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Temperature dependence of relaxation spectra for highly hydrated gluten networks

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ABSTRACT

In the present investigation, the temperature dependence (0–50 °C) of the relaxation spectrum of hydrated gluten was studied using novel numerical algorithms. Tikhonov regularization, in conjunction with the L-curve criterion for optimal calculation of the regularization parameter, was used to generate the relaxation spectrum from stress relaxation measurements on shear. The methodology used revealed six molecular events with baseline resolution that could be grouped into fast- and slow-relaxation regimes. The fast-relaxation regime exhibited strong temperature dependence whereas the slow one is temperature independent indicating on the whole two dominant mechanisms of interactions. The "loop and train" structural model for gluten interactions was found adequate to describe the relaxation events in this system, with the fast regime being assigned to interactions due to hydrogen bonding whereas the slow one to permanent cross-linking of the entire network. Findings of the present investigation provide fundamental understanding and give new insights into the complexity of interactions and relaxation modes of hydrated gluten.

1. Introduction

Characterization of biopolymers becomes increasingly important as emerging applications in microencapsulation, nanotechnology, biodegradable packaging and soft-solid "intelligent" gels require detailed information on their structural behavior (Kasapis, 2008; Sozer and Kokini, 2009). In particular, mechanical measurements within the linear regime of biopolymer networks provide information on the rotation of groups or atoms about individual bonds (conformational rearrangements) and the degree of interaction of these macromolecules with the solvent or neighboring co-solutes (Ferry, 1980). Short- and long-range interactions among polymeric species establish the character of the mechanical response of that system (Rubinstein and Colby, 2003). Understanding the structural behavior of biomaterials requires probing the relaxation patterns of molecular events, and a fundamentally valid approach to achieve this is *via* the concept of the mechanical relaxation spectrum (Malkin, 2006). From the relaxation spectrum with interconversions we can also obtain other viscoelastic functions that further enhance our knowledge of the structure-function relationships of materials (Emri et al., 2005; Ferry, 1980).

An experimental methodology to obtain the relaxation spectrum that is frequently used to characterize solid-like viscoelastic specimens is the stress relaxation function on shear in the linear regime. Such a function after sudden application of strain is given by:

$$\sigma(t) = \sigma_e + \int_0^{+\infty} \sigma(\tau)\exp\left(-\frac{t}{\tau}\right)\mathrm{d}\tau \quad (1)$$

where $\sigma(t)$ is the gradual relaxation of stress to the equilibrium stress (σ_e, complete material relaxation means that $\sigma_e = 0$), with $\sigma(\tau)$ being the distribution function of the elements with relaxation time, τ; the relaxation function $\sigma(\tau)$, therefore, must be calculated from measurements of $\sigma(t)$.

The stress relaxation "problem" is formulated through the use of mechanical analogies (Maxwell model), hence the continuous integral transformation can be represented with a discrete sum of exponents (Malkin, 2006):

$$\sigma(t) = \sum_0^N \sigma_n e^{-t/\tau_n} \quad (2)$$

with τ_n corresponding to the relaxation times with amplitude σ_n and N being the number of the different relaxing elements. Thus the integral (1) can be written in terms of the generic form of the first-kind Fredholm equation:

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$$g(s) = \int_a^b K(s,t)f(t)\mathrm{d}t, \alpha \le s \le \beta \quad (3)$$

where, $K(s,t)$ is the kernel $\exp(-t/s)$ that describes the system, $g(s)$ is the measured signal, and $f(t)$ is the unknown integral solution. The objective in this type of analysis is to determine the spectral function $f(t)$ that represents the relaxation spectrum $\sigma(\tau)$ of the material.

