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Interactions of milk proteins with low and high acyl gellan: Effect on microstructure and textural properties of acidified milk

Patrizia Buldo [a, *], Connie Benfeldt [b], Juan Pablo Carey [a], Ditte Marie Folkenberg [c], Hanne Bak Jensen [b], Sander Sieuwerts [d], Kalliopi Vlachvei [a], Richard Ipsen [a]

[a] University of Copenhagen, Rolighedsvej 30, 1958, Frederiksberg C, Denmark
[b] Arla Foods Ingredients, Sønderhøj 10-12, 8260, Viby J, Denmark
[c] Chr. Hansen A/S, Bøge Allé 10-12, 2970, Hørsholm, Denmark
[d] Arla Foods, Sønderhøj 14, 8260, Viby J, Denmark

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ABSTRACT

The effect of addition of low and high acyl gellan (LAG/HAG) at three different concentrations on the microstructure and texture of acidified milk systems with different casein to whey protein ratios was investigated. The systems with added LAG exhibited a continuous gel network whereas micro-phase separation occurred when HAG was added. The continuous gel network was indicative of complexation between the polysaccharides and casein, which in turn led to enhanced textural properties and reduced syneresis. Those properties were reduced by decreasing the casein to whey protein ratio as fewer complexes were formed. HAG appeared to be incompatible with casein micelles, which resulted in a micro-phase separated system. This caused decreased textural properties but a higher physical stability due to HAG present in the serum phase available to bind water. By decreasing the casein to whey protein ratio the textural properties were improved at expense of the physical stability, indicating a preferential binding between HAG and whey proteins.

1. Introduction

Gellan is a gelling linear anionic exopolysaccharide produced by *Sphingomonas elodea* (ATCC 31461), often referred as gellan gum or Kelcogel (CPKelco, USA). It is a tetrasaccharide polymer containing two residues of β-D-glucose, α-L-rhamnose, and β-D-glucoronic acid, with two side groups, acetyl and L-glyceryl. L-glyceryl is present on O(2) of the 3-linked glucose, and acetyl on O(6) of the same residue but only on half of the repeating units (Jansson, Lindberg, & Sandford, 1983; Kuo, Mort, & Dell, 1986; O'Neill, Selvendran, & Morris, 1983). By removal of the side groups, a deacylated gellan is formed, also known as low-acyl gellan (LAG), to distinguish it from the native form, high-acyl gellan (HAG). The gelation process is based on cold gelation (set temperature is between 30 and 45 °C) and presence of mono- and divalent ions is required (Valli & Clark, 2010). It is generally assumed that gelation occurs by transition from a disordered random coil to an ordered 3-fold left-handed double helix conformation, followed by association of the double helices (Chandrasekaran & Radha, 1995; Morris, Nishinari, & Rinaudo, 2012). Gelation temperature is mainly influenced by presence of different cations and by the concentration of the polymer (Miyoshi, Takaya, & Nishinari, 1996; Sworn, Sanderson, & Gibson, 1995). By lowering the pH, or by adding salts, junction zones between the helices are formed via hydrogen bonds. Gellan is a multi-functional polysaccharide and, additional to being a gelling agent, it is also used as a texturizing, stabilizing, film-forming and flavor releasing agent. In the food industry, gellan is used for water-based gels as well as in confectionary, dairy products, bakery fillings, low fat spreads, sauces and beverages (Valli & Clark, 2010). Generally, a very low amount (<1%) of gellan is required to achieve the desired gel properties. In fermented dairy products, to avoid a grainy texture, the amount of added LAG is recommended to not exceed 0.06%, whereas HAG should be limited to 0.1% (Valli & Clark, 2010). The textural properties of the gel formed by HAG and LAG are very different, with HAG forming a soft and elastic gel, contrary to LAG forming a firm, brittle and heat stable gel (Valli & Clark, 2010). The differences in gel textural properties are a consequence of the presence of the acyl groups, which interfere with the aggregation of

* Corresponding author. Present address: Chr. Hansen A/S, Bøge Allé 10-12, 2970, Hørsholm, Denmark. Tel.: +45 53393170 (mobile).
E-mail address: dkpabu@chr-hansen.com (P. Buldo).

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double helices, thus resulting in a soft, elastic and thermo-reversible gel (Valli & Clark, 2010). Proteins can interact with both coil and helix polysaccharide. At low pH, proteins interact with the junction zones in the helices.

Based on the charge of the biopolymers in the system, thermodynamic in-/compatibility, often referred to as non-absorbing or absorbing polysaccharides, can occur. Similar charge of proteins and polysaccharides will lead to electrostatic repulsions, hence to phase separation in the system, driven by volume exclusion/depletion effects. Contrarily, if the biopolymers in the system carry opposite charges, electrostatic interactions can occur. Based on the affinity, charge density and conformation of the biopolymers, different types of aggregates, coacervates, complexes, and gel can be formed (Turgeon & Laneuville, 2009, chap. 11). Both coacervates and complexes initially form soluble aggregates; and at environmental pH approaching the pI of the protein, some combinations of biopolymers form complexes and other coacervates. Coacervates are liquid spherical vesicles; whereas complexes are fractal aggregates. Further aggregation of complexes can result in formation of interpolymeric complexes or co-precipitates, which are insoluble. Complexes as well as interpolymeric complexes can contribute to texture and stability, and gellan is known to form complexes due to its stiff structure (Turgeon & Laneuville, 2009, chap. 11). Very few studies have been performed on addition of gellan in a fermented milk system, and these mainly focus on LAG which has been shown to induce a weak gel with increased stability (Kiani, Mousavi, Djomeh, & Yarmand, 2008; Kiani, Mousavi, Razavi, & Morris, 2010). However, the protein composition of the system, such as whey protein concentrate (WPC) or caseinate, affects the texture and the microstructure of the acidified gel and LAG shows incompatibility for WPC, contrary to the caseinate which forms complexes with LAG (Picone & da Cunha, 2010). The present study, for the first time to our knowledge, investigates the effect that both LAG and HAG exert on the microstructure, textural properties and physical stability of acidified milk systems, as a function of the ratio of casein to whey proteins.

