Bull. Korean Fish. Soc. 22(6), 391~396, 1990

韓水誌 22(6), 391~396, 1990

Kinetic Study of Milk Gellation by the Electrical Resistance Measurement

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Changes in electric resistance was measured to carry out the kinetic analysis of milk gellation upon addition of rennet. Using pasteurized milk and commercial rennin, kinetic properties were investigated during milk gellation in terms of initial hydrolysis and coagulation steps. Specially designed reactor with two platinum electrodes was used throughout the experiments. As a function of either milk concentrations or reaction temperatures, gel time exhibited directly proportional relations; on the contrary, gel time was inversely proportional to enzyme concentration. Activation energies for enzymatic degradation and cogulation were 16.3, 4.6 and 34, 8.6 Kcal/mol, repectively. This simple analytical method proved to be very effective to characterize the mechanism of milk gellation. Moreover, unlike other methods, this method reguired simple apparatus and short time of analysis.

INTRODUCTION

As progress of economic growth has become substantially fast, diet patterns have gone through a lot of changes. As a result, food processing industries have been forced to develop new type of processed foods. Recently serious oversupply problem has arisen by large increase in milk production. To solve this imminent crisis, it is urgently reguired to find a way to achieve products of superior quality as well as products of various kinds, which may contribute to more effective utilization of milk and increase in milk consumption. Unfortunately, at the present time, our dairy processing technology remained in the beginning stage; thereby, rate of milk consumption has been improving very slowly. Just recently, a few dairy products of new type has been introduced, but new types of dairy products using gelling property of milk need to be developed in terms of diversifying the product types with superior quality. To achieve this goal, kinetical analysis of milk gellation has to be investigated.

In this respect, Blair and Oosthuzen (1961) tried to analyze the reaction of casein with enzyme during the initial stage of milk gellation using Ostwald viscometer. The same mechanism was studied by Blair and Tuszynski (1967) using Torson meter. Garnier (1963) and Foltmann (1959) investigated gelling mechanism using chemical methods; in contrast, Humme (1972), Payens (1976, 1978) and Surkov et al. (1982) used photometric methods. By Boussemaer (1961) and Castelain (1982), universal testing machine was employed to measure changes in elasticity from which they tried to establish models for reaction mechanism. More recently, Lee (1986) explained the reaction mechanism in terms of viscoelastic changes using mechanical spectrometer. Unlike all these works, Hori (1985) determined the rate of milk gellation by measuring changes in thermal diffusivities using platinum wires.

In this study, simple and time-saving analytical method was used to measure changes in electrical resistance for characterization of gelling mechanism. Upon gellation of milk, effects of milk and

enzyme concentrations as well as reaction temperatures were evaluated by measuring changes in electrical resistance.

MATERIALS AND METHODS

Materials

Milk

Pasteurized skim milk (DIFCO Lab.) was used throughout the experiment. This milk was stored at 5℃ and diluted with a given concentration of calcium chloride solution just before the experiment. To 1.0 *l* of the milk preparation, 300 *mg* of sodium azide was added to prevent from contamination during reactions followed by the adjustment of pH to 6.5

Enzymes

Rennin of powder form (Tokyo Kasei Co.) was dissolved in 0.02M sodium acetate solution to give final concentration of 16% by weight. Enzyme stock solution was stored at 5℃ and diluted just before each experiment. Coagulation activity was determined by the method of Berridge (1942).

Skim milk was dissolved in 0.01 M calcium chloride solution to give final concentration of 12% by weight. Aliquots of 10 *ml* was transfered to test tubes and placed in 30℃ water bath for thermal equilibration. To the test tube, 1.0 *ml* of enzyme dilutions was added and reaction time was read. After closing the cap, test tubes were inverted for mixing. Clottig time was recorded when firt formation of clot was observed. Enzyme dilution which produced clotting time of 1~2 min. Unit of activity was calculated as follows:

$$\text{Coagulation activity } (ml/mg.\text{sec}) = \frac{1}{[\text{Enzyme}] \times \text{Clotting time}}$$

Measurement apparatus

Changes in electrical conductivity during milk gellation were monitered using specially designed reactor (Fig. 1). To maintain constant temperature, reactor was covered with water jacket. Two platinum electrodes were installed through the side of reactor from the opposite directions. The gap between two electrodes can be adjusted as necessary. Resistance between the electrodes was measured using Dynascan voltmeter (Model 177. VTVM, Dynascan Co. U. S. A.).

