, 13951–13955.
- (24) Dachkevitch, T.; Autran, J.-C. *J. Cereal Chem.* **1989**, 66 (6), 448–456.
- (25) Gosline, J. *Rubber Chem. Technol.* **1987**, 60, 417–438.
- (26) Tatham, A.; Hayes, L.; Shewry, P.; Urry, D. *Biochim. Biophys. Acta* **2001**, 1548, 187–193.
- (27) Weegels, P.; Hamer, R. In *Interactions: The keys to cereal quality*; Hamer, R., Hoseney, R., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1998; pp 95–130.
- (28) Kasarda, D. *Cereal Foods World* **1999**, 44 (8), 566–571.
- (29) Shewry, P.; Popineau, Y.; Lafiandra, D.; Belton, P. *Trends Food Sci. Technol.* **2001**, 11, 433–441.
- (30) Olemskoi, A.; Krakovsky, I. *Physica A* **2001**, 291, 79–8.
- (31) Hedden, R.; Wong, C.; Cohen, C. *Macromolecules* **1999**, 32, 5154–5158.
- (32) Orwoll, R. *Rubber Chem. Technol.* **1977**, 50, 452–479.
- (33) Okay, O.; Durmaz, S.; Erman, B. *Macromolecules* **2000**, 33 (13), 4822–4827.