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Properties of gels from whey protein concentrate and honey at different pHs

Diego K. Yamul, Cecilia E. Lupano*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, UNLP-CONICET, 47 y 116, 1900 La Plata, Argentina

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Abstract

Structural and functional properties of heat-induced gels from whey protein concentrate (WPC)-honey prepared at pHs 3.75, 4.2 and 7.0 were analyzed. Gel structure was observed by scanning electron microscopy, and the apparent transition temperature for protein denaturation was determined by differential scanning calorimetry. The solubility of the protein components in different extraction media, and the water-holding capacity, firmness, elasticity, relaxation time, adhesivity, cohesiveness and color of gels were determined. Results show that disulfide interchange reactions are important in determining the elasticity, water-holding capacity, relaxation time and cohesiveness of WPC gels. Honey decreases the relaxation time of gels prepared at pHs 7.0 and 4.2, and increases the browning and the water-holding capacity of gels, the apparent transition temperature of WPC dispersions at the three pHs assayed, and the adhesivity of acidic gels. The solubility of the protein constituents of gels in a pH 8.0 buffer increases slightly at honey concentrations of 27.5% or more, which correlates with a decrease in the gel cohesiveness, having these gels a structure with smaller pores. The products obtained could be utilized in the formulation of different desserts, such as flans and cake and tart fillings.

Keywords: Whey protein concentrate; Gels; Honey

1. Introduction

Whey protein concentrates (WPC) and isolates (WPI) have received considerable attention as potential food ingredients because of their excellent nutritional value and wide range of functionality (Morr, 1984). The capacity of whey proteins to form gels upon heating is one of the main functional properties expected from these proteins (Cheftel & Lorient, 1982; Dumay, 1988). The process of gelation in food systems is normally carried out in several stages including conformational changes of protein molecules, their aggregation, and formation of a three-dimensional gel network from interacting aggregates. In thermotropic gelation of globular proteins, thermal denaturation is of critical importance (Tolstoguzov, 1991). Proteins maintain their native structure by chemical forces such as hydrophobic, ionic, hydrogen, and disulfide bonds. The chemical bonds are highly dependent upon the environment. As environment conditions change, some of the original bonds may be altered, new bonds may form, and the proteins then assume new conformations (Ju, Hettiarachchy, & Kilara, 1999). The formation of a gel depends thus on several factors such as protein concentration and environmental characteristics as pH, ionic strength, and the presence of other food components (Gault & Fauquant, 1992; Tolstoguzov, 1993).

The major whey proteins [β-lactoglobulin (β-Lg) and α-lactalbumin (α-La)] consist of a chain of amino acids folded into a compact three-dimensional structure, maintained by many weak noncovalent bonds and some disulfide bonds (Steventon, Gladden, & Fryer, 1991). β-Lg exists as a 36.7 Kda dimer in solutions above its pI 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 & Ha, 1993). The molecular mechanism of thermal denaturation of β-Lg at neutral pH includes dimer dissociation, loss of helix stability at about 65 °C and disulphide-linked aggregation, transition to a molten globule-like state at about 77 °C and

* Corresponding author. Tel./fax: +54-221-425-4853.
E-mail address: cel@quimica.unlp.edu.ar (C.E. Lupano).

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post molten globule transitions at higher temperatures (Holt, 2000). 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 (Shimada & Cheftel, 1988). Also, when pH approaches the pI, the charge of the proteins is progressively neutralized, favoring protein aggregation. Thus, the structure of gels is very dependent on the pH at which they are prepared (Lupano, Dumay, & Cheftel, 1992; Lupano, Renzi, & Romera, 1996).

Argentine produces a high amount of honey. Most of this honey is exported in bulk, and it is desirable to find new possibilities for this product as a natural ingredient in formulated foods. Honey has the advantage of its natural origin, and confers a particular flavor to the foods in which is included. Historically, honey has been used for thousands of years. The addition of honey to sweet solutions enhances their sweet intensity, due to honey carbohydrate composition (Cardetti, 1998). Also, the addition of honey is expected to change the functional properties of gels. There are some studies concerning the effect of sugars on the denaturation and functional properties of whey proteins (Dumay, 1988; Kulmyrzaev, Bryant, & McClements, 2000; Spiegel, 1999). Lactose strongly slows down the denaturation of β-Lg (Spiegel, 1999). Also, the addition of sucrose slows down the gelation and increases the temperature of the start of gelation of β-Lg (Dumay, 1988). However, no information is available concerning the effect of a complex sweetener as honey on the structural and functional properties of WPC gels. The products obtained could be utilized in the formulation of different desserts, such as flans and cake and tart fillings; however, microbiological studies must be performed before their incorporation into foods, especially in the case of gels prepared at neutral pH.

