Rheological and Physical Properties of Derivitized Whey Protein Isolate Powders

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Pregelatinized starch is employed in many food applications due to the instantaneous nature of thickening and stability imparted by modification. Proteins, however, have been excluded as a viscosifying agent due to requisite thermal treatments required to create structure. Whey protein isolate gels were produced while manipulating heating time, pH, and mineral type/content, producing a variety of gel types/networks. Gels were frozen, freeze-dried, and ground into a powder. Once reconstituted in deionized water, gel powders were evaluated based on solubility studies, rotational viscometry, and electrophoresis. The protein powder exhibiting the largest apparent viscosity, highest degree of hydrolysis, and greatest solubility was selected for pH and temperature stability analyses and small amplitude oscillatory rheology. This processing technique manipulates WPI into a product capable of forming cold-set weak gel structures suitable for thickening over a wide range of temperature and pH food systems.

Keywords: *Whey protein, gelation, cold-set gels, freeze-drying, acid hydrolysis*

INTRODUCTION

Whey protein possesses an unsurpassed nutritional quality and inherent functional properties that can be manipulated to meet the demands of a thickener application. When heated, whey proteins aggregate, and under suitable conditions gels may form. These heat-induced whey protein aggregates or gels can be used in the food industry to alter texture and water-binding properties. Under appropriate ionic strength, pH, and thermal gelation conditions, whey protein gels are capable of immobilizing large quantities of water and other ingredients. The adjustment of these physical conditions determines the structural network, water-holding, and rheological properties possessed by the gels.

The thermal gelation of WPI has been extensively studied (Mulvihill and Donovan, 1987; Donovan and Mulvihill, 1987; Paulsson et al., 1986; Xiong, 1992) and is a two-stage process involving an initial unfolding and subsequent aggregation of protein molecules (Verheul et al., 1998a; de Wit and Klarenbeek, 1984). When native whey proteins are heated above 65−70 °C, they partially unfold as physical forces favoring unfolding, mainly configurational entropy, increase above the forces favoring folding (Bryant and Mc Clements, 1998). Once whey proteins have been heated to unfolding temperatures, they may either aggregate or remain unfolded as individual molecules, depending on the balance of attractive and repulsive interactions. In general, changes in the solution environment may alter protein−protein and protein−solvent interactions by shifting the balance of attractive and repulsive forces (predominately disulfide bonding, electrostatic and hydrophobic interactions, and hydrogen bonding). This shift consequently affects the rates of unfolding and aggregation, resulting in different gel structures (Tang et al., 1995). Protein unfolding and aggregation are particularly sensitive to pH and ionic strength due to the dependence on electrostatic interactions.

Networks formed during WPI gelation contribute to texture, water holding, and appearance of the gel. Gels are grouped as particulate, fine-stranded, or mixed networks; and pH, solutes, and gelation kinetics determine the type of gel matrix formed. Protein molecules are charged at pH values significantly higher or lower than their isoelectric points (pI), and an appreciable electrostatic repulsion opposing intermolecular protein−protein interactions is present (Foegeding et al., 1998).

At low salt concentrations, the electrostatic repulsion can be so significant that protein−protein interactions are limited and gelation is prevented (McClements et al., 1993). As the salt concentration is increased (<0.1 M), the charges are partially shielded and a transparent filament gel is formed (Doi, 1993). Gels produced under these ionic conditions contain a fine-stranded microstructure and translucent appearance (McClements and Keogh, 1995). At neutral pH and salt concentrations high enough to completely shield electrostatic repulsion (>0.1 M), attractive forces dominate. Protein aggregation may be so extensive that protein molecules form large particles, typically a few micrometers in diameter (Bryant and Mc Clements, 1998). The resulting turbid, particulate gels have an opaque milky-white appearance due to large aggregates scattering light. Mixed gels possess physical and functional properties of both fine-stranded and particulate gels and are produced with intermediate salt concentrations (Foegeding et al., 1998). The condensing of linear strands into larger aggregates is thought to be the causal mechanism for mixed gel formation (Foegeding et al., 1998).

The textures of each gel type can be characterized by rheological properties, fracture stress and deformation, and physically by appearance and water-holding analyses (Table 1). Holding protein concentration and pro-

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Table 1. Network Types and Physical Properties of Whey Protein Gels[a]

gel type	fracture rheology: stress	fracture rheology: deformation	appearance	water holding
stranded				
I. pH > pI	+++[b]	+++	translucent	+++
II. pH < pI	+	+	translucent	?
mixed	++++	++	cloudy/opaque	++
particulate	+++	+++	opaque	+

[a] Adapted from Foegeding et al., 1998. [b] The number of "+" symbols represents the relative magnitude of a property among gel types, with the greater number of symbols indicating a greater magnitude, and "?" indicates no relevant data.

cessing temperature constant, the value of these parameters will vary according to processing conditions such as heating time and solution conditions, including pH and salt. For example, fine-stranded gels formed above or below the pI have a similar translucent appearance. However, stranded gels formed above the pI (FS−I) are strong and elastic, whereas those formed below the pI (FS−II) have a weak, brittle structure (Foegeding et al., 1998). Therefore, while FS−I gels and FS−II gels possess the same appearance, their rheological properties are different due to varying amounts of intermolecular interactions at reduced pH. In addition, WPI gels with well cross-linked, fine-stranded (FS−I) microstructures hold water better than gels with particulate microstructures (Bowland and Foegeding, 1995; Verheul et al., 1998b). The increasing salt concentrations found in particulate gels result in an increased effective matrix pore size; consequently, water-holding capacities of the gels decrease as salt concentrations increase (Bowland and Foegeding, 1995). Mixed gels have intermediate water-holding properties with large fracture stresses and low deformability (Foegeding et al., 1998). Manipulation of gelation conditions can yield gels with numerous attributes suitable for the wide variety of applications required by the food industry.

