Monitoring of Making Cheese Process by Acoustic Non-linear Parameter

Takahisa NISHIZU and Yoshio IKEDA

Agricultural Process Engineering Laboratory
Graduate School of Agricultural Science, Kyoto University
Sakyo-ku, Kyoto 606-01, JAPAN

INTRODUCTION

Qualities of cheese are influenced by coagulation or gelation process of raw milk with addition of an enzyme. The cutting time for the curd plays the most important role in the cheese making process. The milk coagulation information is useful for making cheese qualities stable or for controlling their physical properties. Some attempts for a monitor of a milk coagulation have been made by the cheese industry. The measurement of the milk viscosity or stress-strain relation during the coagulation is the most simple method among them. But this method is not acceptable by the cheese industry because it is a destructive way. Recently a hot-wire method, a near-infrared spectroscopy and an optical technique with light scatter in medium have been reported and some of them are applied in the cheese industry.

Utilization of ultrasonic technique has been also tried. This method does not need the expensive instruments and the complicated measurement system. Ultrasonic wave invades through the milk curd more deeply than light wave. Therefore the ultrasonic method should give the interior information of the milk curd. The acoustic velocity and the absorption coefficient are often used as the acoustic parameters of food stuff. But these parameters are not available to monitor a state of milk coagulation because they reflect only static properties of water that is main component of the milk curd and they do not change during the milk coagulation.

In order to solve this problem, we applied an acoustic non-linear parameter B/A to a monitor of milk coagulation and examined its possibility.

ACOUSTIC PARAMETER

Acoustic Wave

Sound is a longitudinal wave. Condensation and rarefaction of density are induced by sound pressure and they propagate spatially through material. Therefore sound propagation phenomena expresses the pressure-density relation of the medium. Generally speaking, only two thermodynamic state variables can change independently. Using density and entropy as two independent parameters, pressure can be expressed as the following equation :

$$P = P(\rho, s) \tag{1}$$

Since this local compression-expansion cycles very fast, we can regard this cycle as adiabatic change. Consequently from Eq. (1) sound pressure depends on only density. If Eq. (1) is expressed in Taylor's series around ρ_0, the following equation is obtained:

$$P = P(\rho_0) + \frac{\rho - \rho_0}{1!}\left(\frac{\partial P}{\partial \rho}\right)_{s,\rho_0} + \frac{(\rho - \rho_0)^2}{2!}\left(\frac{\partial^2 P}{\partial \rho^2}\right)_{s,\rho_0} + \frac{(\rho - \rho_0)^3}{3!}\left(\frac{\partial^3 P}{\partial \rho^3}\right)_{s,\rho_0} + \cdots \quad (2)$$

where P, ρ and s are pressure, density and entropy respectively, and the suffix "0" indicates equilibrium value.

Sound Velocity

The terms higher than the second order in Eq. (2) are neglected because the first order term is much larger than the second one for liquid or solid medium :

$$P = P(\rho_0) + \frac{\rho - \rho_0}{1!}\left(\frac{\partial P}{\partial \rho}\right)_{s,\rho_0}$$

$$\Leftrightarrow \quad P = P(\rho_0) + \frac{\lambda}{\rho}(\rho - \rho_0) \quad (3)$$

This is a linear elastic equation. We now substitute this relation into a wave equation of an isotropic elastic body. As a result the following equation is obtained:

$$c = \sqrt{\frac{(\lambda + 2\mu)}{\rho_0}} \quad (4)$$

where c, λ, μ and K are sound velocity, two Lame constants and bulk modulus respectively. For liquid medium $\lambda \doteqdot 0$, and for food stuff such as gel $\mu << \lambda$. Therefore Eq. (4) can be approximated by the following equation :

$$c = \sqrt{\frac{\lambda}{\rho_0}} = \sqrt{\frac{K}{\rho_0}} \quad (5)$$

These calculated values agree with the experimental results. This shows that sound velocity is a linear elastic parameter.

Acoustic Non-linear Parameter B/A

Neglecting the terms higher than the third order in Eq. (2) gives the following equation :

$$P = P(\rho_0) + \frac{\rho - \rho_0}{1!}\left(\frac{\partial P}{\partial \rho}\right)_{s,\rho_0} + \frac{(\rho - \rho_0)^2}{2!}\left(\frac{\partial^2 P}{\partial \rho^2}\right)_{s,\rho_0}$$

$$\Leftrightarrow \quad P = P(\rho_0) + \rho_0\left(\frac{\partial P}{\partial \rho}\right)_{s,\rho_0}\left(\frac{\rho - \rho_0}{\rho_0}\right) + \rho_0^2\left(\frac{\partial^2 P}{\partial \rho^2}\right)_{s,\rho_0}\left(\frac{\rho - \rho_0}{\rho_0}\right)^2 \quad (6)$$

We call the coefficient of the first and second order in Eq. (6) as A and B respectively. The acoustic non-linear parameter B/A is defined as a ratio of B/A :

$$\frac{B}{A} = 2\rho_0 c_0\left(\frac{\partial c}{\partial P}\right)_{s,\rho_0} \quad (7)$$

This parameter indicates the degree of non-linearity of the pressure-density relationship. B/A is one of the acoustic parameters and a material constant according to the definition.

