

Rheological Properties of Chymosin Induced Gels From Ultrafiltered Milk

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ABSTRACT

This paper presents a study of the enzymatic reaction, the coagulation process and their interaction for renneting of milk concentrated up to 5-fold by ultrafiltration.

INTRODUCTION

Coagulation of casein micelles in milk by the enzyme chymosin is a fundamental step in the production of almost all cheeses. The coagulation process consists of two overlapping steps: 1) cleavage of the phe₁₀₅-met₁₀₆ bond of κ -casein by chymosin whereby a highly negatively charged peptide (CMP) is released and the micelles are rendered unstable, and, 2) the modified micelles aggregate and form a gel. In recent years technology has been developed for making cheese from milk that has been concentrated by ultrafiltration (UF), a process that selectively concentrates protein and fat in milk. Consequently, there is a need for a detailed knowledge on the chymosin gelation properties of ultrafiltered milk.

MATERIALS AND METHODS

Milk and Retentate

Raw milk was standardized to approx. 1.5 % fat and pasteurized (72°C/15 sec or 95°C/15 sec in a plate heat exchange) before ultrafiltration. The milk was concentrated up to 5-fold (5x) by ultrafiltration using an Alfa-Laval UFS-1 (Alfa-Laval, Århus, Denmark), and then diluted with permeate to give various protein concentrations. To prevent

bacterial growth 0.02 % NaN₃ was added. The samples were cooled to 5°C and kept at this temperature until the next day.

Assessment of Renneting Reaction

Dynamic testing, using the Bohlin VOR Rheometer¹, was used to follow the coagulation. The oscillation software was used with the following settings: Measuring system: C 25, torque element: 1.36 g cm, measurement interval: 20 sec., frequency: 1 Hz, and strain: 0.01. A HPLC gel filtration method based on the work of Hooydonk and Olieman² was used to determine caseino macro peptide (CMP), and thereby follow the enzymatic reaction.

Compositional Analysis

Protein, fat, calcium and phosphorus were measured as described by Qvist *et al.*³.

Experimental

The samples were equilibrated at 30°C for 30 min before addition of chymosin and mixed by stirring for about 30 s. Oil was layered on top of the samples in the rheometer cup to avoid drying⁴. Rennet concentration was 0.0132 CHU chymosin/ml and temperature was 30°C, unless otherwise indicated.

RESULTS

Degree of concentration

Table 1 shows the composition of the milk and milk retentates.

Table 1. The protein and fat content, n=4.

%	Degree of concentration				
	Milk	2x	3x	4x	5x
protein	3.5	6.2	9.2	12.2	14.7
fat	1.7	3.0	4.5	6.0	7.3

Figure 1 shows the large difference in the gelation of milk and 5-fold concentrated milk. It can be seen that the phase angel drops earlier after rennet addition for 5x that for milk, indicating that 5x had a shorter coagulation time than milk.

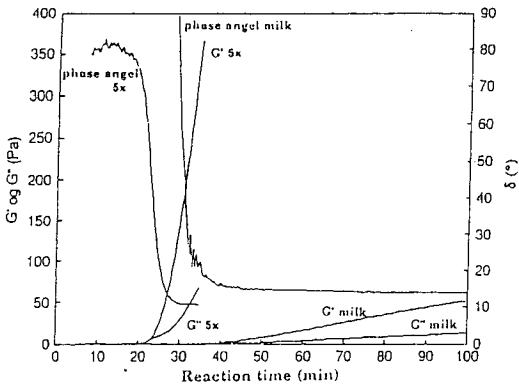


Figure 1. The storage modulus G' , the loss modulus G'' and phase angel δ as a function of reaction time.

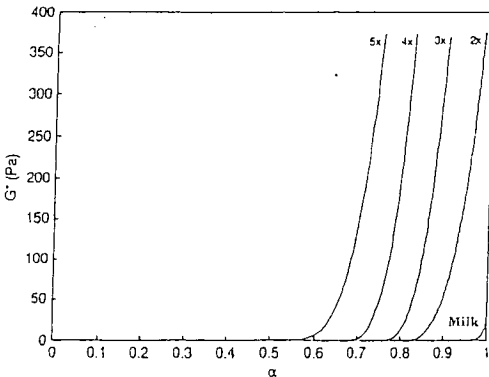


Figure 2. The complex modulus G^* as a function of the extent of the enzymatic reaction, α .