In this study, structural and functional properties of WPC gels prepared at different pHs and with different honey concentrations were studied, and the correlation between the structure and the gel properties was analyzed.

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). 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. Honey was harvested in the Province of Buenos Aires and contained 16.9% moisture, 76.3% glucose and fructose, and 1.7% sucrose. All chemicals used were of analytical grade.

2.2. Heating and gelation of WPC and WPC-honey dispersions

Aqueous dispersions (10.0% protein; 0, 10, 20, 27.5, 32.5, and 37.5% honey, w/w) of WPC or WPC-honey were adjusted to pH 3.75, 4.2 and 7.0 with 1–3 N HCL or 1N 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). After the 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 (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-honey 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 (T_p) and the enthalpy (ΔH_p) for protein denaturation were computed from the endothermic peaks. At least two determinations were made for each condition.

2.4. 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) (B), or in the same buffer containing 0.5% sodium dodecyl sulfate (SDS) and 8 M urea (BSU) (Lupano et al., 1992, 1996; Shimada & Cheftel, 1988). Gels of pH 7.00 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 17,400 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 $E^{1\%}_{1cm}$ of 8.636 for DW solutions (Lupano et al., 1996) and 10.2 for pH 8.0 solutions (Lupano et al., 1996; Shimada & Cheftel, 1988).

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 3×100% 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 Pelco, and observed with a JEOL 35 CF scanning electron microscope, at an acceleration voltage of 5 kV. Magnification: 4000×. Three-four images of each sample were captured for analysis.

2.6. Water-holding capacity (WHC) 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, 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.

2.7. 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., England) in the compression mode. Compression was exerted by a cilindric 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. 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. Relaxation time τ was taken as the time at which $F=(F_0+F_{20})/2$ (Lupano et al., 1992; Peleg, 1979). The measurements of gel adhesiveness and gel cohesiveness were performed with two compression cycles. Gel adhesiveness 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). For each type of gel, the average (±standard deviation) of three determinations was calculated.

2.8. Color

Superficial gel color was measured with a colorimeter Minolta (Japan), and Hunter parameters were determined. Values are the average (±standard deviation) of two or three determinations.

3. Results and discussion

3.1. Differential scanning calorimetry

Fig. 1a depicts the apparent transition temperature for protein denaturation (T_p) of WPC dispersions as a function of honey content. The T_p was higher at acid pH than at neutral pH, confirming previous results (Lupano et al., 1992). Honey increased the T_p at all pHs assayed, suggesting a protective effect against protein denaturation. Sugars can increase the thermal denaturation temperature of β-Lg, the major whey protein. This effect could be due mainly to the ability of some sugars, as sucrose, to increase the surface free energy between water and an hydrophobic surface, such as the area exposed to the solvent in protein unfolding (Kulmyrzaev et al., 2000). The effect of the environment composition in the T_p was observed in previous results, which show that dialysis decreases the T_p for whey protein denaturation (Lupano et al., 1992), indicating a protective effect of salts, whereas cassava starch has no effect (Lupano & González, 1999) and gluten shifts a little the T_p to lower temperatures (Lupano, 2000a). A