Numerical treatment of this procedure is not a straightforward task since the Fredholm integral equation is a classical example of an ill-posed problem that requires a special mathematical approach (Groetsch, 1984). Ill-posed problems in mechanical spectroscopy and numerical methods to solve them have been discussed in the literature (Brabec et al., 1997; Elster et al., 1991; Friedrich et al., 1996; Honerkamp, 1989; Honerkamp and Weese, 1993). Previously, we described the protocol available for the calculation of relaxation spectra in biopolymer gels (Kontogiorgos et al., 2009). The objective of the present work is to utilize this type of analysis in order to probe molecular events in the hydrated gluten network using relaxation spectra and regularization tools.

2. Materials and methods

2.1. Sample preparation

Samples of hydrated gluten (40% w/w protein solids, 60% w/w deionized water) from Sigma–Aldrich (St. Louis, MO) were prepared as described previously (Kontogiorgos et al., 2007), wrapped thoroughly with a plastic membrane and left to hydrate at 4 °C for 30 min.

2.2. Stress relaxation measurements

Stress relaxation measurements were performed with the Advanced Rheometrics Expansion System (ARES, TA Instruments, New Castle, DE), which is a controlled strain rheometer. ARES is equipped with a mechanical chiller for temperature regulation (Polycold Gas Chiller, Polycold Systems International, CA) and controlled using the operational software accompanying the instrument (TA Orchestrator).

Experimental protocol of the present investigation includes the following steps.

(i) At the end of the hydration period, samples were loaded onto the preheated platen of the rheometer (0, 10, 20, 30, 40 or 50 °C) employing parallel plate geometry of 40 mm diameter and 2 mm gap. Preliminary time sweeps in dynamic oscillation on shear, which were carried out at 1 rad/s and 3% strain, showed that the elastic (storage modulus; G') and viscous (loss modulus; G'') components of the network reached a "pseudo-equilibrium" plateau within 10 min. Therefore, samples were left to equilibrate for 10 min prior to measurement.

(ii) Strain sweeps in dynamic oscillation on shear were carried out at 0, 10, 20, 30, 40 and 50 °C to identify the linear viscoelastic region (LVR) of the sample under test conditions and an angular frequency of 1 rad/s.

(iii) Stress relaxation tests were carried out using the % instantaneous strain at each different temperature as calculated in the previous step. Thirty minutes relaxation following application of the instantaneous strain was found to be appropriate to obtain reproducible and highly resolved relaxation spectra with this particular system (Kontogiorgos et al., 2009). Data of stress relaxation modulus ($G'(t)$) were collected in a logarithmic mode with respect to the timescale of observation.

A thin layer of low viscosity silicone oil (dimethylpolysiloxane, Sigma–Aldrich, St. Louis, MO) was also applied to minimize moisture loss during the course of stress relaxation testing. Mechanical measurements were performed in triplicate at each temperature using different samples, and representative figures that depict accurately the behavior of the material are illustrated.

2.3. Numerical computation

Numerical computation was performed in MATLAB (v7.0 R14 Service Pack 2, The Mathworks Inc., MA) in three steps (Kontogiorgos et al., 2009). Discretization of kernel $K(s,t)$ (Eq. (3)) to create matrix A was performed with the *discr.m* script that is published elsewhere (Kontogiorgos et al., 2009). Following creation of matrix A, Hansen's regularization tools package were used (Hansen, 1994, 2002). Specifically, scripts *csvd.m* (calculation of the singular value decomposition of matrix A), *l_curve.m* (creation of the L-plot and calculation of the optimum regularization parameter) and *tikhonov.m* (calculation of the spectrum) were used to perform data analysis.

3. Results and discussion

3.1. Stress relaxation measurements

The objective of the experimental work was to probe molecular relaxations and explore their temperature dependence hence stress relaxation tests were conducted in the linear viscoelastic region (LVR) of gluten networks. LVR varies with temperature, and it must be calculated for all temperatures used presently. Depending on temperature, this spreads up to 20% deformation and the value of 3% used for stress relaxation measurements is well within the acceptable limits (Lefebvre et al., 2000). Furthermore, ice formation as monitored by the development of storage modulus begins at about −5 °C and concludes at −13 °C (Jiang et al., 2008; Kontogiorgos and Goff, 2006), therefore, signals obtained at 0 °C represent molecular relaxations of the hydrated gluten network.

