fered marginally ($F = 5.4$) at $P \leq 0.05$. Independent tests on the slope and y intercept indicated that the slope did not differ from 1 ($t = 2.35$, $P \leq 0.05$) and the intercept did not differ from 0 ($t = 0.123$, $P \leq 0.05$). A pairwise T test on the data did not indicate a difference ($t = 1.65$ at $P \leq 0.1$). It is therefore apparent that the two methods gave results which were in fair agreement (on the alkaline extract). The microbiological method has better precision than the HPLC method. This is to be expected since the values for the microbiological method is estimated from the mean of five different dilutions of the sample.

The recovery by the HPLC method on the millet sample was 100.1% (coefficient of variation CV = 4.5%, $n = 10$) and for the microbiological method 101.1% (CV = 6.6%, $n = 5$). The HPLC method has a detection limit of 0.5 mg of niacin/100 g of sample when a 5-g sample is extracted and diluted to 100 mL.

A chromatogram can be completed within ca. 1 h. The HPLC method would, therefore, have an advantage when a small number of samples must be analyzed. For a large number of samples, the HPLC method would only be able to compete with the microbiological method, in the number of samples analyzed, if an automatic sampler is used to enable the chromatograph to be run overnight.

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Progel and Gel Formation and Reversibility of Gelation of Whey, Soybean, and Albumen Protein Gels

Tom Beveridge,*[1] Linda Jones, and Marvin A. Tung

Dynamic shear measurements were used to determine the kinetics of development of structural rigidity in thermally produced egg white (EW), whey protein concentrate (WPC), and Promine D (PD) gels by measuring the change in the storage modulus (G') during heating and cooling. EW showed rapid and extensive G' value development with heating time whereas G' development in WPC was much slower and less extensive. When cooled, both protein systems showed a reversible cooling "set" with the WPC "set" approximately 10× greater than that of EW. Thermal development of G' values could be described by a first-order kinetic equation of the form $G' = G'_\infty(1 - e^{-kt})$. G' development in PD gels was characterized by the presence of considerable elasticity in the aqueous suspensions prior to heat application and rapid development of G' values on heat application. This development could not be described as first order and no cooling set was observed in PD gel samples. Scanning electron microscopy showed EW and WPC gels to consist of a network of spherical particles apparently adhering together. PD gels consisted of very large particles embedded in a gel matrix.

The basic texture and structure of many foods depends upon the formation of relatively rigid structures by protein through the process of thermal gelation. Despite this obvious importance of protein thermal gelation to food formation, little is known of gel formation following initial denaturation (Shimada and Matsushita, 1980, 1981) and few methods exist to accurately measure gel formation (Hermansson, 1979). Potentially, valuable information on the degree and mechanism of gelation would be available if it were possible to follow the time course of gelation (Catsimpoolas and Meyer, 1970), but following formation of structure in a heated protein solution or suspension is difficult. Commonly, rheological instruments are designed to measure the "gel strength" of the preformed gel (Beveridge et al., 1980; Schmidt et al., 1979) or the apparent viscosity of heated solutions or suspensions (Hermansson, 1975, 1978; Catsimpoolas and Meyer, 1970). None of these methods are very suitable for following the time course of gelation since either the gel must be performed or the shearing forces generated during measurement destroy the forming structure.

Dynamic shear measurements are made at small total strains and the rheological properties measured are essentially those of the undisturbed material (Elliott and Ganz, 1975), avoiding the problems associated with structural breakdown during measurement. Evaluation of dynamic shear stress response to small amplitude oscillation requires evaluation of two moduli, the storage modulus (G'), a measure of energy stored due to elastic deformation of the sample, and the loss modulus (G''), a measure of energy dissipated as heat due to viscous flow within the sample. The sinusoidally varying shear stress and strain signals are separated by a phase difference, the tangent of which, the loss tangent, is numerically equal to the ratio G''/G'. In principle, as gel networks form, the sample becomes more elastic in nature and G' values will rise while the loss tangent values fall.

School of Food Science, Macdonald Campus of McGill University, Ste Anne de Bellevue, Quebec, Canada H9X 1C0 (T.B.), and Department of Food Science, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5 (L.J. and M.A.T.).

[1] Present address: Agriculture Canada Research Station, Summerland, British Columbia, Canada V0H 1Z0.

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In gel property measurement, it is highly desirable that the gels be formed within the instrument (Elliott and Ganz, 1975; Gill and Tung, 1978). This paper describes an attempt to utilize dynamic shear measurements to follow gel development during heating and cooling of egg albumen, whey, and soybean protein concentrates, forming gels within the instrument. The effects of solids content and temperature, cooling effects, gelation reversibility, gel ultrastructure, and the kinetics of gel development are examined.