For many foods, proteins may prove unsuitable as food texturizers due to the thermal processing required to produce structure. Starch thickeners introduce problems associated with retrogradation, namely a time-dependent functionality (Eliasson and Gudmundsson, 1996). In addition, dairy-based materials able to mimic the thickening functionality of gelatin, hydrocolloids, and other thickeners such as starches in dairy systems has been a focus of current research (Hoch, 1997). Therefore, a protein ingredient capable of gelling at ambient temperatures would be advantageous for many food applications. The pH, heating time, mineral type, and content of WPI solutions were manipulated to produce a variety of gel types/networks through thermal gelation. A technique was identified to produce a gel, which upon additional processing yields a protein-based thickener not requiring heat or the addition of salts to function.

MATERIALS AND METHODS

Materials. A commercial whey protein isolate (WPI) powder (lot no. JE057-7-420), containing approximately 91.2% (w/w) protein (N × 6.38, micro-Kjeldahl, AOAC, 1984), was used for the experiments (Bipro, produced by Davisco International Inc.). All chemicals, NaCl, $CaCl_2$, NaOH, and HCl, were purchased from Fisher Scientific Company.

Methods. *Preparation of Protein Dispersions and Gels.* WPI powder was hydrated (12% w/w) in 0−50 mM NaCl and/or 0−20 mM $CaCl_2$ solutions made with double-distilled water for 1 h by slow stirring, degassed under vacuum for 1 h to remove visible air bubbles, and brought to the necessary volume with double-distilled water. The pH for FS−II gel protein dispersions was adjusted to 4.0 or 3.5 with 6 N HCl or 6 N NaOH. WPI gels were produced by heating the dispersions in aluminum freeze-drier pans (13.5 cm × 13.5 cm × 0.038 cm) at 80 °C for 45 min or 3 h according to the procedure of Foegeding (1992).

Gel-Type Determination. Vane fracture tests and water holding trials were conducted to determine gel type: particulate, mixed, FS−I, or FS−II. The vanes consisted of four identical stainless steel blades (23 gage) fixed to a center shaft. Two vanes with similar heights (H) of 20 mm and varying diameters (D) of 10 and 6 mm were inserted flush with the gel sample in aluminum pans and rotated with a Haake VT550 Viscometer at a speed of 0.5 rpm until fracture. Tests were performed nine times for each gel, and peak torque (M_f) and time at fracture (t_f) were recorded. Stress (σ_f) and vane rotation at fracture (θ_f) (∝ strain) were calculated according to Daubert et al. (1998).

$$\sigma_f = \frac{2M_f}{\pi D^3}\left(\frac{H}{D} + \frac{1}{6}\right)^{-1} \quad (1)$$

$$\theta_f = t_f\left(\frac{\pi}{60\ (s)}\right) \quad (2)$$

Water Holding. A cylindrical gel sample was placed in an inner cell containing a filter membrane. Next, the system was placed in an outer microcentrifuge tube, and the unit was spun in a Beckman Microfuge 11 horizontal-rotor microcentrifuge (Beckman Instruments, Inc.) at 153g (2000 rpm) for 10 min. Water holding was determined by weight difference of the inner cell prior to and after centrifugation according to the procedure of Kocher and Foegeding (1993).

Gel Powder Production. After thermal gelation, gels were held at 25 °C until internal temperature was less than 40 °C. Gels were then placed at −5 °C for 16−18 h. Frozen gels were dried in a 10-145-MR-TR mechanically refrigerated freeze-mobile freeze-dryer with tray drying chamber (Virtis Research Equipment). Shelf temperature was held between 26.7 and 37.8 °C with the 10-701 control unit (Virtis Research Equipment) with a thermocouple inserted into the center of the gel. Gels were dried until the moisture content was below 5%, as determined by a Computrac Max-50 Moisture Analyzer (Arizona Instrument Corp.). Dried gels were crushed by hand, stored in airtight bags for 18 h, and ground using a ZM-1 Brinkman Centrifugal Grinding Mill with a 24-tooth stainless steel rotor (Brinkman Instruments Co.) at 10,000 rpm for 60 s and a screen size of 50 mm. Average particle size of ground protein powder was determined by a centrifugal particle size analyzer SA-CP4 (V1.0) (Shimadzu Corporation). Protein gel powders were stored at 25 °C in airtight bags.

Solubility Determination. Milled protein powders were dispersed (1% w/w) in deionized water and the pH adjusted with either 6 N NaOH or 6 N HCl to 7.0. Dispersions were centrifuged at 20,000g for 15 min, and absorbance was measured at 280 nm on a sample aliquot diluted 1:10 (vol/vol) in dissociating buffer (50 mM EDTA, 8 M urea at pH 10). Solubility was obtained from the absorbance ratio of the supernatant and the dispersion before centrifugation (Britten et al., 1994). The term "soluble" was used to define samples having over 70% suspended particles (as compared to native WPI) after centrifugation at pH 7.0.

