Gelation of Whey Protein Concentrate in Acidic Conditions: Effect of pH

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A whey protein concentrate was obtained in acidic conditions, and heat-induced gels were prepared from it at pHs between 3.5 and 4.25. Whey protein concentrate and gels were characterized through solubility assays in different extraction solutions and SDS−PAGE of the soluble protein components. Transmittance at 660 nm of heated protein dispersions and water-holding capacity of gels were also performed. Results show that protein solubility and water-holding capacity of gels decrease when pH approaches the p*I*, and gels present different electrophoretic patterns. Solubility of the protein components of gels in the presence or absence of denaturing and reducing agents indicates that noncovalent bonds are responsible for the maintenance of gel structure at pHs 3.5−4.0, but in the gel prepared at pH 4.25, disulfide bonds would also be involved in the structure of the gel.

Keywords: *Gelation of whey proteins; whey proteins; gelation in acidic conditions*

INTRODUCTION

Whey protein concentrates or isolates are major sources of nutritional and functional ingredients for the food industry, gelation being one of the main functional properties of these proteins (Cheftel and Lorient, 1982; Dumay, 1988). Whey proteins (*β*-lactoglobulin (*β*-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA)) consist of chains of amino acids folded into a compact three-dimensional structure, maintained by many weak noncovalent bonds and, in the case of *β*-Lg, a few disulfide bonds. As a consequence of this weak bonding, these proteins have labile structures, which can be unfolded as a result of changes in temperature or pH (Steventon et al., 1991). On heating, the reactive nonpolar side chains and, in some cases, such as in *β*-Lg denaturation, sulfhydryl groups are exposed. Denaturation is almost always followed by aggregation. There are two types of bonding in these systems: a few covalent bonds, such as those formed in *β*-Lg aggregation between exposed sulfhydryl groups on adjacent denatured molecules, and numerous noncovalent interactions, such as van der Waals and hydrogen bonds as well as hydrophobic interactions (Steventon et al., 1991).

Reactivity of SH groups, which enhances both the oxidation of sulfhydryl groups into disulfide bonds and sulfhydryl−disulfide interchange reactions, decreases significantly under acidic conditions, and thus, mainly noncovalent interactions are involved in the structure of acid gels, whereas at neutral pH intermolecular sulfhydryl−disulfide interchange reactions are favored (Shimada and Cheftel, 1988). As a consequence, gels prepared at acid pH are different from those prepared at neutral pH (Shimada and Cheftel, 1988; Lupano et al., 1992). Moreover, *β*-Lg exists as a 36.7 kDa dimer in solutions above its p*I* of 5.2, but below pH 3.5 and above pH 7.5, the dimer dissociates to a slightly expanded monomer, and between pHs 3.5 and 5.2, the dimer polymerizes to a 147 kDa octamer (Morr and Ha, 1993). Also, when pH approaches the p*I*, the charge of the proteins is progressively neutralized, favoring protein aggregation. Thus, the structure of gels prepared at pHs between 3.5 and 5.2 is expected to be different from that of gels prepared at neutral pH or at pH below 3.5.

Several studies were performed on gelling properties of whey proteins in acidic conditions (Shimada and Cheftel, 1988; Stading and Hermansson, 1991; Quéguiner et al., 1992; Lupano et al., 1992; Lupano, 1994). However, little is known concerning the gel structure and properties at pHs between 3.5 and 4.5.

The objective of this work was to analyze the modifications in the gel characteristics when pH varies between 3.5 and 4.25. Solubility of the protein constituents in different media, in the presence or absence of denaturing and reducing agents, was studied.

MATERIALS AND METHODS

Materials. Whey protein concentrate (WPC) was prepared from sweet whey powder, which was a gift from Unión Gandarense SACIA. Sweet whey powder contained 3.8% moisture, 10.9% proteins, 2.8% lipids, 9.5% ash, and 73.0% lactose (estimating by difference). The powder was dispersed in distilled water (800 g dispersion, 50%, w/w) and then centrifuged at 3300*g* during 45 min. Lipids were removed from the surface with a little spoon. Supernatant was adjusted to pH 3.75 with 2 N HCl, dialyzed against 3 × 4 L of distilled water at 4 °C (cutoff about 10 000), and freeze-dried. All chemicals used were of analytical grade.

