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Whey protein concentrate gels with honey and wheat flour

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Abstract

Structural and functional properties of whey protein concentrate (WPC) gels with different honey and wheat flour contents, prepared at pHs 3.75, 4.2 and 7.0, were analysed. Gel structure was observed by scanning electron microscopy. The apparent transition temperatures for protein denaturation and starch gelatinization were determined by differential scanning calorimetry. Gels were characterised through solubility assays in different extraction solutions and polyacrylamide gel electrophoresis of the soluble protein components. The firmness, elasticity, relaxation time, adhesivity and cohesiveness of gels were determined, and the water-holding capacity and superficial colour of gels were also studied. Results suggest that wheat flour could interact with whey proteins, and produces a decrease in the protein solubility of WPC gels, and in the temperature of whey protein denaturation. The effect of wheat flour on the functional properties of WPC gels was different at acidic than at neutral pH: the presence of wheat flour produced an increase in the relaxation time and in the cohesiveness of gels prepared at pH 3.75, whereas at neutral pH a decrease in both properties was observed. Honey and flour content increased the water-holding capacity and browning of WPC gels.

Keywords: Whey protein concentrate; Gelation; Wheat flour; Honey

1. Introduction

Whey protein concentrates (WPC) are widely used in the food industry because of their nutritional and functional properties. An important functional property of whey proteins is the ability, under appropriate conditions, to form heat induced gel structures capable of immobilise large quantities of water and other food components (Hermansson & Akesson, 1975). The formation of a gel is the result of an equilibrium between intermolecular attractive forces, 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).

Reactivity of SH groups, which enhances both the oxidation of sulphydryl groups into disulphide bonds and sulphydryl–disulphide 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 sulphydryl–disulphide interchange reactions are favoured (Shimada & Cheftel, 1988). As a consequence, gels prepared at acid pH are different from those prepared at neutral pH (Lupano, Dumay, & Cheftel, 1992, 1996; Shimada & Cheftel, 1988). Also, when pH approaches the pI, the charge of the proteins is progressively neutralised, favouring protein aggregation (Lupano et al., 1992, Lupano, Renzi, & Romera, 1996). The susceptibility of whey proteins to denaturation is largely determined by the pH (de Wit, 1981), being their maximum stability in distilled water at pH 3–4 (Hegg, 1980).

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As a consequence, the temperature of whey protein denaturation at acidic pH is higher than that at neutral pH (Lupano et al., 1992).

Several studies have been performed concerning the effect of the presence of other food components on WPC gels: on mixed gels WPC–cassava starch, WPC–gluten, and WPC–honey (Lupano, 2000; Lupano & González, 1999; Yamul & Lupano, 2003). The presence of other components can affect the susceptibility of whey proteins to thermal denaturation. It was observed that cassava starch does not modify the thermal stability of whey proteins (Lupano & González, 1999), whereas gluten decreases slightly the temperature of whey protein denaturation (Erdogdu, Czuchajowska, & Pomeranz, 1995; Lupano, 2000). Honey, on the other hand, increases the temperature of whey protein denaturation at both acidic and neutral pH, suggesting a protective effect against protein denaturation (Yamul & Lupano, 2003).

No information is available concerning WPC gels containing wheat flour and honey. This study would provide useful information about the role of each component on the gel structure and properties. These gels could be used as dessert fillings, having the advantage of combine the high nutritional quality of whey proteins with the functional properties of gluten, and the delicate taste of honey.

2. Materials and methods

2.1. Materials

WPC was prepared by large scale ultrafiltration by Williner S.A. (Rafaela, Santa Fe, Argentina). WPC contained 49.3% protein [calculated as [total nitrogen (8.0%) − nonprotein nitrogen (0.3%)] × 6.38], 5.1% moisture, 6.0% ash, 5.6% lipids and 32.3% lactose (estimated by difference). The nitrogen solubility index was 80.9% at pH 7.0 and 70.8% at pH 4.75. Analytical methods were described by Lupano et al. (1996). Honey was harvested in the Province of Buenos Aires and contained 16.9% moisture, 76.3% glucose plus fructose, and 1.7% sucrose. Wheat flour was from S.A. Miguel Campodónico Ltda (La Plata, Buenos Aires, Argentina) and contained 10% of proteins and 13% moisture.

2.2. Preparation of WPC–honey–wheat flour dispersions and gels

Aqueous dispersions (10.0% whey protein; 0%, 10%, 20% or 25% honey; 0%, 10% and 20% wheat flour, w/w) of WPC–honey–wheat flour were adjusted to pH 3.75, 4.2 and 7.0 with 1–3 N HCl or 1N NaOH. Homogeneous dispersions were placed into 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). After heating, the tubes were cooled rapidly to room temperature in tap water and kept at 4 °C for at least 15 h before analysis. Gels were equilibrated at room temperature before all functional determinations. Samples for differential scanning calorimetry (DSC) were prepared in the same way but without heating.

2.3. Differential scanning calorimetry

A differential scanning calorimeter (Rheometric Scientific Ltd., Epsom, Surrey, UK) calibrated with indium was used. Homogeneous samples of 8–15 mg of WPC and WPC–honey–wheat flour dispersions were placed in aluminum DSC hermetic pans. An empty double pan was used as reference. Sample and reference were heated between 25 and 120 °C at a heating rate of 10 °C/min. The apparent transition temperature for protein denaturation (T_p) and for starch gelatinization (T_s) were computed from the endothermic peaks. At least two independent replicates were obtained.

2.4. Determination of the protein solubility of gels

In order to analyse which kind of bonds are involved in the maintenance of the gel structure, gels 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 sulphate (SDS) and 8 M urea (BSU) (Lupano et al., 1992, 1996; Shimada & Cheftel, 1988). These solvents can disrupt different kind of bonds: B can disrupt electrostatic bonds, whereas BSU can disrupt hydrophobic and hydrogen bonds. Gel samples (0.1% total protein, w/v) were homogenised at room temperature with an Ultra-Turrax T25 (IKA Labortechnik, Stauffen, Germany) at 8000 rpm for 1 min, and then centrifuged at 17,400 rpm for 30 min. Protein solubility was determined from supernatants and expressed as 100 × protein content in the supernatant/total protein content. Three independent replicates were carried out with each solvent. Protein concentration was determined with a Beckman DU 650 Spectrophotometer (California, USA) at 280 nm with apparent $E^{1\%}_{1\,cm}$ values of 8.636 for DW solutions, determined by measuring the absorbance at 280 nm and the protein content by the Kjeldahl method ($N \times 6.38$) in the same protein solution (Lupano et al., 1996; Yamul & Lupano, 2003) and 10.2 for pH 8.0 solutions (Lupano et al., 1996; Shimada & Cheftel, 1988; Yamul & Lupano, 2003).