EXPERIMENTAL SECTION

Egg white was obtained from eggs purchased locally and yolk and white separated. The albumen was blended gently by brief, intermittent operation of a Waring blender so as to avoid foaming and then feeze-dried. The freeze-dried powder was stored in a sealed container at 4 °C. Whey protein concentrate (WPC) was a commercially available product of Dairyland Products, Inc., Savage, MN (WPA-36A), and Promine D was a commercially available soybean protein concentrate obtained from Food Products, Ltd., Montreal, Canada. The composition of these three products as determined from standard procedures (Association of Official Analytical Chemists, 1975) is as follows: albumen, protein ($N \times 6.25$) 81.7%, moisture 6.4%, ash 5.4%, and pH of aqueous solution 9.3; WPA-36A, protein ($N \times 6.38$) 30.6%, moisture 9.5%, fat 0.5%, ash 6.0%, and pH of aqueous suspension 6.6–6.7; Promine D, protein ($N \times 5.7$) 85.2%, mositure 9.0%, fat 0.9%, ash 4.2%, and pH of aqueous suspension 6.8–6.9. Solutions or suspensions were made on a weight percent basis in distilled water.

Temperature Control. Gels were evaluated for dynamic shear stress response to small amplitude oscillation with a Model R.18 Weissenberg rheogoniometer (Sangamo Controls, Ltd., Bognor Regis, England) equipped with a modified Ferranti-Shirley lower platen combined with the 7.5-cm flat upper platen supplied by the instrument manufacturer. The lower plateau was modified to allow simultaneous connection through two Y-fittings to a Haake Model FE circulating heating bath and a Haake Model FP circulating heated-refrigerated bath, set at 25 °C. Circulation of either heating or cooling (25 °C) fluid (50:50 water–ethylene glycol automotive antifreeze) was controlled by means of screw clamps situated at the Y-fittings. This provided a parallel configuration with a rapidly responding temperature control, which allowed the temperature to be changed in a stepwise manner.

The temperature profile within the gap under conditions of actual use was measured with an iron, iron–constantan, plastic-coated thermocouple located in the approximate center of the sample cylinder formed between the two platens and connected to a C.W. Brabender digital temperature indicator. Temperatures within the gap are shown in Figure 1 for both the heating and cooling cycles.

Gel Formation. Gels were formed in situ between the plates of the rheogoniometer. A masking tape dyke was formed around the outer circumference of the lower platen, and sample (~7 mL) was pipetted on the center. The upper platen was lowered into approximate position, the assembly was allowed to equilibrate (25 °C) for 10 min, and the gap was set at 1 mm. The sample was trapped within the gap by careful introduction of sufficient oil at 25 °C (25–30 mL, paraffin oil, Saybolt viscosity 125/135, Fisher Scientific Co.) to just cover the upper platen, oscillation was begun, and initial readings were obtained for the sample. At zero time, heating fluid was allowed to circulate through the lower platen, causing gelation. At the end of the heating period, clamps were adjusted to allow cooling fluid to flow through the lower platen. Oscillatory input was continued throughout the experimental period.

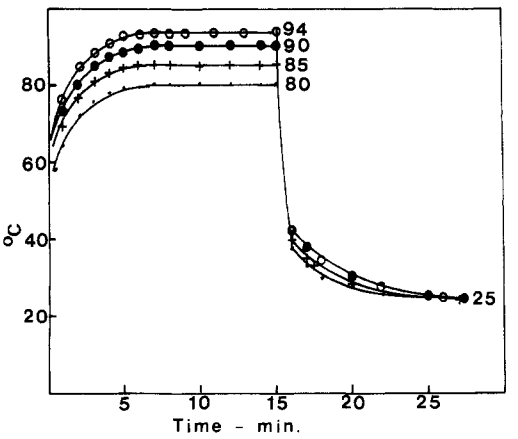

Figure 1. Gap temperature profiles for egg albumen (8%) and WPC (22.8%) as the average of two runs each. Gap was 1.0 mm at 25 °C and temperatures were obtained as shown.

Rheological Evaluation. Dynamic measurements were made at strain amplitudes (maximum linear upper platen displacement divided by sample thickness) of about 0.06 at a fixed freqeuncy of 0.948 Hz. The 7.5-cm flat upper platen was constrained by a torsion bar of torsion constant 875 dyn cm μm^{-1}. Since sample thickness decreased as temperature increased due to thermal expansion of the metal platens, the gap was set at 25 °C with water in the gap. The temperature was raised incrementally by 10 to 95 °C and a gap measurement made at each temperature after a 20-min equilibration period. This allowed calibration of the gap change with temperature and calculation of the appropriate sample thickness when results were obtained with samples gelled at high temperature. The shear strain input signal, the resultant shear stress output signal, and the phase difference between the two sinusoidally varying functions were monitored with a Tronotec Model 703A digital analyzer (Tronotec, Inc., Franklin, NJ). Calculation of rheological parameters was done according to Walters (1968) using the formulae appropriate to the parallel plate geometry.

