



## Functional properties of whey proteins microparticulated at low pH

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### ABSTRACT

The main aim of the study was to assess the effect of microparticulation at low pH on the functionality of heat-denatured whey proteins (WP). Spray-dried, microparticulated WP (MWP) powders were produced from 7% (wt/wt) WP dispersions at pH 3, acidified with citric or lactic acid, and microfluidized with or without heat denaturation. Nonmicroparticulated, spray-dried powders produced at neutral pH or pH 3 served as controls. The powders were examined for their functional and physicochemical properties. Denatured MWP had an approximately 2 orders of magnitude reduction in particle size compared with those produced at neutral pH, with high colloidal stability indicated by substantially improved solubility. The detection of monomeric forms of WP in PAGE also confirmed the particle size reduction. Microparticulated WP exhibited enhanced heat stability, as indicated by thermograms, along with better emulsifying properties compared with those produced at neutral pH. However, MWP powders created weaker heat-induced gels at neutral pH compared with controls. However, they created comparatively strong cold acid-set gels. At low pH, a combination of heat and high hydrodynamic pressure produced WP microaggregates with improved colloidal stability that affects other functionalities.

**Key words:** whey protein, low pH, dynamic high pressure, functional property

### INTRODUCTION

Our previous studies showed that microparticulation of whey proteins (WP) using combined heat and high pressure shearing (microfluidization) could successfully modulate and stabilize WP against heat at neutral pH (Dissanayake and Vasiljevic, 2009; Dissanayake et al., 2010). In addition to enhanced heat stability, denatured microparticulated whey proteins (MWP) exhibited

different functionalities from those of native WP, such as improved emulsifying activity and gelling properties (Dissanayake and Vasiljevic, 2009). Although microparticulation produced microaggregates by dispersing heat-denatured WP aggregates, the particle size of these aggregates was not small enough to prevent sedimentation. This may have adverse effects on most other WP functionalities, restricting their successful application in various heterogeneous food systems.

Whey proteins at acidic pH are more stable than those at neutral pH against heat-induced aggregation (de la Fuente et al., 2002). Consequently, they form heat-set gels that are relatively brittle and weak. Compared with these gels, those formed at a neutral pH exhibit strong and elastic fracture properties (Havea et al., 2009). The weak and brittle nature of acid gels primarily arises due to the overall positive charge of WP at acidic pH with enhanced intermolecular repulsive electrostatic interactions and inhibition or suppression of thiol group activity (Morr and Ha, 1993; Spiegel and Huss, 2002; Damodaran, 2008). Lactose, a factor that governs the WP gel structure at neutral pH due to its influence on the denaturation rate of  $\beta$ -LG and the particle size of aggregates (Spiegel, 1999), was reported to have little or no effect on gels formed at acidic pH (Spiegel and Huss, 2002). In addition, most WP products contain a considerable amount of calcium, which is associated with the creation of a strong gel network due to additional cross-linking via formation of calcium bridges between protein molecules (Havea and Sing, 2003). Introducing a calcium-chelating agent such as citrate ions may result in relatively weaker WP gels and dispersions with enhanced heat stability (De Rham and Chanton, 1984). Understanding the relative influence of different acidulants on WP gelation would be beneficial for manufacturing of MWP at low pH. The variations in gel quality due to different acidulant effects may be related to specific anion effects, such as size of the ion and the charge density, which may determine their ability to perturb the solvent (water) structure (Resch et al., 2005b). For example, large ions with low surface charge density generally exert the greatest disruption in the hydrogen-bonded structure of water, whereas smaller ions with greater charge density tend to have

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the smallest effect on the disorder of the solvent, which in turn influences the hydrophobic interactions within the protein molecule in solution (Resch et al., 2005b).

The principal aim of the current study was to produce heat-stable MWP powders with improved colloidal stability via reduction of particle size. The study was carried out at low pH to create a WP gel network with relatively weaker molecular linkages that would be easily dispersed by mechanical forces used in high-pressure homogenization or microfluidization. In addition, the influence of change in the ionic environment was assessed by using different acidulants for acidification of samples.

