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Ohmic Heating for Milk Pasteurization:

Effect of Electric Current on Nonthermal Injury to *Streptococcus thermophilus*

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Abstract. *Experiments on milk pasteurization were conducted with cells of Streptococcus thermophilus 21072 to confirm the lethal effect of electric current using combination treatment (sublethal ohmic and conventional heating treatment) and control treatment (conventional heating treatment only). Bacteria counts and calculated decimal reduction times (D value) of combination treatment indicated that combination treatment had significantly higher lethality than that of control treatment under the condition of the same temperature histories ($p<0.05$). However, no pasteurizing effect of the electric current on bacteria cells was observed during the sublethal ohmic treatment. The results suggested that sublethal ohmic treatment decreased the heat resistance of bacteria probably due to nonthermal injury of bacteria caused by the electric current.*

Keywords. milk, pasteurization, ohmic heating, electric current, nonthermal injur

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Introduction

Food sterilization is one of the most important processes in food industries. Novel low-energy or efficient methods of sterilization continue to attract interest of food engineers. Anderson and Finklestein (1919) initially examined milk sterilization with electric fields. In recent years, ohmic heating and high-voltage pulsed electric field processes have been studied as alternative methods to conventional heating sterilization (Castro et al., 2004; Cho et al., 1999; Gilliland and Speck, 1967a, 1967b; Imai et al., 1995; Pareilleux and Sicard, 1970; Tsong, 1991).

Although the effect of electricity on microorganisms has been studied, the thermal (ohmic heating) effect of electricity has never been satisfactorily explained. Microbial inactivation during low-voltage electrical treatment has been attributed primarily to heating since the interaction between electric fields and microorganisms has not been elucidated.

Palaniappan et al. (1992) reported that there was no difference between the effects of ohmic heating and conventional heating under the condition of identical thermal histories on the death kinetics of yeast cells. They found, however, that mild electrical pretreatment of *Escherichia coli* decreased the subsequent inactivation requirement in certain cases. In other studies, an inactivation effect on bacteria at sublethal temperatures was observed using an alternating low-voltage current (Pareilleux and Sicard, 1970; Shimada and Shimahara, 1985). Cho et al. (1999) found that the kinetics of inactivation of *Bacillus subtilis* spores can be accelerated by ohmic treatment. Recent studies suggest that mild electroporation might occur during ohmic heating and high-voltage electrical treatment (Imai et al., 1995; Kulshrestha et al., 1999; Yoon et al., 2002). Yoon et al. (2002) reported that leakage of intracellular constituents of *S. cerevisiae* is greater under the condition of ohmic heating than under the condition of conventional heating in boiling water. We reported that death rates of viable aerobes and *Streptococcus thermophilus 2646* resulting from ohmic heating indicated that ohmic heating had significantly higher lethality than that of conventional heating under the condition of the same temperature histories ($p < 0.05$) (unpublished data).

There is little information on the effect of the electrical current during ohmic heating on microbes. Accordingly, the aim of this study was to determine the effect of electric current on pasteurization of microorganisms during ohmic heating.

Materials and methods

Bacterial strains and materials

Streptococcus thermophilus 21072 was cultivated in 12.6% (in weight) skim milk and the culture was incubated at 37°C for 48 h. The concentrated culture was suspended in commercial whole milk (purchased at a supermarket in Sapporo, Hokkaido, Japan) to acquire ca. 10^7 cells/mL.

Experimental setup

The ohmic heating setup consisted of a wide-band function generator (NF Electronic Instruments, FG-143, Japan), a precision power amplifier (NF Electronic Instruments, 4510, Japan), two digital multimeters (Iwatsu SC-303A GP-IB unit, Voac7411, Japan), a data logger (Eto Denki co. Thermodac model 5020A, Japan), a computer, and a heating unit (Fig.1). The heating unit consists of titanium (Ti) electrodes for contact with the sample and a thermocouple (k-type) inserted in an acryl box. The distance between the two electrodes was 30 mm, and the size of each electrode contacting with the sample was 101×85 mm. Time and temperature data

were collected with a data logger linked to a computer, and the voltage and current data were collected with two digital multimeters linked to the same computer. As control, conventional heating was performed in a water bath with an aluminum vessel of 300 mL in capacity.