Analytical. Moisture was determined by accurately weighing samples of WPC into separate dishes. Samples were dried during 3 h at 100 °C, cooled in a desiccator, and reweighed (AOAC, 1984). Total nitrogen was determined by the Kjeldahl method, and nonprotein nitrogen (NPN) was determined as soluble N in 12% TCA (Morr et al., 1973). Fat was determined by dispersing 1.5 g of WPC in 25 mL of distilled water before measuring the fat content in milkfat bottles utilizing the Gerber procedure (CAA, 1992). Ash was determined by igniting WPC at 550 °C (AOAC, 1984).

Nitrogen Solubility Index (NSI). NSI was determined at different pHs by a modification of the AACC method (AACC, 1983): 1 g of WPC was dispersed in about 40 mL of distilled water, and pH was adjusted to 3.75, 4.75, and 7.0 with NaOH or HCl of the appropriate strength. Samples were stirred with

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S0021-8561(96)00051-9 CCC: $12.00

a mechanical stirrer for 120 min at 30 °C by immersing beakers in a water bath. Dispersions were transferred to 50 mL vol flasks and centrifuged at 17400*g* for 15 min. N was determined in the supernatants by the Kjeldahl method. NSI was expressed as g of soluble N/100 g of total N.

Solubility of WPC Powders and Gels. Samples were dispersed in distilled water (DW), in a pH 8.0 buffer (0.086 M Tris, 0.09 M glycine, 4 mM Na_2EDTA) (B), or in the same buffer containing 0.5% sodium dodecyl sulfate (SDS) and 8 M urea (BSU). Gels prepared at pH 4.25 were also dispersed in BSU plus 1% β-mercaptoethanol (ME) (v/v) (Shimada and Cheftel, 1988). Samples (0.1% protein, w/v) were homogenized by magnetic stirring (WPC powders) or with a Virtis 23 homogenizer (The Virtis Co. Inc., Gardiner, NY) at room temperature for 1 min (WPC gels) and then centrifuged at 17400*g* for 15 min. Protein solubility was determined from supernatants and expressed as 100 × protein content in the supernatant/total protein content. Three independent extractions were carried out with each solvent. Average values (±standard deviation) were reported. Protein concentration was determined spectrophotometrically at 280 nm with an apparent $E^{1\%}_{1cm}$ of 8.636 for DW dispersions (determined by the Kjeldahl method) and 10.2 for dispersions at pH 8.0 (Shimada and Cheftel, 1988).

Electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to Laemmli (1970) as modified by Petruccelli and Añón (1994), using a linear gradient separating gel (5−15% in polyacrylamide) with an acrylamide:bisacrylamide ratio of 75:2. A continuous dissociating buffer system was used, containing 0.375 M Tris-HCl, 0.1% SDS, pH 8.8, for the separating gel and 0.025 M Tris-HCl, 0.192 M glycine, 0.1% SDS, pH 8.3, for the run buffer. Protein solutions (about 10 mg mL^{-1}) were diluted with an equal volume of a pH 8.0 buffer (0.01 M Tris-HCl, 0.001 M EDTA, 1% SDS, and about 30% glycerol, v/v), with or without 5% ME, v/v. Except in the cases specified in the text, samples were heated in a boiling water bath for 5 min before electrophoresis. Low MW markers (Pharmacia calibration kit) used included phosphorylase *b* (94 000), albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100), and α-lactalbumin (14 400). The relative intensity of the stained bands was determined with a scanner densitometer Shimadzu dual wavelength TLC Scanner CS-910 (sample wavelength 570 nm and reference wavelength 395 nm).