Sulfhydryl Analysis. This analysis was performed by a modification of the procedure of Beveridge and Arntfield (1979). Duplicate 1-mL samples of whey protein concentrate (20%) were pipetted into 15 × 150 mm screw-capped tubes, capped tightly, and heated in a thermostated water bath. After heating, the tubes were cooled in tap water and opened, and 10 mL of 0.1 M phosphate buffer, pH 8, containing 1.2 g/L Na_2EDTA and 6 g/L sodium dodecyl sulfate (SDS) was added followed by 0.1 mL of DNTB solution (4 mg/mL 5,5-dithiobis(2-nitrobenzoic acid) in 0.1 M phosphate buffer, pH 8). Color was developed for 5 min, and then the solution was transferred to 50-mL centrifuge tubes and centrifuged at 27000*g* (Sorval RC-2B) for 20 min at 15 °C. The slightly opalescent supernatant was read at 412 nm in a Unicam SP 800 B spectrophotometer in 1-cm cells. To correct for this slight opalescence, a blank tube in which 0.1 M phosphate buffer was substituted for the DTNB solution was carried through in parallel. Egg white (1%) was treated in a similar fashion; however, since no turbidity developed during heating, centrifugation and the extra blanks were unnecessary and were omitted. The molar absorptivity, 13 600 (Ellman, 1959), was used to determine the concentration of sulfydryl in the sample.

Scanning Electron Microscopy. Gel pieces (approximately 2 × 2 × 4 mm) were fixed overnight in 4%

glutaraldehyde in 0.07 M phosphate buffer, pH 7.0, followed by a phosphate buffer rinse (0.07 M, pH 7.0, 3 times, 10 min). Osmium tetraoxide fixation (1% in phosphate buffer, 3 times, 10 min) was followed by ethanol dehydration (50, 70, and 80% in distilled deionized water 5 min, 90%, 2 times, 10 min, and 100% 3 times, 10 min) and then transferred to amyl acetate (25, 50, and 75% in ethanol, 10 min each, and 100% for 1 h). Critical point drying in liquid CO_2 completed the process. The dried pieces were mounted on aluminum stubs with epoxy, coated with a gold–platinum alloy in a sputter coating device (Technics, Inc.). A Cambridge Stereoscan scanning electron microscope operated at 20 or 40 kV was used to examine the gels.

RESULTS

Three protein systems exhibiting three completely different gel forms were selected for this initial study. Heating aqueous solutions of freeze-dried egg albumen resulted in formation of cloudy, but translucent, cohesive, and very elastic gels rather than the more traditional opaque white gels because of the high pH (9.3) of the solutions. WPC formed a distinct, visually strong appearing gel on heating. However, when examined in the fingers, the gelled material lacked the springy, rubber elastic character of the gelled albumen, consisting instead of drier, crumbly, dense material having some elastic character but overall more pâté-like. Promine D thickened on heating to form a soft gel, lacking the springy elastic character of albumen gels and being much more paste-like, more crumbly, and less cohesive than WPC gels.

Preliminary experiments with egg albumen showed that sample dehydration and particularly skinning over of the sample at the edge between the two platens precluded simple application of heat to the sample in the gap even if a film of oil was applied to the exposed, gelled sample surface. However, it proved possible to trap the aqueous sample within the gap by forming an oil bath around the sample and upper platen, effectively eliminating skinning and reducing evaporation to low levels. During heating, gas bubbles became evident in that portion of the sample that had overflowed from between the platens. The gas source is not known but probably arises from water vapor generation as sample temperature is raised. The problem became very serious as temperature increased beyond 90 °C. Because of possible sample dehydration, to limit the influence of gas bubble formation and to conserve time, temperatures above 90 °C and heating times beyond 30 min were not routinely attempted.

Gel Formation. Protein gel formation in egg albumen and WPC as a function of time and temperature, measured in terms of the storage modulus, is shown in Figure 2. Similar data for Promine D are given in Figure 3, which also shows the effects of solids concentration. The increasingly elastic character of all gels is clearly shown, being characterized by increasing values of G', the rate and extent of such increases being temperature dependent. However, the behaviors of the three gelling materials contrast sharply with each other when the patterns of gelation with heating time and temperature are compared.

