Research Journal of Dairy Sciences 2 (2): 30-34, 2008
ISSN: 1993-5277

Rheological Properties on Acidification of Whey Protein Isolate as Affected by Complex Coacervate Formation with κ-carrageenan

John S. Mounsey
Teagasc, Moorepark Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

Abstract: Protein-polysaccharide mixtures are used to enhance the texture of dairy products. This study was undertaken to examine the aggregation and gelation of 1% (protein basis) preheated (80°C for 30 min) Whey Protein Isolate (WPI) on acidification in the pH range 3.0-7.0 and characterise the acid gelling properties of complex coacervates formed between WPI and 0.5% κ-carrageenan. The particle size of preheated WPI increased from 47.7 nm at pH 7 to a peak of 1031 nm at pH 4.75 before decreasing at lower pH values. On acidification using Glucono Delta-Lactone (GDL), preheated WPI commenced gelation at pH 6, had a peak in storage modulus of 71.9 Pa at pH 4.75 before weakening considerably at lower pH values. The zeta potential of the preheated WPI went from -40 mV at pH 7.0 to +35.1mV at pH 3.5. The unheated 1% WPI and 0.5% κ-carrageenan mixture formed a gel at pH ~ 6.0, which continued to increase in strength on acidification reaching a G' of 267 Pa at pH 3.5. Results showed the formation of complex coacervates can enhance the acid gelation properties of WPI without the requirement of a preheating step for the WPI.

Key words: Whey Protein Isolate (WPI), κ-carrageenan, acid gelation, complex coacervates

INTRODUCTION

The structure of dairy protein products such as yoghurt is determined by the ability of heated whey proteins to undergo secondary aggregation during acidification (Vasbinder *et al*., 2003). Whey protein ingredients such as Whey Protein Isolate (WPI) are used as food ingredients due to their nutritional and functional properties. WPI is composed predominantly by β-lactoglobulin (β-lg, ~80%) and α-lactalbumin (> 15%) with trace amounts of other whey proteins (bovine serum albumin and immunoglobulins). These are globular proteins with molecular weights ranging from 14-1000 kDa. The isoelectric points of the principal proteins are 5.2 forβ-Lg and from 4.2-4.5 for α-lactalbumin (Bryant and McClements, 1998). The gelation properties of these complex protein systems have been widely studied (Bryant and McClements, 1998; Cavallieria and Cunha, 2008; Puyol *et al*., 2001; Mounsey and O'Kennedy, 2007).

In recent years, considerable interest has also been devoted to the study of polysaccharide-protein mixtures, particularly as a way to optimise new food ingredients. Studies have been made on the gel formation of whey proteins in combination with polysaccharides (Doyle *et al*., 2008; Tavares and Lopes da Silva, 2003). One widely used polysaccharide is κ-carrageenan, a high-molecular weight sulphated polysaccharide which exists as random coils at high temperatures with a coil-to-helix transition occurs on cooling below a certain temperature (Viebke *et al*., 1995). Recent work has shown that insoluble complexes were formed between β-lg and κ-carrageenan via complex coacervation at pH values, close to and below the isoelectric point of the protein because of the electrostatic attraction between the positively charged protein and the negatively charged hydrocolloid (Doyle *et al*., 2008).

The aim of this study was to compare the acid gelation properties of complex coacervates of WPI and κ-carrageenan with the acid gelling properties of preheated WPI.

MATERIALS AND METHODS

WPI powder (92%, w/w protein) was obtained from Davisco Foods International (Le Sueur, MN, USA). A commercial source of κ-carrageenan (Fairgel C80; 80-90% κ-carrageenan on a carrageenan basis) extracted from the tropical seaweed Eucheuma cottonii, was obtained from Scotcol Biopolymers (Stirling, UK). The κ-carrageenan was composed of 19.5% ash (8.5% K, 3.56% Ca, 2.85% Na and 0.02% P), 9.2% moisture and <0.2%, protein. All other chemicals were analytical reagent grade and supplied by BDH (Poole, England). Distilled deionised water was used in all cases for dispersion of samples.

