Gelation of Whey Protein Concentrate−Cassava Starch in Acidic Conditions

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Whey protein concentrate (WPC)−cassava starch (CS) gels were prepared by heating WPC−CS dispersions (0−12.5% protein−0−4.2% starch, w/w; pH 3.75 and 4.2). Gels were characterized by measures of water-holding capacity (WHC), estimation of the relative size and/or density distribution of the gel particles, and light microscopy. Differential scanning calorimetry (DSC) of WPC−CS dispersions was also performed. Results show that CS increased the WHC of gels. Mixed gels presented separate zones of gelatinized starch and aggregated protein and a higher proportion of large/high-density particles. DSC assays showed that starch gelatinization preceded protein denaturation during heating. Starch gelatinization shifted to higher temperatures in dispersions containing WPC, due to the presence of whey proteins, lactose, and calcium.

Keywords: *Gelation; whey protein−cassava starch gelation; 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). On heating, the native nonpolar side chain of whey proteins and, in some cases, such as in β-lactoglobulin (β-Lg) denaturation, sulfhydryl groups are exposed (Steventon et al., 1991). 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 sulfhydryl−disulfide interchange reactions are favored (Shimada and Cheftel, 1988). Moreover, β-Lg, the major whey protein, exists as a 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 pH 3.5 and 5.2 the dimer polymerizes to an octamer (Morr and Ha, 1993). Also, when pH approaches the p*I*, the charge of the proteins is progressively neutralized, favoring protein aggregation. As a consequence, the structure of gels prepared at pH between 3.5 and 5.2 is expected to be different from that of gels prepared at pH below 3.5 or above 5.2 (Lupano et al., 1996).

Functional properties of food proteins are also strongly dependent on protein interactions with other food components, such as polysaccharides (Tolstoguzov, 1993). The behavior of mixed polymer solutions is determined predominantly by the energies of interaction between chains. When the net energy of interaction is favorable, the two polymers may associate into a single gellike phase or insoluble precipitate. In most other cases the interactions are unfavorable and can lead to mutual exclusion of each component from the "polymer domain" of the other, with increase in the effective concentration of both. At sufficiently high concentration the system can separate into two phases, giving a route to production of multitextured gels (Morris, 1991; Tolstoguzov, 1991, 1993).

Cassava, in the form of flour and starch, is increasingly being used as a raw material for processing into a range of food products (Moorthy et al., 1996). Tuber starches (e.g., potato and cassava), contrary to cereal starches, undergo very rapid and extensive swelling starting at temperatures between 50 and 60 °C (Aguilera and Baffico, 1997).

Some studies have been carried out on mixed systems of whey protein−cassava starch at pH 5.75 (Aguilera and Rojas, 1996; Aguilera and Baffico, 1997). However, no information is available concerning the properties of these mixed gels at pH below the p*I* of the β-Lg. Viscous pastes prepared by thermocoagulation of aqueous dispersions of whey protein concentrates or isolates at pH 3.7−4.2 have been developed as fat substitutes to be incorporated into foods such as ice cream, mayonnaise, salad dressing, yogurt, and various types of spreads (Quéguiner et al., 1992). Mixed gels of whey protein concentrate−cassava starch (WPC−CS) prepared at these acidic pH values could be utilized in salad dressings or lemon pie fillings.

The objective of this work was to characterize the gelation of mixed systems of WPC−CS at two pH values below the p*I* of the β-Lg. The pH was chosen in the zone at which large modifications in gel characteristics are obtained with even minor changes in pH. The effects of protein and starch concentrations on gel characteristics were also studied.

MATERIALS AND METHODS

Materials. WPC was prepared by large scale ultrafiltration and was a gift from Williner S.A. (Rafaela, Santa Fe, Argentina). Starch utilized was commercial CS (Dietética Científica S.A., Remedios Escalada, Provincia de Buenos Aires, Argentina). WPC contained 3.1% moisture, 4.5% lipids, 4.9% ash, 0.7% calcium, 47.5% protein [calculated as [total N (8.13) − nonprotein N (0.69)] × 6.38], and 38.7% lactose (estimated by difference), on dry basis. CS contained 14.5% moisture and

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97.2% starch, on dry basis. All chemicals used were of analytical grade.

