LINEAR VISCOELASTICITY OF GLUTEN: DECOUPLING OF RELAXATION MECHANISMS

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Abstract

The influence of water content on the relaxation dynamics of mesoporous gluten networks has been explored in a wide range of temperatures. The systems were investigated in the linear viscoelastic region by means of stress relaxation, creep and numerical analysis of data. Time-temperature superposition principle and sticky reptation dynamics have been used to provide molecular interpretation of gluten relaxation. Overall, hydration influences relaxation behaviour of the system, which can be linked to changes in the secondary structure of gluten proteins with increase in water content. Relaxation spectra calculated with Tikhonov regularization revealed the remarkable influence of water on the long times relaxation processes of the material. Creep measurements and extraction of dynamic data with direct conversion of creep data via Laplace transform augmented the experimental timeframe of observations to low frequencies unattainable by standard frequency sweeps. The predominance of loss modulus at long times is attributed to migration of water within the nanopores of the structure. Samples also exhibit self-similar relaxation a characteristic of systems existing at a critical state. Two relaxation mechanisms can be distinguished: one arising from viscoelastic relaxation of protein chains and an additional stemming from poroelastic relaxation owing to migration of water in the system.
1. Introduction

Presence of nanocapillaries in materials gives rise to intricate thermodynamics, and a range of different types of spatial confinement and pore-wall interactions can be used to assist tuning the physical properties of soft matter. Gluten is a mesoporous biological material with average pore diameter of about 5 nm and confinement of water in the nanopores depresses melting point of confined water by more than 10 °C (Kontogiorgos and Goff, 2006). It consists of more than fifty proteins with distinct structures resulting in a particularly complex biological material that presents difficulties when is subjected to rheological examination.

Viscoelasticity and macromolecular relaxations of hydrated biological structures is largely determined by their water content, interactions with other constituents, pH and ionic strength. Gluten viscoelasticity is frequently described with the "loops and trains" model (LP-model) (Belton, 1999), which is essentially the Lodge model adapted to gluten. In the Lodge model, junctions zone of the network break and reform with a particular duration, as a result of transient binding between the polymer chains. The transient binding in the LP-model is formed via hydrogen interactions between amino acids of the polypeptide chains. The strength and extend of the interactions depends on the level of hydration thus having direct influence on viscoelasticity. At low water contents hydrogen interactions occur primarily between the amino acids of proteins while as hydration increases the water-protein hydrogen bonding is enhanced.

It is well documented in the literature that hydration of gluten proteins induces conformational changes to their structure. In particular, β-sheet to α-helix ratio increases in response to hydration whereas at even higher hydration levels β-sheets are replaced
with β-turns (Almutawah et al., 2007; Belton et al., 1995; Popineau et al., 1994; Wang et al., 2001; Wellner et al., 1996). The interplay of interactions between β-sheets, α-helices and β-turns modulates the rigidity of the network as intermolecular hydrogen interactions occurring between β-sheets will have greater number of neighbouring binding partners compared to, for instance, α-helices or β-turns (Belton, 1999). Temperature-induced conformational changes further complicate the landscape with additional formation of intermolecular β-sheets by loss of α-helices or interchange of disulfide linkages on heating (Georget and Belton, 2006). The cooperation of supramolecular forces, temperature and time on gluten viscoelasticity have been recently put under rheological scrutiny revealing that the importance of hydrogen bonding precedes over disulfide cross links (Kontogiorgos et al., 2016). Additionally, in the mesoporous structure of gluten, water is associated with the protein walls of the nanocapillaries with different strength and molecular mobility (Bosmans et al., 2012; Kontogiorgos et al., 2007). Such structures may allow water migration between neighbouring nanopores that further influences viscoelasticity.

It is evident from the above discussion that water levels would play critical role in the viscoelasticity of gluten networks and other similar hydrated biopolymer systems due to chain conformational changes and water resettling within the pores of the structures. In our previous investigations, we have focused on the influence of protein composition (Kontogiorgos and Dahunsi, 2014) and supramolecular forces (Kontogiorgos et al., 2016) on the relaxation dynamics of model gluten networks focusing, however, at one hydration level. The aims of the present investigation are to build on our previous findings and by
using gluten as model system to explore the influence of hydration and decouple the mechanisms that contribute to the relaxation dynamics in hydrated gluten.

