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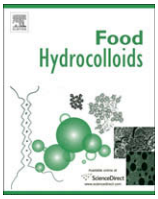

Viscoelastic behaviour of heat-treated whey protein concentrate suspensions

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ABSTRACT

The viscoelastic behaviour of heat-treated whey protein concentrate (WPC) suspensions was studied. Suspensions with total protein (TP) content of 5% and 9% w/v prepared from a commercial WPC with 38% w/w protein were treated at two temperatures (72.5 and 77.5 °C) during selected times to obtain 60% of denatured protein content. Unheated WPC suspensions were used as control. Frequency sweeps were performed in the range of 0.01–10 Hz at 20 °C. Mechanical spectra of WPC suspensions were similar to viscoelastic fluids. However, unheated WPC suspensions showed some mechanical characteristics of colloidal crystals, like little dependency of elastic (G') and viscous (G'') moduli with TP content, solid-like mechanical spectra, and difficulty to flow at low content of TP. At a selected frequency of 1 Hz, viscous modulus was more frequency (ω) dependant on experimental conditions (TP and temperature) than elastic modulus. Mechanical spectra were modelled using power law equations ($G' = a\omega^x$, $G'' = b\omega^y$), but only parameters of heat-treated WPC suspensions containing 9% of TP showed temperature dependency. Characteristic relaxation times (τ_c) were calculated as the inverse of the crossover frequency, where $G'' = G'$. Heat treatments produced a decrease in the extent of the elastic behaviour because heat-treated WPC suspensions presented higher phase angle and smaller τ_c values than unheated WPC suspensions for the same TP content. The τ_c decreased when treatment temperature increased but only in heat-treated WPC suspensions with 5% of TP.

1. Introduction

Whey proteins are well known for combining high nutritional value with functional properties, such as gelation, thermal stability, foam formation or emulsification. Numerous treatments are available to improve whey proteins functionality including enzymatic hydrolysis and heat-induced polymerization (Foegeding, Davis, Doucet, & McGuffey, 2002). Whey proteins are commercialised as isolates (WPI) and concentrates (WPC) and they can be different in functional properties due to differences in milk composition as well as in the manufacturing conditions (Huffman, 1996; Morr & Foegeding, 1990).

Rheological properties of polymers or aggregates are related to the size and shape of molecules (Vardhanabhuti & Foegeding, 1999). The major whey proteins β-lactoglobulin and α-lactalbumin are small and globular in shape; therefore, native whey protein solutions exhibit low viscosity. When globular proteins in aqueous solution are heated above a certain temperature, complex changes on the molecular conformation are produced that lead the proteins to exhibit hydrophobic areas on its surface (Gimel, Durand, & Nicolai, 1994) and induce the formation of aggregates. If protein concentration is sufficiently high a gel may be formed, otherwise aggregates remain in solution. These aggregates or polymers are larger in size as well as more asymmetric in shape and have a larger effective volume fraction than native molecules, resulting in an increase in the viscosity of the suspensions (Bryant & McClements, 1998; Mleko & Foegeding, 1999; Vardhanabhuti & Foegeding, 1999). Suspensions of soluble whey protein aggregates can be used in food processing, for instance as thickening agents.

The formation of soluble whey protein aggregates can be affected by the relative concentrations of each protein and also by mineral, lactose, and total solids contents (Bernal & Jelen, 1985; Garret, Stairs, & Annet, 1988; Hongsprabhas & Barbut, 1996; Xiong, Dawso, & Wan, 1993; de Wit, 1981). Also, the structure of protein aggregates can be influenced by electrostatic interactions (Le Bon, Nicolai, & Durand, 1999). This is due to the intricate balance between attractive and repulsive forces between particles that is determined by the ionic strength and the pH of the solution (Gimel et al., 1994). Temperature and total protein content may also influence the aggregation mechanism and produce differences in the structure of aggregates (Le Bon et al., 1999). Therefore, the behaviour of soluble whey protein aggregates will depend on the conditions of the heat treatment

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as well as on the nature and relative composition of the WPI or WPC used to prepare the suspensions.

Foods can be subject to rheological tests in the linear viscoelastic period to understand the relationship between structural and rheological properties (Ferry, 1970). The response of a material subjected to harmonically varying stress or strain is used to construct a mechanical spectra that can provide information about the viscous and elastic nature of the sample over a range of frequencies (Steffe, 1992). Few studies have reported the rheological behaviour of whey protein aggregates remaining in suspension, despite the extensive study of whey proteins denaturation and aggregation (Bryant & McClements, 2000; Mleko & Foegeding, 1999; Vardhanabhuti & Foegeding, 1999). The knowledge of the mechanical properties of whey protein aggregates is necessary to improve the texture of food products (Le Bon et al., 1999). Only some studies about the characterization of the mechanical behaviour of β-lactoglobulin, bovine serum albumin, α-lactalbumin, and WPI suspensions have been found in literature (Ikeda & Nishinari, 2001a, 2001b; Ikeda, Nishinari, & Foegeding, 2001). For this reason, it is interesting to study the mechanical behaviour of suspensions of soluble whey protein aggregates prepared from a commercial WPC treated under specified conditions of temperature, time, and total protein content in natural pH.

In this study, the viscoelastic behaviour of heat-treated WPC suspensions was analysed performing frequency sweeps in the linear viscoelastic period.