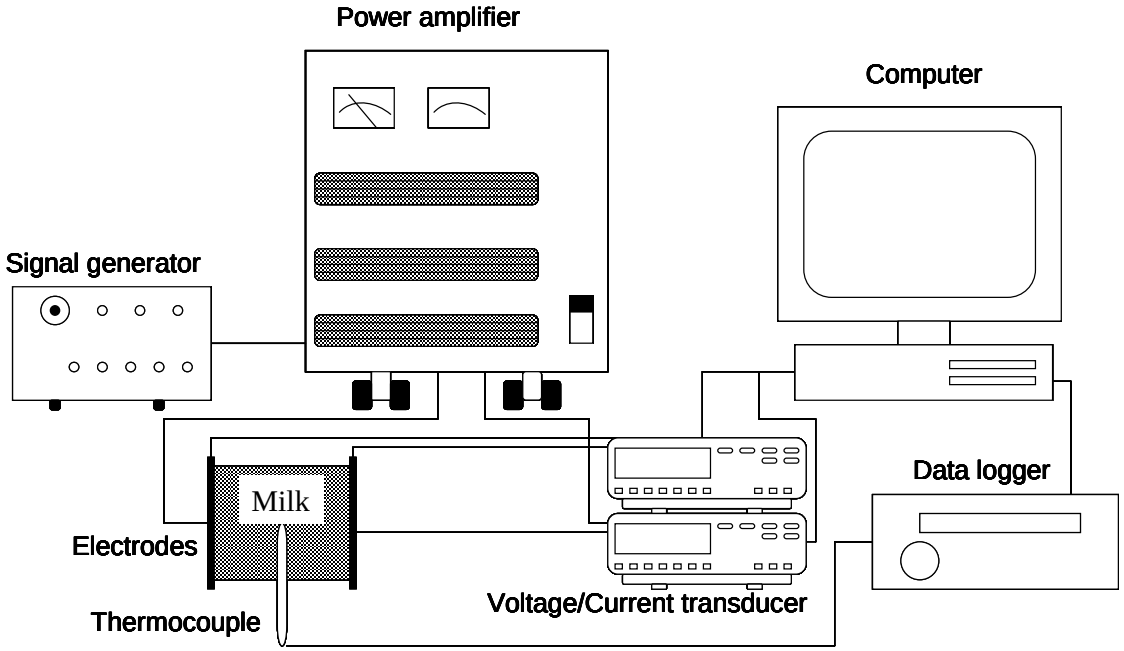

Figure1. Schematic diagram of the experimental setup.

Combination treatment and control treatment

The death rates of *Streptococcus thermophilus 21072* cultured in milk were evaluated during combination treatment (sublethal ohmic and conventional heating treatment) and control treatment (conventional heating treatment). Figure 2 shows the procedure used for combination treatment. In the case of sublethal ohmic treatment, a 250-ml milk sample was subjected to an alternating current of 7 A with a frequency of 20 kHz for 1 min until the temperature increased from 10°C to 42°C and then immediately cooled to 10°C in a cold water bath for 5 min. Changes in voltage and current during sublethal ohmic heating are shown in Figure 3. Heating and cooling histories during sublethal ohmic treatment are shown in Figure 4. To perform long sublethal ohmic treatment, the operations of ohmic heating and cooling were repeated 12 times. After the 12th sublethal treatment, the sample was immediately subjected to conventional heating for 5 min until the temperature increased to 70°C and was held at 70°C for 6 min. For control treatment, a 250-ml sample was heated for 5 min from 10°C to 70°C and was held at 70°C for 6 min. Conventional heating in the combination treatment performed by exactly the same procedure as that of control treatment in order to match the time-temperature histories within ±0.5°C. Figure 5 shows a comparison of thermal histories during conventional heating for combination and control treatments.