Stress relaxation measurements were obtained next and plotted in semi-logarithmic scale (Fig. 1). Plotting data in semi-logarithmic mode is necessary so as to determine whether the sample has

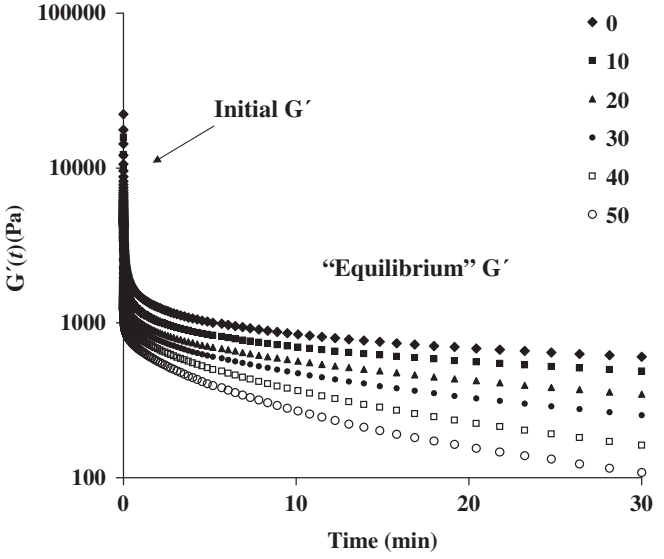

Fig. 1. Stress relaxation measurements for hydrated gluten at various temperatures (0–50 °C) showing the initial stress relaxation modulus that decays fast in the first few minutes and reaches a pseudo-equilibrium plateau.

reached a pseudo-equilibrium $G'(t)$ value. Long baselines are necessary for the numerical algorithms to calculate accurately the relaxation spectrum. However, time dependant effects, slippage or aging of the material will change eventually the three-dimensional morphology, hence prolonged experimentation is not feasible. In the present investigation, samples relaxed until the transient lost more than 95% of its initial $G'(t)$ value, and this can be considered as a "fully" decayed process. As shown in Fig. 1, temperature increase results in modulus decrease and in the fifty degrees range of operation, modulus values fall about an order of magnitude.

Decrease in rigidity reflects changes in the relaxation modes of the sample and these will be discussed in detail in the following section. Furthermore, traces of stress relaxation modulus exhibit a gradient as a function of temperature, an outcome that may introduce errors in the analysis. This is an inevitable consequence of temperature increase and can be alleviated only by extending the duration of relaxation to several hours. We have previously shown that relaxation spectra obtained with such subtle variations in the baseline (fully or partially decayed) can also resolve the majority of the peaks so as to identify the molecular processes that are primarily responsible for relaxation in the sample (Kontogiorgos et al., 2009). Taking into consideration that all samples were investigated within LVR, these minor changes in the baseline are unlikely to interfere with the resolution of spectra and interpretation of the data described next.

3.2. Calculation of relaxation spectra

After acquiring stress relaxation data, Hansen's algorithms that employ Tikhonov regularization were used to convert data to the relaxation spectrum of gluten. To obtain the optimum spectral resolution using this procedure it is necessary to calculate the optimum regularization parameter, λ. The regularization parameter controls the filtering of the spectrum noise or in other words the balance between the regularization error and the loss of resolution (smoothness) of the solution (Hansen, 1992, 1994). The most appropriate tool to analyze this type of problem is the so-called L-curve method, which is a plot of the solution and residual norms for all valid regularization parameters (Hansen, 1992). The vertical part of the curve corresponds to solutions that are sensitive to perturbation errors whereas the horizontal part to solutions where the regularization (calculation) error dominates. The optimum regularization parameter λ is located at the corner of the curve and this value is used to calculate the optimum least square solution of the spectrum with the Tikhonov minimization.

