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# The rheological properties of calcium-induced milk gels

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ABSTRACT

The study investigated the calcium-induced gelation of milk during heat treatment. Rheological measurements showed that the addition of 10–20 mM calcium chloride caused thickening or gelation of milk on heating at 70 °C. Thickening was observed with 10 mM addition, while gelation was evident with ⩾12.5 mM additions, as indicated by an increase in the storage modulus ($G'$) of the calcium-added milk. The final $G'$ and breaking stress of milk gels made from ⩾12.5 mM added calcium increased with calcium addition. Pre-heat treatment significantly affected the strength of calcium-induced milk gels. Strong milk gels were obtained by the addition of 20 mM calcium chloride to pre-heated milk and holding at 70 °C for 60 min followed by cooling to 20 °C. The technology of making calcium-induced milk gels can be exploited commercially to make non-fermented dairy gels.



## 1. Introduction

Recently, calcium supplementation of milk has become a common practice in the dairy industry. It is usually performed by adding calcium salts to milk. Heat treatment of milk is a common industrial process, with the types of heat treatment employed varying from mild (pasteurization) to severe (in-container sterilisation), depending on the purpose of the heating. Mild heat treatments are performed to destroy pathogens and reduce spoilage micro-organisms in milk, while more severe treatments are employed to prepare milk for yogurt making or to prepare shelf-stable milk products. Heat processing of calcium-added milk is challenging because adding soluble calcium salts increases the ionic calcium concentration [$Ca^{2+}$] of milk and decreases milk pH (Omoarukhe et al., 2010; Ramasubramanian et al., 2008), both of which can lead to the coagulation of milk during heat treatment. Especially in milk with high protein content (such as milk concentrates), calcium-induced heat coagulation is a very negative consequence of heat processing that needs to be controlled.

Studies on the effect of ionic calcium on the coagulation of milk during heat treatment are extensive (On-Nom et al., 2012; Singh et al., 2007 and Vyas and Tong, 2004). A wide variety of techniques have been employed to study the coagulation of calcium-added milk during heat treatment. These include the subjective measurement of Heat Coagulation Time (HCT), and objective measurements of apparent viscosities of thickened milk samples and nitrogen contents of sedimented milk samples to monitor changes in physical properties, including gelation of calcium-added milk samples during heat treatment. The coagulation of calcium-added skim milk (10–20 mM calcium chloride added) at 60–120 °C was reported by On-Nom et al. (2012). Changes in the apparent viscosity of calcium-added milk or micellar casein solutions (5–20 mM calcium added) following heat treatment have been reported (Augustin and Clarke, 1990; Beliciu and Moraru, 2011 and McKinnon et al., 2009) using flow behaviour studies. The increase in firmness of thermal milk gels (made by heating a mixture of milk and vegetable shortening at 100 °C for 10 min) by the addition of calcium chloride (5–250 mM) has been reported (Kalab and Emmons, 1972; Kalab et al., 1972). These studies do not report the calcium content of the calcium-added milk samples and the rheological changes that occur in milk during gelation. Further, to date, a non-destructive rheological approach using Small Amplitude Oscillatory Shear (SAOS) measurements, has not been employed to study the effect of calcium and heat treatment on milk.

Previously (Ramasubramanian et al., 2012), we reported the coagulation of milk by calcium chloride (20–200 mM) at 70 °C, and introduced a calcium–milk coagulum which resulted from the coagulation of milk by 50 mM ionic calcium during heat treatment at 70 °C. We indicated that calcium chloride additions at less than 20 mM did not cause coagulation of milk on heating at 70 °C.

The aim of the present study was to monitor physically the changes that occur in heated milk with low levels (⩽20 mM) of calcium chloride addition, and to investigate the thickening of milk and the formation of calcium-induced milk gels rather than coagula, through the addition of calcium chloride. A non-destructive rheological approach based on SAOS was used to study the

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formation of calcium-induced milk gels and thickened milks with up to 20 mM added calcium chloride.

## 2. Materials and methods

### 2.1. Manufacture of calcium-induced milk gels on the rheometer

Commercial homogenised pasteurised or UHT whole milk containing 3.2% protein and 3.5% fat were used in the study. Ionic calcium was added as calcium chloride dihydrate (molecular weight 147.02). Commercial milk samples were obtained from a local supermarket on the day of the experiment. All chemicals were obtained from Sigma Chemicals Ltd. (North Ryde, NSW, Australia).

Commercial homogenised pasteurised milk (100 mL) was heated at 90 °C for 10 min and then cooled to room temperature (~22 °C). Calcium chloride at 10, 12.5, 15, 17.5 or 20 mM was then added to the milk sample at room temperature and the milk sample was mixed thoroughly for 30 s. The formation of gels in calcium-added milk during heating and that of ultra-high-temperature treated (UHT) milk (commercial UHT milk) was then monitored using rheological methods.

