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Relaxation dynamics in hydrated gluten networks

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ABSTRACT

Stress relaxation in hydrated gluten networks was investigated by means of rheometry and confocal laser scanning microscopy. Stress relaxation was followed for 30 min over a wide temperature range (0 −70 °C). Temperature played a significant role in relaxation, allowing the construction of mastercurves and calculation of shift factors. This approach revealed a continuous relaxation with absence of plateau modulus, typical of polydisperse materials of low molecular weight. Calculation of stress relaxation spectra identified three relaxation regimes. Stress relaxation is independent of compositional differences, although, confocal microscopy showed the influence of protein composition on the morphology of the networks. Utilization of concepts from macromolecular dynamics and poroelasticity allowed a first insight to the mechanisms of relaxation. Reptation of chains in combination with water migration from the pores of the network seems to play major role in the relaxation mechanisms. Description of relaxation phenomena under such a theoretical framework allows better understanding of the rheological properties of gluten with the aim to improve its industrial performance.

1. Introduction

Thermal, dielectric or mechanical relaxations are frequently used to examine various aspects of polymeric melts and solutions or cross-linked polymers and gels. The objective of such a treatment is to gain insight into macromolecular rearrangements, thus allowing the understanding of physicochemical properties of materials (Rubinstein and Colby, 2003). In rheology, relaxation is the attempt of a deformed specimen to reach a steady state following application of strain. The objective is to calculate the relaxation time τ, a fundamental dynamic property of viscoelastic bodies that gives information on chain rearrangements as macromolecules arrive at a state of lower energy. Examination of mechanical relaxations in viscoelastic materials proceeds *via* calculation of the relaxation spectrum, as it is responsive to changes in the fine structure of polymers such as molecular weight distribution, branching, network formation or composition.

Gluten is a complex material of great financial significance for the food industry. With its unique functional properties, it also forms the basis of production of novel biomaterials such as biodegradable plastics, films or foams. Understanding relaxation behavior of gluten proteins is of paramount importance, as it enables improvement of the industrial performance of flours. Characterization of relaxation behavior of hydrated gluten has received attention (Li et al., 2003; Ng and McKinley, 2008; Rao et al., 2000) but detailed analysis on the contribution of individual protein fractions to the relaxation is lacking. Furthermore, the interrelationships between composition, temperature, relaxation time and microstructure are still unclear. The present work aims to unveil the relaxation behavior of hydrated gluten composites and give a fundamental description of the dynamic processes involved.

2. Materials and methods

2.1. Materials and chemicals

General-purpose wheat flour was purchased from the local market for gluten isolation. Sodium chloride, acetic acid, dialysis membrane tubing (MwCO 12000) and formaldehyde (37–40%) were purchased from Sigma–Aldrich (St. Luis, MO) and ethanol from Fisher Scientific (Loughborough, UK).

2.2. Protein isolation

Wheat flour was mixed with 0.2 M NaCl in a ratio of 1.5:1 (flour:saline water) to create dough. The dough was wrapped with cling film and allowed to hydrate for 30 min at 4 °C. Following the end of the hydration period, the dough was washed under running deionized water until a clear solution was observed (indicating starch removal). The resulting material was subsequently freeze-dried and ground into powder.

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To isolate the gliadin fraction, gluten powder was dispersed (5% w/v) in 0.5 M NaCl for 30 min at room temperature to remove albumins. The resulting dispersion was centrifuged at 5000*g* for 15 min. The supernatant was discarded and the resulting pellet was rinsed with deionized water and dispersed in 70% v/v ethanol (1:1 ratio) overnight at room temperature. The resulting solution was centrifuged at 5000*g* for 15 min and the supernatant containing the gliadin fraction was concentrated using a rotary evaporator at 35 °C. The concentrated gliadin fraction was freeze-dried and ground into powder. The pellet from the centrifugation step was rinsed with deionised water, dispersed in 0.1 M acetic acid (1:1 ratio) and stirred overnight at room temperature. The supernatant containing the glutenin fraction was further centrifuged at 5000*g* for 15 min and extensively dialyzed (3 days) to remove acetic acid before being freeze-dried and finally ground into powder.

2.3. Sample characterization

Samples obtained from the above isolation procedures were subjected to proximate analysis (International Laboratory Services, Shadlow, UK). Electrophoresis was conducted under reducing conditions (dithiothreitol) using the Novex® NuPAGE® SDS-PAGE Gel System (Life Technologies Ltd, Paisley, UK). The proteins were separated on gradient precast gels (4–12%) and stained with Coomassie Blue stain.

2.4. Sample preparation and stress relaxation measurements

Isolated gluten (Glut), glutenin-enriched (Gln), gliadin-enriched (Gli) fractions and their mixtures in ratios of 3:1, 1:1 and 1:3 (Gln:Gli) were prepared for stress relaxation measurements. Samples contained 40% w/w total protein solids (60% w/w deionized water), as described elsewhere (Kontogiorgos et al., 2007).