Fig. 2 reproduces the result of calculations of the optimum regularization parameter using the L-curve method for all different temperatures (0–50 °C). As it is evident, parameter λ varies significantly with changes in temperature and calculation of a different regularization parameter for every dataset is imperative so as to obtain meaningful and comparable relaxation spectra. Another parameter that must be optimized is the range of the relaxation space where the relaxation times of the material will lie. A heuristic approach was adopted to calculate the optimum relaxation space by varying the value of its width until a stable and well resolved spectrum was obtained. The best resolved spectra were obtained when the range of the relaxation space spanned from 10^{-3} to 10^{4} s. It has been previously suggested that artifacts due to inertia may affect the spectra especially peaks at the short relaxation times (Li et al., 2003). The instrument we used to obtain stress relaxation data virtually eliminates the need to correct for motor friction and inertia as the sensitivity and the torque resolution is in the nNm range, as opposed to that of instruments with inferior technical specifications employed in previous investigations.

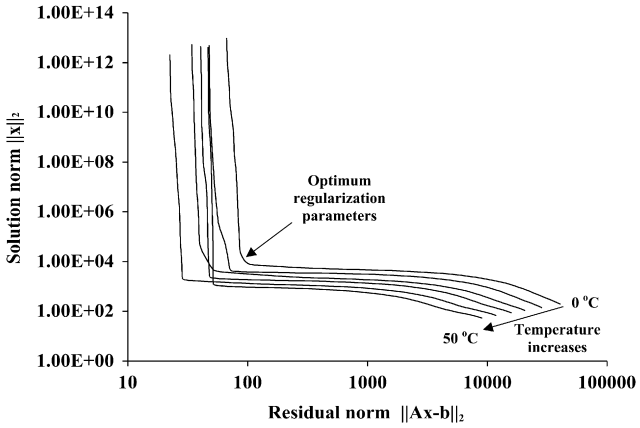

Fig. 2. L-curve plots for optimum calculation of the regularization parameter for the different temperatures the hydrated gluten networks were exposed (0–50 °C), with the optimum λ being located at the corner of the L-curves.

Following optimization of the parameters, the relaxation spectra of gluten were calculated (Figs. 3 and 4). Two relaxation regimes can be identified for this material at all temperatures, one at short (<1 s) and another at long relaxation times (>1 s), which are qualitatively in agreement with previously reported relaxation spectra for various gluten samples (Bellido and Hatcher, 2009; Bohlin and Carlson, 1981; Li et al., 2003; Rao et al., 2000). Elements with fast relaxation times return quickly to equilibrium after the initial excitation whereas those with slow relaxation times require longer times to reach the initial state. The methodology adopted in the present investigation using state-of-the-art algorithms returns highly resolved spectra with baseline resolution. This could not be achieved with previously reported methodologies where the spectra were mostly comprised of poorly resolved peaks.

In the short relaxation times regime, we can identify three dominant relaxation processes. Fast relaxing species exhibit strong temperature dependence and as the temperature increases their intensity decreases (arbitrary units on y-axis). Reduction in the peak magnitude corresponds to a decrease in the amount of species that can be excited from the applied stress. This strong temperature dependence (one log cycle from 0 to 50 °C) suggests that a smaller number of protein chains respond to the application of stress as the temperature increases or, in other words, more chains remain at the equilibrium state. At long times, three additional relaxation events that are mostly unaffected by temperature changes can be observed. These peaks correspond to species with slow molecular rearrangements and require long time to return to equilibrium. This implies that a material with high polydispersity and network structure like gluten exhibits long relaxation events (Ferry, 1980). The fact that the long relaxation regime is not sensitive to changes in temperature suggests that rheological behavior could be controlled by the elements in the short relaxation regime with respect to temperature changes. Furthermore, increase in temperature could favor entropically the opening of relatively larger gluten polymers and therefore interaction with other protein chains, a process that could also contribute to the observed variations in the spectra. In both cases, these events are important for the optimum mixing behavior of flours if one considers that stress relaxation phenomena directly correspond to their mixing characteristics (Rao et al., 2000). Chemical identification of the peaks and subsequent intelligent modification of gluten could help to improve the rheological performance of flours.