Differential Scanning Calorimetry. A Perkin-Elmer DSC 7 (The Perkin-Elmer Corporation) differential scanning calorimeter utilizing N_2 gas was used to assess the thermal denaturation of the gel powders for each processing condition. The DSC was calibrated with indium and allowed to equilibrate for 1 h prior to analysis. Dried protein powder was hydrated (10% w/w), and 20 μL was loaded into an aluminum capsule and hermetically sealed. A similarly weighted, aluminum pan was filled with deionized water and used as the reference. The scanning temperature was raised from 5 to 110

°C at a rate of 5 °C/min. Temperature at peak height and peak area were recorded. Native protein % was calculated as

$$\left(\frac{\text{peak height sample}}{\text{peak height native WPI}}\right) \times 100 \quad (3)$$

Electrophoresis. Protein samples were analyzed for hydrolysis by SDS−PAGE (sodium dodecyl sulfate−polyacrylamide gel electrophoresis) with a Novex Midget electrophoresis unit (Novex, Novel Experimental Technology). All procedures were followed according to the NuPAGE gel procedure for a Bis-Tris (MES) gel utilizing dithiothreitol (DTT) as the reducing agent. A 30 μL aliquot of 0.1% protein was injected into each well of the 10-welled, 4−12% gradient polyacrylamide gel (Novex, Novel Experimental Technology). The gel was stained with a 0.1% Coomassie Blue solution (45% water, 45% methanol, 10% acetic acid) (Pierce Chemical Company, Rockford IL). A Molecular Dynamics personal densitometer was used to determine the major protein species hydrolyzed by the various processing conditions. In addition, the densitometer allowed quantification of the hydrolyzed protein bands and their molecular weight range based on the MultiMark multicolored standard used (Novex, Novel Experimental Technology). Colored standards were myosin at 185 kDa, phosphorylase B at 98 kDa, glutamate dehydrogenase at 52 kDa, carbonic anhydrase at 31 kDa, myoglobin (red) at 19 kDa, myoglobin (blue) at 17 kDa, lysozyme at 11 kDa, aprotinin at 6 kDa, and insulin at 3 kDa.

Rheological Analysis. The protein powders were hydrated (10% w/w) and tested rheologically. Once solutions were placed in the measuring cup and the bob immersed in the sample, solutions were coated with a thin layer of paraffin oil from Fisher Scientific Company to prevent moisture loss. Shear rate ramps, temperature ramps, and pH stability tests were conducted on a Bohlin VOR Controlled Strain Rheometer (Bohlin Reoligi AB) using a concentric cylinder geometry (C14).

Shear Rate Ramps. Selected shear rates were ramped from 0.9 to 116.0 s^{-1} and back to 0.9 s^{-1} for 650 s as apparent viscosity was recorded. Fresh samples were sheared at temperatures of 25, 50, and 75 °C.

Temperature Ramps. Temperatures were ramped from 5 to 90 °C and back to 5 °C at a shear rate of 46 s^{-1} for 2220 s, and apparent viscosity was noted. Although a layer of paraffin oil was applied to the surface of the sample before temperature ramps were performed, a moisture analysis was conducted before and after ramps to establish apparent viscosity differences were a characteristic of the material and not a consequence of drying. Only data on samples below 1% moisture difference before and after analysis were kept.

pH Stability. Apparent viscosity was measured at pH 4.0 and 8.0 at 25 °C as shear rates were ramped from 0.9 to 116.0 s^{-1} and back to 0.9 s^{-1} for 650 s.

Frequency Sweeps. Analysis was performed on a StressTech Controlled Stress Rheometer (ReoLogica Instruments AB) using a concentric cylinder geometry (CC25). Stress was held at 1.0 Pa, and frequency was oscillated from 0.1 to 20.0 Hz and alternated between 25 and 90 °C for a total of four sweeps each for 688 s. All tests were performed within the identified linear viscoelastic region for the material at the temperatures tested.

Statistical Analysis. Significant differences ($p \leq 0.05$) were determined by analysis of variance using the general linear models and least square means procedure of the statistical analysis system (SAS Institute, Inc., 1998).

RESULTS AND DISCUSSION

WPI Gelation. Whey protein solutions underwent thermal gelation as the first processing step of the WPI. Gels were prepared according to Foegeding (1992), with the heating time extended from 30 to 45 min ensuring adequate heat transfer in the aluminum freeze-drier pans. During preliminary rheological studies of the FS−II gels, the apparent viscosity of the protein powders in solution (10% w/w) sharply increased at 88 °C. If held at that temperature longer than 5 min the solution would gel, indicating a substantial portion of the protein had not denatured or had undergone reversible denaturation. Therefore, a 3-h gelation time (at 80 °C) was applied to all gel-type solutions to promote more extensive protein denaturation and enhanced heat stability.

Table 2. Dried WPI Gel Powder Sample Identification, Thermal Gel Time, Concentration of Salts Used in Gelation, pH, and Solubility

sample ID	gel time	pH	mM NaCl	mM $CaCl_2$	gel type	corrected solubility[a]
7H	45 min	3.93	50	0	FS−II	61.4
7G		3.99	20	0	(pH < 4)	70.0
7I		3.98	0	0		67.8
7K	45 min	3.45	50	0	FS−II	61.1
7J		3.40	20	0	(pH<3.5)	71.2
7L		3.40	0	0		70.3
7F	45 min	6.77	50	0	FS−I	6.7
7E		6.77	20	0		8.0
7D	45 min	6.77	25	5	mixed	6.0
7C		6.77	20	5		23.3
7B	45 min	6.77	40	5	particulate	14.6
7A		6.77	30	10		6.4
1	3 h	3.88	50	0	FS−II	68.8
2		3.94	20	0	(pH<4)	68.6
3		3.83	0	0		74.9
4	3 h	3.37	50	0	FS−II	78.3
5		3.28	20	0	(pH<3.5)	79.2
6		3.36	0	0		88.2
7	3 h	6.82	50	0	FS−I	5.3
8		6.82	20	0		4.1
9	3 h	6.82	25	5	mixed	13.7
10		6.82	20	5		11.8
11	3 h	6.82	40	5	particulate	9.4
12		6.82	30	10		22.6

[a] Corrected solubility (in percent) based on native WPI = 100% soluble. Method adapted from Britten et al., 1994.

