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Mechanical and novel optical techniques for rheological characterisation of cereal beta-glucan

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Abstract: The increasing research interest on the rheological properties of cereal β -glucan is because of a deepening scientific evidence of the health functionality of this soluble dietary fibre. The review outlines the existing methods for mechanical rheometry and the novel microrheological approach based on optical measurements. Further emphasis is on the studies that relate the viscoelastic parameters of β -glucan obtained by particle tracking microrheology with conventional rheology. It is expected that researchers will take advantage of the recent advancements in microrheology techniques to gain newer insights in the behaviour of the bioactive nutrient undersimulated physiological conditions.

Keywords: Beta-glucan; Rheology; Gel; Thixotropy; Light Scattering; Microrheology.

1. Introduction

Cereal β -glucan, which mainly occurs in the cell walls of oat and barley, is a viscous-gelling soluble dietary fibre with scientifically substantiated health benefits such as lowering blood cholesterol and glucose levels [1,2,3]. Thecell wall polysaccharide exhibits all the physiological benefits of dietary fibres, and also the thickening and gelling characteristics of food hydrocolloids. The molecular weight (MW), structural features, concentration and solubility of β -glucan influence its solution viscosity and gelation behaviour, which affect the aforementioned functionalities and physiological benefits. Although the physiological responses are positively correlated to viscosity of β -glucan, the relationship with its gelling capacity is not fully understood.

Gelation rheology of β -glucan has been extensively studied for concentrated solutions, but the dilute concentration regime is largely unexplored due to the limitations of oscillatory rheometry at low concentrations [4]. Freshly prepared dilute solutions of β -glucan display negligible association of the polymer molecules, as evaluated by light scattering techniques [5]. However, a better understanding of β -glucan viscoelasticity in dilute solutions is necessary to appreciate the physiological consequences of gelation. The present review focuses on various mechanical and optical methods utilised for rheological characterisation of β -glucan, which encompass different concentration regimes and frequency ranges.

2. Rotational and oscillatory rheology

Rheology is the study of flow and deformation of materials their semi-solid, fluid, or in a transient state [6,7]. Conventional rheology includes both fundamental and empirical techniques. The fundamental methods, which are based on the established principles and equations of physics, account for both the magnitude and direction of the applied stress and the resulting strain. Empirical methods, which include texture

profile analysis (TPA), extensiograph, farinograph, and rapid visco-analyser (RVA), are generally applied when the shape or the composition of samples preclude the fundamental methods.Moreover, the results obtained in empirical testing are dependent on the set-up of the particular experiment, and are thus not quantitatively reproducible elsewhere.

Mechanical rheometers determine the fundamental rheological characteristics of materialsby measuring their response to the applied force or deformation [6].Rotational testing methods are employed to ascertain the viscous flow characteristics such as shear-thinning and thixotropy, and the gelation phenomenon is studied with oscillatory rheometry.

2.1 Rheology of viscous fluids

Viscosity, defined as the resistance of a fluid to flow, is ascertained by measuring the strain resulting from the shear stress applied in a controlled manner in rotational rheometry experiments [6,7]. The most significant factors influencing the viscosity of fluids are temperature, molecular structure, pressure and time. It is important to note that viscosity is also a function of the rate of shear, and is independent of the shear rate only for Newtonian liquids such as water and glycerol [8]. In the case of non-Newtonian fluids, which include most of the dispersions, emulsions, and polymeric food materials, viscosity also changes with shear rate. Shear-thinningbehaviour, in which viscosity is proportionately lowered with the applied shear rate, is more common in everyday materials. In the case of shear-thickening, for instance in corn starch mixed with water, the apparent viscosity increases with shear rate.

2.2 Thixotropy and hysteresis loops

Thixotropy was defined byBarnes [8,9]as time-dependent reversible modification in microstructure produced by externally enforced stresses operating in the non-linear viscoelastic region. The phenomenon is analogous to the heat-induced melting of gels that regain their elasticity with time on cooling, but the deformation is accomplished by shearing and not by heating above themelting temperature range. The term was originally derived from two Greek words *thixis* (mixing or agitation) and *trepo* (rotating or changing). This material property has been extensivelyapplied in industrial products such as paints, adhesives and lacquers.