## MATERIALS AND METHODS

### *Materials and Proximate Composition*

Whey protein retentate samples used in the study were kindly provided by Warrnambool Cheese and Butter Factory (Warrnambool, Victoria, Australia). They contained approximately 30% total solids and were collected from 2 batches produced on separate days. Initial compositional analysis of these samples was carried out following established AOAC methodology during our earlier studies (Dissanayake and Vasiljevic, 2009). Whey retentates contained an average of 70.10% moisture, 24.53% protein, 1.51% fat, 1.89% lactose, 1.00% ash, and 470.2 mg/L calcium. All chemicals used in the study were of analytical grade.

### *Production of WP Powders and Sample Preparation*

Seven types of MWP powders were prepared with replicates from 7% (wt/wt) WP dispersions. This concentration was the maximum achievable protein concentration that could have been subjected to the described process. The experimental design applied in this study is depicted in Figure 1. Briefly, 4 microparticulated samples were prepared by adjusting the pH of dispersions to 3, using citric or lactic acid, and then spray-drying the dispersions using a pilot-scale spray dryer (SL-10 Mini-Maxi Pilot Spray Dryer, Saurin Enterprises Pty. Ltd., Melbourne, Australia) after 5 passes through a microfluidizer (model 110 Y, Microfluidics, Newton, MA) at 140 MPa, with or without a prior heat treatment of 20 min at 90°C. Three nonmicroparticulated controls were prepared by spray-drying 7% WP dispersions: 2 with the pH adjusted to approximately 3 using citric or lactic acid and the other diluted with Milli-Q water (Millipore, Billerica, MA) without changing the pH (pH ~6). The inlet and the outlet air temperatures of the spray drier were 180 and 80°C, respectively. Subsequently,

hydrated WP dispersions were prepared from spray-dried powders using Milli-Q water with no further pH adjustment at this step. The treatments were designated as follows: untreated control; citric acid-acidified control (**Control-CA**); citric acid-acidified and microfluidized (**M-CA**); citric acid-acidified, heated, and microfluidized (**HTM-CA**); lactic acid-acidified control (**Control-LA**); lactic acid-acidified and microfluidized (**M-LA**); lactic acid-acidified, heated and microfluidized (**HTM-LA**). The analysis of physical, chemical, and thermal properties of the dispersions was conducted as follows.

### **PAGE**

Protein profiling of the samples was carried out by electrophoretic analysis of the powder dispersions according to the method described in Dissanayake and Vasiljevic (2009). Native PAGE was performed to observe the extent of protein denaturation in the samples, whereas reducing and nonreducing SDS-PAGE were performed to observe the types of interactions that prevailed in protein aggregates.  $\beta$ -Mercaptoethanol was used as a reducing agent in reducing SDS-PAGE. The concentration of whey protein samples used in the SDS-PAGE was 1 mg/mL.

### *Thermal Analysis*

Thermal analysis of 12% (wt/wt) WP dispersions was carried out using a differential scanning calorimeter (DSC 7, Perkin Elmer, Norwalk, CT) and the Pyris Manager software (version 5.0002). Samples (approximately 30  $\mu$ L) of 12% (wt/wt) WP dispersions were accurately weighed in aluminum pans and hermetically sealed. An empty pan of equal weight was used as a reference. The scanning temperature was increased from 25 to 140°C at a rate of 10°C/min (Dissanayake and Vasiljevic, 2009).

### *Particle Size Analysis*

The particle size distribution pattern of approximately 1% (wt/wt) WP dispersions was obtained using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). Approximately 1% (wt/wt) WP dispersions were prepared from the spray-dried powders and hydrated overnight at 4°C. After hydration, dispersions were diluted 1/1,000 with Milli-Q water and vortexed before determination of the particle size. The refractive index of solvent (water) and the dispersed phase (WP) were 1.33 and 1.52, respectively. For each replicate, the average of 2 mean particle diameter readings was obtained.

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