VISCOELASTIC PROPERTIES OF WHEY PROTEIN CONCENTRATE GELS WITH HONEY AND WHEAT FLOUR AT DIFFERENT pH

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ABSTRACT

Viscoelasticity of heat-induced gels from whey protein concentrate, with different contents of honey and wheat flour, and prepared at pH 3.75, 4.2 and 7.0, was studied by using dynamic rheological assays. The elastic modulus of gels prepared at neutral pH was higher than the corresponding to acidic gels, probably due to the fact that sulphydryl-disulfide interchange reactions are favored at neutral pH. Honey decreased the elastic modulus and increased the viscous modulus and the complex viscosity in all conditions assayed. Wheat flour increased the elastic modulus, and all samples exhibited a gel-type behavior except at high honey content.

PRACTICAL APPLICATIONS

Honey and wheat flour modifies the properties of whey protein concentrate gels. Both components have opposite effects: honey increases the viscous-like behavior and wheat flour the solid-like behavior of gels in all conditions assayed. The different characteristics of gels prepared at different pHs and with different amounts of honey and wheat flour could be used in different formulated foods, as desserts.

KEYWORDS

Honey, viscoelasticity, wheat flour, whey protein concentrate

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INTRODUCTION

A gel is a solid-in-liquid colloid in which the solid phase forms a network structure immobilizing the liquid and producing solid-like properties (Krieger 1983). The viscoelastic properties of a gel depend on the three-dimensional network structure, the nature of cross-links and the length of the molecular chains between cross-linking points (Katsuta and Kinsella 1990). The nature of cross-links in protein gels has been discussed by several authors (Clark *et al.* 1983; Morris 1985; Clark and Lee-Tuffnel 1986; Oakenfull 1987). The consensus view is that, with the exception of disulfide bonds in some protein gels, the molecules are held together by a combination of weak intermolecular forces; i.e., hydrogen bonds, electrostatic forces, Van der Waals forces and hydrophobic interactions. These cross-links in gels are not permanent but may continuously break, reform (Oakenfull 1987) and rupture when gels are shared (Mitchell 1980). Zheng *et al.* (1993) have indicated that the contribution of covalent and non-covalent bonds to the gel texture and viscoelasticity is different. Disulfide bonds play an important role in stabilizing the gel and increasing the gel matrix hardness, whereas hydrogen and hydrophobic interactions are especially responsible for keeping the structure and for viscosity increase.

During protein gelation, denatured protein molecules interact with each other to form polymeric molecules. A gelling biopolymer system is a solution of reactive polymers referred to as the sol, which becomes progressively cross-linked. As the gelation reaction proceeds, a point is reached when the average molecular weight diverges to infinite and an “infinitely extended” network appears, referred to as the gel phase. The abrupt appearance of the gel is called the sol-gel transition or the gel time (t^*) (Steventon *et al.* 1991). The properties of gels can be studied by oscillatory rheometry, which measures the elastic and viscous contributions to the overall system rheology in terms of the storage (G') and loss (G'') moduli (Ferry 1980). The storage modulus is a measure of the energy stored in the material and recovered from it per cycle. It is dependent upon what rearrangements can take place within the period of oscillation; this estimates the solid behavior. On the other hand, the loss modulus measures the energy dissipated or lost (as heat) per cycle of sinusoidal deformation, and indicates the liquid or viscous behavior. Complex viscosity is defined as:

$$\eta^* = \left\{\left[(G')^2 + (G'')^2\right]^{1/2}\right\} \Big/ \omega \qquad (1)$$

where ω is the radial oscillation frequency (rad/s) (Sopade *et al.* 2004).

Between the popular foods based on gelation are gelatin desserts, cooked egg white, frankfurters, surimi-based seafood analogs and fruit jellies (Krieger

1983). Whey protein concentrate (WPC) is an important food ingredient with the ability to form gels upon heating. These gels incorporate both elastic and viscous properties, which are perceived as a texture attribute attractive to the consumer (Steventon *et al.* 1991).

Foods are complex systems, in which many ingredients interact with each other. The rheological behavior of gel proteins depends on several factors, as the interactions between the protein constituents and the interaction of proteins with other components. Honey is a natural mixture of sugars which is used in almost every country in the world. Many formulated foods have refined sugars as additives; however, the new healthy trends among consumers have focused their attention on honey as a natural sweetener. Thus, it is desirable to find new possibilities for this product as a natural ingredient in formulated foods. Wheat flour and its products are eaten all over the world in every meal and provide as an excellent source of carbohydrates. The gluten network which is formed upon applying mechanical energy to a mix of water and wheat flour, has a special viscoelastic behavior. Thus, it is expected that the addition of wheat flour to WPC gels modifies its rheological behavior.

