Effect of microfluidization on casein micelle size of bovine milk

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Abstract. The properties of milk are likely to be dependent on the casein micelle size, and various processing technologies produce particular change in the average size of casein micelles. The main objective of this study was to manipulate casein micelle size by subjecting milk to microfluidizer. The experiment was performed as a complete block randomised design with three replications. The sample was passed through the microfluidizer at the set pressure of 83, 97, 112 and 126 MPa for one, two, three, four, five and six cycles, except for the 112 MPa. The results showed that microfluidized milk has smaller size by 3% with pressure up to 126 MPa. However, at each pressure, no further reduction was observed after increasing the passed up to 6 cycles. Although the average casein micelle size was similar, elevating pressure resulted in narrower size distribution. In contrast, increasing the number of cycles had little effect on casein micelle distribution. The finding from this study can be applied for future work to characterize the fundamental and functional properties of the treated milk.

1. Introduction
Milk has so many benefits in human life as it contains the most nutrition food in nature. Different types of milk will have wide variation in nutrient proportions, however, generally milk containing high amount of proteins, lipids and minerals, along with carbohydrates and vitamins. Whole milk is composed of 87.42% water and 12.58% total solids, which compromised 3.86% lipid components and 8.73% of solids non fat. Lactose, nitrogenous matter, salts and other solids account for 4.67%, 3.28%, 0.66% and 0.17%, respectively, of the solids non fat [1].

Caseins as a dominant protein are a family of phosphorylated proteins. Caseins are about 80% of the protein in bovine milk, represents 4 gene products: \(\alpha_s1\)-, \(\alpha_s2\)-, \(\beta\)- and \(\kappa\)- caseins in the molar ratio 4:1:4:1 [2] and a trace amount of gamma casein [3]. Caseins also contain a large proportion of minerals (calcium and phosphate), which are required for bone growth for the newborns [4]. Most of caseins exist as casein micelles; they tend to associate because of their high hydrophobic bonding. The smallest aggregates can contain 10-20 moles casein [5].

Various processing technologies produce particular change in the average micelles sizes (CMs) [6, 7, 8]. As the properties of milk are likely to be dependent on the micelle size, the micelle size modification might affect the behaviour of protein during processing [9, 10]. In the past, most of the works are focused on the casein micelles model and the composition. Only little is known about the main effect of casein micelle size and on how the manipulation in their micelle structural properties contribute to the functionality of protein in general.

Homogenization in milk processing is generally applied to produce more consistent raw milk. Naturally fat milk will separate and form the top layer of cream, as the fat has a less dense than the...
milk serum. Initially homogenisation refers to break down the fat globules, and allow the smaller particles distribute consistently in milk [11]. Other research suggested that high pressure homogenisation can also be used to produce particular change in the molecular structure of protein, that can alter the milk constituent and properties [11, 12]. Along with homogenisation, microfluidisation is the most common method used in high shear processing. The samples are processed in an inline homogeniser and micro channels of a Microfluidizer, for homogenisation [13, 14], dispersion [15], emulsification [16, 17, 18] and particle size reduction [19, 20].

The equipments were originally used only for pharmaceutical emulsions, however nowadays there are widely used in dairy production [20]. When compared to conventional homogenised milk, microfluidized milk have smaller particle size and narrow size distribution [19], but resulting in larger particles in yoghurt [21]. Although Olson et al [21] observed that the particles size only decreased at the pressure < 100 MPa, others found that the decrease still continued at 150 MPa [21]. Other study had analysed functions to predict average diameter and size distribution of the microfluidized fat globules in dairy model emulsions [20].

Fat globules and emulsions prepared by microfluidization have been repeatedly documented in literatures. The structure of protein differed from those produced by valve-homogeniser. The change in particle size distribution was best measured by dynamic light scattering (DLS), as the instrument can measure the size smaller than 100 nm [22]. The results provided good overviews of using the device for the reduction of casein micelles size.

