Oscillatory Rheological Comparison of the Gelling Characteristics of Egg White, Whey Protein Concentrates, Whey Protein Isolate, and β-Lactoglobulin

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A Bohlin rheometer was used in the oscillatory mode to compare the gelation characteristics of egg white, two commercially available whey protein concentrates, a commercially available whey protein isolate, and β-lactoglobulin. At a given protein concentration egg white had a lower gelation temperature, a higher initial gelation rate, a higher gel stiffness (G'), and, at concentrations ≤16% protein, a higher value of the ratio $G'(80\ °C)/G'(20\ °C)$ than had the three commercial whey protein products. Furthermore, egg white had a much lower minimum protein concentration for gelation. Whey protein concentrate and isolate preparations with increased salt contents could match egg white in terms of gel stiffness and could almost match egg white in terms of initial gelation rate. However, further development work is needed to produce whey protein products that match egg white in terms of the other three properties mentioned above. Cations tested all increased G' of whey protein isolate gels in the order $Mg^{2+} > Fe^{3+} > Ca^{2+} > K^{+} > Na^{+}$.

Keywords: *Gelation; whey proteins; rheology*

INTRODUCTION

Whey protein products are widely used in foods because of their nutritional value and functional properties. Different functional demands are made of whey proteins in different foods. The heat denaturation and heat gelation of whey proteins are important functional characteristics in some confectionery products, meat products, bakery products, and textured products and in a range of new dairy analogue products formulated especially to take advantage of these characteristics (de Wit, 1989). In many of these food applications whey protein products compete with whole egg or egg white products; some of the problems of replacing egg white with whey proteins have been discussed previously (Melachouris, 1984; Kinsella and Whitehead, 1989). For effective application of whey proteins, differences in the heat gelation characteristics of egg white and whey proteins must be recognized; in particular, the different gelation temperatures and the effects of physicochemical variables on gelation must be understood (Kinsella and Whitehead, 1989). Little detailed information is available in this area. A recent study (Hsieh *et al.*, 1993) has compared the gel point temperatures of various whey and egg proteins.

A range of physicochemical variables have been shown to affect the progress of gelation and the final gel properties of various whey protein products. Tang *et al.* (1993) showed that protein concentration, temperature, pH, salt type, and salt concentration all had a marked effect on the gelation time of a commercially available whey protein concentrate (WPC). Stading and Hermannson (1991) demonstrated that pH had a complex effect on the rheological properties of whey protein gels, with two maxima in G' at pH 4 and 7. Several groups of workers have demonstrated the marked effect of both salt type and salt concentration on the rheological properties of whey protein gels, with optimum gel stiffness or strength usually occurring at a relatively low salt concentration and with higher salt concentrations resulting in weaker gels because of the occurrence of coagulation rather than gelation (Schmidt *et al.*, 1978, 1979; Mulvihill and Kinsella, 1988; Kuhn and Foegeding, 1991). Different whey protein preparations differ markedly in their response to some of the above physicochemical variables. The aim of this work was to compare the gelation properties of various whey protein products with those of egg white protein and to explore methods for eliminating some of the differences in gelation characteristics.

MATERIALS AND METHODS

Protein Samples. Two commercially available New Zealand WPC powders, WPC(A) and WPC(B), one whey protein isolate (WPI) powder (Le Sueur Isolates, Le Sueur, MN), and one commercially available egg white powder (egg white powder type 110, Henningsens Foods, White Plains, NY) were provided by the New Zealand Dairy Research Institute (NZDRI). The WPC(A) sample contained 79.8% protein, 4.4% moisture, 7.1% fat, 4.6% lactose, 0.16% sodium, 0.69% potassium, 0.33% calcium, 0.26% phosphorus, and other components. The WPC(B) powder contained 76.0% protein, 4.5% moisture, 4.9% fat, 6.3% lactose, 1.6% sodium, 0.12% potassium, 0.22% calcium, 0.33% phosphate, and other components. The WPI powder contained 93.6% protein, 4.2% moisture, 0.86% fat, 1.04% lactose, 0.51% sodium, 0.12% potassium, 0.2% calcium, <0.005% phosphate, and other components. The spray-dried egg white powder contained 85.8% protein, 7.4% moisture, and other components. The proportions of the major whey proteins in WPC(A) and WPC(B) were identical to those found in raw whey, and only a trace of the whey protein separated as insoluble material when 10% protein solutions at pH 7.00 were centrifuged for 5 min at 550*g*.