Three schools of thought are available to describe the molecular processes that occur in gluten network after application of stress so as to interpret the relaxation processes, namely, the "loop and

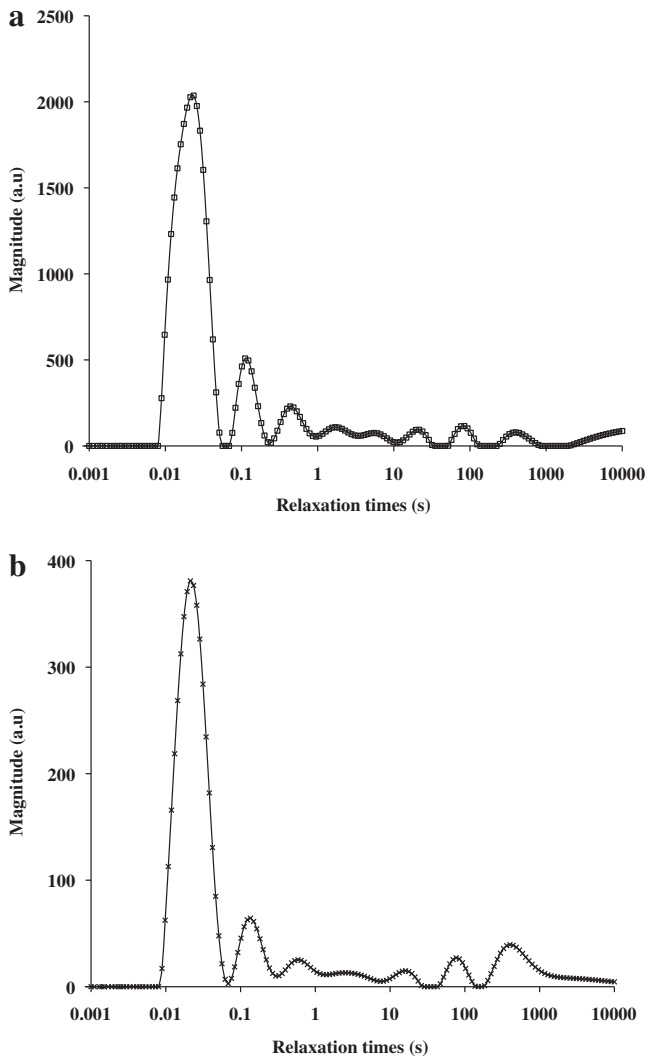

Fig. 3. Representative relaxation spectra of hydrated gluten at two different temperatures: (a) 0 °C and (b) 50 °C, with six major relaxation events that can be resolved between 0.01–1 s and 10–1000 s.

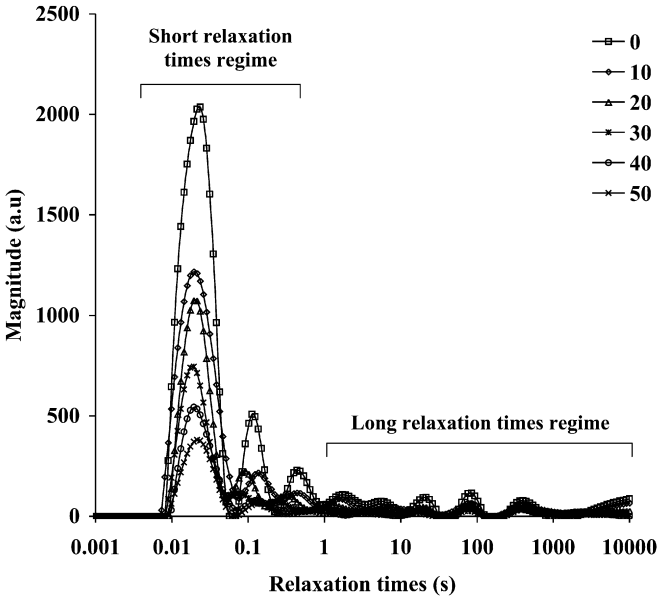

Fig. 4. Temperature dependence of relaxation spectra of hydrated gluten networks from 0 to 50 °C exhibiting short and a long-relaxation time regimes.

