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Increasing the heat stability of whey protein-rich emulsions by combining the functional role of WPM and caseins

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ABSTRACT

The heat stability of whey protein emulsions remains a real challenge due to the rapid denaturation/aggregation of native whey proteins on heating. The use of heat-stable Pickering-like whey protein microgels (WPM) makes it possible to develop heat-stable emulsions in a large range of whey protein concentrations. In this study, emulsion heat stability was evaluated with a special focus on the contribution of WPM adsorbed at the fat droplet surface and in the continuous phase of the emulsion. Dairy emulsions were prepared with 30% milk fat and 70% suspension of WPM in the dispersed phase of milk. The protein interfacial load and the composition of the fat droplet surface were determined immediately after emulsion formation, and the heat stability of the emulsions at 120 °C was assessed visually and at microscopic scale. WPM are heat stable in the continuous phase of the emulsion, but the presence of WPM at the surface of the fat droplets is responsible for a rapid gelation of the emulsions. In the heated emulsions, the fat droplets seemed to be crosslinked by WPM. The presence of caseins instead of WPM at the fat droplet surface allowed the heat stability of the emulsion to recover at low and high whey protein concentrations. This study shows that it is possible to prepare heat-stable whey protein–rich emulsions by using whey proteins previously aggregated as heat-stable WPM and a sufficient amount of caseins in order to fully cover the fat droplet surface. These results will contribute to the development of heat-stable whey protein–rich emulsions.

1. Introduction

Consumers' demands in terms of food constantly evolve at the pace of scientific knowledge, with a growth in demand for more natural (Li & Nie, 2016) and healthy products (Mollet & Rowland, 2002). Due to their functional properties and exceptional biological value, e.g. richness in essential amino acids, binding properties for vitamins, minerals and fatty acids (Morr & Ha, 1993; Smithers, 2008), there is a trend to increase the use of whey proteins in a large range of food products such as emulsion beverages (Çakir-Fuller, 2015). However, these products are heat sensitive due to the rapid denaturation/aggregation of whey proteins on heating (Griffin, Griffin, Martin, & Price, 1993; Roefs & De Kruif, 1994; Sawyer, 1968; Verheul, Roefs, & de Kruifs, 1998). In liquid dairy emulsions, heat treatments can cause fat droplet aggregation and even the gelation of the emulsions if the whey protein concentration and/or the fat droplet volume fraction are too high (Çakir-Fuller, 2015; Demetriades, Coupland, & McClements, 1997; Euston, Finnigan, & Hirst, 2000; Hunt & Dalgleish, 1995; Jost, Baechler, & Masson, 1986; Yamauchi, Shimizu, & Kamiya, 1980; Yost & Kinsella, 1992).

The pre-denaturation of whey protein into aggregates was shown to improve emulsion heat stability (Çakir-Fuller, 2015). The heat stability of whey protein stabilized emulsions is affected both by the heat stability of the proteins adsorbed at the fat droplet surface and by the concentration and heat stability of the proteins (native or aggregated) in the continuous phase (Çakir-Fuller, 2015; Chevallier et al., 2016; Euston et al., 2000). The presence in the continuous phase of the emulsion of whey protein aggregates instead of native whey proteins improves emulsion heat stability at a high protein concentration (Çakir-Fuller, 2015; Chevallier et al., 2016). The ability of adsorbed whey protein aggregates to stabilize fat droplets on heating was less studied. Under certain

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conditions, the whey protein microgels (WPM) spread at the fat droplet interface (Destribat et al., 2014), and their structure can be disintegrated into small aggregates (Murphy, Farkas & Jones, 2016). These rearrangements or preferential orientations of the aggregates at the fat droplet surface could modify their behavior on heating. Emulsions stabilized by soft WPM particles but produced differently than in the previous studies (Destribatet al., 2014; Murphy, Farkas & Jones, 2016) exhibited a heat-induced aggregation of neighboring fat droplets by a mechanism of fusion of adsorbed particles (Sarkar et al., 2016). The presence of strand-shaped whey protein aggregates at the fat droplet surface was also shown to alter emulsion heat stability (Chevallier et al., 2016).

In this study, the heat stability of designed Pickering-like emulsions prepared with heat-stable WPM (Bovetto, Schmitt, Beaulieu, Carlier, & Unterhaslberger, 2007; Donato, Schmitt, Bovetto, & Rouvet, 2009; Moitzi et al., 2011; Murphy, Cho, Farkas, & Jones, 2015; Phan-Xuan et al., 2011, 2014; Schmitt, Bovay, Vuilliomenet, Rouvet, & Bovetto, 2011; Schmitt et al., 2009, 2010) was evaluated with a specific emphasis on the contributions of the WPM adsorbed at the fat droplet surface and the WPM dispersed in the continuous phase of the emulsion. The objective was to develop heat-stable fluid emulsions in a large range of whey protein concentrations and in the dispersed phase of milk, i.e. in the presence of salts and lactose.

2. Materials and methods

2.1. Materials

Whey protein powder, sodium caseinate powder and milk ultrafiltration permeate powder were provided by dairy companies (confidential origin). Anhydrous milk fat (melting point 32 °C) was from Corman (Corman, Limbourg, Belgium).

