Effects of Salts on the Properties of Sols and Gels Prepared from Whey Protein Isolate and Process Whey Protein

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ABSTRACT

Process whey protein was prepared by heat treatment of whey protein isolate under salt-free conditions. Addition of sodium chloride, trisodium cit-rate, potassium chloride, or calcium chloride to a process whey protein solution increased the viscosity and resulted in gelation at 25°C. In contrast, the viscosity of whey protein isolate solutions was not changed. The properties of whey protein isolate and process whey protein gels prepared by heating at 80°C for 60 min with 25 different salts were measured and compared. Although process whey protein produced gels with the addition of any of the salts examined, whey protein isolate solution did not gel with some salts. The tendency of both protein solutions to gel apparently depended on the lyotropic nature of the cation. Inorganic salts produced firmer gels than did organic salts. The gel strength and water-holding capacity of both proteins decreased as salt concentration increased. When the transparent gels prepared from process whey protein were heated again, the gel firmness increased, adhesiveness decreased, and transparency and cohesiveness slightly decreased, depending on the temperature and duration of the second heating.

(**Key words**: whey protein isolate, process whey protein, gelation)

Abbreviation key: **I** = ionic strength, **PWPI** = process whey protein, **WPC** = whey protein concentrate, **WPI** = whey protein isolate.

INTRODUCTION

Whey proteins from bovine milk, produced in the casein and cheese manufacturing processes, are isolated and concentrated using UF or ion-exchange chromatography. The resulting whey protein concentrate (**WPC**) and whey protein isolate (**WPI**) have many applications, including use in bakery, dairy, beverage, meat, and fish products and in infant formulas (3, 13). The properties of thermally induced gels made from whey proteins are influenced by several factors, including protein concentration, pH, and temperature and duration of heat treatment (12, 15, 17, 18). Various salts also strongly affect the gelation of food proteins, including whey proteins, and the properties of the resulting gels, including the gel structure, firmness, and water-holding capacity. Extensive studies have been carried out on the effect of calcium on whey protein (10).

Under specific heating conditions, whey proteins form a transparent gel, although heat treatment usually produces a turbid suspension or gel. A WPI that has been preheated under salt-free conditions, referred to as process whey protein (**PWPI**), also produces a transparent gel upon reheating in the presence of NaCl. The PWPI has properties similar to those of WPI over a wider range of conditions and also has other novel characteristics as a food ingredient (5). The PWPI can produce a transparent gel or a viscous liquid even at 25°C (9) and can produce either a stable emulsion or an emulsion gel after heat treatment (our unpublished data). Because PWPI can be obtained in large quantities by a simple procedure, PWPI is already being used in several processed foods (8).

The PWPI appears to consist of soluble linear aggregates of heat-denatured whey protein molecules (5). A fine-stranded protein matrix was observed in a WPI gel that was prepared by heating a PWPI sample after addition of $CaCl_2$ (1). Similar fine-stranded structures were observed in a gel prepared from β-LG at pH 4 to 6 and in the gels prepared from WPI at pH 4 to 6 (11) and at pH 5.3 (16). Such fine-stranded structures have been observed in heated ovalbumin and BSA (2, 6). Those fine-stranded structures (1, 2, 6) might correspond to linear aggregates of heat-denatured globular protein molecules.

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After the addition of salts, including NaCl, the PWPI solution produced gels or sols by heat treatment or incubation at room temperature (5). The PWPI can be used as an additive in processed food. However, processed foods contain many inorganic and organic salts (i.e., NaCl and sodium citrate) derived from raw materials, food additives, and food ingredients. Therefore, it is important to investigate the effects of salts on the rheological properties of the gels prepared from WPI and PWPI solutions. In this study, 25 typical food or food additive salts were added to WPI and PWPI, and the rheological properties of the resulting sols and gels were measured.

MATERIALS AND METHODS

Materials

Cheese WPI (Daiichilacto; Daiichi-Kasei Co. Ltd., Kyoto, Japan) containing 92.4% protein (as determined by the Kjeldahl method; N × 6.38), 1.6% ash, 0.16% lactose, 0.8% lipid, and 6.4% moisture was prepared by ion-exchange chromatography, UF, and spray-drying. The protein fraction contained 62.0% β-LG, 16.8% α-LA, 5.2% BSA, and 16.0% other proteins as measured by ion-exchange chromatography (LC-6A; Shimadzu Corporation, Kyoto, Japan, and Ultron MR-300 DEAE column; Shinwa Kako Co., Ltd., Kyoto, Japan). The pH of this WPI sample was adjusted to 7.0 by addition of 1.0 *N* NaOH, and the protein concentration was adjusted to 90 mg/ml. The electric conductivity of this WPI solution was the same as that of 2.4 m*M* NaCl. Sodium chloride, sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, anhydrous sodium sulfate, sodium lactate, sodium acetate trihydrate, sodium DL-malate, sodium tartrate dihydrate, trisodium citrate dihydrate, sodium L-(+)-ascorbate, KCl, dipotassium hydrogen phosphate, $CaCl_2$, calcium DL-lactate hexahydrate, and $MgCl_2 \cdot 6H_2O$ were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Monobasic potassium dihydrogen phosphate, KNO_3, and $MgSO_4$ were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Calcium gluconate monohydrate was food additive grade (PMP Fermentation Products, Inc., Peoria, IL). Anhydrous sodium pyrophosphate (assay minimum 97.9%), sodium tripolyphosphate (assay minimum 83.5%), sodium tetrapolyphosphate (assay minimum 51%), sodium hexametaphosphate (assay minimum 79.4%), potassium pyrophosphate (assay minimum 95.0%), and potassium polyphosphate were also food additive grade (Daiichi-Kasei Co., Ltd., Kyoto, Japan). Sodium dihydrogen phosphate and disodium hydrogen phosphate were used to prepare buffered saline (pH 7.0), and potassium dihydrogen phosphate and dipotassium hydrogen phosphate were used for buffered saline (pH 7.0). Both solutions were 2.3 *M*.

Protein Concentration

Protein concentrations of WPI and PWPI solutions at pH 7.5 were measured at 280 nm using an extinction coefficient of 11.7 at 1% (wt/wt) and a 1-cm light path (7).

Preparation of WPI and PWPI Solutions

The PWPI solution was prepared from WPI solution as described previously (5). Briefly, after the protein concentration and pH of the WPI solution were adjusted to 90 mg/ml and 7.0, respectively, by addition of 2*N* NaOH, the WPI solution was heated at 90°C for 30 min. The sample was then cooled with tap water for 2 h. A transparent, slightly viscous liquid (PWPI; 90 mg/ml) was obtained. Both WPI and PWPI were prepared fresh each day in the same manner.

Viscosity Measurement After Salt Addition

A concentrated salt solution was added to the PWPI solution (90 mg/ml), and the protein concentration of this solution was adjusted to 70 mg/ml by the addition of distilled and deionized water. Sufficient concentrated salt solutions were added to adjust the concentrations of NaCl, KCl, or sodium citrate to 100, 300, or 500 m*M*, respectively, and the concentration of $CaCl_2$ to 7.5, 10, 15, or 20 m*M* in the PWPI. The viscosity of each solution was measured at 25°C using a B-type rotational viscometer and a Couette type rotor (VDA-L; Shibaura System Co. Ltd., Tokyo, Japan). The rotation of the rotor (Shibaura No. 2 rotor, 17 mm i.d. and 6 mm thick) was 6, 30, or 60 rpm.

