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Small-Strain Dynamic Rheology of Food Protein Networks

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ABSTRACT: Small-amplitude oscillatory shear analyses of samples containing protein are useful for determining the nature of the protein matrix without damaging it. G' (elastic or storage modulus), G'' (viscous or loss modulus), and tan δ (loss tangent, the ratio of G'' to G') give information on the properties of the network. Strain, frequency, time, and temperature sweeps provide information on the linear viscoelastic region, structural assembly, and thermal characteristics. The gelation point may be determined by locating the time at which tan δ is independent of frequency or the temperature at which G' becomes greater than G''. The logarithm of η^* (complex viscosity) may be plotted against the reciprocal of the absolute temperature, with the slope being proportional to the activation energy. Dynamic tests of protein-containing samples reveal a great deal about their rheological characteristics.

KEYWORDS: protein, rheology, small-amplitude oscillatory shear

INTRODUCTION

Viscoelastic materials exhibit elastic solid and viscous liquid behavior. When deformation of such a material is sufficiently small, it may exhibit linear viscoelasticity, in which the measured properties are independent of the magnitude of the input variable.[1] Small-amplitude oscillatory shear analyses are a type of dynamic rheological test in which stress and strain are varied harmonically with time in the linear viscoelastic region (LVR). These are nondestructive tests that provide information on polymer bonding characteristics and can be applied to biopolymers, that is, protein networks in food. When used in conjunction with other analytical techniques, a clear picture of biopolymer behavior at the molecular level may be obtained. The equation governing the procedure is

$$|G^*|^2 = (G')^2 + (G'')^2$$

where G^* is the complex modulus, a measure of the deformation of the sample; G' is the elastic modulus (or storage modulus), a measure of the energy stored and recovered per oscillation; and G'' is the viscous modulus (or loss modulus), a measure of the energy dissipated and lost as heat per oscillation. The complex viscosity η^* is the ratio of G^* to frequency ω and is a measure of the resistance to flow. The loss tangent, or tan δ, is the ratio of G'' to G' and is indicative of liquid-like behavior when much greater than 1.0.[2] The two types of rheometers available are controlled strain, with measurement of torque, and controlled stress, with measurement of angular motion. Rheometers with cone-and-plate, parallel plate, and concentric cylinder measuring geometries are typically used for food analysis.[3]

The common tests performed using small-amplitude oscillatory shear analyses are strain sweeps (or stress sweeps, for controlled stress rheometers), frequency sweeps, time sweeps, and temperature sweeps. Structural information is provided when these tests are applied to proteins in food. Protein gels may have noncovalent linkages such as hydrogen bonds and hydrophobic interactions, covalent bonds such as disulfide bonds, and physical entanglements of the protein chains. Reviews of the use of small-amplitude oscillatory shear in food rheology were published 10 years ago by Gunasekaran and Ak[4] and Tunick,[2] and Kavanagh and Ross-Murphy[5] reviewed the theory underlying the technique. This paper will briefly review and outline the principles and applications of these techniques, along with some representative examples from the author and others.

STRAIN SWEEPS

The LVR may be determined by increasing strain levels at a constant frequency and locating the plateau in which G' does not deviate by a significant amount from a constant value. A percent strain in that region and the same frequency are then used for subsequent analyses. Stress sweeps are used to locate the LVR when controlled stress tests are made. An example is shown in Figure 1, in which a plot of the logarithms of G' and strain of a cheese sample shows a relatively flat region until the structure starts to become affected and log G' drops off. Frequency, time, or temperature sweeps should then be run using a strain value in the flat region, in this case 0.1−0.5%.

