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Investigating rennet coagulation properties of recombined highly concentrated micellar casein concentrate and cream for use in cheese making

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ABSTRACT

Highly concentrated micellar casein concentrate (HC-MCC) contains ~18% casein with ~70% of whey proteins removed by microfiltration and diafiltration of skim milk, followed by vacuum evaporation for further concentration. When blended with cream, HC-MCC forms recombined concentrated milk (RCM), which could be used as a starting material in cheese making. Our objective was to investigate the rennet coagulation properties of RCM while varying parameters such as casein level, pH, rennet level, and coagulation temperature. The HC-MCC was mixed with cream using low shear at 50°C for 10 min, followed by cooling to 31, 28, or 25°C and adding rennet, and rheological properties were determined. Rennet coagulation time [RCT, the time at which storage modulus (G′) = loss modulus (G″)] decreased from 8.7 to 7.4 min as casein level increased from 3.2 to 5.7%, without a significant additional difference in RCT at casein levels >5.7%. The initial G″ (G''_0) increased about 10-fold when casein levels were increased from 3.2 to 10.9%, whereas no change in initial G′ (G'_0) was observed. When G′ was measured relative to RCT (i.e., 1, 1.5, or 2 times RCT after RCT was reached, and expressed as G'_1, $G'_{1.5}$, and G'_2), log relationship was found between relative G′ and casein level ($R^2 > 0.94$). Lowering coagulation temperature from 31 to 25°C increased G''_0 by 6 fold and extended RCT from 7.4 to 9.5 min. After coagulation, relative G′ was initially higher at the lower temperature with G'_1 of 3.6 Pa at 25°C and 2.0 Pa at 31°C, but delayed in further development with G'_2 of 0.8 kPa at 25°C and 1.1 kPa at 31°C. Lowering pH of RCM from 6.6 to 6.2 resulted in reduced RCT from 11.9 to 6.5 min with increased relative G′ after coagulation. When less rennet was used, RCT increased in a linear inverse relationship without changes in relative G′ or G″. The microstructure of RCM coagulum (~11% casein), observed using transmission electron microscopy, confirmed that RCM curd had a rigid protein matrix containing extensively cross-linked protein strands.
Key words: microfiltration, micellar casein, microstructure, coagulation

INTRODUCTION

Cheese manufacturing using concentrated milk is typically performed in industry with milk that has been processed by UF to increase cheese yield and milk processing capacity (Ernstrom et al., 1980; Kosikowski et al., 1985; Govindasamy-Lucey et al., 2004). However, concentrating milk using microfiltration (**MF**) is potentially more suitable for cheese making compared with UF milk as it allows removal of whey proteins before cheese making. These could be used to manufacture milk-derived whey protein concentrates (Evans et al., 2009, 2010) that have potentially higher value because of their stronger foaming ability, gel strength, solubility, and emulsifying ability than whey protein concentrates derived after cheese making (Burrington, 2013). The use of MF concentrated milk has been studied in the manufacture of Cheddar (St-Gelais et al., 1995; Neocleous et al., 2002a,b), Mozzarella (Garem et al., 2000; Brandsma and Rizvi, 2001), and cheese without a standard of identity (i.e., pizza cheese; Govindasamy-Lucey et al., 2007).

Through MF, diafiltration, and vacuum evaporation of skim milk, a highly concentrated micellar casein concentrate (**HC-MCC**; ~20% wt/wt protein) can been manufactured that when mixed with cream forms recombined concentrated milk (**RCM**; Lu et al., 2015). Such RCM is potentially suitable for use in cheese making because it has low whey protein levels (<2% wt/wt), which from a theoretical point of view should result in cheese texture and flavor that is more comparable to that obtained with whole milk. However, both HC-MCC and RCM undergo cold gelation if protein levels are too high, which greatly affect their use as food ingredients (Lu et al., 2015). This can be avoided

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during cheese making provided the casein content of RCM is no more than 12% (Lu et al., 2016).

