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Effect of carrageenan on the formation of rennet-induced casein micelle gels

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ABSTRACT

Casein micelles (CMs) and carrageenan (CG) mixtures were prepared in simulated milk ultrafiltrate. Rennet-induced gelation in the CM/CG mixtures was studied by accessing the kinetics of released caseinomacropeptide (CMP) and the rheological properties of CM/CG mixtures. Particle size distributions, ζ-potential and ionic strength measurements, and microstructure observations were performed to understand the underlying mechanisms. At low CG concentrations, κ-CG, which had a tail-adsorption to the CM surface, affected the second phase of renneting through repulsive interactions. In mixtures containing ι-CG and λ-CG, both the first and second phase of renneting were affected. Flat-adsorption and ring-adsorption of ι-CG and λ-CG to the CM surface, respectively, increased the negative charges and covered more enzymatic action sites, thereby decreasing the maximum concentration of released CMP, increasing coagulation time, and weakening gel strength. At high CG concentrations, free CG molecules contributed to rennet-induced gelation, especially in the presence of κ-CG and ι-CG, which significantly accelerated the second phase of renneting through the self-association of CG double helices. Our findings suggested that ι-CG and λ-CG lead to soft cheese curds and improved low-fat cheese quality.

1. Introduction

As a result of an increasing consumer demand for low-fat products, the manufacture of low-fat and reduced-fat cheeses has significantly increased in the past two decades (Aryana & Haque, 2001; Mistry, 2001; Olson & Johnson, 1990). Fat removal from cheese, however, results in several undesirable texture and flavor characteristics, such as excessive firmness, rubberiness, and bitterness (Drake & Swanson, 1995; Fife, McMahon, & Oberg, 1996; Sipahioglu, Alvarez, & Solano-Lopez, 1999). Therefore, several methods have been studied to improve cheese quality. One of those methods consists of replacing milk fat with carbohydrate-based substances, which improve cheese texture and mouthfeel (Walter & Cox, 1992).

Carrageenan (CG) is the most suitable structure-forming carbohydrate-based substance in the dairy industry (Ermak & Khotimchenko, 1997; Hansen, 1994; Piculell, 1995). Kailasapathy (1998) reported that CG increased the yield of Caerphilly cheese. Totosaus and Guemes-Vera (2008), who worked with low-fat Oaxaca cheese, reported that κ-CG increased its meltability and λ-CG increased its moisture content and yield. CG is a high molecular weight, sulfated, linear polysaccharide extracted from red seaweed (Millane, Chandrasekaran, & Arnott, 1998). There are three main types of CG based on the number and position of sulfate groups on the galactose/anhydrogalactose chain: κ-CG, ι-CG and λ-CG, which contain one, two, and three sulfate groups per disaccharide repeating unit, respectively (Imeson, 2000). During heat treatments and under specific ionic environment, κ-CG and ι-CG undergo a transition from a coil (disordered) to a helical (ordered) conformation (Spagnuolo, Dalgleish, Goff, & Morris, 2005). On the other hand, λ-CG always maintains a coil conformation (Chronakis, Doublier, & Piculell, 2000). Even though several studies have focused on the CG applications in the food industry, few studies have assessed the effect of CG on cheese quality.

Rennet-induced gelation, which is an essential step during cheese manufacture, leads to structural changes that affect cheese quality (Bönisch, Heidebach, & Kulozik, 2008; Everett & Olson, 2000; Sandra & Dalgleish, 2007). Rennet-induced gelation, which is an enzymatic coagulation, takes place in two phases. In the

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primary phase, κ-casein is hydrolyzed by rennet at the Phe105-Met106 bond with the release of caseinomacropeptide (CMP), resulting in reductions in both micellar charge and steric stabilization (de Kruif, 1999; Walstra, 1990). In the secondary phase, casein micelles (CMs) destabilize and aggregate as a result of κ-casein hydrolysis (Dalgleish, 1980; Fox, Guinee, Cogan, & McSweeney, 2000). Ng-Kwai-Hang, Politis, Cue, and Marziali (1989) reported that faster coagulation rate and firmer curd result in lower cheese yield. Therefore, the assessment of gel formation allows an understanding of the mechanism involved in cheese curd formation.

In milk, processing conditions and ingredients affect the interfacial composition and lead to an increase in inter-particle interactions, which affect rennet-induced gelation (Sandra & Dalgleish, 2007; Titapiccolo, Corredig, & Alexander, 2010). High fat levels contributed to increased gel elasticity (Gaygadzhiev, Corredig, & Alexander, 2009), reduced gel time, and increased curd firming rate (Guinee et al., 1997). When milk is subjected to high temperatures or high pressures, complexes are formed between κ-casein and denatured whey protein, thereby decreasing the overall rate of coagulation (Needs, Stenning, Gill, Ferragut, & Rich, 2000; Pires, Gatti, Orellana, & Morales, 2004). Rennet-induced gels from recombined high total milk solids subjected to heat treatment reduce gelation time and weaken gel strength, a phenomenon that is attributed to whey protein denaturation (Pomprasirt, Singh, & Lucey, 1998). Previous studies have reported that CG interacts with CM through electrostatic interactions; conformation, charge density, and CG concentration are the main factors that affect those interactions (Langendorff et al., 2000; Martin, Goff, Smith, & Dalgleish, 2006; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Spagnuolo et al., 2005). Even though several studies have focused on CM/CG systems (Arltoft, Ipsen, Madsen, & de Vries, 2007; Ji, Corredig, & Goff, 2008; Schorsch, Jones, & Norton, 2000), there is conflicting evidence. Vega, Dalgleish, and Goff (2005) reported that the casein micellar structure plays an important role in the interaction between milk proteins with polysaccharides. However, the effects of CG on CM/CG systems and on rennet-induced gelation in native CM have not been assessed.