A sample of purified β-lactoglobulin was prepared from Cheddar cheese whey according to the thermal separation method of Pearce (1983). The dried preparation contained 86.6% protein, 3.5% moisture, 0.75% fat, 3.0% lactose, 4.0% ash, and other components. Separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis indicated that 92% of the protein was β-lactoglobulin.

Preparation of Protein Solutions. Protein powders were reconstituted either with distilled water or with salt solutions of fixed concentration by mixing with a magnetic stirrer until dissolution was complete. The pHs of protein solutions were adjusted from their initial values to pH 7.00 by adding either 1 M HCl or 1 M NaOH. Generally, a sample solution with a

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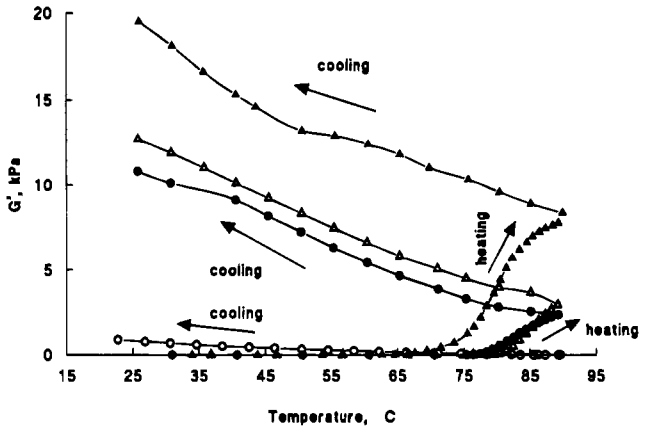

Figure 1. Storage modulus (G') vs temperature for solutions of WPI (○), WPC(A) (●), WPC(B) (△), and egg white (▲) containing 12% protein during a temperature sweep experiment with heating at 1 °C/min and subsequent cooling at 2 °C/min. The gelation was conducted at pH 7, 1 Hz, and 0.01 maximum shear strain.

higher than desired protein concentration was prepared at first. After pH adjustment, this was then diluted to the desired protein concentration, centrifuged for 5 min at 550g to remove foam, dispersed air bubbles, and insoluble material, and stored at 5.5 °C prior to rheological measurements. To produce a sample of reduced mineral content, 550 g of WPC(B) solution containing 20% protein was batch dialyzed against 20 L of distilled water at 20 °C for 12 h. After dialysis and pH adjustment, the sample was finally diluted to the desired protein concentration.

Oscillatory Rheological Measurements. The Bohlin VOR Rheometer System (Bohlin Rheologi AB, Lund, Sweden) was used in its oscillatory mode. The C25 concentric cylinders measuring system, consisting of a 25 mm diameter fixed bob and a 27.5 mm diameter rotating cup, was used in all experiments. All measurements were made with 13 mL of protein solution, which was always covered with a thin layer of liquid paraffin to prevent evaporation of water and consequent surface drying of the sample. Unless otherwise stated, a maximum shear strain of 0.01 was used in all experiments since this is well within the linear viscoelastic region during gelation (Tang *et al.*, 1993). Other experimental details for constant-temperature gelation experiments were as described in Tang *et al.* (1993). Temperature sweep experiments were performed by heating at a rate of 1 °C/min and subsequently cooling at a rate of 2 °C/min.

In some experiments protein gelation was performed by heating the sample at 80 °C for 45 min in the rheometer. The sample was then cooled by adjusting the rheometer water bath setpoint to 20 °C. The water bath took 15 min to reach 20 °C, and the sample temperature lagged ~2 min behind the water bath temperature.

Statistical Planning of Experiments. The preparation and rheological testing of protein solutions were ordered chronologically by randomization and blocking to eliminate systematic error. Duplicate experiments, including both sample preparation and rheological measurements, showed excellent repeatability.