2. Materials and Methods

2.1. Materials

Commercial WPC obtained from cheese whey provided by a local industry was used (Milkaut S.A., Frank, Santa Fe, Argentina).

2.2. Physicochemical Analysis of the WPC

The initial composition of the WPC was determined in laboratory with standardized techniques. The total protein content was considered from total nitrogen content determined by the Kjeldahl method, using a Büchi 430 automatic digestor (Büchi, Flawil, Switzerland), a Büchi 322 distillation unit, and a Mettler DL40RC automatic titrator (Mettler Instrumente AG, Greifensee, Switzerland). Ash content was determined after an overnight incineration in a muffle furnace at 540 °C. Moisture content was measured with a CEM AVC 80 microwave (CEM, Mattheus, NC). Fat content was determined using the Standard International Dairy Federation method (IDF, 1969). Lactose content was defined as the difference between the mass of the sample and the amount of protein, ash, moisture and fat. All compositional analyses were determined in triplicate. The initial composition of the WPC was: lactose 48.8%, protein 38.3%, ash 7.5%, moisture 3.2%, and fat 2.2%.

2.3. Whey Protein Concentrate Suspensions

WPC suspensions at natural pH were prepared to reach 5% and 9% w/v of total protein (TP) content. WPC at an appropriate amount was weighed and dissolved in distilled water with vigorous agitation. The total solid content of the suspensions with 5 and 9% w/v was 12.6 and 22.7% w/v, respectively. Suspensions were prepared three times for each heat treatment.

2.4. Heat Treatments

Glass tubes (160 by 16 mm) containing 10 mL of WPC suspensions were heat-treated at 72.5 and 77.5 °C for different times selected based on previous kinetic studies (Meza, Verdini, & Rubiolo, 2006) to produce 60% of soluble protein aggregates expressed as percentage of denatured protein (DP) content, using a Haake DC 30/Haake W26 recirculating thermostatized bath (Haake Inc. Instruments, Paramus, NJ). Tubes were cooled in an ice water bath for 3 min, stored in refrigerator at 5 °C overnight, and placed in a recirculating thermostatized bath at 20 °C for later analysis. Unheated WPC suspensions were stored at 5 °C overnight and used as control samples. Codes of samples are shown in Table 1.

2.5. Determination of Denatured Protein Content

The soluble protein (SP) content was determined after isoelectric precipitation of denatured/aggregated whey protein (Li-Chan, 1983; Verheul, Roefs, & de Kruif, 1998; de Wit, 1990; de Wit, Hontelez-Backx, & Adamse, 1988). The pH of the suspensions was adjusted to 4.6 ± 0.1 using 0.1 and 1 N of HCl and NaOH solutions. An aliquot of each whey protein suspension was centrifuged for 30 min at 5 °C and 26,000 g in a Biofuge 28RS centrifuge (Heraeus Sepatech, Osterode, Germany). The SP content in the supernatants was determined by measuring absorption at 280 nm after appropriate dilution in a dissociating buffer (EDTA 50 mM, urea 8 M, pH 10) using a Genesys 5 spectrophotometer (Milton Roy Company, Rochester, NY) (Giroux & Britten, 2004; Hudson, Daubert, & Foegeding, 2000). Insoluble protein content of suspensions at pH 4.6 was defined as the difference between TP and SP contents and was used to estimate the extent of denaturation/aggregation of whey proteins (Anandharamakrishnan, Rielly, & Stapley, 2008; Law & Leaver, 1997; Li-Chan, 1983; Morr, 1990). The percentage of DP content was calculated with the following equation (Morr, 1990):

$$\mathrm{DP} = \frac{(\mathrm{TP}-\mathrm{SP})}{\mathrm{TP}} \times 100(\%).$$

2.6. Rheological Measurements

Frequency sweeps were performed in the range of 0.01–10 Hz at 20.0 ± 0.5 °C using a stress controlled rheometer RheoStress 80 (Haake Inc. Instruments, Karlsruhe, Germany) with a cone and plate geometry test fixture. Diameter and angle of the cone were 60 mm and 0.04 rad, respectively. Samples were covered with a thin film of silicone oil to prevent water vaporization during measurements.

The region of linear viscoelasticity was determined prior to each frequency sweep performing stress sweeps to verify the linear relationship between stress and strain. Elastic modulus (G'), viscous modulus (G''), complex viscosity (η^*) and phase angle (δ) were measured at fixed stress amplitude depending on the sample. Values of G' and G'' at a selected frequency of 1 Hz were used for statistical analysis.

2.7. Data Analysis of the Mechanical Spectrum

2.7.1. Modelling of the Mechanical Spectrum

Frequency (ω) dependence of G' and G'' was modelled with the power law parameters a, b, x and y described by the Eqs. 1 and 2.

$$G' = a\omega^x \tag{1}$$

$$G'' = b\omega^y \tag{2}$$

2.7.2. Determination of the Characteristic Relaxation Times

Using data modelled with the power law equations (Eqs. 1 and 2) the crossover frequency, where $G'' = G'$, was determined. The crossover point is a useful criterion for comparing products or treatments (Steffe, 1992). Characteristic relaxation times (τ_c) were calculated as the inverse of crossover frequencies (ω_c) (Kulmyrzaev & McClements, 2000; Sopade & Halle, 2004; Wientjes, Duits,

Table 1
Heat treatment conditions (time and temperature) and percentage of denatured protein content of WPC suspensions

Samples	Total protein content (% w/v)	Temperature (°C)	Time (min)	Denatured protein content (%)
5-unheated	5	—	—	52.8 ± 1.5^a
9-unheated	9	—	—	47.0 ± 1.1^b
5-72.5	5	72.5	15	60.9 ± 0.3^c
9-72.5	9		40	61.7 ± 0.1^c
5-77.5	5	77.5	6	60.8 ± 0.5^c
9-77.5	9		12	61.6 ± 0.3^c

Mean values and standard deviations of three samples.
Values with different letters are significantly different ($P < 0.05$).