2.5. Scanning electron microscopy

Samples were fixed in triplicate in 2.5% glutaraldehyde, dehydrated in a grade acetone series, 25, 50, 70,

90, and 3 three times 100% v/v, and dried at the critical point (Sorrivas de Lozano & Morales, 1983). Dried samples were coated with gold (about 300 Å) in a Sputter coater model 3 PELCO 91000 (Ted Pella Inc., CA, USA), and observed with a JEOL 35 CF (Tokio, Japan) scanning electron microscope, at an acceleration voltage of 5 kV. Magnification: 1000×.

2.6. Electrophoresis

In order to analyse the protein species which are involved in the maintenance of the gel structure, gel protein extracted either by DW, B or BSU were analysed through sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS–PAGE), according to the method of Laemmli (1970). A linear gradient separating gel (5–15% in polyacrylamide, with an acrylamide: bisacrylamide ratio of 75:2), with 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 whey protein/mL) were diluted with an equal volume of sample buffer (0.01 M Tris–HCl, 0.001 M EDTA, 1% SDS, 5% β-mercaptoethanol (β-ME), v/v, and about 30% of glycerol, v/v, pH 8.0). Low MW markers (Amersham Pharmacia Biotech, Inc. calibration kit, Uppsala, Sweden) 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 Gel Doc 1000 Image Analysis System (Bio Rad, Richmond, CA, USA). The results were analysed by using the Molecular Analyst software Version 1.5 (Bio Rad, Richmond, CA, USA). Two independent replicates of each sample were analysed.

2.7. Water-holding capacity of gels

A disk of gel of about 2-mm height and 2.2-cm diameter was cut into two pieces. Each piece was placed on a nylon plain membrane (5.0-μm pores, Microseparations Inc., Westboro, MA) 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). Water-holding capacity (WHC) was expressed as percent of the initial water remaining in the gel after centrifugation. At least two independent replicates were obtained.

2.8. Determination of gel properties

Rheological analyses were performed on gel sections (22 mm diameter × 20 mm height) using a TA.XT2 Texture analyzer (Stable Micro Systems Ltd., Surrey, UK) in the compression mode. Compression was exerted by a cylindric probe with a flat section (75-mm diameter) at a displacement speed of 1 mm/s. Gel firmness was defined as the force F_0 (Newtons) measured at 20% (4 mm) compression (Lupano et al., 1992). This compression was maintained for 20 min, and the force F_{20} exerted on the probe was measured. Gel elasticity was calculated as F_{20}/F_0, and relaxation time τ was taken as the time at which $F = (F_0 + F_{20})/2$ (Lupano et al., 1992; Peleg, 1979; Yamul & Lupano, 2003). The measurements of gel adhesivity and gel cohesiveness were performed with two compression cycles. Gel adhesivity was calculated as the negative force area obtained after the first compression cycle, representing the work necessary to pull the compressing plunger away from the sample. Gel cohesiveness was calculated as the ratio of the positive force area during the second compression to that during the first compression (A_2/A_1) (Bourne, 1978; Yamul & Lupano, 2003). For each type of gel, at least three independent replicates were obtained.

2.9. Colour

Superficial gel colour was measured with a Chroma meter CR 300 Minolta (Osaka, Japan), and Hunter parameters were determined. The hue angle $h°$ was calculated from the arctangent of b^*/a^*. As $a^* < 0$, $h° = 180 + \arctan\ b^*/a^*$ (McGuire, 1992). Values are the average (±standard error) of two or three independent replicates.

2.10. Statistics

In order to estimate the influence of the factors pH, flour and honey on the gel characteristics, an analysis of variance (ANOVA) of the data was performed by using the Systat 5.0 statistical software. The influence of the extraction media on gel protein solubility was also considered as a factor in the solubility assays. Each factor presented three levels, except in the temperature of starch gelatinization, in which the factors pH and flour had two levels. Independent replicates of each sample were performed.

3. Results and discussion

3.1. Differential scanning calorimetry

Fig. 1 shows some thermograms obtained when WPC dispersions with 20% wheat flour and different honey contents, prepared at different pHs, were heated in the DSC apparatus. Two separate endothermic peaks were observed in WPC–flour mixtures prepared at acidic pH; the lower temperature peak corresponding to starch

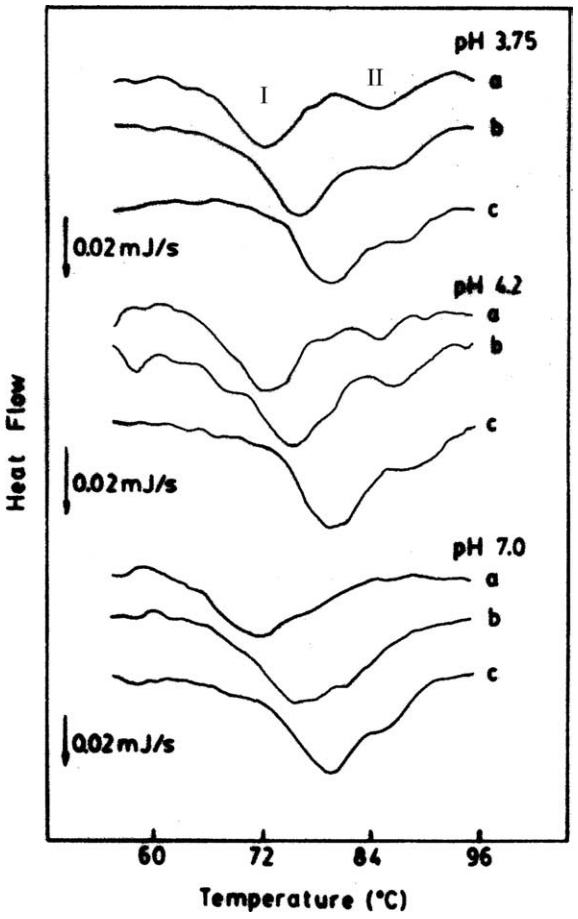

Fig. 1. DSC thermograms of WPC–wheat flour dispersions. Whey protein concentration: 10%, w/w. Wheat flour concentration: 20%, w/w. Honey content: (a) 0%; (b) 10%; (c) 20%, w/w. I, starch gelatinization; II, protein denaturation.

gelatinization and the higher temperature peak to protein denaturation. This indicates that starch gelatinization occurs first, followed by protein denaturation, as was already observed in WPC–cassava starch systems (Lupano & González, 1999). The peaks corresponding to protein denaturation and starch gelatinization overlaps at pH 7.0.