Preparation of Heat-induced Gels

The final concentrations of salts in WPI and PWPI solutions (pH 7.0) were adjusted to 100, 200, 300, 400, or 500 m*M* by addition of NaCl, sodium-buffered saline, sodium sulfate, sodium lactate, sodium acetate, sodium malate, sodium tartrate, sodium citrate, sodium ascorbate, KCl, potassium-buffered saline, or KNO_3. When $CaCl_2$, calcium lactate, calcium

gluconate, $MgCl_2$, or $MgSO_4$ was added, concentrations were adjusted to 7.5, 10, 15, and 20 m*M*, respectively. Sodium pyrophosphate, sodium tripolyphosphate, sodium tetrapolyphosphate, sodium hexametaphosphate, potassium pyrophosphate, and potassium polyphosphate were adjusted to 1.0, 1.5, 2.0, 3.0, and 5.0% (wt/wt), respectively. The protein concentration was adjusted to 70 mg/ml, and, because the pH values of the samples shifted slightly after the addition of salt, the pH value of each sample was confirmed and recorded. Each solution was poured into vinyl chloride tubing (22 mm i.d. × 200 mm long; Kureha Kagaku Kogyo Co., Tokyo, Japan) and heated at 80°C for 1 h in a water bath. The samples were then cooled with tap water for 2 h. The gels were carefully removed from the tubing and cut into 20-mm lengths. The breaking strength, strain, compressive hardness, and water-holding capacity of the gels were immediately measured.

Heating of PWPI Gels

The effect of heating on the PWPI gels prepared by the first heating of PWPI solution was investigated. The PWPI solution (90 mg/ml, protein concentration; 40 m*M*, NaCl, and pH 7.0), placed in a tube as just described, was heated at 80°C for 60 min and cooled with tap water for 2 h. A transparent gel was obtained by this treatment. After cooling, the tube containing PWPI gel was immediately heated again at 80, 90, or 100°C for 60 min or at 121°C for 4 or 20 min and then cooled with tap water for 2 h. The gel was carefully removed from the tube and cut into 20-mm lengths. The texture parameters of the gel were measured immediately.

Measurements of Gel Properties

Breaking strength (grams), compression (millimeters) at fracture and compressive hardness (grams) of the gels (cylindrical shape, 22 mm in diameter and 20 mm in height) were measured with a Fudoh rheometer NRM-2010J-CW (Fudoh Kogyo Co., Ltd., Tokyo, Japan) using a flat plunger 50 mm in diameter. The gel was placed on a glass plate that was moved upward at 6 cm/min to compress the gel. Breaking strength was measured using a force-compression curve at the point that gave the maximum force. The total compression was 80%, to measure breaking strength and strain, or 20%, to measure compressive hardness. The data were plotted with a Rheo Plotter FR-801 (Fudoh Kogyo) and analyzed with built-in software designed for analysis of breaking strength and strain and compressive hardness.

The water-holding capacity of the gels was measured in terms of the water released from the gels onto filter paper (number 40 for paper chromatography; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The gel was placed on the filter paper at 25°C and allowed to stand for 10 min. The exuded area of water was not round but instead was elliptical. To obtain a relative index of the area, the long diameter multiplied by the short diameter of the exuded area of water was calculated. This value, expresses as square centimeters, was used to estimate the water-holding capacity of the gel. All experiments were performed using six samples, and the mean was calculated after the maximum and minimum values were deleted.

Cohesiveness, hardness (Newtons per square meter), and adhesiveness (milli-Newtons per meters) of cylinders of gels (22 mm in diameter and 20 mm in height) were measured with a Rheoner RE-3305 (Yamaden Co., Ltd., Tokyo, Japan) interfaced with a personal computer (PC-386LS, MS-DOS machine; Seiko-Epson Co., Ltd., Tokyo, Japan) using a flat plunger with a 30 mm diameter. Gumminess (Newtons per square meter) was calculated as hardness multiplied by cohesiveness. The gel was placed on a glass plate that was moved upward at 0.5 mm/s to compress the gel by 40%. After compression, the gel was moved downward at the same rate. The data were analyzed with software (TAS-3305-16) designed for texture analysis (Yamaden Co., Ltd.).

RESULTS AND DISCUSSION

Salt Effects on Viscosity and Gelation

The viscosity of the PWPI solution increased at 25°C upon NaCl addition, and a gel was then formed, which is consistent with previous results (5, 9). Figure 1 shows the changes in the viscosities of WPI and PWPI solutions following the addition of NaCl, sodium citrate, KCl, and $CaCl_2$ at 25°C.

After salt addition, pH values were 6.6 to 6.9 for NaCl, 7.1 to 7.4 for sodium citrate, 6.6 to 6.9 for KCl, and 6.6 to 6.8 for $CaCl_2$ (Table 1). The apparent viscosity of WPI solution without salt was <5 mPa·s, and there was no change in viscosity with time. The apparent viscosity decreased slightly with the addition of NaCl (500 m*M*), sodium citrate (500 m*M*), KCl (500 m*M*), or $CaCl_2$ (20 m*M*). The decline in viscosity of the WPI solutions might have been caused by the salts weakening the electrostatic interaction between the protein molecules. In contrast, the apparent viscosity of PWPI solution increased with the addition of salts over time, and this increase depended on both the salt concentration and the dura-