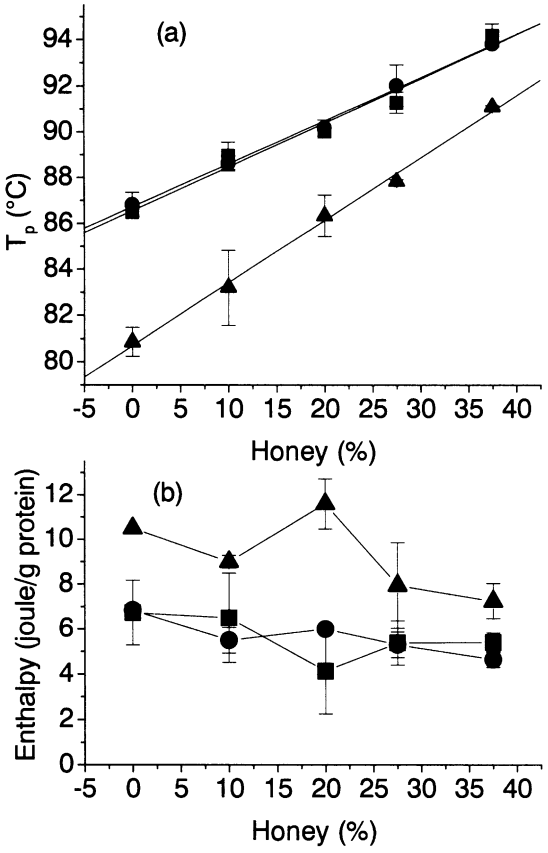

Fig. 1. (a) Apparent transition temperature (T_p) and (b) enthalpy (ΔH_p) for protein denaturation of WPC dispersions as a function of honey content. Protein concentration: 10%, w/w. (■) pH 3.75; (●) pH 4.20; (▲) pH 7.00. Bars show standard deviation ($LSD_{0.05\ T_p}=1.33$; $LSD_{0.05\ \Delta H_p}=2.79$).

linear relationship was observed between the T_p and the honey concentration at the three pHs assayed. Table 1 shows the slope and the correlation coefficient of the straight lines obtained at the three pHs. The effect of honey, reflected by the slope of the straight lines, was more important in gels prepared at pH 7.0, at which the sensitivity of whey proteins to thermal denaturation is higher. As the presence of honey increased the T_p mainly at neutral pH, the difference between the T_p at neutral and acid pH decreased as honey content increased (Fig. 1a).

The enthalpy for protein denaturation (ΔH_p) of WPC dispersions as a function of honey content is shown in Fig. 1b. The analysis of variance showed that there were not differences at a level of significance of 5% between samples with different honey content. The enthalpy values were higher at pH 7.00 than at acidic pH, confirming previous results (Lupano et al., 1992).

Table 1
Slope and correlation coefficient of the straight lines of T_p of WPC-honey dispersions vs honey content

pH of dispersions	Slope	Correlation coefficient
3.75	0.192±0.018	0.986
4.20	0.187±0.007	0.998
7.00	0.272±0.010	0.998

3.2. Structure evaluation by SEM

Fig. 2 shows the structure of gels observed by SEM. Gels prepared at pH 4.2 without honey presented a structure more aggregated with big pores when it is compared with gels prepared at pH 3.75 or 7.0. Honey decreased the size of the pores making the structure more homogeneous, mainly in gels prepared at pH 4.2.

3.3. Protein solubility of gels

The solubility of the protein constituents of WPC gels as a function of honey concentration is shown in Fig. 3. Honey practically did not modify the solubility of the gel protein constituents, except in the case of gels prepared at pH 4.2, in which the solubility in B increased slightly in gels with honey contents of 27.5% or more, when compared with the same gels but without honey. This suggests that honey decreases the stabilization of the gel structure through hydrogen bonds mainly in gels prepared at pH 4.2. This fact can be explained by considering that honey could form hydrogen bonds with whey proteins, thus decreasing the hydrogen bonds between protein molecules, being this effect more important in gels prepared at pH 4.2. These gels had a more homogeneous structure, with smaller pores than those of the same gels without honey, as was observed by SEM.