Stress relaxation measurements were performed between 0 and 70 °C using a Bohlin Gemini 200HR-nano rotational rheometer (Malvern Instruments, Malvern, UK) equipped with serrated plate-serrated plate geometry (25 mm diameter and 900 μm gap). Experimental protocol of the present investigation included the following steps:

i) Shear strain amplitude sweep experiments were performed between 0 and 70 °C using angular frequency of 6.28 rad/s to determine the linear viscoelastic region (LVR) of the samples. 2% strain was found to be within the LVR of all samples and to give satisfactory signal-to-noise ratio.
ii) Time sweeps in dynamic oscillation on shear were executed at 6.28 rad/s and 2% strain for 60 min revealing that storage (G') and loss moduli (G'') reach pseudo-equilibrium within 10 min. Therefore, samples were left to equilibrate for 10 min before measurements to dissipate stresses that were created during loading.
iii) Stress relaxation tests were carried out using 2% instantaneous strain for each sample. Reproducible and highly resolved relaxation spectra were obtained with 30 min relaxation following application of the instantaneous strain (Kontogiorgos et al., 2009). Data of stress relaxation modulus ($G(t)$) were collected in logarithmic mode with respect to the time scale of observation. Strain rise time was 20 ms and data point collection started after 30 ms.

A thin layer of low viscosity silicone oil (polydimethylsiloxane, Sigma–Aldrich, St. Louis, MO) was also applied to minimize moisture loss during the course of experiments. Mechanical measurements were performed ten times for each sample and averaged curves are reported. Nonlinear regression was performed with GraphPad Prism v.6 (Graph-Pad Software, San Diego, USA).

2.5. Numerical computation

Numerical computation was performed in MATLAB (v7.0 R14 Service Pack 2, The Mathworks Inc., MA) as previously described (Kontogiorgos et al., 2009). The first step involves discretization of the stress relaxation function to create matrix *A* and was performed with the *discr.m* script published elsewhere (Kontogiorgos et al., 2009). Following that step, algorithms *csvd.m* for calculation of the singular value decomposition of matrix *A* and *l_curve.m* for computation of the optimum regularization parameter were used from Hansen's regularization tools package (Hansen, 1994). Finally, the algorithm *NLCSmoothReg.m* was used for the calculation of the spectra (Wendlandt, 2005). Numerical integration of the spectra was carried out using trapezoidal numerical integration in MATLAB with the function *trapz*.

2.6. Confocal laser scanning microscopy

Samples prepared for stress relaxation measurements were embedded at ambient temperature in CRYO-M-BED resin (Bright, Huntingdon, UK), quenched frozen and sectioned at −20 °C using a cryostat (Bright Starlet 2212, Huntingdon, UK) to obtain sections with thickness between 8 and 12 μm. Sectioned samples were collected on a slide and fixed immediately using 10% v/v PBS-buffered (phosphate buffer saline) formalin solution for 15 min. Slides were then rinsed three times for 5 min in 1xPBS and left to dry at room temperature. Sections were imaged under a confocal laser scanning microscope using intrinsic protein fluorescence without further treatment (Zeiss LSM510 META Upright, Heidelberg, Germany) at an excitation wavelength of 488 nm and emission wavelengths above 515 nm equipped with argon laser (488 nm) operating at 5% output. z-Stacks with resolution 1024x1024 pixels were captured using the Zen 2009 software. z-Projected images have been produced using "maximum intensity" projection in ImageJ v1.74b.

3. Results and discussion

3.1. Material characterization

The isolation procedure adopted in the present investigation resulted in samples with the following compositional characteristics on a wet basis: gluten: 83% protein, 3% fat, 8% moisture, 5% carbohydrates, gliadin: 84% protein, 2% fat, 10% moisture, 3% carbohydrates and glutenin: 84% protein, 2% fat, 9% moisture, 4% carbohydrates. All samples are of high purity with minimal amount of fat and carbohydrates that is very important for material characterization purposes. The presence of starch is of particular importance as it could function as a non-interacting filler and change substantially the mechanical responses of the samples. The carbohydrate concentration in our samples was estimated by difference and should be attributed to non-starch carbohydrates (arabinoxylans, cellulosic oligomers, neutral sugars) and a minimal amount of starch that remained after washing the flour. Starch granules (5–20 μm typical range of wheat starch) do not seem to be an integral structural element in our samples (Fig. 5). Additionally, the stress relaxation modulus requires at least a 20% step in starch content to show measurable changes in gluten-starch mixtures (Uthayakumaran et al., 2002). In consequence, the relaxation behavior that is described in the following sections can be solely attributed to proteins in the network and not to starch or lipids that are present as contaminants during the isolation steps.