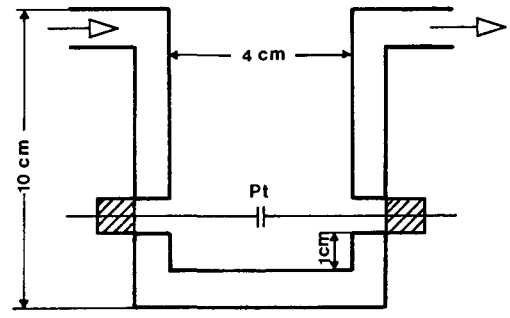

Fig. 1. Conductivity sensor for the measurements of milk coaulation.

Estimate of coefficient for reaction velocity

Enzymatic gellation of milk was thought to be taken place in two steps: degradation of k-casein and subsequent coagulation step.

$$C_1 \xrightarrow[K_1]{\text{Enzymatic degradation}} C_2 \xrightarrow[K_2]{\text{Coagulation}} C_3$$

k-Casein in milk Degraded casein Coagulated casein

The method of Lee (1986) was employed to determine the rate constants of reaction.

Experimental Procedures

Substantial amounts of Ca^{++} are present in recombined milk solution; as a result, some electric conductivity can be exerted. However, immobilization of Ca^{++} ion resulting from milk gellation decreases electrical conductivity; therefore, it might be possible to monitor the progress of gellation by measuring the changes in resistance of milk solution.

Skim milk was dissolved in calcium chloride solution at 45℃. Final concentration of skim milk and calcium chloride were varied. The solution was stirred at low speed to prevent foaming and then placed in the water bath (30± 0.5℃) for 12 h. This substrate solution was thermally equilibrated to reaction temperature before the addition of enzyme. A given amount of enzyme was added to the substrate followed by 20 sec. agitation. After the mixture was placed in the reactor, reactor was co-

vered with paraffin film to prevent evaporation and hardening of surface during the reaction. Initially, reactor with reaction mixture was calibrated by adjusting the gap between platinum electrodes to give zero resistance. The increase in resistance was measured as a function of time. Using this apparatus, reaction time which exhibited the first increase in resistance was designated as gel time (t_g).

RESULTS AND DISCUSSION

Extents of immobilization of Ca^{++} ions resulting from milk gellation will change the resistance of reaction mixture. Thus, the effect of temperature, concentration of milk and enzyme evaluated by measuring the changes in resistance of reaction mixture.

Effect of Milk Concentration

Effect of milk concentration was determined using different concentration of skim milk containing 10 mM $CaCl_2$: 4, 6, 8, 10, 12, and 14% by weight. To 1.0 *l* of substrate, 0.2 *ml* of stock enzyme solution (16% by weight) was added. Reaction temperature and pH were 30℃ and pH 6.2, respectively.

Results were shown in Fig. 2. Changes in resistance were expressed as ratio between resistance at time t (R) and maximum resistance (R_∞). As progression of reation, R/R_∞ showed slight differences with different concentrations of milk, but exhibited the same tendency. This phenomena were coincided with the results reported by the rheological method of Lee (1986) and Johnston (1984). In addition, gel time was a linear function of milk concentrations as show in Fig. 3. In this case, slope was 0.15± 0.11 min/*g*. This value was somewhat different from the published results of Bousseman (1981) and Johnston (1986), who reported sloped of 0.39± 1 and 0.1± 0.002 min/*g*, respectively. This discrepancy might be resulting from types of milk and enzymes used.