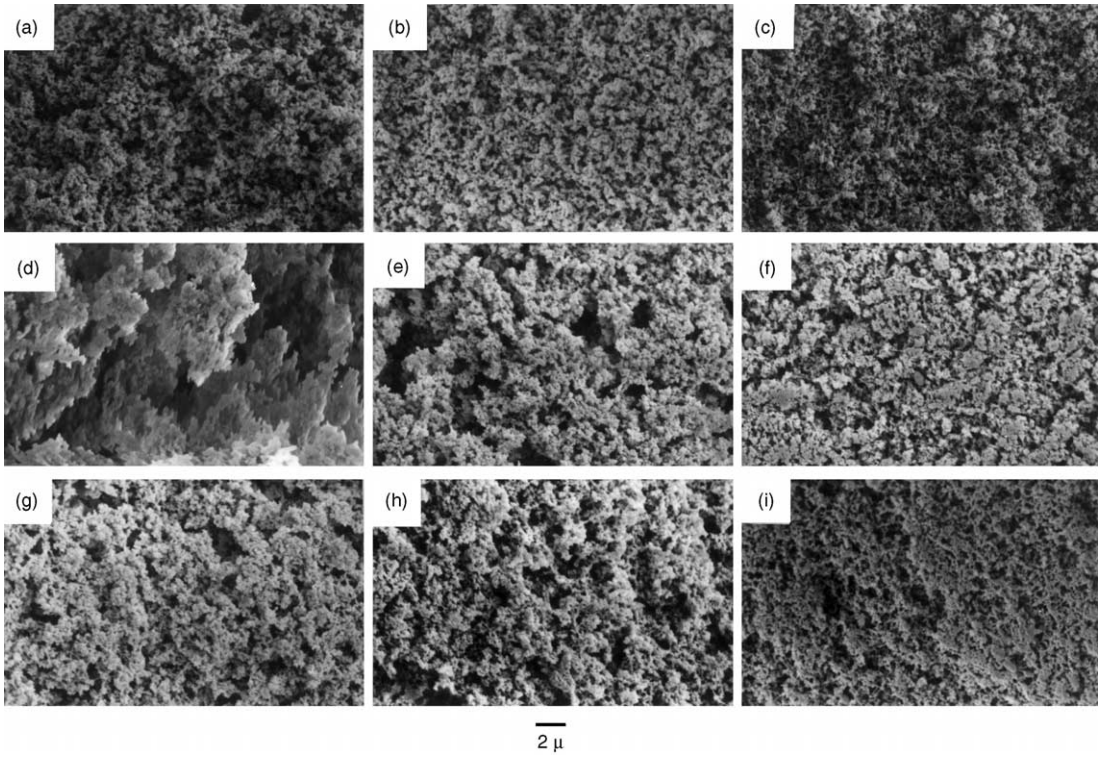

Fig. 2. Scanning electron microscopy of WPC gels. Protein content of gels: 10%, w/w. Honey content of gels: (a, d, g) 0%; (b, e, h) 10%; (c, f, i) 37.5%. pH of gels: (a, b, c) 3.75; (d, e, f) 4.20; (g, h, i) 7.00. Magnification: 4000×.

