International Food Research Journal 20(5): 2167-2171 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Influence of pH and protein concentration on rheological properties of whey protein dispersions

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Article history

Received: 14 March 2013
Received in revised form: 17 June 2013
Accepted: 18 June 2013

Keywords

Whey proteins
Shear
Viscosity
Gel

Abstract

This experiment was carried out to study the flow properties of whey protein (WP) dispersions as influenced by pH and protein concentration. All WP dispersions showed shear thinning behaviour around the ambient temperature which was not affected by pH at low protein concentrations (10%). Consistency coefficient of the dispersions was significantly ($P < 0.05$) affected by pH while the flow behaviour index was influenced by both, the pH and protein concentration. The storage moduli of heat induced gels increased with increase in protein concentration. The heat induced gels formed at low pH (4) were less elastic than those formed at pH 5 and 6.

Introduction

Whey proteins (WP) have found use as a food ingredient not only due to their nutritional benefits but also owing to their technological properties. The overall functionality of WP is governed by the physico-chemical properties of individual proteins present in WP as well as the intrinsic (food properties) and extrinsic (environmental) conditions. Viscosity and heat induced gelation are important functional properties of WP that govern their utility as food ingredients.

Viscosity, resistance to flow under an applied force, is related to the consumer perception of some liquid and semi-solid type foods such as beverages, gravies, soups and sauces. The thickening ability of WP is a useful functional property in such food products and the awareness of the viscous behaviour is practically important in relation to processing, designing processing conditions and development of food products (Huang and Kinsella, 1986). The molecular properties of proteins including the size and shape as well as protein-solvent interactions, hydrodynamic volume and the molecular flexibility in the hydrated state affect the viscosity (Genovese *et al*., 2007). The denatured dispersions of randomly coiled molecules show greater viscosity than solutions of compact folded globular molecules of the same molecular weight (Damodaran *et al*., 2008). In general, very dilute solutions of WP behave as Newtonian fluids, while at higher concentrations the flow is pseudoplastic (Carr *et al*., 2003).

The capability of undenatured WP to form stable heat induced gels is also important in food formulations and product development as these gels can act as a basic medium to hold other components such as water, lipids, sugars and flavour in heterogeneous food systems while providing textural and thickening attributes (Matsudomi *et al*., 1993; Resch *et al*., 2004). Compared to pre-gelatinized starches and hydrocolloids, which also have the capacity of holding water and increasing viscosity, WP add superior nutritional benefits (Resch *et al*., 2005). Thermal gelation involves initial unfolding and subsequent aggregation of proteins depending on the balance of attractive and repulsive forces (Hudson *et al*., 2000). A proper balance of protein-protein as well as protein-water interactions fundamentally controls gelation mechanism and the gel appearance. The physical characteristics of these gels can be altered by manipulating protein concentration, ionic strength, heating time and temperature, solvent condition and quality and addition of other macromolecules or filler particles (Verheul *et al*., 1998). Inconsistent gelling properties of commercially available WP products necessitate the need for developing a fundamental understanding of gelling behaviour of WP under different conditions.

Several conditions have been studied in order to manipulate the functionality of WP. For instance, a recent study revealed a two order magnitude of particle size reduction of microparticulated WP at low pH with substantially enhanced solubility and heat stability in comparison to that at neutral pH (Dissanayake *et al*., 2012). However, the effect of these conditions on the rheological properties is not

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well documented. Therefore, the main objective of this study was to examine the flow and viscoelastic properties of heat treated WP dispersions as a function of pH and protein concentration.

Materials and Methods

Raw materials

Whey protein retentates (~30% total solids) and corresponding whey permeates were provided by Warrnambool Cheese and Butter Factory (Warrnambool, Victoria, Australia). The compositional analysis of the retentate was carried out following established AOAC methodology and reported previously (Dissanayake and Vasiljevic, 2009).