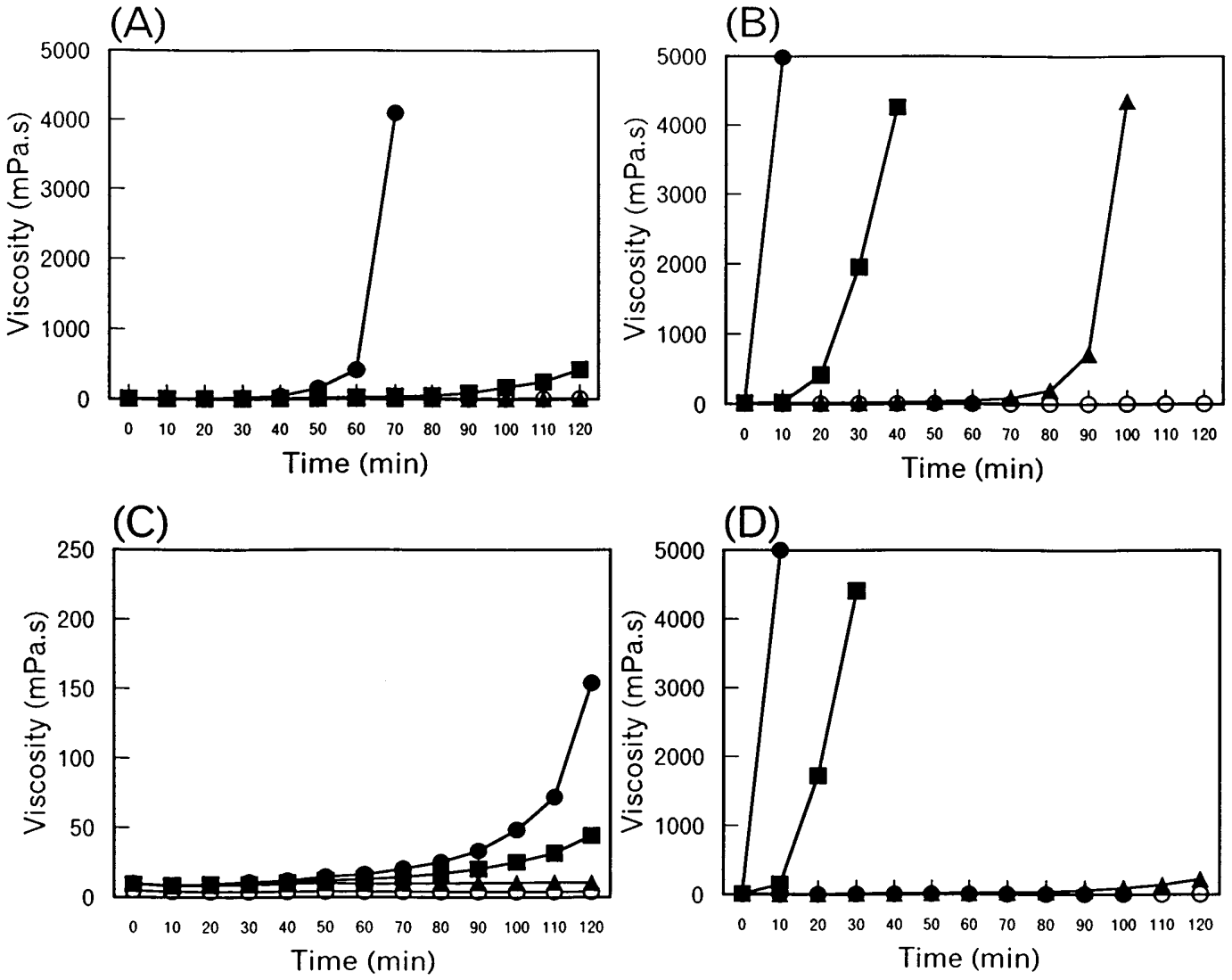

Figure 1. Change in the viscosity of whey protein isolate (WPI; open symbols) and process whey protein (PWPI; closed symbols) with four types of salt: NaCl (A), trisodium citrate (B), KCl (C), and $CaCl_2$ (D). For A, B, C, and D, protein concentration was 70 mg/ml; pH was 7.0, and temperature was 25°C. A, B, and C: 500 m*M* (○; ●) 300 m*M* (□, ■), and 100 m*M* (△, ▲); for D, 20 m*M* (○; ●), 15 m*M* (▲), 10 m*M* (♦), and 7.5 m*M*.

tion of incubation. The viscosities of PWPI solutions increased over time to reach beyond the measurement limit of the viscometer (>100 Pa·s), and the solutions finally gelled. With NaCl, incubation of the PWPI solution for 80 min at 500 m*M* [ionic strength (**I**) = 0.5] produced a gel, and incubation for 120 min at either 300 m*M* (I = 0.3) or 100 m*M* (I = 0.1) gave sols (Figure 1A). With sodium citrate, incubation for 20, 50, or 110 min at 500 m*M* (I = 3.0), 300 m*M* (I = 1.8), or 100 m*M* (I = 0.6) produced a gel (Figure 1B). With $CaCl_2$, incubation of the PWPI solution for 20 or 40 min at 20 m*M* (I = 0.06) or 15 m*M* (I = 0.45) produced a gel, and incubation for 120 min at 10 m*M* (I = 0.03) or 7.5 m*M* (I = 0.0225) produced sols (Figure 1D). Although the viscosity of PWPI solution increased slightly with the addition of KCl, it did not gel, even after incubation for 120 min at 500 m*M* (I = 0.5) (Figure 1C). The transparency of the gels decreased slightly with the addition of NaCl, sodium citrate, or $CaCl_2$.

With chloride salts, $CaCl_2$ was the most effective in increasing viscosity and gelation, followed by sodium and potassium. This pattern is consistent with the lyotropic nature of the cations (Ca > Na > K). These

TABLE 1. pH[1] and appearance of whey protein isolate (WPI) and process whey protein (PWPI) heated for 60 min at 80°C with 25 inorganic and organic salts.