RESULTS

Temperature Sweep Experiments. Temperature sweep experiments indicated the gelation temperatures of various solutions. At 12% protein, egg white solutions began gelling at 65 °C, while each of the WPC solutions began gelling at 75 °C (Figure 1). These results are in agreement with those reported by Melachouris (1984). Egg white solution gelled faster than WPC, and egg white gels had higher G' values than WPC gels during both heating and cooling. WPI solutions at 12% protein did not form gels during heating to 90 °C and formed only soft gels with low G' values after subsequent cooling.

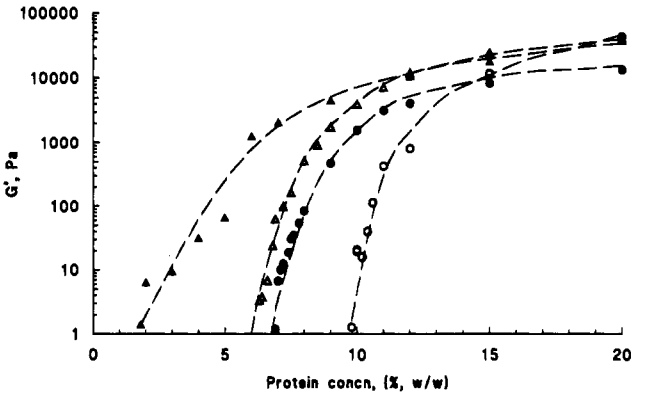

Figure 2. Storage modulus (G') after 11.11 h at 80 °C vs protein concentration for WPI with 0.1 M NaCl added (○), WPC(A) (●), WPC(B) (△), and egg white (▲). Experiments were performed at pH 7, 0.1 Hz, and 0.002 maximum shear strain.

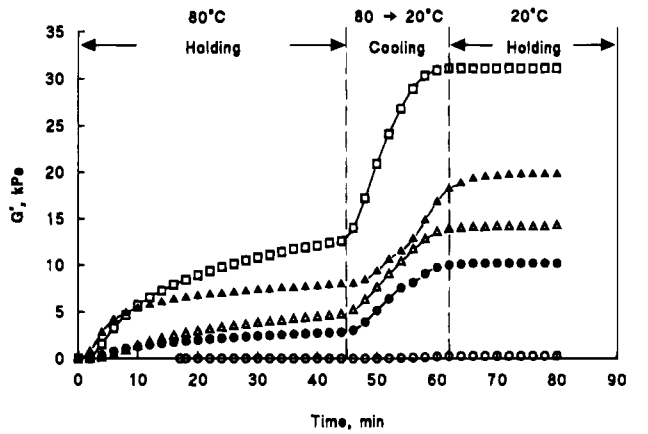

Figure 3. Storage modulus (G') vs time for solutions of WPI (○), WPC(A) (●), WPC(B) (△), egg white (▲), and β-lactoglobulin (□) containing 12% protein during heating and subsequent cooling at pH 7, 1 Hz, and 0.01 maximum shear strain.

Minimum Protein Concentration for Gelation. Protein solutions with a range of concentrations up to 20% were subjected to heating at 80 °C and pH 7 for 11.11 h, and G' was then measured at 80 °C (Figure 2). Egg white solution was able to form gels at much lower protein concentrations (minimum 1.8%) than WPC solutions (minimum 6%). WPC(B) had a slightly lower minimum protein concentration for gelation than WPC-(A). Even in the presence of 0.01 M NaCl WPI solutions needed to contain at least 9.8% protein before gelation would occur. The G' of egg white gels was higher than that of WPC(B) gels below 12% protein, but the two were equal above 12% protein. The G' of WPC(B) gels was always higher than that of WPC(A) gels.

Formation and Cold Stiffness of Egg White and Whey Protein Gels. Figure 3 shows the formation of gels from 12% protein solutions during heating at 80 °C. WPI formed a very soft gel with very low G' values. The magnitude of G' at the end of heating increased in the order WPI < WPC(A) < WPC(B) < egg white < β-lactoglobulin. However, the initial rate of increase in G' was higher for egg white than for any of the whey proteins.