Sample preparation

Three different concentrations WP dispersions (10, 17.5 and 25%, w/w) were prepared for this study. The WP retentate (protein content 25%) was adjusted to 10 and 17.5% protein concentration using the corresponding whey permeates while the corresponding control was the retentate without any dilution. Each concentration of WP dispersion thus prepared was further subdivided into 3 sets and pH was adjusted to 4 and 5 using concentrated HCl while the natural pH of the WP retentate (pH 6) was the corresponding control. In all, 9 sets of WP dispersions were prepared for the rheological studies. The WP retentate collected on two different days served as two replicates.

Measurement of flow and viscoelastic behaviour of WP dispersions

Flow behavior of WP dispersions was studied using a CS/CR rheometer (MCR 301, Anton Paar, GmbH, Germany) equipped with a software (Rheoplus/32 v2.81, Anton Paar). Each WP dispersion (~ 3.9 g) was introduced into a double gap cylinder measuring system (DG26.7- SN7721, Anton Parr) and a thin layer of low-density oil was spread on top of the sample to minimize moisture loss. The mixture was pre-sheared for 5 s at a constant shear rate of 500/s at 20°C and held for 30 s to equilibrate before data collection. Apparent viscosity of the samples was recorded at shear rates over the range of 0.1 – 100/s for 5 min at 20°C. The behaviour of flow curves thus obtained was explained using Power Law (Ostwald model); $\sigma = k\dot{\gamma}^{n}$, where, k , n , σ, and $\dot{\gamma}$ were consistency coefficient (Pa.s^n), flow behavior index (dimensionless), shear stress (Pa), and shear rate (per s), respectively.

Changes in viscoelastic properties during heat gelation of WP dispersions were also investigated using the double-gap geometry described above at constant strain (1%) and frequency (1 Hz). The gelation was carried out by heating the samples from 20 – 90°C (2°C/min) and holding at 90°C for 10 min followed by cooling to 4°C (2°C/min) and aging at 4°C for about 4 h.

Statistical analysis

The experiments were arranged as a randomized block full factorial design. The results were analysed using General Linear Model of SAS statistical programme (SAS, 1996). The three levels of concentration (10, 17.5 and 25%) and pH (4, 5 and 6) were used as the main factors and the replication was the block. All experiments were replicated at least once and sub-sampled ($n \geq 4$). The level of significance was set at $p = 0.05$.

Results and Discussion

Flow behaviour of WP dispersions

Figure 1 A-C presents the changes in apparent viscosity of WP dispersions during the shear rate sweep. WP dispersions behaved as non-Newtonian fluids particularly at low protein concentration (10.5%) regardless of their pH (Figure 1A), exhibiting shear thinning behaviour (Rao, 1999), as was observed by the decrease in apparent viscosity with increasing shear rate. The pseudo-plastic (shear thinning) character of the WP dispersions may have resulted from the disruption of weak inter-particle linkages at sufficiently high enough hydrodynamic forces which were generated when shearing (Rao, 1999) as well as the affinity of protein molecules to align with the direction of flow (Damodaran, 2008). Polymer molecules in dilute solutions are known to behave independent of their environmental conditions, their viscosity depending on the dimensions of the polymer such as hydrodynamic volume and the radius of gyration (Rao, 1999). Yet, WP being compact globular proteins may not have any substantial difference in their dimensions in the presence of vast amount of solvent. Whey proteins, especially β-Lg, have been reported to behave as Newtonian fluids at protein concentrations up to 5% (w/w) but exhibit shear thinning (non-Newtonian) at higher protein concentrations (Carr *et al*., 2003).

