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Effect of Salts and Temperature upon the Rate and Extent of Aggregation of Casein during Acidification of Milk

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산에 의한 우유단백질의 응고속도에 염과 온도가 미치는 영향

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Abstract

The rate and extent of coagulation of milk using fast acidification with 0.1 N HCl were monitored by changes in viscosity and turbidity at various temperatures and pH. Also the gelation rate of milk using slow acidification with D-glucono-δ-lactone was measured in a small strain rheological scanner. Coagulation of milk casein occurred in a specific pH range and was accompanied by an abrupt increase in viscosity at pH 5.0. Acid coagulation rate was enhanced by increasing temperature from 20 to 50℃, and the maximum rate was shown around pH 5.0. The addition of salt ($CaCl_2$) reduced the maximum coagulation rate at all temperature ranges and shifted the pH ranges for maximum coagulation rate and the onset pH of coagulation. The onset of gelation and the rate of network formation during slow acidification were facilitated by Cl^- ion, but suppressed by SCN^- ion, as indicated by the rate of rigidity development. The susceptibility to syneresis was greater in the gel made at lower temperature and around pH 4.6, while preheated milk at 90℃ for 5 min prior to acidification showed the same syneresis profile at all heating temperatures (60~90℃).

Key words: acid coagulation, gelation rate

Introduction

Acid-induced gelation of milk protein is very important in the manufacture of dairy products such as sour cream, yogurt and cottage cheese(1,2). Upon lowering the pH of milk, colloidal calcium phosphate is solubilized from the micelles, micellar disintegration takes place and the caseins associate to form a network, coagulum or gel(3,4). Both are considered to be irreversible and are related to protein-protein or protein-water interaction. However, the rate of acidification affects this sequence and determines the nature of the curd. That is, the rate of acidification determines whether a gel-like structure or a precipitated form. Also, various factors which alter the behavior of micelles upon acidification may affect the kinetics of coagulation and thereby greatly affect the physical properties of the resultant coagulum or gel.

The rate of network formation of casein in greatly affected by the prevailing temperature since there is no network coagulum formed below 5℃ at pH 4~5. Heat treatment alters the protein-ion equilibrium and gel formation results from altered protein-ion environment(2). Apparently higher temperatures favor rapid protein-protein association and increase the amount of whey protein precipitated with casein, producing a softer and more fragile curd(5).

The fact that casein particles can be flocculated at their isoelectric point demonstrates that the charge is of primary importance for their interactions. Exposure of casein micelles to relatively low concentrations of NaCl causes micellar dissociation and reduces its heat stability(6). Hermansson(7) proposed that at higher salt concentration, ionic effects on protein-solvent and protein-protein interactions in solution follow the lyotropic series. The sensitivity of salt, pH and temperature upon the network formation is thought to reflect the

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Corresponding author: Byung-Yong Kim, Department of Food Processing, College of Industry, Kyung Hee University, Yongin-kun, Kiheung-myun, Kyungki-do 449-701, Korea dependency of electrostatic repulsion on the stoichiometric net protein charge(8).

Numerous rheological studies have been conducted in the attempt to more clearly understand the functions and effects of salts and temperature on gel stability and strength. The present study was conducted to monitor the rate and extent of network formation of molk casein upon the addition of salts at various temperatures.

Materials and Methods

Material

Fresh pasteurized skim milk used in this study was purchased daily. D-glucono-δ-lactone (GDL) and HCl used as acid precursors, calcium chloride and calcium nitrate were purchased from Sigma Chemical Co.. Calcium thiocyanate was obtained from Fluka Chemical Co..

Acidification of milk

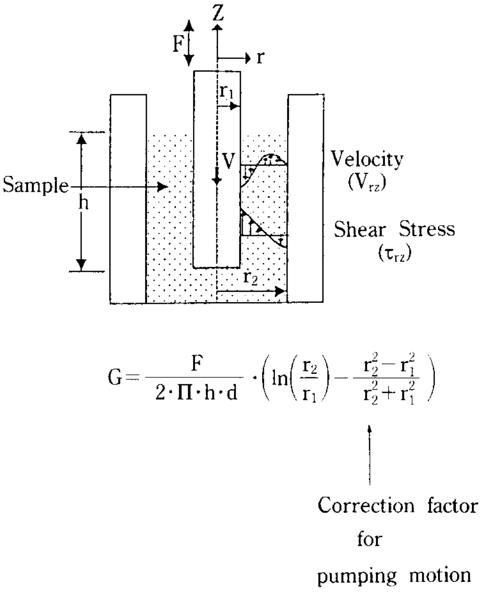
Slow acidification using GDL followed the procedure as described by Kim and Kinsella (1989). Milk (100 m*l*) was acidified with GDL (1g) at low temperature (4℃) and different concentration of salt solution (10~50 mM) was added to acidified milk before subjected to rigidity scanning measurement. For the fast acidification milk (100 m*l*) was acidified with aliquots of HCl (0.1 N) to give specific pH values as directly monitored by a pH electrode in the sample. This preparation was subjected to viscosity and coagulation rate measurement.