Gel-Type Determination. Table 2 serves as a sample identification key, detailing salt concentrations and solution pH for all gels produced. Vane fracture tests and water-holding analyses were conducted to distinguish protein gels rheologically and structurally. The vane attachment was used to quantify the stress and deformation (strain) in order to place the whey protein gels in distinct categories for comparative and classification purposes. Water-holding analysis was used to qualify water-holding trends, related to compositional characteristics of the various gel types.

Vane. Vane attachments sheared gels to fracture, offering a simple approach for gel classification. The peak fracture stress provided an indication of gel strength, while vane rotation at fracture furnished an understanding of gel deformation characteristics. Vane experiments indicated that thermal treatment time influenced stress at fracture values for all gel types ($p \leq 0.05$). When comparing gelation times in Figure 1 parts a and b, the lower fracture stresses for 45-min gels may result from inadequate heating time and/or temperature (80 °C) to completely cure the gel structure. This effect may be due to an underestimation of the heat-transfer effects of the aluminum gel pans or increased solution volume. The significance in the stress difference for the FS−II gels may also be attributed to enhanced thermal stability of whey protein solutions below pH 4.0, thereby requiring longer thermal treatments to denature the proteins and produce networks. This speculation is supported by Jelen and Buchheim (1984), who demonstrated that below pH 3.5 protein solutions resisted coagulation for an average of 30 min

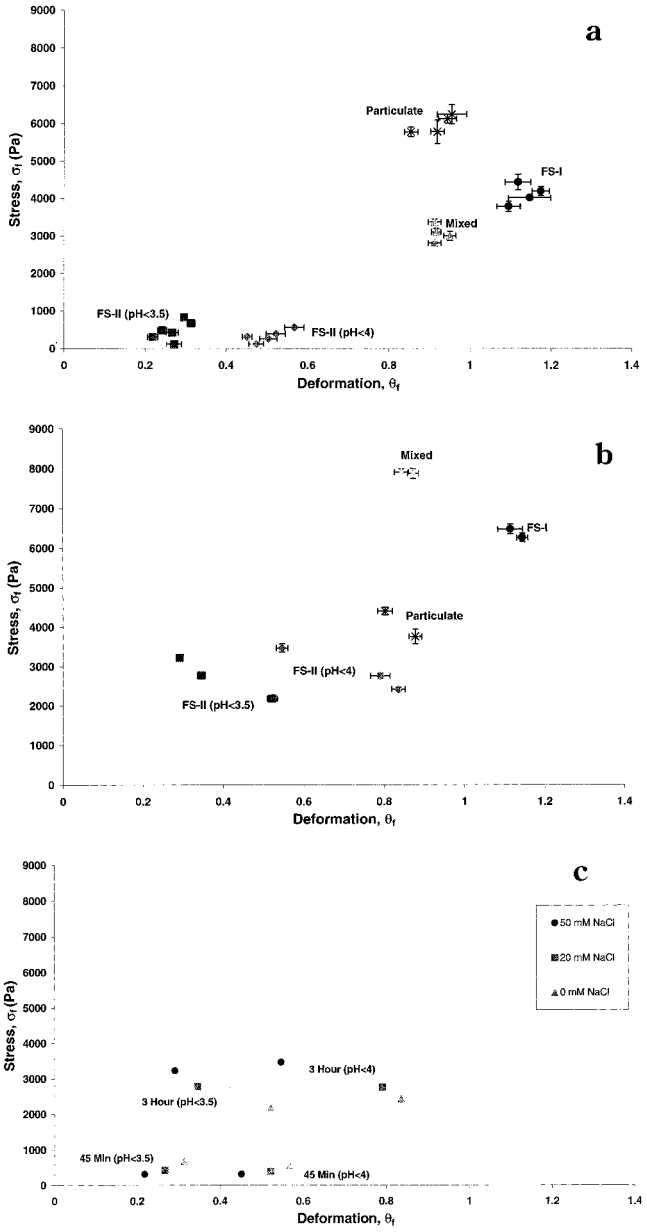

Figure 1. (a) Gel-type determination for 45 min gels using vane fracture. (b) Gel-type determination for 3 h gels using vane fracture. (c) Vane of 45 min and 3 h FS−II gels.

at 80 °C. In addition, Harwalkar (1980a) noted slower thermal denaturation rates of whey proteins at low pH than those reported at near neutral pH. Patocka et al. (1987) found β-LG was virtually heat stable below pH 3.8−3.6. Therefore, little protein denaturation occurred at the 45-min thermal treatment due to the stabilizing effect of low pH. An increase in heating time resulted in more denaturation and complexing between proteins, culminating in a higher fracture stress.

Although FS−I and FS−II gels had the same protein concentration and ionic strength, the fracture stress and deformation differences were significant ($p \leq 0.05$). Errington and Foegeding (1998) noted that gels formed below pH 4.0 were weak and brittle (FS−II gels), in contrast to the same formulation of salt and protein formed at pH above 6.0 (FS−I gels). This decrease in deformability of the FS−II gels was the result of fewer intermolecular disulfide linkages due to minimal sulfhydryl oxidation and sulfhydryl−disulfide exchange at low pH. This hypothesis helps in the understanding of the larger deformation noted in the pH <4.0 FS−II samples and the pH <3.5 FS−II gels in Figure 1 parts a and b. A lower solution pH resulted in fewer disulfide bonds and, therefore, diminished deformability.