Whey protein dispersions at pH 4 showed higher initial viscosities than at pH 5 and 6 when protein concentration was raised to 17.5% (Figure 1B) that remained high throughout the shear rate ramp. The WP dispersion at pH 5 had an intermediate effect on apparent viscosity while at pH 6 the dispersions

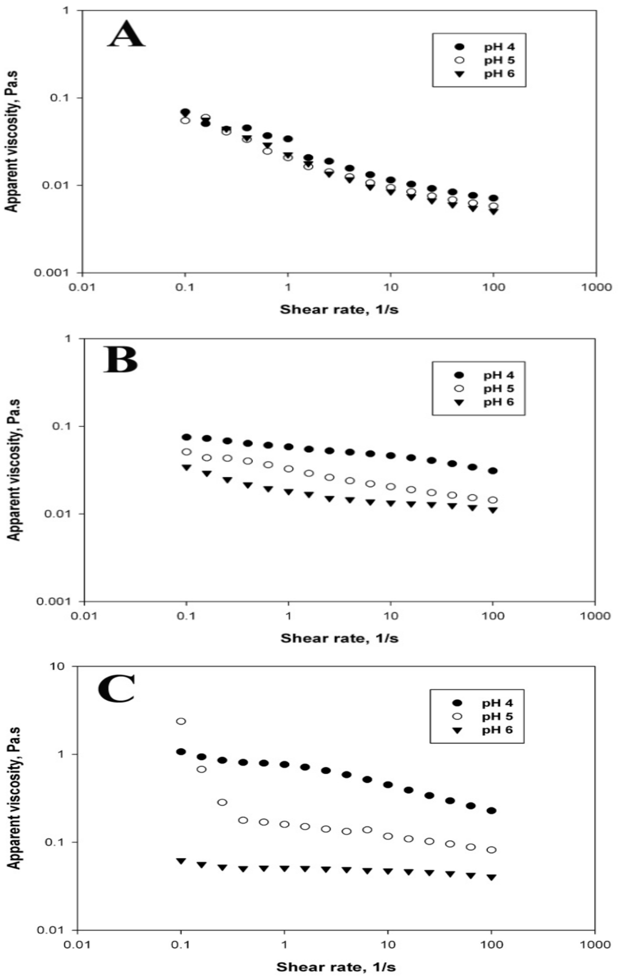

Figure 1. Apparent viscosity of (A) 10%, (B) 17.5% and (C) 25% protein (w/w) concentrations of WP dispersions at pH 4, 5 or 6 during controlled shear rate sweep (0.1 – 100/s) at 20°C

displayed the lowest viscosity during the shear rate sweep. The higher viscosity observed at pH 4 indicated higher resistance offered by WP to induced flow. The charged nature of WP could have contributed to variation in rheological behaviour at varying pH of the medium. At acidic pH, the degree of hydration of proteins could increase due to greater net positive charge on protein molecules leading to a greater affinity for water molecules. Additionally, the increased intra-molecular repulsion between WP under acidic conditions could facilitate partial unfolding of proteins in conjunction with hydrodynamic forces which may also have contributed to the increased viscosity of the dispersion. Solutions of randomly coiled molecules frequently display greater viscosity than solutions of compactly folded macromolecules of same molecular weight (Damodaran, 2008). Similarly the lowest apparent viscosity of WP dispersion at pH 6 may have resulted from enhanced repulsive forces predominating at lower shear rates and likely weak interactions among protein aggregates that are easily disrupted with increase in shear.

Table 1. Rheological parameters of whey protein dispersions, containing 10, 17.5 or 25% (w/w) protein, at pH 4, 5 or 6 obtained by fitting the experimental data to Power Law model

Concentration (%, w/w)	pH	†*k* (mPa.s^{n})	††*n*	R^2
10	4	19.5^{a}	0.76^{b}	0.98
	5	16.5^{a}	0.73^{a}	0.97
	6	27.8^{a}	0.64^{a}	0.97
17.5	4	59.8^{a}	0.87^{bc}	0.99
	5	31.0^{a}	0.82^{b}	0.99
	6	27.8^{a}	0.78^{b}	0.99
25	4	911.3^{c}	0.75^{b}	0.98
	5	237.0^{b}	0.77^{b}	0.94
	6	55.3^{a}	0.94^{c}	0.99
*SEM		45.0	0.03	0.02

Values are means of at least 4 independent observations (n = 4).
Means in the same column with different alphabets are significantly different ($p < 0.05$).
†k – consistency coefficient
††n – flow behavior index
*SEM denotes pooled standard error of the mean, $p < 0.05$.