Effect of Enzyme Concentration

Enzyme concentration affected enzymatic degradation and gel time during the initial reaction period, but did not have any significant effect on coagulation process. As shown in Fig. 4, gel time was drastically increased as increasing dilution of enzyme solutions, indicating that gel time was directly proportional to the inverse of enzyme concentrations. Boussemaer (1981) and Lee (1986) reported the similar results to this one; though, Tokita (1983) produced somewhat different result.

In addition, the maximum resistances were not affected by enzyme concentrations. At high concentration of enzyme, enzymes might hydrolyze proteins very fast; in the mean time, matrix formed could be degraded substantially fast by the same enzymes. With very low enzyme concentrations, the activity of enzymes was limiting factor for gellation because enzymatic degradation would be repressed by syneresis.

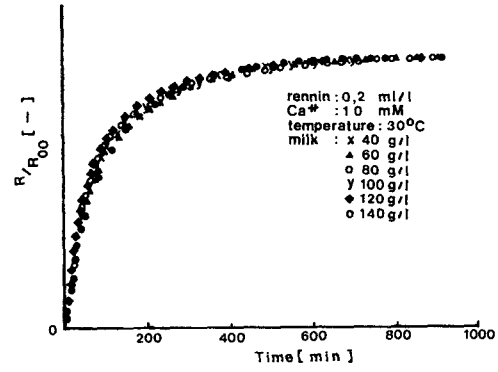

Fig. 2. Variation of registance as a function of time.

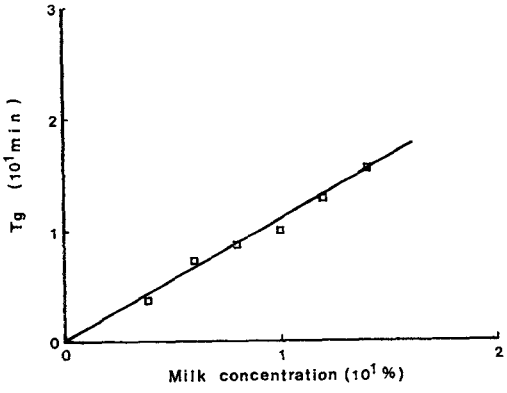

Fig. 3. Variation of gel points as a function of milk concentrations.

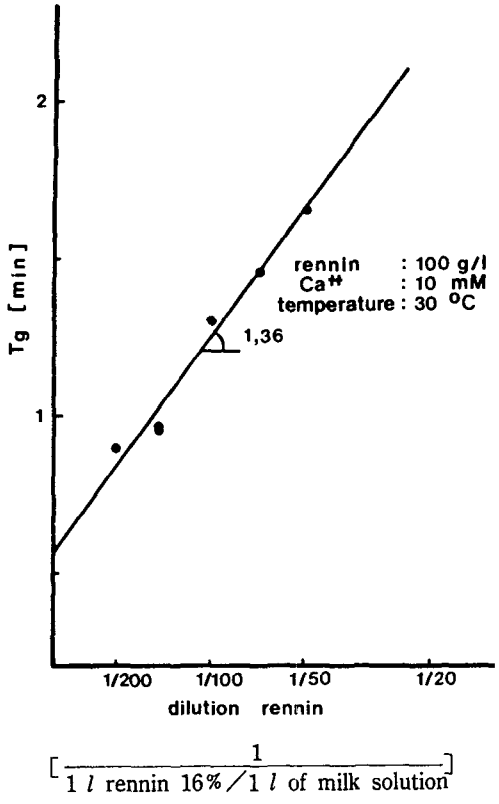

Fig. 4. Variation of gel time as a function of enzyme concentrations.