With egg albumen, development of elasticity within the forming network is easily detected within 5 min of heat application and elasticity continues to develop throughout the heating period. When the gels were cooled to 25 °C a further abrupt marked increase in elasticity occurs, the amount of which depends upon gelation temperature. After 30 min at 80 °C, the "set" is only about 1.3 times the G' value obtained after 30 min of heating, whereas at 30 min at 85 °C the set is about 1.9 times and after 30 min at 90 °C it is about 2.1 times the G' value.

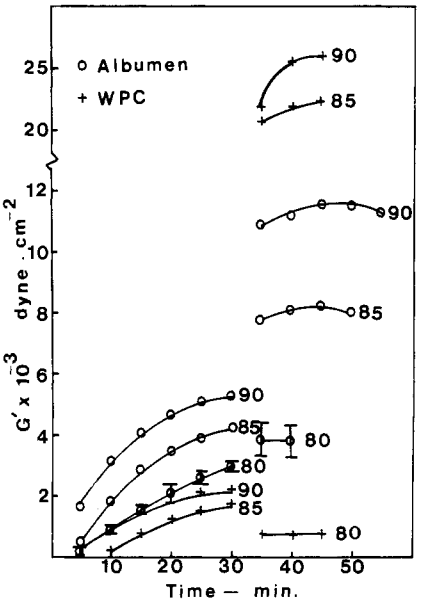

Figure 2. Effect of gelation temperature on the storage modulus of heat-set 7.4% egg albumen and 22.8% WPC. Temperatures (°C) as noted; vertical bars represent ±1 standard deviation (n = 8 runs), n = 2 in the cooling region. All other points in this and other figures represent the average of duplicate or triplicate runs.

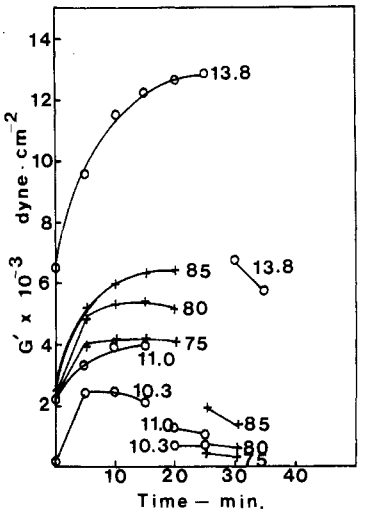

Figure 3. Effect of temperature and solid content on the storage modulus of heat-set Promine D. Runs at 75, 80, and 85 °C (×) contained 11.5% solids; other runs at indicated solids were at 85 °C. Two curves (13.8 and 10.3%) represent single runs.

With WPC, there is a continuous increase in the storage modulus with heating time, as would be expected considering the results obtained with egg albumen. However, the onset of detectable structure development is delayed considerably and elasticity development over the entire heating period is much reduced compared to that of egg white. For example, 22.8% WPC (6.9% protein) gelled at 85 °C required almost 10 min to produce detectable structures and attained a G' value less than 2000 dyn cm^{-2} after 30 min of heating. Egg albumen (6.0% protein), subjected to the same heating regime, produced clear evidence of structure formation within 5 min and a G' value in excess of 4000 dyn cm^{-2} after the heating period (Figure 2). A second remarkable characteristic of WPC compared to egg albumen is the enormous amout of set attained on cooling. With WPC this set is about 10 times the G' value obtained after 30 min of heating. At 80 °C elasticity could only be detected after cooling to 25 °C (Figure 2).

Table I. Parameters of the Kinetic Model $G' = G'_\infty(1 - e^{-kt})$ for Heat Coagulation of Egg Albumen[a]

temp, °C	solids, % (w/w)	r	k, min^{-1}	G'_∞, dyn cm^{-2}	G'_{30}, dyn cm^{-2}	G'_{cool}, dyn cm^{-2}
80	7.4	0.99	0.0248	5450	2860	3880
85	7.4	0.99	0.0592	5000	4153	8230
90	7.4	0.99	0.0869	5650	5233	11600
85	5.7	0.99	0.0043	1850	224	800
85	6.5	0.99	0.0231	3300	1013	2680
85	8.0	0.99	0.0724	7500	6645	14500
85	9.1	0.99	0.0877	13500	12527	18600

[a] G'_{cool} = highest modulus of elasticity attained upon cooling to 25 °C. G'_{30} = value of G' calculated from model at t = 30 min.