The change in apparent viscosity during shearing of WP dispersions containing 25% protein (Figure 1C) differed from that at lower concentrations (Figure 1A and B) since the highest initial yield point was observed at pH 5. However, with the application of shear force, the apparent viscosity declined sharply indicating the development of brittleness in this system. Such high values of initial viscosity may be attributed to elevated protein-protein interactions through hydrophobic and ionic surface patches present in WP at pH 5 when electrostatic repulsion is at minimum. In addition, the protein-protein attraction may have been facilitated via increased volume fraction of dispersed particles at a lower water content that minimizes the inter particle distances (Patocka *et al.*, 2006). Moreover, WP dispersions at pH 4 maintained the overall maximum viscosity throughout the shear rate sweep which was similar to that observed for WP dispersions with 17.5% protein.

Table 1 shows the consistency coefficient (*k*, mPa.s^{n}) and flow behaviour index (*n*) obtained by fitting data to the Power Law model. The results showed that pH had a significant ($p < 0.05$) effect on the consistency coefficients of WP dispersions (k values). The *k* value or consistency index is directly proportional to the resistance of a material to flow (Rao, 1999). The *k* values were higher at pH 6 at low protein concentration while at higher protein concentrations, particularly at 25%, the *k* values were significantly ($p < 0.05$) higher at pH 4 than at pH 5 and 6. The high *k* values were consistent with the high

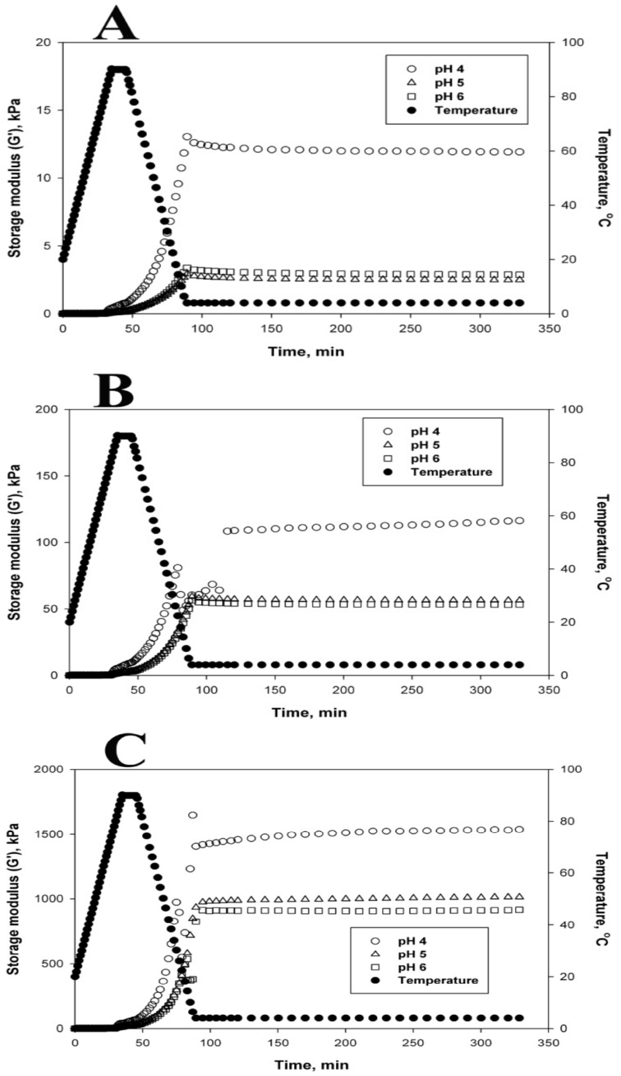

Figure 2. Storage modulus (G′) at constant strain (1%) and frequency (1 Hz) of (A) 10%, (B) 17.5% and (C) 25% (protein, w/w) WP dispersions at pH 4, 5 or 6 during heating (20-90°C), holding (90°C for 10 min), cooling (90 – 4°C) and aging (4°C for 4 h)

