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Model mixtures evidence the respective roles of whey protein particles and casein micelles during acid gelation

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ABSTRACT

In acidified milk, heat-induced whey protein aggregates and casein micelle particles assemble to form a soft gel. The present study was set to evaluate the respective roles of whey protein aggregates (WPIA) and native casein micelles (NMC) during acid gelation by means of changing their ratio in model systems. NMC and WPIA were dispersed in milk permeate at different weight ratios ranging from 0% to 100% NMC for a total protein concentration of ~45 g kg^{-1}. Acidification was performed at 35 °C by addition of glucono-δ-lactone to achieve the same final pH of 4.5 in 6 h. Acid-induced gelation of these systems was followed using small deformation rheology followed by large deformation test and whey retention measurement at pH 4.5, while their microstructure was investigated microscopically. The results showed that higher content in WPIA promoted faster gelation and led to more elastic gels with smaller pore size and increased whey retention. The effects were particularly dramatic up to ~10% w/w WPIA, where the aggregates were about equimolar to the casein micelles and covered ~8% of the micellar surface. The results were discussed in terms of the physical interactions between two populations of colloids of different abilities for acid gelation. It seemed likely that a preferred interaction exists between the casein micelles and the aggregates, and directs the structural and mechanical properties of the acid gel.

1. Introduction

Milk is a colloidal suspension that consists of casein micelles, whey proteins, lactose and salts (Walstra & Jenness, 1984). In their native state, casein micelles are hydrated particles with an average diameter of ~200 nm containing several thousand of α_{s1}-, α_{s2}-, β- and κ-casein molecules and minerals, essentially calcium phosphate (de Kruif, 1999). In physiological conditions, a "hairy layer" of the hydrophilic parts of κ-casein, located at the surface of casein micelles, is responsible for steric and electro-repulsive stabilization of the casein micelles dispersion (Panouillé, Durand, Nicolai, Larquet, & Boisset, 2005). The uses of milk in dairy processing are mainly determined by its ability to either keep liquid or to transform into gels, hence by the stability of the casein micelles upon environmental change (Horne, 2003). The acidification of milk causes structural and compositional changes in the casein micelles such as the collapse of the κ-casein hairy layer at pH ~6.0 and the decrease of electrostatic repulsion as they reach their isoelectric pH (pI) of ~4.9, where destabilization eventually occurs (Lucey & Singh, 1998). Although the intimate structure of the casein micelles varies throughout acidification, it is believed that their individuality is maintained (Ouanezar, Guyomarc'h, & Bouchoux, 2012).

Heat treatment of skim milk at temperature above ~60 °C causes the irreversible denaturation of the whey proteins and the formation of small colloidal aggregates of whey protein and κ-casein via covalent disulphide bonds and hydrophobic interactions (Singh & Creamer, 1991). In milk, these aggregates can bind onto the surface of the casein micelle either on heating (Jang & Swaisgood, 1990; Kalab, Allan-Wojtas, & Phipps-Todd, 1983; Lucey, Tamehana, Singh, & Munro, 1998) or, possibly, during the early stages of acidification (Alexander & Dalgleish, 2005; Donato, Alexander, & Dalgleish, 2007; Guyomarc'h, Jemin, Le Tilly, Madec, & Famelart, 2009). These aggregates are thought to impact the destabilization of casein micelles due to their higher pI and surface hydrophobicity (Guyomarc'h, Renan, Chatriot, Gamerre, & Famelart, 2007; Morand, Dekkari, Guyomarc'h, & Famelart, 2012; Morand, Guyomarc'h, Legland, & Famelart, 2012). They are also thought to act as bridges between the casein micelles in the acid gel network

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(Alexander & Dalgleish, 2007; Famelart, Tomazewski, Piot, & Pezennec, 2004; Guyomarc'h, Queguiner, Law, Horne, & Dalgleish, 2003). In their study, Lucey et al. (1998) showed that the presence of whey protein aggregates in skim milk correlated with the increase in the pH of gelation and produced different gel networks with enhanced firmness and water-holding capacity. Vasbinder, van de Velde, and de Kruif (2004) demonstrated that the casein micelles and the aggregates assemble into a co-gel, and Guyomarc'h et al. (2009) furthermore showed that neither the casein micelle fraction nor the aggregates can alone form an acid gel with G' values of the same magnitude of heated milk. Thus, the whey protein aggregates and the casein micelles interact to form acid gels, however the mechanisms that drive this interaction need to be further investigated.

In this study, our objective was to investigate how these two particles physically assemble to form the acid gel. To do that, model systems were prepared as carefully characterized binary suspensions of native micellar casein (NMC) and whey protein aggregates (WPIA) dispersed at different ratios in the aqueous phase of milk. The consequences of changing the NMC/WPIA ratio, hence the respective numbers, volume fractions and total surfaces of the two types of particle, were measured with respect to the microstructural and rheological properties of the resulting gels.