A challenge of cheese making using concentrated milk such as RCM is that as milk concentration increases, rennet coagulation time shortens, and both curd firming rate and curd hardness increase (Sharma et al., 1993; Orme, 1998). Having a harder coagulum causes problems of cutting using conventional cheese making machinery. Cheese making using highly concentrated milk (~7 to 8×) requires specialized processing equipment, and thus it is not widely used in industry (Brandsma and Rizvi, 1999; Fox et al., 2000; Brandsma and Rizvi, 2001). Instead, cheese making using less concentrated milk (up to 1.8×) is widely applied due to the convenience of not requiring specialized equipment or large changes in cheese making procedures (Neocleous et al., 2002a,b; Govindasamy-Lucey et al., 2004), and the negative effect of higher levels of UF concentration on cheese texture and flavor development (Creamer et al., 1987; Lelievre et al., 1990; Bastian et al., 1991) does not occur. Our objective was to characterize the rennet coagulation properties of RCM made by mixing HC-MCC with cream under varying conditions, such as casein level, rennet level, coagulation temperature, and pH. Transmission electron microscopy was used to understand the microstructure of RCM before and after rennet addition.

MATERIALS AND METHODS

HC-MCC and RCM

The HC-MCC was manufactured at the Institute for Dairy Ingredient Processing at South Dakota State University (Brookings, SD) as described by Lu et al. (2015). In brief, pasteurized skim milk was concentrated to ~12.5% solids using MF with diafiltration and then further condensed through vacuum evaporation to form HC-MCC. Frozen HC-MCC was shipped to Utah State University (Logan) and stored frozen until needed. Compositions of 2 batches of HC-MCC are shown in Table 1.

One hundred gram aliquots of RCM were prepared by mixing thawed HC-MCC with cream (32% to 44% fat; Aggie Creamery, Utah State University, Logan, or Dean Foods, Dallas, TX) that had been warmed to 50°C and skim milk (Dean Foods), in proportions necessary to achieve the desired casein level and protein:fat ratio as described by Lu et al. (2016). The RCM was mixed at low shear using a magnetic stirrer (~800 rpm) at 50°C for 10 min. Typically, RCM had a pH of 6.4 to 6.6. After cooling to 31°C, adjustments to pH were made using 1 *N* HCl or NaOH (J. T. Baker, Phillipsburg, NJ), and the RCM was tempered to the required temperature before renneting. Protein content of RCM was tested in triplicate using a rapid protein analyzer (Sprint, CEM, Matthews, NC).

Rheological Properties. For each rheological test (performed in triplicate), a 100-g aliquot of RCM was prepared with protein:fat ratio of 0.8, and estimated casein levels of 3.2, 5.7, 8.4, or 10.9%; pH 6.2, 6.4, or 6.6; and tempered at 25, 28, or 31°C. Pasteurized whole milk was also used as a control for comparison. Then 20 μL of double-strength [nominal 650 international milk clotting units (**MCU**)/mL] chymosin rennet (Maxiren, DSM Food Specialties, Eagleville, PA) diluted in 1 mL of distilled water was added, yielding test aliquots containing 130 MCU/kg. In addition, for RCM containing ~11% casein, varying amounts of chymosin were added to also produce test mixtures containing 33, 44, and 65 MCU/kg.

Immediately, 7.5 mL of renneted RCM or milk control was poured into the coaxial cylinder of a magnetic bearing rheometer (model AR-G2, TA Instruments, New Castle, DE) at the same temperature. Samples were covered with a solvent trap to prevent evaporation and a time sweep was performed using a strain of 0.01 and frequency of 1 Hz. Based on the time from rennet addition, RCT was determined as the time when storage modulus (G′) equaled loss modulus (G″). Measurements of gel development were continued until twice the RCT.

Transmission Electron Microscopy. An aliquot of RCM was prepared with ~11% casein and casein:fat ratio of 0.8 and cooled to 31°C along with an aliquot of pasteurized whole milk (Aggie Creamery) with 3.1% protein and standardized to protein:fat ratio of 0.8 as a control. Then 5 g of RCM or milk was transferred to a 50-mL test tube and mixed with 0.2 mL of 50% (wt/wt) glutaraldehyde (Electron Microscopy Services, Hatfield, PA) by gently inverting the tube several times and holding for 5 min at 31°C to chemically fix the protein. This was followed by addition of 5.2 mL of

Table 1. Composition of highly concentrated micellar casein concentrate (HC-MCC) made using microfiltration and vacuum evaporation

Component	HC-MCC	
	Batch I	Batch II
TS (%)	30.14	27.04
Fat (%)	0.94	0.67
Total N (%)	23.02	18.63
Noncasein N (%)	2.30	1.62
NPN (%)	0.32	0.21
Casein N/total N	0.90	0.91
Ash (%)	2.33	1.93
Calcium (%)	0.72	0.54