Viscosity measurement

The apparent viscosity of milk solution (8 m*l*) following acidification with 0.1 N HCl was measured at the specific shear rate (1890 sec^{-1}) using a Haake viscometer (Model RV100) with NV sensor system. Measurement of viscosity of all samples was carried out at 25℃ exactly after 5 minutes to avoid errors due to settling of the precipitate. The viscosity of acidified milk was used as an indicator of coagulum formation.

Coagulation rate measurement

Milk (100 μ*l*) was diluted 1000 fold into different salt solutions in a 100 m*l* beaker. Three milliliter of diluted milk was transferred to a cuvette and then an aliquot of 0.1 N HCl 0.5~5 μ*l* was added for different pH ranges. The change in turbidity at 400 nm was recorded as a function of pH, and the rate of coagulation was calculated from the slope of turbidity vs time recording measured between turbidities 0.05 and 0.055.



$$G = \frac{F}{2 \cdot \Pi \cdot h \cdot d} \cdot \left(\ln\left(\frac{r_2}{r_1}\right) - \frac{r_2^2 - r_1^2}{r_2^2 + r_1^2} \right)$$

Fig. 1. Pochettino type thermal scanning rigidity monitor

Gelation rate measurement

Structure formation in acidified milk with GDL and salt combinations at specific temperature was studied by using a small strain rheological scanner (Fig. 1). The apparatus consisted of a cylindrical brass bob (dia. = 3.33 cm and height = 3.81 cm) and a temperature controlled water jacket cup (dia. = 4.92 cm). The bob was attached by a string to a tension load cell mounted in the crosshead of Instron (Instron Engineering Corp. Canton, MA). A bob was suspended inside the cup and cyclic movements of the crosshead were controlled with 0.0105 sec^{-1} shear rate. The crosshead speed was 0.5 mm/sec and chart speed 20 mm/min. Shear modulus (modulus of rigidity) was calculated from shear stress divided by shear strain using the following equation which considered the pumping effect due to the forces at the bottom of the bob(10,11).

$$G = \$F/(2\pi.h.d)] \times [\ln(r_2/r_1) - (r_2^2 - r_1^2)/(r_2^2 + r_1^2)],$$

where F is the cyclic axial force amplitude of the bob, r_1 and r_2 are the bob and cup radii, d is the rod cyclic displacement amplitude, h is the length of bob in contact with the test sample and G is the shear modulus (Pa).

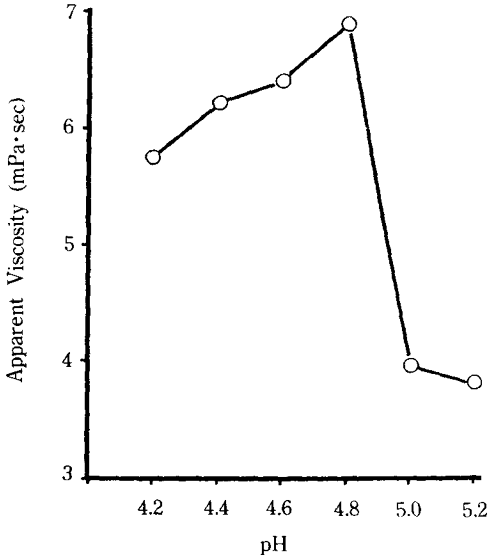


Fig. 2. Effect of pH on the apparent viscosity during acidification

Viscosity was measured using a Haake viscometer at 1890 sec^{-1} shear rate and at 25℃