Table II. Parameters of the Kinetic Model $G' = G'_\infty(1 - e^{-kt})$ for Heat Coagulation of Whey Protein Concentrate

temp, °C	solids, % (w/w)	r	k, min^{-1}	G'_∞, dyn cm^{-2}	G'_{30}, dyn cm^{-2}	G'_{cool}, dyn cm^{-2}
80	22.8					866
85	22.8	0.99	0.0314	2600	1586	24200
90	22.8	0.99	0.0530	2700	2149	26900
85	17.2					589
85	28.6	0.99	0.0318	7100	4365	80800
85	34.3	0.99	0.0224	17000	8318	144000

Experimentally, Promine D proved the most difficult of the three materials to handle. The dried powder occluded considerable air during preparation of aqueous slurries and this was removed by centrifugation or careful vacuum degassing of the suspension, making preparation of exact duplicates difficult. Furthermore, it was frequently found that two samples taken one after another from the same preparation did not give a duplicate response. Vigorous mixing immediately before sampling reduced, but did not eliminate, this problem. Also, G' values obtained after initial sample loading fell steadily over a 5-min period of applied oscillatory input, attaining an apparent equilibrium value after "settling in". In all cases, in the work described, heat was applied only after this initial period.

As expected from previous results, G' increased markedly on application of heat, the rate and extent of the increase being affected both by the temperature and by the solid content (Figure 3). In contrast to egg albumen and WPC, Promine D has considerable elastic character within aqueous suspensions before heating and this was detected prior to heat application; hence, most of the curves of Figure 3 do not pass through zero. Also, no evidence of the cooling set seen in the other samples was detected in Promine D; in fact, the exact opposite occurred. This lack of set may be characteristic of Promine D gels, but usually, on lifting the upper platen from the surface of the gel, a smooth, shiny, upper surface, which showed no tendency to adhere to the upper platen, was found. Also, in several cases, obvious contamination of the upper gel surface with oil from the oil bath was seen. It seems likely that the cooling behavior described here is due to the lack of adhesion between the upper platen and the gel, occasionally allowing oil to penetrate when the gel shrinks slightly and the gap widens during the cooling period.

In many cases, the thermal denaturation of proteins, as measured by solubility loss or sulfydryl group exposure, can be adequately described by first-order kinetics (Wu and Inglett, 1974). In the present case, the time course of development of rheologically significant structures was determined, and modeling this early development would allow description with a few numerical parameters. An inverted exponential equation (Simon, 1972)

$$G' = G'_\infty(1 - e^{-kt}) \quad (1)$$

where G' is the storage modulus at time t, G'_∞ is a constant reflecting the value of G' at infinite t, and k is another constant, having the units of inverse time and reflecting the rate of structure development, is a suitable choice for a first-order reaction. This equation can easily be cast in linear form (eq 2) that can, in principle, be fitted to data

$$\ln(1 - G'/G'_\infty) = -kt \quad (2)$$

by standard linear least-squares techniques. To overcome the difficulty presented by two unknown constants (G'_∞, k), an initial trial value of G'_∞ was selected and k and the correlation coefficient, r, of the regression line were calculated. The G'_∞ was incremented by 50 dyn cm^{-2}, and a new k and r were calculated. The procedure was successively repeated, incrementing G'_∞ in the direction of increasing r until a maximum in r was obtained. The values of G'_∞ and k yielding maximum r were accepted.

The results of calculations on data relating G' and solids content along with calculations on the data of Figure 2 are shown in Tables I and II. In all cases, high correlation coefficients indicated the data was well described by the adopted model. The temperature effect seen earlier (Figure 2) was evident here, but consideration of the G'_∞ values predicted by the model suggests that thermally induced structural development ultimately arrives at a similar point regardless of temperature. Rate constants rise with temperatures as expected and an Arrhenius activation energy of 32 000 cal mol^{-1} may be estimated from the egg albumen data while a value of 30 600 cal mol^{-1} may be obtained for WPC from the rate constants obtained at 85 and 90 °C. Both values are in reasonable agreement with gluten denaturation data obtained by baking-test or solubility methods (Wu and Inglett, 1974).

With egg albumen, G'_∞, G'_{30}, G'_{cool}, and k all rise with increasing solids, and as expected, from consistency or shear press measurements on egg white (Beveridge et al., 1980), gelatin (Ward and Sanders, 1958), rapeseed (Arntfield, 1977), soybean (Circle et al., 1964), and caseinate and whey protein dissersions (Hermansson, 1972), the relationship between solid content and the elasticity parameters calculated here is nonlinear even over the very narrow concentration range (3.4%) examined. With WPC, the increase in G'_∞, G'_{30}, and G'_{cool} with increasing solids is much more rapid than that observed with egg albumen, and in contrast to egg albumen, the k values remained initially constant (22.8 and 28.6% solids) and then dropped (34.3% solids).