2. Materials and Methods

2.1 Materials and sample preparation

Gluten was purchased from Sigma-Aldrich (Poole, UK) and the samples were prepared at three levels of hydration: 70-30 (HW), 60-40 (MW), and 50-50 (LW) %w/w where the first number corresponds to water content and the second to the protein solids in the samples. Samples were labeled as HW, MW or LW for high, medium or low water content, respectively. Following mixing, samples were left to hydrate for 30 min before loading on to the rheometer as described elsewhere in detail (Kontogiorgos et al., 2007).

2.2 Stress relaxation and creep measurements

Stress relaxation measurements on shear were performed between 10–60 °C using a rotational rheometer (Kinexus pro+, Malvern Instruments, Malvern, UK) equipped with serrated plate geometry (25 mm diameter and 1 mm gap). The experimental settings have been described in our previous work in detail (Kontogiorgos et al., 2016). Briefly, after sample loading the specimens were left to relax for 15 min before measurements to dissipate stresses that were created during loading. Shear strain amplitude sweep experiments were then performed in the linear viscoelastic range of the samples (LVR) at 2% instantaneous strain for 30 min. Creep was conducted in the LVR of the samples in the same temperature range and with the same geometry as the stress relaxation measurements. An instantaneous stress of 20 Pa was applied and creep was carried out for 30 min. A thin layer of low viscosity silicone oil (polydimethylsiloxane, Sigma-
Aldrich, St.Louis, MO) and a solvent trap were used to minimize moisture loss from the edges of the geometry during measurement. Nonlinear regression was performed with GraphPad Prism v.6 (Graph-Pad Software, SanDiego, USA).

2.3 Computation of relaxation spectra

Calculation of relaxation spectra was performed in MATLAB (v7.0 R14 Service Pack 2, The Mathworks Inc., MA), as described previously (Kontogiorgos et al., 2016) using Tikhonov regularization and the L-curve criterion to locate the optimum regularization parameter, \( \lambda \). Discretization of stress relaxation function was performed between 0 and 30 min (minimum and maximum experimental time).

2.4 Conversion of creep data to mechanical spectra

Conversion of creep data to dynamic moduli proceeds with the application of Laplace transform to the equation of motion of stress-controlled rheometers:

\[
\mu \frac{d^2 \gamma}{dt^2} + \int_0^t G(t - \tau) \frac{d\gamma}{d\tau} d\tau = \sigma_m(t) \tag{1}
\]

where \( \mu \) is a positive constant representing instrumental inertia, which is given from rheometer manufacturer, \( \gamma(t) \) is the strain measured from creep test, \( G(t) \) is the relaxation modulus of the material and \( \sigma_m(t) \) is the stress input given from the rheometer. Since the term of Boltzmann superposition has the form of convolution, Laplace transform can decompose the Laplace transform of creep compliance or equivalently that of relaxation modulus because of the exact relation of:

\[
sf(s) = \frac{1}{sG(s)} \tag{2}
\]

Numerical calculations proceed with Laplace transform of strain \( \hat{\gamma}(s) \) from experimental data and conversion of \( sG(s) \) to dynamic moduli (Kim et al., 2014; Kwon et al., 2016).
3. Results and Discussion

3.1 Stress relaxation measurements

The present investigation begins with exploration of the influence of water content on the stress relaxation behavior of gluten networks. Three levels of water were used and stress relaxation measurements were performed in the LVR between 10 and 60°C for all samples (Fig. 1 a-c). The maximum (70% w/w) and minimum (50% w/w) hydration levels were chosen according to the levels of water that network is able to retain. At higher (i.e., >70% w/w) or lower hydration levels (i.e., <50 %w/w) water is either not retained in the structure or does not adequately hydrate proteins thus resulting in formation of anisotropic networks. Consequently, the present work reports on the entire range of hydration levels that can be used to create macroscopically isotropic gluten networks.