The apparent transition temperature for protein denaturation (T_p) of WPC dispersions containing different amounts of wheat flour as a function of honey content is shown in Fig. 2(a)–(c). The analysis of variance showed that there are significative differences ($P < 0.01$) between samples prepared at different pHs, and with different flour and honey content. Also, significative interactions were found between the factors pH–flour content and pH–honey content ($P < 0.01$). Honey increased the T_p at all conditions assayed, whereas wheat flour decreased T_p at acidic pH. At neutral pH a higher sensitivity of whey proteins to thermal denaturation was observed, confirming existing knowledge (Hegg, 1980). In gels containing both honey and wheat flour, two opposite effects would take place: the decrease in T_p due to the presence of wheat flour, and the increase in T_p produced by honey, which, according to Kulmyrzaev, Bryant, and McClements (2000), would increase the surface free energy between water and an hydrophobic surface, such as the area exposed to the solvent during protein unfolding.

The effect of honey concentration on the gelatinization temperature (T_s) is presented in Fig. 2(d)–(f). The analysis of variance showed that pH did not modify the T_s at any of the conditions assayed ($P > 0.05$), but T_s increased with honey concentration at both neutral and acidic pHs ($P < 0.01$). It must be taken into account that the water content of samples with wheat flour and honey was lower than the corresponding to samples without these components. Also, those components like honey, which has the ability of strongly hold water, delay the starch gelatinization because they compete with starch for the available water molecules. T_s increased when wheat flour content increased ($P < 0.01$), and significative interactions ($P < 0.05$) were found between flour and honey. According to Ghiasi, Hoseney, and Varriano-Marston (1982) water migrates during starch gelatinization, and in complex food systems other components such as flour proteins can severely limit such migrations (Jovanovich et al., 2003; Manley, 2000). As a consequence, when wheat flour content increases from 10% to 20%, less water is available for starch gelatinization and, hence, higher values of T_s are expected. This effect was more important at higher honey contents, probably due to the fact that honey also decreases the water availability to starch gelatinization.

3.2. Scanning electron microscopy

Fig. 3 shows the structure of gels observed by scanning electron microscopy (SEM). Starch granules are surrounded by gluten and whey proteins in gels containing wheat flour (Fig. 3(d)–(i)). However, a continuous gluten network was not observed in these gels, confirming the fact that whey proteins break the gluten structure (Lupano, 2000). Whey proteins could be able to form hydrogen and disulphide bonds with gluten proteins, interfering with the gluten network formation. On the other hand, gluten network can be observed mainly in acid gels (Fig. 3) in which the possibility of whey proteins to participate in sulphydryl–disulphide interchange reactions with gluten proteins practically does not occur. Also, gluten was better observed in acid gels without honey, probably due to the sugar interference in the formation of gluten.

3.3. Protein solubility of gels

Fig. 4 depicts the protein solubility of gels as a function of honey content. Samples with different pH, flour

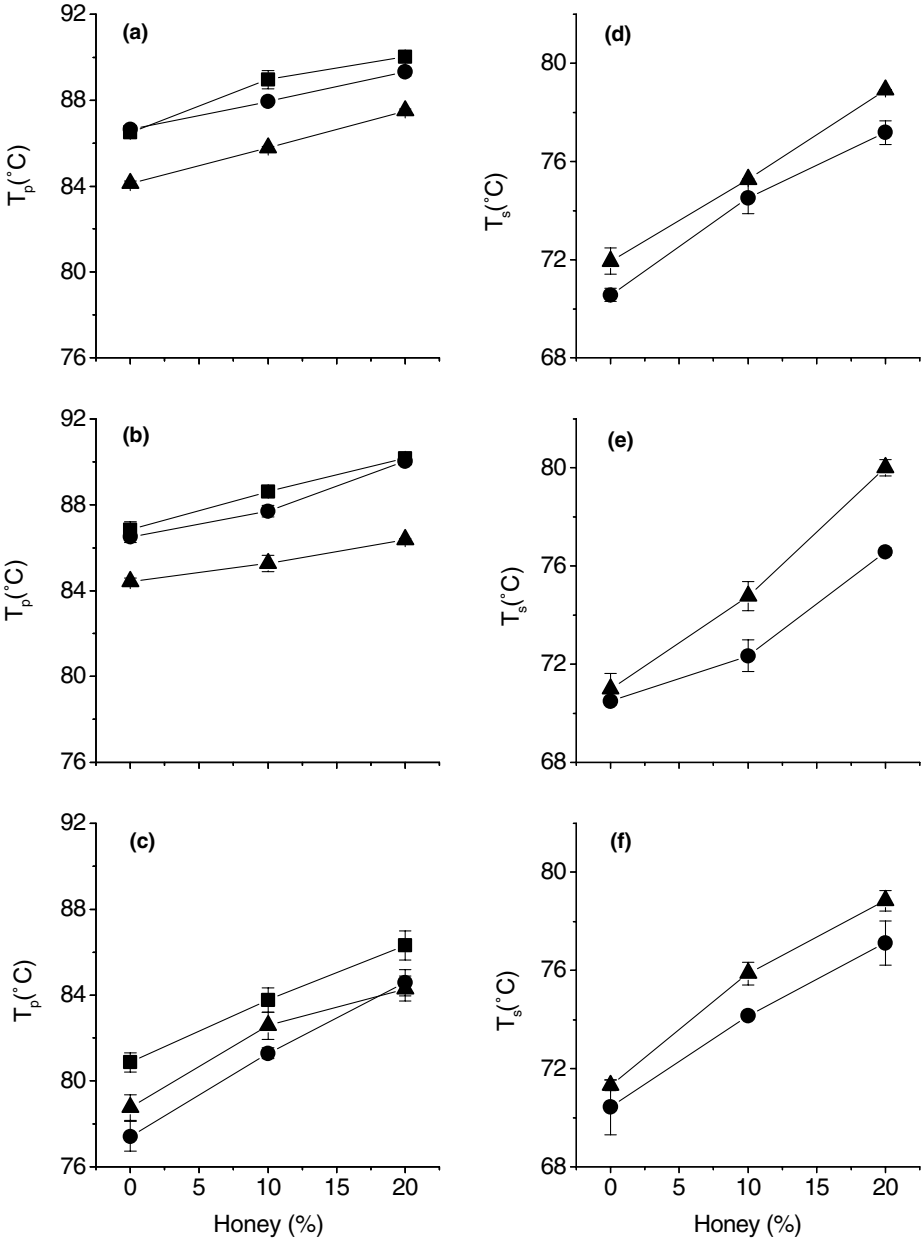

Fig. 2. Apparent transition temperature (T_p) for protein denaturation and starch gelatinization (T_s) of WPC dispersions as a function of honey content, expressed as percentage of the total mass of the sample. Whey protein concentration: 10%, w/w. (a),(d) pH 3.75, (b),(e) pH 4.2, (c),(f) pH 7.0. Wheat flour content: (■), 0%; (●), 10%; (▲), 20%.

content, honey content, or extraction medium presented different protein solubility ($P < 0.01$). Significative interactions were found between the factors pH–extraction medium, pH–flour content, pH–honey content, extraction medium–flour content, extraction medium–honey content ($P < 0.01$), flour content–honey content ($P < 0.05$), pH–flour content–extraction medium, pH–honey content–extraction medium ($P < 0.01$), pH–flour content–honey content ($P < 0.05$), and pH–flour content–honey content–extraction medium ($P < 0.05$).