The stress difference between the FS−I and FS−II gels may be a result of acid hydrolysis of proteins at low pH. Britten et al. (1994) demonstrated that gels formed at pH 4.0 had fewer large aggregates formed with increasing hydrolysis. At that pH, peptides are believed to interfere with the formation of large aggregates, resulting in a large proportion of small aggregates. In addition, Langton and Hermansson (1992) documented gels prepared at pH 3.5 have networks of short thin strands (~4 nm in diameter) with several strands intersecting at one junction, while gel networks formed at pH 7.0 and 6.5 were composed of longer strands of equal or greater thickness than those formed at pH 3.5. These influences would change the intermolecular interactions and thereby alter the stress required to fracture the matrix, providing additional support for the lower fracture stresses recorded for the FS−II gels at pH values less than 3.5 and 4.0.

There were no obvious trends for various salt concentrations for stress and deformation at fracture in the mixed, particulate, or FS−I gels for either gelation time. However, in the FS−II gels (Figure 1c), a pattern of increasing deformation and increasing stress with decreasing salt concentrations for the 45 min gels at pH values of less than 4.0 and 3.5 was observed. In the 3-h gels, however, there was a shift in the pattern, increasing deformation and decreasing stress with decreasing salt concentrations at both pH values. This shift in salt trends from the 45-min to the 3-h gel time may be attributed to an increase in charge shielding over the longer incubation time. The solutions with higher salt concentrations have more time to orient themselves around the positively charged protein molecules while decreasing the repulsion of like charges between protein species and permitting aggregation. Aggregation of proteins results in a more "particulate" type of FS−II gel structure, presented by gels with low deformability and higher fracture stress. This observation was supported by Harwalker and Kalab (1985) who found those protein solutions at low pH but high ionic strengths resulted in lower ionic forces but enhanced hydrophobic interactions, increasing coagulation and, therefore, gelation.

Water Holding. Water holding analysis was conducted to further validate gel-type classification. Water holding could not be preformed on the "pudding-like" FS−II gels as the coring of gel samples was not permitted due to weak structure. The order of increasing water holding was determined as FS−I gels > mixed gels > particulate gels.

On the basis of vane and water-holding experiments, a spectrum of gels with varying structural and rheological characteristics was categorized. Gels possessing similar rheological profiles and network configuration were grouped into five gel categories: mixed, particulate, FS−I, FS−II (pH < 3.5), and FS−II (pH < 4.0).

Powder Characterization. *Particle Size.* After thermal gelation, water-holding, and vane experimentation, gels were frozen, freeze-dried, and ground. Particle size analysis was conducted on the resulting hydrated protein powders, and an average particle size of 25.3 μm was determined. Particle size is thought to influence solubility measurements. If dried protein gels could

Table 3. Percent Native Protein and Temperature of Denaturation As Determined by DSC for Protein Gel Powders Exhibiting Solubility of 70% or Greater

sample ID	% native protein[a]	denaturation temperature (°C)
native	100.00	76.94
3	26.21	87.67
4	10.49	86.69
5	6.58	86.05
6	4.69	88.29
7G	42.04	87.55
7J	41.01	87.16
7L	44.80	87.16

[a] Calculations based on area under curve and native WPI = 100% undenatured.

effectively be ground to a small enough particle size, hydration would be essentially instantaneous and solubility may increase. Continued research will investigate the exact role of particle size and functionality.

Solubility. Solubility is an important characteristic for functional application of proteins in food systems, serving as a prerequisite for derived functional properties such as emulsification, foaming, and gelation (de Wit, 1990). A solubility test, therefore, served as a means of elimination of gel powders based on suitability for further analyses and applicability to industrial use. Powders with low solubility could not be analyzed effectively by gel electrophoresis, DSC, or rheologically and were, therefore, excluded from further evaluation. Seven powders exhibiting solubility values of ≥70% at pH 7.0 were further evaluated (Table 2). Therefore, all subsequent discussion of experimental analysis was only conducted on the soluble gel powders.

Differential Scanning Calorimetry. DSC was conducted to determine the percent denaturation and the shift in denaturation temperature (from native WPI) imposed by each gelation process. According to Table 3, no trends in denaturation temperature and salt concentration were observed. However, increasing salt (samples 6 → 3) did have a stabilizing effect on the protein, decreasing the denaturation and resulting in a higher calculated % native protein. In the 3-h gelation treatment, sample 4, made with 50 mM NaCl, had a native protein concentration of 10.5% while sample 5, made with 20 mM NaCl, was 6.6% and sample 6, made with 0 mM NaCl, was 4.7%. This trend was also noted by Harwalkar and Kalab (1985), who documented higher ionic strength (Γ/2) values increased the rate of denaturation, but a protective effect from denaturation was noticed when Γ/2 was increased from 0 to 0.1. This response may be due to the increase in hydration usually exhibited by globular proteins around Γ/2 of 0.15 (Damodaran, 1996). The thermal stability could arise from the withdrawal of hydrophobic groups of protein from water leading to a compact form. At lower ionic strength, the selective binding of chloride ions by the protein cationic sites may give rise to a more compressed form. The experimental NaCl concentrations in this research were all within the ionic strength range of 0−0.1, providing a protective effect and decreasing the denaturation with increasing salt.