In this study, we examined the influence of honey and wheat flour on the rheological properties of WPC gels.

EXPERIMENTAL

Materials

The WPC was prepared by large-scale ultrafiltration (Williner S.A., Rafaela, Santa Fe, Argentina), and contained 49.3% protein, 1.7% nonprotein nitrogen, 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. Wheat flour contained 10% proteins and 13% moisture. All chemicals used were of analytical grade.

Rheology

Tests were carried out in a Haake CV 20 Rheometer (Karlsruhe, Germany) using a 1-mm gap parallel-plate sensor. Aqueous dispersions (10.0% protein; 0, 10 and 20% honey; 0, 10 and 20% wheat flour, w/w) of WPC or WPC–honey–wheat flour were adjusted to pH 3.75, 4.2 and 7.0 with 1–3 N HCL or 1 N NaOH. Dispersions (1.0 mL) were placed on the lower plate, which was thermostated at 90C, except for when other temperatures were indicated in the text. Low viscosity silicone was added around the plate edges to prevent dehydration. With the exception of the time scanning, samples were left at 90C for 45 min to allow gelation. The equipment was driven through the Haake software osc. 2.0. The experimental procedures allowed recording of the complex modulus (G^*), storage modulus (G'), loss modulus (G''), tangent of the deformation angle or loss tangent ($\tan \Phi = G''/G'$) and complex viscosity (η^*) as a function of the time and frequency of oscillation. Values corresponding to gels prepared at pH 7.0 with 20% wheat flour and 0% honey could not be measured because, due to its hardness, they were out of the measure range of the equipment.

The linear viscoelasticity range of the dispersions was determined by measuring G^* as a function of deformation ($\omega = 1$ rad/s).

$$G^* = (G')^2 + (G'')^2 \tag{2}$$

Based on these results, frequency and time scans of the samples were conducted at the same deformation ($d = 10\%$), within the linear viscoelasticity range.

The variation of G' and G'' with temperature was also tested, measuring the mentioned parameters at 60, 70, 80 and 90C ($\omega = 1$ rad/s).

In order to measure the variation of G' as a function of treatment time, the lower plate was thermostated at 90C, and the measures were immediately started. The gel time (t^*) was calculated as the time when $\tan \Phi = 1$, that is when $G' = G''$ (cross-over time). At least three replications were made for each condition.

The tangent of the deformation angle ($\tan \Phi = G''/G'$) was also analyzed ($\omega = 1$ rad/s) as a function of pH, honey and wheat flour content.

The measurement of G', G'' and η^* as a function of frequency was carried out at the gelation temperature of 90C. Experimental data were fitted by the following expression (Steffe 1996; Dello Staffolo *et al.* 2004; Meza *et al.* 2009):

$$G' = a\omega^b \tag{3}$$

where a and b are parameters that characterize the rheological behavior and were obtained by the nonlinear model method using the SYSTAT software (SYSTAT, Inc., Evanston, IL; SYSTAT 1990).

Statistics

An analysis of variance of the data was performed, using a SYSTAT statistical computer program. A least significant difference test with a confidence interval of 95% was used to compare the means.

RESULTS AND DISCUSSION

Gelling Temperature Tests

Figure 1 shows the variation of *G′* with temperature of WPC gels containing different amounts of honey and wheat flour. The value of *G′* at 60C was similar in all conditions assayed. However, at temperatures from 70C, *G′* increased in most conditions, and samples containing wheat flour showed higher *G′* than those with no flour. Similar results were obtained by Sittikijyothin *et al.* (2007) on β-lactoglobulin gels with tara gum.