Measurement of B/A

Eq. (7) shows that B/A is given by measuring the change of sound velocity with the adiabatic or isoentropical changes of pressure. It is difficult to change the hydraulic pressure isoentropically in the laboratory.

We can expand Eq. (7) into the following equation :

$$\frac{B}{A} = 2\rho_0 c_0 \left(\frac{\partial c}{\partial P}\right)_{T,\rho_0} + \frac{2c_0 T\varepsilon}{C_P}\left(\frac{\partial c}{\partial P}\right)_{P,\rho_0} \tag{8}$$

where T, ε and C_p are absolute temperature, coefficient of thermal expansion and specific heat respectively. The first and second terms represent B/A in an isothermal process and in a constant-pressure process respectively. They can be measured in the laboratory. Because the second term is much smaller than the first one, the second term can be neglected and the following equation is obtained :

$$\frac{B}{A} \approx 2\rho_0 c_0 \left(\frac{\partial c}{\partial P}\right)_{T,\rho_0} \tag{9}$$

Eq. (9) shows that the changes of sound velocity when applying the hydraulic pressure to the medium at the constant temperature give an approximate value of B/A.

Fig. 1 shows the sound velocity of water changes under various pressure. This relation is linear and its slope corresponds to $\partial c / \partial p$.

MONITOR OF MILK COAGULATION PROCESS

Since B/A is a material constant, B/A should have some informations to identify the medium. Fig. 2 shows the relationship between milk fat content and B/A of raw milk. Milk fat content can be predicted by interpolating the B/A value into this linear relation.

B/A is very sensitive to the state of water. Water in liquid state consists of icelike structures and free water. B/A of bound water and free water are about 0.4 and 8.0 respectively. When water's temperature is decreased, the B/A value also decreases. This is because the amount of bound water increases with the decrease of temperature.

A milk coagulation process has two phases. In the first phase there is the enzymatic hydrolysis of κ-casein into the insoluble para-κ-casein and casein-macropeptide. In the second phase the caseinmicelles are aggregated together into the curds. If the state of water in milk changes during the milk coagulation process or if the pressure-density relation of milk changes during forming the caseinmicelle networks in milk, the state of milk during coagulation can be determined quantitatively by the measurement values of B/A.

EXPERIMENT

B/A Measurement System

Fig. 3 shows the experimental setup. In the pressure chamber there are an ultrasonic transducer(PANAMETRICS/V303, ϕ12.7mm, 1MHz) and a reflector. We measured a flight time and a path length for an ultrasonic pulse to go and return back, and then determined a sound velocity with them.

We put 90 ml of milk into the pressure vessel and left it in a constant-temperature bath(Tokyo-Rika-Kikai/NTT-1200, control accuracy ±0.02K) for a while. After that we measured the sound velocity with increasing the inner pressure by putting high pressured nitrogen into the chamber. From these data we calculated dc/dp and then determined B/A. The applied pressure should be as small as possible because the pressurize process may have some effects to the medium. In this study the maximum pressure was 1MPa(gauge pressure) in consideration of the accuracy of measurement.

Materials

10g of skim milk(Snow Brand, Co.) were dissolved in 90g of calcium chloride solution ranged from 0.001M to 0.02M.

We used pepsin(Nacalai Tesque, 10000IU/g) as enzyme for milk coagulation and prepared 4, 20 and 50IU/ml of pepsin solution. In our experiments 0.9ml of pepsin solution were added into 90ml of skim milk solution. The temperature of pepsin solution was kept as same as milk temperature in order to prevent the temperature changes of milk after addition of enzyme.

Viscosity Change of Milk during Coagulation

In a preliminary test we traced the change of milk viscosity after addition of pepsin with a rotor-type viscometer(TOKIMEC/B8L). As a result the data varied widely and were not reproducible. This is because the rotating rod destroyed the curd in measuring the viscosity. Therefore we concluded that it is difficult to measure the reliable values of viscosity. In this report we put a wooden stick into the coagulated milk and judged subjectively the state of coagulation from the nature of matter adhered to the stick. After the beginning of milk coagulation, the curd is formed and its hardness increases with time. In this report we focused on verifying whether B/A changes or not during the curd-hardening process(phase 2).