Heating and Gelation of WPC Dispersions. Aqueous dispersions (10% protein, w/w) of WPC were adjusted to pH 3.50, 3.75, 4.00, or 4.25 with 0.1−2 N HCl or NaOH. Dispersions were partially deaerated by centrifugation at 1000*g* for 1 min (Xiong and Kinsella, 1990), carefully resuspended with a glass rod, and placed in glass tubes (2.2 cm i.d. × 6 cm height) with tightly closed stoppers. Gelation was then carried out by heating the tubes in a water bath at 87 °C for 45 min as described by Shimada and Cheftel (1988). Tubes were then cooled rapidly to room temperature in tap water and kept at 4 °C for at least 15 h before all analyses.

Water-Holding Capacity (WHC) of Gels. Gel (1.0−1.6 g) equilibrated at room temperature was placed on a nylon plain membrane (5.0 μm pores; Micronsep) maintained in the middle position of a 50 mL centrifuge tube. Water loss was determined by weighing before and after centrifugation at 120*g* for 5 min (Quéguiner et al., 1989). WHC was expressed as percent of the initial water remaining in the gel after centrifugation.

Transmittance of Protein Dispersions (T_{660}). Aqueous dispersions (0.1% protein, w/v) of WPC were adjusted to pH 3.50, 3.75, 4.00, or 4.25 with 0.025−0.1 N HCl or NaOH and heated in a water bath at 87 °C during 45 min. Tubes were then cooled rapidly to room temperature in tap water, and dispersions were resuspended if necessary. The gel absorbance (*A*) was measured at 660 nm against distilled water with a spectrophotometer (Beckman DU-650). Gel transmittance (*T*%) was calculated as $T\% = 2 - \log A$. Each value is the mean (±standard deviation) of three independent determinations.

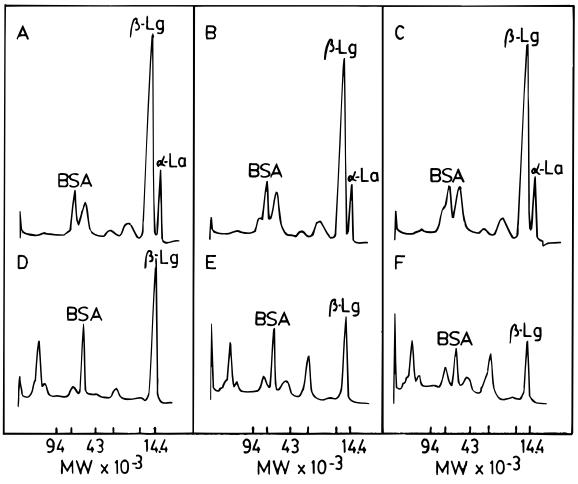

Figure 1. Scanned SDS−PAGE patterns of WPC. Samples were heated (100 °C, 5 min) before electrophoresis: (A−C) samples were treated with β-mercaptoethanol (ME) before electrophoresis and (D−F) samples were not treated with ME before electrophoresis. Extraction solutions: (A, D) distilled water, (B, E) buffer (pH 8.0), and (C, F) buffer (pH 8.0), SDS, urea.

RESULTS AND DISCUSSION

Characterization of WPC Powder. The composition of WPC obtained in the present study was 4.9% moisture, 11.6% lipids, 1.9% ash, 49.3% proteins (calculated as (total N (8.62) − nonprotein N (0.90)) × 6.38), and about 27% lactose (estimating by difference). The spontaneous pH of a WPC dispersion in distilled water (10% protein, w/w) was 4.1. Results are in agreement with other WPCs prepared by ultrafiltration (UF), as reported by Morr et al. (1973), but protein concentration was higher and ash content was lower than a WPC prepared by UF in acidic conditions, with a heating step before or after UF (Modler and Harwalkar, 1981). On the other hand, the protein content of the WPC utilized in the present study was lower than that of eight commercial WPCs, as reported by Morr and Foegeding (1990), which ranged from 72 to 77%.