In this study, we will attempt to develop methods to manipulate the casein micelle size. Although various study reported the casein micelle size of by applying pH [23], partial renneting [24, 25], limited research has been performed which relates to the application of a Microfluidizer. The research findings are expected to solve many unknown size-property relationship, thus can provide enormous opportunities to develop new generation dairy products with variable functionality.

2. Materials and methods
In attempt to manipulate the size of casein micelles, a Microfluidizer was employed, with operating pressure ranged from 0 - 126 MPa. Further treatments were conducted by recirculating the sample through the homogeniser for up to six cycles. Then, the average size and size distribution of the casein micelles were determined.

2.1. Microfluidizer
Pasteurised skim milk was homogenised using a Microfluidizer (Model B12-04DJCM3, Watts Fluidair Inc, Kittery, Maine) operating in continuously or recycles. The sample was passed through the microfluidizer at the set 12, 14, 16 and 18 KPsi (83, 97, 112 and 126 MPa, respectively) pressure for one cycle. The portion of the microfluidized sample was taken for size analysis, and the remaining was passed through a Microfluidizer again for second pass, the third and the fourth pass at 83, 97 and 126 MPa.

2.2. Experimental Design and Statistics
All experiments were performed as a complete block randomised design with three replications. Statistical analyses to determine any treatment effects were done using Minitab 16.0. The corresponding data were determined by analysis of variance (ANOVA) using the General Linear Model. Tukey simultaneous test at the level of P = 0.05 was carried out to assess whether different treatments resulted in statistically significant differences.

3. Results and discussion
Microfluidized treatment significantly reduced the particles size of milk from 246 ± 0.1 nm to 239 ± 1.5 nm (Figure 1). However, elevated pressure from 83 to 126 MPa for one pass had no significant effect on the average size. This meant that casein micelle size did not change if the milk was subjected
to a Microfluidizer with pressure less than 130 MPa. The average size for all treated milk was around 240 nm, only < 10 nm different with the untreated sample.

![Figure 1](image1.png)

**Figure 1.** The particle size of microfluidized milk at pressure of 0 - 126 MPa

Although the average size of casein after treatment 83 - 126 MPa was similar, the size distribution in intensity (%) at lower and higher pressure treatment (112 and 126 MPa), was different (Figure 2). The micelle size distribution in milk treated at higher pressure became narrower than that of the untreated milk. The distribution was shifted towards smaller size.

![Figure 2](image2.png)

**Figure 2.** The size distribution of microfluidised milk at pressure of 0 - 126 MPa

In the multi-passes treatment, the results revealed that more cycle have no greater impact on the reduction of casein micelles size of microfluidized milk (Figure 3). The size decreased only up to 15
nm at 126 MPa at single pass. Further increasing the number of passes did not significantly change the size.

Figure 3. The particle size of microfluidized milk at pressure of 83 - 126 MPa as a factor of number of passes

Treatment of skim milk at 83 - 126 MPa reduced casein micelles in all 6 passes, however, only little differences was observed between micelle size distribution of treated and untreated milk (Figure 4). At all milk samples the size of micelles being present is < 800 nm.

These data suggest that elevating the pressure could lessen the size, however, no further reduction was observed after increasing up to 6 passes. Treatment up to 150 MPa had little effect on casein micelles size [8, 26, 27]. Other study found that only large micelles (300 - 600 nm) might dissociate at this pressure treatment [28]. As the maximum pressure of the Microfluidizer is 126 MPa, other method such as high pressure homogenisation (HPH) application can be used for higher pressure treatment.
Figure 4. The size distribution of microfluidized milk at pressure of 83 - 126 MPa as a factor of number of passes

4. Conclusions
It is concluded that casein micelle size can be manipulated by the application of Microfluidizer. The CM size decreased at pressure of 83 MPa. However, there was no significant different amongst the size, even though the pressure was increased up to 126 MPa. Similarly, the number of passes up to 3 cycles did not affect the CM size. The findings suggested that applying Microfluidizer at 83 MPa for one cycle was enough to manipulate the CM size.

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