Hydration influences elasticity of the material particularly for LW samples where the network stiffens remarkably compared to its counterparts (Fig1 a, b vs. c). Temperature increase reduces relaxation modulus with diminishing influence beyond 50°C at all hydration levels an observation that has been previously assigned to gluten network restructuring (Kontogiorgos and Dahunsi, 2014; Kontogiorgos et al., 2016; Tsiami, Bot and Agterof, 1997; Tsiami, Bot, Agterof, et al., 1997). The onset of the terminal regime is affected by temperature of the matrix where at temperatures above 30°C the slow relaxation processes become particularly evident. The onset of long relaxation times is influenced by the rest time of the material on the geometry before measurement (Ng and McKinley, 2008), temperature, and on the specific molecular interactions within gluten (Kontogiorgos et al., 2016). Generally, onset time decreases
with temperature from about 800 s at 10 °C to 200 s at 60 °C whereas hydration levels do not seem to play a role on the onset of slow relaxation modes. Early studies on the nature of long processes have been attributed to the relaxation of the entire gluten network due to its interconnectivity with disulfide linkages (Li et al., 2003) that could be generalized as hindered motions of interacting polymer chains (Li et al., 2010). More recently weakening of hydrogen bonding with temperature increase and water migration (poroelastic relaxation) in the nanopores of the material have been also shown to play determinant role for the long relaxation processes of this material (Kontogiorgos et al., 2016). As it will be shown later, by following the slow modes with creep measurements, poroelastic relaxation seems to be the determinant cause for the emergence and development of the long relaxation events. The next step in our investigation was to proceed with the construction of master curves of viscoelasticity for all samples using the time temperature superposition principle to explore gluten network dynamics at timeframes beyond those experimentally attainable.

3.2 Time-temperature superposition and relaxation spectra

Time-temperature superposition principle has been successfully applied on several occasions before for gluten specimens (Dahesh et al., 2016; Kontogiorgos and Dahunsi, 2014; Kontogiorgos et al., 2016; Tsiami, Bot, Agterof, et al., 1997). In the present work, we have used the method of reduced variables to calculate the horizontal shift factors ($a_T = t/t_o$) at reference temperature of 20 °C. Vertical shift factors are usually needed when the material density changes during the course of rheological examination. All samples studied are highly hydrated and density changes in the temperature range we
carried out the measurements will be dominated by changes in water density, which are negligible between 10-60 °C (0.999-0.983 g/mL). In addition, the temperature dependence of the vertical shift factor is generally weaker than the horizontal, thus it was taken to be equal to unity. Construction of master curves extended the window of observation for about four logarithmic cycles giving additional information on the fast and slow relaxation processes of the samples (Fig. 2). In particular, hydration seems to affect dramatically the fast relaxation processes (log (t/τ) < -1) that represent the Rouse-like modes of relaxation of the protein chains (Ng and McKinley, 2008) and essentially vanish (i.e., shift at shorter times) for MW and HW samples. Utilization of the LT-model (Belton, 1999) provides molecular insights to the behavior of the fast relaxation processes that is linked to changes in the protein structure with increase in water content. At low hydration levels the individual protein chains interact directly via hydrogen bonding, particularly of glutamine residues, creating “trains”. The “trains” behave as the spring of the Maxwell model that attempts to instantaneously return the system to equilibrium. Increase in hydration results in changes in secondary protein structure from α-helices to β-sheets, and with further increase of hydration to β-turns, thus introducing water molecules between protein chains, as has been described in the introduction. This state of affairs creates “loops” that weaken direct hydrogen bonding between proteins thus decreasing its elasticity. These regions represent the dashpot element in the Maxwell representation that exhibits viscous behavior. Increase of the “viscous” elements in the system shift Rouse-like modes to much shorter times that is not possible to observe with our current experimental protocol for samples HW and MW in contrast to LW. Indeed, in our previous investigation Rouse-like modes
for MW samples have been observed at shorter times at about log (t/\(a_T\)) < -2 s (Kontogiorgos et al., 2016). The power-law relaxation region is evident for all samples (log (t/\(a_T\)) > ~1 s for the LW) and persists until the onset of the terminal regime.