viscosity values of the WP dispersion (Figure 1 A-C) at pH 4 and high protein concentrations (17.5 and 25%). The interaction between pH and concentration significantly affected ($p < 0.05$) both the *k* and *n* values of WP dispersions. On the other hand, the flow behaviour index (*n*) had a similar pattern for samples containing 10 or 17.5% proteins, deviating more from Newtonian flow with elevation of pH. This was however reversed for the concentrated sample (25%) with the highest n value obtained at pH 6 which could indicate enhanced flow due to greater intra-molecular repulsions.

Viscoelastic properties during heat-induced gelation of WP dispersions

Figure 2 A-C presents the change in storage modulus (G′) of WP dispersions during heat-induced gelation. G′ is a measure of the elastic character of viscoelastic gel systems (Doublier *et al*., 1992). Storage modulus of all WP dispersions tested increased during heating and cooling. During aging there was no change in G′ of these dispersions. However, the storage modulus of the heat treated WP dispersion increased from 11.9 kPa at 10% protein concentration to 116.4 kPa and 1538 kPa at 17.5 and 25% concentration, respectively. This affirmed the significance of protein concentration in controlling the firmness of gels.

Regardless of concentration, the G′ values of gels formed at pH 4 was higher than those formed at pH 5 or 6 indicating that the WP dispersions at pH 4 showed greater structure forming capability. This could be as a consequence of intra-molecular repulsions of WP at pH 4 that in turn could have favoured the rapid unfolding during heating which was further enhanced by the subsequent protein-water interactions under a net positive charge resulting in cohesive viscoelastic networks mainly via exposed hydrophobic groups and also with other molecular interactions. Since the thiol groups are inactive at pH 4 (de la Fuente *et al*., 2002), it could be deduced that the facilitated non-covalent interactions were mainly responsible for creating firmer gels at pH 4. These findings were in line with the observations of Havea *et al*. (2009), who reported that firmer (higher storage modulus) WP gels formed through non-covalent interactions. The increased G′ of all gels during cooling indicated that hydrogen bond formation and electrostatic interactions, that are stable at lower temperatures (Damodaran, 2008), were facilitated.

When compared to the gels formed at pH 4, those formed at pH 5 or 6 possessed comparatively lower storage moduli indicative of softer elastic gels. It has been observed that at pH values above the isoelectric point of WP, activated thiol groups form covalent disulfide bonds and these gels are relatively elastic (Corredig and Dalgleish, 2005; Havea *et al*., 2009). Additionally, the net negative charge present on protein molecules could provide them with adequate time for a proper alignment before they associated via different interactions resulting in elastic gels (Morr and Ha, 1993). At both pH 5 and 6, WP gels had similar gel strength. However, the slightly increased G′ of gels at pH 6 at 10% protein concentration (Figure 2A) could be due to enhanced covalent bond formation at pH 6 than at pH 5.

Conclusions

The results of this study indicated that WP dispersions at pH 4 showed higher initial viscosities than at pH 5 and 6 when the protein concentration of the dispersions was raised to 17.5%, and remained high subsequent to the application of shear. The consistency coefficient of WP dispersions was affected by the pH as well as the protein concentration of the WP dispersions. At low protein concentration the consistency coefficient was at maximum at pH 6 while at higher concentrations it reached the maximum at pH 4. The heat induced gel formed by WP dispersions at pH 4 exhibited greater structure and strength that increased with increasing protein concentration.

Acknowledgments

Authors gratefully acknowledge the financial and technical support from Victoria University. The Warrnambool Cheese and Butter Factory (Warrnambool, Victoria, Australia) is sincerely appreciated for the provision of required raw materials.

References

Corredig, M. and Dalgleish, D. G. 2005. Whey protein aggregation studies by ultrasonic spectroscopy. In Dickinson. E. (Ed). Food Colloids: Interactions, Microstructure and Processing, p. 230-236. Cambridge: The Royal Society of Chemistry.