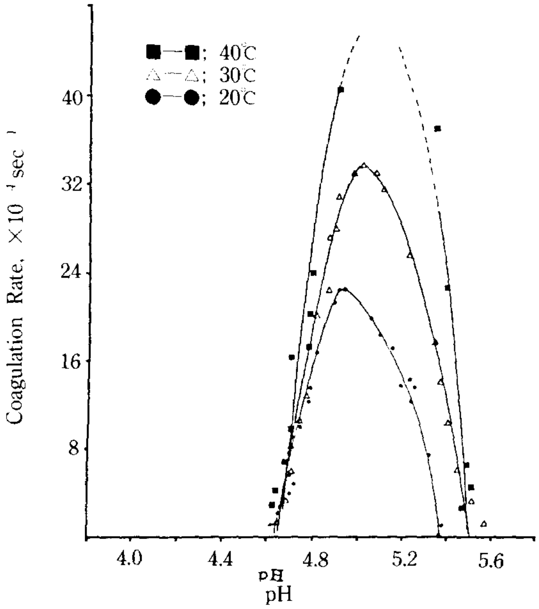


Fig. 3. The relationship between the pH-coagulation rates of casein micelles at various temperatures

The rates of coagulation were determined from the slopes of turbidity at 400 nm vs time recording measured between turbidities 0.05 and 0.055

Gel firmness measurement

Three 50 ml milks with GDL (0.5g) were gelled and were subjected to penetrometric test using a probe 0.95 cm in diameter at a speed 100 mm/min. The probe travelled 1.5 cm from the surface of the sample and firmness was expressed as gf/probe.

Susceptibility of syneresis measurement

Three 50 ml milks with GDL were gelled in 100 ml beaker and were subjected to measurement of syneresis by the drainage test(12). The curd type gel was cut into four sections with blade and transferred into a graduated cylinder. Relative volumes of the whey drained were plotted against the time intervals. The shape of the curve and the relative volume of whey drained during 40 min were used as a measure of syneresis.

Results and Discussion

Viscosity measurement of acidified milk

The change in viscosity of acidified milk as a function of pH at 25℃ is shown in Fig. 2. There was little change in viscosity initially as the pH of milk was decreased to 5.2. However, there was an abrupt increase in viscosity between pH 5.0 and 4.8, while a further decrease in pH from 4.8 to 4.2 was associated with only a slight decrease in viscosity. The small viscosity changes during initial acidification of milk to pH 5.2 suggested the still homogeneous distribution of micelles, and the abrupt increase in viscosity from pH 5.0 to 4.8 probably reflected the disintegration of the casein micelles associated with dissolution of colloidal calcium phosphate and subsequent aggregation of the caseins(4). Upon acidification of the milk to below pH 4.8, caseins associated and became entangled to form a network or coagulum(13). When this system was subjected to shear deformation at a constant shear rate, material resistance to flow decreased and showed shear thinning. Thus, it can be postulated that the smaller decrease in viscosity below pH 4.8 might be caused by smaller hydrodynamic volume of aggregated casein particle as pH was decreased.

Effect of temperature and anions coagulation process

Hyperbolic pH-coagulation rate profile for coagulation of casein micelle without the addition of salt was obtained at various temperatures (Fig. 3). While the onset of coagulation at 20℃ started to 30℃. The maximum rate of casein coagulation increased from 23×10^{-4} sec^{-1} at pH 4.9 to $34\times10^{-}$ sec^{-1} at pH 5.0, as the temperature was increased from 20 to 30℃. Increasing

