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Gelation of mixed systems whey protein concentrate–gluten in acidic conditions

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Abstract

Gels of whey protein concentrate (WPC)–gluten were prepared by heating WPC–gluten dispersions (10% whey protein/0–5–10% gluten protein, w/w; pH 3.75 or 4.2). Gels were characterized through solubility assays in different extraction solutions, measures of water-holding capacity (WHC), firmness, elasticity and relaxation time, and light microscopy. Differential scanning calorimetry (DSC) of WPC–gluten dispersions was also performed. Gluten increases the firmness and elasticity of gels, mainly at pH 4.2. The WHC also increases with gluten content, being higher at pH 3.75 than at pH 4.2. Solubility assays indicate that electrostatic forces, hydrophobic and H bindings would be involved in maintaining the gel structure of WPC gels at pH 3.75 and 4.2, whereas in mixed gels of WPC–gluten, the principal forces responsible for the maintenance of the gel structure at these pHs would be hydrophobic and H bindings, and in gels prepared at pH 4.2 also disulfide bonds, but in a minor extent. The presence of gluten shifts the apparent transition temperature for whey protein denaturation towards lower temperatures. Gels with gluten present a smooth network with gaps and a more elastic appearance, as observed by light microscopy.

Keywords: Gelation; Whey protein–gluten gelation; Gelation in acidic conditions

1. Introduction

Because of its high nutritional value and functionality, whey protein concentrates or isolates has become major sources of functional ingredients for the food industry. One of the main functional properties expected from whey proteins is their capacity to form gels upon heating (Cheftel & Lorient, 1982; Dumay, 1988). The formation of a gel is the result of an equilibrium between intermolecular attractive forces, represented by covalent and noncovalent interactions (disulfide bonds, hydrophobic interactions and van der Waals and hydrogen bonds), and intermolecular repulsive forces between charges of the same sign. This equilibrium depends mainly on protein concentration and environment characteristics, such as pH, ionic strength, and the presence of other food components (Gault & Fauquant, 1992; Tolstoguzov, 1993). The structure of whey protein gels depends on the pH at which they have been performed (Lupano, Renzi & Romera, 1996). This is due because the reactivity of SH groups decreases significantly under acidic conditions and, thus, mainly noncovalent interactions are involved in the structure of acid gels, whereas at neutral pH intermolecular sulphydryl–disulfide interchange reactions are favored (Lupano, Dumay & Cheftel, 1992; Shimada & Cheftel, 1988). Also, when pH approaches the p*I* of 5.2 of the β-lactoglobulin (β-Lg), the major whey protein, the charge of the protein is progressively neutralized, favoring protein aggregation (Lupano & González, 1999; Lupano et al., 1996). Moreover, β-Lg exists as a dimer in solutions above its p*I*, 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 an octamer (Morr & Ha, 1993). Thus, gel characteristics are very different at acidic pH when compare with gels prepared at neutral pH (Lupano et al., 1992).

Gluten proteins, on the other hand, are responsible for the elasticity and the extensibility of the dough.

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Dairy products are traditionally used as components of bread baking formulas, and there are several studies of the effect of whey and WPC on the properties of wheat-based products (Erdogdu-Arnoczky, Czuchajowska & Pomeranz, 1996; Kadharmestan, Baik & Czuchajowska, 1998). However, no information is available concerning the properties of mixed gels WPC–gluten at acidic pH. These gels would combine the gelling capacity and nutritional value of whey proteins with the rheological properties of gluten, and could be utilized in desserts, especially in lemon flavored ones.

2. Materials and methods

2.1. Materials

WPC was prepared by large scale ultrafiltration and was a gift from Williner S.A. (Rafaela, Santa Fe, Argentina). Gluten utilized was gluten Windmill (The Netherlands), and contained 72.7% protein (N×5.6) on dry basis, and 8.1% moisture. WPC contained 3.6% moisture, 6.1% lipids, 5.3% ash, 0.85% calcium, 45.5% protein [calculated as [total N (12.4%)−nonprotein N (0.8%)]×6.38], and 37.8% lactose (estimated by difference), on dry basis. All chemicals used were of analytical grade.