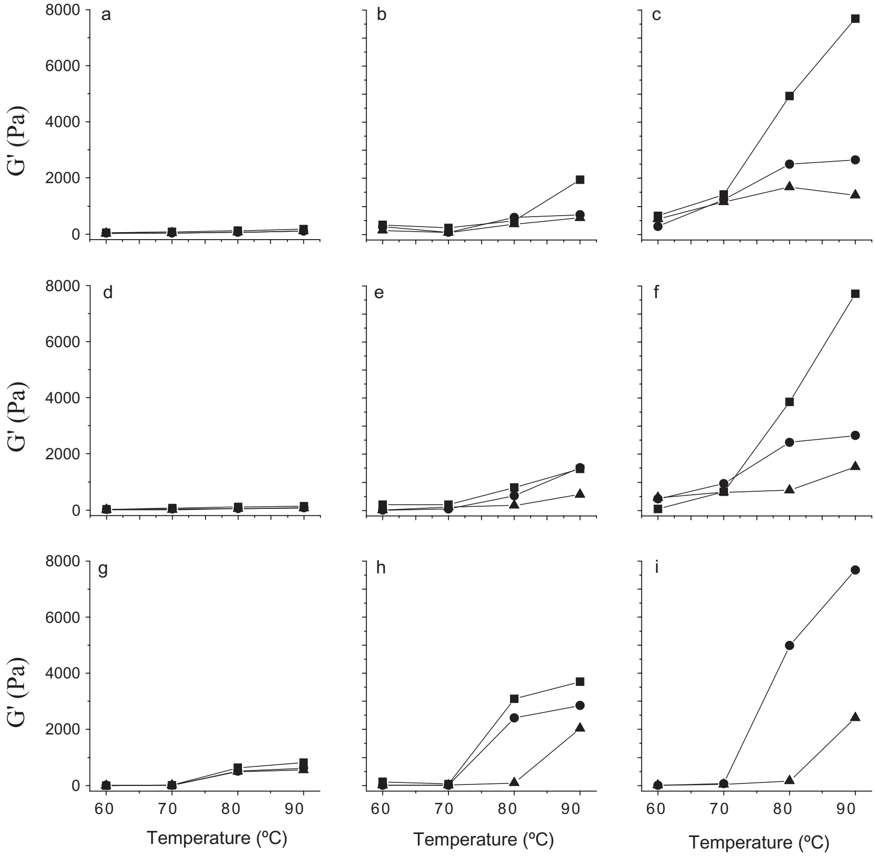

FIG. 1. ELASTIC MODULUS (*G′*) OF WHEY PROTEIN CONCENTRATE GELS AS A FUNCTION OF TEMPERATURE

Protein content of gels: 10%, w/w. pH of gels: (a, b, c) 3.75; (d, e, f) 4.2; (g, h, i) 7.0. Honey content of gels: (■) 0%, (●) 10%, (▲) 20%. Wheat flour content: (a, d, g) 0%; (b, e, h) 10%; (c, f, i) 20%.

According to Parris and Baginsky (1991), the unfolding of whey proteins starts at 40C and continues slowly to reach 10% of denaturation at 62C, and 95% at 85C. This fact can explain the increase in G' with temperature, as only at temperatures higher than 70C the whey proteins, especially the β-lactoglobulin, the main gelling whey protein, are denatured enough to form a gel. The increase in G' was higher in gels prepared at pH 7.0. In these gels, sulphydryl-disulfide interchange reactions are favored, as was discussed in previous works (Shimada and Cheftel 1988; Lupano *et al.* 1992; Yamul and Lupano 2005).

In gels containing wheat flour, it must be considered that not only would the whey proteins contribute to the G' value, but also the gluten proteins and starch that gelatinizes during the gel preparation. Hence, it is not surprising that samples with 20% wheat flour showed the highest increase in G'. A similar increase in G' was observed by de Jong *et al.* (2009) in whey protein – polysaccharide cold set gels.

With respect to honey, G' decreased when honey content increased. Honey has the capacity to form hydrogen bonds with the molecules of water, limiting the amount of water available for starch gelatinization; also, honey sugars interfere with the gelatinized starch structure, reducing its solid behavior and thus decreasing the value of G'. The viscous properties of honey and the protective effect it has on the thermal protein denaturation, which was discussed in a previous work (Yamul and Lupano 2003), would also contribute to the decrease in G'.

The effect of temperature on G'' is shown in Fig. 2. Honey produced an increase of G'' in almost all conditions assayed. Wheat flour, on the other hand, only produced a little increase of G'' in some cases.

These results indicate that wheat flour modifies mainly the G' value of the gels, and honey counteracts, to a different extent, the effect of wheat flour.

Frequency Sweep Tests

The dynamic spectrum (G', G'' and η^* versus oscillation frequency) of gels prepared at different pHs and with different honey and wheat flour contents, are shown in Figs. 3–5. In agreement with results discussed earlier, an increase in G' with the increase in wheat flour content, and a decrease in the same parameter when honey content increases (Fig. 3), was observed. Also, honey produced an increase in G'' values over all the frequency range assayed (Fig. 4).

The complex viscosity (η^*) indicates the total resistance of the sample to the dynamic shear stress. This parameter decreased linearly with the oscillation frequency in all conditions assayed (Fig. 5).