On cooling to 20 °C all of the protein gels except WPI showed a substantial increase in G' (Figure 3). To

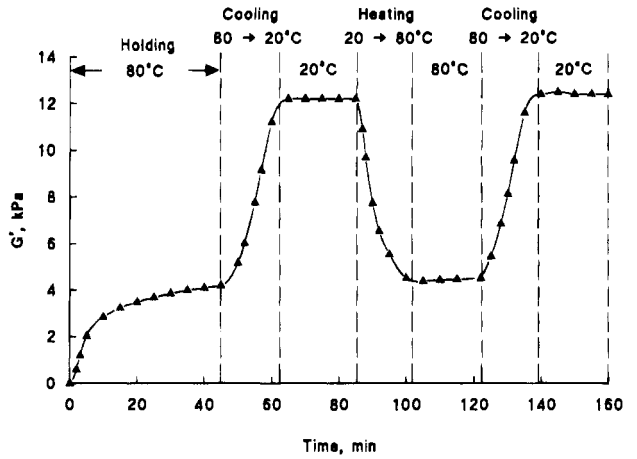

Figure 4. Storage modulus (G') of an egg white protein solution containing 12% protein vs time during heating and cooling cycles at pH 7, 1 Hz, and 0.01 maximum shear strain.

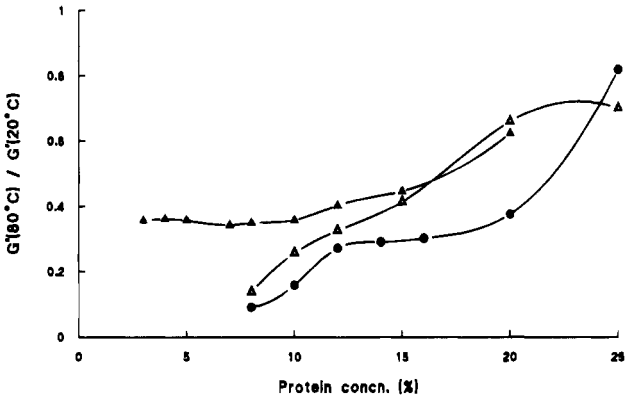

Figure 5. Effects of protein concentration on G'(80 °C)/G'(20 °C) for WPC(A) (●), WPC(B) (△), and egg white gels (▲) after heating at 80 °C and pH 7 for 45 min, with subsequent cooling to 20 °C. Measurements were performed at 1 Hz and 0.01 maximum shear strain.

determine whether the increase in G' due to cooling was reversible or irreversible, several cooling and heating cycles were conducted on the formed gels. The increase in G' seen when egg white, WPC(A), WPC(B), and WPI gels were cooled was found to be almost completely reversible; egg white results are shown in Figure 4. This reversibility is similar to that observed by Beveridge *et al.* (1984).

The ratio of storage modulus at 80 °C to that at 20 °C is plotted as a function of protein concentration in Figure 5. G'(80 °C)/G'(20 °C) was higher for egg white than for either of the WPCs below 16% protein. This ratio is important in the use of gelling proteins in bakery products.

Effects of pH. WPC(A) gels were stiff and opaque at pH 7 and gradually changed to transparent, elastic soft gels as pH was increased from 7 to 9. Between pH 4 and 7 white particulate gels or coagula were formed. At and below pH 4 brittle, sticky gels were formed.

WPI gels exhibited a maximum gel stiffness at about pH 5.5. Between pH 4 and 6 WPI gels were white, particulate, and sponge-like. These gels were very porous in appearance and lost moisture very readily on gentle squeezing without fracturing, yet they had a high stiffness (high G'). WPI gels were transparent, elastic, and soft between pH 6 and 7. No gel formed above pH 7. Below pH 4 transparent or semitransparent, brittle, sticky WPI gels were formed.

Table 1. Effects of KCl on the Storage Modulus (G') of WPC(B) Gels[a]

KCl concn (M)	G'(80 °C)[b] (kPa)	G'(20 °C)[b] (kPa)	G'(80 °C)/ G'(20 °C)
0	0.93	3.7	0.25
0.01	1.4	4.9	0.27
0.05	2.6	9.1	0.29
0.09	3.3	11.5	0.28
0.1	3.8	12.8	0.30
0.12	3.6	12.6	0.29
0.15	2.7	10.3	0.26

[a] WPC(B) solutions containing 10% protein were heated at 80 °C and pH 7 for 45 min to form gels. [b] G' was measured at 1 Hz and 0.01 maximum shear strain.