2.2. Heating and gelation of WPC and WPC–gluten dispersions

Aqueous dispersions (10.0% WPC protein; 0, 5.0, and 10.0% gluten protein, w/w) of WPC or WPC–gluten were adjusted to pH 3.75 or 4.2 with 1–2 N HCl or 1 N NaOH. Dispersions were placed in glass tubes (2.2 cm i.d.×6 cm height) with tightly closed stoppers. Gelation was 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 analysis. Samples for differential scanning calorimetry (DSC) were prepared in the same manner but without heating.

2.3. Water-holding capacity (WHC) of gels

Gel (0.8–1.1 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, Dumay, Cavalier & Cheftel, 1989). WHC was expressed as percent of the initial water remaining in the gel after centrifugation. Values are the average (±standard deviation) of at least two determinations.

2.4. Light microscopy

Gel was extended on a slide and observed with a microscope Leitz (Germany), at a magnification of 320×.

2.5. Differential scanning calorimetry (DSC)

A Rheometric Scientific differential scanning calorimeter (Rheometric Scientific Ltd., Epsom, Surrey, UK) calibrated with indium was used. Samples of 8–15 mg of WPC and WPC–gluten dispersions were placed in aluminum DSC hermetic pans. An empty double pan was used as reference. Sample and reference were heated between 30 and 120°C at a heating rate of 10°C/min. The enthalpy of protein denaturation (ΔH_p) and the apparent transition temperature (T_p) were computed from the endothermic peaks. Values are the average (±standard deviation) of at least two determinations.

2.6. Determination of gel properties

Rheological analyses were performed on gel sections (22 mm ϕ×20 mm height) using an Instron Universal Tester model 1011 (Instron Corporation, MA) in the compression mode. Compression was exerted by a cilindric probe with a flat section (3.5 cm diameter) at a displacement speed of 10 mm min^{-1}. Gel firmness was defined as the force F_0 (Newtons) measured at 20% (4 mm) compression. This compression was maintained for 25 min, and the force F_{25} exerted on the probe was measured. Gel elasticity was taken as F_{25}/F_0. Relaxation time τ was taken as the time at which $F = (F_0 + F_{25})/2$ (Lupano et al., 1992; Peleg, 1979). For each type of gel, the average (±standard deviation) of three determinations was calculated.

2.7. Determination of the protein solubility of gels

Samples were dispersed either in distilled water (DW), in a pH 8.0 buffer (0.086 M Tris-0.09 M glycine-4 mM Na_2EDTA, pH 8.0 buffer) (B), or in the same buffer containing 0.5% sodium dodecyl sulfate (SDS) and 8 M urea (BSU), according to Shimada and Cheftel (1988), Lupano et al. (1992), and Lupano et al. (1996). Gels prepared with 10% gluten proteins were also dispersed in BSU plus 1% β-mercaptoethanol (ME) (v/v). Samples (0.1% protein, w/v) were homogenized at room temperature with an Ultra-Turrax at 8000 rpm for 1 min, and then centrifuged at 15 400 *g* for 30 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 $E^{1\%}_{1\,cm}$ of 8.636 for DW

solutions (Lupano et al., 1996) and 10.2 for pH 8.0 solutions (Shimada & Cheftel 1988).

2.8. Statistics

An analysis of variance (ANOVA) of the data was performed, and a least significative difference (LSD) test with a confidence interval of 95% was used to compare the means.

3. Results and discussion

3.1. Differential scanning calorimetry

One endothermic peak corresponding to whey protein denaturation was observed when WPC-gluten dispersions were heated in a DSC apparatus. The apparent transition temperature (T_p) and the enthalpy (ΔH_p) for protein denaturation are shown in Table 1. No differences in ΔH_p between different samples were observed. On the other hand, T_p shifted a little to lower temperatures in the presence of gluten, observing differences at a level of significance of 5% between samples with and without gluten (Table 1). This behaviour has not been observed in mixed systems WPC-cassava starch (Lupano & González, 1999), which indicates that the effect of the adition of gluten on WPC gels is different than the effect of cassava starch. This shift was also observed by Erdogdu, Czuchajowska and Pomeranz (1995) in gluten and commercial whey protein concentrate mixtures. A decrease in the temperature of protein denaturation was reported by Tolstoguzov (1993) in complexes of a small globular protein with anionic polysaccharides. This author suggests that, presumably, an increase in dimension of the junction zones between the polypeptides and the polysaccharide chains leads to the shift in equilibrium between the native and the denatured forms of the protein towards the latter. In the present study, it is possible that gluten modifies the thermal stability or the environment of whey proteins, turning them more sensitive to the heat treatment.

