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Whey protein particles modulate mechanical properties of gels at high protein concentrations

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A B S T R A C T

We have studied the influence of dense whey protein particles on the mechanical properties of whey protein isolate (WPI) gels at high protein concentrations (16–22% (w/w)). Incorporation of dense whey protein particles in the gel, while keeping the total protein concentration constant, leads to a considerably lower storage modulus. By adding protein particles, the total protein concentration of the WPI gels could be increased by 25–55% (w/w), without increasing the storage modulus of the gel. The large deformation properties of the WPI gels were also influenced by the presence of dense protein particles. Gels containing protein particles fractured at a lower strain than pure WPI gels at the same protein concentration. We conclude that protein particles can be used to modulate mechanical properties of WPI gels and are promising candidates for the development of high protein foods with improved textural properties.

1. Introduction

The ability of whey proteins to form gels under specific conditions is an important functional property of whey proteins, thereby giving several food products desirable textural and sensory characteristics (Barbut, 1995; Foegeding, Davis, Doucet, & McGuffey, 2002; Kinsella & Whitehead, 1989; Langton & Hermansson, 1992). The physicochemical properties of whey protein gels, i.e. rheological, optical and water holding properties, are mainly determined by the molecular interactions and microstructure of the gels and those can be tailored by adjusting the protein concentration, pH and ionic strength of the solution (Barbut, 1995; Foegeding, Bowland, & Hardin, 1995; Langton & Hermansson, 1992; Shimada & Cheftel, 1988; Verheul & Roefs, 1998a, 1998b). Heat-induced gelation of whey proteins occurs at high enough protein concentrations, at temperatures above the denaturation temperature of whey proteins. Heating whey protein solutions at lower protein concentrations leads to formation of protein aggregates. Through addition of salt or decreasing the pH towards acidic pH values, these pre-heated protein solutions may gel, which is known as cold gelation of proteins (Barbut & Foegeding, 1993; Elofsson, Dejmek, Paulsson, & Burling, 1997; Ju & Kilara, 1998).

In addition to their functional properties, whey proteins are also used in food products, for their high nutritional value and balanced amino acid composition (Ha & Zemel, 2003; de Wit, 1998). These properties make them attractive for use in high protein foods. Recent work has demonstrated several health related benefits of high protein foods. In particular, it has been found that there is a strong satiating effect of proteins as compared to carbohydrates and fats (Anderson & Moore, 2004; Bertenshaw, Lluch, & Yeomans, 2008; Johnston, Tjonn, & Swan, 2004; Paddon-Jones et al., 2008; Westerterp-Plantenga et al., 2006). For this reason, it is believed that elevated protein concentrations in a diet may help to lose weight and maintain a healthy body weight. However, products with an increased protein content often display inferior sensory properties. These negative effects were also reported for protein gels. For example, Kangli, Matsumura, and Mori (1991) studied the effect of protein concentration on the texture of soy protein gels formed by heat treatment, and found a strong influence of protein concentration on the 'hardness' and 'toughness' perception. Gels formed at higher protein concentrations were described as firm, tough and hard to fracture. Similar results, for soy and whey protein gels, have been reported in other studies as well (Ju & Kilara, 1998;

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Mleko, 1999; Puppo & Añón, 1998; Twomey, Keogh, Mehra, & O'Kennedy, 1997).

One way to reduce or even eliminate these negative textural properties can be the incorporation of dense protein particles into the protein gel. Mixed gels, containing protein as continuous phase (or matrix) and oil (or other types of particles) as dispersed phase (or filler), have been studied extensively (Chen & Dickinson, 1998; Chen, Dickinson, Langton, & Hermansson, 2000; McClements, Monahan, & Kinsella, 1993; Mor-Rosenberg, Shoemaker, & Rosenberg, 2004; Yost & Kinsella, 1993). The mechanical properties of the mixed gels are determined by several factors such as, the mechanical properties of the matrix and particles, the volume fraction and size distribution of the particles and the interaction between the matrix and particles (Chen & Dickinson, 1998; Chen et al., 2000; Dickinson, 2012; McClements et al., 1993; van Vliet, 1988; Yost & Kinsella, 1993). Incorporation of protein stabilized oil droplets into a heat-induced protein gel has been reported to increase the rigidity of the gel, as a result of interaction between the proteins in the matrix and the proteins on the surface of the oil droplets (Chen & Dickinson, 1998; van Vliet, 1988; Yost & Kinsella, 1993). In this case, the particles are classified as 'active fillers'. Oppositely, if the oil droplets were covered by a non-ionic surfactant, lower gel rigidities were reported by increasing the volume fraction of particles (Chen & Dickinson, 1999). This was attributed to the weak interaction between the matrix and filler particles and in this case the particles are classified as 'inactive fillers'. Mixed protein gels, containing protein aggregates or particles, were also studied to improve textural and mechanical properties (Beuschel, Culbertson, Partridge, & Smith, 1992; Purwanti, Moerkens, van der Goot, & Boom, 2012; Purwanti et al., 2011; Renard, Lavenant, Sanchez, Hemar, & Horne, 2002; Renard, Robert, Faucheron, & Sanchez, 1999; Sanchez, Pouliot, Renard, & Paquin, 1999). It was reported that in the presence of whey protein aggregates, softer whey protein gels were formed (Beuschel et al., 1992; Sanchez et al., 1999). Recently, whey protein particles (average diameter $\sim$ 70 μm) formed under high shear treatment, referred to as 'microparticulated whey proteins', were incorporated in WPI gels and their effect on the textural properties were studied at relatively high total protein concentrations (15–20% w/w) (Purwanti et al., 2012). Protein gels containing those protein particles were reported to be weaker than WPI gels without particles at the same total protein concentration. Increasing the volume fraction of the whey protein particles led to a further decrease in the gel strength. However, due to their large swelling capacity and thereby low internal protein density, the increase in total protein concentration of the gels was limited.