		100 m*M*		200 m*M*		300 m*M*		400 m*M*		500 m*M*	
Salts	Samples	pH	GA[2]	pH	GA	pH	GA	pH	GA	pH	GA
NaCl	WPI	6.85	PP	6.78	PP	6.74	PP	6.72	PP	6.71	PP
	PWPI	6.69	OG	6.63	OG	6.57	OG	6.57	OG	6.56	OG
Sodium-buffered[3] saline	WPI	7.08	TG	7.05	OG	7.03	OG	7.01	OG	7.02	OG
	PWPI	7.05	TLG	7.05	OG	7.00	OG	6.99	OG	6.97	OG
Na_2SO_4	WPI	6.83	OG	6.79	PP	6.77	PP	6.75	PP	6.75	PP
	PWPI	6.77	OG	6.71	OG	6.65	OG	6.62	OG	6.58	PP
Sodium lactate	WPI	6.90	TPS	6.85	OS	6.86	OS	6.85	OS	6.86	OS
	PWPI	6.81	TPS	6.77	TG	6.76	TG	6.76	OG	6.77	OG
Sodium acetate	WPI	6.95	OG	6.96	OG	6.99	PP	7.01	PP	7.05	PP
	PWPI	6.86	TLG	6.89	OG	6.95	OG	6.98	OG	7.00	OG
Sodium malate	WPI	6.96	OS	6.95	OS	6.96	PP	6.98	PP	7.00	PP
	PWPI	6.84	TLG	6.88	TLG	6.92	TLG	6.94	OG	6.99	PP
Sodium tartrate	WPI	6.90	TPS	6.88	TPS	6.88	TLS	6.89	PP	6.90	PP
	PWPI	6.76	TLG	6.76	TLG	6.73	TLG	6.74	OG	6.74	PP
Sodium citrate	WPI	7.19	OG	7.22	PP	7.29	PP	7.32	PP	7.37	PP
	PWPI	7.11	TLG	7.20	OG	7.26	OG	7.31	OG	7.35	PP
Sodium ascorbate	WPI	6.94	OG	6.92	OG	6.93	OG	6.96	OG	6.96	PP
	PWPI	6.75	TG	6.73	OG	6.70	OG	6.68	OG	6.69	OG
KCl	WPI	6.86	PP	6.81	PP	6.79	PP	6.78	PP	6.77	PP
	PWPI	6.74	OS	6.68	OG	6.66	OG	6.65	OG	6.64	OG
Potassium-buffered saline[4]	WPI	7.00	TLG	6.96	OG	6.96	OG	6.95	OG	6.95	PP
	PWPI	6.87	TLG	6.87	OG	6.86	OG	6.86	OG	6.86	OG
KNO_3	WPI	6.97	OG	6.91	OG	6.90	OG	6.90	OG	6.89	OG
	PWPI	6.75	OG	6.72	OG	6.73	OG	6.72	OG	6.72	OG
		7.5 m*M*		10 m*M*		15 m*M*		20 m*M*			
		pH	GA	pH	GA	pH	GA	pH	GA		
$CaCl_2$	WPI	6.82	TPS	6.79	TPS	6.74	OS	6.69	PP		
	PWPI	6.78	OG	6.73	OG	6.66	OG	6.59	PP		
Calcium lactate	WPI	6.84	OS	6.80	OS	6.77	OS	6.74	OS		
	PWPI	6.74	TLG	6.63	OG	6.54	OG	6.43	OG		
Calcium gluconate	WPI	6.81	PP	6.81	PP	6.79	PP	6.76	PP		
	PWPI	6.81	OS	6.77	OG	6.72	OG	6.68	OG		
$MgCl_2$	WPI	6.85	TLS	6.81	OG	6.81	PP	6.80	PP		
	PWPI	6.73	TLS	6.71	OG	6.56	OG	6.47	OG		
$MgSO_4$	WPI	6.88	TG	6.87	TLG	6.86	PP	6.84	PP		
	PWPI	6.80	TPS	6.71	TLG	6.63	OG	6.31	OG		
		1% (wt/wt)		1.5% (wt/wt)		2% (wt/wt)		3% (wt/wt)		5% (wt/wt)	
		pH	GA	pH	GA	pH	GA	pH	GA	pH	GA
Sodium pyrophosphate	WPI	8.55	TG	8.63	TG	8.74	TLG	8.27	OG	8.93	OG
	PWPI	8.53	TPS	8.65	TLG	8.77	OG	8.12	OG	9.22	PP
Sodium tripolyphosphate	WPI	8.13	TG	8.17	TG	8.27	TG	8.32	OG	8.38	OG
	PWPI	8.12	TPS	8.23	TG	8.29	TLG	8.52	OG	8.58	OG
Sodium tetrapolyphosphate	WPI	7.27	TG	7.24	TLG	7.21	OG	7.17	OG	7.11	OG
	PWPI	7.10	TPS	7.12	TG	7.12	TLG	7.11	OG	7.06	OG
Sodium hexametaphosphate	WPI	6.55	TG	6.37	TG	6.32	OG	6.14	OG	5.93	PP
	PWPI	6.62	TPS	6.37	TLG	6.30	TLG	6.12	OG	5.98	PP
Potassium pyrophosphate	WPI	8.70	TPS	8.90	TG	9.01	TG	9.15	OG	9.33	OG
	PWPI	8.68	TPS	8.90	TG	9.03	TLG	9.17	OG	9.36	OG
Potassium polyphosphate	WPI	8.15	TG	8.34	TG	8.42	TLG	8.52	OG	8.61	OG
	PWPI	8.06	TPS	8.30	TG	8.40	TLG	8.51	OG	8.60	OG

[1]Initial pH was 7.0.

[2]Appearance of gel: TPS = transparent sol; TLS = translucent sol; OS = opaque sol; TG = transparent gel; TLG = translucent gel; OG = opaque gel; and PP = precipitate.

[3]Sodium-buffered saline; sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate (pH 7.0).

[4]Potassium-buffered saline; monobasic potassium dihydrogen phosphate and dipotassium hydrogen phosphate (pH 7.0).

results suggest that electrostatic interaction may be important in the dissociation and association of denatured whey proteins. Because the rate of the increase in the viscosity of PWPI solutions after the addition of salts depended on the temperature (8), hydrophobic interactions between PWPI molecules may also affect the association of denatured PWPI protein molecules and the formation of a network structure. The PWPI consists of soluble linear aggregates of heat-denatured whey proteins formed by heating under salt-free or low salt conditions [(5, 7) and our unpublished results]. The soluble linear aggregates of PWPI that are heated at pH 7.0 are negatively charged, and the association of these aggregates is hindered by electrostatic repulsive force, especially at pH values above the pI of whey proteins. When salts are added to PWPI solutions, the electrostatic repulsive forces are weakened by shielding effects with counter ions. The linear aggregates then associate because of hydrophobic interaction and other forces, and the viscosity increases with the eventual formation of a gel network. These changes apparently depend on the salt concentration, the temperature, and the lyotropic nature of the cation. Calcium salts were effective in increasing the viscosity and gelation rate, because Ca^{2+} may form crosslinks between protein molecules.

Effects of Salts on WPI and PWPI

Table 1 shows the pH values when inorganic or organic salts (25 different types of salt) were added to WPI and PWPI solutions (protein concentration, 70 mg/ml), and the appearance of the solution after heating at 80°C for 60 min. In some samples, the pH changed slightly with the addition of salt. The pH of WPI and PWPI ranged from 6.56 to 7.37 after salt addition. When calcium and magnesium salts were added to WPI and PWPI, the pH fell to 6.31 to 6.88. With sodium tetrapolyphosphate, pH values were between 7.06 and 7.27; pH values were between 5.93 and 6.62 with sodium hexametaphosphate, and pH values were between 8.06 and 9.36 with other salts. The pH value of each protein solution was not adjusted back to 7.0 so as to maintain a constant salt concentration during heating.

When sodium-buffered saline, Na_2SO_4, or NaCl was added to WPI solutions, gels were obtained by the addition of sodium-buffered saline or Na_2SO_4 on heating, and a precipitate was formed by NaCl addition. Similarly, PBS and KNO_3 induced turbid gels upon heating, but KCl produced only a precipitate upon heating. The effect of potassium on the formation of gel and coagulum was similar to that of sodium. Sols were obtained by the addition of $CaCl_2$, and translucent or turbid gels were obtained by the addition of $MgCl_2$ at low concentration. Polyphosphates gave transparent, translucent, or turbid gels of WPI on heating, depending on pH and salt concentration, which is consistent with our previous results (5).

The addition of the organic salts (sodium ascorbate, sodium acetate, or sodium citrate) to WPI produced turbid gels upon heating; sodium tartrate, sodium malate, and sodium lactate produced sols or precipitates. Calcium lactate and calcium gluconate produced a turbid sol and precipitate, respectively.

Addition of inorganic sodium, potassium, and calcium salts to PWPI produced translucent or turbid gels, and all types of the polyphosphates examined in this study produced transparent sols of PWPI when added at ≥1% (wt/wt) and transparent, translucent, or turbid gels when added at >1.5%. The effects of pH values and salt concentration on the transparency of the sample after heating were similar to those with WPI. Addition of organic Na salts to PWPI gave transparent, translucent, or turbid gels upon heating. Sodium lactate was the most effective in producing a transparent product, followed by sodium malate, sodium tartrate, sodium ascorbate, sodium acetate, and sodium citrate. The addition of $CaCl_2$ or calcium lactate to PWPI gave translucent or turbid gels; calcium gluconate yielded turbid sols or gels; and $MgCl_2$ or $MgSO_4$ produced transparent sols, translucent sols, translucent gels, or turbid gels, depending on the salt concentration. The effectiveness of cations in the gelation of PWPI was in the order Ca, Mg, Na, and K, which corresponds to the order of the lyotropic nature of the cations and is the same as that observed for WPI.

