J. Dairy Sci. 98:1–14
http://dx.doi.org/10.3168/jds.2015-9482

Solubilization of rehydrated frozen highly concentrated micellar casein for use in liquid food applications

Y. Lu,* D. J. McMahon,*[1] L. E. Metzger,† A. Kommineni,† and A. H. Vollmer*
*Western Dairy Center, Utah State University, Logan 84322
†Midwest Dairy Foods Research Center, South Dakota State University, Brookings 57007

ABSTRACT

Highly concentrated micellar casein concentrate (HC-MCC), a potential ingredient of protein-fortified food, is a gel at cold temperature. It contains ~17 to 21% casein, with most serum proteins and lactose removed by microfiltration and diafiltration, and it is then further concentrated using vacuum evaporation. The HC-MCC can be stored frozen, and our objective was to determine the conditions needed to obtain complete solubility of thawed HC-MCC in water and to understand its gelation upon cooling. Dispersibility (ability to pass through a 250-μm mesh sieve), suspendability (percentage of protein not sedimented at 80 × *g* within 5 min), and solubility (percentage of protein not sedimented at 20,000 × *g* within 5 min) were measured at 4, 12, or 20°C after various mixing conditions. Gelation upon cooling from 50 to 5°C was monitored based on storage (G′) and loss (G″) moduli. The gelled HC-MCC was also examined by transmission electron microscopy. Thawed HC-MCC was added to water to reach a protein concentration of 3% and mixed using high shear (7,500 rpm) for 1 min or low shear (800 rpm) for 30 min at 4, 12, 20, or 50°C and at pH 6.4 to 7.2. The HC-MCC completely dispersed at 50°C, or at ≤20°C followed by overnight storage at 4°C. Suspendability at 50°C was ~90% whereas mixing at ≤20°C followed by overnight storage resulted in only ~57% suspendability. Solubility followed a similar trend with ~83% at 50°C and only ~29% at ≤20°C. Mixing HC-MCC with 60 m*M* trisodium citrate increased dispersibility to 99% and suspendability and solubility to 81% at 20°C. Cold-gelling temperature, defined as the temperature at which G′ = G″ when cooling from 50 to 5°C, was positively correlated with protein level in HC-MCC. Gelation occurred at 38, 28, and 7°C with 23, 20, and 17% of protein, respectively. Gelation was reversible upon heating, although after a second cooling cycle the HC-MCC gel had lower G′. In micrographs of gelled HC-MCC, the casein micelles were observed to be within the normal size range but packed very closely together, with only ~20 to 50 nm of space between them. We proposed that cold-gelation of HC-MCC occurs when the kinetic energy of the casein micelles is sufficiently reduced to inhibit their mobility in relation to adjacent casein micelles. Understanding solubilization of rehydrated frozen HC-MCC and its rheological properties can help in designing process systems for using HC-MCC as a potential ingredient in liquid food.
Key words: solubility, micellar casein, microfiltration

INTRODUCTION

Casein is a food ingredient that is widely used in the dairy, bakery, meat, beverage, and nutraceutical industry based on its diverse functions in emulsifying, foaming, whipping, water-binding, and cheesemaking. Texture properties and nutritional value of casein further support its application as a food additive (Fox, 2001; Fox and Kelly, 2004; Séverin and Wenshui, 2005). Traditionally, casein or caseinate was manufactured in industry by either isoelectric precipitation or by chymosin coagulation (Fox, 2001). Through these processes, casein micelles have irreversibly changed their native colloidal structure into spherical or linear aggregates (Farrell et al., 1988; Oommen, 2004; McMahon and Oommen, 2013).

Since the 1990s, microfiltration (**MF**) of skim milk has been applied to produce micellar casein concentrate (**MCC**), with casein levels ranging from 7 to 20% and concomitant serum protein removal ranging from 46 to 79% based on the composition of MCC, or 60 to 95% based on the serum protein level in MF permeate (Pierre et al., 1992; Garem et al., 2000; Brandsma and Rizvi, 2001; Schuck et al., 2002; Fox and Kelly, 2004; Nelson and Barbano, 2005; Hurt et al., 2010; Marella et al., 2013). Typically, MCC manufactured using MF contains only 7 to 10% casein, which translates into a 3- to 4-fold concentration (St-Gelais et al., 1995; Jost et al., 1999; Nelson and Barbano, 2005; Beckman et

Received February 19, 2015.
Accepted May 2, 2015.
[1]Corresponding author: donald.mcmahon@usu.edu

al., 2010; Hurt et al., 2010; Amelia et al., 2013; Beckman and Barbano, 2013; Hurt et al., 2015). Higher concentrations of 7- to 8-fold are achievable when milk is acidified (Brandsma and Rizvi, 1999; Brandsma and Rizvi, 2001) or when concentrated further using ultrafiltration or vacuum evaporation (unpublished data, L. E. Metzger; Amelia and Barbano, 2013).