Only two of the curves obtained for Promine D (Figure 3) could be fitted to a kinetic model of the form $G' = G'_\infty(1$

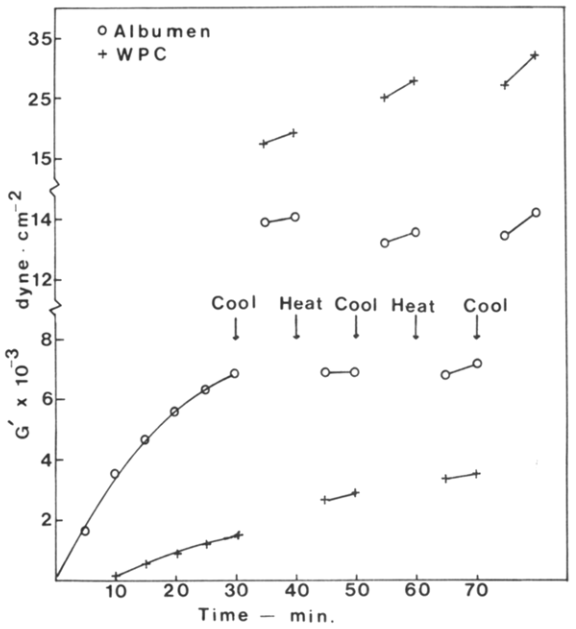

Figure 4. Reversibility of gelation in egg albumen (8.0%) and whey protein concentrate (22.8%). Gelation temperature is 85 °C, and after the initial 30-min heating, the temperature was cycled stepwise between 85 and 25 °C. Three temperature cycles are shown. Note compression and scale changes on the verticle axis. Arrows indicate points of cooling and subsequent reheating.

$- e^{-kt}) - G'_e$, a slightly modified version of the model used previously, where G'_e is the G' value measured initially at equilibrium prior to heat application. At 11.5%, 85 °C, the values $G'_\infty = 4050$ dyn cm^{-2}, $k = 0.218$ min^{-1}, and $r = 0.99$ were obtained when $G'_e = 2390$ dyn cm^{-2}, and at 13.8%, 85 °C, the values $G'_\infty = 6650$ dyn cm^{-2}, $k = 0.143$ min^{-1}, and $r = 0.99$ were obtained when $G'_e = 6410$ dyn cm^{-2}. All other curves shown proved impossible to fit according to the required criteria.

Reversibility of Gelation. Egg white coagulum is routinely characterized as a disulfide cross-linked, thermoirreversible protein gel (Shimada and Matsushita, 1981; Egelandsdal, 1980; Kalab and Harwalker, 1973) appreciably soluble only in concentrated solutions of dissociating reagents containing disulfide bond splitting reagents (Beveridge et al., 1980; Buttkus, 1974). While the setting behavior on cooling has been observed in the viscoamylograph patterns of plant protein concentrates (Martinez, 1979), this behavior was ascribed to nonprotein constituents. Catsimpoolis and Meyer (1970) observed apparently similar behavior with soybean globulins and noted that the set obtained on cooling was reversible if the gel was reheated.

Figure 4 clearly demonstrates that in both egg albumen and WPC gels, that portion of the total elasticity formed on cooling is reversible when the gel is reheated. With egg albumen the reversibility was quantative but with WPC the reversal was not quite as complete—a small portion (~5%) of the total elasticity obtained on first cooling was not returned—and the continued elasticity development throughout the heating–cooling cycles was in contrast to that of egg white. In both systems, reversiblity measurements were completed through three temperature cycles and probably more cycles could have been obtained.

An attempt to detect a "melting point" for this thermoreversible portion of total elasticity by incrementally heating a cooled gel failed to discern a clear melting point in either system. Rather, elasticity fell in an approximately exponential fashion with increasing temperature, the elasticity reaching to within 5% of the value at gelation

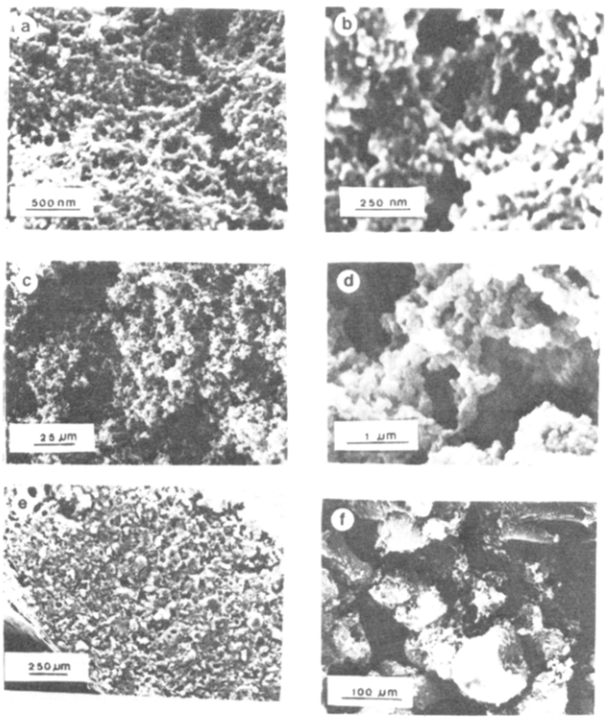

Figure 5. Scanning electron microscopy of thermally produced protein gels. (a and b) 6% (w/w) albumen heated 30 min at 80 °C; (c and d) 22.8% (w/w) whey protein concentrate heated 30 min at 85 °C; (e and f) 11.5% (w/w) Promine D heated 30 min at 80 °C.

temperature at 60 °C. This is very similar to consistency data reported by Catsimpoolas and Meyer (1970) for soybean globulins in a similar experiment.