Jongschaap, & Mellema, 2000). Values of τ_c were used for statistical analysis.

2.8. Statistical Analysis

Analysis of variance was used ($P < 0.05$) and when the effect of the factors was significant, the test of multiple ranks HSD of Tukey was applied (95% confidence level). The statistical analysis was performed using Minitab 13.20 (Minitab Inc., State College, PA).

3. Results and Discussion

3.1. Analysis of Mechanical Spectrum

Mechanical spectrum of unheated WPC suspensions are shown in Fig. 1. At low frequencies a more fluid-like behaviour was observed, where G'' was higher than G'. The crossover between G'' and G' curves was observed at the end of the first decade of the frequency range of 0.01–0.1 Hz. At this point, the relationship between the viscously dissipated and stored energy per deformation cycle is equal to 1 ($\tan\delta = G''/G'$). At frequencies larger than the crossover frequency (ω_c), a solid-like behaviour is observed. According to Steffe (1992), this description of the mechanical spectra is characteristic of the viscoelastic fluids. Similar results were observed by Ikeda et al. (2001) that analysed the mechanical spectra of WPI suspensions prepared in a concentration range of 0.1%–10% w/w WPI in 0.1 M NaCl, with a crossover between G'' and G' at the lower end of the frequency range (<0.3 Hz).

Independency of G' and a low dependency of G'' with TP content was observed in unheated WPC suspensions (Fig. 1). Ikeda et al. (2001) found that dynamic viscoelastic properties of β-lactoglobulin solutions appeared little concentration dependent in a concentration range of 0.01%–7% w/w in 0.1 M NaCl, and the same behaviour was observed in WPI suspensions prepared in a concentration range of 0.1%–10% w/w in 0.1 M NaCl. The little concentration dependence of modulus in a certain concentration region has been observed in some native globular protein aqueous colloids, and interpreted in terms of the formation of colloid crystals (Ikeda & Nishinari, 2001a, 2001b; Ikeda et al., 2001).

Mechanical spectrum of heat-treated WPC suspensions presented similar description to unheated WPC suspensions, but showed a dependence of G'' and ω_c with the treatment temperature (Fig. 2a, b).

A shear-thickening behaviour was observed in both unheated (Fig. 1) and heat-treated WPC suspensions, where η^* increases with frequency. Some researchers observed a shear-thinning behaviour, where η^* decreases with frequency, in both WPI and WPC suspensions prepared with different total protein contents (Ikeda et al., 2001; Lizarraga, De Piante Vicin, Gonzalez, Rubiolo, & Santiago, 2006). However, other research groups observed a shear-thickening behaviour. Walkenström, Nielsen, Windhab, and Hermansson (1999) analysed the effects of the flow behaviour at different temperatures on WPI suspensions, pure or mixed with xanthan, and observed that all shear-treated 10% w/w WPI suspensions showed a shear-thickening behaviour independently of process conditions. Mleko and Foegeding (1999), who have studied whey

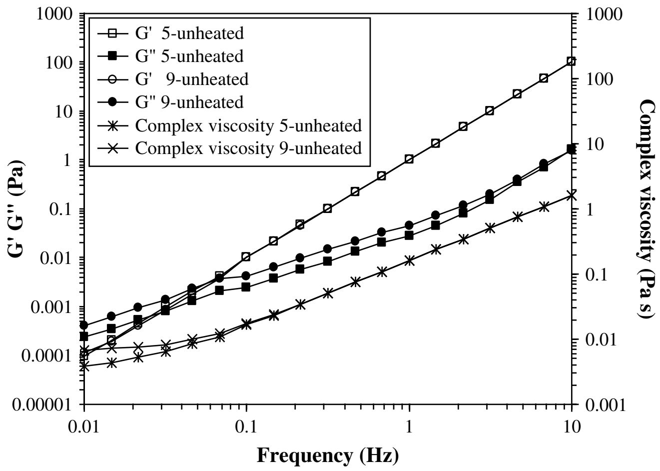

Fig. 1. Mechanical spectra of unheated WPC suspensions.