Another observation from DSC analysis was a decrease in percent native protein with an increased gelation time. The 45-min gel powders (7G, 7J, and 7L) had a native protein concentration of approximately 42%, while the 3-h treatment had concentrations that ranged from 26% to 2.5% depending on pH and ionic strength. In addition, an upward shift (from native WPI) in denaturation temperature of the 45-min and 3-h gels was noted and may be attributed to the increased heat stability imparted by the reduced pH of the gelation conditions. DSC studies by de Wit and Klarenbeek (1984) showed greater thermal stability at pH 3.0 than at neutral pH evidenced by the increased transition temperature (e.g., denaturation temperature). In addition, Harwalkar (1980b) found, via optical rotation, denaturation of whey proteins at a solution pH of 2.5 was detectable only at 75 °C or above. At higher pH (e.g., at pH 7), initiation of thermal denaturation was detected at a much lower temperature, 65 °C, exemplifying the thermal stability and higher transition temperatures noted in the proteins below pH 4.0.

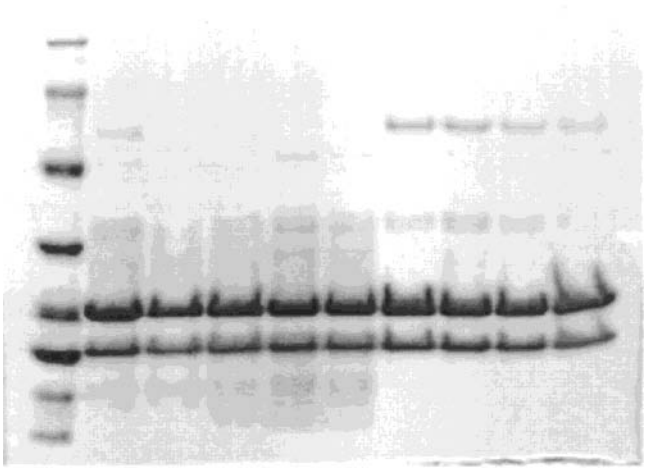

Figure 2. SDS−PAGE electrophoresis gel of soluble protein gel powders.

Electrophoresis. SDS−PAGE electrophoresis was conducted on the soluble WPI gel powders heated (80 °C for 45 min and 3 h) in HCl at pH values below 4.0 or 3.5 with varying NaCl concentrations (Figure 2). Estimated molecular weights and composition determined via densitometer measurements of proteins, protein fragments, and derivatives are presented in Table 4. Control banding patterns of WPI (lane 7) showed peaks corresponding to α-LA (14 kDa) and β-LG (18 kDa) and a minor peak of BSA (66 kDa). The peak at 48 kDa in the native WPI may be due to hydrolysis of the larger molecular weight BSA or association of the smaller proteins, β-LG and α-LA, not effected by the denaturant SDS and dithiothreitol (DTT). All peaks present in native WPI decreased with increased heating time at low pH values (pH <3.5 and pH <4.0), with new bands appearing between α-LA and β-LG while some migrated ahead of α-LA. Harwalkar (1980b) obtained similar electrophoretic patterns with a 2.0% TCA-insoluble fraction of heated whey. These results were attributed to structural alterations in α-LA, β-LG, or both due to low pH thermal treatments. In addition, Lupano (1994) observed by SDS−PAGE many bands in the molecular mass range of 3.5−7 kDa after β-LG (0.5% in 0.02 M HCl; pH 2.5) was heated at 90 °C for 1 h. The heat treatment in acidic conditions produce partial denaturation and cleavage of some peptide bonds.

Further electrophoretic observations show a shift in fragment distribution between 26 and 18 kDa for sample 6. The shift in molecular weight may be attributed to deamidation of the Asn and Gln in β-LG. Ahern and Klibanov (1985) observed deamidation at similar acidic pH values. Harwalkar (1980b) found

Table 4. Densitometer Values (in Percent) of Electrophoresis Bands

		molecular weight of native and hydrolyzed/polymerized protein fragments									
lane[a]	sample ID	82 kDa	66 kDa	58 kDa	48 kDa	36 kDa	26 kDa	18 kDa	14 kDa	7 kDa	4 kDa
2	3		1.74	0.68		4.77	1.30	58.82	31.66	1.03	
3	4						0.53	66.00	31.81	1.67	
4	5			0.49		2.60		61.01	32.24	3.66	
5	6	0.44	1.05		5.47	3.80	53.16		30.56	4.52	1.00
6	6	0.22	0.76		4.25	2.48	55.90		29.30	4.95	2.14
7	native		6.08		3.17			58.66	32.09		
8	7G		5.61	0.59	4.60			56.78	32.43		
9	7J		3.53		3.08			61.08	32.31		
10	7L		2.66		1.76			63.94	31.65		

[a] Lane 1 was a MultiMark molecular weight marker.

proteins thermally treated at pH 2.5 were unchanged in molecular size but altered in structure as determined by increased optical rotation measurements. This chemical alteration may have caused an increase in net charge of the β-LG and a structural change, altering the SDS binding and resulting in β-LG banding as a larger protein.

A low percentage of a high molecular mass band (82 kDa) was observed for sample 6, which may indicate the presence of covalently bound aggregates. While low pH inhibits disulfide interchange reactions, Darbre (1986) presented evidence for their presence at low pH values, although the process is not rapid. Thus, a certain degree of disulfide interchange reactions could be expected, mainly for the 3-h thermal treatment samples. However, this observation was not made for FS−II (pH <3.5) gels containing NaCl, due to the stabilizing effect of salts.