Microscopy. Visualization of albumen gel ultrastructure revealed a network of small spherical particles of aggregated protein, apparently adhering together and having a tendency to appear as chain or strand-like structures within the gel matrix (Figure 5a). Visualization of albumen gels formed with solids contents ranging from 6 to 10% at temperatures of 80, 85, and 90 °C over times from 10 to 30 min gave no discernible differences ascribable to temperature or time of heating. High-magnification examination of the spherical aggregates revealed the presence of a coating or bridging material apparently cementing aggregates together (Figure 5b). This bridging material may represent the deposition of soluble protein between the particles, perhaps by disulfide interchange, or perhaps as an artifact generated by glutaraldehyde gelation of soluble protein as suggested by Hermansson and Buchheim (1980).

The SEM images obtained for WPC gels showed the presence of much larger spherical protein aggregates (Figure 5c,d). At low magnification, a granular-appearing protein matrix was observed, similar to images pubished by Hermansson (1979) for WPC gels. Examination at higher magnification revealed the matrix to consist of spherical protein aggregates adhering together in a manner somewhat similar to that observed in egg albumen.

Promine D consists of rather large, roughly spherical protein bodies that do not dissolve completely when the dry materials is suspended in water. Water-suspended particles are easily viewed under the light microscope, and scanning electron microscopy of dry soy proteinate has shown it to consist of whole, broken, and collapsed spherical particles (Wolf and Baker, 1980). A low-magnification scanning electron micrograph (Figure 5e) of an aqueous Promine D gel cleaved to reveal the internal

Table III. Sulfhydryl Levels (μM/g of Protein) after Heating of Egg Albumen or Whey Protein Concentrate

	heating time, min	temp, °C 80	85	90
egg albumen	0	56.9	56.9	55.9
	10	36.7	30.0	25.9
	20	30.0	17.8	13.4
	30	26.4	13.4	13.4
whey protein concentrate	0	15.0	15.0	14.7
	10	15.5	15.8	15.5
	20	15.0	14.7	13.9
	30	14.2	13.4	12.5

structure shows it to consist of many large particles adhering together and apparently embedded in background matrix. Higher magnification (Figure 5f) revealed broken and collapsed proteinate spheres cemented together by heat-induced soluble protein gel present in rather limited quantities. Undoubtedly, the presence of these large protein spheres in close proximity to each other account for the high initial G' values noted earlier for Promine D suspensions. Also, they may account for the falling or tailing of G' at longer heating times since working of these spherical particles against each other by the continuous oscillatory input would be expected to break any gel cementing particles together—particularly in cases where the solids concentrations (soluble protein concentrations) and temperatures were low, since these conditions would favor the slow development of weak gels.

Sulfhydryl Analysis. The changes in levels of sulfhydryl in the heated protein solutions are shown for egg albumen and WPC in Table III. Analysis of Promine D was not attempted because of the insolubility of the material. Increased loss of sulfhydryl occurs with both increasing time and temperature of heating in both systems, but the losses are much more extensive in egg albumin than in WPC.

DISCUSSION

It has been more than 10 years since Catsimpoolas and Meyer (1970) demonstrated for soybean globulins that the gelation process involved the irreversible conversion of a protein sol to a viscous progel by thermal distruption of native protein structures, followed by gel setting on cooling. Thermal reversibility of this latter step was attributed to the formation of a multiplicity of hydrogen bonds since these are favored by lower temperatures. That analogous behavior occurs during the gelation of other proteins, including those termed "thermally irreversible", is clearly suggested by the present work. However, caution must be exercised in interpreting the increase of G' values obtained during the cooling set. The sample gap widens by about 10% during cooling and since the sample adheres to the platens, a strained or "tight network" state must exist in the sample (McIntire, 1980). This could contribute significantly to the increased G' values observed on cooling and would also be reversible. It is impossible to separate the proportion of the cooling set attributable to sample strain and that which may arise from hydrogen bonding. But, clearly, the amount of set can vary enormously depending on the sample, and it seems likely that the set observed may be characteristic of the material empirically and remain valuable for descriptive purposes. One of the major reasons for studying protein gelation is the hope of future substitution of one protein for another in food systems. It seems unlikely that whey protein with a gelation profile such as that demonstrated here will successfully replace egg white in any functional role considering the marked differences existing between their gelation profiles. This will be true irrespective of whether or not the two gelled systems give the same response in a more arbitrary gel measuring system. It is entirely possible that development of appropriate texture in foods by protein gelation will depend upon the extent of progel formation, the degree of progel–gel transformation, or on an appropriate balance between the two.