Hydration influences the emergence of the terminal processes in the order of HW < MW < LW with onset appearing at log (t/\(a_T\)) at ~ 5, 6 or 7, respectively (Fig 2) an observation that was not evident from stress relaxation curves (Fig 1). In that case, it takes longer time for proteins to “unzip” and relax to equilibrium state due to enhanced interactions in the “train” regions as opposed to their “loop-rich” counterparts at higher hydration levels.

This behaviour can be formally described by utilizing tube dynamics and the sticky reptation approach (Leibler et al., 1991; Xu and Craig, 2011). In the sticky Rouse model the longest time for Rouse-like relaxation will be proportional to the number of elastically active moieties of the protein chains and localized rearrangements of amino acids. These are influenced by the number of disulfide cross-linking (Kontogiorgos et al., 2016) and water content, as we have shown in the present investigation. In the transition zone reptation commences and the modulus is mostly influenced by hydrogen-bonded protein chains that do not form disulfide linkages. In this region hydrogen-bonded segments of the chains, are the loci that store elastic energy. The continuous process of engagement-disengagement of the transient binding associations results in absence of a rubbery plateau and is attributed to the polydispersity of the material (Figure 2). Increase in water content reduces the strength of the inter-protein interactions (i.e., elastically active associations) resulting in decrease of modulus and overall faster relaxation to lower residual stresses (Figure 2). In the terminal region, proteins are released from the tube with the remaining stress being proportional to the number of proteins that remain in
the tube. As it will be shown below, there is an additional mechanism that contributes to
the terminal relaxation.

To advance our understanding on the influence of temperature on the relaxation
mechanisms of the samples, the horizontal shift factors were plotted as a function of T-T₀
(Fig 2., top right inset). Up to 40 °C (T-T₀ = 20 °C) samples show gradual shift in aᵣ, as
temperature increase weakens hydrogen bonding, resulting in faster relaxation dynamics.
However, above 40 °C there is a step-change of about two logarithmic cycles in log aᵣ
particularly for MW and LW samples. Changes in the relaxation mechanisms at 40 °C has
been previously observed (Kontogiorgos et al., 2016) indicating temperature associated
structural changes in the network. Arrhenius relationship can be used to describe the
temperature dependence of aᵣ as:

\[
\log aᵣ = \frac{E_a}{R} \left( \frac{1}{T} - \frac{1}{T₀} \right) \quad (3)
\]

where \(E_a\) is the relaxation activation energy and \(R\) the gas constant. Plotting \(\log aᵣ\) vs. \(1/T\)
(Fig 2, bottom left inset) results in activation energies of 53, 69 or 71 kJ/mol for HW,
MW or LW samples, respectively. Consequently, decrease in water content in the
samples increases the energy that is required for the chains to overcome the energy
barrier for molecular motion. This outcome is in line with the previous discussion on the
enhancement of intermolecular strength of protein interactions at low water contents due
to the proximity of the protein chains to each other.

The next step in the investigation dealt with the calculation of the continuous
relaxation spectra of the materials. The generalized function of the stress relaxation curve
is given by Fredholm integrals of the first kind as:

\[
g(s) = \int_{0}^{a} K(s, t)H(t)dt, \ 0 \leq s \leq a \quad (4)
\]
where $K(s, t)$ is the kernel $\exp(-t/s)$ that describes the decay, $g(s)$ is the relaxation modulus, $G(t)$, and $H(t)$ is the continuous relaxation spectrum of the material. Integration limits are between time zero and the maximum experimental time ($\alpha = 30$ min). Curves plotted in Fig 2 were analyzed using Tikhonov minimization yielding the relaxation spectra of the networks (Fig 3). Relaxation spectra of gluten composites and other similar biopolymer systems correspond to networks that exist in a critical state generally revealing three characteristic regions: “glassy”, power law and terminal (Gabriele et al., 2001; Kontogiorgos et al., 2016; Meerts et al., 2016; Ng and McKinley, 2008). Polymeric systems during sol-gel transition exhibit distinct rheological properties at the critical point, one of them being self-similar relaxation (Winter, 2002). At the critical point, the junction zones have not yet been established and return to the fluid state could be possible with reversal of the gelation condition (e.g., gelation reaction, temperature modulation etc.). In the case of gluten, critical state is easily perceived due to the transient interactions of hydrogen bonds that break and reform with a characteristic lifetime thus making the system to exist in an interminable critical state. The entire network keeps its solid-like character due to disulfide cross linking, absence of which results in fluid flow.