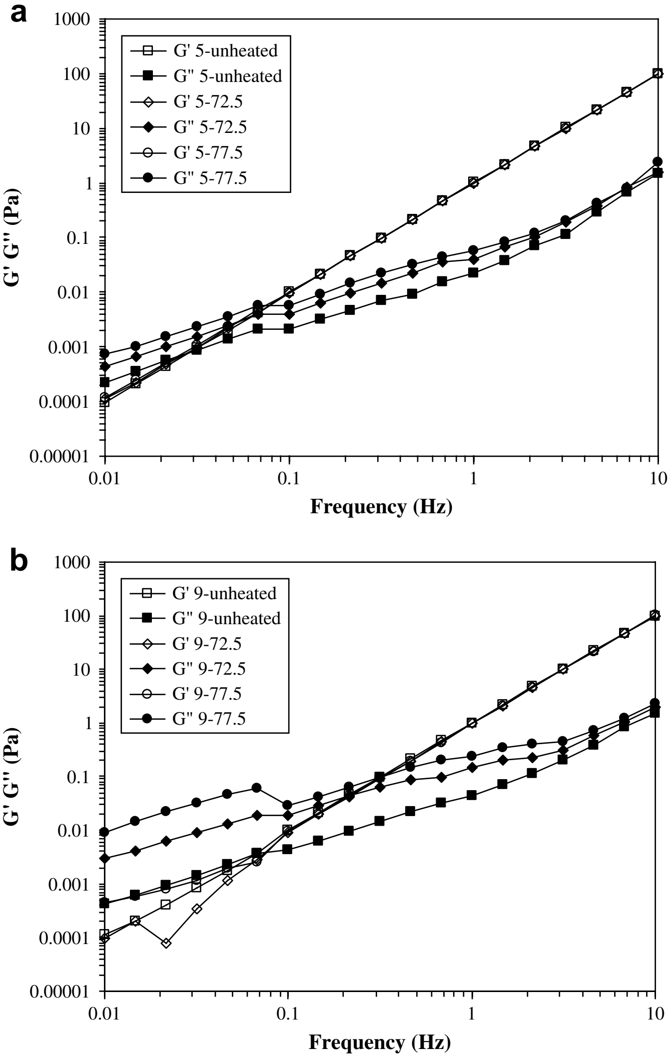

Fig. 2. Mechanical spectra of heat-treated WPC suspensions. (a) 5% and (b) 9% of total protein content.

protein polymers obtained by two-step heating of WPI suspensions, observed a shear-thickening behaviour in 4% w/w WPI suspensions heated at pH 8.0, held at pH 8.0, and adjusted to 2.5% w/w of protein for the second heating. Krešić, Lelas, Jambrak, Herceg, and Brnčić (2007) analysed the influence of novel food processing technologies (high pressure, ultrasound and tribomechanical activation) on the rheological properties of WPC and WPI suspensions with 10% w/w of protein and observed a shear-thickening behaviour in control and treated suspensions, where an increase in shear rate resulted in an increase in apparent viscosity. In addition, Mleko (2004), who has studied the rheological properties of commercial whey products (powdered whey, demineralised powdered whey, WPC and WPI) with wide range of protein content, found that whey protein suspensions showed shear-thinning or shear-thickening behaviour depending on the sample.

Although the behaviour observed in WPC suspensions it is not clear, the shear-thickening may arise from a structuring of the WPC aggregates during the oscillatory shear flow in the rheometer (Walkenström et al., 1999), taking into consideration that shear-thickening is usually an indication that the applied force is causing the material to adopt a more ordered structure or that there are greater interactions between particles (Mleko & Foegeding, 1999).

Early oscillatory-shear studies demonstrated the remarkable fact that crystal order can be induced in suspensions of spheres with near hard-sphere interactions even when the equilibrium structure is liquid-like (Ackerson & Pusey, 1988). Structure formation during flow is strongly affected by the delicate balance among interparticle forces, Brownian motion and hydrodynamic interactions. The resulting nonequilibrium microstructure is in turn a principal determinant of the suspension rheology. At high shear rates, clustering due to strong hydrodynamic forces leads to shear-thickening behaviour. Oscillatory-shear flow generates metastable ordering in suspensions with equilibrium liquid structure (Vermant & Solomon, 2005).

Changes in phase angle (δ) of WPC suspensions are shown in Fig. 3a, b. The pattern of response as frequency changes was similar in all the suspensions. At very low frequencies δ was relatively high showing the dominant effect of the viscous component. As frequency increased δ levelled and at very high frequencies δ decreased showing the dominant effect of the elastic component. Unheated WPC suspensions presented lower values of δ than heat-treated WPC suspensions. These results indicate that heat treatments produced an increase in the extent of viscous behaviour. Also, an almost purely viscous behaviour at very low frequencies, with δ equal or close to 90°, was reached in heat-treated WPC suspensions, containing 9% of TP (Fig. 3b).