The protein solubility of gels with wheat flour was lower than that of the same gels without flour, at all conditions assayed. Wheat proteins are less soluble than whey proteins, but the ratio wheat protein:whey protein in gels was 1:10 or 2:10; hence, the lower solubility of wheat proteins is not sufficient to explain the decrease in the protein solubility of gels containing wheat flour. It is possibly that wheat flour interacts with whey proteins decreasing their solubility, as was suggested by results of DSC and SEM.

The high solubility in BSU of protein components in the acidic gels indicates that noncovalent bonds are the responsible of the maintenance of the structure in these gels, in agreement with previous results

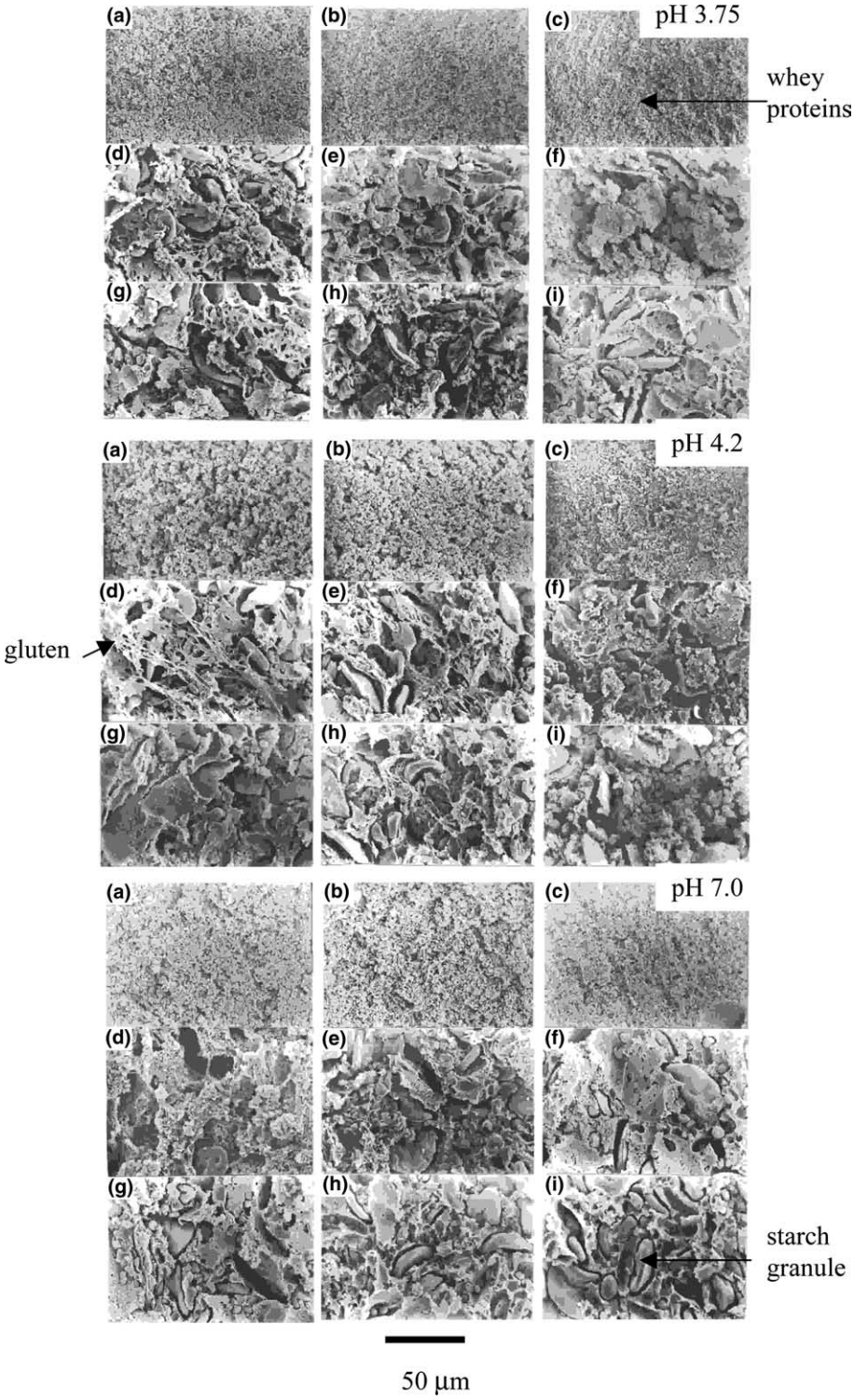

Fig. 3. Scanning electron microscopy of WPC gels. Whey protein content of gels: 10%, w/w. Wheat flour content: (a)–(c), 0%; (d)–(f), 10%; (g)–(i), 20%, w/w. Honey content: (a),(d),(g) 0%; (b),(e),(h) 10%; (c) 37.5%; (f),(i) 25%, w/w.

obtained in gels without wheat flour (Lupano et al., 1992, 1996; Puppo, Lupano, & Añón, 1995). This fact was expected because sulphydryl–disulphide interchange reactions practically do not take place at acid pH. Protein solubility in B was higher in gels prepared at pH 3.75 with respect to gels prepared at pH 4.2. As B can disrupt ionic bonds, these results suggest that these bonds are more important in the