Heating and Gelation of WPC, CS, and WPC−CS Dispersions. Aqueous dispersions (0, 7.5, 10.0, and 12.5% protein; 0, 2.1, and 4.2% starch, w/w) of WPC, CS, or WPC−CS were adjusted to pH 3.75 or 4.2 with 0.5−5 N HCl or 0.05 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. Aqueous dispersions of CS−calcium (4.2% starch−0.15% calcium, w/w) and CS−lactose (4.2% starch−8.7% lactose, w/w) were also utilized in DSC assays.

Water-Holding Capacity (WHC) of Gels. Gel (0.3−0.9 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. Values are the average (± standard deviation) of at least two determinations.

Light Microscopy. Gel was extended on a slide, stained with bromophenol blue (2.5 mg/10 mL) (Quéguiner et al., 1992) during 10 min, and carefully washed with distilled water. Samples were observed with a microscope Leitz (Germany), at a magnification of 320×.

Relative Distribution of Gel Particles According to Their Size and/or Density. Particles of WPC and WPC−CS gels were separated into three classes (large, medium, and small), according to their capacity to sediment through concentrated solutions of glycerol (Quéguiner et al., 1992). The original method was modified as follows: aqueous dispersions (2% protein, w/w) were obtained by dispersing WPC and WPC−CS gels with a glass rod followed by magnetic stirring for 30 min. Samples (0.3 g) were introduced into Eppendorf tubes containing 1 mL of distilled water (tube C), 1 mL of glycerol/distilled water 50:50 (tube B), or 1 mL of glycerol/distilled water 90:10 (tube A). Tubes were then centrifuged at 1100*g* for 10 min. The sediments were washed twice with 0.5−1 mL of distilled water and centrifuged at 9300*g* for 30 min. The dry weight of the washed sediments was determined. The relative proportions of the three classes of insoluble particles are expressed as follows: proportion of large and/or high-density particles = A/C; proportion of medium particles = $(B - A)/C$; proportion of small or low-density particles = $(C - B)/C$, where A = grams of dry solids sedimented in 90% glycerol, B = grams of dry solids sedimented in 50% glycerol, and C = grams of dry solids sedimented in water (Quéguiner et al., 1992). Values are the average (± standard deviation) of at least two determinations.

DSC. A Rheometric Scientific differential scanning calorimeter (Reometric Scientific Ltd., Epsom, Surrey, U.K.) calibrated with indium was used. Samples of 7−17 mg of WPC, CS, and WPC−CS dispersions were placed in aluminum DSC hermetic pans. An empty double pan was used as reference. Sample and reference were heated between 20 and 120 °C at a heating rate of 10 °C/min. The enthalpies of protein denaturation (ΔH_p) and starch gelatinization (ΔH_s) and the apparent transition temperature (T_p and T_s, for protein and starch, respectively) were computed from the endothermic peaks. Values are the average (± standard deviation) of at least three determinations.

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 of WHC and DSC assays.

RESULTS AND DISCUSSION

WHC of Gels. Figure 1 shows the WHC of heat-induced gels as a function of starch content. Gels prepared at pH 3.75 presented values of WHC >85% at both protein concentrations (7.5 and 10.0%) and at all starch contents. High values of WHC of whey protein gels prepared at pH 3.75 have been reported previously (Lupano et al., 1992, 1996) and are associated with less aggregated structures. Also, gels prepared with WPC alone at pH 3.75 presented a higher WHC than gels prepared at pH 4.2, in agreement with previous results (Lupano et al., 1996).

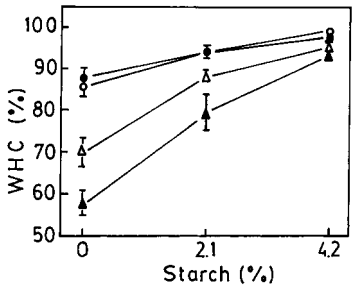

Figure 1. WHC of gels from WPC−CS as a function of CS content: (●) 7.5% protein, w/w, pH 3.75; (○) 10% protein, w/w, pH 3.75; (▲) 7.5% protein, w/w, pH 4.2; (△) 10% protein, pH 4.2. ($LSD_{0.05}$ = 3.7).