In addition, spectral analysis reveals some new striking features as water content decreases in the system. Relaxation spectra present a minimum for HW samples at about $2 \times 10^4$ s that with reduction of water it gradually shifts outside our experimental window ($>10^6$ s) (Fig 3) indicating that the long relaxation processes are highly dependent on the water content in the networks. In our previous work, we have proposed two mechanisms of relaxation in gluten, namely viscoelastic and poroelastic (Kontogiorgos et al., 2016). The latter refers to the migration of water within the nanopores of the material. It has
been previously shown that modification of intermolecular interactions (Kontogiorgos et al., 2016) or gluten composition (Kontogiorgos and Dahunsi, 2014) does not affect the long relaxation processes of the material. Complementing past observations with those from the current investigation, it emerges that the long relaxation processes are only modified with changes in water content in the material. Relaxations caused by the internal flow of solvent in the hydrogel have been previously described in the literature for other hydrogels (Chan et al., 2012; Strange et al., 2013). It has been also suggested that for whey protein gels viscoelastic relaxation should be considered at short times for complete description of the relaxation processes (Mercadé-Prieto et al., 2016). This analysis presents an important development in our understanding of the viscoelasticity of this material as we have shown a clear relationship between water content and relaxation behaviour. The mesoporous structure should be also taken into account as the physically confined water in nanopores contributes to relieving the stress in the material. Consequently, tuning the mechanical properties of gluten networks should mostly focus on the water content in the structure.

As it transpired that water is the determinant factor for the mechanical properties of the material, we proceeded to collect further evidence on the role of hydration on the terminal relaxation processes. This was achieved with creep measurements in the LVR of the material that are described in the next section.

3.3 Creep measurements

In the next step of our investigation we have undertaken creep measurements at the two extremes of hydration (HW or LW) followed by conversion of creep data to mechanical spectra. Creep measurements are more appropriate than dynamic and stress
relaxation for the measurement of long-term behavior of viscoelastic materials. In addition, with conversion of creep curves to mechanical spectra we can retrieve information about material properties from experimentally inaccessible frequencies. Isothermal creep curves for LW and HW samples after application of instantaneous shear stress in the LVR of the materials are presented in Fig 4.

Four distinct regions are distinguished, which is typical for polymeric materials with structure. The initial strain of the curves corresponds to the elastic response of the material after application of stress. The second region extends to about 0.02 s after the application of the instantaneous stress where a peak is observed due to “ringing” (i.e., damped oscillations), as a result of the coupling of instrument inertia and sample elasticity. In this region, the material begins to weaken under the effect of stress which is temperature dependent with higher temperatures resulting in greater structural losses. This region is followed by the steady state creep phase up to about 100 s. During this phase, competing mechanisms of strain hardening and recovery may be present, something that is particularly important for gluten polymers. In the final region beyond 100 s, strain curves for both samples show an upward trend. It is evident that HW samples form weaker structures compared to LW counterparts at all temperatures as higher protein hydration results in fewer direct intermolecular protein interactions and greater molecular mobility of the chains. At long times the strain development is described by (Kim et al., 2014; Kwon et al., 2016):

\[ \gamma(t) \approx A t^\nu \text{ for } t > t_{\text{max}} \]
The parameter $A$ and the terminal exponent $v$, are determined by regression analysis with the data from the terminal regime ($t \sim t_{\text{max}}$). Additionally, in the terminal regime, raw data of strain show:

\[ \frac{d \log y}{d \log t} \approx v \text{ for } t \sim t_{\text{max}} \] (6)

When $v = 1$ the material behaves as a viscoelastic fluid whereas when $v = 0$ as a viscoelastic solid. Terminal exponents as a function of temperature for HW and LW samples range between 0 and 1 (Fig. 4b, inset) something that has been previously observed for viscoelastic dispersions of xanthan (Kim et al., 2014). It is seen that higher water contents and temperatures enhance the liquid-like character of the samples. However, at temperatures above 40 °C a decrease of $v$ is observed that is congruent with the stress relaxation measurements. Denaturation of gluten proteins occurs over very broad temperature range commencing at about 60 °C and continues unabated to 85 °C due to heterogeneous protein composition (Leon et al., 2003). As creep and stress relaxation measurements have been terminated at 60 °C, the networks should not exhibit any measurable protein conformational changes in the window of temperatures we operated. As a result, the observed changes in viscoelasticity should be attributed to the mechanisms that have been described above.