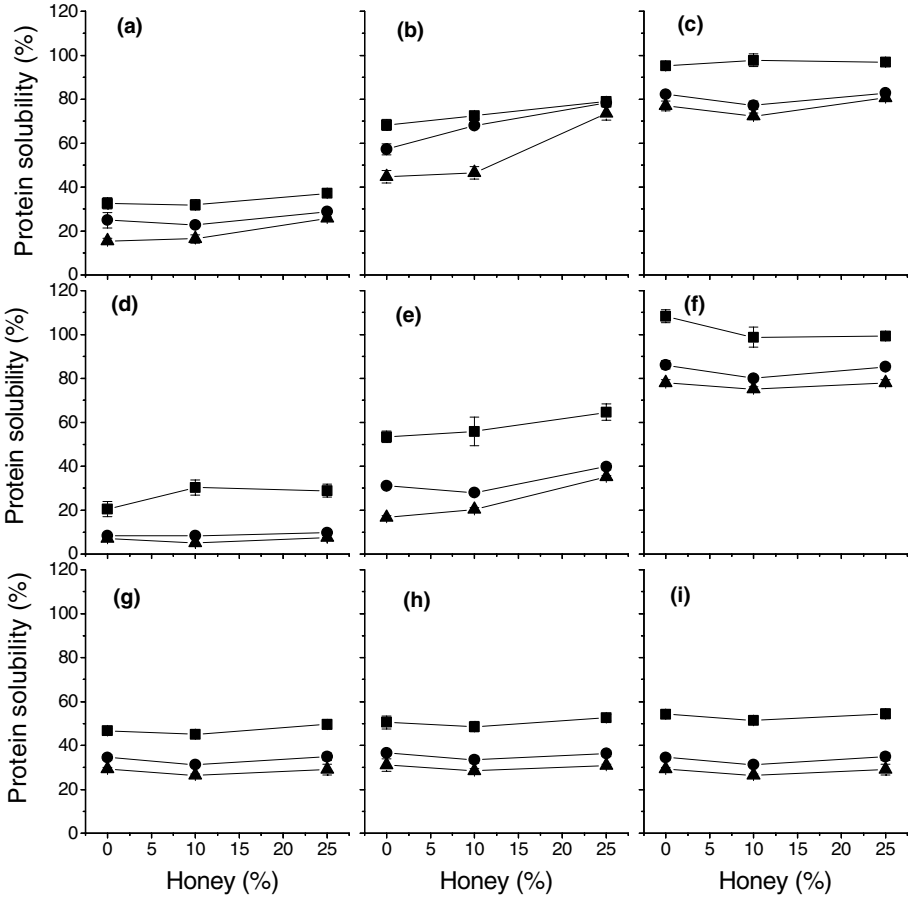

Fig. 4. Solubility of the protein constituents of WPC gels as a function of honey content. Whey protein content of gels: 10%, w/w. Protein concentration of all solubilisation assays: 0.1% w/v. pH of gels: (a)–(c), 3.75; (d)–(f), 4.2; (g)–(i), 7.0. Extraction solutions: (a),(d),(g) distilled water; (b),(e),(h) standard buffer, pH 8.0 (B); (c),(f),(i) standard buffer containing 8 M urea and 0.5% SDS (BSU). Wheat flour content: (■), 0%; (●), 10%; (▲), 20%.

maintenance of the gel structure at pH 3.75 than at pH 4.2.

Sulphydryl–disulphide interchange reactions are favoured at pH 7.0. As the extraction media assayed cannot disrupt disulphide bonds, it is expected that the gel protein constituents have a low solubility at neutral pH, as was observed in Fig. 4(g)–(i).

3.4. Electrophoresis (SDS–PAGE) of WPC gels

Fig. 5 shows the electrophoretic patterns of protein species extracted with DW, B and BSU from gels prepared at different pHs and wheat flour contents. Samples were treated with β-ME before electrophoresis. The patterns reflect the differences in protein solubility in these extraction solutions. In gels prepared at pH 3.75, the solubility of β-lactoglobulin (β-Lg) increased as the extraction solution changed from distilled water to B, and from B to BSU, whereas the solubility of α-lactalbumin (α-La) increased mainly when the extraction solution changed from distilled water to B. This indicates that β-Lg would contribute to the maintenance of the gel structure through electrostatic, hydrophobic and hydrogen bonds, whereas α-La mainly through electrostatic bonds. Also, it can be observed that the peaks corresponding to (α-La) and the monomer of β-Lg tend to decrease as the flour content of gel increases. These results are in accordance with the solubility assays, and indicate that there would be an interaction between flour and whey proteins, as was discussed earlier.

The peaks of gels prepared at pH 4.2 were smaller than the corresponding to gels prepared at pH 3.75. It was observed that the peaks of α-La and β-Lg increased when the solution extraction changes from distilled water to B, and from B to BSU, suggesting that these proteins could contribute to the maintenance of the gel structure through electrostatic, hydrophobic and hydrogen bonds.

Finally, all patterns of gels prepared at pH 7.0 presented very small peaks, in agreement with solubility assays, indicating that disulphide bonds are very

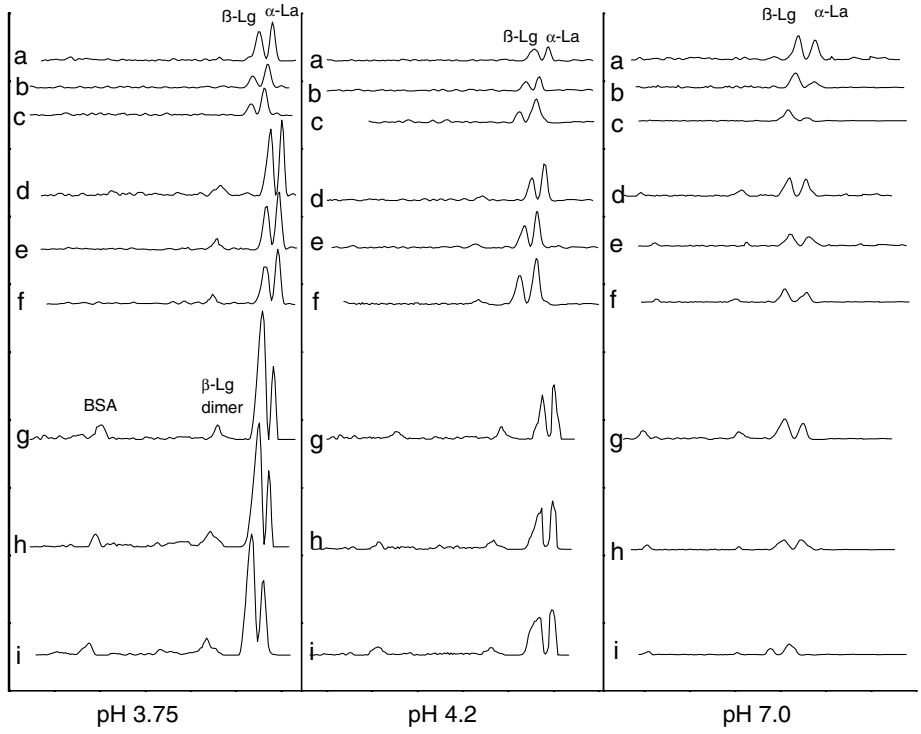

Fig. 5. Electrophoretic patterns of heat-induced gels from WPC–flour. Samples were treated with β-mercaptoethanol before electrophoresis. Whey protein concentration of gels: 10%, w/w. Extraction solutions: (a)–(c) distilled water; (d)–(f) standard buffer, pH 8.0 (B); (g)–(i) standard buffer containing 8 M urea and 0.5% SDS (BSU). Wheat flour content: (a),(d),(g) 0%; (b),(e),(h) 10%; (c),(f),(i) 20%.

important in the maintenance of the structure of these gels.

