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Mixtures of whey protein microgels and soluble aggregates as building blocks to control rheology and structure of acid induced cold-set gels

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ABSTRACT

Whey proteins (WP) today offer an extremely high potential for innovative development of functional and nutritious food products. Acid cold-set gels present an interesting approach of gelation at low temperature upon acidification of preformed whey protein (WP) aggregates. In the present work, we aimed to demonstrate how structure and rheological properties of acid gels can be controlled by combining two types of WP aggregates with different structural and chemical properties. Whey protein microgels (WPM) and soluble aggregates (WPSA) were generated upon heating WP isolate in specific pH conditions and temperature, leading to Z-average hydrodynamic diameters close to 270 nm for WPM and 100 nm for WPSA. Mixtures of WPM and WPSA were prepared at different weight ratios ranging from 100% WPM to 100% WPSA. The total protein concentration was set to 4 or 8%wt. Acidification was performed at 40 °C by addition of 1%wt glucono-δ-lactone (GDL). Gelation was followed using turbidimetry and small deformation rheology as function of pH. Microstructures of the gel were investigated at different length scales using various microscopy techniques (CLSM, SEM, AFM). When the WPM/WPSA ratio decreased, the pH of gelation and the gel strength increased because of the different structure and chemical reactivity of the two types of WP aggregates. The final pH had a strong impact on the structure of the gels. When final pH decreased below pH 4.3, a structure change was suggested by turbidimetry measurements. This resulted in a non self-supporting gel or in a decrease of gel strength. For pH above 4.3, self supporting gel were obtained. The rheological properties of the gel could therefore be modulated depending on the properties of the building blocks used (WPM versus WPSA). Interestingly, the gel microstructures observed for WPM/WPSA mixtures or WPM were comparable to those of acidified skimmed milk gels ranging from coarse structures with clumps of aggregates or to homogeneous fine networks (WPSA only) that have been described for WP gels obtained upon direct heating at various pH.

1. Introduction

Whey proteins are currently used as an ingredient to improve nutritional and functional properties of food (de Wit, 1998). In particular, gelation properties of whey proteins are of interest to promote whey proteins functionalities. Gelation can occur under heat treatment at temperature higher than 60 °C, where whey proteins undergo structural changes that can lead under specific medium conditions to the formation of a tridimensional network called heat set gel. An alternative method is the preparation of so-called, cold-set gel (Alting, Visschers, & Hugenhoultz, 2001). This gelation process consists of two consecutive steps. First, the protein solution is heated under conditions to generate small stable soluble aggregates. Then, gelation is induced either by adding salt (salt set gels) (Barbut & Foegeding, 1993), by lowering the pH (acid-set gels) (Alting, de Jongh, Visschers, & Simons, 2002). This step can be performed at different temperatures which were shown to increase kinetics of aggregation as it increases (Ako, Durand, & Nicolai, 2010). Cold gelation of protein has been reported for β-lactoglobulin (Resch, Daubert, & Foegeding, 2005), whey protein isolate (Ju & Kilara, 1998, 2006), whey protein concentrate (Thomsen, 1994) and recently for soy protein (Maltais, Remondetto, Gonzalez, & Subirade, 2005). Compared to acid induced skimmed milk gels used in yoghurt-making technology, structural and mechanical properties of whey proteins acid set gels remarkably differ. Acid set gels made from whey proteins can form a fine stranded network structure as function of pH (Barbut & Foegeding, 1993) showing strong elastic properties with high gel hardness (Alting, Hamer, De Kruif, & Visschers 2003; Rabiey & Britten, 2009). In contrast, milk gels are often considered as particle gels (Horne, 2003) with lower gel hardness. Such particulate microstructures can only be obtained with whey proteins by heating solution adjusted in pH region close

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to isoelectric point of whey protein (pH 5.2) (Krebs, Delvin, & Donald, 2007; Langton & Hermansson, 1992).

In the first step of preparation of cold-set gels, the generation of soluble aggregates is performed in conditions allowing the formation of non-sedimentable aggregates stable in solution. Such type of aggregates can be obtained after heating whey proteins at pH far from isoelectric point of protein in low ionic strength at protein concentration lower than gelling concentration (estimated around 10–12%wt for whey protein isolate) (Bryant & McClements, 2000; Hongsprabhas & Barbut, 1997). In these conditions, whey proteins have a high net surface charge and repulsive force preventing random aggregation and resulting in the formation of soluble linear or curved ellipsoid-shaped aggregates (Le Bon, Nicolai, & Durand, 1999). The heat treatment (time/temperature) is chosen to minimize residual native protein amount which can impact the final gel structure (Barbut & Foegeding, 1993; Vardhanabhuti, Foegeding, McGuffey, Daubert, & Swaisgood, 2001). In the second step, gelation is induced by decreasing the electrostatic repulsion between the soluble aggregates (Alting et al., 2002) in presence of salts or by decreasing the pH of the solution. Aggregation of the soluble aggregates through physical interaction is then promoted leading to open thermodynamically unstable cluster of aggregates (Alting et al., 2002) up to the gel point defined by the formation of a fine stranded structure. These clusters can be then stabilized by the formation of additional covalent disulfides bonds and/or local rearrangements (Cavallieri & Lopes da Cunha, 2008). Rearrangements of the aggregates can occur which leads to locally denser cluster and larger pores that can lead to more turbid gel and more permeable microstructure (Alting et al., 2003).