In Figure 2 the complex modulus, G^* , is

shown as a function of the relative extent of the enzymatic reaction, α .

$$\alpha(t) = 1 - e^{-k_1 t} \quad (1)$$

k_1 = the first-order reaction rate constant for the enzymatic reaction; t = reaction time.

It can be seen that curd formation starts at a much lower α -value for 5-fold concentrated milk than for milk.

For milk we measured an asymptotic gel strength modulus, G_∞ of ~ 130 Pa after ~ 10 hours. Zoon *et al.*⁵ found, for the storage modulus G' , a plateau value of ~ 125 Pa after about 9 hours.

We did not succeed in determining G_∞ for concentrated milk. Here, a wall slip between sample and rheometer cup always occurred, presumably because of sample contraction during gel consolidation. We then took an indirect approach based on the assumption that development of gel modulus follow the Scott Blair model⁶ in order to obtain G_∞ . We observed a significant disagreement between the predicted and the measured modulus for concentrated milk, and consequently we had to abandon this method to. Instead we chose to used the modulus at the inflection point, G_{inf} , and the maximum rate of increase of G , $(\Delta G/\Delta t)_{inf}$, observed at this point, to characterise the gelation.

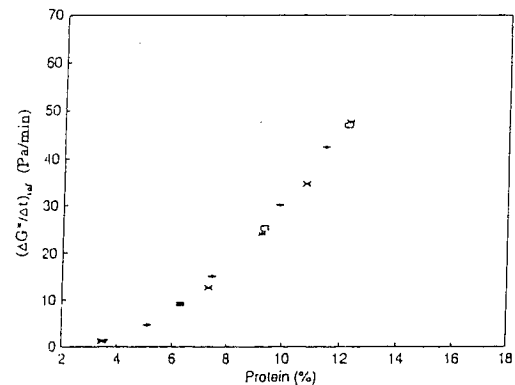


Figure 3. The max rate of development of the complex modulus G^* as a function of protein content (data from four experiments).

We found a strong relation between the max rate of change of the complex modulus

and the protein content, as it can be seen in Figure 3, the same was valid for the storage modulus and the loss modulus.

Heat treatment of milk before UF

The enzymatic reaction was only affected by the level of heat treatment to a limited extent. When the temperature was increased from 72°C to 95°C the first order reaction rate constant decreased 4-5 % for both milk and 5-fold concentrated milk. Van Hooydonk *et al.*⁵ found similar results for milk.

Gel formation in milk was strongly affected by the level of heat treatment. G^*_{inf} was 29 Pa, for milk heated to 72°C/15 sec, but only 13 Pa for milk heated to 95°C/15 sec. In 5-fold concentrated milk, level of heat treatment had little effect on gel formation.

Reaction temperature

Table 2 shows the increase in the rate of gel formation at inflection point with increasing temperature for 5-fold concentrated milk.

Table 2. The rate of gel formation at inflection point for the complex modulus G^* , the storage modulus G' and the loss modulus G'' , $n=2$.

	$(\Delta G^*/\Delta t)_{inf}$ Pa/min.	$(\Delta G'/\Delta t)_{inf}$ Pa/min.	$(\Delta G''/\Delta t)_{inf}$ Pa/min.
25°C	79.7	77.6	12.1
30°C	119.6	118.8	22.0
34°C	153.7	154.9	31.8
38°C	176.4	174.4	42.5

From the Arrhenius plot of the first-order reaction rate constant for the enzymatic reaction, a value of 27.6 kJ/mole was found for the energy of activation, which correspond to a Q_{10} -value of 1.4. The Arrhenius plot of the rate of gel formation $(\Delta G^*/\Delta t)_{inf}$ at inflection point gave a value of 47.6 kJ/mole for the energy of activation, and a corresponding Q_{10} -value of 1.9.

DISCUSSION

It was observed that a gel was formed sooner after chymosin addition, and at a lower relative degree of the enzymatic process, with increasing degree of concentration. Also, the rate of gelation increased with degree of concentration and reaction temperature. Gel formation in unconcentrated milk was strongly reduced by using a heat treatment of 95°C/15 sec, but for 5-fold concentrated milk the ability to form a gel was only slightly reduced. For 5-fold concentrated milk the enzymatic reaction had a activation free energy of 27.6 kJ/mole and for the gelation process a activation free energy of 47.6 kJ/mole. The final gel was increasingly elastic in character with increasing degree of concentration.

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