From the expression that relates G' with frequency (Eq. 3), it is possible to calculate the a and b values. The model satisfactorily fitted experimental

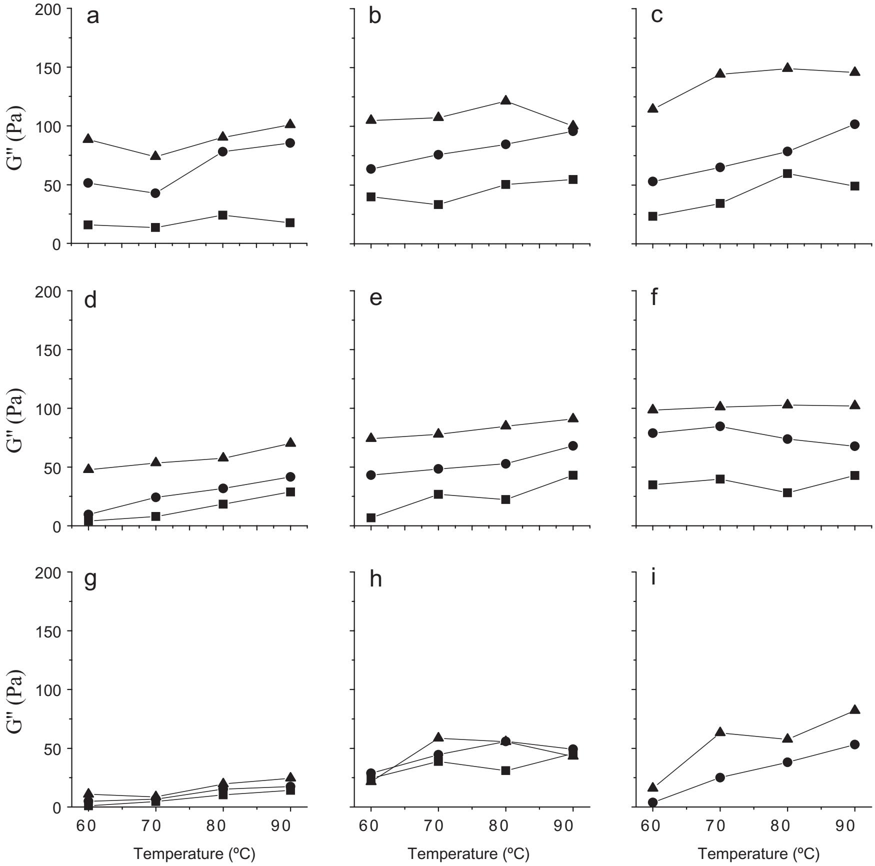

FIG. 2. VISCOUS MODULUS (G'') OF WHEY PROTEIN CONCENTRATE GELS AS A FUNCTION OF TEMPERATURE

Protein content of gels: 10%, w/w. pH of gels: (a, b, c) 3.75; (d, e, f) 4.2; (g, h, i) 7.0. Honey content of gels: (■) 0%, (●) 10%, (▲) 20%. Wheat flour content: (a, d, g) 0%; (b, e, h) 10%; (c, f, i) 20%.

data obtaining a minimum R^2 of 0.914. Table 1 shows the *a* and *b* values estimated at each pH, honey and wheat flour content. These values were between those corresponding to typical gels (5,626 Pa · s and 0.0371, respectively) and concentrated solutions (16.26 Pa·s and 0.84, respectively) (Steffe 1996). Honey decreased the *a* values in gels with or without wheat flour ($P < 0.01$), indicating that this component increases the solution-type behavior. On the contrary, wheat flour presented the opposite effect, increasing the *a* values, that is, the gel-type behavior of these samples ($P < 0.01$).