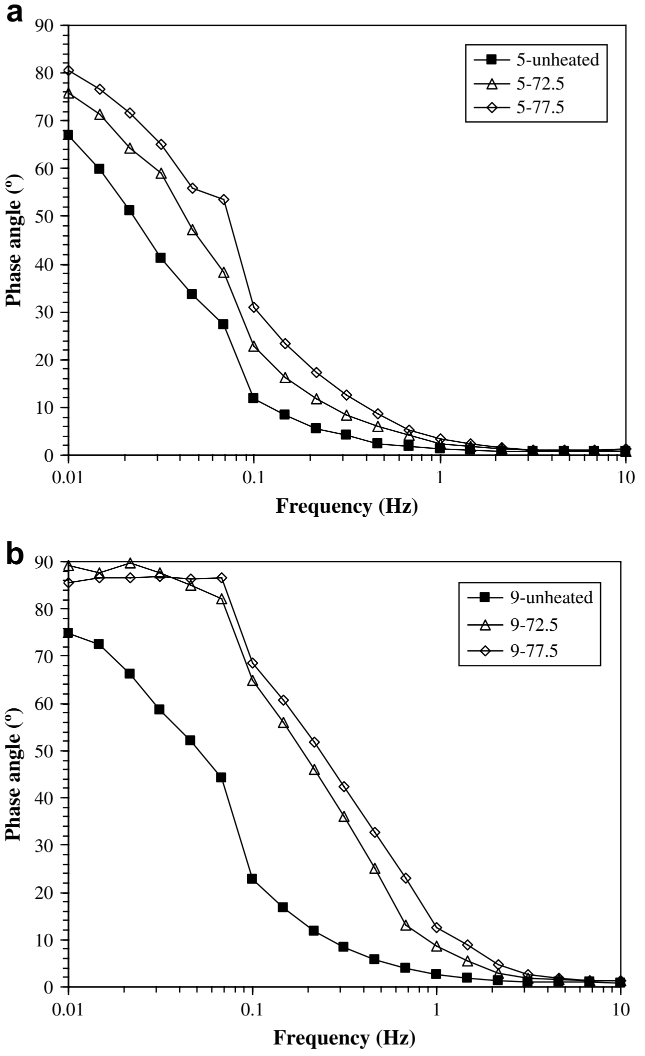

Fig. 3. Dependency of the phase angle (δ) with frequency (ω) of WPC suspensions. (a) 5% and (b) 9% of total protein content.

3.2. Elastic and Viscous Moduli at 1Hz

Values of G' and G'' at 1 Hz are shown in Table 2. In both unheated WPC suspensions (5% and 9% TP), G' (1 Hz) was higher than G'' (1 Hz), which means that at this selected frequency unheated WPC suspensions behaved as a solid material. There were no significant differences in G' (1 Hz) between unheated WPC suspensions containing 5% and 9% of TP. However, G'' (1 Hz) was higher in unheated WPC suspensions containing 9% than in 5% of TP, probably due to the higher content of total solids.

Elastic modulus at 1 Hz of heat-treated WPC suspensions containing 5% of TP was similar to unheated WPC suspensions when the heat treatment temperature was 72.5 °C and decreased in suspensions treated at 77.5 °C. Heat-treated WPC suspensions containing 9% of TP showed lower G'(1 Hz) values than unheated WPC suspensions for both heat treatment temperatures and G' (1 Hz) values decreased as temperature increased from 72.5 to 77.5 °C. In contrast, for suspensions with the same TP content G'' (1 Hz) values increased in all heat-treated WPC suspension when treatment temperature increased. Values of G' (1 Hz) were always superior than G'' (1 Hz), indicating that at this frequency heat-treated WPC suspensions behave as a solid material.

Both G' (1 Hz) and G'' (1 Hz) showed dependency on studied experimental conditions (TP and temperature), but G'' (1 Hz) was more sensitive to their effect. Consequently, at this selected frequency heat treatments affected more extensively liquid-like than solid-like behaviour. These observations are consistent with the analysis of the mechanical spectra (Section 3.1) where G'' was more influenced than G' by studied experimental conditions.

Table 2
Elastic (G') and viscous (G'') moduli of WPC suspensions at 1 Hz

Samples	G' (Pa)	G'' (Pa)
5-unheated	1.008 ± 0.001^a	0.025 ± 0.003^a
9- unheated	1.009 ± 0.001^a	0.043 ± 0.004^b
5-72.5	1.006 ± 0.000^a	0.040 ± 0.002^b
9-72.5	0.982 ± 0.000^b	0.147 ± 0.001^c
5-77.5	1.000 ± 0.001^c	0.059 ± 0.002^d
9-77.5	0.973 ± 0.003^d	0.229 ± 0.001^e

Mean values and standard deviations of three samples.
Values with different letters in each column are significantly different ($P < 0.05$).

3.3. Modelling of the Mechanical Spectra

Power law parameters used to model the frequency dependence of G' and G'' are shown in Table 3. Coefficients a and b represent the magnitude of G' and G'' respectively at a given frequency and exponents x and y represent the slopes of the relationships between modulus and frequency. For instance, an exponent x near zero means that G' does not change with frequency, representing a characteristic behaviour of a fully cured gel (Resch & Daubert, 2002).

In our case, parameter a was higher than b in both unheated and heat-treated WPC suspensions, indicating that G' predominates in the mayor part of the spectra. This solid-like mechanical spectra has been observed in some native globular protein aqueous colloids and interpreted in terms of the formation of colloid crystals (Ikeda & Nishinari, 2001a, 2001b; Ikeda et al., 2001).

Slope x was always significantly higher than y, indicating that G' increases faster than G'' as frequency increased in all studied suspensions. It is interesting to note that the magnitude of x is approximately 2 (Table 3), revealing that G' increases dramatically with frequency. Because $\eta^* = (G^*/\omega)$ and $G^* = [(G'')^2 + (G')^2]^{1/2}$, it is possible that the shear-thickening behaviour of η^* observed in unheated WPC suspensions (Fig. 1) and in heat-treated WPC suspensions is the result of the dramatic increase of G' (Rao & Tattiyakul, 1999). This behaviour may arise from a structuring of the WPC aggregates during the oscillatory shear flow in the rheometer (Walkenström et al., 1999) that may be responsible for the rapid increase in G' with frequency.