As reviewed by Barnes [8,9], a narrower understanding of the concept restricts thixotropy to viscosity loss on shearing, which is subsequently regained with time when stress is discontinued upon resting. For a more detailed understanding, a reference to microstructure and non-linear viscoelasticity is essential. Microstructure refersto theextent of flocculation and spatial arrangement of particles, average alignment of fibre strands,formation of molecular networks, and the degree of polymer entanglements.Maximum microstructure, which is characterised by a high viscosity and a high elasticity as well in most instances, corresponds to high entanglement density, spatial arrangement and general alignment of the fibre strands. A change in microstructure requires definite amount of time, and is often the net effect of disintegration under shear and aggregation due to contact between molecules.In a non-linear viscoelastic response such as thixotropy, the microstructure not only responds to the applied stress but also deforms due to the stress, and thus a finite amount of time is required to recover the structural elements.

Rotational-shear loop tests, which substantiate the development of microstructure, are conducted by increasing the shear-rate from zero to a maximum value, and then returning to the initial value in a time-dependent manner [9]. Scott-Blair [10]has postulated that if the recovery of the microstructure is instantaneous, the phenomenon is described as shear-thinning; if the build-up is gradual, typically ranging from seconds to a few hours, then it is termed as thixotropy.For essentially shear-thinning samples, the up and down curves (shear stress or viscosity,vs. shear rate) are superimposed. Thixotropy is quantified by the area between the up and down curves, which is commonly referred to as hysteresis, in a time-dependent loop test [9,11,12].

2.3 Flow characteristics of β-glucan

In steady-shear rheological tests, aqueous dispersions of β -glucan that are freshly prepared or tempered, initially display a Newtonian region at low shear rates and shear-thinning at high shear rates [3]. Many researchers have established that the apparent viscosity, shear-thinning and viscoelastic characteristics of such dispersions increase with both the molecular weight and the concentration of β -glucan in the solution. This behaviour is characteristic of random coil polysaccharides that form molecular entanglements withincreasing MW and concentration. These entanglements, however, are not a simple overlap of contiguous molecules,

which is feasible at much lower molecular weights, but are somewhat comparable in strength to chemical crosslinks in polymers [8].

Comparative studiesby Papageorgiou et al.[13] and Mikkelsen et al.[14], among others, have demonstrated that the apparent viscosity of oat β -glucanis higher than that of barley β -glucanat various shear rates, temperatures and concentration levels. Furthermore, the flow behaviour of barley β -glucan is predominantly Newtonian, whereas shear thinning is more conspicuous in oat β -glucansolutions of analogous concentrations [3,13,14]. This is ascribed to the fine-structure and relatively higher molecular weight of oat β -glucan, which ensues a high entanglement density. Shear-thinning is due to a disruption of these entanglements at high shear which progressively decreases the resistance to flow, and the effect has been observed to be more pronounced at low temperatures and also at high concentrations. A change in the flow behaviour from shear-thinning to Newtonian can be attributed to a reduction in the molecular weight below the critical level at which molecular entanglement occurs.

Thixotropy, which correlates with shear-thinning, was noticeable when concentrated oat [11] and barely [12] β -glucan solutions were subjected to loop tests with different cycle times. The up and down curves superimposed for very short total cycle time (2 min), but an hysteresis caused by molecular aggregation upon rest was evident for cycle times close to one hour.

2.4 Rheology of gels

Gel formation is quite common in food, and occurs in many familiar products including custard, gelatin, surimi, fruit jellies, tarts and puddings [6,7]. Gels consist of a continuous solid network immobilizing a liquid medium, and are considered as liquids behaving like solids. Gels, which exist as a free-flowing solution, dispersion, or suspension in their initials states, are essentially viscoelasticfluid systems that do not display any steady state flow. Gelation is caused by cross-linking, either of a chemical nature through covalent bonding or by a physical interaction between polymer chains. In food systems, polysaccharides and proteins are the macromolecules involved in gel network formation. Such polysaccharides, however, are not structurally well organized like the insoluble cellulose molecule, but invariably have some irregularities that facilitate a partial association [15]. In the case of β -glucan, the β -(1-3) linkages randomly interrupt the β -(1-4) linked cellulosicoligomers of β -D-glucopyranosyl units, thus imparting solubility to the molecule.

Gelling properties are evaluated in small-scale oscillatory testing, which does not adversely impact the microstructure of the gel [6,7]. A sinusoidal stress is applied, and the resulting sinusoidal strain is measured along with the accompanying phase lag to obtain the values of G', G'' and tan δ . G' (the storage or elastic modulus) represents the elastic behaviour of a sample, since it quantifies the deformation energy stored during the process of shearing.G'' (the loss or viscous modulus), which denotes the energy dissipated as heat during shear, represents its viscous behaviour. The loss tangent (or the loss factor), tan δ , is the ratio of G''/G'.