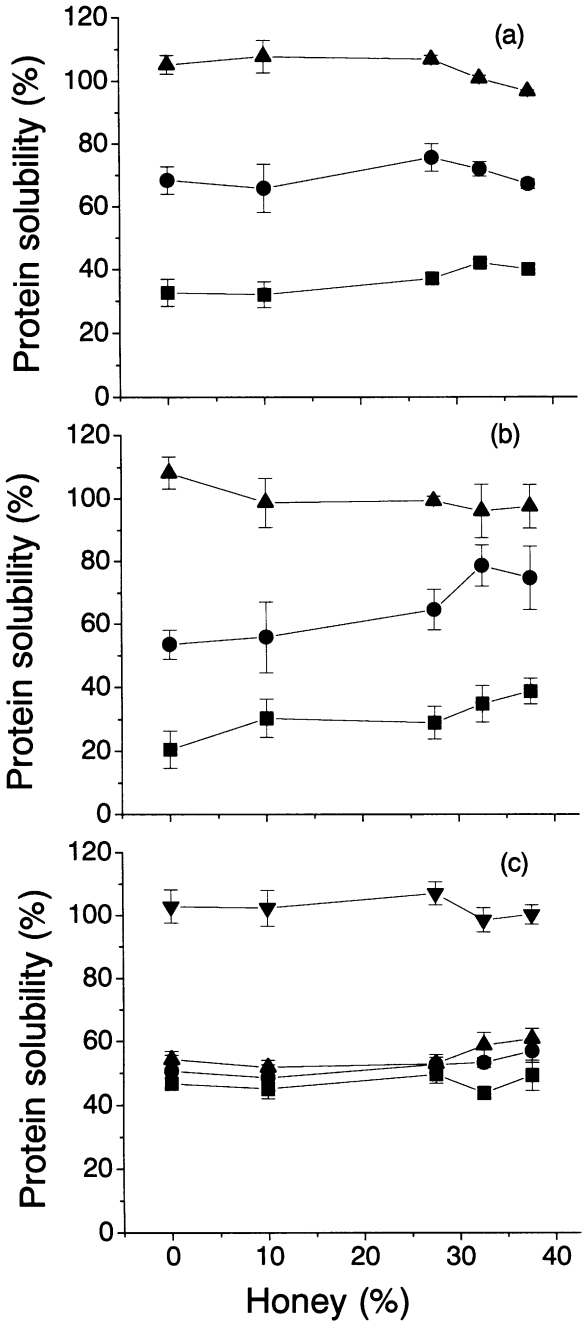

Fig. 3. Solubility of the protein constituents of WPC gels as a function of honey content. Protein content of gels: 10%, w/w. Protein concentration for all solubilization assays: 0.1%, w/v. pH of gels: (a) 3.75; (b) 4.20; (c) 7.00. Extraction solutions: (■) distilled water; (●) standard buffer, pH 8.0 (B); (▲) standard buffer containing 8 M urea and 0.5% SDS (BSU); (▼) standard buffer containing 8 M urea, 0.5% SDS and 1% β-mercaptoethanol (BSUM). Bars show standard deviation ($LSD_{0.05} = 20.6$).

3.4. Water-holding capacity

Fig. 4 shows the water-holding capacity of WPC-honey gels prepared at different pHs as a function of honey content. The water-holding capacity of gels without honey was higher in gels prepared at pH 7.0, and lower in gels prepared at pH 4.2, which have a more aggregated structure, in agreement with previous reports (Lupano, 2000a; Lupano & González, 1999; Lupano et al., 1996). Water-holding capacity increased with honey content at all pHs assayed, reaching similar values at honey concentrations high enough. This is probably due to the ability of honey to form hydrogen bonds with the molecules of water, like other compounds as cassava starch and gluten, which have a similar behavior (Lupano, 2000a, 2000b; Lupano & González, 1999). A linear relationship was observed between the water-holding capacity and the honey content (Table 2), showing that the effect of honey was more important in gels with less water-holding capacity.

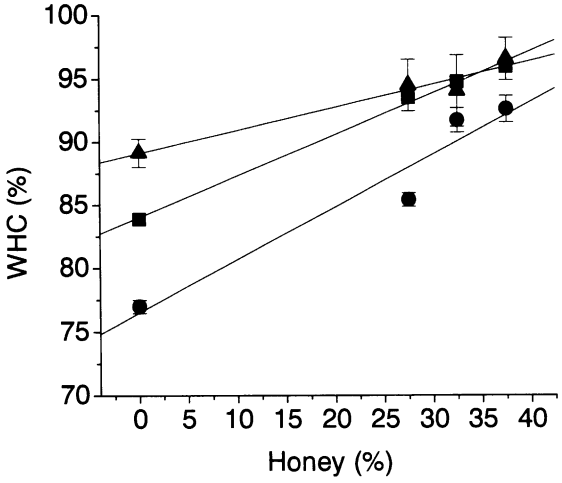

Fig. 4. Water-holding capacity (WHC) of WPC gels as a function of honey content. Protein content of gels: 10%, w/w. pH of gels: (■) 3.75; (●) 4.20; (▲) 7.00. Bars show standard deviation ($LSD_{0.05} = 3.78$).

Table 2
Slope and correlation coefficient of the straight lines of the water-holding capacity of WPC gels vs honey content

pH of gels	Slope	Correlation coefficient
3.75	0.328±0.015	0.998
4.20	0.417±0.076	0.968
7.00	0.182±0.030	0.974

3.5. Gel properties

Fig. 5 shows the firmness, elasticity and relaxation time of WPC gels as a function of honey concentration.

Gels without honey prepared both at acidic and neutral pHs presented a similar firmness, although the firmness of the gel prepared at pH 3.75 was a little higher (Fig. 5a). These results agree with previous data (Lupano, 2000a, 2000b; Lupano et al., 1992), and with results reported by Shimada and Cheftel (1988), who

found that gels of whey protein isolate prepared at neutral pH and at pH 3.5 have a similar firmness, observing an increase in the firmness of gels prepared at pH 6.5 and a decrease in the firmness of gels prepared at pH higher than 7.5. On the contrary, Tang, McCarthy, and Munro (1995) found that firmness of WPC gels presented a maximum at pH 7.5 and decreases to minimum values at pHs lower than 6. Honey did not modify the firmness of gels prepared at pH 7.0 and 4.2, but slightly increased the firmness of gels prepared at pH 3.75 (Fig. 5a).

Fig. 5b depicts the elasticity of WPC gels prepared at different pHs as a function of honey concentration. Elasticity of gels without honey is in accordance with previous results (Lupano et al., 1992). As was expected, elasticity was higher in gels prepared at pH 7.0, at which sulphydryl-disulphide interchange reactions are favored. Honey did not modify appreciably the elasticity of WPC gels, although a little decrease in elasticity was observed in gels prepared at pH 7.0 with 37.5% honey, and in gels prepared at pH 4.2 with honey contents of 20% or more.