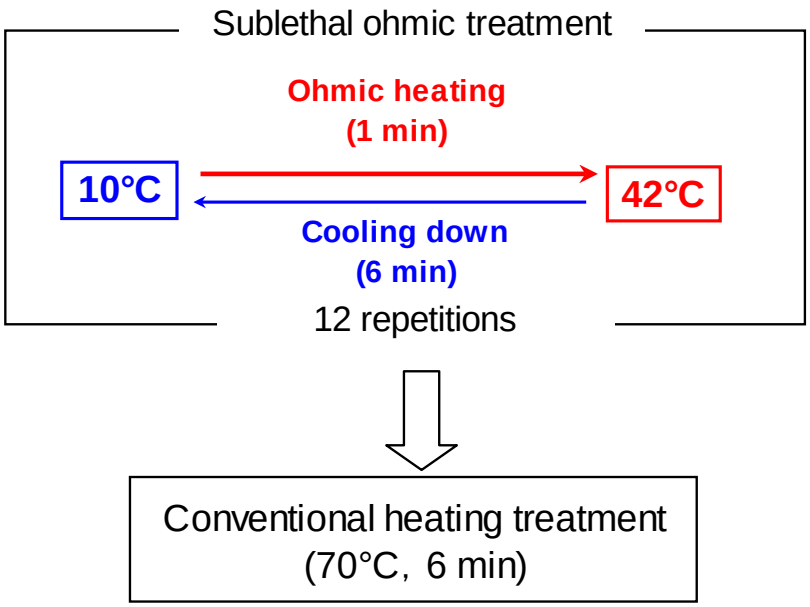

Figure 2. Procedure for combination treatment of sublethal ohmic treatment and conventional heating treatment.

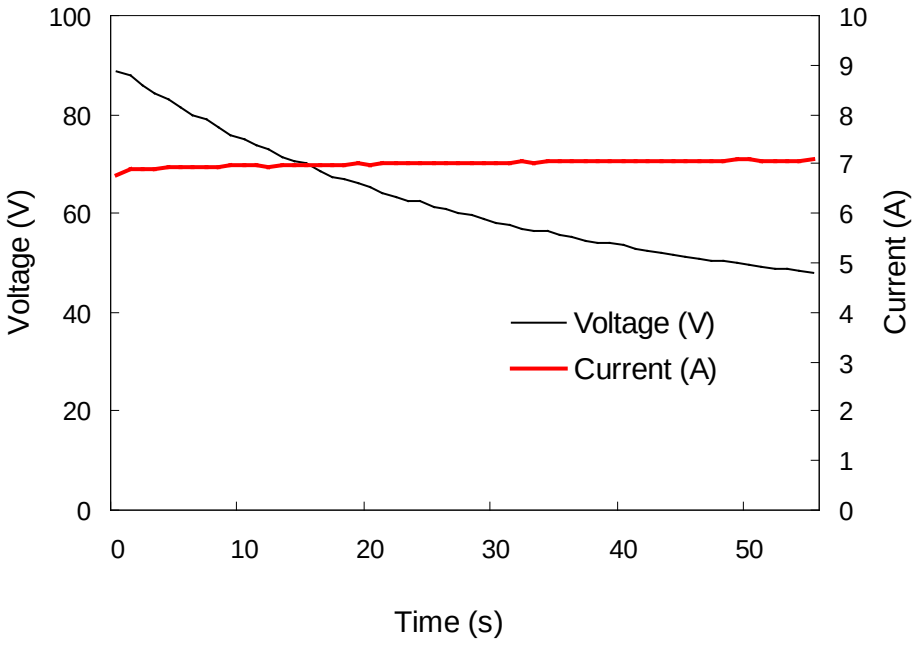

Figure 3. Voltage and current histories during sublethal ohmic heating.