Preparation of solutions: A stock solution of κ-carrageenan was prepared by dispersing (1%) in distilled, deionised water and dissolved by heating in a water bath at 80°C for 2 h. A stock solution of WPI (10%) was dispersed in water with stirring at 22°C. Another stock solution was prepared by heating some of the WPI (10%) in a water bath at 80°C for 30 min before cooling in water to 22°C. A series of dispersions of 1% WPI and 0.5% κ-carrageenan were prepared by combining the above stock solutions and adjusting the pH to values in the range 3.0-7.0. Dispersions of 1% WPI were prepared by diluting the preheated WPI stock solution to 1% protein.

Particle size and viscosity measurements: Particle size analysis was performed on undiluted samples using a Zetasizer Nano system (Malvern Instruments, Inc., Worcester, UK). The size measurements were undertaken at 25°C and at a scattering angle of 12°. The viscosity of samples were determined and factored into the size calculation. The viscosity of the samples was measured using a Vibro Viscometer (Model AX-SV-34) (A and D Co. Ltd. Tokyo, Japan). Sample volumes of 40 mL were applied to the sample container at 22°C. The Vibro viscometer measures the driving electric current to vibrate the sensor plates with a uniform frequency (30 Hz) and amplitude and then the viscosity is given by the positive correlation between the driving electric current and the viscosity. Three viscosity (mPa s) readings per sample were recorded at 25°C. The refractive index of protein (1.45) and water (1.33) at 25°C was taken as applicable. The cumulative method was used to find the mean average (z-average) or the size of a particle that corresponds to the mean of the intensity distribution.

Turbidity measurements: The turbidity of dispersions (absorbance in a 10 mm light path) was measured using 10×10 mm polystyrene spectrophotometer cuvettes (Sigma Aldrich Co., St Louis, USA) a Hitachi U-1100 spectrophotometer at 600 nm and 25°C.

Zeta potential measurements: Zeta potential measurements were conducted by laser doppler electrophoresis using the Nano-S Zetasizer (Malvern Instruments Limited, Malvern, Worcestershire, U.K.) equipped with a 2 mW Helium Neon laser with an output of 633 nm. Zeta potential is a measure of the net charge on a particle and depends on the charge on the particle plus the charge of associated with any ions that move along with the particle in an electric field. An applied potential of 150 V and a modulation frequency of 250 Hz were used. The instrument was calibrated with a standard carboxyl modified polystyrene latex solution, with a surface potential of -55 mV, provided by Malvern Instruments. Zeta potential was calculated from the Helmholtz-Smoluchowski equation: zeta potential (δ) = $\mu 4\pi\eta/E$, where, μ is the electrophoretic mobility or velocity at unit potential gradient, η is the viscosity of the liquid and E is the dielectric constant. The dielectric constant and viscosity of water at 25°C was taken as applicable. Samples were measured 20 times. The sample count rate was generally 2,000 times higher than the background count for distilled and deionised water (filtered). For zeta potential determination, the results were expressed in absolute values (mV).

Acid gel formation of WPI/WPI and κ-carrageenan dispersions: The storage modulus (G'), loss modulus (G'') and tan δ (loss tangent; G''/ G') during acidification of the β-lg dispersions were determined at 25°C using a Bohlin CVO Rheometer (Bohlin Cirencester, UK) as used as previously described (Mounsey *et al*., 2005). Sufficient GDL to achieve a pH of 3.5 in 160 min was added to the dispersions (0.5% GDL for 1% WPI dispersions and 1% GDL for 1% WPI/0.5% κ-carrageenan dispersions). To prevent evaporation, n-tetradecane was applied on the surface of the protein dispersion. The gel point was defined as the pH where the G' reached a value greater than 1.0 Pa and the final storage modulus was determined after 160 min.

Statistical analysis: The preparation of all solutions and subsequent analyses on them were performed in triplicate. Analysis of Variance (ANOVA) was carried out using SigmaStat (version 3.0; Jandel Scientific, Corte Madera, CA, USA). Tukeys multiple-comparison test was used to determine differences between treatment means. Treatment means were considered significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of pH on the particle size, turbidity, zeta potential and viscosity of 1% WPI (pre-denatured at 80°C for 30 min at a concentration of 10%).