If a test piece is deformed to a certain extent, after which the deformation is maintained at a constant level, the stress built up during deformation gradually relaxes (Bloksma, 1978). Relaxation time of gels without honey decreased with pH (Fig. 5c), in agreement with previous results (Lupano, 2000a; Lupano et al., 1992). Fig. 5c also shows that relaxation time decreased with honey

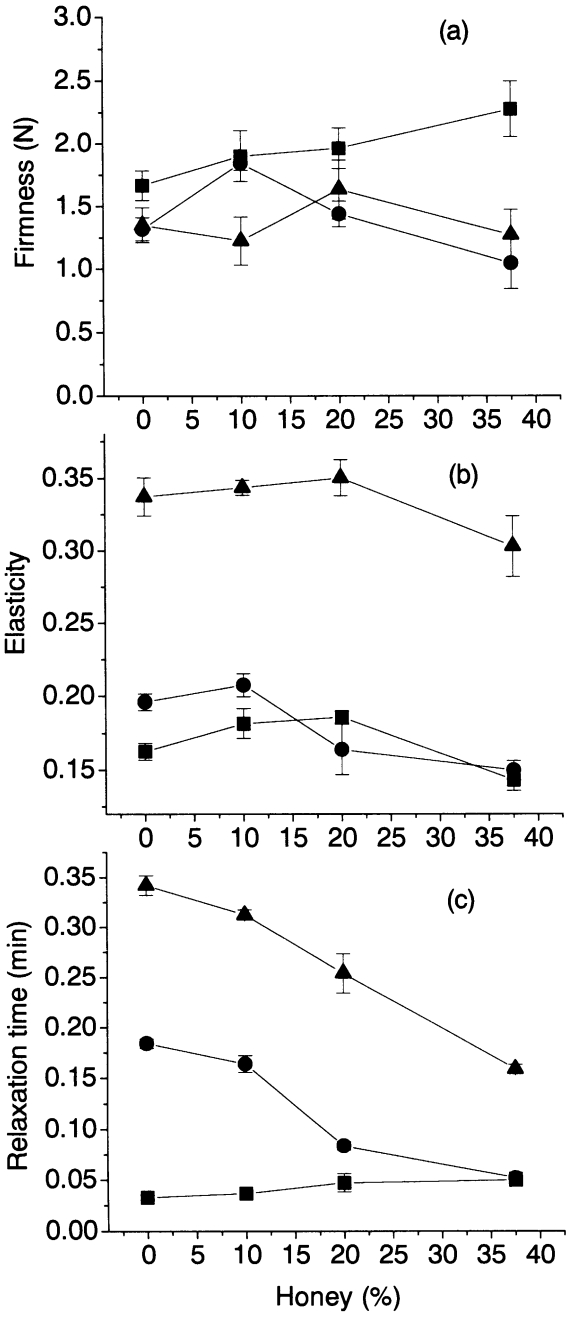

Fig. 5. (a) Firmness, (b) elasticity and (c) relaxation time of WPC gels as a function of honey content. Protein content of gels: 10%, w/w. pH of gels: (■) 3.75; (●) 4.20; (▲) 7.00. Bars show standard deviation ($LSD_{0.05}$ firmness = 0.27; $LSD_{0.05}$ elasticity = 0; $LSD_{0.05}$ relaxation time = 0).