The nitrogen solubility index at pHs 3.75, 4.75, and 7.0 was 85.6, 69.9, and 94.9, respectively. Results at pHs 3.75 and 7.0 were similar to those corresponding to an acidic whey protein isolate (WPI), as reported in a previous paper (Lupano et al., 1992). The value obtained at pH 4.75, near the p*I*, was a little lower than that obtained with the acidic WPI, which was 77.5 (Lupano et al., 1992), indicating that proteins in the present work would be a little more denatured.

Protein solubilities of the WPC in B and BSU were above 90% (93.1% ± 1.6% and 111.2% ± 2.4%, respectively), indicating the presence of only a few insoluble aggregates. Protein solubility in BSU was higher than 100%. This behavior was also observed in a previous paper (Lupano et al., 1992), the reason for this high value being unknown. Protein solubility in distilled water was a little lower, 84.1% ± 1.3%, probably because the pH of aqueous dispersions of WPC was near the p*I* of β-Lg. The least significant difference ($LSD_{0.05}$) between solubility values was 3.7. Similar results of protein solubility were obtained previously with whey protein isolates (Lupano et al., 1992).

Electrophoresis of WPC Powder. The electrophoretic patterns of B and BSU extracts of WPC treated with ME before electrophoresis were very similar (Figure 1). Protein constituents migrated a little more in samples not treated with ME than in treated ones. This

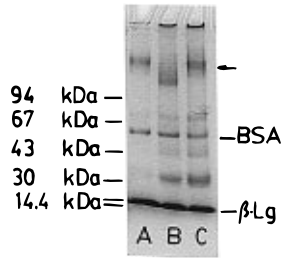

Figure 2. SDS−PAGE of WPC. Samples were not treated with ME nor heated before electrophoresis. Extraction solutions: (A) distilled water, (B) buffer (pH 8.0), and (C) buffer (pH 8.0), SDS, urea.

behavior had be observed in previous experiments (data not shown). The band corresponding to α-La was absent in samples not treated with ME (Figure 1D−F), which suggests that it would participate in the formation of protein constituents of higher MW through disulfide bonds. Also, the peak corresponding to β-Lg decreased in the B and BSU extracts. This behavior was not observed when sample was treated with ME before electrophoresis (Figure 1A−C). The B and BSU extracts were heated at pH 8.0 before electrophoresis, which would favor the formation of disulfide bonds.

High molecular weight aggregates were observed in samples not treated with ME before electrophoresis, which practically disappeared when samples were treated with ME (Figure 1), indicating that they would be formed through disulfide bonds. If samples were heated before electrophoresis, this band was observed in the same position in DW, B, and BSU extracts (Figure 1). However, if samples were not heated before electrophoresis, as was the case of samples shown in Figure 2, the band was shift toward a low molecular weight zone in the case of the B extract but not in DW and BSU extracts (arrow in Figure 2). One possibility would be that proteins were less aggregated in B extracts. Probably in this extraction solution sulfhydryl and other reactive groups were not at the surface of the molecule; and when the sample is heated reactive groups would be exposed favoring the formation of higher MW aggregates. Another possibility is that the aggregate would have a more compact conformation in B than in DW or BSU, but when the sample is heated in the presence of SDS, it would adopt a similar conformation in the three extraction media.

Protein Solubility of WPC Gels. The solubility of the protein constituents of WPC gels in DW and B decreased with increasing pH in the range 3.5−4.25 (Figure 3). This result was expected because the positive charge of the protein is progressively neutralized when the pH approaches the p*I*, favoring protein aggregation.