Strain sweeps provide information on the properties of protein dispersions. Hsu[6] concluded that the constancy of G' in the LVR in soy protein isolate dispersions at room temperature (0.8−8.8% concentration, pH 6.8−7.2) was due to a stable dispersion of particles that were not agglomerated. The G' values decreased above 2% strain, suggesting interference across the weak network with the structure of the protein spheres. Uthayakumaran et al.[7] found that the upper limit of the LVR of gluten dough at 25 °C (45% gluten) was an order of magnitude larger than that of flour dough (60% flour with all gluten extracted). The upper limit decreased exponentially when they increased the starch concentration, whereas G' and G'' increased. They concluded that

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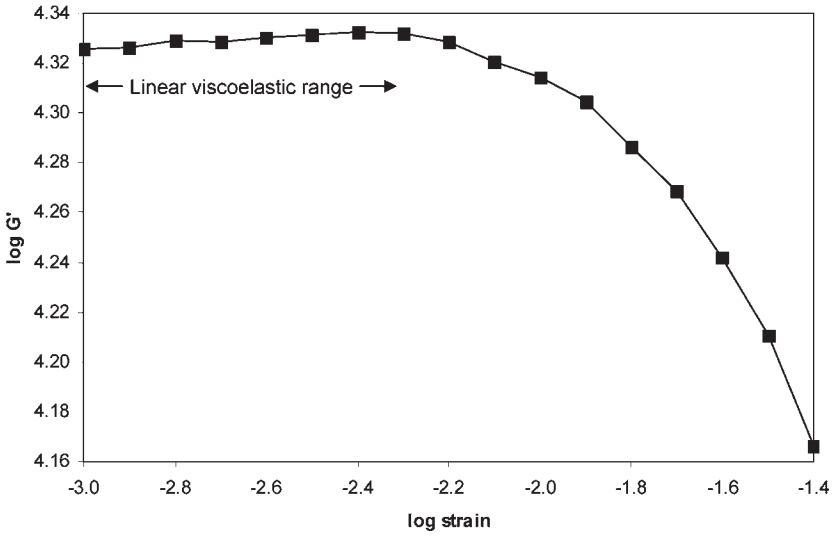

Figure 1. Strain sweep of Cheddar cheese made from goat's milk after 1 month of storage, demonstrating change in elastic modulus (G') with strain. The linear viscoelastic range corresponds to 0.1−0.5% strain.

dough may be considered as a concentrated dispersion of starch granules within a protein phase.

Some food protein networks may not exhibit a linear viscoelastic region. Strain sweeps at 1 and 10^{-3} Hz of natural (not stabilized with emulsifier) and smooth (stabilized) peanut butters by Citerne et al.[8] did not reveal a linear range, apparently because of structural breakdown through the strain sweep. Conclusions could still be drawn regarding the material, such as time-dependent effects of thixotropy and the time of structural breakdown.

FREQUENCY SWEEPS

In a frequency sweep, ω is varied while the strain is held constant. The test determines the response of the structure to different experimental times: a relatively low ω such as 1 Hz could be considered a long time (1 s), as opposed to a relatively high ω such as 100 Hz, which would be a short time (0.01 s). Bonds between particles in a sample may be made and broken during the observation time, either spontaneously or from applied forces. Bond-breaking and bond-making lead to structural changes that affect rheological properties.[9] In addition, simple entanglements of polymer strands provide a response. Figure 2 shows a frequency sweep of an extruded whey protein concentrate, which in this case was a sticky soft solid produced by extrusion at 50 °C and containing 52% moisture. G'' increased at a faster rate than G', resulting in a crossover at 63 rad/s. The sample could be considered to have exhibited solid-like behavior below this frequency and liquid-like behavior above it. Therefore, at long experimental times elastic behavior was dominant, and at higher frequencies and short times, viscous behavior exceeded elastic. In frequency sweep studies of soy protein isolate gels (120 mg/g, pH 7.6), Renkema et al.[10] found high tan δ values at low frequencies in pH 7.6 and 5.2 gels at 95 °C, implying that bonds between protein molecules could be broken and reformed more easily than at pH 3.8. They concluded that rearrangements caused changes in the protein network. They also related G' to gel stiffness, which varied when pH, NaCl concentration, and temperature were altered.

