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Partial renneting of pasteurised bovine milk: Casein micelle size, heat and storage stability

Hotnida Sinaga [a,b], Nidhi Bansal [a], Bhesh Bhandari [a,*]

[a] School of Agriculture and Food Sciences, The University of Queensland, QLD 4072, Australia
[b] Department of Food Science and Technology, University of North Sumatera, Medan, Indonesia

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ABSTRACT

The effects of partial renneting at low temperature on the casein micelle (CM) size and the storage stability of milk were investigated. Low chymosin concentrations (≤0.03 IMCU mL^{-1}) was applied to pasteurised skim milk at 4 °C and enzyme activity was terminated by thermal application at 60 °C/3 min and 85 °C/30 min, referred to as low heat (LHT) and high heat (HHT) treatment milk, respectively. The addition of rennet with concentrations of 0.01, 0.02 and 0.03 IMCU mL^{-1} for 15 min resulted in κ-casein hydrolysis of 10, 20 and 25%, respectively. Moreover, mean CM size of milk was reduced by up to 10 nm. For LHT milk, the renneted micelles appeared to be stable for up to 17 days, especially in response to the application of 0.01 IMCU mL^{-1} and at a storage temperature of 4 °C. Severe heating at 85 °C/30 min to inactivate the enzyme caused an increase in CM size.

1. Introduction

Many studies have been done and reported on the effects of the addition of rennet to skim milk, especially in cheese manufacturing. Conventionally, this enzyme is a stomach extract that contains mainly chymosin, with a small amount of pepsin (Mohanty, Mukhopadhyay, Grover, & Batish, 1999). Nowadays, the enzyme is manufactured from plants, fungi and bacteria, which have met the needs for an alternative milk clotting enzyme. Throughout this study, "rennet" and "chymosin" are used as interchangeable terms and refer to an enzyme with 100% pure chymosin.

The κ-casein in a casein micelle (CM) is mostly on the micelle surface, and composed of 169 amino acid residues. The first 105 residues contain N-terminal and are located inside the CM known as para-κ-casein. The remaining 64 residues (residues 106–169 which, located in the exterior part and protrude into the solution of the protein) are widely known as the hairy layer. This hairy layer is a macropeptide, also recognised as caseinomacropeptide (CMP) or glycomacropeptide (GMP), as this κ-casein part contains carbohydrates. Although the proportion of κ-casein in the micelles is only 10–15% (Ekstrand & Larsson-Raznikiewicz, 1978; Qi, 2007), κ-casein is responsible not only for CM size but also for micelle stability. This is due to the extension of the hairy layer into milk solution, preventing individual CM from approaching each other. It is well known that milk protein, especially κ-casein of CM, is the primary target of enzyme actions (Hidalgo, Pires, & Risso, 2010). Given that the κ-casein is the protected layer of the CM, the removal of κ-casein will affect the stability of the micelles. Therefore, κ-casein structure plays an important role in understanding the mechanisms of enzyme action. Two separate but overlapping phenomena are observed when rennet is introduced to milk as enzymatic (primary) and non-enzymatic (secondary) (Anema, Lee, & Klostermeyer, 2007; StGelais & Savoie, 1993). In the first phenomenon, rennet action cleaves the chymosin-sensitive residues. In bovine milk, the enzyme splits off residues between Phe 105 and Met 106, resulting in the separation of κ-casein into para-κ-casein and caseinomacropeptide (CMP). As a result, para-κ-casein remains attached to the micelles, whereas the CMP is released into the solution. Following the liberation of CMP, scattering light measurements have shown that CM size decreases in this stage (Horne & Davidson, 1993; Walstra, Bloomfield, Wei, & Jenness, 1981).

* Corresponding author.
E-mail address: b.bhandari@uq.edu.au (B. Bhandari).

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In the non-enzymatic phenomenon, the induction of gel formation occurs when the degree of κ-casein hydrolysis reaches 65–90% (Salvatore, Pirisi, & Corredig, 2011; Sandra, Alexander, & Dalgleish, 2007; Sandra, Ho, Alexander, & Corredig, 2012). When sufficient protective layer has been "destroyed", the milk loses its liquid infrastructure, because the unstable micelles tend to get closer to each other and form a gel network. Consequently, aggregation of CM occurs.

Partial renneting is defined as the disruption of the enzyme process by stopping the reaction for a defined period of time, with or without the application of low incubation temperature. Partial renneting can allow specific reactions to be followed during the course of experiments, or allow control of cleavage extension of the κ-casein hairy layer. In the past, partial renneting was used to compare acid milk gels with strictly acid or rennet gels, with or without heat treatment, prior to enzyme addition (Gastaldi et al., 2003; Li & Dalgleish, 2006; Renan, Guyomarc'h, Chatriot, Gamerre, & Famelart, 2007). Partial renneting was also performed to modify the texture of low fat ice cream (Chang, Marshall, & Heymann, 1995). Despite many reviews related to rennet action for cheese production, very little research has been done on the effects of partial renneting to modify CM size and on the stability of such partially enzyme treated milk during storage.