These results show that the addition of salts is needed for the gelation of PWPI, but the type of salt needed is not as limited as with WPI. Any cation, including Ca and Mg, can produce gelation of PWPI. This property makes PWP useful as an ingredient in processed foods.

Properties of WPI and PWPI Gels

The properties of WPI and PWP gels were measured. Table 2 shows breaking strength, Table 3 shows compression, Table 4 shows compressive hardness, and Table 5 shows water-holding capacity. With the WPI gel, the breaking strength, compression at fracture, compressive hardness, and water-holding capacity depended on salt concentration. This relationship

may have resulted from a change in the WPI gel from a fine structure to a coarse structure as salt concentration increased. This tendency was evident, except for sodium ascorbate, KNO_3, and polyphosphates. The strength of WPI gels decreased as pH declined. The strength of a heat-induced gel increased as salt concentration increased. Above the salt concentration giving the maximum point of gel strength, gel

TABLE 2. Breaking strength (grams) of whey protein isolate (WPI) and process whey protein (PWPI) heated for 60 min at 80°C with 25 inorganic and organic salts.

Salts	Samples	100 m*M*		200 m*M*		300 m*M*		400 m*M*		500 m*M*	
		(g)									
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
NaCl	PWPI	2868	201	3496	369	3271	388	2720	17	2636	355
Sodium-buffered	WPI	2095	237	1848	79	1140	20	811	14	593	90
Saline[1]	PWPI	2338	193	2813	275	3028	41	3005	122	2761	161
Na_2SO_4	WPI	1345	17	...[2]	...	...	...	...	...	...	...
	PWPI	3856	284	2933	102	1938	149	1735	58	...	...
Sodium lactate	PWPI	...	...	751	33	663	58	780	72	751	33
Sodium	WPI	2841	112	1626	118	...	...	...	...	...	...
acetate	PWPI	3950	449	3173	327	3400	147	3210	213	3620	121
Sodium malate	PWPI	728	61	731	66	820	18	700	20	...	...
Sodium tartrate	PWPI	465	51	628	27	681	71	648	23	...	...
Sodium	WPI	860	54	...	...	...	...	...	...	...	...
citrate	PWPI	523	47	668	89	908	81	590	21	...	...
Sodium	WPI	1877	155	1485	85	967	49	612	143	...	...
ascorbate	PWPI	1955	438	3867	476	3587	99	3405	220	2512	180
KCl	PWPI	...	...	616	75	648	43	523	38	701	40
Potassium-	WPI	1985	130	2133	61	2098	93	1640	124	...	...
buffered saline[3]	PWPI	2350	406	2798	176	2676	157	2183	223	1921	92
KNO_3	WPI	1713	257	1723	83	605	39	548	10	512	126
	PWPI	2358	84	2570	44	2417	176	2280	133	2430	147
		7.5 m*M*		10 m*M*		15 m*M*		20 m*M*			
$CaCl_2$	PWPI	3551	40	4863	144	3451	208	...	...		
Calcium lactate	PWPI	4488	437	4881	93	3450	461	3320	333		
Calcium gluconate	PWPI	...	...	1231	43	2023	42	2058	175		
$MgCl_2$	WPI	...	...	616	76	...	...	...	...		
	PWPI	...	...	3768	162	4023	400	3576	133		
$MgSO_4$	WPI	1228	64	1423	116	...	...	...	...		
	PWPI	...	...	3433	305	3508	83	3726	228		
		1% (wt/wt)		1.5% (wt/wt)		2% (wt/wt)		3% (wt/wt)		5% (wt/wt)	
Sodium	WPI	898	220	1338	187	1435	111	786	30	943	135
pyrophosphate	PWPI	...	...	827	222	1761	133	2545	342	...	...
Sodium	WPI	1200	21	1275	0	1075	212	1098	63	1680	395
tripolyphosphate	PWPI	...	...	1136	297	2171	249	2853	388	2913	303
Sodium	WPI	1397	60	2500	84	2887	328	3020	28	1297	88
tetrapolyphosphate	PWPI	...	...	1728	477	2303	30	3648	446	3975	235
Sodium	WPI	2448	448	3521	162	3296	130	2515	199	...	...
hexametaphosphate	PWPI	...	...	2183	73	2837	208	3428	226	...	...
Potassium	WPI	...	...	1185	169	1341	272	1273	30	1250	177
pyrophosphate	PWPI	...	...	978	68	1301	199	1766	364	2371	215
Potassium	WPI	610	14	1350	124	1530	52	1200	84	1645	14
polyphosphate	PWPI	...	...	898	154	1666	546	2175	182	3062	92

[1]Sodium-buffered saline; sodium dihydrogen phosphate or disodium hydrogen phosphate dodecahydrate (pH 7.0).

[2]Not determined because the sample was not a self-supported gel.

[3]Potassium-buffered saline; monobasic potassium dihydrogen phosphate or dipotassium hydrogen phosphate (pH 7.0).

strength decreased. In a previous study (14), the maximum compressive strength of heat-induced β-LG gel was at 200 m*M* NaCl and 10 m*M* $CaCl_2$; higher salt concentrations resulted in soft gels with a low water-holding capacity. Results were similar in our study using WPI.

TABLE 3. Compression (millimeters) at fracture of whey protein isolate (WPI) and process whey protein (PWPI) heated for 60 min at 80°C with 25 different inorganic and organic salts.