train" model (Belton, 1999, 2005; Li et al., 2003), the "particulate network" model (Don et al., 2003; Lefebvre et al., 2003; Lefebvre and van Vliet, 2003) and the "bond-breaking-slippage" model (Singh and MacRitchie, 2001; Termonia and Smith, 1987). Our research on thermophysical, mechanical and microstructural properties of hydrated gluten systems has put forward a novel model for gluten network microstructure suggesting that the entangled protein network creates a nano-capillary sheet arrangement (Fig. 5). This model was based on the behavior of ice melting in the hydrated gluten matrix by applying first thermodynamic principles from the Defay–Prigogine theory on the behavior of liquids inside porous materials (Kontogiorgos and Goff, 2006). Furthermore, detailed SEM and TEM micrographs of hydrated gluten and its protein fractions could not identify a particulate network at micro- or nano-scale in which, as has been suggested (Don et al., 2003; Lefebvre et al., 2003; Lefebvre and van Vliet, 2003), protein particles are aggregated by hydrogen and hydrophobic interactions (Jiang et al., 2008; Kontogiorgos and Goff, 2006; Kontogiorgos et al., 2007). On the other hand, the "bond-breaking-slippage" model has been put forward to describe the behavior of gluten at large deformations where stresses cause breakage of covalent bonds and the chains can slip one past another (Singh and MacRitchie, 2001). However, it is unlikely that small deformations in the LVR of the material, like those employed in the present study, will cause bond breakage so as to influence the mechanical behavior of the material.

In "loop and train" model, "trains" represent areas in the structure where the HMW protein–protein interactions prevail, whereas "loops" are areas with enhanced water–protein interactions (Belton, 1999). Upon deformation, loops will be deformed first followed by trains and when the stress is removed these components will eventually relax. If we further add other protein fractions that interact with the HMW subunits *via* hydrogen bonding and disulfide bridges then we reach a state of interactions that define the overall relaxation behavior of the network. It must also be pointed out that sample preparation in the present study involves significantly lower mixing energy (with spatula), as compared to that required for bread dough formation. Under these mild conditions and high water content, the HMW fraction of gluten is not expected to adopt the conformation found in bread dough where both high energy of mixing and significantly lower amounts of water will unfold HMW fractions. On balance, under the present environment (high water content, mild mixing), we feel that the structural model of "loops and trains" is more appropriate to interpret the relaxation behavior of gluten. Following this line of thought, trains are more difficult to deform than loops and therefore have rapid relaxation times. Thus peaks at short relaxation times may be assigned to the behavior of the train regions of the relatively larger gluten polymers. It has also been suggested that the peaks in the fast relaxation regime, which is also observed in soluble gliadins and glutenins, could be assigned to proteins that do not participate in network formation (Belton, 1999, 2005; Li et al., 2003).

Hydrogen bonding plays a central role on the relaxation behavior of gluten in the "loops and train" structural model. Increase in temperature weakens the strength of hydrogen bonding between the relatively larger gluten polymers as well as to the rest of the structure. This leads to a decrease in the intensity of the short relaxation regimes (Fig. 4). Similarly, in Fig. 1 the relaxation modulus decreases as the temperature increases. On the other hand, the long relaxation regime indicates the presence of a strong network structure that should be due to disulfide bridges or other