3.5. Water-holding capacity

The WHC of WPC–honey–wheat flour gels is shown in Fig. 6. The analysis of variance showed that the WHC of gels with different pH, flour or honey content is significatively different ($P < 0.01$). Significative interactions between the factors pH–flour content, pH–honey content, flour content–honey content, and pH–flour content–honey content were found ($P < 0.01$).

The WHC increased with honey content at all pHs assayed. The lowest values of WHC corresponded to gels without flour, prepared at pH 4.2. The effect of pH agrees with previous results (Yamul & Lupano, 2003). The WHC of gels with flour was near 100%, probably due to the ability of starch to hold water when gelatinization takes place, and the presence of gluten proteins which can form hydrogen bonds with water (1/3 of the aminoacid residues of gluten proteins is glu-

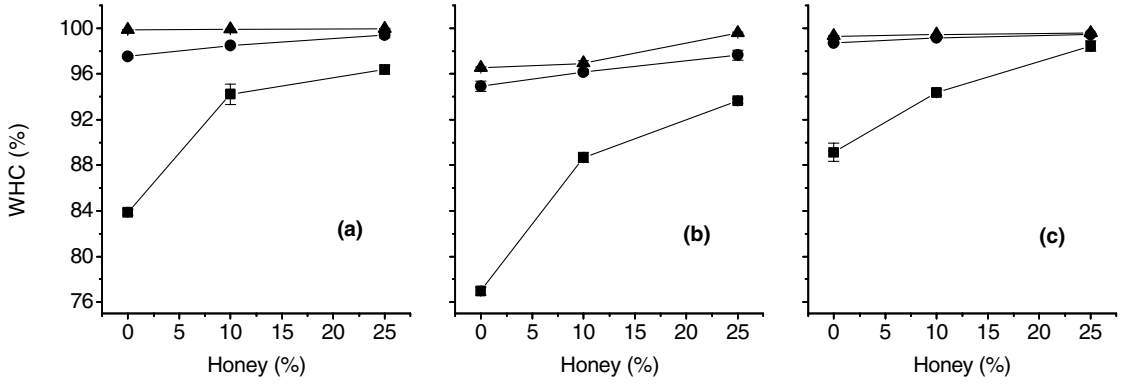

Fig. 6. Water-holding capacity (WHC) of WPC gels as a function of honey content, expressed as percentage of the total mass of the sample. (a) pH 3.75, (b) pH 4.2, (c) pH 7.0. Whey protein content of gels: 10%, w/w. Flour content of gels: (■) 0%; (●) 10%; (▲) 20%, w/w.

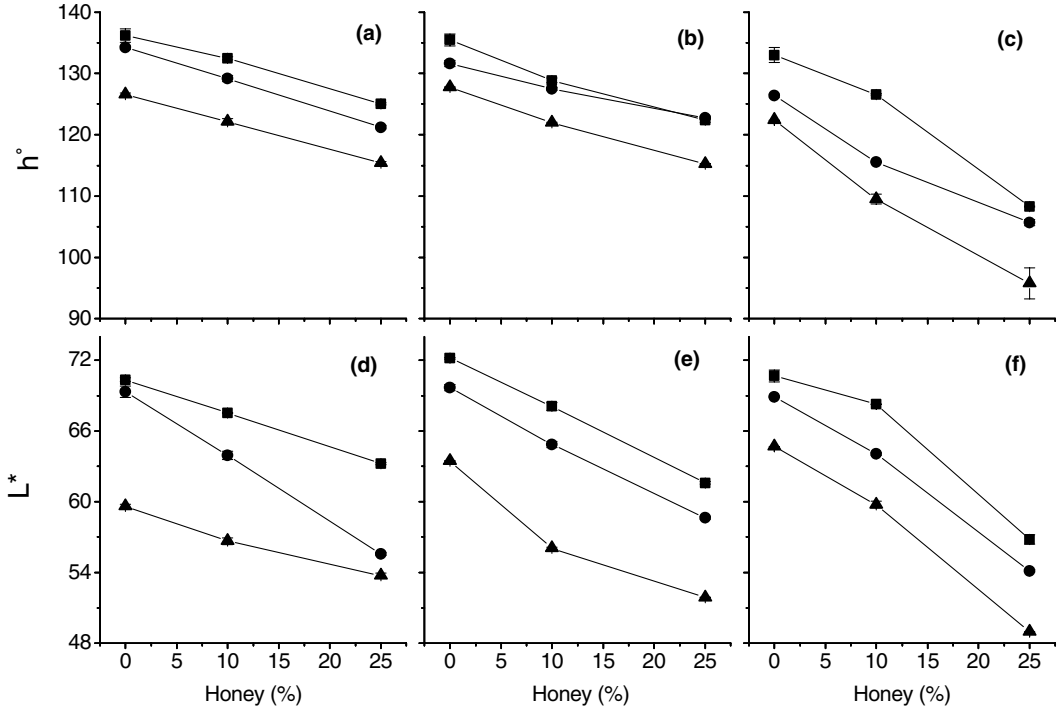

Fig. 7. Parameters $h°$ and $L*$, of WPC gels as a function of honey content, expressed as percentage of the total mass of the sample. Whey protein content of gels: 10%, w/w. Flour content of gels: (■), 0%; (●), 10%; (▲), 20%, w/w. (a),(d) pH 3.75; (b),(e) pH 4.2; (c),(f) pH 7.0.

tamin). In fact, gluten proteins can absorb twice its weight of water (Manley, 2000).

3.6. Colour

Fig. 7 shows the parameters $h°$ and $L*$ of WPC–honey–flour gels. Significative differences ($P < 0.01$) in these two parameters were found between gels prepared at different pHs and with different flour and honey content. Significative interactions ($P < 0.01$) between pH–flour content, pH–honey content, flour content–honey content, and pH–flour content–honey content, were also found.

The hue angle $h°$ was considered instead of $a*$ and $b*$, as suggested by McGuire (1992). Confirming previous results (Yamul & Lupano, 2003), honey decreased $L*$ (lightness) of WPC gels at all pHs assayed, being the gels containing higher honey and flour content those that present the highest browning. The $h°$ values were between 90° (yellow) and 180° (bluish-green). When honey content increased, gel colour approaches to yellow zone, being this effect more important at neutral pH, which favors the Maillard reactions. Flour content also modified the colour of WPC gels towards the yellow zone (Fig. 7).

3.7. Gel properties

Fig. 8 shows the firmness, elasticity and relaxation time of WPC–honey–flour gels. Gels with different pH, flour or honey contents presented different firmness, elasticity and relaxation time ($P < 0.01$). Significative interactions between the factors pH–flour content and pH–honey content were found in the firmness, elasticity and relaxation time, and between flour content–honey content and pH–flour content–honey content in the firmness ($P < 0.05$), elasticity and relaxation time ($P < 0.01$).