Figure 3 also shows that in all cases protein constituents were more soluble in B than in DW. This agrees with results reported in a previous paper, in which acidic gels from a whey protein isolate prepared with different protein and calcium concentrations were analyzed (Lupano et al., 1992). The low solubility in DW and the high solubility in B of gel protein constituents in the gel prepared at pH 3.5, and the fact that SDS and urea, which cause the disruption of hydrophobic and H bindings, practically did not increase the protein solubility at this pH, indicate that electrostatic forces would be involved in maintaining the gel structure at this pH. Protein solubility in B progressively decreased in gels prepared at pHs between 3.5 and 4.0, but protein constituents were completely solubilized by BSU at

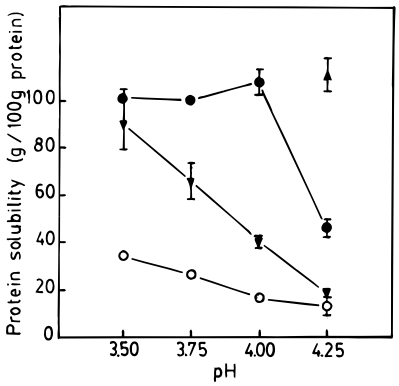

Figure 3. Solubility of the protein constituents of heat-induced (87 °C, 45 min) gels from WPC vs pH of the gels. Protein concentration of gels: 10%, w/w. Protein concentration of all solubilization assays: 0.1%, w/v. Extraction solutions: (○) distilled water, (▼) buffer (pH 8.0), (●) buffer (pH 8.0), SDS, urea, and (▲) buffer (pH 8.0), SDS, urea, ME. The bars show standard deviation. $LSD_{0.05} = 6.9$.

these pHs (Figure 3). This buffer contained urea and SDS; thus, hydrophobic and H bindings would be the principal forces responsible for the maintenance of the gel structure at pHs 3.75 and 4.0. Results also indicate that mainly noncovalent bonds are involved in the structure of gels prepared at pHs between 3.5 and 4.0. This fact was expected because sulfhydryl−disulfide interchange reactions do not occur, at least to a high extent, at acidic pH and agrees with results obtained in acid gels prepared with whey protein isolates at pH 3.75 (Lupano et al., 1992) and with soy protein isolate gels at pHs between 2.5 and 3.5 (Puppo et al., 1995).

Gel prepared at pH 4.25 had a protein solubility in BSU of about 45% but was completely soluble in BSU with 1% ME (Figure 3). This fact indicates that in this case there would be disulfide bonds involved in the maintenance of the gel structure, probably because the pH of the gel was near the p*I* of β-Lg and protein−protein interactions are expected to be increased, favoring the contact between sulfhydryl groups.

Electrophoresis of WPC Gels. Figure 4 shows the electrophoretic patterns of proteins extracted with DW, B, and BSU from gels prepared at different pHs. Samples were treated with ME before electrophoresis. The patterns reflect the differences in protein solubility in these extraction solutions, as shown in Figure 3. The peak of β-Lg decreased more rapidly than the one corresponding to α-La in the B extracts, when pH of the gel increased from 3.5 to 4.25 (Figure 4B,E,G,I). This suggests that β-Lg would be more involved than α-La in the maintenance of the gel structure when pH approaches the isoelectric point. Figure 4 also shows that α-La was more soluble in distilled water than β-Lg. The electrophoretic patterns of BSU extracts from gels of pHs 3.75 and 4.0 and the electrophoretic pattern of BSU plus ME corresponding to the gel of pH 4.25 were similar to the BSU extract of the gel of pH 3.5 (data not shown).

Peaks assigned to BSA and dimers and trimers of β-Lg (MW 67, 37, and 55 kDa, respectively) decreased progressively when the pH of gels increased between 3.5 and 4.25 in B extracts and were practically absent in the DW extracts (Figure 4).