Another possible explanation of the 82 kDa band for sample 6 is β-elimination of cystine residues in BSA. These reactions are usually carried out by heating the solution with K^+, ^-OH, or Na^+ and involve the loss of a proton at a β-carbon and the formation of an alkene (Fessenden and Fessenden, 1993). However, β-elimination has also been noted in acidic systems by Patrick and Swaisgood (1976). The result of β-elimination is the formation of π bonds and, therefore, an alteration in structure. This structural modification may affect DTT binding and thus result in different banding patterns for those BSA proteins having undergone this elimination reaction.

In addition, trends in salt concentration and acid hydrolysis were noted from electrophoretic results. An increase in ionic strength of the protein powders for the 3-h heating time (samples 6 → 3) had a stabilizing effect on the rate of acid hydrolysis, yielding fewer low molecular weight bands. Inorganic salts, such as NaCl, in low ionic strength aqueous solutions can increase the stability of folded conformations to minimize the protein surface in contact with the solvent (Creighton, 1993). It has also been found that salt bridges can form at low ionic strengths (<0.1 M) which can increase the stability of the folded state because ionization and conformational stability are linked functions. The greater the stabilizing effect of a salt bridge, the less susceptible it is to disruption by extremes of pH (Creighton, 1993). The enhanced stability of the folded protein conformation with increasing salt can decrease the interaction with the acidic environment, thereby decreasing the hydrolysis.

Selected Gel Powder. *Shear Ramps at Various Temperatures.* On the basis of solubility and apparent viscosity (Figure 3), sample 6 was selected as the best possibility for a thickener and marked for further study.

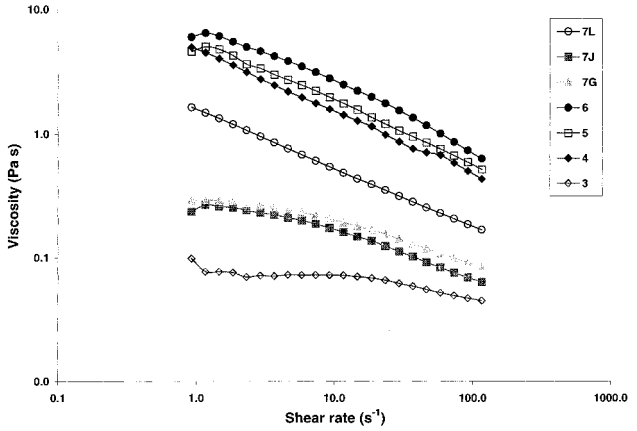

Figure 3. Apparent viscosity of soluble gels under shear at 25 °C.

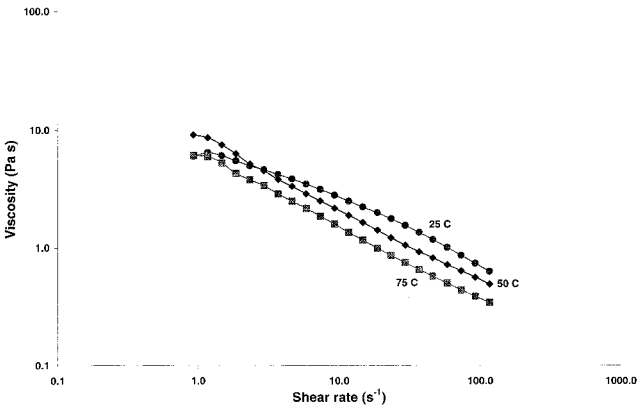

Figure 4. Temperature effects of averaged apparent viscosity from shear ramps for sample 6.

Further shear ramps were performed at 25, 50, and 75 °C to evaluate the influence of temperature on the apparent viscosity of sample 6 (Figure 4). Sample 6 displayed little variation in apparent viscosity over the selected temperatures.

Temperature Ramps. In Figure 5, sample 6 temperature ramps revealed little variation in the apparent viscosity from 10 to 55 °C; however, from 55 to 90 °C there is an increase of 1.3 Pa s. The increase in viscosity at the higher temperatures may be attributed to the additional denaturation of native or renatured protein structures. This theory is supported by the slightly higher viscosity recorded for the 90−5 °C temperature ramp. The higher apparent viscosity may be due to protein unfolding and interaction, resulting in a larger hydrodynamic radius of the particles and increased viscosity.

pH Stability. Shear ramps were conducted on sample 6 at pH values of 4 and 8 to determine pH effects on

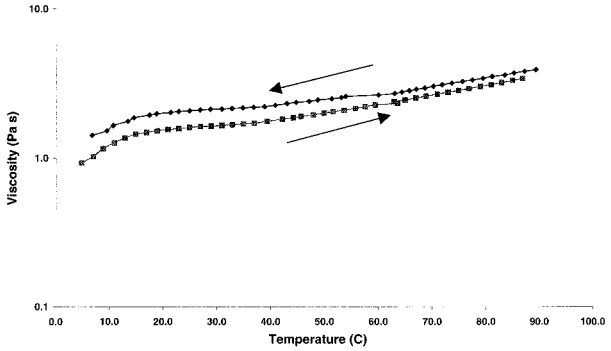

Figure 5. Temperature ramp (5−90 and back to 5 °C) for sample 6 at 46 s^{-1}.