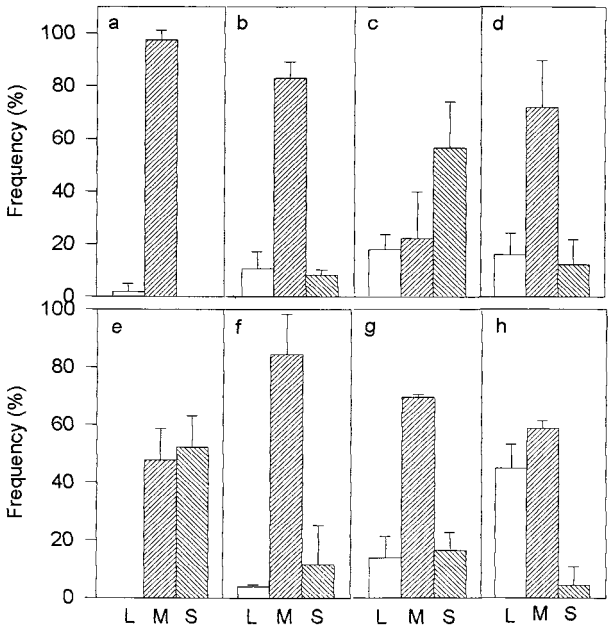

Figure 2. Relative size and/or density distribution of particles of WPC−CS gels: (L) large; (M) medium; (S) small. Composition of gels: (a, e) 7.5% protein−0% starch, w/w; (b, f) 7.5% protein−4.2% starch, w/w; (c, g) 10% protein−0% starch, w/w; (d, h) 10% protein−4.2% starch, w/w. pH of gels: (a−d) 3.75; (e−h) 4.2.

Gels with 10% protein presented higher WHC values than gels with 7.5% protein at pH 4.2. This is probably due to the fact that when protein concentration increases from 7.5 to 10%, the number of globular proteins such of β-Lg, which are able to form hydrogen bonds with the molecules of water, also increases.

Starch increased the WHC of gels in all cases (Figure 1). Gels with a starch content of 4.2% had very high WHCs, independent of their protein concentration and pH. These results are expected because of the ability of starch to form hydrogen bonds.

Relative Size and/or Density of the Gel Particles. The relative size and/or density distribution of the particles of heat-induced gels is presented in Figure 2. Gels were dispersed in distilled water before doing