The whey protein powder contained 88.8% proteins (wt/wt) determined by Kjeldahl method, < 4% lactose and < 3% minerals (Ca^{2+}: 0.31%; Mg^{2+}: 0.02%; Na^{+}: 0.09%; K^{+}: 0.16% determined by atomic adsorption). The protein content of the whey protein powder was composed of whey proteins (82%) and caseins (18%) (quantified by SDS-PAGE under reducing conditions using standard calibration curves; Fig. 8B, track 6), mainly α-caseins and κ-caseins (identified by mass spectrometry). Sodium caseinate powder had a dry matter > 94%, 87.6% protein (wt/wt) of which 99.2% casein, < 0.4% lactose and < 4.5% minerals (Na^{+}: 1.2%). The mineral content of the milk ultrafiltration permeate powder was composed of Ca^{2+}: 0.31%; Mg^{2+}: 0.12%; Na^{+}: 0.63%; K^{+}: 2.74% (determined by atomic adsorption).

2.2. Suspensions of whey protein microgels

Whey protein microgels (WPM) were produced according to Bovetto, Schmitt, Beaulieu, Carlier, & Unterhaslberger (2007). The whey protein powder was dissolved at 4% (wt/wt) proteins in ultrapure water. The solution was adjusted to pH 5.8 and was gently stirred at room temperature for at least 4 h. The protein solution preheated to 45 °C (15 min) was heated up to 80 °C in 15 s with a home-made tubular heat exchanger. The heated solution was transferred in a glass bottle immersed in a water bath at 80 °C. The solution was kept at this temperature without stirring for 1 h. The obtained suspension was called WPM suspension. The proportion of WPM in the WPM suspensions was determined by quantifying the total protein content and the protein content in the supernatant after centrifugation as performed by Schmitt et al. (2011). The protein composition of the WPM suspension was determined after protein separation by SDS-PAGE (see below). The proteins recovered in the supernatant were a fraction of protein not included in the WPM.

After heating, the WPM were recovered by centrifugation at 27,000 × g for 15 min at 20 °C using an Avanti J-26S XP centrifuge (Bekman Coulter, USA) and the pellet was redispersed and centrifuged twice with ultrapure water (Donato, Schmitt, Bovetto, & Rouvet, 2009). Then, the washed pellet was dispersed at around 6% protein (wt/wt) in a milk ultrafiltration permeate solution prepared to obtain a composition similar to the soluble phase of milk. This suspension was called WPM suspension after centrifugation.

The WPM suspension was concentrated twice by ultrafiltration with a Millipore filtration system (PROLAB Millipore MSP 006239, Millipore, France) using a 10 kDa spiral organic membrane (Helicon, Millipore, France). A range of WPM suspensions was prepared by diluting the concentrated protein suspension with concentrated milk ultrafiltration permeate solutions. The concentration of milk permeate solutions was chosen to obtain protein suspensions after dilution with a soluble phase similar to that of milk. The protein concentration of the diluted WPM suspensions was between 2.7 and 6.5% (wt/wt).

In the WPM suspension at 2.7% protein (wt/wt), 0.23% sodium caseinate (wt/wt) was added in order to increase the amount of caseins.

Further experiments and characterization were carried out with the WPM suspensions dispersed in the milk ultrafiltration permeate.

2.3. Preparation of emulsions

Oil-in-water emulsions were prepared with 30% (wt/wt) anhydrous milk fat and 70% (wt/wt) protein suspension. To avoid the formation of fat crystals, the anhydrous milk fat and protein suspensions were heated to 60 °C and then mixed and pre-emulsified with a rotor stator homogenizer (Heidolph Silent Crusher M, Schwabach, Germany) set at 18,000 rpm for 5 min. The coarse emulsion was then passed through a homogenizer (Stansted, Harlow, Essex, UK) at a pressure of 5 MPa (5 passes in order to have stable fat droplet size distribution in the emulsion). Each emulsion was prepared in duplicate.

In order to measure the heat stability of the emulsions, a series of 3 glass tubes per emulsion was filled with 2 mL of emulsion. The first tube was used for particle size measurements. In the second tube, 20 μl of Nile Red (0.125% in propylene glycol) was added to the emulsion to clearly visualize fat release (oiling off). Volumes of 20 μl of Nile Red and 10 μl of Fast Green (1% in water) were added to the third tube that was used for confocal laser scanning microscopy (CLSM) analysis. The glass tubes were immersed in an oil bath set at 120 °C (Huber, Germany) and were respectively heated for 1, 2, 3, 5, 10, 20 and 30 min. Immediately after heating, the tubes were cooled and a visual analysis was performed to detect any traces of heat-induced modifications (thickening, gelation, fat release).

Exactly the same protocol was performed for measuring the heat stability of the protein suspensions except that only 2 glass tubes were prepared per suspension. The first glass tube was filled with the protein suspension only and 10 μl of Fast Green (1% in water) was added to the second tube for CLSM analysis.

2.4. Size distribution of protein particles

A Zetasizer Nano ZS (Malvern Instrument, Worcestershire, UK) was used to determine the size (Z-average hydrodynamic diameter) and size distribution of the particles in the protein suspensions before and after heat treatment by using dynamic light scattering. The protein suspensions were diluted 100 times with milk ultrafitration permeate solution to avoid multiple scattering effects. The diluted protein suspensions were placed in a plastic cell and

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