The next step of the investigation was the conversion of creep data to dynamic data yielding mechanical spectra with experimentally inaccessible angular frequencies (0.0001 – 0.1 rad/sec) (Fig. 5). Conversion of creep data and recovery of mechanical spectra is in agreement with previously published mechanical spectra of gluten with conventional dynamic measurements on shear (Janssen et al., 1996; Meerts et al., 2016; Tsiami, Bot, Agterof, et al., 1997). At long times (0.0001- 0.001 rad/s) loss modulus
dominates over storage something that is particularly noticeable in HW samples. Combining information from three different rheological experimental treatments it emerges that at long times the liquid like character controls the mechanical properties of gluten composites. We attribute this behaviour to migration of water within the nanoporous structure of the material that occurs in the terminal region of viscoelastic relaxations. In addition, stress relaxation measurements that do not involve application of stress for prolonged periods of time have also shown that the long relaxation peaks are manipulated with changes in water content (Figure 4). At intermediate angular frequencies (0.001-0.1 rad/s) storage modulus overtakes loss modulus whereas the latter exhibits a peak. The reversible associations between protein chains prolong the relaxation of the system and such systems exhibit two maxima in the $G''(\omega)$ function. The low frequency peak is attributed to the disengagement of the proteins from the tube by diffusion. This peak appears at frequencies $1/\tau$ where $\tau$ is the terminal relaxation time (Fig 1). Using data of Figure 1 onset of terminal relaxation ranges between 800 and 200 s (depending on the temperature and water content) yielding angular frequencies between 0.0078 and 0.0314 rad/s, which is within the range of $G''(\omega)$ peaks presented in Figure 5. The high frequency peak is generally difficult to be observed (~3 or 30 rad/s HW or LW samples, respectively) and it attributed to dissipative processes of small peptides or oligomers of gluten. At $\omega > 0.1$ rad/s viscoelastic moduli enter the power law region which is a behaviour for systems existing in a critical gel state (Ng and McKinley, 2008). In critical gels the storage and loss moduli should scale as (Winter, 2002):

$$G'(\omega) \sim \omega^n$$ and $$G''(\omega) \sim \omega^n$$  (7)
where \( n \) is the gel exponent and \( \omega \) is the angular frequency. When \( n \to 1 \) the material behaves as a soft gel whereas when \( n \to 0 \) it has characteristics of stiff network. Equation 7 indicates that at the gel point storage and loss moduli curves should run parallel to each other \( i.e., \) exhibiting the same exponent, \( n \). Curve fitting between 1-10 rad/s yield \( n \) values that are presented in Table 1. Close inspection of the table reveals that temperature increase and/or decrease in moisture stiffens the network. These slopes agree particularly well with previously observed in the literature as 0.22 (Meerts et al., 2016), 0.22-0.35 (Kontogiorgos et al., 2016) or 0.17 (Ng and McKinley, 2008). It can be seen that increase in temperature augments the differences in the \( n \) values obtained from storage or loss moduli, however, they are characteristically close (~0.3) particularly for the HW samples. The behaviour of the exponents shows that gluten indeed displays characteristics of a system existing near the sol-gel transition although disulfide cross-linking should be responsible for the deviations from the theory \( i.e., \) exact agreement between \( n \) values).

