Food Hydrocolloids 47 (2015) 41–50

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Enhanced heat stability of high protein emulsion systems provided by microparticulated whey proteins

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ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form
19 November 2014
Accepted 6 January 2015
Available online 20 January 2015

Keywords:
Whey protein
Emulsion
Microparticulation
Heat stability

ABSTRACT

There is a desire to incorporate increasingly high concentrations of whey proteins into nutritional beverages to improve their nutritional content and amino acid profile. However, typical thermal treatments for sterility or extended shelf life cause undesirable aggregation/gelation of the whey proteins. To address this instability issue at high protein concentration, there is a need to develop new protein ingredient technologies that can provide resistance to heat-induced aggregation. In this study, the heat stability characteristics of model food emulsions containing standard or microparticulated whey protein concentrate (WPC80) were compared. Oil-in-water emulsions [10% (wt/wt) sunflower oil] containing increasing concentrations of protein (from 2 to 12 wt%) were prepared at pH 7.0. The effects of retorting at 120 °C for 10 min on particle size distribution, rheological properties, susceptibility to heat-induced coagulation, microstructure and surface protein concentration of the standard and microparticulated WPCs were compared. Also, various levels of NaCl were added to examine the heat stability of micro-aggregated WPC in the presence of additional salts.

Microparticulated WPC emulsions showed significantly enhanced heat stability compared with standard WPC emulsions. Emulsions with up to 11 wt% protein and no visible aggregation or gelation after retorting were produced using microparticulated WPC. For standard WPC emulsions under the same heating conditions, large aggregates were formed and there was a change in flow behaviour to non-Newtonian at 3 wt% protein. With this specific technology, high protein whey-based nutritional beverages can be produced using conventional thermal treatments.

1. Introduction

Whey proteins are used extensively in various food products for their high nutritional value and versatile functional properties (Morr & Ha, 1993). They are used mostly in the form of whey protein concentrates (WPCs), which are produced using membrane-based separation processes such as ultrafiltration and diafiltration (de la Fuente, Singh, & Hemar, 2002). Whey proteins contain all of the essential amino acids, making them nutritionally superior compared with other dietary proteins (de Wit, 1998). They have been widely used in body-building supplements and in infant formulae because of their nutritional benefits. More recently, whey protein has been shown to have positive effects on weight management and satiety (Little & Phillips, 2009). Because of their enhanced dietary properties, there is a global trend to incorporate whey proteins into nutritional beverages. Although whey proteins have significant potential for use in beverage formulations at high concentrations, the main limiting factor for their use is heat-induced destabilisation. Nutritional beverages, like many other liquid food products, are subjected to heat treatment during processing to ensure product safety and extended shelf life.

The heating of whey-protein-based emulsions may cause protein denaturation, aggregation and flocculation, resulting in destabilisation of the emulsion and causing phase separation or protein precipitation (Dybowska, 2011). Whey proteins, including β-lactoglobulin (β-lg), α-lactalbumin (α-la) and bovine serum albumin (BSA), are very sensitive to heat treatment; they typically form aggregates or gels above 70 °C (Hoffmann & van Mil, 1997). On heating to 70 °C under neutral pH conditions, the globular conformation of whey proteins is reversibly changed to a more random structure, resulting in the exposure of previously buried hydrophobic groups. Subsequently, aggregates are formed via intermolecular sulphydryl–disulphide interchange reactions, free thiol oxidation and non-covalent interactions (McSwiney, Singh,

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http://dx.doi.org/10.1016/j.foodhyd.2015.01.003
0268-005X/

Campanella, & Creamer, 1994; Mulvihill & Kinsella, 1987). Above a critical globular protein concentration, at which the kinetics of aggregation and the structure of the aggregates are governed by the balance between attractive and repulsive interactions, heat-induced gelation of the whey proteins occurs (Renard, Lavenant, Sanchez, Hemar, & Horne, 2002). Several authors, including Bowland, Foegeding, and Hamann (1995), Clark, Kavanagh, and Ross-Murphy (2001), de la Fuente et al. (2002) and Singh and Havea (2003), have explained that the formation of heat-induced whey protein gels is irreversible mainly because of disulphide bridges and hydrophobic interactions.

Much lower concentrations of protein are sufficient to form gels for emulsions stabilised by whey proteins than for the protein solution alone, because the emulsified droplets can become incorporated into the gel matrix. Hunt and Dalgleish (1995) reported that a gel structure can be formed by heating (at 90 °C) emulsions made with whey protein isolate (WPI) at concentrations as low as 2%. Monahan, McClements, and German (1996) found that emulsions (19% oil, wt/wt) stabilised by WPI (1%, wt/wt) demonstrated droplet aggregation on heating at 75 °C for 30 min. They showed that the aggregation was due mainly to non-covalent interactions between unfolded protein molecules adsorbed on different droplets and that the interactions were further strengthened by disulphide bonds. Demetriades, Coupland, and McClements (1997) studied the heat stability of emulsions (19.6% oil, wt/wt) stabilised by WPI (2%, wt/wt) with pH values in the range 3.0–7.0 and with added NaCl concentrations in the range 0–100 mM. The emulsions flocculated when heated at 70–80 °C for 30 min at pH 7.0, especially at high salt concentration, but similar heating at pH 3.0 did not cause flocculation.