2. Materials and methods

2.1. Determination of the overlap concentration of gellan in milk

The overlap concentration corresponds to the critical concentration of polysaccharide at which its mobility is limited by the formation of an entangled network (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). To identify the overlap concentration of gellan in milk, two gellan types in their pure form, LAG (Mw: $2-3 \times 10^5$ Da) and HAG (Mw: $1-2 \times 10^6$ Da) (CPKelco, San Diego, USA), were used in different concentrations. Medium heat skim milk powder (SMP, Arla Foods Ingredients, Viby, Denmark) containing 36% protein (Nx6.8), 52% lactose, maximum 1.25% fat, 8% minerals (ash), and 4% moisture, was reconstituted in demineralize water. HAG was added to reconstituted SMP (3.5% total protein) at 0.02, 0.03, 0.05, 0.08, 0.10% (w/w). LAG was added to reconstituted SMP (3.5% total protein) at 0.01, 0.02, 0.03, 0.04, 0.05, 0.06% (w/w). The obtained solutions were stirred with a magnetic stirrer (RCT basic, IKA Werkr, Staufen, Germany) for 2 min at ~1000 rpm, and then a pasteurization step (90 °C for 20 min) in a water bath followed. After pasteurization, the samples were cooled to 25 °C in an ice bath. The overlap concentration in milk for both gellan types was identified by a flow test using a rheometer (AR G2, TA Instruments, New Castle, USA) equipped with a conical concentric steel cylinder (15 mm stator inner radius; 14 mm rotor outer radius; 42 mm cylinder height; 7000 μm gap). After loading the sample, an equilibration step of 2 min at 25 °C was performed, after that a shear rate sweep from 1E-3 to 10 1/s over 5 min was applied. The zero-shear viscosity, or Newtonian viscosity, was determined as the average value of the first five measurements. The log of the gellan concentration versus the log of the zero shear viscosity was plotted, and the change in slope of the curve was considered to coincide with the overlap concentration for gellan (data not shown) (Morris et al., 1981). Three separate batches were made, and for each batch duplicate measurements were taken.

2.2. Acidified milk

Ten low fat stirred yoghurt systems were manufactured at pilot plant scale. The protein content was kept constant at 3.5%. Five systems contained only SMP and hence had unchanged casein to whey protein ratio, ~80:20. The rest of the systems had decreased casein to whey protein ratio, as 1% protein from SMP was substituted by WPC (Arla Foods Ingredients, Viby, Denmark). The WPC ingredient used contained 80–84% protein (Nx6.38), maximum 9% of lactose, maximum 10% fat, maximum 3.5% ash, and maximum 6% moisture. The milk bases (3L) were prepared by adding SMP, and WPC were needed, to demineralize water and mixing (Janke & Kunkel, IKA, Labortechnik, Staufen, Germany) the solution at 600 rpm for 3 min (4 min when WPC was added) at room temperature, followed by overnight hydration at 5 °C. Before pasteurization (90 °C for 20 min) in a water bath, two concentrations of gellan (at and above the overlap concentration), 0.02% and 0.04% for LAG, and 0.03% and 0.05% for HAG were added to the milk bases. The milk bases were cooled to 43 °C in an ice bath and 1.5% (w/w) of GDL (D-(+)-gluconic acid δ-lactone 99.0%, Alfa Aesar, Karlsruhe, Germany) was used for the chemical acidification. The acidification step was considered terminated when the pH of the coagulum reached 4.5. The coagulum was manually broken with a stainless steel perforated disk and subsequently a post treatment unit (FH Scandinox A/S, Tarm, Denmark) was used for cooling the yoghurt to ~15 °C, and to smoothen the texture of the coagulum by applying a back pressure of 2 bars. The yoghurts were packed in 250 ml plastic cups, and stored at 5 °C for at least four days before performing the analytical measurements. For each system, yoghurt production was conducted at least in triplicate.

2.3. Syneresis and water holding capacity (WHC)

Syneresis was measured as the spontaneous whey formed on the surface of the yoghurt after 5 days of storage. The separated whey was collected from the yoghurt cup (which was placed at 45° for 1 min), and it was measured as % (w/w) (Eq. (1)).

$$\text{Syneresis}\ (\%) = (\text{g of expelled whey})/(\text{g of yoghurt})*100 \quad (1)$$

WHC measurements were performed by centrifuging 20 *g* of yoghurt at 3913× *g* at 5 °C for 10 min. The separated serum was weighed, and the amount of serum retained in the network was calculated as % (w/w) (Eq. (2)).

$$\text{WHC}\ (\%) = (\text{g of yoghurt} - \text{g of expelled whey})/(\text{g of yoghurt})*100 \quad (2)$$

2.4. Particle size distribution

Particle size distribution measurements were performed on the yoghurt with a Mastersizer 2000 (Malvern Mastersizer Micro Particle Sizer, Malvern Instruments Ltd., Worcestershire, UK). The refractive index of the real part was set to 1.53 and the one of the dispersant phase, water, was set to 1.33, the particles absorption