The particle size (nm) and turbidity (Absorbance at 600 nm) of 1% (protein basis) WPI (preheated at 80°C for 30 min and a concentration of 10% protein) was plotted in the pH range 3-7 (Fig. 1). The preheated WPI had a particle size of 47.7 nm and a turbidity of 0.108 at pH 7, which is evidence of the denaturation and aggregation of the proteins due to heat. Previous work showed that 0.5% WPI heated at 85°C and at pH 6.6 had a particle size of ~90 nm following heating (O'Kennedy and Mounsey, 2006).

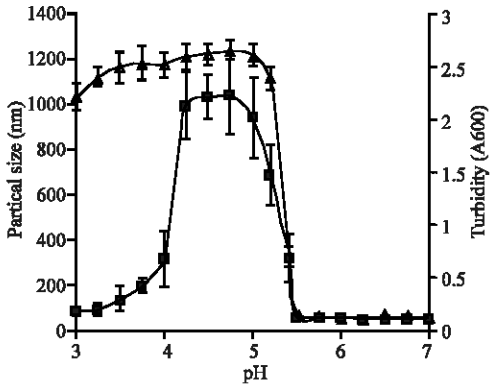

Fig. 1: Particle size (■; nm) and turbidity (Δ; Absorbance at 600 nm) of 1% (protein basis) WPI (preheated at 80°C for 30 min and a concentration of 10% protein) at 25°C. Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means

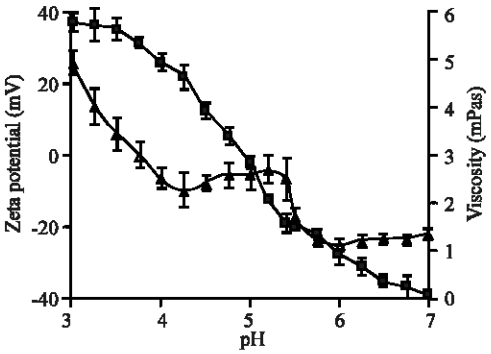

Fig. 2: Zeta potential (■; mV) and viscosity (Δ; mPa s) of 1% (protein basis) WPI (preheated at 80°C for 30 min and a concentration of 10% protein) at 25°C. Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means

The WPI preparation showed little change in particle size and turbidity between pH 7 and 5.5. However, at lower pH values both particle size and turbidity increased significantly ($p \leq 0.05$) with a peak in particle size (1031 nm) and turbidity (2.64) at pH 4.75. The particle size was reduced at lower pH values with a size of 79.8 nm at pH 3.0. The turbidity showed a lower extent of reduction at lower pH values. Previous research has shown a peak in turbidity and particle size of undenatured β-lg (the principle protein in WPI) around pH 5.0 (Mounsey *et al*., 2008). This increase in protein aggregation and particulation at pH values close to the isoelectric point of the whey protein has been attributed to a reduction in the electrostatic repulsion between the protein molecules (Guzey and McClements, 2006; Schmitt *et al*., 1999). The reduction in particle size at lower pH values (3.0-4.0) of the pre-denatured WPI particles from the peak size compared to at the peak of pH 4.75 is evidence of disaggregation, possibly due to an increase in electrostatic repulsion at the lower pH values.