Gel Type. Frequency sweeps over at least three decades of frequency may be used to provide an indication of the type of gel formed in the sample. Protein gels may be classified as entangled networks (of biopolymers), chemical (cross-linked) gels, or physical (noncovalent linkages) gels.[11] Entangled networks are soft gels with strong G' versus ω dependence and a $G'-G''$ crossover, meaning that they are liquid-like at low frequencies and solid-like at higher frequencies. Cross-linked gels are strong and have permanent covalent networks and little frequency dependence. Physical gels, which are intermediate between strong and weak gels, have some frequency dependence and no $G'-G''$ crossover.[12] The pertinent equation is

$$\log G' = n \log \omega + K$$

where n and K are constants, n being the degree of frequency dependence.[13] The value of n is 0 for a perfectly cross-linked (covalent) gel and is a positive number for a physical gel.[11,13] Figure 3 shows the frequency dependence of 20% solutions of egg albumen (pH 6.96, mixed at 20 °C and allowed to stand for 1 h) and whey protein isolate (pH 6.34, same conditions as above) at 80 °C. The n value for egg albumen is 0.114, indicating a strong cross-linked gel, and the n value for whey protein isolate is relatively high at 0.454, indicating a weak physical gel.

In frequency sweeps from 0.01 to 5 Hz at 20 °C, Stading and Hermansson[11] found that 10−12% solutions of β-lactoglobulin preheated to 90−95 °C formed physical gels at pH 4−6 and covalent gels at low and high pH. The intermediate pH samples were coarsely aggregated gels, and the others were fine-stranded gels with flexible or rigid strands, making this technique a way of distinguishing between the two.

TIME SWEEPS

When the frequency, strain, and temperature are held constant, the resulting plot is a time sweep. The procedure is often used for monitoring reactions and structural assembly or degradation. A classic application of this technique is the coagulation of milk. Figure 4 shows a time sweep of 20 mL of milk (pH 6.65) to which a 100 μL aliquot of 2.5% rennet had been added. G' and G'' increase gradually until the 7 min mark, when both values increase sharply, indicating the start of curd formation. G' eventually predominates, demonstrating solid-like behavior.

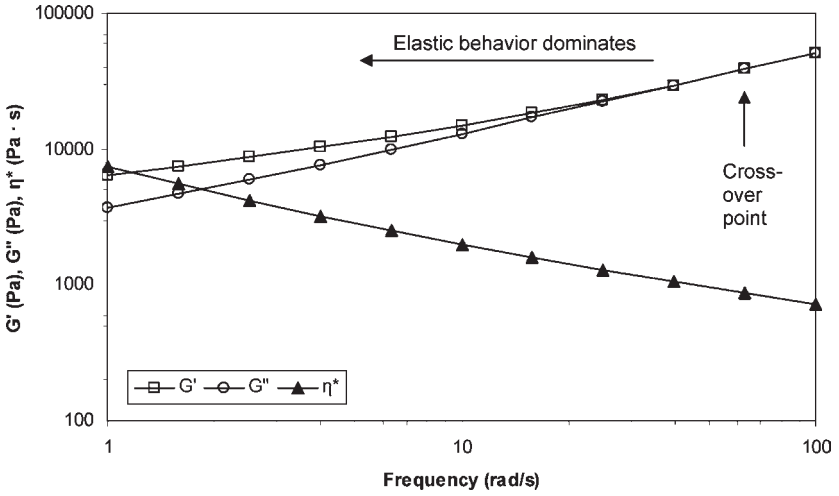

Figure 2. Frequency sweep of extruded whey protein concentrate, showing elastic modulus (G'), viscous modulus (G''), and complex viscosity (η^*). Elastic behavior dominated until the crossover point at 63 rad/s, at which G'' became greater than G'.