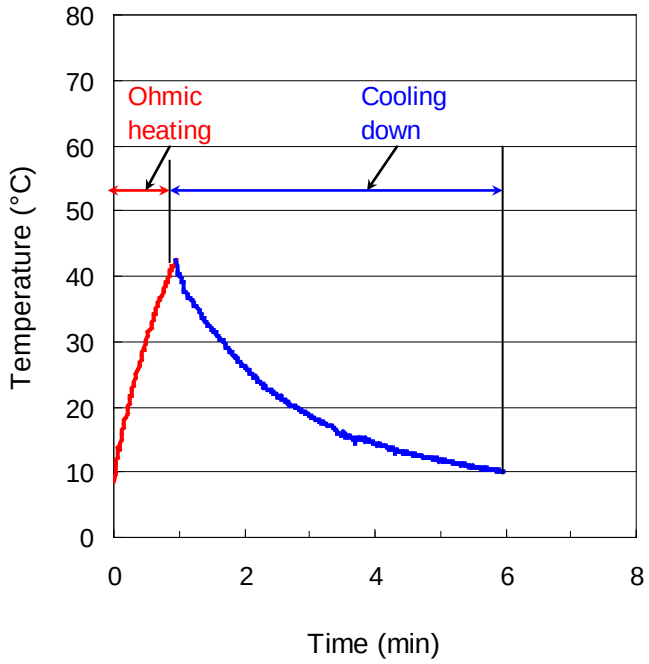

Figure 4. Heating and cooling history during sublethal ohmic heating.

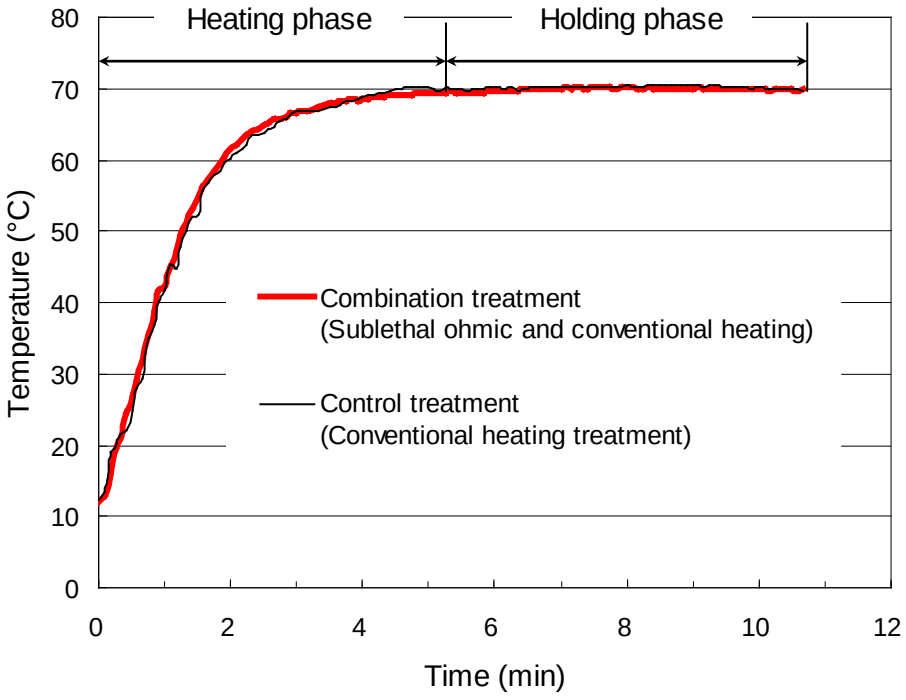

Figure 5. Comparison of temperature histories during conventional heating for combination and control treatments.