Table 1
Apparent transition temperature (T_p) and enthalpy (ΔH_p) for whey protein denaturation of WPC–gluten dispersions[a,b]

Gluten protein (%)	0	10	0	10
PH	3.75	3.75	4.2	4.2
T_p (°C)[c]	87.52±0.43	86.76±0.35	87.46±0.07	86.82±0.30
ΔH_p (J/g)[d]	7.70±0.74	8.13±1.00	7.68±1.03	8.33±0.72

[a] Values are the average (±standard deviation) of at least two determinations.
[b] Whey protein concentration: 10%.
[c] $LSD_{0.05} = 0.39$.
[d] $LSD_{0.05} = 1.33$.

3.2. Protein solubility of gels

The solubility in distilled water (DW) of the protein constituents of WPC gels prepared at pH 3.75 was higher than the corresponding to gels prepared at pH 4.2 (34.1 and 18.5%, respectively) in agreement with previous results (Lupano et al., 1996). The presence of gluten practically did not modify the solubility of gels in DW (Fig. 1A and B).

Protein constituents were more soluble in B than in DW, except in the gel prepared at pH 3.75 with 10% gluten proteins, in which the solubility in B of the protein constituents was similar to their solubility in DW (Fig. 1A). Gels with gluten, particularly those prepared at pH 3.75, presented a lower protein solubility in B than gels without gluten. These results suggest that the increase in the solubility of gels in B is due mainly to WPC protein constituents.

Protein solubility in BSU was higher than 90% in all gels, except in those gels with 10% gluten proteins, indicating that noncovalent interactions are the main forces responsible for the maintenance of the gel structure. The solubility in BSUM of gel prepared at pH 4.2 with 10% gluten proteins was higher than its solubility in BSU, which suggests the presence of disulfide bonds in these gels, which were broken by ME. This effect was not observed in the gel with 10% gluten proteins prepared at pH 3.75 (Fig. 1). This is consistent with the significant decrease in the reactivity of SH groups at acid pH (Lupano et al., 1992; Shimada & Cheftel, 1988).

Results indicate that electrostatic forces, hydrophobic and H bindings would be involved in maintaining the gel structure of WPC gels at pH 3.75 and 4.2, whereas in mixed gels of WPC–gluten, the principal

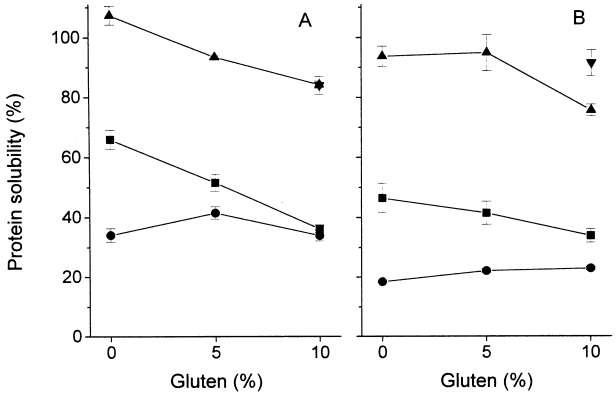

Fig. 1. Solubility of protein constituents of heat-induced gels from WPC–gluten, as a function of gluten protein content. Whey protein content of gels: 10%, w/w; pH of gels: (A) 3.75; (B) 4.2. Protein concentration for all solubilization assays: 0.1%, w/v. Extraction solutions: (●) distilled water; (■) standard buffer, pH 8.0; (▲) standard buffer containing 8 M urea and 0.5% SDS; (▼) standard buffer containing 8 M urea, 0.5% SDS and 1% β-mercaptoethanol. $LSD_{0.05} = 11.23$.

forces responsible for the maintenance of the gel structure at pHs 3.75 and 4.2 would be hydrophobic and H bindings, and in gels prepared at pH 4.2 also disulfide bonds, but to a minor extent.

