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Aggregated whey proteins and trace of caseins synergistically improve the heat stability of whey protein-rich emulsions

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ABSTRACT

Heat treatments are used to extend the shelf life of manufactured food emulsions, which in turn require excellent heat stability. Whey protein aggregation prior to homogenization is a means to modify emulsion heat stability but the underlying mechanism of heat stabilization has hardly been studied in an industrial context where whey protein ingredients contain caseins. Emulsions were prepared with 30% anhydrous milk fat and 70% whey protein/casein solutions with protein concentrations ranging from 3 to 6%. The proteins were either unheated (WP/Cas samples) or heat-aggregated (A-WP/Cas samples). After homogenization, the fat droplet interface was characterized and emulsion stability was analyzed visually and at microscopic level. WP/Cas emulsions were heat stable at low protein concentrations but exhibited a gradual decrease in heat stability when the protein concentration increased (>3%). This instability was due to the co-gelation of the protein-coated fat droplets and the proteins in the dispersing phase. In contrast, A-WP/Cas emulsions were rapidly heat destabilized at low protein concentrations (<4%) but were more heat stable than WP/Cas emulsions at higher protein concentrations. The stability of the A-WP/Cas emulsions at protein concentrations higher than 4% was correlated with the heat stability of the whey protein aggregates in the dispersing phase and the decrease in the proportion of whey protein aggregates at the oil/water interface due to increasing competition with caseins present in the whey protein ingredient. This study contributes to greater understanding of the functional role of aggregated whey proteins and residual caseins in emulsions stabilized by industrial WP ingredients.

1. Introduction

Heat treatments are carried out in the food industry to deactivate enzymes and to reduce microbial load with the aim of extending the shelf-life of food products. However, heat treatments can cause detrimental changes in some products such as liquid protein-stabilized emulsions due to the heat instability of the proteins adsorbed at the surface of fat droplets and in the dispersing phase (Çakir- Fuller, 2015; Dybowska, 2011; Euston, Finnigan, & Hirst, 2000). Heating leads to the flocculation and coalescence of the fat droplets and in extreme cases to gelation of the emulsion (Çakir- Fuller, 2015; Demetriades, Coupland, & McClements, 1997; Euston et al., 2000; Hunt & Dalgleish, 1995; Jost, Baechler, & Masson, 1986; Yamauchi, Shimizu, & Kamiya, 1980; Yost & Kinsella, 1992). To prevent such instability, manufacturers used to add non-protein additives (such as carrageenans, pectins, mono and diglycerides of fatty acids) that are able to keep the fat droplets away from each other. However, consumers are increasingly demanding more natural food products, whether additive-free or 100% natural (Li & Nie, 2016). Identification of alternative ways to protect protein-coated emulsions against instability without using additives is an active research area in the dairy industry in order to satisfy new market trends (clean label tendency).

Whey proteins are natural surfactants used to emulsify and stabilize dairy emulsions. However, when heated above 70 °C whey proteins unfold, exposing reactive groups that are responsible for protein aggregation (Griffin, Griffin, Martin, & Price, 1993; Roefs & De Kruif, 1994; Sawyer, 1968; Verheul, Roefs, & de Kruifs, 1998) and emulsion instability (Çakir- Fuller, 2015; Dybowska, 2011; Euston et al., 2000; Hunt & Dalgleish, 1995; McClements, 2004; Surel

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et al., 2014). It has been shown that the heat stability of whey protein-stabilized emulsions is dependent on the concentration of native whey protein in the dispersing phase (Çakir- Fuller, 2015; Dybowska, 2011; Euston et al., 2000). Nicolai and Durand (2013), suggesting that controlled whey protein aggregation before emulsification is a way to improve emulsion stability. Heat-induced aggregates, known as whey protein microgels, have been used as effective food-grade particles to stabilize emulsions due to the "Pickering-like mechanism" (Destribats et al., 2014). In addition, microparticulated whey protein stabilized emulsions are more resistant to heat treatment than emulsions stabilized by native proteins at high protein concentrations (Çakir- Fuller, 2015).

Whey protein ingredients produced on an industrial scale by using membrane filtration techniques contain residual amounts of caseins in addition to globular whey proteins (Coppola, Molitor, Rankin, & Lucey, 2014). Caseins are heat stable and they improve emulsion heat stability at low protein concentrations by forming long dangling tails at the surface of fat droplets (Dickinson & Parkinson, 2004; Hunt & Dalgleish, 1995; McCarthy, Noel, Kelly, O'Mahony, & Fenelon, 2014; Parkinson & Dickinson, 2004). However, the mechanism of heat stabilization of whey protein-stabilized emulsions over a wide range of protein concentrations has not been completely clarified, especially in an industrial context where whey protein ingredients contain variable amounts of caseins. Since emulsions having high fat and protein contents are the most susceptible to heat destabilization, this study focused on emulsions having 30% (w/w) fat and containing up to 6% (w/w) proteins in the aqueous phase. The heat stability of whey protein/casein (WP/Cas) emulsions and aggregated whey protein/casein (heat aggregated prior to emulsification) (A-WP/Cas) emulsions was investigated on both the macroscopic and microscopic scales in order to distinguish the influences of the fat droplet interface and the dispersing phase in the overall mechanism of emulsion heat stability.