Different factors can affect the structure and mechanical properties of the gel, including the aggregate shape (Alting et al, 2004) and size (Ju & Kilara, 2006; Mleko, 1999). It has been suggested that the increase in aggregate size increased gel strength. However, changes in the aggregates sizes and shape were mostly performed by varying the heating temperature and time (Hongsprabhas & Barbut, 1997; Ju & Kilara, 2006), resulting therefore in a different amount of residual native proteins and concentration of aggregates. Recently, Ako et al. (2010) showed separately the influence of aggregates size and concentration on the microstructure of NaCl induced gel of β-lactoglobulin aggregates observed by light scattering and confocal laser scanning microscopy. Aggregates were obtained after prolonged heating at 80 °C at pH 7.0 to minimize residual native protein amount. The authors have shown that the aggregates size does not impact the final microstructure of salt-induced gel but rather plays a role in the kinetics of aggregation. The concentration of aggregates however impacts the kinetics and the final microstructure with an increase of apparent molar mass of the particle forming the gel, as concentration decreases.

In all studies mentioned, main limitation in cold-set gel process remains the formation of stable aggregates in the first stage. Recent research has established conditions to generate whey protein aggregates called whey protein microgels by applying heat treatment on protein solution adjusted in pH range between 5.7–5.9 (Donato, Schmitt, Bovetto, & Rouvet, 2009; Schmitt et al., 2009). WPM can be defined as microgels of whey protein aggregates with a defined size between 200 and 400 nm, a low polydispersity index and surface charge of ~ -30 mV at pH > 5. These aggregates showed a larger size, a slightly lower surface charge, a lower surface hydrophobicity and a lower content of accessible thiol groups than soluble aggregates formed at pH close to pH 7.0 (Schmitt et al., 2009). Solubility curves obtained with WPM solution (4%wt) shows a minimum in the pH range 4–5.5 with a minimum in surface charge at pH 4.8. Apart from this pH region, WPM form stable colloidal dispersions with surface charge alternately positive or negative. Electrostatic repulsions between the WPM confer their stability and prevent from precipitation. Detailed analyses by SAXS of the structure of WPM 4%wt as function of pH suggest a swelling behaviour of WPM at pH below or above their pI, as the effective charge of the microgel network is increasing (Schmitt et al., 2010). In the critical pH range of 4.5–5, aggregation of WPM into large aggregates are formed due to screening of electrostatic repulsions.

Up to now, no description in the literature can be found of acid induced gels made out from WPM. The present study was set in to evaluate how structure and rheological properties of acid gels can be modulated by combining two types of WP aggregates with different structural and physico-chemical properties. Whey protein microgels (WPM) and soluble aggregates (WPSA) were generated upon controlled heating conditions. Mixtures of WPM and WPSA were prepared at different weight ratios ranging from 100% WPM to 100% WPSA. The total protein concentration was set to 4 or 8%wt. Acidification was performed by addition of glucono-δ-lactone (GDL), 1%wt at 40 °C. Acid-induced gelation of these systems was followed using turbidimetry and small deformation rheology as function of pH. Microstructures of the gel were investigated at different length scales using various microscopic techniques.

2. Material and methods

2.1. Preparation of aggregates

2.1.1. Preparation of WPM

WPM powder was produced according to Schmitt et al. (2010). For preparation of the WPM dispersions, WPM powder was dispersed at 10%wt (protein basis) in MilliQ® water (18.2 MΩ cm) at room temperature for 1 h under moderate stirring to avoid foam formation. The protein solution was then left at 4 °C overnight under gentle stirring to allow complete hydration. Preliminary trials showed that homogenisation treatment of the WPM dispersion was required at 250 bar was required to recover solubility of WPM powder. A lab scale GEA Niro Soavi homogeneiser (N1001L2K, Parma, Italy) was used to perform this step. The solutions were then pasteurised at 85 °C for 15 min in a water bath and stored at 4 °C.

2.1.2. Preparation of WPSA

The protein solution was prepared by dispersion of WPI powder (Prolacta 90, Lactalis, France) 10%wt (protein basis) in MilliQ® water, and stirring 1 h at room temperature. The protein solution was left at 4 °C overnight under gentle stirring to allow complete hydration. A centrifugation for 1 h at 10 800 g (Sorvall Evolution, rotor SLA 3000, Thermofisher scientific, Zurich, Switzerland) was then performed to remove insoluble fraction (<1%wt total solid). Protein concentration was measured using UV/VIS spectroscopy ($\varepsilon_{280} = 0.9916$ g dL cm^{-1} as determined experimentally) (UVIKON 810, Kontron Instruments, Flowspec, Switzerland). Concentration and pH of the protein solution were then adjusted by addition of NaOH 0.1 M (Merck, Darmstadt, Germany) to obtain 8%wt protein solution at pH 7.6 and at 4%wt protein solution at pH 7. Several 22 mL glass vials (with a screw top and a solid cap with PTFE liner) (Supelco, Bellefonte, PA, USA) were filled with 20 mL of protein solution and heated under stirring (300 rpm) in a water bath at 85 °C for 1 h (time to reach the temperature was 3 min). After heat treatment, the samples were rapidly cooled in ice.

2.1.3. Mixtures of WPM/WPSA solution

Mixtures of WPM and WPSA were prepared at different WPM/WPSA weight ratios of total protein concentration ranging from 100% WPM to 100% WPSA (noted respectively WPM/WPSA 100/0, 80/20, 60/40, 20/80, 0/100). Total protein concentration was set to 4 or 8%wt. WPM and WPSA concentrations were adjusted from the