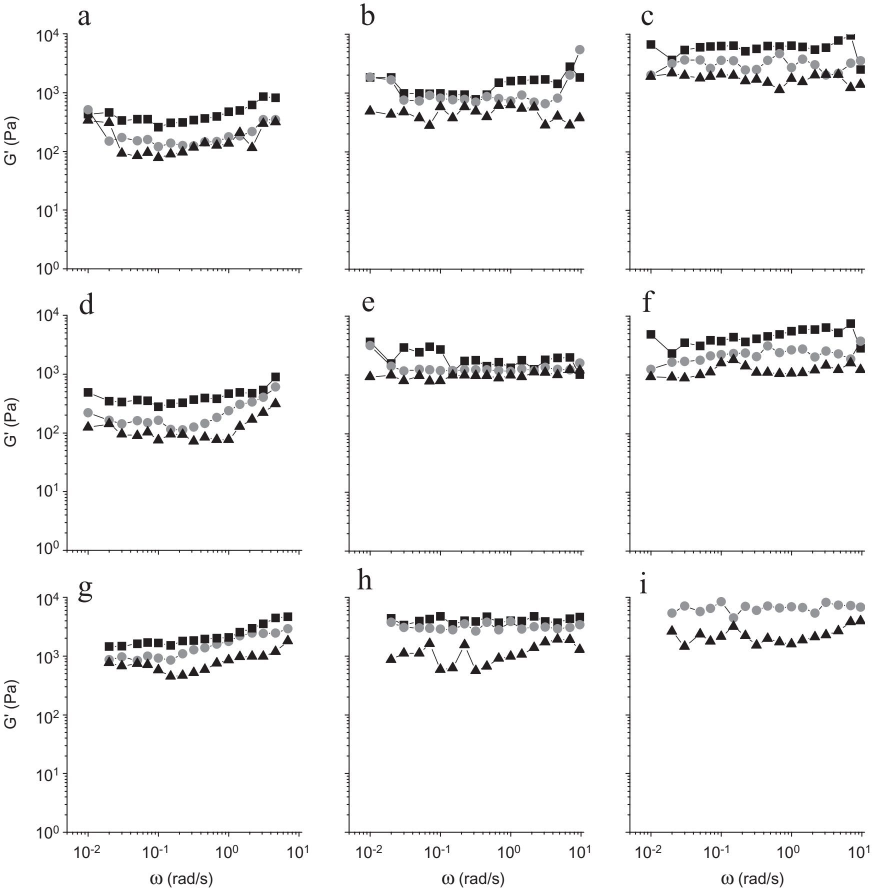

FIG. 3. ELASTIC MODULUS (G') OF WHEY PROTEIN CONCENTRATE GELS AS A FUNCTION OF THE OSCILLATION FREQUENCY

Protein content of gels: 10%, w/w. pH of gels: (a, b, c) 3.75; (d, e, f) 4.2; (g, h, i) 7.0. Honey content of gels: (■) 0%, (●) 10%, (▲) 20%. Wheat flour content: (a, d, g) 0%; (b, e, h) 10%; (c, f, i) 20%.

Significant differences ($P < 0.01$) in the *a* values were also observed between gels prepared at a different pH. These results agree with those discussed in the previous section. No significant differences were found in the *b* values of gels of different pH and with different contents of honey or wheat flour ($P > 0.05$).

When the results of Figs. 3 and 4 were compared, it was observed that except in some gels with high honey content, G' was higher than G'' over all the oscillation frequency range studied. Thus, according to the model of

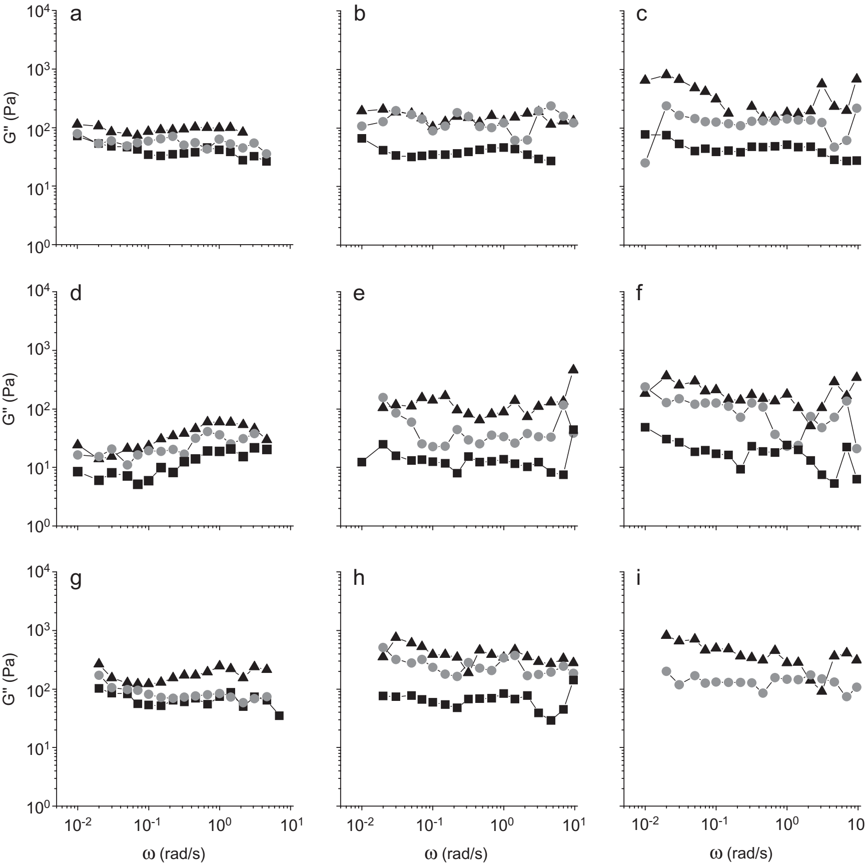

FIG. 4. VISCOUS MODULUS (G'') OF WHEY PROTEIN CONCENTRATE GELS AS A FUNCTION OF THE OSCILLATION FREQUENCY

Protein content of gels: 10%, w/w. pH of gels: (a, b, c) 3.75; (d, e, f) 4.2; (g, h, i) 7.0. Honey content of gels: (■) 0%, (●) 10%, (▲) 20%. Wheat flour content: (a, d, g) 0%; (b, e, h) 10%; (c, f, i) 20%.