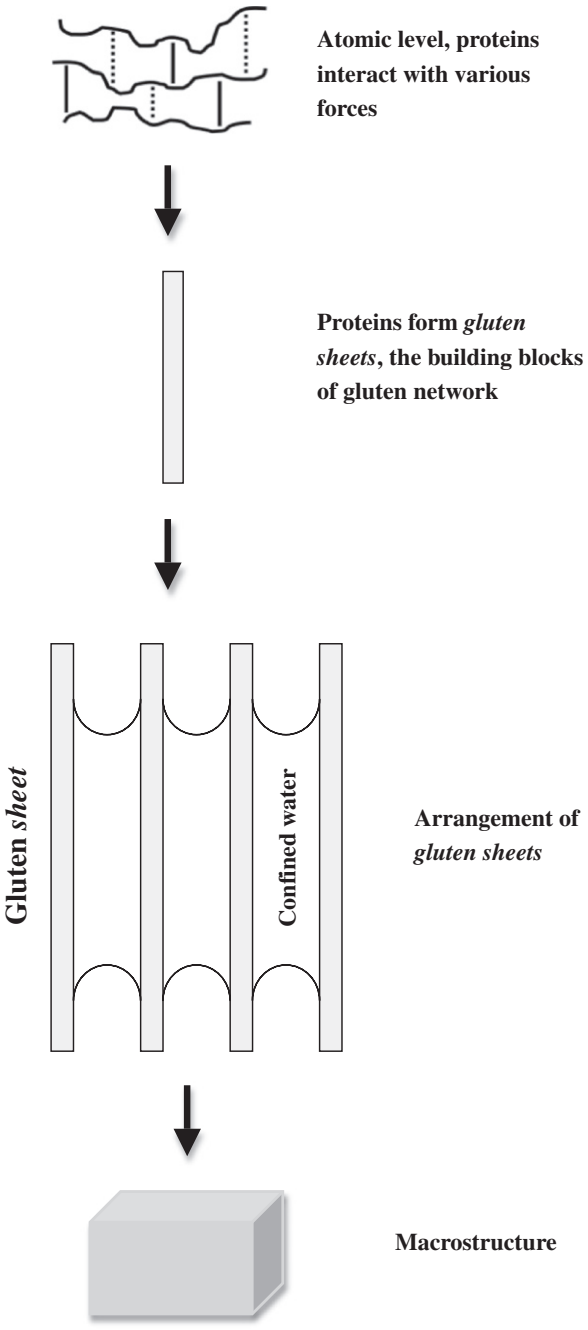

Fig. 5. Model showing the structural hierarchy in gluten microstructure. Adopted with modifications from Kontogiorgos and Goff (2006). At the atomic level protein chains interact with various forces and create a *gluten sheet*, the building block of the network. The sheets are arranged in a manner that they form nano-capillaries and result in the gluten macrostructure.

permanent cross-links showing a relative temperature independence (Figs. 3 and 4). Peaks at long relaxation times are typical of polydispersed polymers with a "permanent" network structure within the experimental timescale of observation (Ferry, 1980; Li et al., 2003) and correspond to the relaxation of the entire network to the equilibrium state.

Taking everything into account, it appears that the "loop and train" model on gluten interactions can be adequately used to interpret the relaxation behavior of the system. It must be stressed, however, that work on the chemical fingerprints of the peaks would further elucidate the exact nature of the relaxation behaviour. Identification of molecular processes provides fundamental understanding of the components that are responsible for the relaxation behavior of gluten, which could assist improving the industrial performance of flours.

4. Conclusions

The temperature dependence of the relaxation spectrum of highly hydrated gluten networks was investigated by means of stress relaxation measurements. Regularization algorithms were employed to analyze the experimental data and generate the relaxation spectrum. This type of numerical treatment results in a multimodal spectrum that can be divided into fast and slow relaxation regimes. For the first time, six major molecular events were resolved for this material and the "loop and train" interaction model was considered to interpret the relaxation peaks. The fast-relaxation regime was attributed to the relatively larger gluten proteins that are held together by hydrogen bonding. In contrast, the long-relaxation regime was assigned to the relaxation of the entire network that is held together primarily by covalent cross-links. The present methodology treats in detail stress relaxation data hence being able to unveil new insights into the mechanical and structural properties of the gluten network.

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