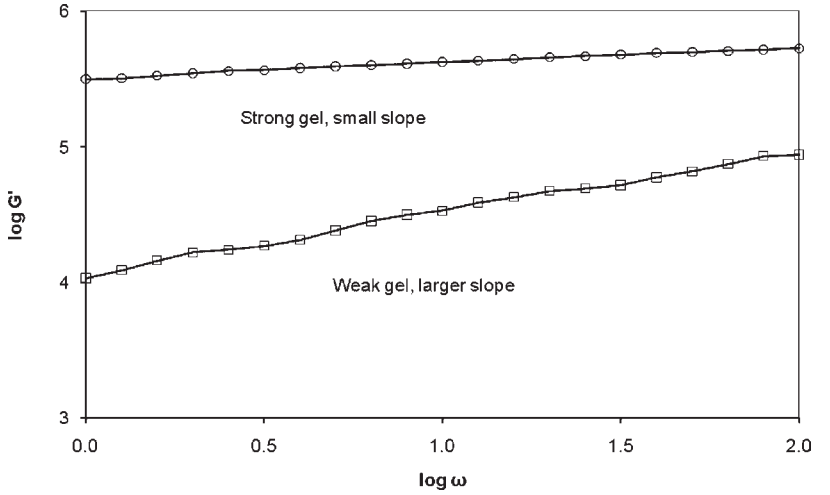

Figure 3. Frequency sweep at 80 °C of 20% solutions (mixed at 20 °C and allowed to stand for 1 h) of egg albumen (circles, pH 6.96) and whey protein isolate (squares, pH 6.34), demonstrating frequency (ω) dependence of elastic modulus (G'). For egg albumen, $\log G' = 0.114 \log \omega + 5.51$. For whey protein isolate, $\log G' = 0.454 \log \omega + 4.06$.

Time sweeps are also used to determine structural arrangement and kinetics of protein matrices. In a study of dispersions of cross-linked waxy maize starch and whey protein isolate, Ravindra et al.[14] observed a slight decrease in tan δ over 4 h, attributed to gradual but limited structural assembly. By varying the starch mass fraction and plotting against G', they observed three zones in the resulting curve, which they assumed to be a continuous whey protein network interrupted by maize starch granules, a continuous maize phase interrupted by whey protein aggregates, and two continuous noninterrupted phases. Zhang et al.[15] performed time sweeps to determine the gelation kinetics of chickpea protein isolate dispersions. By using a nonlinear model, they related G' to the rate constant.

TEMPERATURE SWEEPS

Temperature sweeps are used to observe behavior upon heating. Figure 5 shows temperature sweeps of calcium caseinate (pH 6.87, mixed at 20 °C and allowed to stand for 1 h). Hydrophobic interactions started to take place at low temperatures, and above 40 °C there was aggregation and formation of a rigid gel, leading to a sharp increase in G' and G''. Madeka and Kokini[16] performed temperature sweeps on zein; a 15% solution exhibited a decrease in G' and G'' from 50 to 120 °C, indicating entangled polymer flow. Above 122 °C, G' increased sharply and G'' decreased due to cross-linking. By testing at various moisture contents and using frequency sweep data, a state diagram for zein was obtained. Badii and Howell[17] related G' to muscle toughening in temperature sweeps from 25 to 90 °C at 1 rad/s of frozen cod and haddock fillets (90 g in 10 mL of water). G' values for white muscle were higher than those for dark muscle, indicating that white muscle contained more covalent and noncovalent aggregates resulting from more extensive protein denaturation.

Activation Energy. When a protein network undergoes a transition as the temperature increases, the activation energy may be determined from temperature sweeps by using the Arrhenius equation

$$\eta^* = A \exp(-E_a/RT)$$

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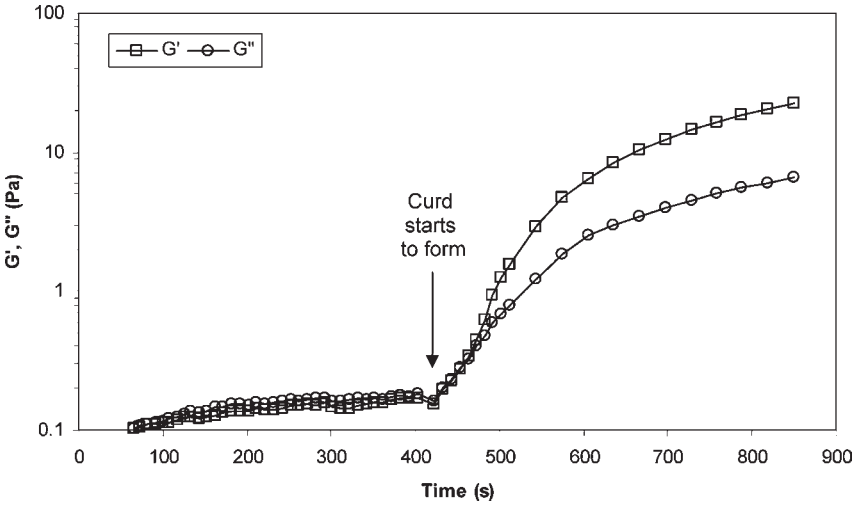