As a method for following the thermal gelation process in protein materials, small amplitude oscillatory testing is clearly capable of characterizing the process in a unique way. At the same time, under appropriate conditions, modeling allows simple numerical description. However, one problem associated with the method is the presence of the oil bath surrounding the sample and the tendency of the sample, on cooling, to shrink and suck oil into the free space created between the sample and upper platen. This was not a serious problem with the albumen and whey protein samples used here but occurred sufficiently often to be an annoyance. However, with samples with reduced tendency to stick to the upper platen (like Promine D), the problem became serious and data collection, on cooling, became difficult. Thus, while the method is clearly capable of providing valuable information on the progress of gelation, further development to overcome these technical difficulties is required.

Different rheological behavior observed during the thermal gelation between the three materials examined is associated with markedly different electron microscopic images. In both egg albumen and WPC gel network is made up of spherical protein aggregates as expected (Hermansson, 1979; Kalab and Harwalker, 1973), differing principally in size; however, possible correlations between rheological properties and aggregate size requires further study. The observation that heating times and temperatures within the ranges employed did not affect the appearance of the aggregates in either egg albumin or WPC gels is in agreement with previous observations by Beveridge and Arntfield (1979) that turbidity development in heated egg albumen solutions is rapidly completed in the first 5 min of heating and remains stable to further heating. Visual and tactile observations of egg albumin and WPC gels formed within the rheogoniometer gap indicated that rheogoniomeric detection of rigidity approximately coincided with the completion of turbidity development and the appearance of self-supporting gels. The structural aggregates are formed very early during gel formation, prior to development of the self-supporting macroscopic gel, and do not appear to change despite the continuous development of elasticity in the hot system. Gelation of protein occurs through intermolecular disulfide bonds formed by sulfhydryl–disulfide interchange or sulfhydryl oxidation and through intermolecular hydrophobic interactions (Fukushima, 1980; Shimada and Matsushita, 1980, 1981). Involvement of hydrogen bonds or ionic lnks has been discounted since they are weak or nonexistet at gelation temperatures (Catsimpoolas and Meyer, 1970; Bello and Vinogard, 1958). Hydrophobic bonds are weak (1–2 kcal mol^{-1}), and gels formed mainly through hydrophobic interactions, such as those formed by soybean 7S globulins, are soft and inelastic while gels extensively cross-linked by disulfide bonds (11S globulins) are much more elastic. It seems reasonable to suppose that the increasing elasticity observed here during heating—particularly for egg white and WPC gels—is due to disulfide bonds formed by interchange or oxidation of sulfhydryl to disulfide. In the case of these two gels, toughening at low concentration of small molecular weight thiols and softening at high concentrations in WPC (Schmidt et al., 1979) and the solu-

bility properties of egg white coagulum (Buttkus, 1974) combined with the extreme softening of egg albumen gels by KIO_4 (Beveridge et al., 1980) make sulfhydryl–disulfide involvement in gelation of these protein systems probable. In egg albumen, where large elasticity values were obtained with increased heating times, total sulfhydryl dropped drastically over the 30-min heating period, whereas in WPC, where rather limited elasticity developed with heating time, much more moderate losses of total sulfhydryl occurred (Table III).

Elasticity results from energy storage within close-packed spherical aggregates forced to move relative to each other, resulting in aggregate deformation and stretching of interaggregate "bridge" material, because of the imposed oscillatory strain. The basic structure of the gel has already been formed as spherical protein aggregates and the increasing elasicity probably results from increasing numbers of cross-links stiffening the structure of individual aggregates and perhaps with slow deposition of disulfide cross-linked material binding the globules together.

The continuous gelation process of egg white and WPC can, for convenience, be divided into three steps following the initial denaturation and unfolding of the native protein. The first step is turbidity development during the first 3–10-min heating resulting from formation of spherical aggregates. This step is probably directed by hydrophobic interactions modified by the charged state of the protein molecules (Hermansson, 1979). The second step is sulfhydryl–disulfide interchange and sulfhydryl oxidation stiffening the preformed aggregates and perhaps enhancing interaggregate adherence. The third step is the sudden, large increase in elasticity that occurs on cooling due to the rapid formation of multiple hydrogen bonds (Catsimpoolas and Meyer, 1970), causing a marked increase in the rigidity of the aggregates.

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