2. Materials and methods

2.1. Materials

Anhydrous milk fat (fusion point 32 °C) was obtained from Corman (Corman, Limbourg, Belgique). Milk permeate powder and whey protein powder were provided by a dairy company (confidential origin). Whey protein powder contains a high amount of protein (88.8%), 82% which are whey proteins and 18% are caseins (determined by SDS-PAGE quantification under reducing conditions).

2.2. Whey protein solutions

Whey protein/casein (WP/Cas) solutions were prepared by dispersing the whey protein powder at about 3, 4, 5 or 6% (w/w) protein in milk permeate solution reconstituted at 5.6% (w/w). This dispersion phase had a composition similar to that of the dispersing phase of milk. The pH of the WP/Cas solutions was adjusted to 7.

Protein aggregates were prepared according to a protocol adapted from Mahmoudi, Gaillard, Boué, Axelos, and Riaublanc (2010). The WP/Cas solution was reconstituted at 4% (w/w) protein by dispersing the whey protein powder in ultra-pure water. The mixture was gently stirred at room temperature for at least 4 h. The protein concentration of the WP/Cas solution was adjusted to 4% (w/w) with ultra-pure water and the pH of the solution was then set at 7. The WP/Cas solution was heated to 80 °C in less than 1 min with a home-made tubular exchanger immersed in a water bath set at 85 °C and then maintained at this temperature for 15 h without stirring in order to form the aggregates. After heating, around 5% (w/w) of globular whey protein (β-lactoglobulin and α-lactalbumin) was non-aggregated and 60% of the casein was combined with the aggregates. The aggregated whey protein/Cas (A-WP/Cas) solution was concentrated twice by ultrafiltration using a 10 kDa spiral organic membrane (Helicon, Millipore, France) connected to a Millipore filtration system (PROLAB Millipore MSP 006239, Millipore, France). A-WP/Cas solution was diluted at about 3, 4, 5 or 6% protein (w/w) with milk permeate solution (5.6% w/w in the final protein solution). The pH of the A-WP/Cas solutions was adjusted to 7. Sodium azide (0.05% w/v) was then added to protein solutions to prevent bacterial growth.

2.3. Preparation of protein-coated emulsions

Emulsions were prepared in two steps: first, pre-emulsification was performed by mixing protein solutions (70% w/w) with anhydrous milk fat (30% w/w) previously heated to 60 °C using a rotor stator homogenizer (Heidolph Silent Crusher M, Schwabach, Germany) at 18000 rpm for 5 min in order to avoid the presence of fat crystals. The pre-emulsions at 60 °C were then passed through a homogenizer (Stansted, Harlow, Essex, UK) set at a pressure of 50 bars (5 passes). After 5 passes in the homogenizer the dispersion fat globule size no longer evolved. All emulsions were prepared in duplicate.

2.4. Heat stability of protein-coated emulsions and protein solutions

Volumes of 2 mL of emulsion were placed in glass tubes in order to evaluate emulsion heat stability. Three glass tubes were prepared for each emulsion, one containing only the emulsion, the second with the emulsion and 20 μl of Nile Red (0.125% in propylene glycol) to clearly visualize fat release (oiling off) and the third with the emulsion, 20 μl of Nile Red and 10 μl of Fast Green (1% in water) for CLSM observation. Glass tubes were immersed in an oil bath at 120 °C (Huber, Germany). Emulsions were heated for 1, 2, 3, 5, 10, 20 and 30 min. Samples were immediately cooled and were analyzed visually to detect any trace of heat-induced modification (thickening, gelation, fat release).

Protein solutions were analyzed following the same protocol except that only two glass tubes were filled with each protein solution, and 10 μl of Fast Green (1% in water) was added to one of the two glass tubes for CLSM analysis.

2.5. Size distribution of protein particles

The sizes (hydrodynamic diameter) and the size distributions of the particles in WP/Cas and A-WP/Cas solutions were determined before and after heat treatment by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instrument, Worcestershire, UK). Each sample was diluted in sufficient milk permeate solution to avoid multiple particle effects. Measurements were performed in triplicate in a plastic cell at 25 °C in backscattering configuration at 173° over 120 s. Experimental data were analyzed using the general purpose model. Results were expressed as the mean of 10 runs. The hydrodynamic diameter of particles was calculated by the Stockes-Einstein equation using the diffusion coefficient extracted from the fit of the correlation curve. A refractive index of 1.45 was used for intensity-size representation.

2.6. ξ-potential of protein particles

The ξ-potential of WP/Cas and A-WP/Cas solutions diluted 10 times in milk permeate solution at 5.6% was measured in triplicate using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire,