ULTRASONIC CHARACTERISTICS FOR GEL FORMATION IN THE CALIFORNIA MASTITIS TEST

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1. INTRODUCTION

Mastitis is one of the most serious and costly diseases affecting dairy cow production (Heringstad et al., 2000). Measurement of the somatic cell count (SCC) in raw milk is widely accepted as the most useful indirect indicator of mastitis and milk quality. SCC actually only can be counted in laboratories. To achieve a faster, but less accurate result, some farmers use a simple, inexpensive, cow-side test, known as the California Mastitis Test (CMT) (Schalm et al., 1957), to provide a qualitative estimate of SCC in the foremilk of individual cows or quarters. CMT is an indirect method to detect infected quarters on the farm based upon the reaction of special detergents with DNA of somatic cells and increasing the viscosity of the mixture which is proportional to the SCC, and an evaluation of the degree of gel formation is done by gently rotating CMT paddle. However, the interpretation can be subjective, and this might result in false positives and negatives.

CMT was mechanized by a ball viscosimeter (Tolle et al., 1976). Fast analysis devices, or systems, would be highly desirable as they would allow an on-line response to the changes in media components. However, most on-line systems currently used have a low accuracy. The rheology of the gel formed in the CMT was investigated by using both capillary and rotational viscometry (Verbeek et al., 2008) and found that the gel is non-Newtonian, but the initial phase of viscosity increase was not due to shear dependence, but rather due to the gelation reaction. It can therefore be deduced that the rheology of the gel is complicated not only by it being non-Newtonian, but also by the strong dependence on test conditions. These make designing a successful sensor much more challenging.

On this background, ultrasound is becoming an alternative technique for the on-line monitoring of mastitis based on CMT. Most important features of ultrasonic systems are robustness, non-invasiveness, precision, low cost, rapidity and easy automation. Furthermore, ultrasound can be used to analyze opaque materials, offering an alternative to electromagnetic waves based devices. Recently, on-line ultrasonic techniques have been used for monitoring alcoholic fermentations (Becker et al. 2001; Resa et al. 2004), dairy fermentations (Elvira et al. 2002), dough fermentations (Elmehdi et al. 2003) and Escherichia coli growth (Reddy et al. 2001; Sierra et al. 2010).

Understanding the ultrasonic properties of the CMT gel is important in the design and operation of the automated CMT. The purpose of this paper is to investigate ultrasonic characteristic of the CMT gel formation and to determine whether a reliable and inexpensive automated on-line SCC detector based on CMT might be developed. Therefore, in this work, measurements of ultrasonic velocity and attenuation in the CMT gel have been carried out and used to understand ultrasonic velocity and attenuation changes.

2. APPARATUS AND METHOD

An ultrasonic waveform (4 cycles) acquired is shown in fig. 1. The first pulse wave, peak 1a, was the input wave and the second pulse wave, peak 2a, was the output wave. Then the next incoming wave is the input wave obtained by the first pulse wave in fig. 1. From the waveform signal data, the ultrasonic velocity (c, m/s) and attenuation (α, neper/m) of the CMT gel were calculated as follows (Ay et al., 1994):

$$c = 8d/t_r \quad (1)$$

$$\alpha = [1/(2d)] \ln \Delta V \quad (2)$$

where $t_r = \frac{1}{2}[(t_{5a}-t_{1a}) + (t_{5b}-t_{1b})]$, d= distance traversed by the ultrasonic pulse (m), and

$$\Delta V = \frac{1}{8}\left[\frac{V_{1b}}{V_{2b}} + \frac{V_{2b}}{V_{3b}} + \frac{V_{3b}}{V_{4b}} + \frac{V_{4b}}{V_{5b}} + \frac{V_{1a}}{V_{2a}} + \frac{V_{2a}}{V_{3a}} + \frac{V_{3a}}{V_{4a}} + \frac{V_{4a}}{V_{5a}}\right]$$

where V_{1a}, V_{1b}, etc. are signal amplitudes (V) and t_{1a}, t_{1b}, etc. are the time (s) corresponding to different peaks in fig. 1.

