



# Concentrated whey protein particle dispersions: Heat stability and rheological properties

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## ABSTRACT

In this work heat stability and rheological properties of concentrated whey protein particle dispersions in different dispersing media are studied. Whey protein particles (protein content ~20% w/v) having an average size of a few microns were formed using a combination of two-step emulsification and heat-induced gelation. Particles were dispersed (volume fraction of particles ~0.35) in solutions of Na-caseinate, whey protein isolate or gum arabic at different concentrations. The microstructure, particle size distribution and flow behaviour of the dispersions were analyzed before and after heating at 90 °C for 30 min.

All dispersions were liquid-like and no significant change in the microstructure was observed after heat treatment. Viscosity measurements showed that both the type and the concentration of the stabilizer influenced the viscosity changes after heat treatment. When 1% (w/w) gum arabic was used as stabilizer no change in the viscosity was observed after heat treatment. However, when Na-caseinate or whey protein isolate was used, viscosity increased in low-shear regime and shear-thickening was observed in high-shear regime. Heat treatment did not significantly alter the zeta potential of the particles, whereas the size of the particles increased after heating due to swelling.

The results show that swelling of the particles plays a significant role in the heat stability and rheological properties of these dispersions.

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## 1. Introduction

Whey proteins are widely used as food ingredients because of their techno-functional properties and high nutritional value. Although whey proteins are included in many food formulations, the limited heat stability of beta-lactoglobulin in particular may give rise to problems (Bernal & Jelen, 1985; Dissanayake & Vasiljevic, 2009; de Wit, 1990, 1998). Whey proteins denature and aggregate upon heating and may form a gel, depending on conditions. While the ability of whey proteins to thicken and form a gel upon heating can be an advantage for some applications, it can be a disadvantage for some others. Several problems related to food structure and texture may occur for example in protein-enriched foods. In whey protein-enriched drinks undesirable changes such as a turbid appearance or thick, undrinkable texture may form

during the thermal processing due to aggregation (Singh & Nath, 2004). However the development of novel food products with high protein content is of great interest because of health benefits and satiating properties reported for high protein foods (Anderson & Moore, 2004; Bertenshaw, Lluch, & Yeomans, 2008; Campbell & Leidy, 2007; Paddon-Jones et al., 2008; Westerterp-Plantenga et al., 2006).

To eliminate these problems and modify the functional properties of whey proteins, several approaches have been investigated. Some authors have focused on modification of whey proteins through cross-linking by transglutaminase (Lorenzen, 2007; Soeda, Hokazono, Kasagi, & Sakamoto, 2006). These authors report that cross-linked whey protein concentrates have improved heat stability. Recently it was shown in another study that ultrasound treatment of whey proteins after a pre-heating step enhanced the heat stability of whey proteins in subsequent heating steps (Ashokkumar et al., 2009). The viscosity of whey protein concentrate remained low upon heat treatment because aggregates formed during pre-heating were broken down by the ultrasound

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treatment and prevented from reforming in the second heating step.

Recently we have developed a robust method to prepare spherical whey protein particles with high internal protein content (~20% w/w) (Sağlam, Venema, de Vries, Sagis, & van der Linden, 2011). Heat-stable protein particles with controlled size, surface properties and internal density are interesting candidates for the development of novel high protein foods. Formation of heat-stable whey protein particles was investigated in a few other studies. A combination of heat and high-pressure was used to modify whey proteins (Dissanayake & Vasiljevic, 2009). This process resulted in formation of microparticulated whey proteins having increased heat stability compared to native whey proteins. An alternative way to prepare protein particles to enhance heat stability of liquid protein formulations was recently reported (Zhang & Zhong, 2009, 2010). Here whey protein nano-particles were prepared (average diameter smaller than 100 nm) through addition of whey protein isolate solution in a w/o micro-emulsion containing reverse micelles of surfactant and subsequent heating at 90 °C for 20 min. The dispersion of those whey protein nano-particles was transparent and liquid-like after thermal treatment, while WPI formed a gel at the same protein concentration after heat treatment. The emphasis in this work was given to formation of very small particles to be used in clear beverage applications, therefore the protein contents studied was rather low (5% w/v).

Design of protein particles is also an important subject in industries other than the food industry such as the pharmaceutical industry: processes such as spray-drying and jet-milling are used to prepare fine protein particles (Chan, Clark, Gonda, Mumenthaler, & Hsu, 1997; Johnson, 1997). Aqueous phase separation was extensively studied by Morita, Horikiri, Yamahara, Suzuki, and Yoshino (2000) for formation of spherical micron-sized protein particles that might be suitable for encapsulation and delivery of protein drugs. Whey proteins are reported to have good micro-encapsulation properties and several studies focused on preparation of microspheres from whey proteins through emulsification, heat gelation or chemical cross-linking (Heelan & Corrigan, 1998; Je Lee & Rosenberg, 2000).