No significant differences were observed in parameters a, x and b between unheated WPC suspensions due to TP content (5% and 9%). Only parameter y of unheated WPC suspensions containing 9% of TP was lower than that of unheated WPC suspensions with 5% of TP. These results are consistent with the independency of G' and the little dependency of G'' with TP content showed in the analysis of the mechanical spectra of unheated WPC suspensions (Section 3.1).

Only power law parameters of heat-treated WPC suspensions with 9% of TP showed dependency on experimental temperatures (72.5 and 77.5 °C). In this case, a and b increased and x and y decreased when the temperature of heat treatment increased. This results indicate that WPC suspensions with the highest content of TP heated at 77.5 °C presented more elastic and viscous behaviour than suspensions treated at 72.5 °C.

3.4. Analysis of the Characteristic Relaxation Times

Characteristic relaxation times (τ_c) of WPC suspensions are shown in Table 4. Some authors have used the expression "terminal relaxation time" to define τ_c because, in a mechanical spectra the pseudo-terminal region always occurs for relaxation times lower than the reciprocal of the crossover frequency (Franco, Berjano, & Gallegos, 1997). High values of τ_c implies important elastic characteristics in the material.

The τ_c of unheated WPC suspensions with 5% of TP was significantly higher than that of suspensions with 9% of TP, indicating that suspensions with the lower content of TP have a higher range of elastic behaviour in the mechanical spectra. The rheological behaviour of globular protein suspensions is similar to a colloidal crystal. It has been known for years that charged colloidal particles in an aqueous solution can arrange into a crystalline lattice (Lindsay & Chaikin, 1982). Such colloidal system, designated as a colloidal crystal, mechanically responds to oscillating small strains as if it was a solid. However, it is not known with certainty the reason why globular protein suspensions behave as colloidal crystals. These phenomena could be due to the existence of repulsive forces of hydration, where polar interactions orient water molecules on the surface of the protein and generate repulsions between molecules. These polar forces could be far greater than van der Waals attractive or electrostatic repulsive forces (Ikeda & Nishinari, 2001b). Also, it has been observed that colloidal crystals flow relatively easily at high concentrations, but do not flow easily at low concentrations (Inoue & Matsumoto, 1996). In our study, unheated WPC suspensions have a high content of DP (>40%) (Table 1) that may be related to the high temperatures used in some steps of the commercial WPC manufacture, like heating and drying (de la Fuente, Hemar, Tamehana, Munro, & Singh, 2002; de la Fuente, Singh, & Hemar, 2002; Puyol, Cotter, & Mulvihill, 1999; Roufik, Paquin, & Britten, 2005). However, it possible that globular proteins that remain in native state showed some mechanical characteristics of colloidal crystals like the difficulty to flow at low contents of TP represented by high values of τ_c in unheated WPC suspensions containing 5% of TP.

Table 4
Characteristic relaxation times (τ_c) of WPC suspensions

Samples	τ_c (s)
5-unheated	7.25 ± 0.73^a
9-unheated	3.51 ± 0.02^b
5-72.5	3.68 ± 0.29^c
9-72.5	0.64 ± 0.04^d
5-77.5	2.43 ± 0.08^e
9-77.5	0.63 ± 0.05^d

Mean values and standard deviations of three samples.
Values with different letters are significantly different ($P < 0.05$).

Characteristic relaxation times of heat-treated WPC suspensions were smaller than in unheated WPC suspensions for the same content of TP (Table 4), indicating a smaller range of elastic behaviour for heat-treated suspensions. The repulsive forces present in globular protein suspensions are dominated by a molecular surface force (such as the hydration force) that would be lost by denaturation, due to exposure of the hydrophobic core of the protein (Ikeda & Nishinari, 2000). This phenomena could explain the diminution of τ_c in the heat-treated WPC suspensions.

It was observed that only in heat-treated WPC suspensions containing 5% of TP, the τ_c decreased when the temperature of heat treatment increased. The rheological behaviour is related to size, nature and amount of protein aggregates. Kazmierskim and Corredig, 2003 have published that the size of whey protein aggregates is more dependent on the temperature than on the time of heat treatment, and also, aggregates that have been formed at higher

Table 3
Power law parameters for the elastic ($G' = a\omega^x$) and viscous moduli ($G'' = b\omega^y$) of WPC suspensions

Samples	G'			G''		
	a (Pa s)	x	R^2	b (Pa s)	y	R^2
5-unheated	0.025 ± 0.000^a	2.011 ± 0.007^a	0.99	0.005 ± 0.000^a	1.177 ± 0.016^a	0.98
9-unheated	0.024 ± 0.000^a	2.019 ± 0.007^a	0.99	0.008 ± 0.000^a	1.119 ± 0.008^b	0.99
5-72.5	0.025 ± 0.001^a	2.005 ± 0.009^a	0.99	0.008 ± 0.002^a	1.106 ± 0.007^c	0.99
9-72.5	0.019 ± 0.000^b	2.114 ± 0.008^b	0.99	0.033 ± 0.002^b	0.872 ± 0.021^d	0.99
5-77.5	0.026 ± 0.000^a	1.987 ± 0.003^c	0.99	0.011 ± 0.000^c	1.054 ± 0.006^c	0.99
9-77.5	0.041 ± 0.009^a	1.786 ± 0.093^c	0.99	0.066 ± 0.009^d	0.728 ± 0.039^e	0.96

Mean values and standard deviations of three samples.
Values with different letters in each column are significantly different ($P < 0.05$).

temperatures are bigger in size. Aggregates that are larger in size and shape can produce an increase in the viscous component of the suspensions. This behaviour could explain the diminution in τ_c observed in heat-treated WPC suspensions with 5% of TP when the heat treatment temperature increased.