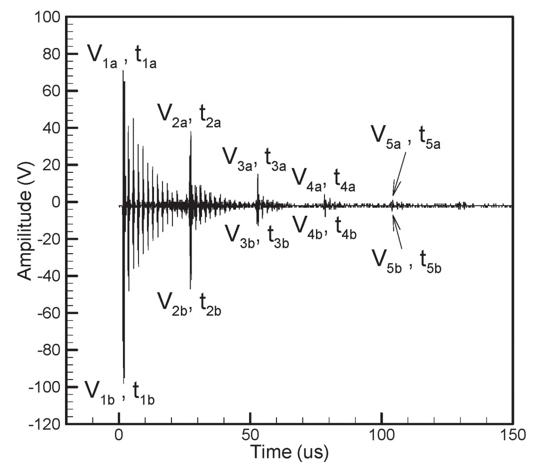

Figure 1. Waveform of ultrasonic pulse travelled through the sample

An ultrasonic pulse echo experimental set up was used. The system was comprised of one 1-MHz central frequency ultrasonic longitudinal wave transducers (Model P/N V314, Panametrics), an ultrasonic pulser-receiver (Model 5072PR, Panametrics), a digital storage oscilloscope (Model 2430, Tektronix) and, via a general purpose interface bus (GPIB), stored in a computer equipped with analysis software to obtain the time of flight, which is the time taken by the ultrasonic pulse to travel through the sample.

The transducer was mounted in the one side of the circular cross section of a cylinder sample tank submerged in water bath at 30±0.1℃ in order to minimize the influence of the temperature on the measurements. The traversing distance between the transducer and the reflective surface of the cylinder was 1.9 cm. Raw milk (6ml) was combined 1:1 with an anionic surfactant (SDS: sodium dodecylsulphate, 0.1g/ml, in distilled water). All experiments were replicated three times. The ultrasonic signals were collected continuously after CMT reagent addition for 10 seconds.

3. RESULTS

The ultrasonic velocity and attenuation in CMT milk gel were measured at SCC=72.5(10^3/ml), 30℃ and f=1MHz as shown in fig. 2 to fig. 3, separately. Dashed lines are linear fitting of the experimental data. These preliminary results indicated that ultrasonic velocity and attenuation increased with time duration in CMT milk, which is sensitive to the degree of mixing during gelation.

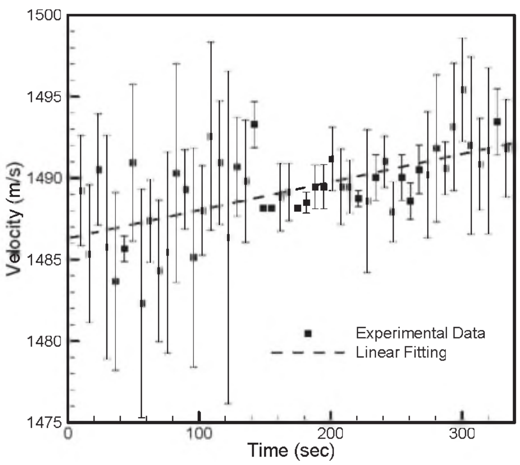

Figure 2. Ultrasonic velocity in CMT milk, at SCC=72.5 (10^3/ml), T = 30°C and f = 1 MHz.

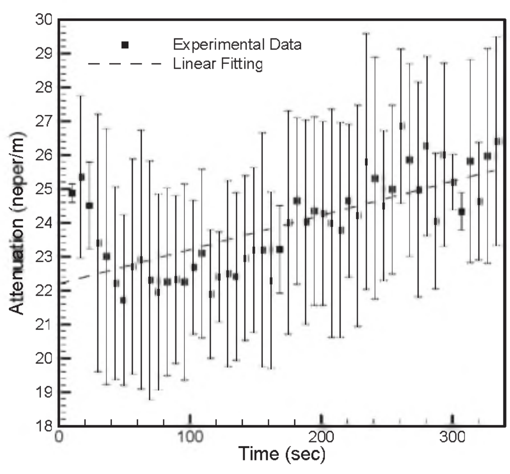

Figure 3. Ultrasonic attenuation in CMT milk, at SCC=72.5 (10^3/ml), T = 30°C and f = 1 MHz.

4. DISCUSSION AND CONCLUSIONS

Due to the more DNA dissolved out of cells depended on the degree of mixing during gelation, the gel viscosity increases with the mixing duration, which induces the increase in the velocity. Results also demonstrate that attenuation increases with mixing duration, probably due to the friction of ultrasound propagated in gelation obviously affected by the gel viscosity, which may tend some ultrasonic energy to be adsorbed and tuned into heat.

However, attenuation is decreased in the initial state and then increased after about 50 seconds. The main reason is that CMT gel is non-homogeneous mixing, which is caused sediment phenomenon by gravity effect without any shear application at the beginning of measurement. Then, mixing effect is continued to increase gel viscosity during gel reaction, which induces the increase in attenuation. CMT relies on the fact that the viscosity of the non-Newtonian gel formed during the test is proportional to the SCC concentration. In this study pulse-echo ultrasonic technique is used to preliminary investigate the ultrasonic characteristics in CMT gelation. However, there is a need for further identifying for SCC concentrations relative to accuracy of CMT rank using ultrasonic technology in the future.

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