3.3. WHC of gels

Fig. 2 shows the WHC of heat-induced gels (10% WPC protein, w/w) as a function of gluten protein content. Gels prepared at pH 3.75 presented values of WHC higher than gels prepared at pH 4.2, in agreement with results reported previously (Lupano & González, 1999; Lupano et al., 1996).

Gluten increased the WHC of gels, obtaining values higher than 90% in all cases. This was expected because 1/3 of the amino acid residues of gluten proteins is glutamin, which explains the ability of these proteins to form hydrogen bonds with molecules of water.

3.4. Rheological properties of gels

Fig. 3 shows the firmness, elasticity and relaxation time of WPC–gluten gels. When comparing these results with data reported previously (Lupano et al., 1992), elasticity of gels prepared at pH 3.75 was similar to that of non-dialysed whey protein isolate (WPI) gels, whereas firmness resulted intermediate between those of gels prepared with dialysed and non-dialysed WPI. This behaviour can be explained by considering the calcium content of gels: calcium content of gels prepared in the present work was about 47 mM, whereas calcium content of gels from non-dialysed WPI was about 12 mM, and calcium content of gels prepared with dialysed WPI was non-detected (Lupano et al., 1992). Results are in agreement with the hypothesis that at low concentrations, calcium enhances protein–protein interactions and contributes positively to the gel network, but at calcium concentration above 20 mM, calcium causes excessive protein aggregation (isoelectric-type) with detrimental effects on gel cohesiveness and firmness (Lupano et al., 1992).

Gels of WPC prepared at pH 4.2 without gluten presented the same firmness but a higher elasticity and a very higher relaxation time than gels prepared at pH 3.75 (Fig. 3). The lower relaxation time of gels prepared at pH 3.75 indicates viscous characteristics in these gels.

The relaxation time of gels prepared at pH 4.2 was higher than the corresponding to gels prepared at pH 3.75 (Fig. 3A). The presence of gluten did not modify

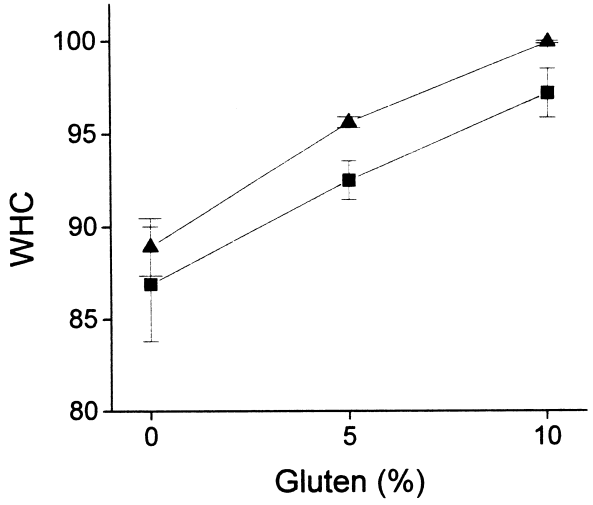

Fig. 2. Water-holding capacity (WHC) of gels from WPC–gluten as a function of gluten protein content. Whey protein content of gels: 10%, w/w; pH of gels: (▲) 3.75, (□) 4.2. The bars show standard deviation. $LSD_{0.05} = 4.17$.