The zeta potential (mV) and viscosity (mPa s) of 1% preheated WPI is shown in the pH range 3-7 (Fig. 2). It has been suggested that the magnitude of the zeta potential (net charge on a particle) indicates the potential stability of a colloidal system (Van Nieuwenhuyzen and Szuhaj, 1998). It was observed that the zeta potential of preheated WPI was significantly ($p \leq 0.05$) reduced from a positive value of +37.2±2.8 mV at pH 7.0 to a point of zero charge at a pH of ~4.9 before reaching a negative charge of -39.5±1.3 mV at pH 7.0. Previous work showed lower zeta potential values (-9.8 mV) for unheated β-lg at pH 7.0 (Mounsey *et al*., 2008). The heat treatment at pH 7.0 in the present research probably exposed charged groups, which increased the negative charge at this pH. The point of zero charge in the heated WPI is lower than that for β-lg (~ pH 5.3) shown previously (Mounsey *et al*., 2008), which is possibly due to the presence of α-lactalbumin in WPI (isoelectric point of pH 4.3). Particles with zeta potentials more positive than +30 mV or more negative than –30 mV are normally considered stable (Van Nieuwenhuyzen and Szuhaj, 1998). The increase in particle size of preheated WPI at pH values below pH 5.5 coincides with a reduction in zeta potential below -20 mV.

The particle size decreased again at pH values below 4.0 where the zeta potential was more positively charged than + 20 mV. These results indicate the importance of electrostatic interactions in determining the particle size of preheated WPI on acidification. The viscosity of preheated WPI showed little change on pH reduction from 7.0 (1.34 ± 0.1 mPa s) to pH 5.75 (1.2 ± 0.2 mPa s). However, on further pH reduction the viscosity was significantly ($p \leq 0.05$) increased, reaching a maximum at pH 3.0 (4.93±0.2 mPa s). Results indicate that the aggregation of preheated WPI at lower pH values as evidenced by increased particle size resulted in increased viscosity at these lower pH values.

Gelation behaviour on acidification of 1% WPI (pre-denatured at 80°C for 30 min at a concentration of 10%): The acid gelation methodology used here is essentially a cold gelation mechanism where pH reduction is used as a driver of whey protein aggregation and eventual gelation. The gelation profile of 1% (w/w) WPI that was heated at 78°C for 30 min, pH 7.0 and subsequently acidified with GDL is outlined (Fig. 3).

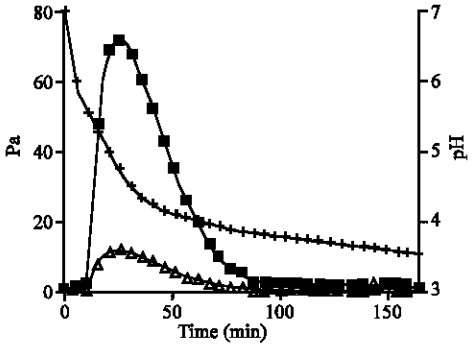

Fig. 3: Acid gelation profile showing the G' (■) and G" (Δ) of 1% (protein basis) WPI (preheated at 80°C for 30 min and a concentration of 10% protein) on acidification with 0.5% GDL at 25°C. Typical pH curve (+) is included. Each curve represents the mean of triplicate trials

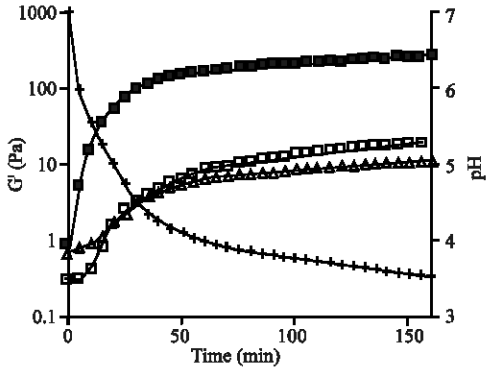

Fig. 4: Acid gelation profile showing the G' on acidification with 1% GDL at 25°C of 1% WPI (unheated) and 0.5% κ-carrageenan (■), 1% WPI (preheated as 10% protein) and 0.5% κ-carrageenan (Δ) or 1% WPI and 0.5% κ-carrageenan preheated together (£). Typical pH curve (+) is included. Each curve represents the mean of triplicate trials