RESULTS AND DISCUSSION

Repeatability of Measurement

The mean sound velocity and the mean B/A of ten samples were 1519.88m/s and 4.83 respectively for distilled water at 308K. Their coefficients of variation(CV) were 0.0038% and 0.279% respectively. In this study these repeatabilities were permissible to examine the experimental results.

Concentration of Pepsin Solution

The concentration of enzyme has some effects on the start time of milk coagulation, the hardening rate of curd and the hardness of curd. We added 4, 20 and 50IU/ml of pepsin solution into the skim milk and measured the changes of B/A with time. The results are shown in Fig. 4. B/A decreased immediately after adding the pepsin solution into the milk and then began to increase slowly. A couple of minutes later the value recovered to the previous level 5.01(ave.). We could not find the difference of B/A values for these 3 cases. The milk began to coagulate 2.5-3min after adding pepsin solution into milk and then continued to become hard. This result means that B/A is not effected on a state or hardness of coagulated milk in a caseinmicelle coagulation process(phase 2).

Concentration of Calcium Chloride Solution

The composition of milk has some influences on the phenomena of milk coagulation too. Especially the amount of calcium ion plays an important role in a milk coagulation process. Casein can not be clotted with little amount of calcium ion. If the concentration calcium ion is increased, the milk coagulation begins earlier and the clotting rate becomes faster and the curd becomes harder. We added 0.01, 0.005 and 0.001M of calcium chloride solution into milk and measured their B/A values with time. We could not find the differences of the B/A values among these three samples(Fig.5). Two samples prepared with 0.01 and 0.005M of calcium chloride solution began to coagulate within 2.5-3min after adding enzyme into them. Meanwhile the remaining sample didn't coagulate within 60min. These results show that B/A keeps constant in a second phase of milk coagulation and its value does not depend on the state of coagulation.

Milk Temperature

A start time of milk coagulation and a hardening rate of milk curd depend on milk temperature. The results are shown in Fig. 6. The shapes of line are almost same as the above-mentioned results. But a required time for milk coagulation was about 4 minutes at 298K and larger than at 308K. B/A at 298K was 4.77(ave.) and smaller than at 308K.

Sound Velocity

The changes of sound velocity with time are shown in Fig. 7. Under a constant temperature, B/A keeps constant before and after adding enzyme into milk. This means that the bulk modulus of milk dose not change during its coagulation. And its sound velocity is near to water's sound velocity. This shows that the sound velocity of coagulated or non-coagulated milk reflects the static properties of water which is a main component of milk. At the lower temperature the sound velocity of milk becomes lower. In such a case sound velocity of water becomes smaller too. These results support that the sound velocity of water in milk is a dominant factor to determine the sound velocity of milk.

B/A Changes Immediately After Addition of Pepsin Solution

B/A of milk decreased once immediately after addition of pepsin solution in every experimental condition. B/A of water decreases when its temperature is decreased. This is because the decrease in temperature increases the amount of bound water whose B/A is smaller than of free water. This decrease of B/A may be caused by small decrease in temperature of sample.

We added pepsin solution into distilled water instead of skim milk and measured the change of B/A with time right after the enzyme addition. This was a control test in order to examine whether the decrease of B/A was caused by the decrease in temperature or not. The result is shown in Fig. 8. Both before and after addition of pepsin solution, B/A had no changes and maintained constant. As a result some phenomena given by mixing skim milk and enzyme solution must cause the decrease of B/A.

The decrease of B/A is happened in the first phase. The milk coagulation began about 3 min after addition of pepsin solution for most of samples. But we could not catch the state changes of milk in the first phase with the wooden stick. Therefore we could not relate the decrease of B/A with any particular cause.

CONCLUSIONS

We measured the acoustic non-linear parameter B/A of milk during its coagulation in the cheese making process. As a result we got the following findings.

1) The coefficient of variation(CV) in measuring B/A was 0.279%.
2) The addition of pepsin solution into milk did not change sound velocity of

milk.

3) Immediately after the addition of pepsin solution, B/A of milk decreased rapidly and then recovered to the previous level.
4) In a curd-hardening process called as the second phase, B/A of milk curd was as same as B/A of skim milk.
5) B/A of milk curd did not depend on the hardness of curd.

In this report we focused on the second phase of a milk coagulation and examined the relationship between the changes of B/A and phenomena in forming milk curd. In conclusion it is difficult to monitor the milk coagulation in the second phase. But in the first phase we observed the rapid decrease of B/A. Therefore we may apply this decrease of B/A to a monitor of an enzyme reaction in the first phase of milk coagulation.