Figure 4. Time sweep of 20 mL of milk (pH 6.65) to which a 100 μL aliquot of 2.5% rennet was added at 0 s, showing changes in elastic modulus (G') and viscous modulus (G''). Curd formation began at 420 s.

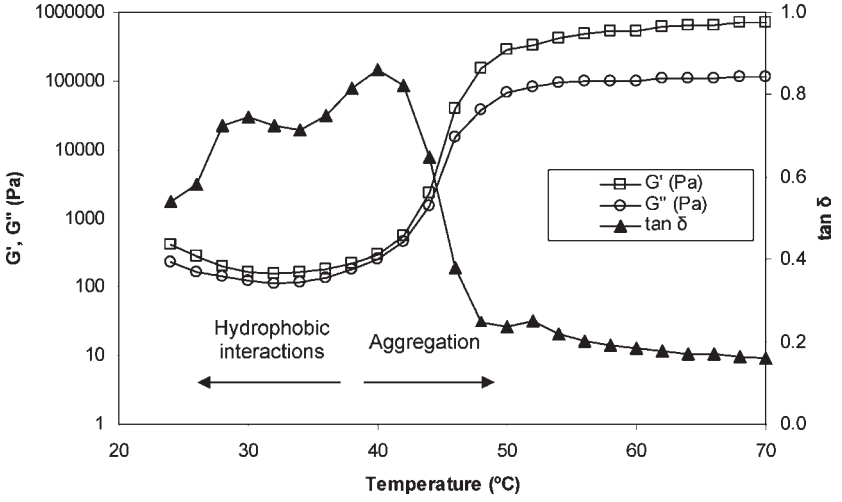

Figure 5. Temperature sweep of a 20% suspension of calcium caseinate (pH 6.87, mixed at 20 °C and allowed to stand for 1 h) showing changes in elastic modulus (G'), viscous modulus (G''), and loss tangent (tan δ).

where A is the pre-exponential factor, E_a is activation energy, R is the gas constant (8.314 J/K mol), and T is temperature in K. When the reciprocal of absolute temperature is plotted against ln η^*, E_a is proportional to the slope of the line. Tunick et al.[18] found that E_a decreased with age of cheese because the protein matrix was breaking down. Figure 6 shows Arrhenius plots of Cheddar, soft goat, and Parmesan cheeses, with E_a values of 142, 60, and 180 kJ/mol, respectively. The results provided a method for quantitating the degree of melting of cheese, with a high E_a indicating rapid liquefying of fat and collapse of the protein matrix.

Temperature sweeps are also employed to determine the glass transition temperature of partially amorphous food such as baked goods and confectionery products, although differential scanning calorimetry is the more commonly used technique. The glass transition is determined at the G'' peak, the tan δ^* peak, or the point at which half of the initial stiffness (G') is lost.[4]

Gel Point. The gel point may be defined as the temperature when an infinite network occurs in the specimen. In small amplitude oscillatory shear, it is the time or temperature at which tan δ is independent of frequency.[19] The gel point of a 20% egg albumen solution (pH 6.96, mixed at 20 °C and allowed to stand for 1 h) was found by performing temperature sweeps at several frequencies (Figure 7). Ovotransferrin denaturation is completed around 60 °C, causing the tan δ value to decrease and level off; the temperature at which these events occur decreases with frequency.[20] Tan δ was the same for all frequencies at 62 °C, indicating that this was the gel point related to ovotransferrin. Michon et al.[21] determined gelation (sol−gel state) and melting (gel−sol state) of gelatin solutions at 1.1−20% w/w by frequency sweeps. Repeating at temperatures between 17 and 35 °C led to the creation of a phase diagram.