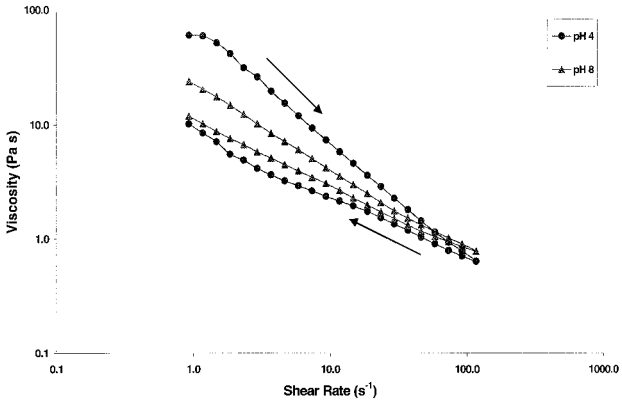

Figure 6. Effects of pH on averaged apparent viscosity for sample 6.

apparent viscosity over the selected shear rates (Figure 6). Sample 6 exhibited little variation in apparent viscosity over the pH values selected for the up curve of the shear ramp. However, on the initial ramp up, the solution at pH 4.0 had a substantially higher apparent viscosity than the sample at pH 8.0. This observation at pH 4.0 may be the result of increased interaction of protein fragments at pH values near the isoelectric point of whey.

Frequency Sweep. Small amplitude oscillatory rheology was performed on sample 6 at temperatures of 25 and 90 °C to examine viscoelasticity. A layer of paraffin oil was applied to the surface of the sample before frequency sweeps were performed to minimize moisture loss, and moisture analysis was conducted before and after testing to demonstrate results were not a consequence of drying. Only data on samples which possessed less than 1% moisture difference before and after analysis was used. Viscoelastic properties are typically described with three parameters, the phase angle (δ), the storage modulus (G'), and the loss modulus (G''). The moduli show distinct behaviors when subjected to a frequency sweep and can reveal general characteristics of the sample. As pictured in Figure 7a, an increased frequency resulted in an increased G' value. The phase angle is equivalent to the $\tan^{-1}(G''/G')$ and ranges from 0° to 90°. The lower the phase angle, the more solidlike the material. According to Figure 7b, for all sweeps the phase angle was below 25°, indicating the storage modulus dominated the loss modulus at every frequency. The coupling of the low phase angle and linearly increasing storage modulus with frequency is indicative of a weak gel network.

Four sweeps were performed on a single solution at temperatures alternating between 25 and 90 °C to

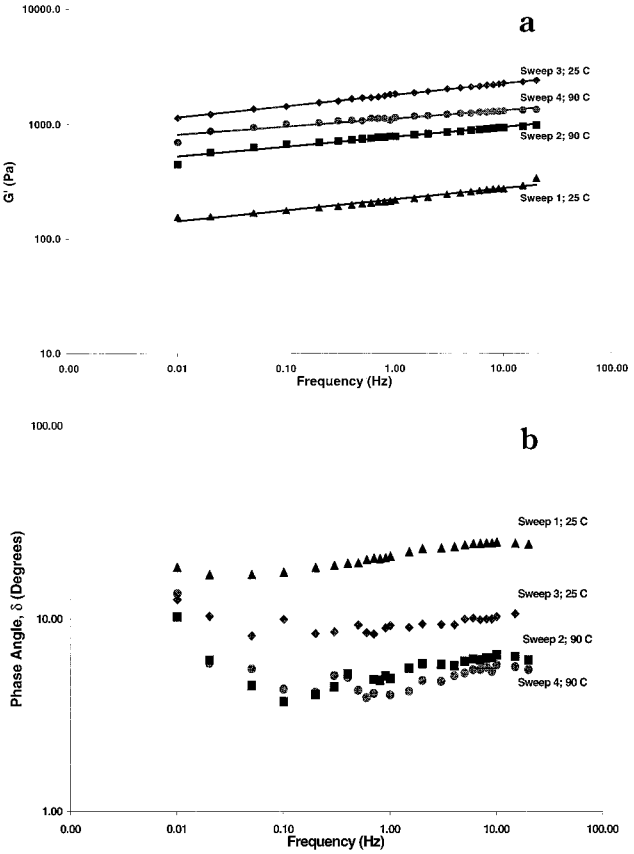

Figure 7. (a) Rheogram illustrating the storage modulus vs frequency for four consecutive frequency sweeps on one aliquot of sample 6 at 25, 90, 25, and 90 °C. (b) Rheogram illustrating the phase angle vs frequency for four consecutive frequency sweeps on one aliquot of sample 6 at 25, 90, 25, and 90 °C.

elucidate temperature effects on gel network interactions. Comparing sweep 1 verses sweep 2 in Figure 7 parts a and b, the storage modulus increased and the phase angle decreased at 90 °C, caused by protein unfolding and increased hydrophobic interactions. As temperature was lowered for sweep 3, the newly unfolded proteins from sweep 2 were able to participate in network formation yielding a higher G'. Sweep 4 and sweep 2, each at 90 °C demonstrated similar rheological behavior, typical of diminished hydrogen bonding at elevated temperatures. Following the initial sweep at 90 °C, subsequent sweeps produced comparable rheological results, supporting the claim of a temperature-independent gel structure (see Figure 4).

A process has been developed to produce whey protein ingredients capable of thickening or creating weak gel structures at ambient and refrigeration temperatures, which is stable over a wide range of typical food temperatures and pH values. This cold-set feature may impart a wide range of potential applications such as malted-milk beverages, protein drinks, and nutritious liquid formulations for athletes, infants, or the elderly.

Abbreviations Used

WPI, whey protein isolate; FS−I, fine-stranded one gel type; FS−II, fine-stranded two gel type; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; DTT, dithiothreitol; β-LG, beta-lactoglogulin; DSC, differential scanning calorimetry; α-LA, alpha-lactalbumin; pI, isoelectric point.

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