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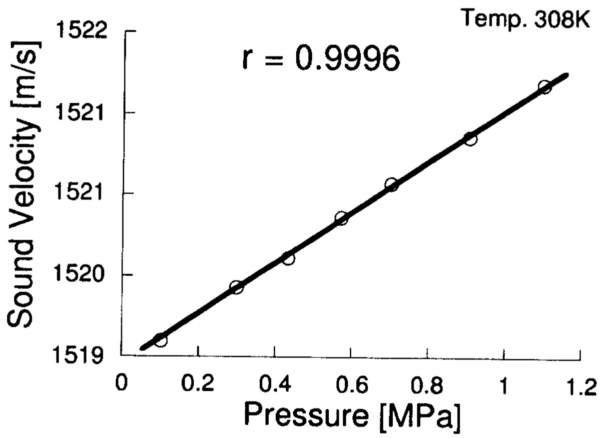

Fig. 1 Relationship between Sound Velocity of Distilled Water and Atmospheric Pressure.

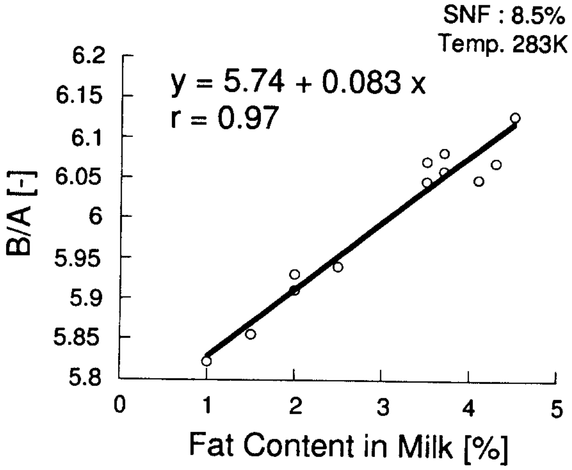

Fig. 2 B/A as Function of Fat in Cow Milk.

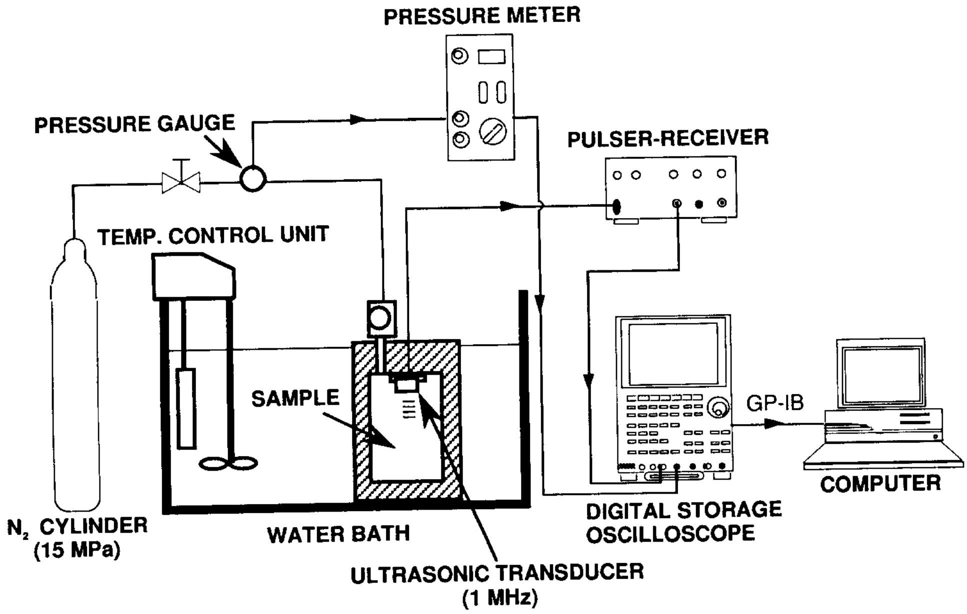

Fig. 3 Schematic Diagram of Experimental Setup for Measuring Acoustic Non-linear Parameter.

