Food Hydrocolloids 34 (2014) 68–77

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Exceptional heat stability of high protein content dispersions containing whey protein particles

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ARTICLE INFO

Article history:
Received 4 June 2012
Accepted 15 December 2012

Keywords:
Whey proteins
Protein particles
Heat stability
Gelation
Protein drinks
Viscosity
High protein foods

ABSTRACT

Due to aggregation and/or gelation during thermal treatment, the amount of whey proteins that can be used in the formulation of high protein foods e.g. protein drinks, is limited. The aim of this study was to replace whey proteins with whey protein particles to increase the total protein content and heat stability. For this purpose whey protein particles with a size of a few micrometers were formed through emulsification and heat gelation of a 25% (w/w) whey protein isolate (WPI) solution at either pH 5.5 or at pH 6.8. Dispersion of whey protein particles formed at pH 5.5 showed an exceptional heat stability (at pH ~ 7); the viscosity of the dispersions containing a total protein concentration around 18% (w/w) did not change after heating at 90 °C for 30 min, while a WPI solution already gelled under same heating conditions at protein concentrations around 11% (w/w). Additionally, no gelation was observed in the dispersions prepared by pH 5.5 particles, when the total protein concentration was increased above 20% (w/w). However, due to the increased particle concentration shear-thickening was observed in these samples. Whey protein particles prepared at pH 6.8 showed rather weak stability against heat treatment, mainly as a result of swelling. Protein particles were not resistant to gastric digestion and complete degradation of the particles was observed after a short incubation time under pancreatic conditions. In conclusion, the use of dense whey protein particles has been shown to be a useful strategy to counter aggregation and/or gelation problems in high protein foods.

1. Introduction

Proteins give rise to a stronger feeling of satiety in comparison to carbohydrates and fats (Anderson & Moore, 2004; Bertenshaw, Lluch, & Yeomans, 2008; Paddon-Jones et al., 2008; Westerterp-Plantenga et al., 2006). Also, high protein diets result in increased energy expenditure following the consumption than low protein diets (Halton & Hu, 2004; Lejeune, Westerterp, Adam, Luscombe-Marsh, & Westerterp-Plantenga, 2006). Therefore high protein foods are considered to be a potential candidate for body-weight control and treatment of obesity. In addition, liquid high protein formulations play an important role in clinical nutrition. Protein energy malnutrition is a common problem among hospitalized patients (Beattie, Prach, Baxter, & Pennington, 2000; McWhirter & Pennington, 1996; Potter, Roberts, McColl, & Reilly, 2001; Sullivan, Sun, & Walls, 1999) and continuation of a balanced oral diet is not usually possible in some of the patients, such as suffering from cancer or AIDS, due to development of anorexia, eating difficulty and reduced intestinal function (McWhirter & Pennington, 1996; Stack, Bell, Burke, & Forse, 1996). Those patients are at the risk of losing weight and developing impaired function in the organs and muscles, therefore there is a need for a dietary supplement, such as a small volume of liquid with high content of nutrients, in particular proteins and minerals (McWhirter & Pennington, 1996; Potter et al., 2001; Stack et al., 1996). High-protein, high-energy liquid supplements were suggested to be beneficial. for example routine consumption of a high-protein energy sip feed supplement (containing approximately 6.25% (w/v) protein) resulted in an improved energy intake and reduced weight loss in elderly patients (Potter et al., 2001). Similar findings were also reported on another study focused on a patient group infected with HIV (Stack et al., 1996).

Therefore, in view of the above issues, the development of novel food products with substantially higher protein content is of

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http://dx.doi.org/10.1016/j.foodhyd.2012.12.016

importance. When developing high protein foods maintaining a desired product structure is often difficult due to unwanted interactions between the ingredients, which may occur especially during heating and storage. Dairy proteins such as caseins and whey proteins are usually used in formulations of liquid products with high protein levels. In the concentrated systems caseins may aggregate during thermal sterilization (de Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2011). Whey proteins have high nutritional value and they are good source of leucine, which is an important amino acid in the muscle protein synthesis (Fujita & Volpi, 2006). Similar to most proteins, whey proteins are also not stable at high protein concentrations: viscosity increase and gelation after heat treatment may result in the formation of undrinkable products.