Results show that the 1% WPI (preheated at 10% protein levels) formed a gel (G' > 1) when a pH of 6 was reached. At all pH values below 6 the G' was higher than the G". The WPI had peak gel strength of 71.8 Pa at pH 4.75. At lower pH values the G' was reduced, reaching a value of ~ 1 Pa at pH 3.6. The present work shows that while the preheated WPI could form a gel on acidification to pH 4.75, the gel dissaggregated at lower pH values. Previous researchers have demonstrated the importance of thiol groups during aggregation and the heating step of cold gelation of β-lg and concluded that disulphide bonds were mainly involved in the polymerisation step prior to gelation (Hoffmann and van Mil, 1997; Hongsprabhas and Barbut, 1997). It is likely that the heating regime in the present work did not facilitate the formation of sufficient irreversible bonds in the form of disulphide bonds. Hence, once the steric repulsion was increased (as indicated by increased zeta potential in Fig. 2) at lower pH values the protein aggregates held together largely by hydrophobic and electrostatic interactions were partly dissociated as evidenced by the particle size reduction shown in Fig. 1. This unstable gel system is unsuitable for practical applications such as yoghurt-like systems where on acidification to pH values of 4 or below, consistent gel properties are required.

Gelation behaviour on acidification of 1% WPI and 0.5% κ-carrageenan: The gelation profile of 1% (w/w) WPI and 0.5% κ-carrageenan with various heat treatments and was acidified with GDL is outlined (Fig. 4).

Results show that the 1% WPI (unheated) and 0.5% κ-carrageenan formed a gel when a pH of ~ 6 was reached. It should be noted that unheated WPI will notgel in the pH range 3-7 due to the absence of research, the acidification of 1% WPI (unheated) in the presence of 0.5% κ-carrageenan resulted in gelation on acidification. Unlike the preheated WPI sample which formed a weak gel with maximum G' at pH 4.75 and dissociated on further acidification, the unheated 1% WPI (unheated) and 0.5% κ-carrageenan formed a gel which continued to increase in strength on acidification reaching a G' of 267 Pa at pH 3.5.

Previous research showed that unheated β-lg and κ-carrageenan formed insoluble complexes via complex coacervation at pH values, close to and below the isoelectric point of the protein (pH 5.2) because of the electrostatic attraction between the positively charged protein and the negatively charged hydrocolloid (Doyle *et al*., 2008). This concurs with the findings of the present work where the interaction between unheated WPI and κ-carrageenan led to the formation of a reasonably strong acid gel. However, the acid gel strength of the WPI and κ-carrageenan mixtures was impaired when the mixture was heated together (80°C for 30 min) with a G' of 19.3 Pa. An even weaker gel was obtained when the WPI was preheated (80°C for 30 min) at protein levels of 10% before addition to the κ-carrageenan (a G' of 11.2 Pa).

Earlier research showed that the addition of κ-carrageenan to reconstituted skim milk at 60°C, which was above the coil to helix transition (~ 37°C), impaired the acid gelation of the system compared to when the κ-carrageenan was added at a lower temperature of 22°C (Mounsey *et al*., 2006). The mechanism of gel formation of the mixture of WPI and κ-carrageenan on acidification was probably due to complex coacervation involved electrostatic attraction between the positively charged protein and the negatively charged hydrocolloid. It is possible that the globular whey protein

(~3 nm in diameter) functioned as a 'seed', upon which κ-carrageenan was deposited once the pH was reduced to around the isoelectric point of the protein, hence the increase in negative charge (at p H values below the isoelectric point of the protein) of β-lg/ κ-carrageenan mixtures shown previously (Doyle *et al*., 2008). It is possible that when the mixtures were heated (i.e., κ-carrageenan in the coil conformation), the κ-carrageenan would have interacted in a different way with the protein, particularly when the WPI was denatured and aggregated. In the heated systems, the κ-carrageenan may have caused weak gelation of the system at pH 7.0, which contributed to impaired secondary gelation on acidification. This gelling effect at pH 7.0 has previously been shown for heated whey protein concentrate-based solutions with added κ-carrageenan (Dybing and Smith, 1998).

CONCLUSION

This research has shown that preheated WPI forms unstable acid gels which disaggregate at lower pH values. Acid-induced gels formed from unheated WPI and κ-carrageenan mixtures had greater acid stability than the preheated WPI and may have application in specialist yoghurt-type food systems where minimisation of thermal treatment is required.