Table 2. Effect of Salts on Storage Modulus of WPI Gels[a]

salt	concn (M)	G'(80 °C)[b] (kPa)	G'(20 °C)[b] (kPa)	G'(80 °C)/ G'(20 °C)
	0	0.01	0.32	0.03
NaCl	0.01	0.24	2.0	0.12
KCl	0.01	0.70	3.3	0.21
$CaCl_2$	0.01	0.73	4.7	0.16
$MgCl_2$	0.01	4.0	14.5	0.28
$FeCl_3$	0.01	3.9	12.1	0.33
CH_3COO Na	0.01	0.16	1.8	0.09
CH_3COOK	0.01	0.28	2.1	0.13
$(CH_3COO)_2Mg$	0.01	6.5	20.2	0.32
$FeC_6H_5O_3$[c]	0.01	1.3	4.1	0.32
$Na_3C_6H_5O_3$[c]	0.0033	0.04	0.76	0.05
$K_3C_6H_5O_3$[c]	0.0033	1.5	4.7	0.31
Na_2HPO_4[d]	0.005	0.06	0.88	0.07
K_2HPO_4[d]	0.005	1.6	4.5	0.34

[a] WPI solutions containing 12% protein were heated at 80 °C, pH 7, for 45 min to form gels. [b] G' was measured at 1 Hz and 0.01 maximum shear strain. [c] Citrate. [d] Orthophosphate.

Egg white gels exhibited maximum G' at pH 4. Below pH 4 brittle, sticky gels were formed. Between pH 4 and 5 strong, particulate white gels were formed. Strong, elastic white gels were formed at and above pH 6.

Effects of Salts. The effects of KCl concentration on G' of WPC(B) gels are shown in Table 1. Small additions of salt caused dramatic increases in G'. However, a maximum G' was reached at about 0.1 M KCl, and G' then decreased with further increases in salt concentration. G'(80 °C)/G'(20 °C) also reached a maximum value of 0.30 at about 0.1 M KCl. However, this is lower than the G'(80 °C)/G'(20 °C) of 0.36 for egg white at 10% protein.

Table 2 shows the effect of different anions and cations on the G' of WPI gels. All added salts caused marked increases in the G' of WPI gels. Divalent (Ca^{2+}, Mg^{2+}) and trivalent (Fe^{3+}) cations had a much greater effect on G' than monovalent cations (Na^+, K^+). Cations listed in increasing order of effectiveness were $Na^+ < K^+ < Ca^{2+} < Fe^{3+} \approx Mg^{2+}$. This order follows the Hofmeister or lyotropic series except that Na^+ follows K^+ in that series. G'(80 °C)/G'(20 °C) tended to be higher for stiff gels than for weak gels. The effect of anions does not appear to be so systematic. For sodium as common cation the anions listed in increasing order of effectiveness were $HPO_4^{2-} \approx cit^{3-} < CH_3COO^- < Cl^-$. This order again follows the Hofmeister series. However, for potassium as common cation the order was $CH_3COO^- < Cl^- < cit^{3-} \approx HPO_4^{2-}$. With magnesium as the cation acetate was more effective than chloride.

Dialysis of a WPC(B) solution to reduce its mineral content caused it to gel much more slowly, and the gel formed had a much lower G' value than was observed

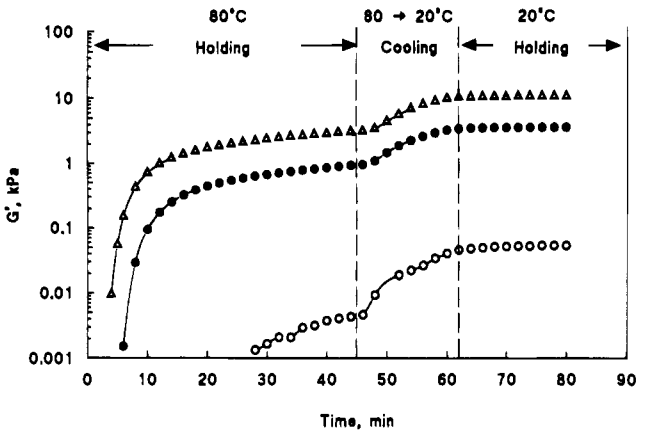

Figure 6. Storage modulus (G') vs time for solutions of WPC(B) containing 10% protein during heating and subsequent cooling at pH 7, 1 Hz, and 0.01 maximum shear strain: WPC(B) (●); WPC(B) after 12 h of dialysis against distilled water (○); WPC(B) after 12 h of dialysis against distilled water followed by 0.1 M NaCl addition (△).