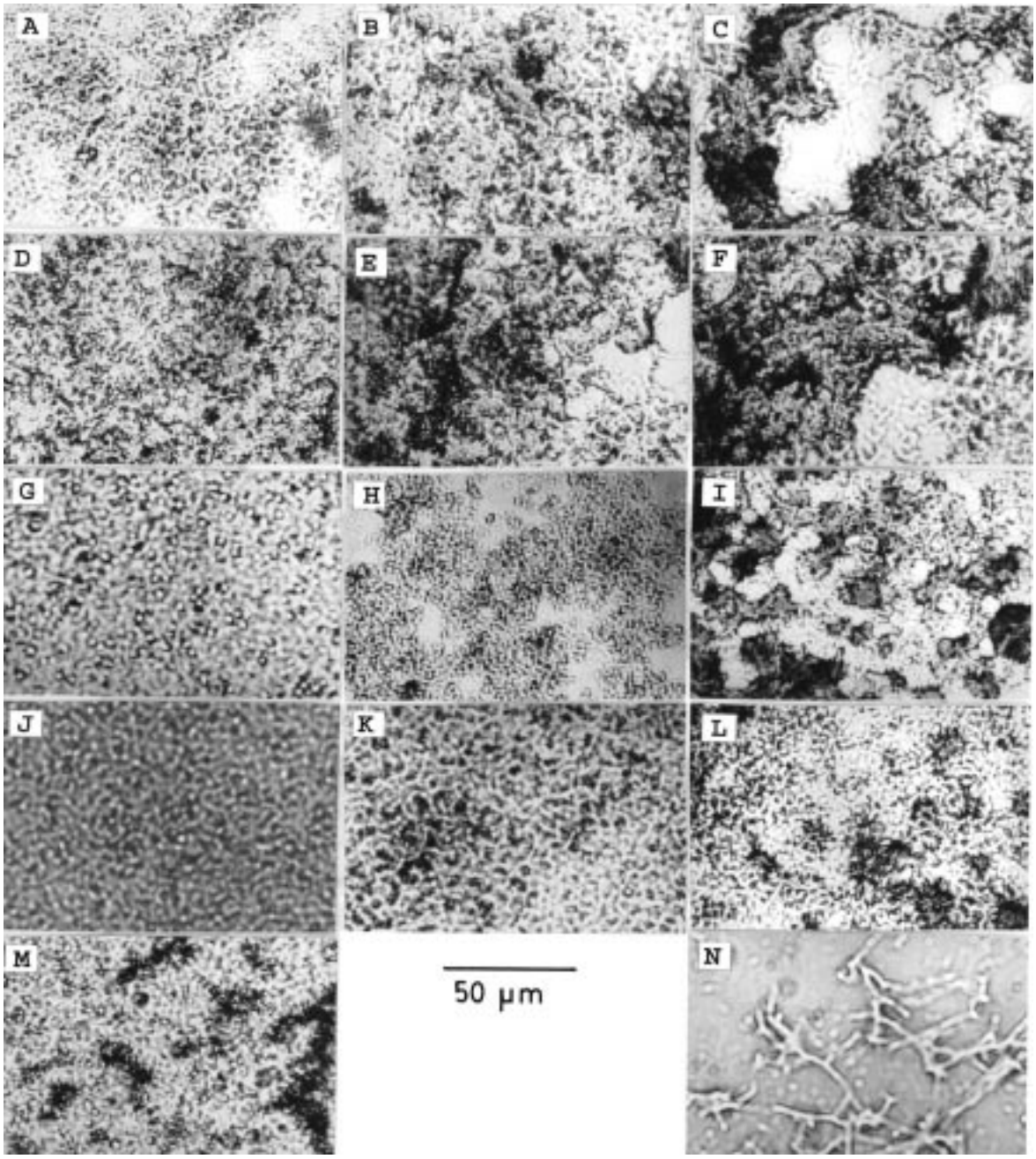

Figure 3. Light microscopy of WPC−CS gels. Gel composition: (A, G) 7.5% protein−0% starch, w/w; (B, H) 7.5% protein−2.1% starch, w/w; (C, I) 7.5% protein−4.2% starch, w/w; (D, J) 10% protein−0% starch, w/w; (E, K) 10% protein−2.1% starch, w/w; (F, L) 10% protein−4.2% starch, w/w; (M) 12.5% protein−0% starch, w/w; (N) 0% protein−4.2% starch, w/w. pH of gels: (A−F) 4.2; (G−N) 3.75.

the measurement. Gels with a smaller particle size are expected to have a smoother texture than gels with a higher particle size. The proportion of large/high-density particles increased when protein concentration increased from 7.5 to 10% (significant differences for $P \leq 0.05$). This is probably due to the more compact structure of gels prepared at 10% protein when compared with gels at 7.5% protein.

Also, gels containing 4.2% starch had a higher proportion of large/high-density particles and a lower proportion of small/low-density particles than gels prepared without starch (significant differences for $P \leq 0.05$) (Figure 2), indicating that mixed gels would be more aggregated than gels containing only WPC.

Gels prepared at 10% protein presented a higher proportion of small/low-density gel particles at pH 3.75 (Figure 2c,d) than at pH 4.2 (Figure 2g,h). This was expected because gels become more aggregated when the pH approaches the p*I*. However, the effect of pH on the particle size/density of gels prepared with 7.5% protein was not clear (Figure 2a,b,e,f).

In sum, results presented in Figure 2 indicate that the proportion of large/high-density particles of WPC gels, which is associated with a less smooth texture, increases with protein concentrate, with the presence of cassava starch, and when the pH approaches the p*I* of β-Lg.

Structure Evaluation by Light Microscopy. Figure 3 shows the structure of gels as observed by light microscopy. The most homogeneous structure corresponded to gels prepared at pH 3.75 with 7.5 and 10% protein, without starch (Figure 3J,G, respectively), the

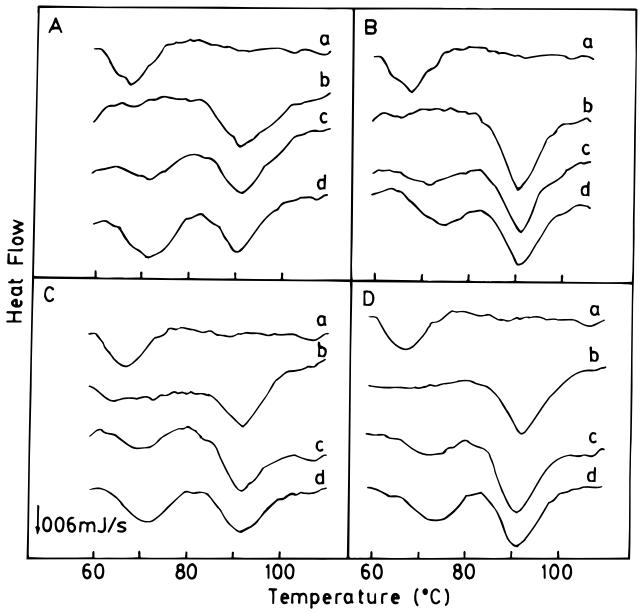

Figure 4. DSC thermograms of WPC−CS dispersions (10 mg): (A, C) 7.5% protein; (B, D) 10% protein. Composition of dispersions: (a) 0% protein−4.2% starch, w/w; (b) 7.5 or 10% protein−0% starch, w/w; (c) 7.5 or 10% protein−2.1% starch, w/w; (d) 7.5 or 10% protein−4.2% starch, w/w. pH of dispersions: (A, B) 3.75; (C, D) 4.2.