The complexity of the relaxation processes can be also visualized with Cole-Cole plots of \( G' \) vs. \( G'' \) (Figure 5a, inset). A semicircle in the Cole–Cole plot denotes a system with a single relaxation time whereas if additional features appear in the right-hand part of the curve \( e.g., \) shoulders or more arcs), they signify the existence of a longer relaxation times with deviation from the ideal Maxwell behaviour, as is the case with our samples. Taking everything into account, we could provide a highly idealized depiction of the processes that are involved during gluten relaxation (Figure 6). At equilibrium (Figure 6a) gluten proteins are fully extended forming nanocapillaries that are able to confine water. Water is physically confined with the aid of capillary forces but also because of gluten-water interactions. Application of instantaneous strain (Figure 6b) disturbs
equilibrium and macromolecular relaxation begins in an attempt to reach equilibrium. The very fast relaxation processes (Figure 6c) correspond to the elastically active moieties of the protein chains and localized rearrangements of amino acids, as described above. In the next stage, reptation of hydrogen-bonded segments of the protein chains takes the lead and dominates over the rest of the processes further relieving the stress in the material that is manifested with further decline in relaxation of modulus (Figure 6d). In this part of the relaxation events, poroelastic relaxation also commences but does not seem to play substantial role in the events. Finally, in the terminal regime confined water disengages from the nanopores and migrates to neighbouring pores and poroelastic relaxation is the dominant mechanism in an attempt to reach the initial equilibrium state (Figure 6e).

4. Conclusions

Relaxation dynamics of mesoporous gluten networks as affected by water content have been investigated by means of stress relaxation, creep and numerical analysis of the ensuing data. Stress relaxation measurements in a wide range of temperatures and application of time-temperature superposition principle enlarged the experimental window of observation allowing construction of master curves of viscoelasticity. Water content played critical role on relaxation dynamics affecting the Rouse-like and terminal relaxation processes. Relaxation spectra revealed that the long times relaxation processes can be only controlled by changes in water content. Probing long-time relaxation processes with creep measurements conversion of the curves to mechanical spectra allowed assessing experimentally inaccessible frequencies. The
networks present characteristics of systems that exist in a critical state throughout all the functionally relevant temperatures and hydration levels. Combination of rheological data revealed that the long relaxation processes should be associated with water migration in the pores of the structure. As a result, the mechanisms of gluten network relaxation can be separated into viscoelastic due to protein relaxation and poroelastic due to water migration in the nanopores.

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5. References


FIGURE LEGENDS

Figure 1: Double logarithmic plots of stress relaxation curves between 10 and 60 °C for all gluten samples: a) HW, b) MW, and c) LW.

Figure 2: Double-logarithmic plots of master curves of stress relaxation modulus against reduced time at $T_o = 20$ °C for all samples. Top right inset shows the temperature dependence of shift factors ($a_T$) plotted against $T-T_o$. Bottom left inset shows the calculations of the activation energy in semi-logarithmic plots of $\log a_T$ vs. $1/T$.

Figure 3: Double-logarithmic plots of relaxation spectra calculated using data from master curves (Fig. 2).

Figure 4: Double logarithmic plots of creep curves between 10 and 60 °C for gluten samples: a) HW, and b) LW, inset shows the development of terminal exponent $\nu$ with temperature.

Figure 5: Mechanical spectra obtained by direct conversion of creep curves in Figure 4. Filled or open symbols indicate storage or viscous modulus, respectively. a) HW samples: square at 20 °C, and triangle at 40 °C, Top-right inset: Cole-Cole plots for LW samples, b) LW samples: circle at 30 °C and diamond at 50 °C.

Figure 6: Idealized depiction of the processes that are involved in gluten relaxation. Strands (brown zig-zag lines) represent gluten walls that form nanocapillaries that confine water (blue circles). a) Equilibrium before application of strain, b) application of instantaneous strain, c) in the first stage, fast relaxation processes correspond mostly to the elastically active protein moieties, d) in the second stage, continuous reptation of hydrogen-bonded segments of the protein chains reduces further relaxation modulus, and
e) in the final stage, poroelastic relaxation is the dominant mechanism. Confined water may also migrate to neighbouring pores whereas gluten proteins are mostly relaxed.

TABLES

Table 1: Slopes of the curves of Figure 5 between 1-10 rad/s that represent the gel exponent $n$ at all temperatures for HW and LW.

<table>
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<th>Temperature (°C)</th>
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<th>Slope G'(ω) LW</th>
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</table>
a.

![Graph](image)

b.

![Graph](image)
Confined water

Gluten walls

\[ \log G(t) \text{ (Pa)} \]

\[ \log (t/\alpha_T) \text{ (s)} \]

\[ \sim 5 \text{ nm} \]