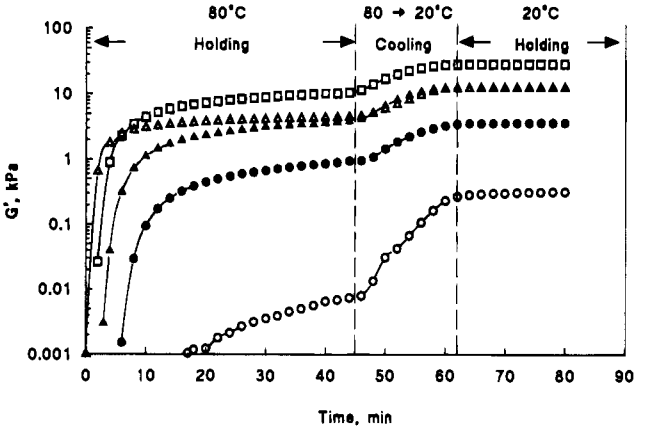

Figure 7. Storage modulus (G') vs time for 10% protein solutions during heating and subsequent cooling at pH 7, 1 Hz, and 0.01 maximum shear strain: WPI (○); WPC(B) (●); egg white (△); WPC(B) with 0.1 M KCl addition (▲); WPI with 0.1 M KCl addition (□).

without dialysis (Figure 6). However, addition of 0.1 M NaCl to the dialyzed protein solution caused a large increase in the rate of gelation and the final G'. Manipulating the salt content is clearly a good method of controlling whey protein gel properties. The final G' values of WPC(B) and WPI gels could be made, respectively, equal to and greater than that of egg white by the addition of 0.1 M KCl (Figure 7). Further, the initial gelation rate of WPI with added salt was very close to that of egg white.

Effect of Lactose. Addition of lactose to WPC(B) solutions containing 12% protein at pH 7, followed by heating at 80 °C for 45 min, produced somewhat weaker gels than when lactose was absent. Addition of 10% lactose produced gels with G' 25% lower at 20 °C and 47% lower at 80 °C than the corresponding values for gels without lactose added.

DISCUSSION

The least heat-stable proteins in egg white are conalbumin, ovalbumin, and the G-globulins with denaturation temperatures of 57.3, 71.5, and 72 °C, respectively, (Froning, 1988). Of the whey proteins, α-lactalbumin has the lowest denaturation temperature (62 °C), but it is the whey protein most thermostable against aggregation because denaturation is highly reversible (deWit and Klarenbeek, 1984). The other major whey proteins have denaturation temperatures of 64 (bovine serum albumin), 72 (immunoglobulin), and 78 °C (β-lactoglobulin) (deWit and Klarenbeek, 1984). When binary egg protein mixtures are heated, denaturation occurs near the denaturation temperature of the least heat-stable protein (Froning, 1988). Since egg white, WPI, and WPC are multicomponent protein mixtures, their heat gelation might be expected to begin near the denaturation temperature of the least heat-stable protein in the mixture. Egg white would thus be expected to begin to gel at a lower temperature than WPC. Figure 1 indicates only a 10 °C difference in denaturation temperature between egg white proteins and whey proteins, whereas Melachouris (1984) and Kinsella and Whitehead (1989) quote a 20 °C difference in gelation temperature. The lower difference in denaturation temperature in this work may be attributed to the WPCs having a higher protein content, an improved salt balance, and a lower level of denaturation. These factors were not reported by either Melachouris (1984) or Kinsella and Whitehead (1989).

The two major egg white proteins, ovalbumin (45 000 Da) and conalbumin (76 000 Da) (together comprising 66% of egg white protein), have much higher molecular masses than the two principal whey proteins, α-lactalbumin (14 200 Da) and β-lactoglobulin (18 600 Da) (together comprising 80% of whey protein) (Froning, 1988; Kinsella and Whitehead, 1989). Linear polymers of high molecular mass form stronger gels and gel at lower concentrations than low molecular mass polymers. The difference in molecular masses is one possible reason why egg white proteins are able to form gels at much lower concentrations than whey proteins. Alternatively, the gelation mechanisms for egg white protein may be different from those for whey protein.