former being less compact than the latter. When protein concentration increased to 12.5%, the structure became less homogeneous, and some protein aggregates were observed (Figure 3M).

Gels prepared at pH 4.2 (Figure 3A−F) were more aggregated than those prepared at pH 3.75 (Figure 3G−M). This was expected because when the pH approaches the p*I*, the charge of the proteins is progressively neutralized, favoring protein aggregation (Lupano et al., 1996).

Separate zones of aggregated protein and gelatinized starch were observed, mainly at the starch concentration of 4.2%, indicating that starch breaks the protein network (Figure 3C,F,I,L). A similar behavior was observed by Aguilera and Rojas (1996) in mixed whey protein isolate−CS gels prepared at pH 5.75. This indicates that there was no cogelation between whey proteins and CS. Starch gelatinization reduces water availability of proteins, thus having an effect on protein concentration.

The nonhomogeneous structure observed in mixed gels would be related to the high proportion of large/high-density particles observed in these gels, as described previously.

DSC. Figure 4 shows a few thermograms obtained when WPC, CS, and WPC−CS dispersions were heated in the DSC apparatus. Two separate endothermic peaks were observed in WPC−CS mixed systems, the lower temperature peak corresponding to starch gelatinization and the higher temperature peak to protein denaturation. This indicates that starch gelatinization occurs first, followed by protein denaturation, in agreement with results obtained at pH 5.75 by Aguilera and Rojas (1996).

Table 1 shows the apparent transition temperature for protein denaturation (T_p). The T_p decreased when the pH decreased from 4.2 to 3.75 (significant differences for $P \leq 0.05$). It is known that the susceptibility of whey proteins to denaturation is largely determined by the pH of the solution (de Wit, 1981) and that small changes in the pH in the range 3.5−4.5 produce considerable modifications in whey protein coagulation (Quéguiner et al., 1992). Hegg (1980) found that the stability of whey proteins in distilled water, as represented by the denaturation temperature, is maximum at pH 3−4 and then decreases when the pH is increased. Bernal and Jelen (1985) reported that the highest denaturation temperature for an acid WPC prepared by ultrafiltration was 88 °C at pH 3.5, but no data were reported at pH 3.75 or 4.2. Quéguiner et al. (1992) found apparent transition temperatures of 80.1−80.2 °C at pH 3.5; 80.5 °C at pH 3.9; and 78.0 °C at pH 4.5, but no data were reported at intermediate pH values. Thus, it is possible that protein stability was a little higher at pH 4.2 than at pH 3.75, as suggested by results obtained in the present study.

The presence of CS did not modify the thermal stability of proteins, as represented by T_p, in the conditions of the present work. Also, an increase in

Table 1. Apparent Transition Temperature (T_p) and Enthalpy (ΔH_p) for Protein Denaturation of WPC−CS Dispersions[a]

	pH 3.75			pH 4.2		
	0% starch	2.1% starch	4.2% starch	0% starch	2.1% starch	4.2% starch
7.5% protein						
T_p (°C)	91.2 ± 0.3	91.2 ± 0.4	90.4 ± 0.7	91.9 ± 0.2	91.7 ± 0.3	91.7 ± 0.5
ΔH_p (J/g)	11.2 ± 1.5	9.1 ± 2.1	8.8 ± 0.5	8.7 ± 1.7	9.2 ± 0.7	7.9 ± 0.5
10% protein						
T_p (°C)	90.8 ± 0.3	90.4 ± 0.9	90.8 ± 0.8	91.8 ± 0.2	91.0 ± 0.4	91.7 ± 0.1
ΔH_p (J/g)	8.9 ± 0.9	8.5 ± 0.4	8.9 ± 0.8	8.1 ± 0.4	8.5 ± 0.9	8.1 ± 0.8

[a] Values are the average of at least three determinations.