High MW aggregates which did not enter the gel were observed in B and BSU extracts of samples not treated with ME before electrophoresis (Figure 5). Also, a peak of MW about 30 kDa, assigned to dimers of β-Lg, was

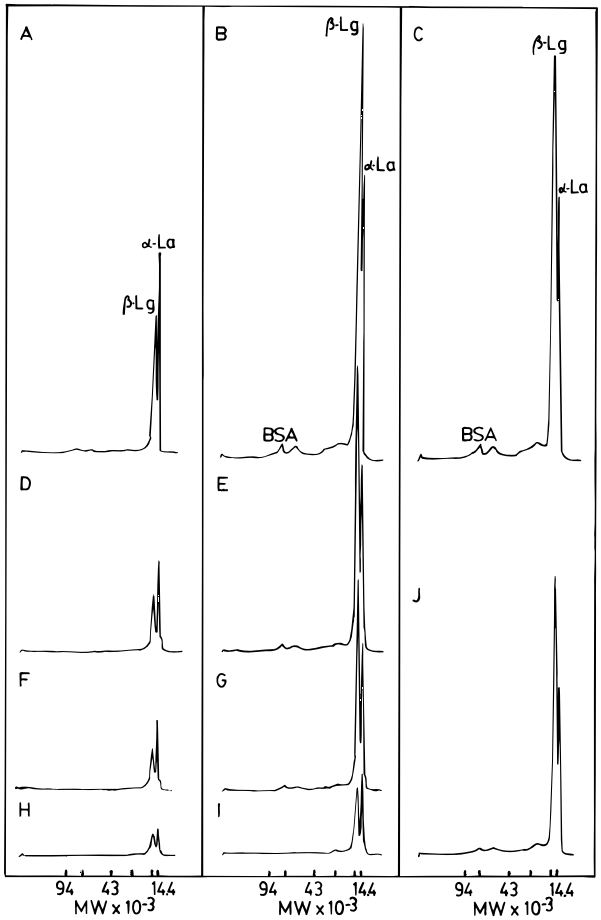

Figure 4. Scanned SDS−PAGE patterns of heat-induced (87 °C, 45 min) gels from WPC. Samples were treated with ME before electrophoresis. Protein concentration of gels: 10%, w/w. pH of gels: (A−C) 3.5, (D, E) 3.75, (F, G) 4.0, and (H−J) 4.25. Extraction solutions: (A, D, F, H) distilled water, (B, E, G, I) buffer (pH 8.0), and (C, J) buffer (pH 8.0), SDS, urea.

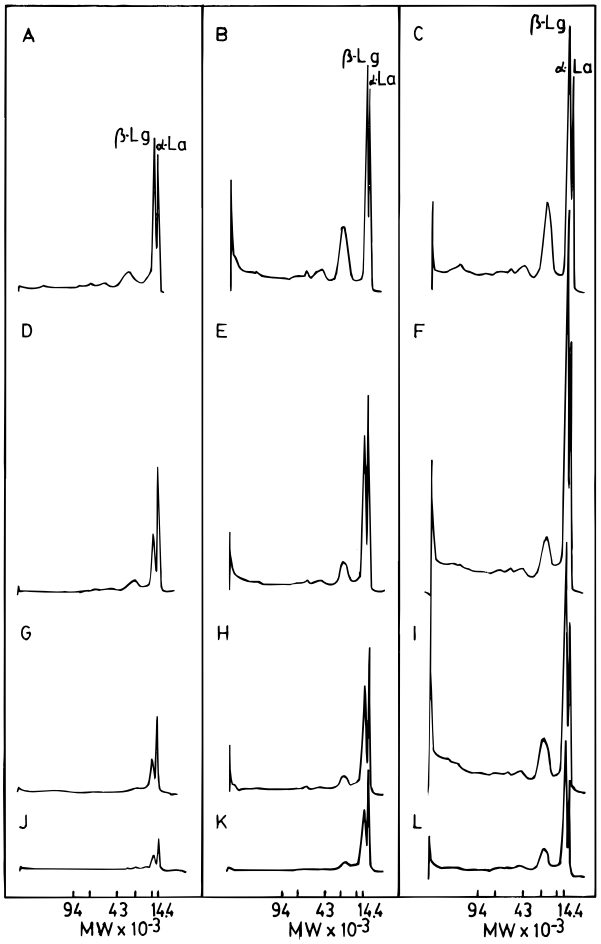

Figure 5. Scanned SDS−PAGE patterns of heat-induced (87 °C, 45 min) gels from WPC. Samples were not treated with ME before electrophoresis. Protein concentration of gels: 10%, w/w. pH of gels: (A−C) 3.5, (D−F) 3.75, (G−I) 4.0, and (J−L) 4.25. Extraction solutions: (A, D, G, J) distilled water, (B, E, H, K) buffer (pH 8.0), and (C, F, I, L) buffer (pH 8.0), SDS, urea.