REFERENCES

Bryant, C.M. and D.J. McClements, 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends Food Sci. Technol., 9: 143-151.

Cavallieria, A. L. F. and R. L. Cunha. 2008. The effects of acidification rate, pH and ageing time on the acidic cold set gelation of whey proteins. Food Hydrocolloids, 22:439-448

Doyle, J., J.S. Mounsey and B.T. O'kennedy, 2008. Complex Coacervation Between B-lactoglobulin and K-carrageenan. In: Gums and Stabilisers for the Food Industry 14 P. Williams, A. and G.O. Phillips, (Eds.), Royal Society of Chemistry, Cambridge.

Dybing, S.T. and D.E. Smith, 1998. The Ability of Phosphates or κ-Carrageenan to Coagulate Whey Proteins and the Possible Uses of Such Coagula in Cheese Manufacture. J. Dairy Sci., 81: 309-317.

Guzey, D. and D.J. McClements, 2006. Characterization of â-lactoglobulin-chitosan interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study. Food Hydrocolloids, 20: 124-131.

Hoffmann, M.A.M. and P.J.J.M. Van Mil, 1997. Heat induced aggregation of β-lactoglobulin: Role of free thiol group and disulphide bonds. J. Agric. Food Chem., 45: 2942-2948.

Hongsprabhas, P. and S. Barbut, 1997. Effects of N-ethylmaleimide and CaCl2 on cold gelation of whey protein isolate. Food Res. Int., 30: 451-455.

Mounsey, J.S. and B.T. O'Kennedy, 2007. Conditions limiting the influence of thiol-disulphide interchange reactions on the heat-induced aggregation kinetics of β-lactoglobulin. Int. Dairy J., 17: 1034-1042.

Mounsey, J.S., B.T. O'Kennedy and P.M. Kelly, 2005. Comparison of re-micellised casein prepared from acid casein with micellar casein prepared by membrane filtration. Lait, 85: 419-430.

Mounsey, J.S., B.T. O'Kennedy, M.A. Fenelon and A. Brodkorb, 2008. The effect of heating on β-lactoglobulin-chitosan mixtures as influenced by pH and ionic strength. Food Hydrocolloids, 22: 65-73.

Mounsey, J.S., B.T. O'Kennedy, P.M. Kelly, L. Pesquera and J.C. Jacquier, 2006. Microgel Evaluation in a Model Food System. In: Gums and Stabilisers for the Food Industry 13: The Proceedings of the 13th Gums and Stabilisers for the Food Industry. Williams, P.a. and G.o. Phillips, (Eds.). Royal Soc. Chem. Cambridge, Pp: 257-267.

O'Kennedy, B.T. and J.S. Mounsey, 2006. Control of Heat-induced Aggregation of Whey proteins using Casein. J. Agric. Food Chem., 54: 5637-5642.

Puyol, P., M.D. Péreza and D.S. Horne, 2001. Heat-induced gelation of Whey Protein Isolates (WPI): Effect of NaCl and protein concentration. Food Hydrocolloids, 15: 233-237.

Schmitt, C., C. Sanchez, F. Thomas and J. Hardy, 1999. Complex coacervation between β-lactoglobulin and acacia gum in aqueous medium. Food Hydrocolloids, 13: 483-496.

Tavares, C. and J.A. Lopes da Silva, 2003. Rheology of galactomannan-whey protein mixed systems Int. Dairy J., 13: 699-706.

Van Nieuwenhuyzen, W. and B.F. Szuhaj, 1998. Effects of lecithins and proteins on the stability of emulsions. Fett/Lipid, 100: 282-291.

Vasbinder, A.J., A.C. Alting, R.W. Visschers and C.G. de Kruif, 2003. Texture of acid milk gels: Formation of disulphide cross-links during acidification. Int. Dairy J., 13: 29-38.

Viebke, C., J. Borgstrom and L. Piculell, 1995. Characterisation of kappa- and iota-carrageenan coils and helices by MALLS/GPC. Carb. Polymers 27: 145-154.