The marked increase in G' on cooling (from 80 to 20 °C in the present study) has been attributed by Beveridge *et al.* (1984) mainly to the formation of a multiplicity of hydrogen bonds at 20 °C since these are favored at lower temperatures. The increased cross-linking by hydrogen bonds at the lower temperature would make the gels much stiffer.

The complex variation in physical appearance and rheological properties of WPC gels with pH has been discussed in detail by Stading and Hermannson (1991). The complex behavior was attributed to variations in electrostatic interactions and disulfide bonding with pH. At certain pH values there was an optimum balance between protein–protein and protein–solvent interactions resulting in maximum gel stiffness.

It is well established that salts have a major influence on the properties of whey protein gels. Increasing levels of either NaCl or $CaCl_2$ cause increases in gel hardness, gel shear stress, and other rheological properties until maximum values of these properties are reached; values then decrease with higher salt concentrations (Schmidt *et al.*, 1978, 1979; Mulvihill and Kinsella, 1988; Kuhn and Foegeding, 1991). The same pattern has been observed for the effects of NaCl and $CaCl_2$ concentrations on G' and G'' (Q. Tang, unpublished work) and was observed here for the effect of KCl concentration on G' (Table 1). A maximum in such a gel property has generally been attributed to an optimum balance between protein–protein and protein–solvent interactions at a particular salt concentration. Divalent cations

had a much greater effect on gel properties than monovalent cations. Kuhn and Foegeding (1991) showed in detail that a range of divalent cations (Ca, Mg, Ba) all caused a similar increase in shear stress and shear strain at failure of WPI gels and that the increase in shear stress at failure was much larger than that caused by a range of monovalent cations (Na, Li, K, Rb, Cs). The effect of anions was not systematically studied here but has been reported for conalbumin (Oe *et al.*, 1987) and β-lactoglobulin (Mulvihill *et al.*, 1990).

The thermal coagulation of whey proteins can be effectively inhibited by various sugars including lactose (Garrett *et al.*, 1988). This inhibition would cause a decrease in the G' values of formed WPC gels. Garrett *et al.* (1988) showed that sucrose promoted the denaturation of whey proteins but inhibited their subsequent coagulation and interpreted their results in terms of the effect of sucrose on "pairwise" hydrophobic interactions involving both solvent and protein. An alternative mechanism by which lactose inhibits whey protein gelation could be the formation of Schiff base addition products between lactose and amine groups on the whey protein.

Whey proteins are often suggested as a replacement for egg white proteins in foods where heat gelation is required, for example, bakery products. Five major differences have been demonstrated here between the gelation properties of egg white proteins and those of whey proteins. At a given protein concentration egg white proteins have a higher initial gelation rate, a higher gel stiffness, a lower gelation temperature, and a higher value of the ratio $G'(80\ °C)/G'(20\ °C)$ (at <16% protein). Further, egg white proteins have a much lower minimum protein concentration for gelation. Whey proteins can be made roughly equivalent to egg white proteins in terms of initial gelation rate and gel stiffness by increasing their salt content. However, further development work is needed to produce whey protein preparations that match egg white in terms of the other three properties listed. These three properties of whey protein cause particular difficulties in the manufacture of some types of cake, e.g., angel food cake (Melachouris, 1984; Kinsella and Whitehead, 1989). At the lower temperatures needed for this type of cake the whey protein gel network is reluctant to form. Also, the network suffers from low strength at high temperatures, allowing shrinkage or collapse of the cake structure during the later stages of baking and during cooling. The result is a low final cake volume, especially at the center of the cake.

It is commercially important to produce WPCs and WPIs with consistent functionality. However, commercial WPCs and WPIs have been reported to be highly variable in functionality (Kinsella and Whitehead, 1989; Morr and Foegeding, 1990). Morr (1992) suggested various approaches that could be taken to reduce the wide variability in properties of commercial WPCs. From the work reported here and other studies it is clear that careful control of WPC mineral content, particularly divalent and trivalent cation content, is crucial if consistent gelling properties are to be obtained.

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