To stop enzyme action in treated milk, it is necessary to inactivate the enzyme at a pre-determined treatment time. Pepsatin has been used to inhibit rennet activity by other researchers (Heinrich & Kulozik, 2011; Li & Dalgleish, 2006; Tuinier, Rolin, & De Kruif, 2002b). However, this compound is not widely available and is expensive for actual application. Therefore, for industrial purposes heat treatment has been the best option to stop the rennet reaction. It is also important to consider that heat, when used to inactivate enzymes, can also cause denaturation of whey protein. Therefore, the use of low heat (to avoid denaturation of whey protein) and high heat (which will denature the whey protein) can be applied to inactivate the enzyme activity and to help understand the differences in their effects.

This study reports on the partial renneting treatments of milk with a low concentration of enzymes at low temperature to slow down the enzymatic reaction, followed by heat application to end the enzyme activity. The aim of the work was to investigate the effects of partial renneting on CM size, and to better understand the stability of treated milk during storage at low (4 °C) and room (23 °C) temperatures.

2. Materials and methods

2.1. Materials

Fresh pasteurised skim milk was purchased from a local supermarket. The initial pH of the milk was about 6.62. Sodium azide 0.02% (*w*/*v*) was added as a preservative to prevent microbial spoilage during the experimental period.

2.2. Sample preparation for rennet addition

Renneting was carried out using low rennet levels of 0.01 to 0.03 IMCU mL^{-1} (Fig. 1). Chymosin (EC 3.4.23.4; Chy-Max Plus; 200 IMCU mL^{-1}) were provided by CHR Hansen Pty Ltd., Melbourne, Australia. Chymosin solution was freshly prepared each day by diluting the chymosin 1:200 (vol vol^{-1}) with filtered MQ water at 4 °C. Skim milk (50 mL) cooled to 4 ± 1 °C in ice water was treated with a suitable quantity of chymosin. The term renneted is used for milk treated with

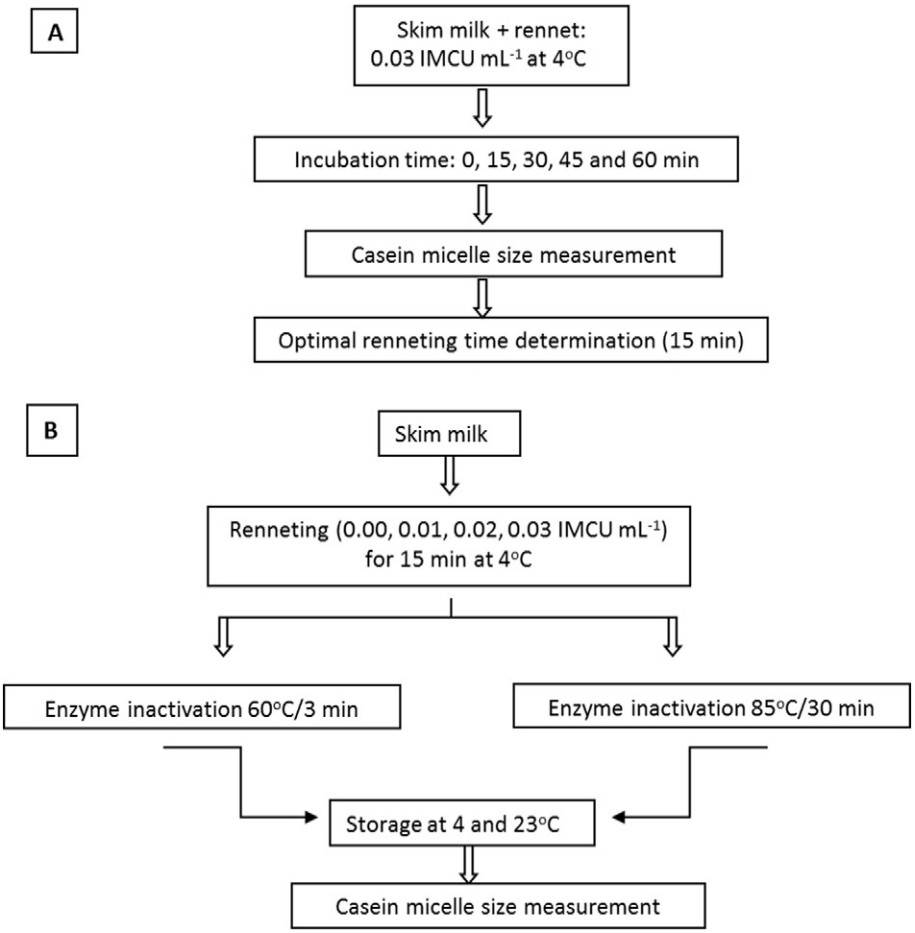

Fig. 1. Experimental protocol for the study of partial renneting: renneting at 4 °C (A) and inactivation of enzyme reactions by the application of low and high heat treatments (B).