Microbiological analysis

The microbiological medium used in this study was purchased from Merck (Darmstadt, Germany). *Streptococcus thermophilus 21072* counts were determined by calculating the colonies from M17 medium containing a diluted sample (1 mL) that had been cultured at 37°C for 72 h. Five independent replications of each experiment were conducted. Decimal reduction times (D value: time in minutes required for log-cycle reduction in bacterial population) were calculated from the slope of the regression line fitted to the survival plots.

Statistics

Statistical analysis was carried out using software (MS-Excel 2000, Microsoft). All data were subjected to variance analysis and a least significant difference test to determine significant differences ($P < 0.05$) between treatments.

Results and discussion

In the holding phase in all experiments, standard deviations of temperature difference between the center temperature of the sample and set temperature were 0.19-0.21 °C (combination treatment) and 0.11-0.18 °C (control treatment). Therefore, it is clear that the temperature was appropriately controlled and that the experimental conditions of the two treatments were identical.

Representative survival counts for *Streptococcus thermophilus 21072* during combination treatment and control treatment are presented in Figure 6. The D values determined from survivor curves are shown in Figure 7. No difference in survival counts was observed between the initial samples and the samples subjected to sublethal ohmic treatment (Fig. 6). However, significant differences in survival counts and D values were found between combination treatment and control treatment during holding time. These results suggested that there was a synergistic effect of sublethal ohmic and conventional heating treatment on bacteria cell death. It is possible that sublethal ohmic treatment decreased heat resistance of bacteria, since no cell death was observed during sublethal ohmic treatment. The mechanism by which an electric current causes bacteria to be weak in heat resistance remains unclear. However, the results of this study suggest that ohmic heating enhances nonthermal injury caused by electric current on bacteria. Ohmic heating is therefore an effective method of pasteurization because of the combination of a nonthermal effect by electric current and lethal thermal effect by Joule heat generation.

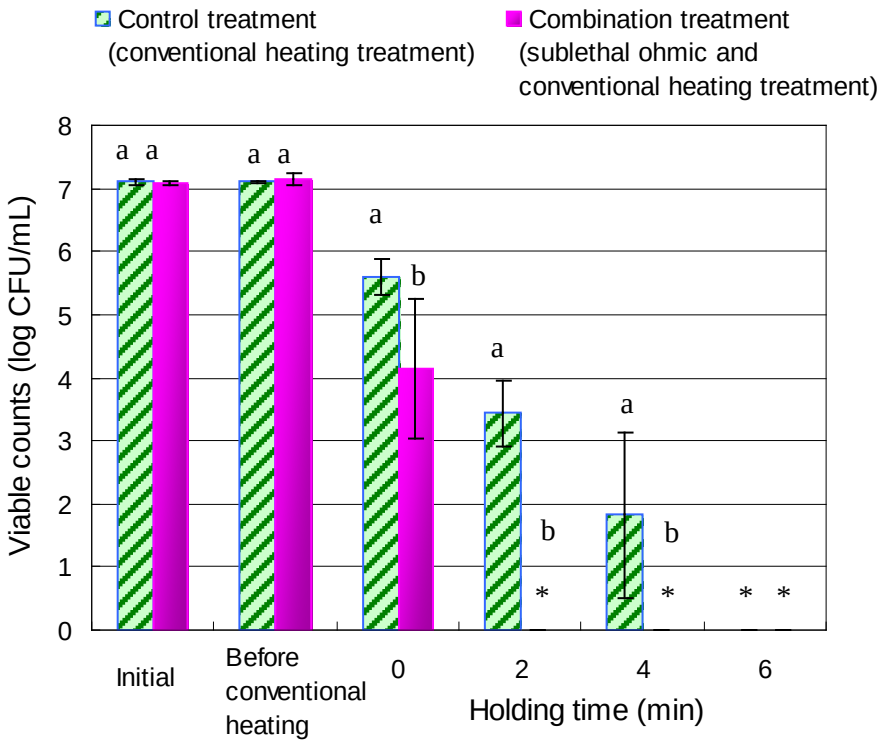

Figure 6. Comparison of survival of *Streptococcus thermophilus* subjected to control treatment and combination treatment.