Giboreau *et al.* (1994), these samples present a behavior according to gel-type materials. These gels reflect the existence of a three-dimensional matrix stable at high oscillation frequencies (Clark and Ross-Murphy 1987; Giboreau *et al.* 1994).

According to Letang *et al.* (1999), the tangent of the deformation angle ($tan\ \Phi = G''/G'$) can be considered as an indicator of the structural organization of a material. Thus, highly structured materials produce, in general, low values of $tan\ \Phi$; values of $tan\ \Phi > 1$ indicate that the viscous behavior

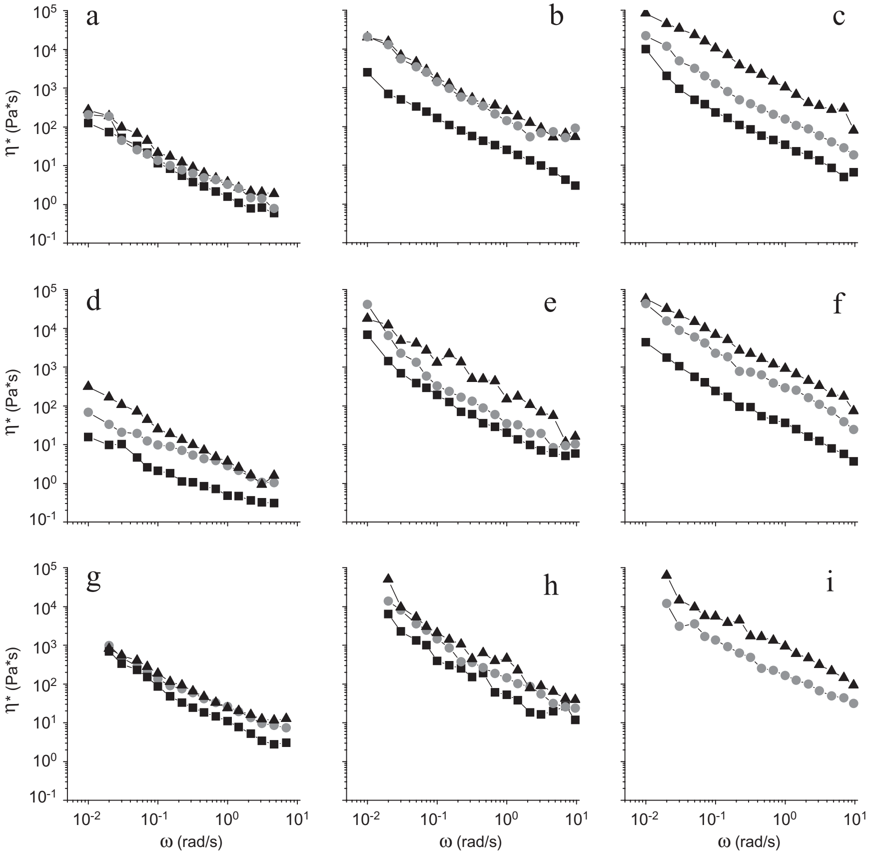

FIG. 5. COMPLEX VISCOSITY (η*) OF WHEY PROTEIN CONCENTRATE GELS AS A FUNCTION OF THE OSCILLATION FREQUENCY

Protein content of gels: 10%, w/w. pH of gels: (a, b, c) 3.75; (d, e, f) 4.2; (g, h, i) 7.0. Honey content of gels: (■) 0%, (●) 10%, (▲) 20%. Wheat flour content: (a, d, g) 0%; (b, e, h) 10%; (c, f, i) 20%.

predominates, whereas values of *tan* $\Phi < 1$ indicate that the elastic behavior predominates in the sample. Results of Figure 6 shows that honey increased ($P < 0.01$) and wheat flour decreased ($P < 0.01$) the *tan* Φ values of WPC gels at the three pHs assayed. Wheat flour would contribute to the gel structure through the starch and gluten proteins. Neutral gels presented values of *tan* $\Phi < 1$, indicating that these gels were mainly elastic. Disulfide bonds, which are favored at this pH, would contribute to this effect. On the contrary, some acidic gels, especially those with high honey and low wheat flour contents,