Honey increased the firmness and decrease the elasticity of gels containing wheat flour, mainly at pH 7.0. Also, honey decreased the relaxation time of gels at pH 4.2 and 7.0, in accordance with previous results (Yamul & Lupano, 2003). On the other hand, wheat flour had a marked effect on the firmness and elasticity, increasing both parameters at all pHs assayed.

The relaxation time increased when the pH increased from 3.75 to 7.0 on gels without honey and flour, in accordance to previous results (Lupano, 2000; Lupano et al., 1992; Yamul & Lupano, 2003). As honey decreases the relaxation time of WPC gels, the effect of pH was less important in gels with higher honey content (Fig. 8). The relaxation time of gels prepared at pH 3.75 increased when wheat flour content increased from 0% to 20%, but no effect was observed in gels prepared at pH 4.2. Similar results were reported in gels prepared with WPC–gluten (Lupano, 2000). On the contrary, the relaxation time of gels prepared at pH 7.0 decreased with wheat flour content. A higher interaction between wheat flour and whey proteins could take place at

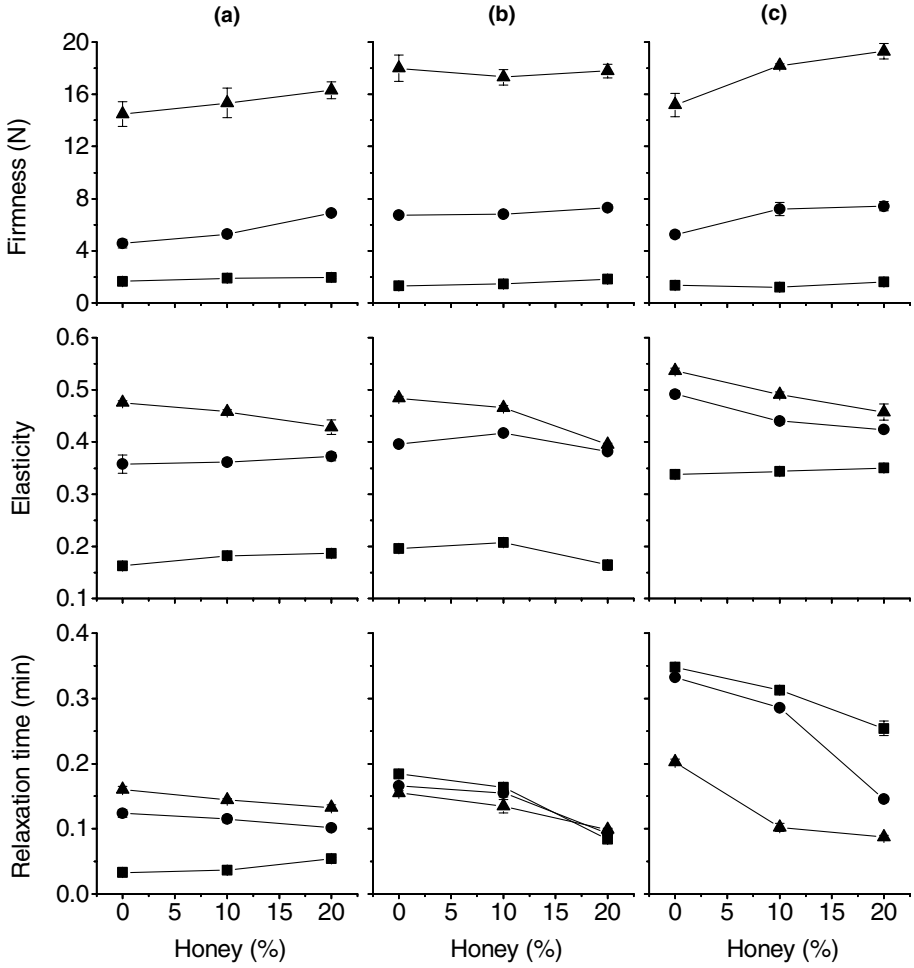

Fig. 8. Firmness, elasticity and relaxation time of WPC gels as a function of honey content, expressed as percentage of the total mass of the sample. Whey protein content of gels: 10%, w/w. Flour content of gels: (■), 0%; (●), 10%; (▲), 20%, w/w. (a) pH 3.75; (b) pH 4.2; (c) pH 7.0.

neutral pH, through noncovalent and covalent bonds, resulting in the fact that the addition of 20% flour would counteract the effect of pH.

Fig. 9 shows the adhesivity and cohesiveness of WPC–honey–flour gels. Significative differences ($P < 0.01$) were found in the adhesivity of gels prepared at different pHs, and with different flour and honey contents, and interactions between pH–flour content, pH–honey content, flour content–honey content, and pH–flour content–honey content, were also found ($P < 0.01$). The adhesion of a material can be described in terms of the sum of two energy contributions, the surface energy and the cohesive energy. The surface energy depends on the type and strength of bonding between the adhesive and the substrate, whilst the cohesive energy represents the energy dissipated in viscoelastic and plastic deformation within the adhesive (Dobraszczyk, 1997). The adhesivity of gels increased with wheat flour content, mainly in gels prepared at pH 7.0 and 4.2. Honey, on the other hand, increased the gel adhesivity at acidic pH, being this effect more evident in gels prepared at pH 4.2 with wheat flour.

Cohesiveness is defined as the ratio of the positive force area during the second compression to that during the first compression. Significative differences were found in the cohesiveness of gels prepared at different pHs, and with different flour and honey contents ($P < 0.01$). Interactions between pH–flour content and pH–honey content ($P < 0.01$) were also found. Cohesiveness was higher in those gels prepared at neutral pH. This can be explained taking into account that disulphide bonds are involved in the maintenance of the structure of neutral gels, whereas noncovalent bonds are responsible for maintenance of the structure of acid gels. Wheat flour increased the cohesiveness of gels prepared at pH 3.75 and 4.2, suggesting an increase in the strength or density of cross-links between molecules in the gel structure. Wheat flour could increase the noncovalent cohesive force that held molecules together in acid gels. On the contrary, wheat flour decreased a little the cohesiveness of pH 7.0 gels, probably interfering with the disulphide bonds formation between whey protein molecules. Honey, on the other hand, tended to decrease the cohesiveness of gels prepared at pH 4.2 and