*: No growth.
Initial: No treatment.
Before conventional heating: After being treated with sublethal ohmic treatment of combination treatment.
Holding time 0: The time when the samples were heated to the set temperature (70°C).

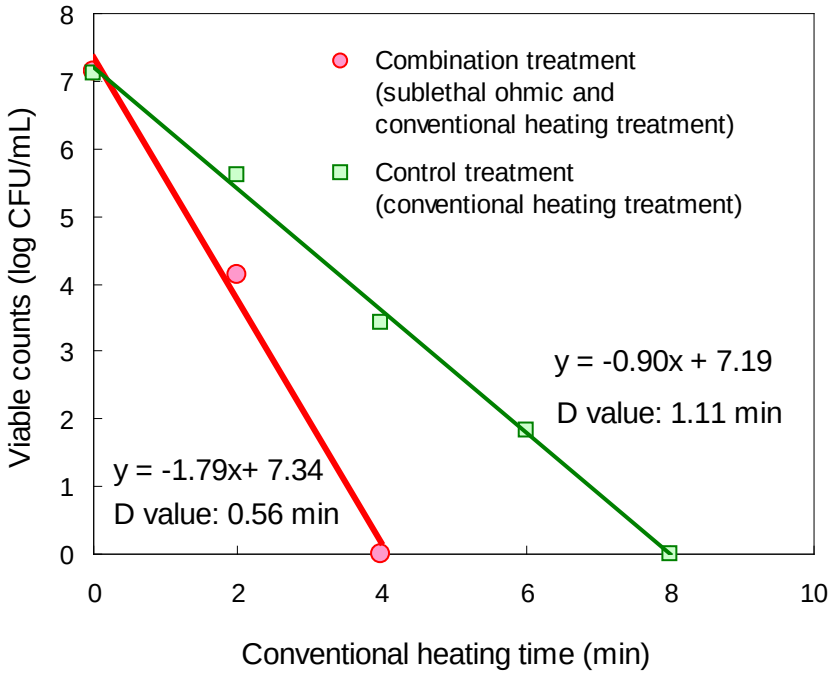

Figure 7. Regression line of heating time and *Stereptococcus thermophilus* counts.

Palaniappan et al. (1992) reported that there was no difference between the effects of ohmic and conventional heat treatments under the condition of identical thermal histories on the death kinetics of yeast cells at currents ranging from 0.5 to 1.0 A of 60 Hz. Moreover, mild electrical pretreatment of *Escherichia coli* decreased the subsequent inactivation requirement only in a few cases. The current and frequency used in this study were 7 A and 20 kHz, respectively, which were higher than those used in the experiments of Palaniappan et al. (1992). Lee and Yoon (2002) reported that intracellular protein leakage is induced by ohmic heating and that the amount of exuded protein increased significantly as the electric field was changed from 10 to 20 V/cm with a frequency of 60 Hz. In similar, more proteins are exuded when higher frequencies (60 kHz) are applied. Accordingly, an additional lethal effect of ohmic heating is possible due to the higher current and frequency. Therefore, we speculate that an effect of ohmic heating on pasteurization can be obtained with appropriate current strength and frequency. Additional studies are needed to prove this hypothesis and verify the nonthermal effect of electricity on bacteria. Further investigation is also needed to elucidate how ohmic heating enhances killing of bacteria.

Conclusions

The results of our comparative study on lethal effects on *Streptococcus thermophilus 21072* show that there was a significant difference between the combination treatment and control treatment on death rates. An electric current could not generate a lethal effect on bacteria but caused a decrease in heat resistance of bacteria due to nonthermal injury. Nonthermal injury caused by an electric current had a significant effect on killing of bacteria during thermal lethal treatment. The results suggest that the mechanism by ohmic heating kills bacteria is probably the combination of the nonthermal effect of the electric current and the lethal thermal effect.

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