TABLE 1.
VALUES OF *a* AND *b*, OBTAINED BY FITTING ($R^2 = 0.914$) THE EXPERIMENTAL DATA WITH THE EXPRESSION $G' = a\omega^{b}$ ($\mathrm{LSD}_{0.05,a} = 247.3$; $\mathrm{LSD}_{0.05,b} = 0.07$)

pH	Wheat flour (%)	Honey (%)	*a*	*b*
3.75	0	0	468.88 ± 9.68	0.28 ± 0.02
		10	178.25 ± 11.96	0.45 ± 0.06
		20	159.95 ± 13.74	0.39 ± 0.08
	10	0	1,519.85 ± 25.60	0.14 ± 0.01
		10	1,345.73 ± 18.34	0.21 ± 0.05
		20	997.29 ± 37.21	0.30 ± 0.01
	20	0	5,592.08 ± 42.63	0.04 ± 0.02
		10	3,244.51 ± 42.72	0.10 ± 0.07
		20	1,815.95 ± 19.54	0.22 ± 0.02
4.2	0	0	494.57 ± 33.37	0.14 ± 0.04
		10	282.26 ± 22.73	0.23 ± 0.07
		20	148.70 ± 15.03	0.35 ± 0.06
	10	0	1,667.86 ± 57.41	0.04 ± 0.01
		10	1,238.96 ± 20.26	0.11 ± 0.04
		20	1,009.67 ± 22.69	0.12 ± 0.03
	20	0	5,277.18 ± 35.31	0.04 ± 0.01
		10	2,611.48 ± 81.84	0.06 ± 0.02
		20	1,111.5 ± 17.03	0.13 ± 0.03
7.0	0	0	2,653.38 ± 99.32	0.25 ± 0.02
		10	1,799.96 ± 44.35	0.27 ± 0.01
		20	794.32 ± 24.92	0.29 ± 0.03
	10	0	5,605.74 ± 84.23	0.09 ± 0.03
		10	2,259.89 ± 37.50	0.13 ± 0.02
		20	1,262.55 ± 88.88	0.17 ± 0.04
	20	10	5,693.07 ± 72.69	0.05 ± 0.01
		20	2,247.07 ± 48.58	0.17 ± 0.04

LSD, least significant difference.

presented values of *tan* $\Phi > 1$, indicating that the viscous behavior predominates in these gels.

Time Sweep Tests

Figure 7 depicts the elastic modulus G' as a function of time, and Table 2 shows the gel time of gels. As expected, G' increased with time. Also, G' increased as wheat flour content increased, whereas honey presented the opposite effect.

Results of Fig. 7 and Table 2 suggest that honey delays the gelation process ($P < 0.01$), but this effect was not evident in gels containing wheat flour. Wheat flour, on the other hand, did not present a clear effect on the gel time.

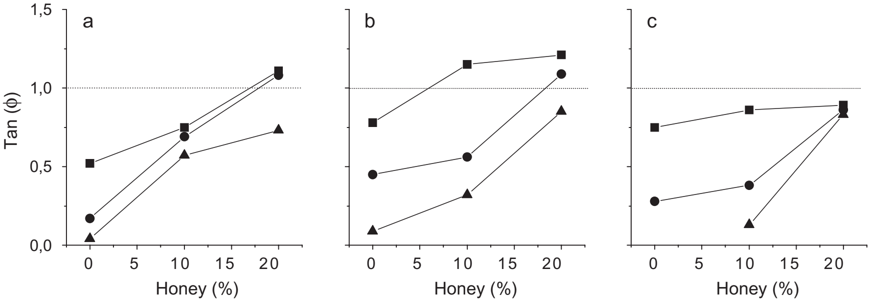

FIG. 6. TANGENT OF THE DEFORMATION ANGLE (*tan* Φ) OF WHEY PROTEIN CONCENTRATE GELS AS A FUNCTION OF HONEY CONTENT
Protein content of gels: 10%, w/w. pH of gels: (a) 3.75; (b) 4.2; (c) 7.0. %. Wheat flour content: (■) 0%, (●) 10%, (▲) 20%. LSD$_{0.05}$ = 0.082.