Damodaran, S. 2008. Amino acids, peptides and proteins. In . Damodaran, S., Parkin, K. L. and Fennema, O. R. (Ed). Fennema's Food Chemistry. 4th edn. p. 217-330. Boca Raton, FL: CRC Press, Taylor and Francis Group.

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

Dissanayake, M. and Vasiljevic, T. 2009. Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. Journal of Dairy Science 92: 1387-1397.

Dissanayake, M., Liyanaarachchi, S. and Vasiljevic, T. 2012. Functional properties of whey proteins microparticulated at low pH. Journal of Dairy Science 95: 1667-1679.

Doublier, J. L., Launay, B. and Cuvelier, G. 1992. Viscoelastic properties of food gels. In Rao, M. A. and Steffe, J. F. (Eds). Viscoelastic properties of foods, p. 371-434. England: Elsevier Science Publishers Ltd.

Carr, A. J., Southward, C. R. and Creamer, L. K. 2003. Protein hydration and viscosity of dairy fluids. In Fox, P. F. and McSweeney, P. L. H. (Eds). Advanced Dairy Chemistry – Proteins, Vol. 1, 3rd edn, Part B. p. 1289-1323. New York: Kluwer Academic/Plenum Publishers.

Genovese, D. B., Lozano, J. E. and Rao, M. A. 2007. The rheology of colloidal and noncolloidal food dispersions. Journal of Food Science 72: R11-R20.

Havea, P., Watkinson, P. and Kuhn-Sherlock, B. 2009. Heat induced whey proteins gels: Protein–protein interactions and functional properties. Journal of Agricultural and Food Chemistry 57: 1506-1512.

Huang, Y. T. and Kinsella, J. E. 1986. Functional properties of phosphorylated yeast proteins: Solubility, water-holding capacity, and viscosity. Journal of Agricultural and Food Chemistry 34: 670-674.

Hudson, H. M., Daubert, C. R. and Foegeding, E. A. 2000. Rheological and physical properties of derivatized whey protein isolate powders. Journal of Agricultural and Food Chemistry 48: 3112-3119.

Matsudomi, N., Oshita, T., Kobayashi, K. and Kinsella, J. E. 1993. α-Lactalbumin enhances the gelation properties of bovine serum albumin. Journal of Agricultural and Food Chemistry 41: 1053-1057.

Morr, C. V. and Ha, E. Y. W. 1993. Whey protein concentrates and isolates-processing and functional properties. Critical Reviews in Food Science and Nutrition 33: 431-476.

Morr, C. V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., Kilara, A., Lewis, B. A. and Mangino, M. E. 1985. A collaborative study to develop a standardized food protein solubility procedure. Journal of Food Science 50: 1715-1718.

Patocka, G., Cervenkova, R., Narine, S. and Jelen, P. 2006. Rheological behaviour of dairy products as affected by soluble whey protein isolate. International Dairy Journal 16: 399-405.

Rao, M. A. 1999. Flow and functional properties models for rheological properties of fluid foods. In Rao, M. A. Ed. Rheology of fluid and semisolid foods, Principles and Applications, p. 25-57. Maryland: Aspen Publishers.

Resch, J. J., Daubert, C. R. and Foegeding, E. A. 2004. The effects of acidulant type on the rheological properties of beta-lactoglobulin gels and powders derived from these gels. Food Hydrocolloids 19: 851-860.

Resch, J. J., Daubert, C. R. and Foegeding, E. A. 2005. β-Lactoglobulin gelation and modification: Effect of selected acidulants and heating conditions. Journal of Food Science 70: 79-86.

SAS. 1996. SAS/STAT Softwares, Changes and enhancements through release 6.11. Cary, NC: SAS Inst. Inc.

Verheul, M., Roefs, S. P. F. M. and de Kruif, K. G. 1998. Kinetics of heat-induced aggregation of β-lactoglobulin. Journal of Agricultural and Food Chemistry 46: 896-903.