2. Materials and methods

2.1. Materials

Native micellar casein (NMC) was prepared as described by Schuck et al. (1994). Briefly, raw milk was skimmed at ~50 °C then microfiltered at that temperature through 0.1 μm cut-off membrane, diafiltered, evapo-concentrated and spray-dried. Total solids include ~815 g kg^{-1} caseins, 85 g kg^{-1} of ashes (including colloidal calcium phosphate associated with the casein micelles), ~70 g kg^{-1} residual whey proteins and peptides, and residual lactose. The microfiltration permeate, containing the native whey proteins, was concentrated by ultrafiltration at ~50 °C, diafiltered and freeze-dried to yield a native whey protein isolate (WPI, 972 g kg^{-1} of proteins on dry basis, of which ~80% are whey proteins, ~10% β-casein and minor amounts of the other caseins (Morand, Guyomarc'h, Pezennec, & Famelart, 2011). The milk ultrafiltration permeate (UFP) resulting from UF-concentration of the whey protein fraction was collected and stored at 5 °C after addition of 0.2 g L^{-1} sodium azide (NaN_3). All other reagents were of analytical grade.

2.2. Preparation of the heat-induced aggregates and native micellar casein suspension

Model whey protein aggregates (WPIA) were prepared as described by Vasbinder et al. (2004). Briefly, a 90 g kg^{-1} solution of WPI in deionized water with 0.2 g kg^{-1} NaN_3 was stirred for 2 h, adjusted at pH 7.5, filtered on 0.45 μm filter syringe and then left at 4 °C overnight for complete hydration. After equilibration at room temperature, the solution was dispensed in sealed 25-g glass tubes, heated at 68.5 °C for 2 h in a water-bath then rapidly cooled in ice water to room temperature. The heat-induced aggregates were then diluted to ~60 g kg^{-1} with milk ultrafiltration permeate (UFP) and were extensively dialyzed (6–8 kDa molecular weight cut-off, Medicell International Ltd., London, UK) against commercial ultra-high temperature (UHT) skimmed milk with 0.2 g kg^{-1} NaN_3, in order to replace their solvent phase with milk permeate. The protein content of the resulting WPIA suspension was determined spectrophotometrically using absorbance at 280 nm and an experimental extinction coefficient value of 1.122 L g^{-1} cm^{-1} measured as in Morand et al. (2011), then standardized to a mother suspension at 45 g kg^{-1} total protein using UFP. Morand et al. (2011) showed that the size, surface hydrophobicity, isoelectric pH and gelation properties of WPIA were in line with those of aggregates containing κ-casein and obtained at 80 °C as in heated milk, while only their free SH groups were more numerous.

A 45 g kg^{-1} suspension of NMC was produced by gradually dispersing the required amount of NMC powder in UFP at 40 °C with gentle stirring for 4 h. The suspension was stored overnight at 4 °C for complete hydration before further processing. The total protein concentration of this mother NMC was checked using the Kjeldahl method (conversion factor 6.38).

Mixtures of NMC and WPIA at ~45 g kg^{-1} total protein and different NMC/WPIA w/w ratios were prepared by mixing appropriate amounts of each mother suspension. The mixtures ranged from CAS0, containing no NMC, to CAS100, containing 100% of NMC (see Table 2 for the calculated composition of each system). All samples were replicated at least two times and analyzed within one week.

2.3. Characterization of particles

2.3.1. Volume fraction and voluminosity of the particles

Voluminosities of the casein micelles and of the heat-induced whey proteins aggregates were determined by diluting each mother suspension in UFP in order to achieve different concentrations. The viscosity of each dilution was measured at 20 °C using a LS30 thermostated viscometer (low shear S30 Contraves, Zurich, Switzerland) equipped with coaxial cylinders. Each dilution was measured in duplicate. The relative viscosity (η_r) was calculated with the following equation:

$$\eta_r = \eta/\eta_0 \quad (1)$$

where η is the viscosity of the suspension (Pa s) and η_0 is the viscosity of the solvent (Pa s). The volume fraction, ϕ_v (mL L^{-1}) of each dilution was calculated from the experimental viscosity data by solving Lee's equation as in Boulet, Britten, and Lamarche (1998) as follows:

$$\eta/\eta_0 = 1 + 2.5*\phi_v + 7.031*\phi_v^2 + 37.371*\phi_v^3 \quad (2)$$

The voluminosity, V_e (mL g^{-1}) was eventually calculated using:

$$V_e = \phi_v/c \quad (3)$$

where c is the protein concentration in g L^{-1}.

2.3.2. Size distribution

Particle size distribution of each type of particle was measured in the mother suspensions by dynamic light scattering (DLS) at a fixed angle of 173° on a Zetasizer nano ZS (λ = 633 nm, Malvern

Table 1
Average properties of the native casein micelles (NMC) and of the whey protein aggregates (WPIA).

	NMC particles	WPIA particles
Hydrodynamic diameter (nm)[a]	208	132
Volume (mL)[b]	1.3E-15	1.8E-16
Surface (nm^2)[b]	5.6E+04	1.5E+04
Voluminosity V_e (mL g^{-1})[c]	4.75	5.51
Number at ϕ_v = 1% (L^{-1})[d]	7.8E+15	5.6E+16

[a] Measured as the mean of the log normal size distribution $P(I)$.
[b] Calculated using the mean diameter of the log normal distribution $P(V)$ i.e. 134 nm for NMC and 70 nm for WPIA.
[c] Measured.
[d] Calculated as the division of ϕ_v by the mean volume of particle.