Salts	Samples	100 m*M*		200 m*M*		300 m*M*		400 m*M*		500 m*M*	
		(mm)									
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
NaCl	PWPI	13.7	0.4	13.7	0.8	12.9	0.4	12.5	0.6	11.8	0.3
Sodium-buffered saline[1]	WPI	12.0	0.1	11.4	0.2	9.9	0.7	9.1	0.0	8.4	1.2
	PWPI	11.4	0.5	13.6	0.7	13.2	0.4	12.6	0.6	11.9	0.7
Na_2SO_4	WPI	10.2	0.3	...	...	...	...	...	...	...	...
	PWPI	12.9	1.0	12.5	0.2	10.1	0.3	10.4	0.6	...	...
Sodium lactate	PWPI	...[2]	...	7.6	0.0	7.3	0.1	8.0	0.4	7.9	0.3
Sodium acetate	WPI	13.5	0.2	10.2	0.9	...	...	...	...	...	...
	PWPI	14.3	0.9	12.2	0.9	12.6	0.1	12.3	0.2	12.4	0.7
Sodium malate	PWPI	7.3	0.2	7.7	0.2	8.3	0.2	8.0	0.1	...	...
Sodium tartrate	PWPI	6.3	0.4	6.7	0.5	7.3	0.2	7.6	0.2	...	...
Sodium citrate	WPI	8.6	0.9	...	...	...	...	...	...	...	...
	PWPI	7.2	0.8	7.6	0.9	9.0	0.1	9.2	0.4	...	...
Sodium ascorbate	WPI	12.3	0.4	11.4	0.4	9.6	0.4	9.1	0.5	...	...
	PWPI	9.1	0.9	13.5	0.1	13.4	0.1	12.8	0.5	11.3	0.0
KCl	PWPI	...	...	8.9	0.7	9.0	0.5	8.1	0.4	8.5	0.0
Potassium-buffered saline[3]	WPI	12.1	0.3	12.2	0.3	12.1	0.5	11.3	0.2	...	...
	PWPI	13.0	0.7	12.4	0.2	12.7	0.5	11.8	0.1	10.8	0.3
KNO_3	WPI	12.2	0.3	10.2	0.1	7.5	0.4	7.8	0.5	8.3	0.6
	PWPI	12.9	0.4	12.1	0.4	12.0	0.6	11.8	0.1	12.3	0.1
		7.5 m*M*		10 m*M*		15 m*M*		20 m*M*			
$CaCl_2$	PWPI	14.2	0.2	3.5	0.5	11.1	0.8	...	...		
Calcium lactate	PWPI	13.9	0.4	13.1	0.4	11.3	0.6	11.0	0.7		
Calcium gluconate	PWPI	...	...	10.6	0.7	11.9	0.4	12.0	0.4		
$MgCl_2$	WPI	...	...	8.3	0.6	...	...	...	...		
	PWPI	...	...	14.1	0.4	13.4	0.2	11.2	0.3		
$MgSO_4$	WPI	8.0	0.1	11.1	0.2	...	...	...	...		
	PWPI	...	...	14.2	0.1	12.6	0.4	12.2	0.1		
		1% (wt/wt)		1.5% (wt/wt)		2% (wt/wt)		3% (wt/wt)		5% (wt/wt)	
Sodium pyrophosphate	WPI	9.4	1.6	9.7	0.2	10.3	0.6	7.9	0.1	9.8	2.1
	PWPI	...	...	9.1	0.1	12.1	0.3	12.7	0.4	...	...
Sodium tripolyphosphate	WPI	10.1	0.1	11.3	0.3	9.5	0.4	8.5	0.5	11.0	0.1
	PWPI	...	...	10.5	0.8	12.6	0.6	13.1	0.8	12.0	0.8
Sodium tetrapolyphosphate	WPI	12.0	0.0	13.6	0.2	13.8	0.0	13.6	0.3	9.0	0.4
	PWPI	...	...	111.3	0.6	12.3	0.4	13.3	0.5	12.9	0.2
Sodium hexametaphosphate	WPI	12.0	0.6	14.2	0.3	13.7	0.4	12.3	0.3	...	...
	PWPI	...	...	11.9	0.4	13.8	0.7	13.8	0.1	...	...
Potassium pyrophosphate	WPI	...	...	10.0	0.3	11.5	0.2	10.3	0.7	9.2	0.8
	PWPI	...	...	9.3	1.8	9.9	0.3	11.9	0.1	11.6	0.7
Potassium polyphosphate	WPI	7.2	2.6	9.7	0.3	10.4	0.4	8.1	0.7	11.0	0.5
	PWPI	...	...	9.0	1.1	11.2	0.6	11.6	0.7	12.6	0.7

[1]Sodium-buffered saline; sodium dihydrogen phosphate or disodium hydrogen phosphate dodecahydrate (pH 7.0).

[2]Not determined because the sample was not a self-supported gel.

[3]Potassium-buffered saline; monobasic potassium dihydrogen phosphate or dipotassium hydrogen phosphate (pH 7.0).

The gels prepared from PWPI with added NaCl, sodium-buffered saline, Na_2SO_4, sodium acetate, and sodium ascorbate showed higher values for breaking strength, compression at fracture, and compressive hardness and lower values for water-holding capacity than those prepared with sodium lactate, sodium malate, sodium tartrate, and sodium citrate. The gels prepared with PBS and KNO_3 gave higher values for

TABLE 4. Compressive hardness (grams) of whey protein isolate (WPI) and process whey protein (PWPI) heated for 60 min at 80°C with 25 different inorganic and organic salts.

Salts	Samples	100 m*M*		200 m*M*		300 m*M*		400 m*M*		500 m*M*	
		(g)									
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
NaCl	PWPI	405	8	577	17	666	23	693	42	686	11
Sodium-buffered	WPI	373	20	496	11	445	0	386	17	340	21
saline[1]	PWPI	481	14	443	5	561	42	553	40	573	21
Na_2SO_4	WPI	468	11	...[2]	...	...	...	...	...	...	...
	PWPI	516	27	651	17	658	14	688	16	...	...
Sodium lactate	PWPI	...	...	308	7	308	7	295	25	305	8
Sodium	WPI	436	10	602	2	...	...	...	...	...	...
acetate	PWPI	475	0	755	0	838	2	855	31	898	15
Sodium malate	PWPI	280	0	280	0	315	0	315	0	...	...
Sodium tartrate	PWPI	317	53	298	17	278	2	283	30	...	...
Sodium	WPI	536	24	...	...	...	...	...	...	...	...
citrate	PWPI	423	40	413	18	443	14	503	28	...	...
Sodium	WPI	420	0	446	23	375	27	366	18	...	...
ascorbate	PWPI	461	72	476	24	563	2	643	20	611	30
KCl	PWPI	...	...	368	18	370	43	408	8	396	5
Potassium-	WPI	483	33	445	8	328	2	301	20	...	...
buffered saline[3]	PWPI	410	8	536	27	526	5	546	17	520	10
KNO_3	WPI	403	17	365	0	330	0	300	8	295	8
	PWPI	376	7	455	10	488	22	471	11	453	10
		7.5 m*M*		10 m*M*		15 m*M*		20 m*M*			
$CaCl_2$	PWPI	511	27	1090	21	1243	17	...	...		
Calcium lactate	PWPI	695	31	1155	25	1308	14	1206	11		
Calcium gluconate	PWPI	...	...	373	5	515	8	526	2		
$MgCl_2$	WPI	...	...	320	8	...	...	...	...		
	PWPI	...	...	588	37	705	20	1058	30		
$MgSO_4$	WPI	533	46	426	150	...	...	...	...		
	PWPI	...	...	345	18	823	35	983	45		
		1% (wt/wt)		1.5% (wt/wt)		2% (wt/wt)		3% (wt/wt)		5% (wt/wt)	
Sodium	WPI	473	24	435	13	443	11	500	40	562	27
pyrophosphate	PWPI	...	...	320	0	370	0	451	30	...	...
Sodium	WPI	410	27	371	11	475	21	480	13	433	40
tripolyphosphate	PWPI	...	...	406	21	376	11	458	32	616	20
Sodium	WPI	428	28	393	31	508	37	543	22	366	5
tetrapolyphosphate	PWPI	...	...	363	11	436	5	558	11	686	37
Sodium	WPI	530	34	435	17	528	2	521	30	...	...
hexametaphosphate	PWPI	...	...	401	10	397	23	547	15	...	...
Potassium	WPI	...	...	391	24	408	10	488	14	561	27
pyrophosphate	PWPI	...	...	66	5	406	5	348	20	478	25
Potassium	WPI	600	49	440	10	415	13	523	11	530	0
polyphosphate	PWPI	...	...	78	2	106	1	264	19	454	8

[1]Sodium-buffered saline; sodium dihydrogen phosphate or disodium hydrogen phosphate dodecahydrate (pH 7.0).