Therefore, increasing the heat stability of whey protein prior to emulsion formation is important for food processors considering the use of whey proteins as functional or nutritional ingredients in emulsion-based products (Dybowska, 2011). Microparticulation of whey proteins has been shown to produce micro-aggregates with enhanced heat stability (Dissanayake & Vasiljevic, 2009). Microparticulation is generally achieved by thermal aggregation or acid precipitation, often combined with high shear and high pressure conditions (Havea, Baldwin, & Carr, 2009). Microparticulated whey protein can be regarded as a combination of native proteins and both soluble and insoluble protein aggregates of controlled size (Renard et al., 2002). The aggregated particles have limited interaction with each other, because free thiol groups are reduced during microparticulation (Dissanayake & Vasiljevic, 2009). Thus, one of the key parameters that relate to the functional properties of microparticulated whey protein is the extent of protein denaturation in the product.

The objective of this study was to examine the heat stability of emulsions stabilised by various concentrations of microparticulated WPCs (MP-WPCs) and standard WPCs. The heat stability of MP-WPC-stabilised emulsions in the presence of different concentrations of NaCl was also examined.

2. Materials and methods

2.1. Materials

Samples of standard whey protein concentrate (WPC 392) and microparticulated whey protein concentrate (WPC 550) were obtained from Fonterra Co-operative Group Ltd, Auckland, New Zealand. The compositions of the WPC powders are given in Table 1. The MP-WPC was manufactured as described by Havea, Grant, Hii, and Wiles (2012). Reverse phase-high performance liquid chromatography (RP-HPLC) was used to determine the extent of denaturation in the MP-WPC using the method described by Elgar et al. (2000). Standard WPC and MP-WPC solutions (1% protein, wt/wt) were centrifuged at 16,000 × *g* for 3 min and the supernatants of the solutions were passed through an HPLC column, with the proteins being separated according to their mass. Only soluble proteins passed through the HPLC column; the aggregated proteins formed a pellet when centrifuged. Integration and comparison of the elution profiles of the WPCs (Fig. 1) enabled the extents of denaturation to be determined. The percentage denaturation was determined as the difference in the total amount of denaturable protein (β-lg, α-la, BSA, immunoglobulin G (IGG) and lactoferrin) between standard WPC and MP-WPC. It should be noted that this method measured the extent of protein denaturation relative to the level of denaturable protein in standard WPC. It did not consider that there could have been some denatured whey protein in the standard WPC (392) depending on the thermalisation temperatures applied during processing (generally <5%).

Table 1
Composition of WPCs used for preparing the emulsions.

	Standard WPC	MP-WPC
Composition (g/100 g)		
Protein	80.3	79.1
Fat	6.2	4.8
Total carbohydrate	7.0	9.3
Moisture	3.7	4.0
Ash	2.8	2.8
Minerals (mg/100 g)		
Calcium	400	347
Phosphorus		290
Sodium	340	220
Potassium		744
Magnesium		63
Chloride		118

Sunflower oil was purchased from Davis Trading Co., Palmerston North, New Zealand. All of the chemicals used were of analytical grade, obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St Louis, MO, USA) unless otherwise specified.

2.2. Emulsion preparation

Oil-in-water emulsions were prepared with either MP-WPC or standard WPC. Dispersions of MP-WPC at protein concentrations from 2 to 12% (wt/wt) and standard WPC at protein concentrations from 2 to 6% (wt/wt) were prepared by hydrating the powders in distilled water at 50 °C for 60 min under continuous stirring. Appropriate quantities of sunflower oil were added to the protein solutions to give 10% (wt/wt) added oil in the final emulsion. The mixture was heated to 60 °C and a coarse emulsion was formed by mixing at 13,500 rev/min for 2 min using an Ultra-Turrax T25 disperser (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The

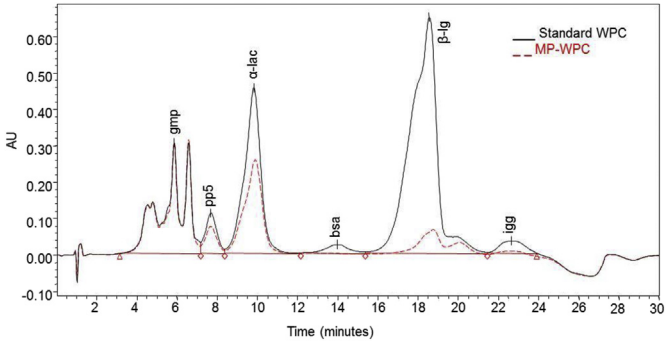

Fig. 1. Protein profiles of the supernatants from standard WPC and MP-WPC solutions.