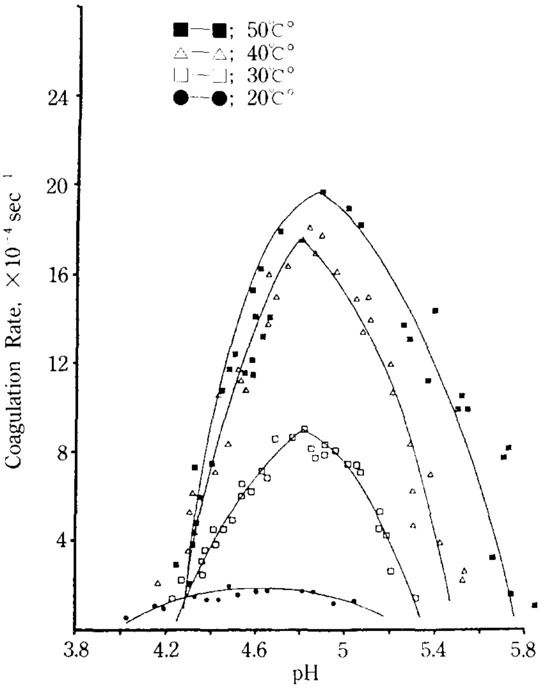


Fig. 4. Effect of $CaCl_2$ on the coagulation rate at various temperatures

the temperature more than 40℃ instantaneously raised the maximum coagulation rate unmeasurably. This also confirmed that the increasing temperature ostensibly enhanced the coagulation rate, as indicated in Fig. 4, due to increases in the frequency of thermal collision of casein micelles(14). The temperature dependence of aggregation reaction is also consistent with the hypothesis that the acidic coagulation of casein micelle involves hydrophobic interactions which increased with temperature(2).

Compared to maximum coagulation rate for control without salt, addition of 10 mM salt ($CaCl_2$) to acidified milk decreased the maximum coagulation rate up to about 10 times at 20℃ and 4 times at 30℃ (Fig. 4). Usually the addition of salt to milk causes micellar dissociation and reduces its heat stability(2). Also the pH of maximum coagulation rate of milk shifted to a lower value when salts were added. pH 5.0 at which maximum coagulation rate occurred for control shifted to pH 4.8. When the temperature decreased from 50℃ to 20℃, the highest pH at which coagulation was initiated decreased significantly. That is, the onset of coagulation at 50℃ started at pH 5.8, but this occurred at pH 5.2 when the temperature was decreased to 20℃. Therefore it appeared that salt affected the pH range for maximum coagulation rate as well as onset pH of coagulation in the coagulation process.

Effect of anions on gelation process

The observed changes in the development of shear modulus in the presence of various anions can be explained in terms of the effects of anions on protein network formation. Onset time of the milk gelation for the control (0.5g GDL/50 ml milk) without salt was 32 min and rigidity was gradualy developed up to 60 min (Fig. 5A). As the amount of $CaCl_2$ was increased to 13.6 or 27.2 mM, the gelation time was reduced from 32 min to 15 and 12 min, respectively and the rate of rigidity development increased, indicating that the addition of $CaCl_2$ facilitated the gelation process. However, the addition of more than 40 mM $CaCl_2$ caused gelation before the temperature reached 50℃ and aggregation was observed after short period.

The addition of 13.6 mM $Ca(NO_3)_2$ to acidified milk reduced onset time of the gelation to 20 min (Fig. 5B). As the concentration of $Ca(NO_3)_2$ was increased to 27.2 and 40.8 mM, the onset of the gelation was retarded to 30 and 47 min, respectively. Also, the rate of net work formation was decreased, as indicated by the rate of development of rigidity at the time intervals.

The addition of 13.6 and 27.2 mM $Ca(SCN)_2$ to acidified milk significantly retarded onset of the gelation to 55 and 160 min, respectively and reduced the degree and the rate of net work formation (Fig. 5C). Even, the gelation did not occur when more than 30 mM $Ca(SCN)_2$ was added to milk system.

According to the above data (Fig. 3A-3C), the addition of different anions to the protein system exerted profound effects upon network formation due to charge balance and conformational stability of protein(15,16). This indicates that the rate of protein-protein interactions that occured between casein micelles are varied depending upon the specific ions followed the Hofmeister series. The acidification of casein micelles reduces the repulsive forces between casein micelles at H^+ ions associate with negatively charged groups. This charge facilitates the network formation. The addition of SCN^- ion to acidified milk significantly retarded the network formation, indicating that SCN^- ion suppressed the rate of destabilization of micelles by H^+ ion and reduced the tendency of casein micelles association to form a network by screening cationic groups of casein milk system. As the anions of the calcium salt solution was changed from SCN^- to NO_3^- and Cl^- ions, the effects of anions on the tendencies of casein micelle to dec-