[2]Not determined because the sample was not a self-supported gel.

[3]Potassium-buffered saline; monobasic potassium dihydrogen phosphate or dipotassium hydrogen phosphate (pH 7.0).

breaking strength, compression at fracture, and compressive hardness and lower values of water-holding capacity than those gels prepared with KCl. Because PWPI solutions immediately gelled after addition of calcium or magnesium salts at high concentrations, these samples could not be poured into a tube. Therefore, gels with a fixed size and shape for measurement could not be prepared. Hence, measurements

TABLE 5. Water-holding capacity (square centimeters) of whey protein isolate (WPI) and prrocess whey protein (PWPI) heated for 60 min at 80°C with 25 different inorganic and organic salts.

Salts	Samples	100 m*M*		200 m*M*		300 m*M*		400 m*M*		500 m*M*	
		(cm^2)									
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
NaCl	PWPI	20.4	0.3	32.7	1.2	36.0	0.0	36.9	1.2	37.2	0.0
Sodium buffered saline[1]	WPI	12.7	0.7	33.0	0.7	42.5	0.4	59.4	1.5	65.8	2.9
	PWPI	11.2	1.4	22.0	0.0	31.8	1.5	34.2	0.0	37.8	0.0
Na_2SO_4	WPI	53.2	0.9	...[2]	...	...	...	...	...	...	...
	PWPI	28.8	0.3	36.8	2.2	39.0	0.0	33.3	0.0	...	...
Sodium lactate	PWPI	...	...	44.5	0.4	53.2	1.0	52.1	1.4	57.3	0.5
Sodium acetate	WPI	22.3	0.3	45.8	0.4	...	...	...	...	...	...
	PWPI	25.2	1.0	38.7	2.1	42.5	0.4	46.9	0.9	47.2	2.4
Sodium malate	PWPI	46.2	3.7	61.9	4.9	60.0	0.0	56.5	2.6	...	...
Sodium tartrate	PWPI	46.9	1.9	73.9	0.0	74.3	0.6	72.6	1.8	...	...
Sodium citrate	WPI	29.7	0.7	...	...	...	...	...	...	...	...
	PWPI	57.0	4.2	66.7	5.1	73.2	0.4	50.2	27.5	...	...
Sodium ascorbate	WPI	24.5	0.7	45.2	1.4	66.3	0.0	80.7	0.0	...	...
	PWPI	23.5	0.6	23.5	2.0	28.6	0.0	31.2	0.7	31.6	1.9
KCl	PWPI	...	...	38.1	1.3	49.6	4.0	52.5	1.0	56.5	2.6
Potassium-buffered saline[3]	WPI	24.2	0.3	46.9	0.0	66.9	0.0	72.2	0.0	...	...
	PWPI	21.6	0.0	34.2	0.0	39.6	0.9	45.8	1.4	46.2	0.9
KNO_3	WPI	29.4	0.3	52.5	0.0	63.4	2.8	70.0	2.8	73.0	7.0
	PWPI	22.0	1.3	29.1	0.0	30.2	0.0	33.6	0.0	35.7	0.4
		7.5 m*M*		10 m*M*		15 m*M*		20 m*M*			
$CaCl_2$	PWP	24.0	0.7	24.0	0.7	23.1	2.0	...	...		
Calcium lactate	PWPI	24.5	0.7	24.5	2.1	27.3	0.4	32.5	7.0		
Calcium gluconate	PWPI	...	...	45.6	7.5	52.0	0.5	52.0	0.4		
$MgCl_2$	WPI	...	...	53.2	0.9	...	...	...	...		
	PWPI	...	...	23.7	0.3	24.0	0.0	29.9	0.3		
$MgSO_4$	WPI	17.2	0.0	37.4	2.1	...	...	...	...		
	PWPI	...	...	20.2	0.0	27.5	0.0	27.2	0.3		
		1% (wt/wt)		1.5% (wt/wt)		2% (wt/wt)		3% (wt/wt)		5% (wt/wt)	
Sodium pyrophosphate	WPI	10.4	2.0	12.2	0.0	23.0	1.3	44.5	2.3	49.0	4.9
	PWPI	...	...	11.3	0.2	14.0	0.5	29.4	0.0	...	...
Sodium tripolyphosphate	WPI	10.2	0.0	13.1	0.2	27.7	0.4	38.4	0.9	51.4	1.4
	PWPI	...	...	9.9	0.0	12.2	0.0	27.2	0.3	35.4	0.8
Sodium tetrapolyphosphate	WPI	10.0	0.6	15.6	1.6	23.0	0.6	36.5	0.8	64.8	1.5
	PWPI	...	...	11.2	0.4	18.2	0.9	27.8	1.8	39.3	2.1
Sodium hexametaphosphate	WPI	9.0	0.0	12.6	0.4	20.0	0.3	38.4	0.8	...	...
	PWPI	...	...	13.3	0.5	19.8	0.6	30.8	0.8	...	...
Potassium pyrophosphate	WPI	...	...	13.8	0.2	12.2	0.0	33.0	0.9	49.6	0.9
	PWPI	...	...	9.0	0.0	11.5	0.0	16.4	0.0	33.3	0.0
Potassium polyphosphate	WPI	10.5	0.4	12.2	0.0	16.0	0.0	37.5	0.4	60.0	0.0
	PWPI	...	...	9.6	0.0	12.2	0.0	24.3	0.7	34.7	0.5

[1]Sodium-buffered saline; sodium dihydrogen phosphate or disodium hydrogen phosphate dodecahydrate (pH 7.0).

[2]Not determined because the sample was not a self-supported gel.

[3]Potassium-buffered saline; monobasic potassium dihydrogen phosphate or dipotassium hydrogen phosphate (pH 7.0).

were carried out only for gels prepared with 20 m*M* salt. Gels prepared with these salts at concentrations <20 m*M* gave high values of breaking strength, compression at fracture, and compressive hardness compared with those prepared with sodium and potassium salts. When salts were added to PWPI and heated, transparent or translucent sols or gels were formed at low concentrations of salt. With increases in salt concentration, opaque gels or precipitates were formed. Breaking strength increased as salt concentration increased and gave a peak at a given salt concentration, which depended on the type of salt.

The effects of polyphosphates on the gel properties were complicated, which may have resulted from the effects of different phosphates on the pH values of the system. The lyotropic nature of the cations seems to influence gel properties based upon the results of gels prepared with NaCl, KCl, $CaCl_2$, and $MgCl_2$.