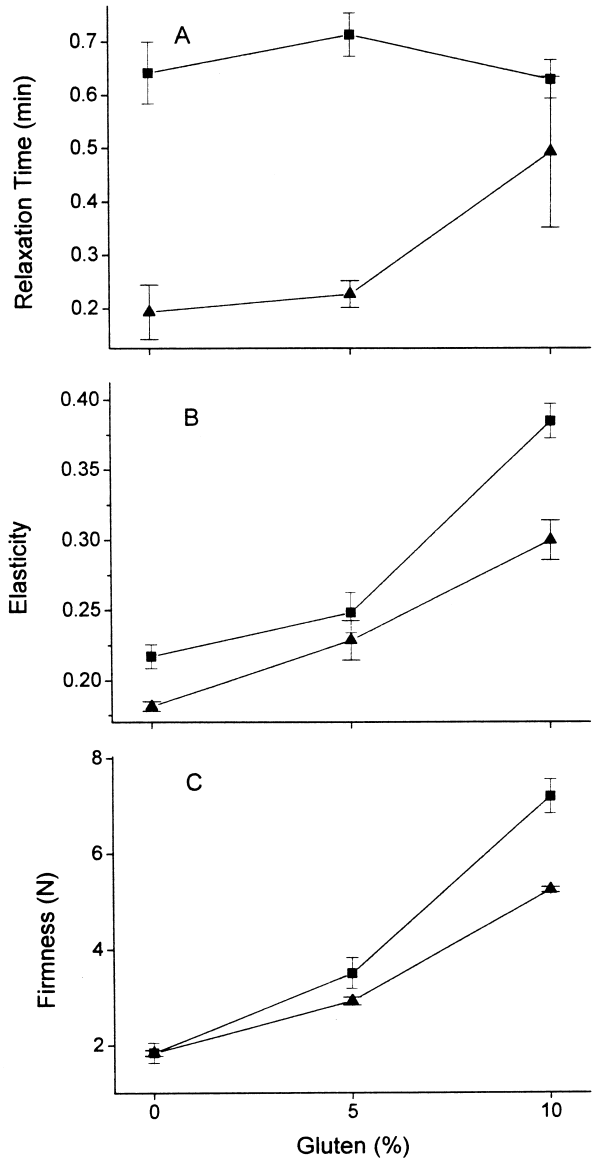

Fig. 3. (A) Relaxation time, (B) elasticity and (C) firmness of gels from WPC–gluten as a function of gluten protein content. Whey protein content:10%, w/w; pH of gels: (▲) 3.75; (■) 4.2. The bars show standard standard deviation. $LSD_{0.05}$ = (A) 0.192; (B) 0.034; (C) 0.888.