very high, especially in the patterns of gels prepared at pH 3.5 (Figure 5A−C). The MW of β-Lg dimers is about 37 kDa, but, as discussed previously, protein constituents migrated a little more in samples not treated with ME, which explains why this peak appeared in the zone of 30 kDa. These peaks were very low in the patterns of samples treated with ME (Figure 4).

Peaks of β-Lg and α-La were present, in contrast to the electrophoretic patterns of WPC samples (Figure 1), in the patterns of gel extracts not treated with ME (Figure 5). These peaks presented a similar behavior as in samples treated with ME (Figure 4).

Transmittance (T_{660}) of Heated Protein Dispersions. Figure 6 shows the transmittance of heated protein dispersions (0.1% protein, w/v) in the function of pH. The T_{660} decreased with increasing pH, significant differences between data being found in the pH range 3.75−4.25.

The transmittance of heated protein dispersions appears to be directly related to protein solubility in pH 8.0 buffer, as can be seen by comparing Figures 3 and 6. In a previous paper it was observed that T_{660} correlated with protein solubility in distilled water at pH 7−8 (Lupano, 1994). This indicates that it would be a relationship between T_{660} and the solubility of protein species at pH 7−8, independent of the ionic strength of the media.

WHC of Gels. Figure 6 shows the WHC of heat-induced gels (10% protein, w/w) in the function of pH.

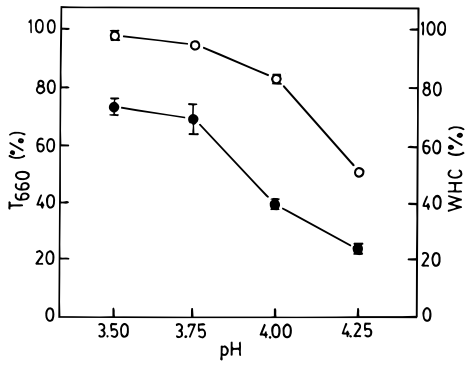

Figure 6. Transmittance at 660 nm (T_{660}) and water-holding capacity (WHC) of heated (87 °C, 45 min) protein dispersions (0.1% protein, w/v) or gels (10% protein, w/w) from WPC, as a function of pH: (●) T_{660} ($LSD_{0.05} = 6.1$) and (○) WHC ($LSD_{0.05} = 3.6$). The bars show standard deviation.

Gels presented values of WHC higher than 90% at pHs 3.5 and 3.75. As in the case of T_{660}, WHC of gels decreased with increasing pH, and significant differences between values were found in the pH range 3.75−4.25. Results indicate that gels become very different from pH 3.75 to 4.25, when pH approaches p*I*.

Conclusions. Extracts of heat-induced gels presented electrophoretic patterns different from those of the whey protein concentrate, indicating that there are

certain protein constituents which are more involved in the maintenance of the gel structure.

Protein solubility and water-holding capacity of gels decreased, and gels presented different electrophoretic patterns when pH approaches the p*I*, suggesting different gel structures. Solubility of the gel protein components in the presence or absence of denaturing and reducing agents indicates that noncovalent bindings are responsible for the maintenance of gel structure at pHs between 3.5 and 4.0, but in the gel prepared at pH 4.25, disulfide bonds would also be involved in the structure of the gel.

Results show that a wide range of gel characteristics could be obtained by varying the pH of gels from 3.5 to 4.25, suggesting different applications of these products as food ingredients.

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Received for review January 23, 1996. Accepted July 15, 1996.[⊗] This investigation was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). C.E.L. is a member of the Researcher Career of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

JF9600519

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.