TABLE 2.
GEL TIME OF WHEY PROTEIN CONCENTRATE GELS WITH DIFFERENT HONEY AND WHEAT FLOUR CONTENT (LSD$_{0.05}$ = 0.123)

pH	Wheat flour (%)	Honey (%)	Gel time (min)
3.75	0	0	0.305 ± 0.138
		10	0.587 ± 0.324
		20	1.029 ± 0.007
	10	0	0.378 ± 0.321
		10	0.757 ± 0.684
		20	0.595 ± 0.114
	20	0	0.328 ± 0.157
		10	0.517 ± 0.230
		20	0.825 ± 0.376
4.2	0	0	0.171 ± 0.005
		10	1.429 ± 0.542
		20	2.232 ± 0.012
	10	0	0.415 ± 0.276
		10	0.987 ± 0.390
		20	0.955 ± 0.429
	20	0	0.618 ± 0.344
		10	0.570 ± 0.325
		20	0.494 ± 0.348
7.0	0	0	1.978 ± 0.623
		10	5.501 ± 0.458
		20	7.327 ± 0.018
	10	0	0.671 ± 0.315
		10	1.523 ± 0.439
		20	2.498 ± 1.403
	20	0	1.007 ± 0.162
		10	0.875 ± 0.125
		20	3.024 ± 0.871

LSD, least significant difference.

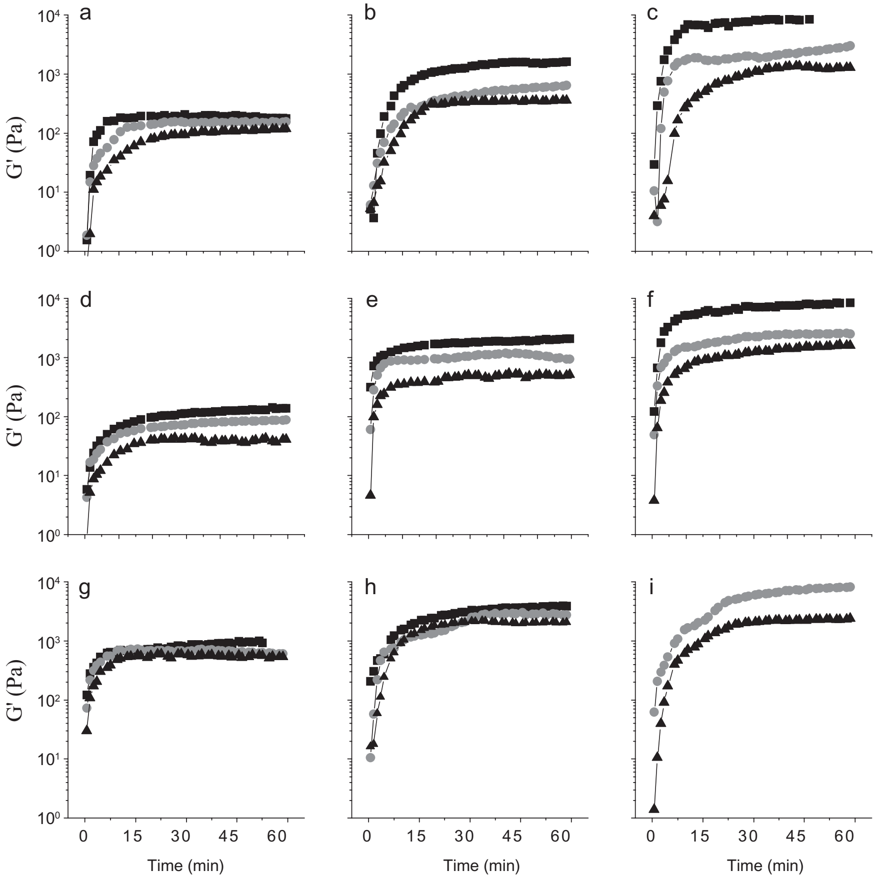

FIG. 7. ELASTIC MODULUS (G') OF WHEY PROTEIN CONCENTRATE GELS AS A FUNCTION OF TIME

Protein content of gels: 10%, w/w. pH of gels: (a, b, c) 3.75; (d, e, f) 4.2; (g, h, i) 7.0. Honey content of gels: (■) 0%, (●) 10%, (▲) 20%. Wheat flour content: (a, d, g) 0%; (b, e, h) 10%; (c, f, i) 20%.

CONCLUSIONS

Honey and wheat flour modifies the rheological properties of WPC gels. Both components have opposite effects: honey increases the viscous-like behavior and wheat flour the solid-like behavior of gels in all conditions assayed. Disulfide bonds, gelatinized starch and the gluten network contribute to the solid-like behavior of neutral gels, whereas gelatinized starch and the gluten proteins determinate the solid-like behavior of acidic gels. The different rheological characteristics of gels prepared at a different pH and with different amounts of honey and wheat flour could be used in different formulated foods.

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