4. Conclusions

Unheated WPC suspensions presented mechanical spectra similar to those of viscoelastic fluids. Nevertheless, those suspensions showed some characteristics of colloidal crystals, like little dependency of moduli with the TP content, solid-like mechanical spectra, and the difficulty to flow at low content of TP.

Heat-treated WPC suspensions also showed a mechanical spectra similar to those of viscoelastic fluids. Also, heat treatments produced a diminution in the extent of elastic behaviour of WPC suspensions, probably due to the lost of the repulsive forces of hydration by heat-denaturation.

Although the DP content of heat-treated WPC suspensions were all equal, differences in the rheological behaviour could be attributed to the formation of different aggregates that are dependent on the TP content and heat treatment temperature.

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References

Ackerson, B. J., & Pusey, P. N. (1988). Shear-induced order in suspensions of hard spheres. *Physical Reviews Letter, 61*, 1033–1036.

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. (2008). Loss of solubility of α-lactalbumin and β-lactoglobulin during the spray drying of whey proteins. Lebensmittel-Wissenschaft und- Technologie. *Lebensmittel-Wissenschaft und-Technologie, 41*, 270–277.

Bernal, V., & Jelen, P. (1985). Thermal stability of whey proteins. A calorimetric study. *Journal of Dairy Science, 68*, 2847–2852.

Bryant, C. M., & McClements, D. J. (1998). Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends in Food Science and Technology, 9*, 143–151.

Bryant, C. M., & McClements, D. J. (2000). Optimizing preparation conditions for heat-denatured whey protein solutions to be used as cold-gelling ingredients. *Journal of Food Science, 65*, 259–263.

Ferry, J. D. (1970). *Viscoelastic properties of polymers* (2nd ed.). New York: John Wiley & Sons.

Foegeding, E. A., Davis, J. P., Doucet, D., & McGuffey, M. K. (2002). Advances in modifying and understanding whey protein functionality. *Trends in Food Science and Technology, 13*, 151–159.

Franco, J. M., Borjano, M., & Gallegos, C. (1997). Linear viscoelasticity of salad dressing emulsions. *Journal of Agricultural and Food Chemistry, 45*, 713–719.

de la Fuente, M. A., Hemar, Y., Tamehana, M., Munro, P. A., & Singh, H. (2002). Process-induced changes in whey proteins during the manufacture of whey protein concentrates. *International Dairy Journal, 12*, 361–369.

de la Fuente, M. A., Singh, H., & Hemar, Y. (2002). Recent advances in the characterization of heat-induced aggregates and intermediates of whey proteins. *Trends in Food Science and Technology, 13*, 262–274.

Garret, J. M., Stairs, R. A., & Annet, R. G. (1988). Thermal denaturation and coagulation of whey proteins: effect of sugars. *Journal of Dairy Science, 71*, 10–16.

Gimel, J. C., Durand, D., & Nicolai, T. (1994). Structure and distribution of aggregates formed after heat-induced denaturation of globular proteins. *Macromolecules, 27*, 583–589.

Giroux, J. H., & Britten, M. (2004). Heat treatment of whey proteins in the presence of anionic surfactants. *Food Hydrocolloids, 18*, 685–692.

Hongsprabhas, P., & Barbut, S. (1996). Ca^{2+}-induced gelation of whey protein isolate: effects of pre-heating. *Food Research International, 29*, 135–139.

Hudson, H. M., Daubert, C. R., & Foegeding, E. A. (2000). Rheological and physical properties of derivitized whey protein isolate powders. *Journal of Agricultural and Food Chemistry, 48*, 3112–3119.

Huffman, L. M. (1996). Processing whey protein for use as a food ingredient. *Food Technology, 50*, 49–52.

IDF. (1969). *Determination of the fat content of cheese and of processed cheese products. IDF Standard 5A. Bruselas*. Belgium: International Dairy Federation.

Ikeda, S., & Nishinari, K. (2000). Intermolecular forces in bovine serum albumin solutions exhibiting solidlike mechanical behaviors. *Biomacromolecules, 1*, 757–763.

Ikeda, S., & Nishinari, K. (2001a). On solid-like rheological behaviors of globular protein solutions. *Food Hydrocolloids, 15*, 401–406.

Ikeda, S., & Nishinari, K. (2001b). Solid-like mechanical behaviors of ovalbumin aqueous solutions. *International Journal of Biological Macromolecules, 28*, 315–320.

Ikeda, S., Nishinari, K., & Foegeding, E. A. (2001). Mechanical characterization of network formation during heat-induced gelation of whey protein dispersions. *Biopolymers, 56*, 109–119.

Inoue, H., & Matsumoto, T. (1996). Viscoelastic characterization of solid-like structure in aqueous colloids of globular proteins. *Colloids Surfaces A, 109*, 89–96.

