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Mixtures of sodium caseinate and whey protein aggregates: Viscosity and acid- or salt-induced gelation

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ABSTRACT

Mixtures of sodium caseinate (SC) and whey protein aggregates (WPA) were investigated at different compositions, protein concentrations and temperatures. Potentiometric titration suggests that no complexes between SC and WPA were formed, at least in the pH range 4–7. The viscosity increased when SC was substituted with large fractal WPA and decreased when substituted with whey protein microgels, and followed a logarithmic mixing rule. Gelation of mixtures of fractal WPA and SC was induced by adding $CaCl_2$ or acidification. Addition of SC did not have an effect on the elastic modulus and the microstructure properties of acid- or salt-induced gels formed by WPA, but the rate of gelation decreased with increasing SC concentration, even though the activation energy remained the same. This effect was attributed to binding of Ca^{2+} to SC and a reduction of the effective amount of Ca^{2+} available for binding to WPA.

1. Introduction

Cows' milk contains on average 2.9% protein that can be divided into whey proteins (~20%) and caseins (~80%) (O'Mahony & Fox, 2013). The two types of proteins are characterised by different morphology and functionality. Whey proteins are compact globular proteins (mainly β-lactoglobulin and α-lactalbumin) that are stable in aqueous solutions due to electrostatic repulsions. However, their heat-induced denaturation leads to irreversible aggregation, resulting in formation of stable solutions of whey protein aggregates (WPA). Aggregates with three different morphologies are formed depending on the pH: dense microgels in a pH range around the isoionic point (between pH 4.3 and 6.1); small strands at higher and lower pH; and long rigid fibrils close to pH 2 (Jung, Savin, Pouzot, Schmitt, & Mezzenga, 2008; Nicolai, Britten, & Schmitt, 2011). Strands and microgels can themselves further randomly associate into larger clusters with a self-similar structure characterised by a fractal dimension $d_f \approx 2$ (Mahmoudi, Mehalebi, Nicolai, Durand, & Riaublanc, 2007; Phan-Xuan et al., 2013). The size of the aggregates at steady state increases with increasing protein concentration and above a critical concentration (C_g) the system gels. The viscosity of WPA solutions increases with increasing concentration depending on the size and morphology of the aggregates (Inthavong, Kharlamova, Chassenieux, & Nicolai, 2016).

Solutions of WPA can be induced to gel by reducing the electrostatic repulsions between the aggregates either by adding salt or by reducing the pH (Alting, Hamer, de Kruif, & Visschers, 2000; Barbut & Foegeding, 1993; Bryant & McClements, 1998). This process is known as cold gelation, because, contrary to heat-induced gelation of native whey proteins, it can occur even at low temperatures. Recently, we have reported on a detailed systematic investigation of gelation of whey protein aggregates induced by addition of $CaCl_2$ (Kharlamova, Nicolai, & Chassenieux, 2018b) or by acidification (Kharlamova, Chassenieux, & Nicolai, 2018a). In both cases the gelation rate increased strongly with increasing temperature and was characterised by an activation energy that was higher for Ca^{2+}-induced gelation (E_a = 210 kJ mol^{-1}) than for acid-induced gelation (E_a = 155 kJ mol^{-1}). The gelation rate also depended strongly on the pH and the concentration of $CaCl_2$. However, the gel stiffness and microstructure were found to depend only on the protein concentration.

Whey protein aggregates (WPA) can be used for gelation and modification of the viscosity in dairy products, where they are usually found in presence of other milk proteins. It is therefore important to investigate the texturising properties of WPA in mixtures with caseins. The objective of the present investigation was to study mixtures of WPA and sodium caseinate (SC), which is a

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common ingredient in dairy products. Elsewhere, we will report on the mixtures of WPA and casein micelles.

SC is obtained by decreasing the pH of casein micelle solutions to the iso-electric point (around pH 4.6), which leads to dissolution of the colloidal calcium phosphate. Washing and increasing the pH by addition of NaOH produces a mixture of casein proteins that organise into small aggregates with a radius of about 11 nm (HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008). The viscosity of SC solutions increases with increasing concentration but is not sensitive to addition of NaCl up to at least 0.25 M or acidification down to pH 5.4 (Thomar, Durand, Benyahia, & Nicolai, 2012). However, SC solutions gel at room temperature when the pH is decreased below 5.1 (Lucey, Van Vliet, Grolle, Geurts, & Walstra, 1997). SC solutions are quite insensitive to heating except at pH $\leq$ 6 at high concentrations (C > 100 g L^{-1}; Thomar & Nicolai, 2016). Addition of $CaCl_2$ does not induce gelation, but adding relatively large amounts of $CaCl_2$ to SC solutions leads to formation of large dense protein domains (Thomar, Nicolai, Benyahia, & Durand, 2013).

The effect of SC on heat-induced aggregation and gelation of native whey proteins has been reported in the literature. It has been demonstrated that at low concentrations SC or individual α- and β-caseins can inhibit heat-induced aggregation and gelation of native whey proteins (Guyomarc'h, Nono, Nicolai, & Durand, 2009; Kehoe & Foegeding, 2011; Morgan, Treweek, Lindner, Price, & Carver, 2005), whereas at high concentrations SC actually favours thermal gelation of whey proteins (Nguyen, Nicolai, Chassenieux, Schmitt, & Bovetto, 2016b; Nguyen et al., 2016a; Picone, Takeuchi, & Cunha, 2011). However, to our knowledge no investigation has been reported so far of the viscosity of stable mixtures of WPA and SC, nor of the salt- or acid-induced gelation of such mixtures.