Previously, we have found that dense protein particles, having approximately 39% (w/v) internal protein concentration can be formed by heat induced gelation of whey proteins at pH 5.5 (Sağlam, Venema, de Vries, van Aelst, & van der Linden, 2012). These particles are rather compact, heat-stable and do not significantly swell upon heat treatment and may be used to modulate the rheological properties of protein gels at high protein concentrations. Here we investigate the influence of these dense whey protein particles on the mechanical properties of whey protein isolate gels (WPI). Mixed systems at different particle volume fractions and whey protein concentrations in the continuous phase were prepared. These systems were heat-treated (90 °C for 30 min) and based on their gelling behavior a state diagram was prepared. The viscoelastic properties of the gels, with and without added protein particles (at the same total protein concentration) were also measured. To study the influence of particle type, we compare the influence of the dense protein particles (39% w/v) formed at pH 5.5, with the influence of less dense particles formed at pH 6.8. The latter have a significantly lower internal protein concentration ($\sim$18.5% w/v) and a markedly different surface morphology compared to the particles prepared at pH 5.5 (Sağlam et al., 2012). Images, as obtained by SEM of both types of particles are available in the supporting information section (Fig. S1).

2. Materials and methods

2.1. Materials

Whey Protein Isolate (WPI, BiPro JE 034-7-440-1) was obtained from Davisco Foods International Inc. (Le Sueur, MN, USA). 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). By acidification (at pH 4.75) and centrifugation, it was found that the percentage of denatured protein was approximately 10% (w/w). 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. Sunflower oil (Reddy, NV Vandemoortele, Breda, The Netherlands) was purchased from a local supermarket.

2.2. Solutions

WPI solutions at different concentrations were prepared by dissolving whey protein isolate powder in demineralized water. The solutions were first stirred at room temperature for at least 2 h and the solutions were kept mildly stirred overnight at 4 °C to allow complete hydration. The pH values of WPI solutions were either left unadjusted (pH 6.8) or were adjusted to pH 5.5 using 6 M HCl. PGPR (2.5% w/w) was dissolved in sunflower oil by stirring for at least for 2 h at room temperature and was stored in a dark cabinet before use.

2.3. Formation of protein particles

Protein particles were prepared according to the method described previously (Sağlam, Venema, de Vries, Sagis, & van der Linden, 2011). In brief, a water in oil (w/o) emulsion was prepared by mixing a 25% (w/w) WPI solution in sunflower oil (containing PGPR 2.5% w/w) with the help of a high speed mixer (Ultraturrax T 25, IKA Werke, Germany). The weight ratio of WPI solution to sunflower oil was 1:9 and the total mixing time of 5 min was applied at 6500 RPM. Directly after preparation, the w/o emulsion was heated at 80 °C for 20 min and subsequently centrifuged (33,768 × g, Avanti J-26 XP, Beckman Coulter, U.S.A) for 1 h to remove the excess oil. The centrifugation step was repeated three times and subsequent washing and dispersing steps were performed using solutions of 1% WPI (w/w).

2.4. Volume fraction of particles

The Einstein expression for the effective viscosity of an emulsion was used to determine the volume fraction (Φ) of protein particles:

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

where η_{eff} and η_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's equation. The viscosities of the diluted samples were determined using a glass capillary viscometer having an internal diameter of 0.53 mm (Ubbelohde). The viscometer was placed in a water bath at