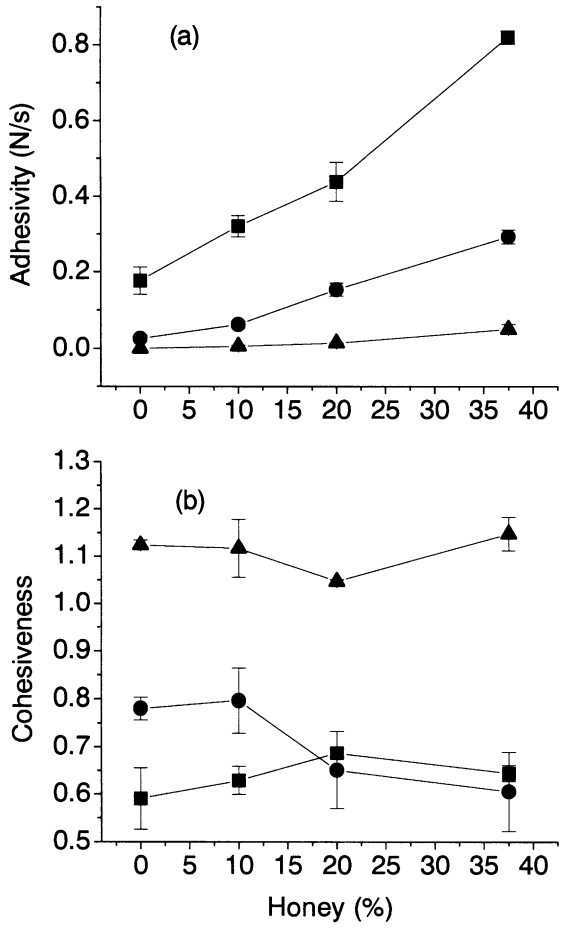

Fig. 6. (a) Adhesivity and (b) cohesiveness of WPC gels as a function of honey content. Protein content of gels: 10%, w/w. pH of gels: (■) 3.75; (●) 4.20; (▲) 7.00. Bars show standard deviation ($LSD_{0.05}$ adhesivity = 0; $LSD_{0.05}$ cohesiveness = 0.093).

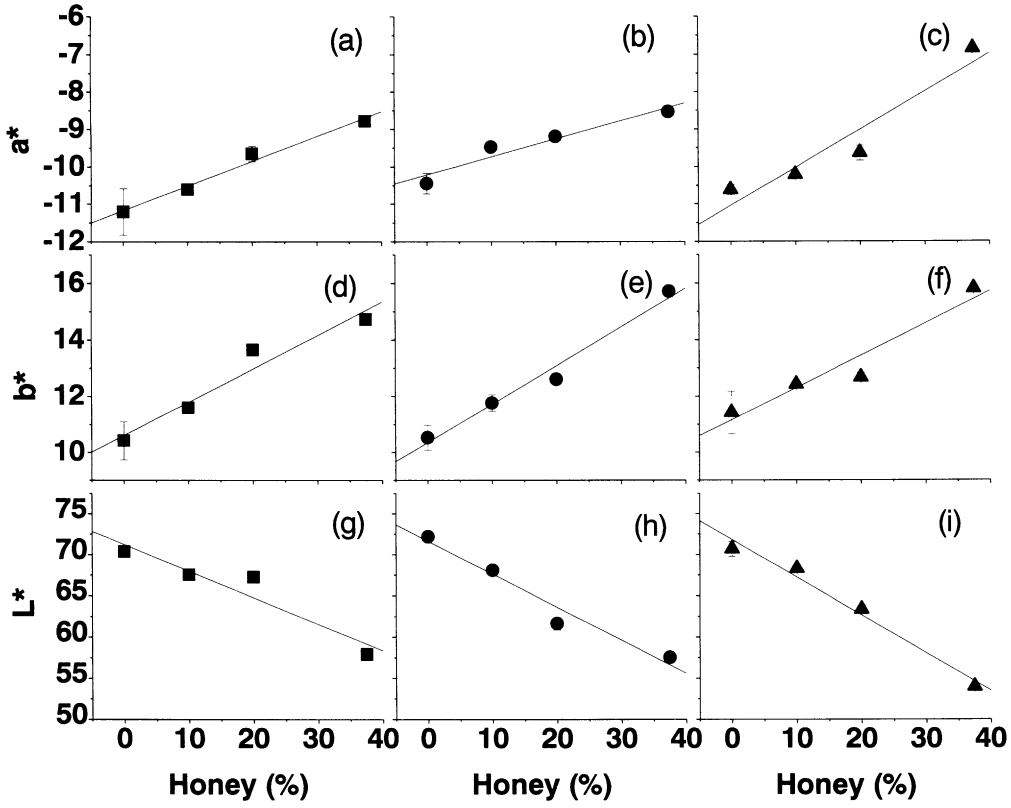

Fig. 7. Hunter parameters of WPC gels as a function of honey content. Protein content of gels: 10%, w/w. pH of gels: (■) 3.75; (●) 4.20; (▲) 7.00. (a, b, c) parameter a^*; (d, e, f) parameter b^*; (g, h, i) parameter L^*. Bars show standard deviation ($LSD_{0.05}$ $a^* = 0.39$; $LSD_{0.05}$ $b^* = 0.63$; $LSD_{0.05}$ $L^* = 0.78$).