### 2.2. Monitoring of gelation in calcium-added milk during heating and measurement of rheological properties

A controlled stress rheometer (AR-G2, TA instruments, UK) with cone and plate geometry was used (Cone angle: 2°; diameter: 40 mM; truncation: 58 μm) to monitor gelation in calcium-added milk samples and to examine properties of the resulting calcium-induced milk gels. An aliquot (0.61 mL) of the calcium-added milk sample was loaded onto the plate, and the cone was lowered onto the milk sample. The outer surface of the sample was lined with a thin layer of oil (polydimethylsiloxane 200®; viscosity 10 cst) and an evaporation trap was used to prevent drying out during heating. Each calcium-added milk sample was first heated from 20 °C to 70 °C (20 °C increase per min) and then held at 70 °C for 60 min, while monitoring the $G'$ and $G''$ changes at a constant strain of 0.05% and a constant frequency of 0.1 Hz.

The calcium-induced milk gels formed on the rheometer were monitored for $G'$ changes as they were cooled from 70 °C to room temperature 20 °C at 2 °C decrease per min at 0.05% strain and 0.1 Hz frequency. The gels were then subjected to a frequency sweep from 0.01 Hz to 10 Hz (at 20 °C; 0.05% strain), followed by a strain sweep from 0.05–300% (at 20 °C; 0.1 Hz frequency) to measure the oscillation stress on the sample.

### 2.3. Batch-manufacture of calcium-induced milk gels and calcium-induced thickened milk

Batches (100 mL) of commercial homogenised pasteurised whole milk were placed in sterilised glass containers. Calcium chloride was then added (0, 10, 12.5, 15, 17.5 or 20 mM) to these batches of milk at room temperature and mixed thoroughly for 30 s. The calcium-added milk samples were then heated to 70 °C and held undisturbed at 70 °C for 10 min in a temperature-controlled water bath (Ratek Ltd. Australia). The effect of pre-heating was investigated by subjecting milk to a heat treatment of 90 °C for 10 min before adding calcium chloride.

Thickened milks or milk gels were formed at the end of heating. The produced thickened milks or calcium-induced milk gels were then cooled to room temperature and stored at 5 °C overnight. Aliquots (0.61 mL) of the calcium-induced thickened milks/calcium-induced milk gels were used for flow behaviour evaluations.

### 2.4. Rheological evaluations of batch-manufactured calcium-induced milk gels and thickened milks

The flow behaviour of batch-manufactured thickened milks or calcium-induced milk gels was observed using the AR-G2 rheometer (TA Instruments, UK). A small amount of sample was placed on the plate of cone and plate geometry (40 mM diameter; 2° cone angle). The flow behaviour of milk and gel samples was observed by increasing the shear rate from 0.1 $s^{-1}$ to 100 $s^{-1}$ and measuring the shear stress (at 20 °C).

### 2.5. pH and [$Ca^{2+}$] in milk samples and calcium-induced milk gels

The pH of the milk samples and calcium-induced milk gels was measured using a TPS pH electrode (TPS Pty Ltd., Springwood, QLD, Australia) at room temperature (~22 °C). The ionic calcium concentration [$Ca^{2+}$] in the milk samples and calcium-induced milk gels was determined at room temperature (~22 °C), by a Sentek calcium ion selective electrode (Sentek Ltd., Essex, UK). The electrode was calibrated with calcium chloride standard solutions of 1–100 mM [$Ca^{2+}$], whose ionic strength had been adjusted to 0.08 by adding 0.1 M KCl. The logarithm of [$Ca^{2+}$] was plotted against mV readings, to obtain a calibration curve. The $R^2$ values were >0.985.

### 2.6. Statistical design and analysis

A complete randomised design was used. All experiments were performed in duplicate. ANOVA at the 95% significance level and correlations were performed using the Minitab-15 statistical software package (Minitab Inc., Chicago).

## 3. Results and discussion

### 3.1. Changes in pH and [$Ca^{2+}$] of milk samples due to calcium chloride addition

The addition of calcium chloride to milk caused a significant ($P < 0.05$) decrease in milk pH and an increase in [$Ca^{2+}$] (Table 1). Although pre-heat treatment (both UHT and 90 °C for 10 min) contributed to a slight reduction in milk pH and increase in [$Ca^{2+}$], the contribution was not significant. The pH drop is a manifestation of the 'calcium equilibrium' that exists in milk (Lewis, 2010). A sudden increase in [$Ca^{2+}$] was noticed when 17.5 mM $Ca^{2+}$ was added. It appears that most of the added calcium entered the micellar phase up to 15 mM addition, but at higher additions, the majority of added calcium was retained in the ionic phase. Such an increase in retainment of calcium in the ionic phase of heat treated calcium-added milk has been reported earlier. The percentage of added calcium remaining in ionic phase of milk was 20% with up to 16 mM added (Philippe et al., 2004), 28.5% with 10 mM added (Seivanen et al., 2008) and 28.3% with 30 mM added (Omoarukhe et al., 2010).

### 3.2. Rheological monitoring of gelation in calcium-added milk samples during heating

The changes in storage modulus ($G'$) of calcium-added milks (commercial pasteurised milk; milk pre-heated to 90 °C for 10 min) during heating from 20 °C to 70 °C are shown in Fig. 1. Some calcium-added milk samples exhibited gelation during this heat treatment regime while others thickened but did not display gelation, as indicated by an increase in $G'$. The temperature–time combination at which the $G'$ of a calcium-added milk sample reached 1 Pa was defined as the 'gelation point' of the sample.