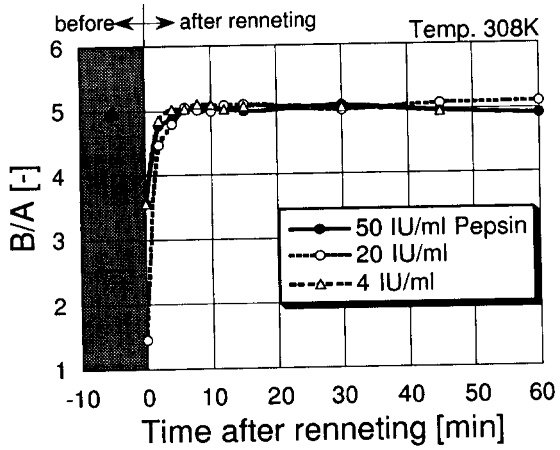

Fig. 4 B/A of Milk during Coagulation at Different Pepsin Concentrations.

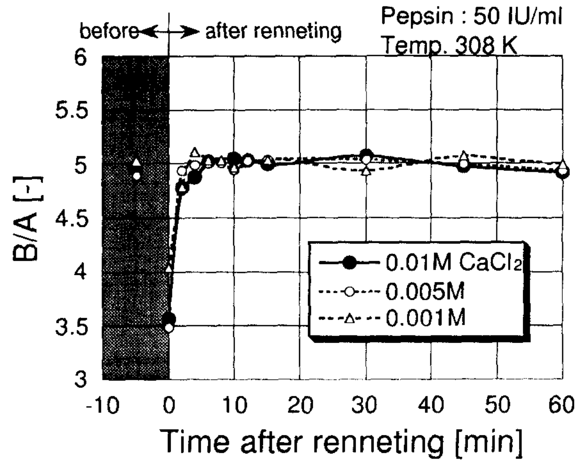

Fig. 5 B/A of Milk during Coagulation at Different Calcium Chloride Concentrations.

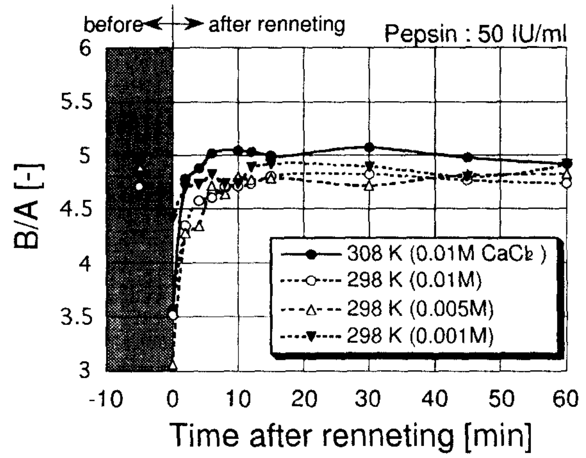

Fig. 6 B/A of Milk during Coagulation at Different Temperatures.

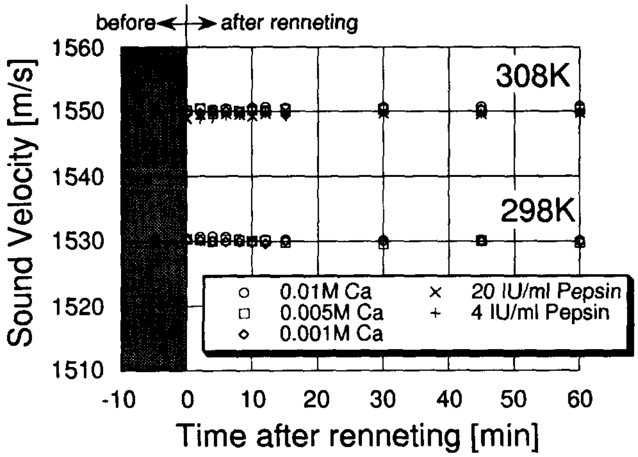

Fig. 7 Sound Velocity of Milk during Coagulation at Different Pepsin Concentrations, Different Calcium Chloride Concentrations and Different Temperatures.

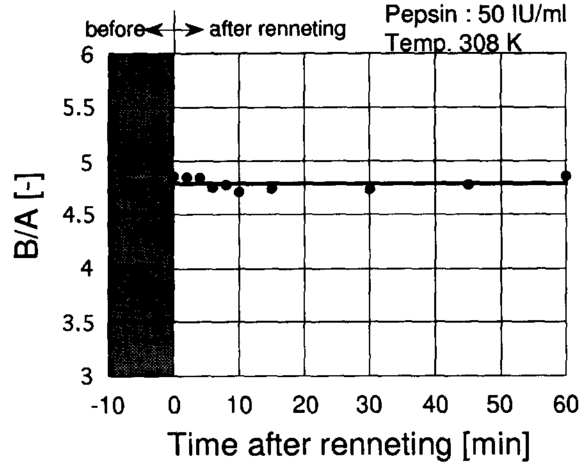

Fig. 8 Change of Water's B/A by Addition of Pepsin.