Here we report on an investigation of the properties of mixtures of WPA and SC. We will first show with potentiometric titration that SC and WPA do not form complexes. Then we discuss how the viscosity of mixtures of WPA and SC depends on the protein concentration and the compositions at conditions where they are stable (pH 6.0–7.0, low ionic strength). Finally, we will look at salt- and acid-induced gelation of the mixtures as a function of the temperature after adding different amounts of $CaCl_2$ or HCl. The results will be compared with previous investigations of pure WPA suspensions to assess the effect of the presence of SC.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of whey protein aggregates

WPA were prepared with WPI powder purchased from Lactalis (Laval, France) containing (w/w) 89% protein, 6% moisture, <0.4% fat, <4% lactose, and 2.0% ash, including 0.25% calcium, according to the manufacturer's specification. The proteins consisted of 70% β-lactoglobulin, 20% α-lactalbumin and 10% other whey proteins and caseins, as determined by size exclusion chromatography (SEC). SEC experiments were carried out at room temperature in water (0.1 M $NaNO_3$ + 50 ppm NaN_3 as a bacteriostatic agent) using a Thermo Sep pump (Thermofisher Scientific, Villebon sur Yvette, France) operating at a flow rate of 1 mL min^{-1} in combination with an automatic injector Gilson 234 (Villiers Le Bel, France) (injection volume = 0.3 mL, polymer concentration ~ 0.2 g L^{-1}). The detection proceeded through a dual flow refractive index detector (RI171 from Shodex, Perkin Elmer Villebon sur Yvette, France) and a UV detector (UV2000 from Spectra Physics) operating at 278 nm. Separation was achieved using a column set constituted of one G2000SWXL TSK column (17 μm, 7.8*300 mm) and of a guard column (Tosoh Europe, Tenssenderlo, Belgium).

The powder was mixed with Milli-Q water and stirred for 4–6 h, then filtered two times through 0.45 μm and 0.2 μm syringe filters (Acrodisc®, Pall Life Sciences, Fajardo, Puerto Rico). The protein concentration (C) was determined by UV absorption at 278 nm using extinction coefficient $\varepsilon = 1.05$ L g^{-1} cm^{-1}. The measurements were conducted on samples diluted to protein concentrations C < 1 g L^{-1} with a spectrophotometer (Varian Cary-50 Bio, Les Ulis, France) at 20 °C in quartz cuvettes with the path length of 1 cm.

The pH of stock solutions (C ≈ 13.5%, w/w) was set from pH ≈ 6.2 to 7 by addition of aliquots of a standard 1 M NaOH (Fisher Scientific, UK) while stirring. At pH 7 the net charge density of the whey proteins in the stock solution was $\alpha = -8.7$, as was reported elsewhere (Kharlamova, Inthavong, Nicolai, & Chassenieux, 2016). Fractal WPA with different sizes were prepared by heating WPI solutions at different concentrations at 80 °C for 24 h. Aggregates with hydrodynamic radius $R_h = 35$ nm were formed at C = 62 g L^{-1}, whereas aggregates with $R_h = 125$ nm or $R_h = 155$ nm were formed at C = 91 g L^{-1}. Close to C_g the size of the aggregates is very sensitive to the concentration and the pH, which explains why different sizes were obtained after heating different batches of WPI at C = 91 g L^{-1}. The size of the aggregates was determined using dynamic light scattering (DLS) as described in Mahmoudi et al. (2007). In brief, the measurements were conducted with an ALV-CGS3 multibit, multitau, full digital correlator combined with a laser emitting vertically polarised light at λ = 632 nm (ALV-Langen), the temperature of 20 °C was controlled within ±0.1 °C by a thermostat bath. DLS measurements were done as a function of the scattering wave vector (q). The correlation functions of the scattered light intensity were analysed in terms of a relaxation time distribution. The z-average diffusion coefficient (D) was calculated from the average relaxation rate and R_h was calculated from D extrapolated to q = 0 using the Stokes–Einstein relation:

$$D = kT/(6\pi\eta R_h) \quad (1)$$

where k is the Boltzmann constant, T is the absolute temperature and η is the viscosity of water.

Microgels with R_h = 125 nm were prepared by heating a WPI solution at protein concentration C = 41 g L^{-1} at pH 6.0 ($\alpha = -5.25$) at 80 °C for 24 h. The pH increased to 6.28 during heating. The fraction of protein that was transformed into microgels was determined by centrifugation as described elsewhere (Phan-Xuan et al., 2011) and was found to be 88%. The microgels were concentrated using ultrafiltration (KrosFlo Research II/i/Tangential Flow Filtration System, Spectrum Laboratories, Inc., Rancho Dominguez, USA) and evaporation under vacuum to a final concentration of 96 g L^{-1}. The pH of concentrated microgels was increased from 6.1 to 7.0 by addition of a standard 0.1 M NaOH solution (Fisher Scientific, UK), which resulted in the increase of the negative value of α to −7.5. The concentration of microgels after ultrafiltration was determined by the turbidity measurements with a UV–visible spectrometer (Varian) at 650 nm using quartz cuvettes with the path length of 2 mm. The calibration curve was prepared by measuring a series of dilutions of initial (non-concentrated) microgel solutions with known protein concentration in the range between 0.02 and 2 g L^{-1}, which gave a linear dependency on concentration.

2.1.2. Preparation of sodium caseinate suspensions

Sodium caseinate powder (380 "Spray Dried", batch 8 O 380 SD) was purchased from Lactalis Ingredients (France). The powder contained (w/w) 92% protein, 6% moisture, <1.0% fat, <0.5% lactose, <4.5% minerals, of which 1.2% sodium and ≤0.1% calcium, according to the manufacturer's specification. SC suspensions were prepared in Milli-Q water under stirring while keeping them in a water bath