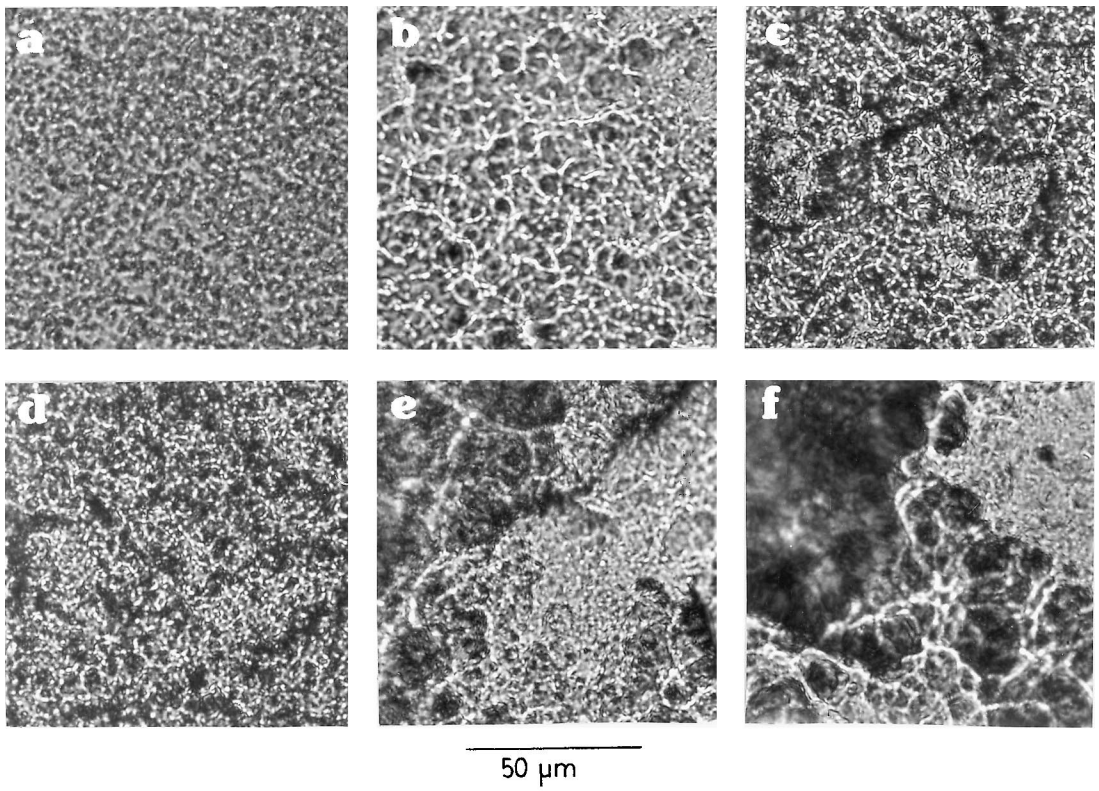

Fig. 4. Light microscopy of WPC–gluten geels. Whey protein content: 10%, w/w. Gluten protein content: (a,d) 0%, w/w; (b,e) 5%, w/w; (c,f) 10%, w/w; pH of gels: (a–c) 3.75; (d–f) 4.2.

the relaxation time of gels prepared at pH 4.2, but increased the corresponding to gels prepared at pH 3.75 (Fig. 3A).

Gluten increased the firmness and elasticity of WPC gels, mainly in gels prepared at pH 4.2 (Fig. 3B and C). Zadow (1981) studied the effect of the addition of whey protein concentrates in proofed bread dough, and observed that the result was a weaker and less elastic dough. This author concluded that the weaker and less elastic properties of this dough are the result of the interference of WPC sulphydryl groups in the normal sulphydryl/disulfide interchange reactions occurring during dough development. Data obtained in the present work are consistent with the results of Zadow even when the system was analyzed in a different manner, by studying the effect of gluten on WPC gels. Sulphydryl/disulfide interchange reactions are inhibited at low pH, thus, they would occur in a higher extent in gels prepared at pH 4.2 than in gels prepared at pH 3.75, explaining the lower firmness and elasticity of the latter gels.

3.5. Structure evaluation by light microscopy

Fig. 4 shows the structure of gels as observed by light microscopy. The most homogeneous structure corresponded to the gel prepared without gluten at pH 3.75, whereas the gel prepared at pH 4.2 presented a more aggregated structure (Fig. 4), in agreement with previous results (Lupano & González, 1999).

The presence of gluten modified completely the gel texture, showing a smooth network with gaps and a more elastic appearance, mainly in gels prepared at pH 4.2 (Fig. 4), in agreement with rheological data.

Belton (1999) proposed a model of gluten in which two classes of protein are considered: linear proteins (mainly high molecular weight glutenin subunits) and globular proteins (gliadins). The linear proteins interact with each other by disulfide and hydrogen bonds. Dough working favours the formation of end to end disulfide bonds in the subunits, increasing the effective molecular weight of the subunit and hence the number of linear–xlinear protein interactions. This, in turn, increases the resistance to deformation and the restoring force after deformation. Gliadins, on the other hand, contribute to resistance to extension by forming a viscous environment, causing also a slowing down of elastic recovery. According to this model, it is possible that whey proteins contribute to the viscous environment like gliadins, having thus a similar effect on the decrease of elasticity, but they would also be able to form hydrogen and disulfide bonds with glutenin subunits, interfering with the normal structure of gluten. It was observed that whey proteins break the gluten

structure, that is, gluten can be dispersed with a glass rod in distilled water by the addition of WPC (results not shown).

4. Conclusions

Results suggest that the main forces responsible for the maintenance of the structure of mixed gels WPC–gluten prepared at pHs 3.75 and 4.2 are hydrophobic interactions and H bindings, and in gels prepared at pH 4.2 also disulfide bonds, but to a minor extent.

Whey proteins would form a viscous environment, being also able to form hydrogen and disulfide bonds with glutenin subunits, mainly at pH 4.2, interfering with the normal structure of gluten. Gluten, in turn, modifies the thermal stability of whey proteins, turning them more sensible to the heat treatment, and increases the firmness and elasticity of WPC gels.

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