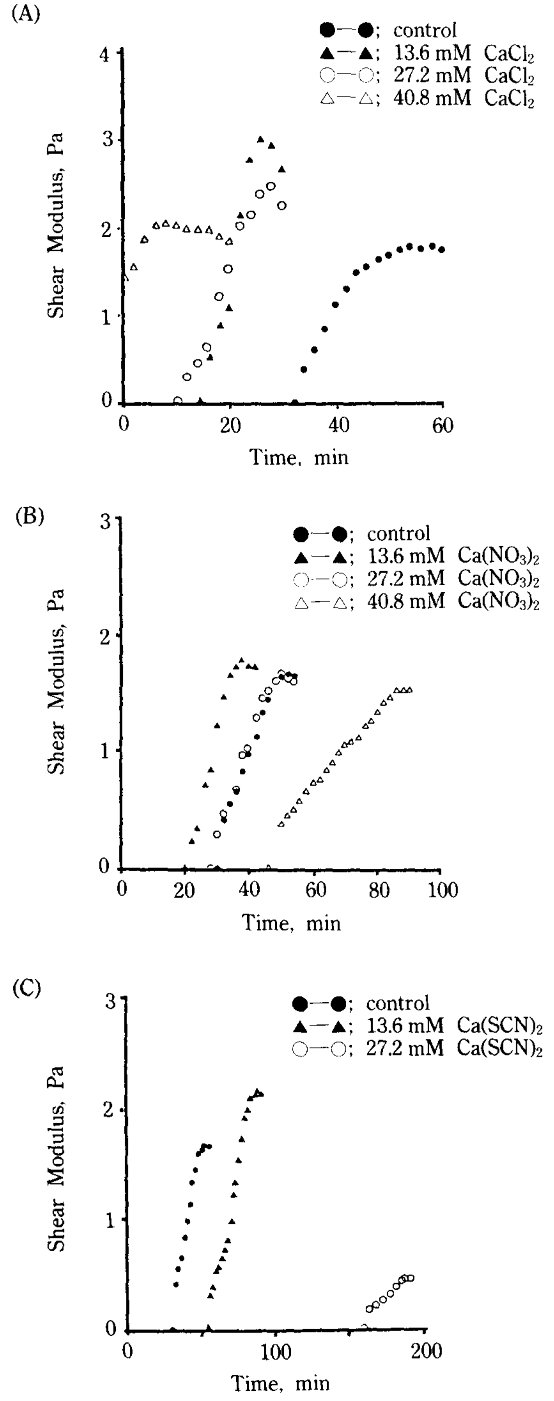


Fig. 5(A). Effect of $CaCl_2$ concentration on the development of shear modulus during sol-gel transformation of milk protein, (B) Effect of $Ca(NO_3)_2$ concentration on the development of shear modulus during sol-gel transformation, (C) Effect of $Ca(SCN)_2$ concentration on the development of shear modulus during sol-gel transformation

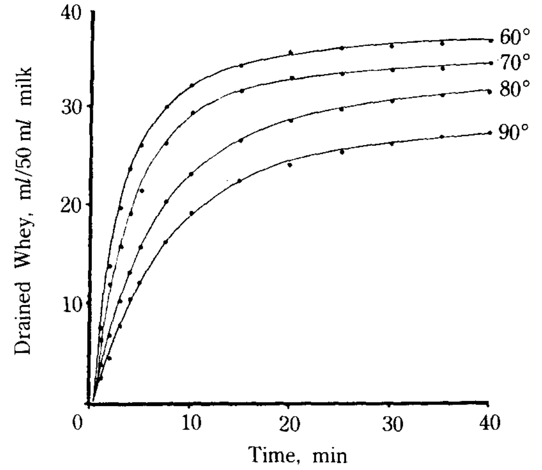


Fig. 6. Separation of whey by draining acidified milk gel obtained at different temperatures for varying periods of time

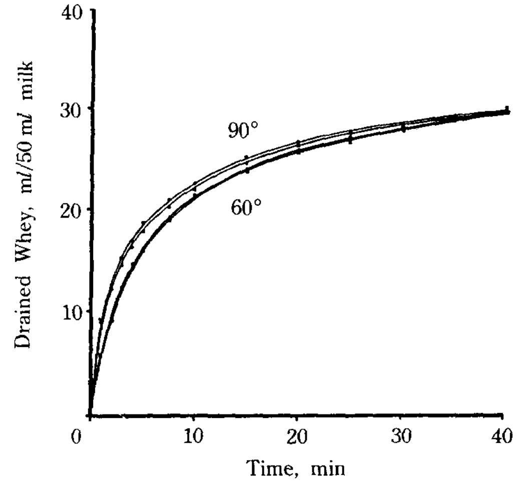


Fig. 7. Effect of preheating (90°C for 5 min) on susceptibility of syneresis in acidified milk obtained at different temperatures

rease the network formation was diminished (Fig. 5A-5C), suggesting that most contribution of Cl^- ion on the network formation. Thus, the process of gelation during acidification is influenced by electrostatic potential for aggregation. That is, the high sensitivity· of the rate of network formation to ions as reflected by rigidity development partly reflects the degree of electrostatic repulsion between protein molecules.