We are interested in formation of heat-stable protein particles with controlled surface properties. Therefore the aim of the present study was to investigate the influence of different stabilizers on the heat stability and rheological behaviour of whey protein particles. For this purpose concentrated whey protein particle dispersions in different dispersing media were prepared and the physical properties of the dispersions were characterized after heat treatment. Changes in the surface properties or in the volume fraction of the particles during heat treatment may lead to differences in the physical properties of the dispersions. Swelling of whey protein gels (Gunasekaran, Ko, & Xiao, 2007; Gunasekaran, Xiao, & Ould Eleya, 2006) and particles prepared from whey proteins (Heelan & Corrigan, 1998) is already reported. Here we will also show that swelling of whey protein particles dominates the viscosity changes observed in the dispersions after heating.

## 2. Experimental

### 2.1. Materials

Whey Protein Isolate (WPI, BiPro JE 034-7-440-1) was obtained from Davisco Foods International Inc. (Le Sueur, MN). The composition of WPI as stated by the manufacturer was 97.9% protein, 0.3% fat, 1.8% ash (dry weight basis) and 4.9% moisture (wet weight basis). Polyglycerol Polyricinoleate (Grindsted PGPR 90, Denmark) was purchased from Danisco and consisted of polyglycerol ester of

poly-condensed ricinoleic acid with added antioxidants Alpha-tocopherol (E 307) and Citric acid as stated by manufacturer. Sodium caseinate (EM 7) was obtained from DMV international (Veghel, the Netherlands). Sunflower oil (Reddy, NV Vande-moortele, Breda) was purchased from a local supermarket. Gum arabic was purchased from Merck (Darmstadt, Germany).

### 2.2. Solutions

Whey protein isolate (WPI), Na-caseinate and gum arabic were dissolved at desired amounts in Millipore water (Millipore Corp., Billerica, MA). The solutions were stirred overnight, and refrigerated before usage. The pH of the solutions was left unadjusted. The pH values of 1%(w/w) WPI, Na-caseinate and gum arabic solutions were 6.8, 6.9 and 5.8 respectively. PGPR (2.5 wt%) was dissolved in sunflower oil by stirring for at least for 2 h at room temperature and stored in a dark cabinet.

### 2.3. Formation of protein particles

Protein particles were prepared according to the method described previously (Sağlam et al., 2011). First a water in oil (w/o) emulsion was prepared by mixing 25% (w/w) WPI solution in sunflower oil (containing PGPR 2.5% w/w) with the help of high speed mixer (Ultra-turrax T 25, IKA Werke, Germany). The total mixing time was kept at 5 min and mixing speed was fixed at 6500 RPM. Directly after preparation, the w/o emulsion was heated at 80 °C for 20 min and subsequently centrifuged (33,768g, Avanti J-26 XP, Beckman Coulter, U.S.A) for 1 h to remove the excess oil. The subsequent washing and dispersing steps were done using solutions of either Na-caseinate, WPI or gum arabic at different concentrations. All the dispersions had a pH value close to 7 after preparation and no further adjustment of the pH was done.

### 2.4. Volume fraction of particles

The volume fraction of the particles could not be accurately adjusted prior to re-dispersing. Therefore the Einstein expression for the effective viscosity of a dispersion was used to determine the volume fraction ( $\Phi$ ) of protein particles in the dispersions:

$$\eta_{\text{eff}} = \eta_c \left( 1 + \frac{5}{2} \Phi \right) \quad (1)$$

where  $\eta_{\text{eff}}$  and  $\eta_c$  are the dynamic viscosities of the dispersion and the continuous phase respectively. The final particle dispersions were diluted 5, 10, 20 and 50 times in order to secure that the dispersions are sufficiently diluted to be able to use Einstein equation. Dynamic viscosities of the diluted samples were determined using a capillary viscometer (Ubbelohde) placed in a water bath at 25 °C. Each dilution was measured three times and average viscosity values were used for further calculations. The experimental error when determining the volume fraction of the particles was found to be around 5%.

### 2.5. Heating experiments

Approximately 20 ml from each sample was transferred into a glass tube and closed tightly. Samples were heated at either 70 °C, 80 °C or 90 °C for 30 min in a temperature-controlled heating plate (RT15, IKA Werke, Germany). Samples were mildly stirred by a magnetic stirrer during heat treatment to avoid particle sedimentation and to facilitate heat transfer. The experiments were performed in duplicate.

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