Table 2. Apparent Transition Temperature (T_s) and Enthalpy (ΔH_s) for Starch Gelatinization of WPC−CS Dispersions[a]

	pH 3.75			pH 4.2		
	0% protein	7.5% protein	10.0% protein	0% protein	7.5% protein	10.0% protein
2.1% starch						
T_s (°C)	nd	70.5 ± 1.1	71.3 ± 0.1	nd	69.2 ± 1.3	71.2 ± 0.8
ΔH_s (J/g)	nd	9.7 ± 1.4	8.9 ± 2.7	nd	14.0 ± 0.8	10.6 ± 1.4
4.2% starch						
T_s (°C)	66.4 ± 0.6	72.2 ± 0.9	73.8 ± 0.3	66.7 ± 0.3	71.8 ± 0.5	73.3 ± 0.8
ΔH_s (J/g)	9.5 ± 1.2	11.9 ± 4.4	12.8 ± 3.7	9.2 ± 1.8	14.0 ± 4.4	17.4 ± 4.6

[a] Values are the average of at least three determinations. nd, not determined.

protein concentration from 7.5 to 10% did not have any effect on T_p (Table 1). These results suggest that neither CS nor whey proteins protect or sensibilize the whey proteins when they are submitted to a heat treatment.

Results of ΔH_p shown in Table 1 are in the same range as those reported in the literature (Quéguiner et al., 1992; Lupano et al., 1992). ΔH_p obtained at pH 3.75 was higher than ΔH_p at pH 4.2 (significant differences for $P \leq 0.05$), in agreement with results obtained by Quéguiner et al. (1992), who reported values of ΔH_p at pH 3.9 higher than those at pH 3.5 and 4.5.

CS did not modify the ΔH_p values under the conditions utilized in this work, indicating that the presence and the gelatinization of the starch did not produce protein denaturation.

Figure 4 shows that peaks of starch gelatinization shifted to higher temperatures in the presence of WPC. Also, T_s increased when CS increased (significant differences for $P \leq 0.05$) (Table 2). This is probably mainly because available water for starch gelatinization decreases as CS content increases or in the presence of WPC. Ghiasi et al. (1982) studied a dough system, which has a relatively low water content, and speculated that water migrates during starch gelatinization, and in complex food systems such as dough, gluten proteins severely limit such migrations. Also, the gelatinization of a dispersion of CS 4.2% (Table 2) presented T_s values lower than those obtained at a higher starch concentration by Moorthy et al. (1996), which agrees with the hypothesis of water availability.

To analyze the contribution of the WPC components to the shift of T_s, DSC assays were also performed with dispersions of CS−calcium and CS−lactose. The concentrations of calcium and lactose were the same as the corresponding WPC dispersions with 10% protein. Values of T_s obtained were 68.70 °C ± 0.22 and 67.79 °C ± 0.37 for CS−calcium and CS−lactose, respectively, indicating that calcium and lactose also contribute to the shift in T_s observed when WPC−CS dispersions were analyzed by DSC. The main shift in T_s was attributed to the presence of proteins.

It is known that nonionic soluble constituents such as sugars elevate the gelatinization temperature, compared to water alone (Xu and Shoemaker, 1986; Biliaderis, 1990), whereas electrolytes exhibit a more complex behavior. At low concentrations, NaCl and $CaCl_2$ slightly increased the gelatinization temperature (Biliaderis, 1990). Wu et al. (1985) found that thermal transitions of starch and fish protein seemed to proceed independently in mixture systems; however, the presence of salt and sucrose, necessitated by the inclusion of fish protein, caused starch gelatinization to shift to higher temperatures, exhibiting additive effects on the transition. This agrees with the results obtained in the present study.

ΔH_s values shown in Table 2 were similar to those found by Moorthy et al. (1996), who reported values of ΔH_s between 11.3 and 14.2 J/g when starch obtained from five varieties of cassava was analyzed.

CONCLUSIONS

Cassava starch increased the WHC of WPC gels and rendered them more aggregated. Starch gelatinizes before protein denaturation takes place during heating and breaks the protein network, no cogelation between whey proteins and CS being observed.

CS does not modify the apparent transition temperature or the ΔH for protein denaturation in the conditions utilized in the present study. On the other hand, starch gelatinization shifts to higher temperatures in dispersions containing WPC, due to the presence of whey proteins, lactose, and calcium.

Gels with different characteristics can be obtained by combining WPC and CS at pH values below the pI of the β-Lg, suggesting different applications in the food industry, such as salad dressings or lemon pie fillings.

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