Kazmierskim, M., & Corredig, M. (2003). Characterization of soluble aggregates from whey protein isolate. *Food Hydrocolloids, 17*, 685–692.

Krešić, G., Lelas, V., Jambrak, A. R., Herceg, Z., & Brnčić, S. R. (2007). Influence of novel food processing technologies on the rheological and thermophysical. properties of whey proteins. *Journal of Food Engineering, 87*, 64–73.

Kulmyrzaev, A., & McClements, D. J. (2000). High frequency dynamic shear rheology of honey. *Journal of Food Engineering, 45*, 219–224.

Law, A. J. R., & Leaver, J. (1997). Effect of protein concentration on rates of thermal denaturation of whey proteins in milk. *Journal of Agricultural and Food Chemistry, 45*, 4255–4261.

Le Bon, C., Nicolai, T., & Durand, D. (1999). Growth and structure of aggregates of heat-denatured β-lactoglobulin. *International Journal of Food Science and Technology, 34*, 451–465.

Li-Chan. (1983). Heat-induced changes in the proteins of whey protein concentrate. *Journal of Food Science, 48*, 47–56.

Lindsay, H. M., & Chaikin, P. M. (1982). Elastic properties of colloidal crystals and glasses. *Journal of Chemistry Physics, 36*, 3774–3781.

Lizarraga, M. S., De Piante Vicin, D., Gonzalez, R., Rubiolo, A., & Santiago, L. G. (2006). Rheological behaviour of whey protein concentrate and λ-carrageenan aqueous mixtures. *Food Hydrocolloids, 20*, 740–748.

Meza, B.E., Verdini, R.A., & Rubiolo, A.C. (2006). Efecto de los tratamientos térmicos y de la congelación en la agregación de suspensiones de concentrado de proteína de suero. Proceedings of XXII Congreso Interamericano de Ingeniería Química and V Congreso Argentino de Ingeniería Química. Buenos Aires, Argentina. Available on CD ROM.

Mleko, S. (2004). Rheological properties of whey products dispersions. *Milchwissenschaft, 59*, 287–290.

Mleko, S., & Foegeding, E. A. (1999). Formation of whey protein polymers: effects of a two-step heating process on rheological properties. *Journal of Texture Studies, 30*, 137–149.

Morr, C. V. (1990). Effect of heating and elevated temperature storage on cheese whey. *Journal of Food Science, 55*, 1177–1179.

Morr, C. V., & Foegeding, E. A. (1990). Composition and functionality of commercial whey and milk protein concentrates and isolates: a status report. *Food Technology, 44*, 100–112.

Puyol, P., Cotter, P. F., & Mulvihill, D. M. (1999). Thermal gelation of commercial whey protein concentrate: influence of pH 4.6 insoluble protein on thermal gelation. *International Journal of Dairy Technology, 52*, 81–91.

Rao, M. A., & Tattiyakul, J. (1999). Granule size and rheological behavior of heated tapioca starch dispersions. *Carbohydrate Polymers, 38*, 123–132.

Resch, J. J., & Daubert, C. R. (2002). Rheological and physicochemical properties of derivatized whey protein concentrate powders. *International Journal of food Properties, 5*, 419–434.

Roufik, S., Paquin, P., & Britten, M. (2005). Use of high-performance size exclusion chromatography to characterize protein aggregation in commercial whey protein concentrates. *International Dairy Journal, 15*, 231–241.

Sopade, P. A., & Halley, P. J. (2004). Dynamic and steady-state rheology of Australian honeys at subzero temperatures. *Journal of Food Process Engineering, 27*, 284–309.

Steffe, J. F. (1992). *Rheological Methods in Food Process Engineering*. East Lansing, MI: Freeman Press.

Vardhanabhuti, B., & Foegeding, E. A. (1999). Rheological properties and characterization of polymerized whey protein isolates. *Journal of Agricultural and Food Chemistry, 47*, 3649–3655.

Verheul, M., Roefs, S. P. F. M., & de Kruif, K. G. (1998). Kinetics of heat-induced aggregation of α-lactoglobulin. *Journal of Agricultural and Food Chemistry, 46*, 896–903.

Vermant, J., & Solomon, M. J. (2005). Flow-induced structure in colloidal suspensions. *Journal of Physics: Condensed Matter, 17*, R187–R216.

Walkenström, M., Nielsen, E., Windhab, A.-M., & Hermansson. (1999). Effects of flow behaviour on the aggregation of whey protein suspensions, pure or mixed with xanthan. *Journal of Food Engineering, 42*, 15–26.

Wientjes, R. H. W., Duits, M. H. G., Jongschaap, R. J. J., & Mellema, J. (2000). Linear rheology of guar gum solutions. *Macromolecules, 33*, 9594–9605.

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

de Wit, J. N. (1990). Thermal stability and functionality of whey proteins. *Journal of Dairy Science, 73*, 3602–3612.

de Wit, J. N., Hontelez-Backx, E., & Adamse, M. (1988). Evaluation of functional properties of whey protein concentrates and whey protein isolates. 3. Functional properties in aqueous solution. *Netherlands Milk Dairy Journal, 42*, 155–172.

Xiong, Y. L., Dawson, K. A., & Wan, L. (1993). Thermal aggregation of β-lactoglobulin: effect of pH, ionic environment, and thiol reagent. *Journal of Dairy Science, 76*, 70–77.