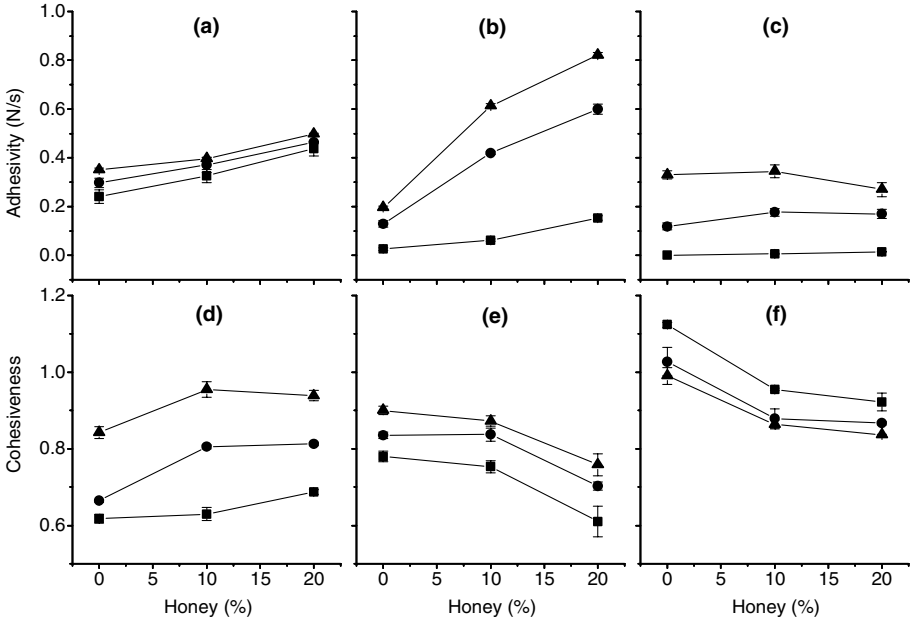

Fig. 9. Adhesivity and cohesiveness of WPC gels as a function of honey content, expressed as percentage of the total mass of the sample. Whey protein content of gels: 10%, w/w. Flour content of gels: (■), 0%; (●), 10%; (▲), 20%, w/w. (a),(d) pH 3.75; (b),(e) pH 4.2; (c),(f) pH 7.0.

7.0, and to increase the cohesiveness of gels prepared at pH 3.75 (Fig. 9).

4. Conclusions

Results suggest that wheat flour interacts with whey proteins, through noncovalent bonds and, at neutral pH, also through disulphide bonds. The presence of flour produces a decrease in the protein solubility of whey protein concentrate gels, and in the temperature of whey protein denaturation, also observing an increase in the firmness, elasticity and adhesivity of whey protein concentrate gels. The effect of wheat flour on the functional properties of whey protein concentrate gels is different at acidic than at neutral pH: the presence of wheat flour produces an increase in the relaxation time and cohesiveness of gels prepared at pH 3.75, whereas at neutral pH it was observed a decrease in both relaxation time and cohesiveness of gels. Honey increases the adhesivity of acidic gels, being this effect more important in gels prepared at pH 4.2 with wheat flour. Honey and flour increases the water-holding capacity and browning of WPC gels.

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References

Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*, 62–66, 72.

de Wit, J. N. (1981). Structure and functional behaviour of whey proteins. *Netherlands Milk Dairy Journal, 35*, 47–64.

Dobraszczyk, B. J. (1997). The rheological basis of dough stickiness. *Journal of Texture Studies, 28*, 139–162.

Erdogdu, N., Czuchajowska, Z., & Pomeranz, Y. (1995). Wheat flour and defattted milk fractions characterized by differential scanning calorimetry II. DSC interaction products. *Cereal Chemistry, 72*, 76–79.

Gault, P., & Fauquant, J. (1992). Aptitude à laa gélification thermique de la β-lactoglobuline: influence du pH, de l'environnement ionique et de la présence des autres protéines du lactosérum. *Lait, 72*, 491–510.

Ghiasi, K., Hoseney, R. C., & Varriano-Marston, E. (1982). Effects of flour components and dough ingredients on starch gelatinization. *Cereal Chemistry, 60*, 58–61.

Hegg, P.-O. (1980). Thermal stability of β-lactoglobulin as a function of pH and the relative concentration of sodium dodecyl sulphate. *Acta Agriculturae Scandinavica, 30*, 401–404.

Hermansson, A. M., & Akesson, C. (1975). Functional properties of added proteins correlated with properties of meat system. Effect of salt on water. Binding properties of model meat systems. *Journal of Food Science, 40*, 603–610.

Kulmyrzaev, A., Bryant, C., & McClements, D. J. (2000). Influence of sucrose on the thermal denaturation, gelation, and emulsion stabilization of whey proteins. *Journal of Agricultural and Food Chemistry, 48*, 1593–1597.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature, 227*, 680–685.

Lupano, C. E. (2000). Gelation of mixed systems whey protein concentrate–gluten in acidic conditions. *Food Research International, 33*, 691–696.

Lupano, C. E., Dumay, E., & Cheftel, J. C. (1992). Gelling properties of whey protein isolate: influence of calcium removal by dialysis or diafiltration at acid or neutral pH. *International Journal of Food Science and Technology, 27*, 615–628.

Lupano, C. E., & González, S. (1999). Gelation of whey protein concentrate–cassava starch in acidic conditions. *Journal of Agricultural and Food Chemistry, 47*, 918–923.

Lupano, C. E., Renzi, L. A., & Romera, V. (1996). Gelation of whey protein concentrate in acidic conditions: effect of pH. *Journal of Agricultural and Food Chemistry, 44*, 3010–3014.

Manley, D. (2000). Wheat flour and vital wheat gluten. In *Technology of biscuits, crackers and cookies* (3rd ed) (pp. 87). Cambridge, England: Woodhead Publishing Limited.

McGuire, R. G. (1992). Reporting of objective color measurements. *HortScience, 27*, 1254–1255.

Peleg, M. (1979). Characterization of the stress relaxation curves of solid foods. *Journal of Food Science, 44*, 277–281.

Puppo, M. C., Lupano, C. E., & Añón, M. C. (1995). Gelation of soybean protein isolates in acidic conditions. Effect of pH and protein concentration. *Journal of Agricultural and Food Chemistry, 43*, 2356–2361.

Quéguiner, C., Dumay, E., Cavalier, C., & Cheftel, J. C. (1989). Reduction of *Streptococcus thermophilus* in a whey protein isolate by low moisture extrusion cooking without loss of functional properties. *International Journal of Food Science and Technology, 24*, 601–612.

Shimada, K., & Cheftel, J. C. (1988). Texture characteristics, protein solubility, and sulphydryl group/disulphide bond contents of heat-induced gels of whey protein isolate. *Journal of Agricultural and Food Chemistry, 36*, 1018–1025.

Sorrivas de Lozano, V. & Morales, A. (1983). *Introducción a la microscopía electrónica*. Bahía Blanca: CRIBABB.

Tolstoguzov, V. B. (1993). Functional properties of food proteins. Role of interactions in protein systems. In K. D. Schwenke & R. Mothes (Eds.), *Food proteins. Structure and functionality* (pp. 203–209). Weinheim: VHC.

Yamul, D. K., & Lupano, C. E. (2003). Properties of gels from whey protein concentrate and honey at different pHs. *Food Research International, 36*, 25–33.