Such highly concentrated MCC (**HC-MCC**) has many prospective applications in food industry, as it offers several potential advantages over caseinate or milk protein concentrate (**MPC**) made using ultrafiltration. (1) Compared with caseinate, casein micelles in HC-MCC still exhibit their native structure (Saboyainsta and Maubois, 2000); (2) HC-MCC has lower levels of serum protein compared with MPC, which in turn may lead to improved heat stability or storage stability by reducing binding of denatured serum protein to casein; and (3) HC-MCC is still hydrated with water. Hence, it may show improved functionality compared with milk protein powders that can lose solubility because of heat exposure during drying as well as during storage (Baldwin and Truong, 2007; Mimouni et al., 2010; Sikand et al., 2011). (4) Additionally, HC-MCC can be stored under refrigeration or frozen (Schokker et al., 2011; Sauer et al., 2012). This is beneficial because bacterial growth is repressed during refrigerated storage at 4°C. For example, Amelia and Barbano (2013) reported that the bacterial count of refrigerated HC-MCC stayed below 20,000 cfu/mL for 16 wk.

It is very difficult to resolubilize MCC that has been spray-dried (Schuck et al., 1999, 2002). Solubility index has been measured by volume of sediment after dispersing a specified amount of powder and centrifugation under well-defined conditions. Thus, solubility is inversely correlated with solubility index. Schuck et al. (1999) reported a solubility index of 15 mL of MCC powder at 24°C, indicating an extremely low solubility compared with low-heat NDM powder with a solubility index of <0.5 mL. Having a nondried form of HC-MCC would be advantageous for use in liquid food systems where high solubility is needed. However, HC-MCC forms into a gel when it is cooled and it is then crucial to disrupt the gel structure to fully disperse the casein micelles.

To test the extent of disruption of the HC-MCC gel, it was necessary to adapt tests used for measuring solubility of milk powders. Many reports have been published on the solubility of dairy protein powders, such as caseinate, MPC, or MCC powder (Schuck et al., 2002; Gaiani et al., 2005, 2007; Fang et al., 2007, Schokker et al., 2011; Hussain et al., 2012; Richard et al., 2013; Chandrapala et al., 2014; Crowley et al., 2015). The International Dairy Federation standard dispersibility test involves pouring reconstituted milk powder through a sieve with a mesh size of 250 μm (Westergaard, 2004). This dispersibility test is used to determine if any of the HC-MCC gel remains in relatively large pieces when dispersed in water. Such macrogel pieces (≥250 μm) would be too big to remain dispersed and would rapidly sediment. Smaller microgel pieces (containing aggregates of casein micelles) could be dispersed but probably not visibly observable. However, these smaller microgel pieces would sediment at centrifugation speeds used by researchers who have studied dispersibility of milk powders, such as 700 × *g* for 10 min (Moughal et al., 2000; Havea, 2006), 750 × *g* for 15 min (Schokker et al., 2011), or 36 × *g* for 10 min followed by 168 × *g* for 10 min (Crowley et al., 2015). In preliminary studies, we observed some sedimentation from pasteurized skim milk when centrifuging conditions were greater than 80 × *g* for 10 min.

The final step in fully solubilizing a dried or a cold-gelled HC-MCC includes disruption of any remaining aggregates into individual casein micelles. This can be measured by centrifuging at a speed at which any particles larger than individual casein micelles would sediment, such as centrifuging at 20,000 × *g* for 5 min. Our objective was to determine the best way to disperse and solubilize cold-gelled HC-MCC for its use in liquid food applications as a function of shear speed and time combinations, mixing temperatures, pH, and extended time. We further investigated the effect of citrate addition on dispersibility, suspendability, and solubility of HC-MCC. To better understand the factors affecting solvation of HC-MCC, we also studied rheological properties and microstructure of HC-MCC gel.

MATERIALS AND METHODS

HC-MCC Manufacture

MF. Pasteurized skim milk (72°C for 20 s) was processed into MCC in a 4-vessel, continuous MF unit (Filtration Engineering Inc., Champlin, MN; Figure 1) at the Institute for Dairy Ingredient Processing, South Dakota State University, Brookings. The 4 vessels were 161 mm in diameter and 965 mm in length and were fitted with polyvinylidene fluoride membranes in spiral wound configuration. The 4 membranes used were FH6438-OS03S, FH6430-OS03S, FH6430-OS03S, and FH6430-OS03S (Parker Process Advanced Filtration Division, Oxnard, CA), respectively, for vessels 1, 2, 3, and 4. The total surface area of the 4 membranes was 57.4 m^2. Immediately before processing skim milk, membranes were subjected to a short clean and sanitization. The short clean consisted of a water rinse to neutral pH, followed by a 30-min 50°C alkaline wash [1.46% (vol/vol) Ultrasil 110 and 0.11% (vol/vol) Ultrasil 01, Ecolab Inc., St. Paul, MN]. Alkaline solution