Increased salt concentration resulted in the formation of a fine network, but further increases in salt concentration resulted in a coarse network, which reduced the water-holding capacity and firmness of the gel. These phenomena were, in principle, the same as those observed with WPI, although PWPI

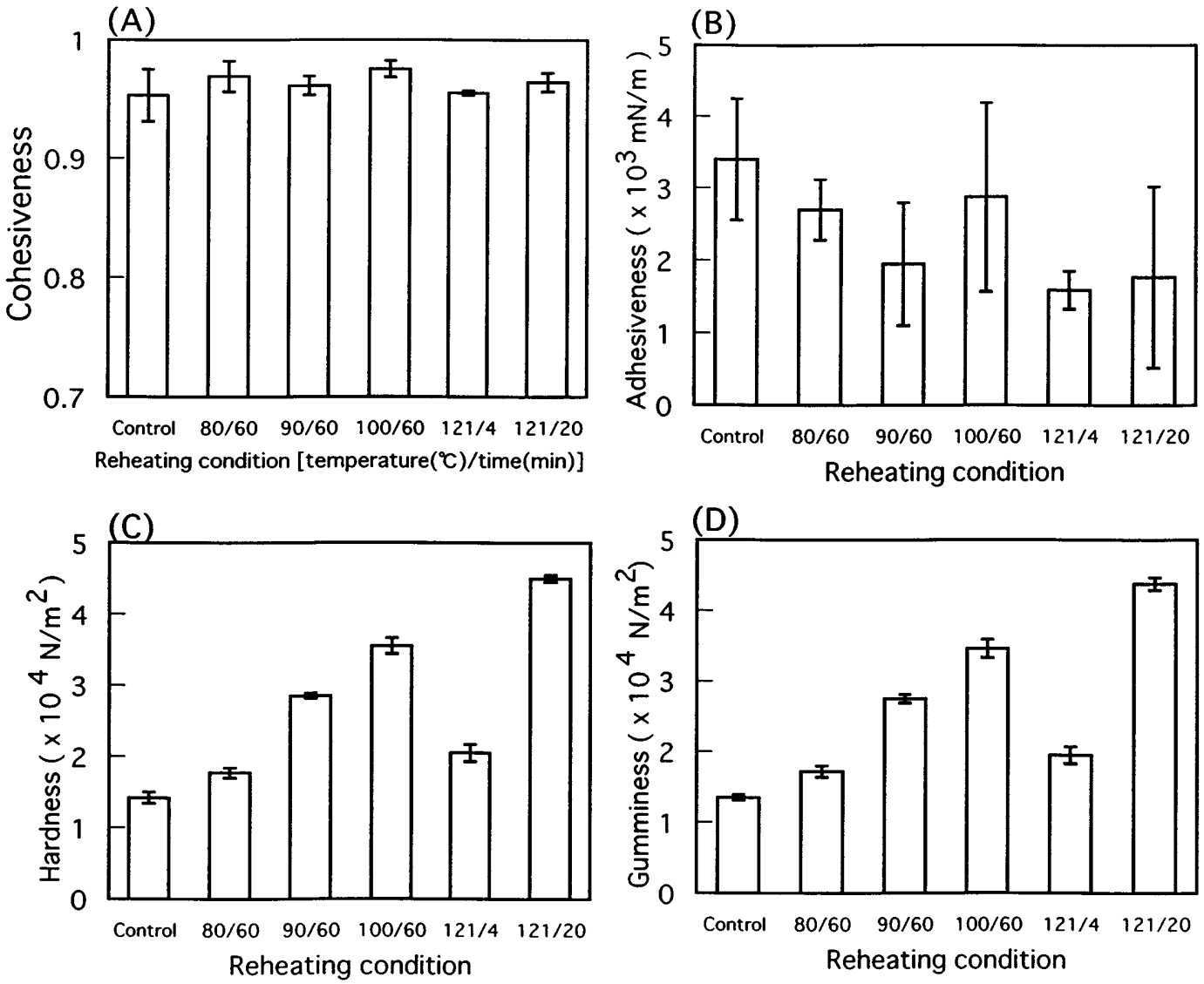

Figure 2. Physical properties of reheated transparent gels prepared from process whey protein. Protein concentration, 90 mg/ml; pH, 7.0; and NaCl concentration, 40 m*M*. First heat treatment was 80°C for 60 min (control). Second heat treatment, 80, 90, or 100°C for 60 min or 121°C for 4 or 20 min. A, Cohesiveness; B, adhesiveness; C, hardness; and D, gumminess.

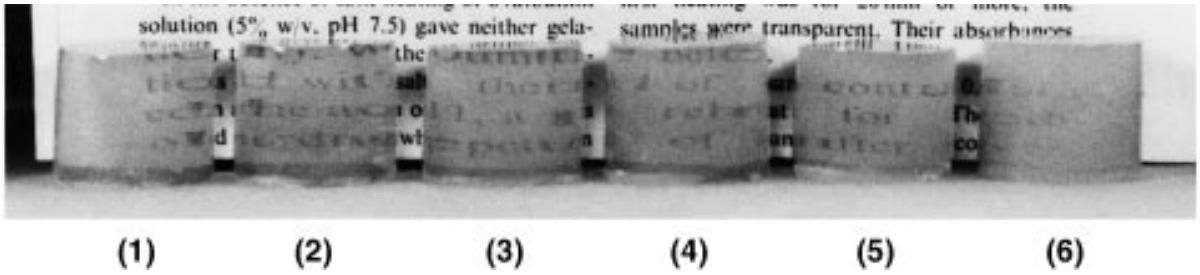

Figure 3. Appearances of reheated transparent gels prepared from process whey protein. The first heat treatment was 80°C for 60 min 1) without reheating, 2) reheated at 80°C for 60 min, 3) reheated at 90°C for 60 min, 4) reheated at 100°C for 60 min, 5) reheated at 121°C for 4 min, and 6) reheated at 121°C for 20 min.

required a much higher salt concentration for such changes in gelation properties. In the case of some salts, the gel properties (e.g., firmness) did not give the maximum in the range of salt concentration measured in this study.

Properties of Reheated Transparent PWPI Gels

Transparent gels, prepared by heating PWPI (protein concentration, 90 mg/ml; pH 7.0; 40 m*M* NaCl) at 80°C for 60 min, were reheated at 80 to 121°C for 4 to 60 min. Textural parameters and the appearance of the reheated gels are shown in Figures 2 and 3. The transparency of reheated gels at room temperature did not change, except that the transparency of gel heated at 121°C for 20 min was slightly reduced. The cohesiveness of the gels did not change with the second heating, but hardness and gumminess apparently increased, and adhesiveness decreased, depending on the heating temperature and duration. These results show that firm gels were obtained by reheating.

When hydrated whey proteins were heated at neutral pH, conformational changes and unfolding of the protein molecules occurred. Hydrophobic areas are exposed on the surface of the protein molecule by heat denaturation, and then whey protein molecules associate by hydrophobic interaction as well as intramolecular bridges (7). When WPI solutions are heated under salt-free or low salt conditions, a transparent sol of soluble linear aggregates is formed by heating because the electrostatic repulsive forces between protein molecules are strong enough to overcome the intermolecular attractive forces among denatured molecules. When the salt concentration is high, the attractive forces increase as the electrostatic repulsion among denatured molecules is reduced by the salt. Denatured molecules then coagulate randomly, and a turbid gel or precipitate is formed.

The PWPI is prepared from WPI by heat treatment under salt-free conditions to produce a transparent viscous liquid that consists of soluble linear aggregates of heat-denatured protein molecules (5, 7, 9). Heating PWPI at pH 7.0 gives a viscous liquid, but not a gel. Because the addition of salts to PWPI leads to gelation or to an increase in viscosity, there may be electrostatic repulsive forces between the linear aggregates.

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