Design of protein particles with controlled size, protein content, and surface properties can be useful to reduce or even eliminate such effects of increased protein content on food structure. Improved heat stability was shown for micro-aggregates formed through heat treatment and high-pressure shearing of whey proteins (Dissanayake & Vasiljevic, 2009). The authors reported that the heat coagulation time at 140 °C was significantly higher for whey protein aggregates in comparison to native whey proteins. In another recent study it was also reported that using whey protein nano-particles can enhance heat stability of liquid protein formulations (Zhang & Zhong, 2009). 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 at 5% (w/v) protein content was reported to be transparent and liquid-like after heat treatment.

The aim of current study was to increase the total protein content that can be included in a liquid formulation without showing gelation after heat treatment. For this purpose native whey proteins were replaced by dense whey protein particles and the effect on the viscosity of dispersions before and after heat treatment was analyzed at neutral (~pH 7) and acidic pH (~pH 3.6). Previous work has shown that whey protein particles prepared at different conditions have different viscosity profiles after heat treatment (Sağlam, Venema, de Vries, van Aelst, & van der Linden, 2012). Therefore we have compared the dispersions of two different types of particles with a reference protein solution consisting only of native WPI. In view of their potential application in foods, we also have checked the in-vitro digestibility of the protein particles under gastric and pancreatic conditions.

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, 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). The protein content analysis of WPI after denatured proteins were removed by acidification (at pH 4.75) and centrifugation showed that the denatured protein ratio 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 (1% w/w and 25% w/w) were prepared by dissolving whey protein isolate powder in Millipore water (Millipore Corp., Billerica, MA, USA). The solutions were first stirred at room temperature for at least 2 h and then solutions were kept slightly stirred overnight at 4 °C for complete hydration. The pH values of WPI solutions were either left unadjusted (pH 6.8) or 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 stored in a dark cabinet.

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). First 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 (Ultra-turrax T 25, IKA Werke, Germany). The weight ratio of WPI solution to sunflower oil was 1:9. 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 (33,768 × *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 done using solutions of 1% WPI (w/w).

2.4. Scanning electron microscopy

The microstructure of protein particles was analyzed by scanning electron microscopy (SEM). Samples were critical-point dried (CPD) prior to SEM analysis. For CPD of the samples clean circular cover slips of 8 mm (Menzel, Braunschweig, Germany) were coated with 0.2% Poly-L-lysine hydrobromide in water (Sigma–Aldrich, Inc., USA) and dried for 1 h. After diluting in MQ (100×), a droplet of the particle dispersion was transferred to the coated cover slips. After waiting 30 min, cover slips were gently rinsed in water and then dehydrated in a series of acetone (30, 50, 70, 100%, 10 min per step). The samples were subsequently critical-point dried with carbon dioxide (CPD 030, BalTec, Liechtenstein). Finally samples were sputter-coated with 2 nm Tungsten (MED 020, Leica, Vienna, Austria) and analyzed with a field emission scanning electron microscope (Magellan 400, FEI, Eindhoven, the Netherlands) at room temperature at a working distance of 4 mm with SE detection at 2 kV. The images were digitally recorded.

2.5. Confocal scanning laser microscopy

Protein particles were diluted in Millipore water (100×) and stained with a few drops of 0.2% (w/w) Rhodamine B (Sigma–Aldrich, Steinheim, Germany) in water prior to microscopy analysis. Samples were placed in a chamber made from a standard microscopy glass slide (Menzel-Glaser, 76 × 26 mm, Thermo Scientific, Braunschweig, Germany) and a cover slip (Menzel-Glaser, 24 × 24 mm, Thermo Scientific, Braunschweig, Germany), separated from each other by two strips of parafilm, giving a volume of ~25 μl. Imaging of the samples was done using the confocal microscope (Zeiss 200M Axiovert, Thornwood, NY, USA) using an oil immersion objective (Plan-Apochromat, 100×/1.4 oil, Zeiss, Thornwood, NY, USA). The samples were analyzed at an excitation wavelength of 550 nm and an emission wavelength of 585 nm and at constant acquisition conditions.