Summary. Small-amplitude oscillatory shear analyses provide a nondestructive method for evaluating structural characteristics of protein networks. Strain sweeps are used to locate the LVR and the nature of dispersions. Frequency sweeps are employed to examine bond-breaking and bond-making, protein chain entanglements, characteristics of a protein gel, and gel point. Reactions are monitored with time sweeps. Temperature sweeps may determine behavior with heating, E_a, and glass transition.

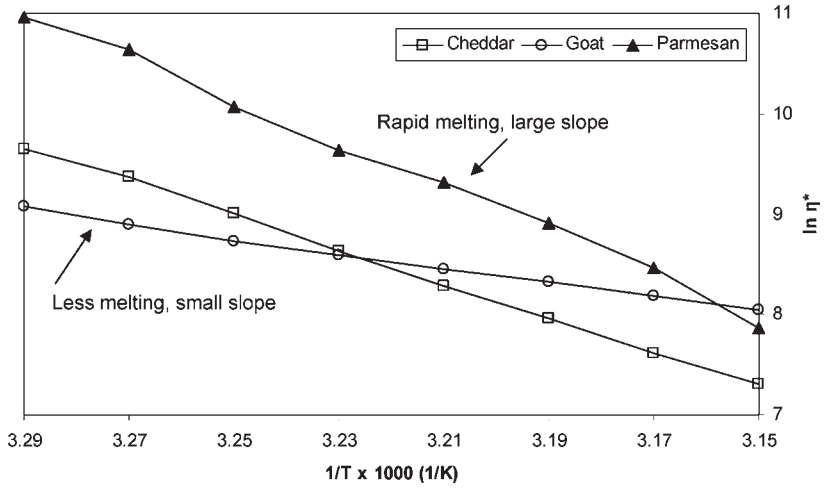

Figure 6. Arrhenius plots of Cheddar, soft goat, and Parmesan cheeses from 30 °C (3.29/K) to 44 °C (3.15/K).

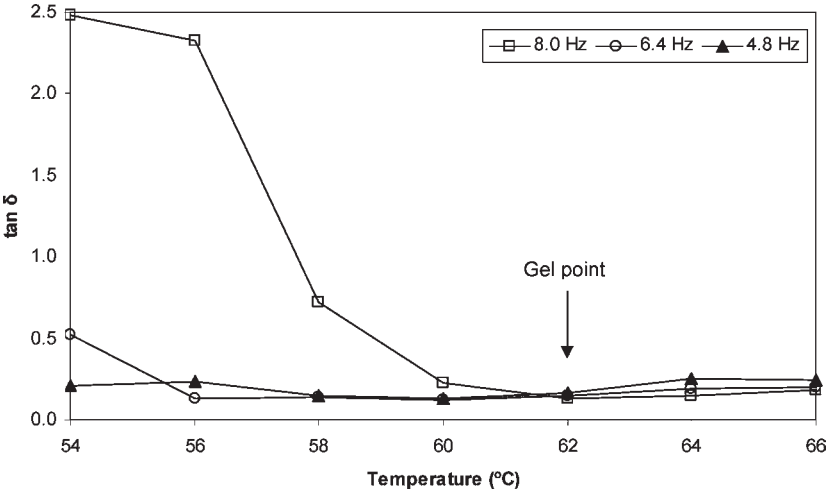

Figure 7. Temperature sweeps of 20% egg albumin solutions (pH 6.96, mixed at 20 °C, allowed to stand for 1 h, heated at 0.8 °C/min) at different frequencies. The gel point, where the loss tangent (tan δ) is independent of frequency, is 62 °C.

Knowledge of the rheology of protein-containing samples allows scientists to determine their suitability in food formulations.

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