In this study, we used native CM, which was obtained by ultracentrifugation, and CG (κ-CG, ι-CG, and λ-CG). The objectives of this study were to assess the effects of CG on rennet-induced gelation and understand the underlying mechanisms. CMP released kinetics and rheology experiments were performed to assess different aspects of gel formation. Particle size distributions, ζ-potential and ionic strength measurements, and microstructure observations supplied more detailed information for understanding the CM/CG systems.

2. Materials and methods

2.1. Materials

Fresh milk was obtained from a local farm (Sino-US Research & Development Center, China Agricultural University, Beijing, China) and centrifuged at 4500 rpm for 20 min to remove fat. The resulting skim milk was ultracentrifuged at 25,000 × *g* and 20 °C for 1 h (Optima L-XP, Beckman, USA) using a Type 45 Ti rotor to separate CM. The resulting supernatants were discarded; the micellar pellets were collected. Three different types of CG (κ-CG, ι-CG, and λ-CG) were purchased from Sigma Chemical Co. (St Louis, Mo, USA) and used without further purification. Rennet Stamix 1150 was supplied by Chr. Hansen (Beijing, China). All other chemicals were of analytical grade. Deionized water (>17 MΩ) was used throughout the experiments. A salt solution that simulated ultrafiltrate milk (SMUF, $I = 0.08$ M and pH = 6.8) was prepared (Jenness & Koops, 1962). Sodium azide (0.02%, w/v) was added to prevent microbial growth.

2.2. Mixture preparation

CM and CG concentrations in the samples were based on their powder contents. CM was dispersed in SMUF to obtain the original CM concentration (26 mg/ml); the concentrations of CG are shown in Table 1. The control sample consisted of CM dispersion in SMUF without CG. The CM dispersion in SMUF was achieved by magnetically stirring at 37 °C for 2 h and allowing the solution to stand at 4 °C overnight for complete hydration. The following day, the CM dispersion was allowed to warm at room temperature. After the addition of different amounts of CG, the CM/CG samples were mixed at 20 °C for 30 min; pH was adjusted to 6.7 with 0.1 M KOH. Electrostatic interactions were assessed by adjusting the amount of NaCl from 0 to 50 mM; pH was measured to ensure the appropriate pH value. The control and CM/CG samples were hermetically sealed, placed in a water bath, heated at 60 °C for 10 min, cooled in ice water, and stored at room temperature.

2.3. Determination of CMP content during renneting

The extent of the enzymatic reaction was determined on the amount of CMP released. Following the addition of rennet, the samples were stirred for 30 s and separated into 4-ml aliquots. After 1, 5, 10, 15, 20, 40, 60, or 120 min, the enzymatic reaction was stopped by adding 365 μl of 4% (w/v) trichloroacetic acid. Samples were mixed, stored overnight at 4 °C, and filtered. The resulting supernatant was transferred to HPLC vials. CMP content was determined using RP-HPLC (LC-20AT, Shimadzu, Japan) according to the method reported by Thomä, Krause, and Kulozik (2006). The kinetics of CMP released during renneting was presented as the percentage of the CMP amount released in the CM/CG samples relative to the control. All measurements were performed in triplicate.

2.4. Rheological measurements

Rheological measurements were performed using an AR2000 controlled-stress rheometer (TA Instruments Inc., New Castle, Del., USA) equipped with an aluminum probe (60 mm diameter) with a gap of 1000 μm. Rennet-induced gelation was monitored at 32 °C. Following rennet addition, the mixtures were stirred for 30 s and immediately measured in the rheometer. The exposed edges of the samples were coated with vegetable oil to avoid potential losses of water. Samples were oscillated at 1 Hz; measurements were performed every 60 s for 1 h; the strain applied was 0.50%, which was within the linear region. Coagulation time was defined as the time point when gels had a storage modulus (G') of ≥1 Pa (Srinivasan & Lucey, 2002). All experiments were repeated three times.

Table 1
The CM/CG samples with different types and concentrations of carrageenans.

Sample number	CG		
	κ-CG	ι-CG	λ-CG
CG-1	0.01%	0.01%	0.01%
CG-2	0.02%	0.025%	0.025%
CG-3	0.03%	0.05%	0.050%
CG-4	0.04%	0.075%	0.075%
CG-5	0.05%	0.10%	0.10%

CM: casein micelles; CG: carrageenan.