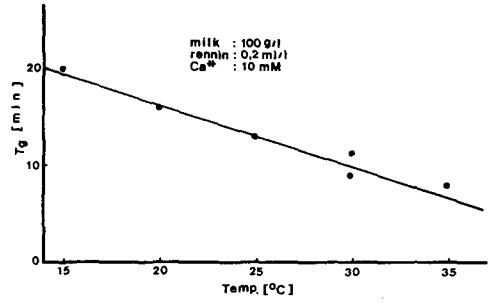

Fig. 5. Variation of gel point as a function of temperature.

Effect of Reaction Temperature

Most enzymatic reaction and gellation were very sensitive to the reaction temperatures. Garnier (1963) reported that increase in reaction temperature accelerated the enzymatic reaction rate. Berridge (1942) also reported the acceleration of milk coagulation by increasing temperature.

To investigate the effect of temperatures, temperature range of 15~35℃ was selected and to 10% milk solution containing 10 mM Ca^{++}, 0.2 *ml* of enzyme solution was added. As show in Fig. 5, gel time were linearly decreased as increasing temperatures with good coefficient of correlation. This was very similar to the result of Lee (1986). Only difference was rather higher gel points compared to Lee's. It might be explained by either differences in types of milk and enzymes or those in measurement techniques. In this case, however, it appeared to be the effect of milk and enzymes.

Constants for enzymatic reaction rate (K_1) and coagulation reaction rate (K_2) were increased as increasing temperatures. Fig. 6 and Fig. 7 presented the relation between inverse of reaction temperatures and constants for reaction rate by Arrhenius equation. Activation energy of enzymatic degradation was 16.3± 4.6 Kcal/mol (coefficient of correlation=0.985; standard deviation=5%), which was lower than 21.1 Kcal/mol of Lee's (1986) and higher than 12.9 and 11.9 Kcal/mol of Mehaia et al. (1982) and Tuszynski (1971), respectively. During the coagulation process, activation energy was 34± 8.6 Kcal/mol with correlation coefficient of 0.9625 and standard deviation of 5%. This value was higher than 38.7 and 45.6 Kcal/mol reported by Tuszynski (1971) and Surkov et al. (1982), respectively. However, it was higher than 17.0 Kcal/mol reported by Bachman et al. (1978a and b).

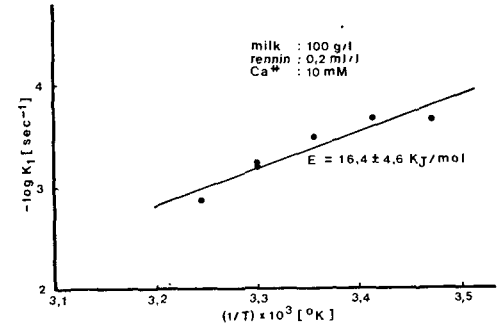

Fig. 6. Variation of initial reaction constant (K_1) as a function of temperature.

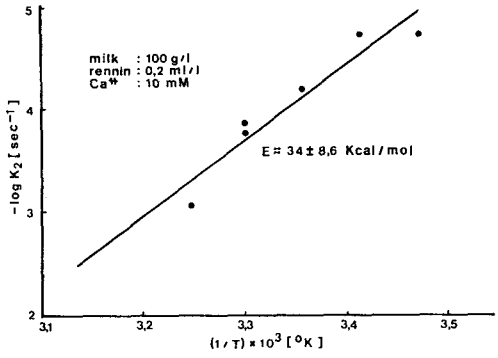

Fig. 7. Variation of coaguration constant (K_2) as a function of temperature.

CONCLUSIONS

Changes in electric resistance was measured to carry out the kinectic analysis of milk gellation with addition of rennet. The results were as follows:

1. Gel time was directly proportional to milk concentrations and inversly proportional to enzyme concentrations.

2. Linear relation was obtained between gel time and reaction temperatures.

3. Activation energies were 16.3± 4.6 and 34± 8.6 Kcal/mol for enzymatic degradation and coagulation, repectively.

4. Measuring changes in electric resistivity had some limits, but could be sucessfully used to analyze biochemical reactions as good as rheological method, if not better.

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Received December 20, 1989
Accepted January 20, 1990