Oscillatory rheology, which isapplied to differentiate between the viscous and elastic characteristics of materials, primarily consists of stress sweepand frequency sweep [8,9,16]. Stress sweep, also referred to as amplitude sweep, ascertains the linear viscoelastic region (LVER)located within the limiting value of shear stress at which the microstructure is deformed.Fundamentally, the amplitude of the applied stress and strain is linear in the LVER, and this is a necessary condition for the reproducibility of rheology results. For practical purposes, the LVER is inferred from the stress value until which the moduli (G' and G'') remain constant. Frequency sweep is performed at a shear stress value in the LVER to yield the plot of G' and G'' against angular frequency (ω). The moduli behaviour and theircrossover, if any, in a typical frequency sweep plot can reveal the structural character of materials, either as gel, paste or solution.

The transformation from a viscous to a gel state can be interpreted from the change in the mechanical spectrum which is defined by the viscoelastic parameters of G', G" and $\eta^*[6]$. On reaching a gel state, the value of G' plateaus (G'_{max}) and becomes independent of ω , while G" remains one decade below G'_{max} on a logarithmic scale. In time sweep experiments, gelation is characterised by a crossover point at which G' exceeds G", signifying the transition from a 'viscous' liquid to an 'elastic' gel.

2.5 Gelation behaviour of β-glucan

Gelling properties of oat and barley β -glucan, the two major cereal sources, were first reported by Doublier and Wood [17]and Bohm and Kulicke[5,15], respectively. Their investigations reaffirmed the significance of molecular weightand the solution concentration in polymer rheology, since an increase in

viscoelasticity due to the aggregation of molecules was discernible only when both the parameters exceed their respective critical values. The induction time for the onset of gelation in typical viscous solutions was observed to be inversely proportional to the concentration. The gelation characteristics were corroborated in frequency and time sweep experiments in which a substantial increase in the values of η^* and G' was recorded. Furthermore, Bohm and Kulicke [5]also noted the gelation to be completely thermo-reversible. The viscoelastic fluid properties were restored upon heating the gel at 80°C and subsequently cooling. No changes in the flow characteristics were detectable on repeating this cycle for even up to 10 times.

In the subsequent research article, Bohm and Kulicke [15]discuss the kinetics and mechanism of barley β -glucan gel formation. In trials with oat, barley and lichenan β -glucan solution of different concentrations and MW, gelation timedeclined with the solution concentration, but was inversely proportional to the molecular weight of β -glucan. This is because a low concentration reduces the probability of contact between the polymer coils, which is a prerequisite for the formation of a gel network. The influence of low molecular weight β -glucan in increasing the gelation rate is possibly due to the higher mobility of shorter polymer chains which facilitates diffusion and inter-chain associations [3].

3. Microrheology methods

Microrheology comprises of both active and passive optical measurement techniques which are increasingly utilised for research on biopolymer characterisation [18,19,20]. Active methods manipulate the added probes, also referred as tracer particles, in the sample by applying optical or magnetic tweezers to determine rheological properties of materials. Atomic force microscopy (AFM) is another active technique that has been newly developed to study the interactive molecular forces and local viscoelastic characteristics. Passive particle tracking techniques, which track the Brownian motion of tracer particles by direct-imaging based video microscopy or dynamic light scattering (DLS), are more frequently employed in biopolymer solutions. Motion of tracer particles in a macromolecular solution, which is thermally driven by Brownian forces, is dependent on the viscoelastic features of the medium [20,21].

In microrheology, the microstructure of the entity is explicitly accounted for [8]. This is in contrast with the typical rheology approach, whichdoes not consider the solute and the solvent as distinct entities, but theentire polymer solution as a homogenous continuum. The microrheological approach facilitates measurement of the viscoelasticity of complex fluids at a micron-scale at considerably high frequencies (above 1000 Hz), without affecting the microstructure of the system sincean external stress is not applied [4,20]. Furthermore, microrheology techniques are highly sensitive even at low solution concentrations, and do not display any inertia effects which are likely in mechanical rheometry. In addition, small sample volume requirements in a micro-litre scale are particularly advantageous for biological systems.