Effect of temperature and pH on gel syneresis and firmness

The initial rate of the whey drainage and the volume

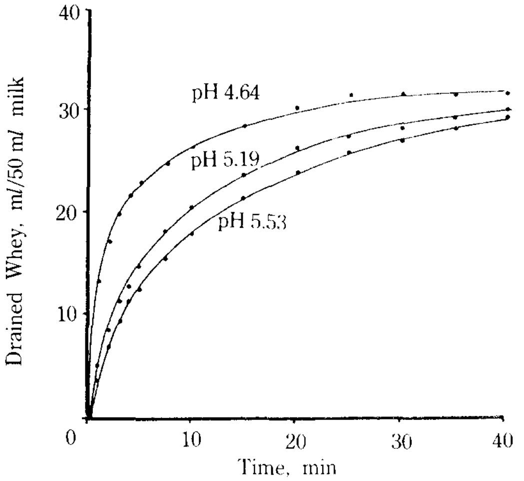


Fig. 8. Separation of whey by draining acidified milk gel obtained at different pH

of whey drained at the end of the test could serve as arbitrary measures of the susceptibility to syneresis (12). The effect of temprature on susceptibility to syneresis of milk gel is shown in Fig. 6. In the drainage test, approximately 60% of the whey was drained within 40 min. The separation of whey proceeded at a rapid rate during the first 10 min and then followed by a slower rate for the rest of period. The drainage test showed the susceptibility to syneresis was greater in the gel made at lower temperature (60°C) than at higher temperature (90°C), indicating the temperature dependence of the rate of syneresis. The gel made at higher temperature (90°C) had a higher firmness (41 gf) and showed a more compact type structure, compared to the gel made at low temperature (60°C), which showed a lower firmness (17 gf) and produced rubber-like weak bodied gel.

The preheating effect (90°C for 5 min) on the rate of syneresis is shown in Fig. 7. Syneresis change for preheated milk had the same profiles as the Fig. 6 at all heating temperature ranges, and showed no difference in gel structure and firmness. Heat treatment before acidification causes interactions between β-lactoglobulin and casein which alter micelle aggregation(17). This structure change in preheated milk affected the rate of network formation, firmness(9) and syneresis profile (Fig. 7), but susceptibility to syneresis of preheated milk was not affected by heating temperatures during acidification.

Compared to gel made from different acidification pH, allowed to develop varying amounts of H^+ ion, was apparently different in susceptibility to syneresis (Fig. 8). Acidification of milk at pH 5.53 showed lower susceptibility to syneresis and produced rubber-like weak bodied gel. As the acidification pH decreased, susceptibility to syneresis gradually increased and showed the maximum value at pH 4.64. At pH 6 no gel formation was observed.

요 약

0.1 N HCl을 이용하여 빠른 산성화를 시켰을 때에 우유의 응고속도와 그 정도는 온도와 pH에 따른 점도 변화와 혼탁도 변화로서 측정하였고, DGL을 이용하여 천천히 산성화 시켰을 때에 우유의 겔화 과정은 rheological scanner로서 측정하였다. 우유 casein의 응고는 pH 5.0에서의 돌연한 점도증가로서 나타났으며 산에 의한 응고형성의 기점 pH로 삼았다. 산에 의한 응고속도는 20°C에서 50°C로 온도가 높아질수록 빨라졌고 최대 응고속도는 pH 5.0에서 일어났다. 염($CaCl_2$)의 첨가는 모든 온도범위에서 최대 응고속도를 감소시켰으며 응고가 시작되는 pH 및 최대 응고속도가 일어나는 pH를 변화시켰다. 천천히 산성화 동안에 생성되는 겔의 초기 형성시간과 조직 형성속도는 Cl 이온에 의해 촉진되고 SCN 이온에 의해 억제되었다. 겔에서 생겨나는 syneresis 현상은 저온으로 갈수록, pH가 4.6에 접근할수록 심했으며, 90°C에서 5분 동안 열처리한 우유에서는 가열온도(60~90°C)에 상관없이 일정하게 나타났다.

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