content in gels prepared at pH 7.0 and 4.2, reaching in the latter similar values to those of gels prepared at pH 3.75. This suggests that honey increase the viscous behavior of WPC gels. Hydrogen bonds between small molecules significantly increase the viscosity of a liquid, and the bonds are weak enough to be temporarily extended, exchanged, or broken (Pomeranz, 1978). Honey, because of its high sugar content, has the possibility to form hydrogen bonds and thus increases the viscous behavior of the gels. Gels prepared at pH 3.75, on the other hand, presented viscous characteristics before the addition of honey; thus, honey does not modify appreciably their relaxation time.

Adhesivity is the work necessary to overcome the attractive forces between the surface of the food and the surface of other material with which the food comes in contact (Larmond, 1976). A theory of adhesion proposes that materials adhere because of interatomic and intermolecular forces, such as covalent or ionic bonds or van der Waals forces. 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). Fig. 6a shows the adhesivity of WPC gels as a function of honey content. Adhesivity of acid gels increased with honey concentration, mainly in gels prepared at pH 3.75. It is possible that, at acid pH, honey sugars may adhere easily to the metal of the probe. In addition, gels prepared at pH 7.0 are more cohesive and, thus, would have a less ability to adhere to other materials. Disulfide bonds would decrease the plastic deformation needed to adhere to a surface.

Cohesiveness is defined as the ratio of the positive force area during the second compression to that during the first compression (Bourne, 1978). Gels prepared at neutral pH had a higher cohesiveness than gels prepared at acidic pH, in agreement with data reported by Tang et al. (1995). This can be explained taking into account that disulfide bonds are involved in the maintenance of the structure of neutral gels, whereas noncovalent bonds are responsible for the maintenance of the structure of acidic gels (Fig. 3) (Lupano et al., 1992). Gels prepared at pH 3.75 are the least cohesive (Fig. 6b). The presence of honey did not modify the cohesiveness of gels prepared at pHs 7.0 and 3.75, whereas honey contents of 20% or more decreased the cohesiveness of gels prepared at pH 4.2 (Fig. 6b). This coincides with the increase in the solubility in B of the protein constituents of these gels.

Table 3
Slope and correlation coefficient of the straight lines of the Hunter parameters a^*, b^* and L^* of WPC gels vs honey content

Hunter parameter	pH of gels	Slope	Correlation coefficient
a^*	3.75	0.066 ± 0.006	0.992
	4.20	0.048 ± 0.009	0.963
	7.00	0.101 ± 0.022	0.955
b^*	3.75	0.118 ± 0.020	0.971
	4.20	0.137 ± 0.014	0.989
	7.00	0.114 ± 0.023	0.962
L^*	3.75	-0.323 ± 0.075	−0.951
	4.20	-0.400 ± 0.060	−0.979
	7.00	-0.459 ± 0.047	−0.990

3.6. Color

The Hunter's parameters a^*, b^* and L^* of WPC-honey gels are shown in Fig. 7. Honey increased the parameters a^* and b^* (reddish and yellowness, respectively) of WPC gels, whereas the parameter L^* (lightness) decreased with the honey content, at all pHs assayed. This is caused by the color of honey and also by the Maillard reactions favored by the presence of honey reducing sugars. A linear relationship was observed between the Hunter parameters and the honey concentration, being the slope of the straight line of the parameter a^* of gels prepared at pH 7.0 higher than the corresponding to gels prepared at acidic pH (Table 3). This indicates that honey increase the browning reactions mainly at pH 7.0, which is expected because Maillard reactions are favored at this pH (Namiki & Hayashi, 1983).

4. Conclusions

Disulfide interchange reactions appear to play an important role in determining the elasticity, water-holding capacity, relaxation time and adhesivity of the WPC gels.

Honey decreases the relaxation time of gels prepared at pHs 7.0 and 4.2, increasing their viscous characteristics, and increases the water-holding capacity of gels at the three pHs assayed and the adhesivity of acidic gels. Also, honey increases the apparent transition temperature of WPC dispersions at the three pHs assayed, and the browning of WPC gels.

The solubility in a pH 8.0 buffer of the protein constituent of gels prepared at pH 4.2 increases slightly at honey concentrations of 27.5% or more, which coincides with a decrease in the cohesiveness and a more homogeneous structure with smaller pores, as observed by SEM.

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