3.1 Direct-imaging technique

Video particle tracking microrheology is based on either fluorescence or bright field video microscopyof a sizable ensemble of embedded probe particles [20,22,23]. The digital images are processed to yield the time-dependent trajectories of the probe particles which are utilised to compute the mean-squared displacement (MSD) averaged for the ensemble. Complex modulus, $G^*(\omega)$, which quantifies the sample viscoelasticity, is derived by applying the Stokes-Einstein equation to MSD { $\Delta r^2(i\omega)$ }data.

$$G^{\bullet}(\omega) = \frac{k_{B}T}{\pi a i \omega \langle \Delta r^{2}(i\omega) \rangle}$$

where Δr is the displacement of a tracer particle, ω is the angular frequency, i is the imaginary unit of complex numbers, a is the diameter of probe particle, T is the absolute temperature, and k_B is the Boltzmann constant.

3.2 Dynamic Light Scattering

In the rapid non-invasive DLS microrheology technique, scattering of light by suspended particles determines the average motion (MSD) of the probe particles added to the solution matrix [18,23,24,25]. The random movement of particles, commonly referred to as Brownian motion, is due to thermally induced collisions with molecules in the liquid interface. Furthermore, since small particles move rapidly, and vice versa, the rate of the intensity fluctuation of the scattered light varies with the size, and this has also been exploited for obtaining the particle size distribution by DLS.Mason and Weitz [26] were the first to

quantitatively estimate the linear viscoelastic moduli (G' and G") of bulk complex fluids by applying DLS to ascertain the MSD. However, the DLS method is not feasible for typical food suspensions such as milk, since the scatters must be adequately dilute to prevent multiple scattering which might distort the results [24].

The selected microrheology probe chemistry must ensure that the particle-matrix interactions are minimised [18]. In their paper on developing the method withbovine serum albumin (BSA) protein solutions, Amin et al.[21]outline the steps for ascertaining the suitability of probes for microrheology measurements. Firstly, the zeta potential difference, which is measure of the effectiveness of the surface charge of a colloid particle, between the dilutions of probe-in-sample and probe-in-solvent (water for β -glucan samples) is determined. If the two differ significantly, an alternate probe with different chemical properties is considered. Furthermore, the size and the concentration of probe are considered appropriate for microrheology measurements if the particle size distribution (PSD) is dominated by single peak intensity corresponding to the nominal probe diameter.Otherwise the concentration of the probes isincreased, failing whichlarger probe diameters investigated.

3.3 Microrheology of β-glucan

Direct-imaging based microrheology was employed by Xu et al.[27]to investigate the evolution of viscoelasticity with increasing concentration in oat β -glucan solutions. Sol-gel transitions in barley β -glucan solutions at different polysaccharide concentrations [4] and molecular characteristics [28] were also investigated with this technique. In the aforementioned experiments, motion of fluorescent polystyrene probes was monitored with phase contrast microscopy to obtain the MSD data.

The results obtained by Moschakis et al.[4] in barley β -glucan solutions, which formed a gel upon storage in isothermal conditions, were found to satisfactorily overlap with dynamic rheometry measurements at the pre-aggregation stages. The trials confirmed the high sensitivity of the microrheological experiments in determining much lower values of G' during the early stages of elasticity development. However, it was also revealed that the particle tracking methodshave limitations in measuring high values of G' beyond the gel point, which is due to the constraints in the movement of the tracer particle in aggregated systems. Furthermore, the microscopically evaluated dynamics of cereal β -glucan gel networks by Moschakis et al. [28] did not fully corroborate with the respective bulk rheology response. There were no other research publications on microrheology of β -glucan solutions at the time of writing this review, and nonehad specifically utilised the DLS microrheology technique for characterisation of the biopolymer.Further research on optimising the DLS technique is mandated so that any possible interaction of microrheology probes with the sample matrix is minimised.

4. Summary and prospects

In this review, the underlying concepts in various rheology and microrheology methods applied to assess the viscosity and gelation characteristics of cereal β -glucan are briefly explained. The studies emphasised are primarily for oat and barley β -glucan at different molecular weight and solution concentrations. At sufficiently high concentrations, an overlap in the mechanical spectrum (G', G'' and η^*), obtained respectively in gelation rheology and direct-imaging microrheology experiments, has greatly enhanced the reported frequency range. However, the optimal concentration regimes are dissimilar for bulk rheology and DLS microrheology, and thus the possibility of such an overlap may be unlikely. Nevertheless, the development of viscoelasticity in dilute β -glucan solutions can be rapidly and more accurately appraised by applying the novel light scattering based microrheology method. Dilute β -glucan solutions are more likely to be encountered in the human intestine during the *in vivo* digestion process because of its relatively low content in cereals and other functional foods, and thus it will be interesting to correlate the physiological effects of the soluble dietary fibre at low concentrations with the viscoelasticity evaluated by the DLS microrheology technique.

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