

## Effects of Ohmic Heating on Microbial Counts and Denaturation of Proteins in Milk

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**The aim of this study was to compare the inactivation effects of ohmic heating (internal heating by electric current) and conventional heating (external heating by hot water) on viable aerobes and *Streptococcus thermophilus* 2646 in milk under identical temperature history conditions. The effects of the two treatments on quality of milk were also compared by assessing degrees of protein denaturation in raw and sterilized milk (raw milk being sterilized by ohmic heating or conventional heating). It was found that microbial counts and calculated decimal reduction time (D value) resulting from ohmic heating were significantly lower than those resulting from conventional heating. There was no difference in degrees of protein denaturation during the two treatments. The results suggested that ohmic heating had not only a thermal-lethal effect, but also a nonthermal-lethal effect on microorganisms, due to the electric current. Based on the results, we propose that ohmic heating can be effectively used to pasteurize milk with no additional protein deterioration.**

Keywords: milk, ohmic heating, microbial counts, decimal reduction time (D value), protein denaturation

### Introduction

Sterilization of food is one of the most important processes in food industries. In recent years, much interest has been shown in technologies utilizing electrical energy in food processing. Results of studies in this area have provided food processors with opportunities to produce new, high-quality and shelf-stable products by alternative sterilization techniques. Most previous studies in this area (Cho *et al.*, 1999; Guillou and El Murr, 2002; Pareilleux and Sicard, 1970; Uemura and Isobe, 2002, 2003) dealt with microbial inactivation. Palaniappan *et al.* (1992) reported no difference between the effects of ohmic heating (internal heating by electric current) and conventional heating (external heating by hot water) under identical thermal history conditions on the death kinetics of yeast cells. They found that mild electrical pretreatment reduced the requirement of heat for subsequent inactivation of *Escherichia coli* at certain temperatures. In other studies, a microbial inactivation effect at sublethal temperatures was observed with an alternating current (10 to 200 mA or 0 to 1200 mA) treatment (Pareilleux and Sicard, 1970; Shimada and Shimahara, 1981, 1982, 1983, 1985a, 1985b).

A number of investigators (Lebovka *et al.*, 2005; Praporscic *et al.*, 2006; Sastry, 2003; Tsong, 1991; Uemura and Isobe, 2002, 2003; Yoon *et al.*, 2002) also suggested that mild electroporation might occur during ohmic heating (less than 500 V/cm) and high-voltage electrical treatment (14 to 16.3 kV/cm). Yoon *et al.* (2002) reported that leakage of intracellular constituents in *Saccharomyces cerevisiae* was greater during ohmic heating than during conventional heating. Although the technology of ohmic heating appears to be promising and highly effective, there is limited information regarding its effects on specific food products compared with effects of conventional pasteurization (Leizerson and Shimoni, 2005; Ozkan *et al.*, 2004; Piette *et al.*, 2004; Shirsat *et al.*, 2004; Vikram *et al.*, 2005).

The objective of this study was to confirm the inactivation effect of the electric current in ohmic heating on microorganisms in milk in comparison with the inactivation effect of conventional heating under identical temperature history conditions. The effect of ohmic heating on quality of milk was also examined by assessing protein denaturation and pH.

### Materials and Methods

*Raw materials and indicator microorganisms* Fresh raw milk was obtained from the Experimental Farm of Hok-

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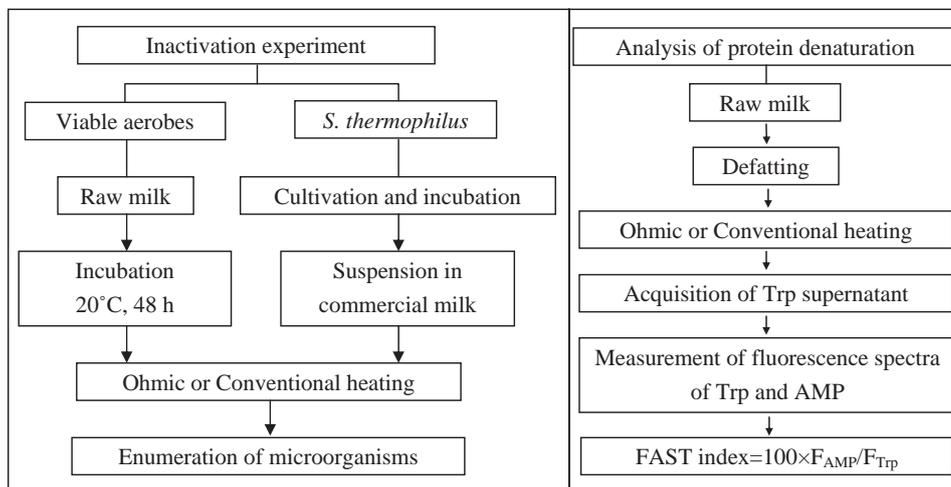


Fig. 1. Experimental procedures.

kaido University, Japan. The raw milk was incubated at 20°C for 48 h in order to obtain samples having ca.  $10^7$  microbial cells/mL of viable aerobes. The incubated milk was used for the inactivation experiment.

*Streptococcus thermophilus* strain 2646 was obtained from Sapporo Research Laboratory of Snow Brand Milk Products Co., Ltd., Sapporo, Japan. This strain was chosen as a representative heat-resistant microorganism. *S. thermophilus* was cultivated in sterilized skim milk (Snow Brand Milk Products) solution (126 g of skim milk powder/1 L of water), and the culture was incubated at 37°C for 24 h. Next, 25 g of the concentrated culture (yoghurt) was suspended in 225 g of commercial whole sterilized milk (Yotsuba Milk Products Co., Ltd., Sapporo, Japan) to acquire ca.  $10^7$  cells/mL. The suspended milk was used for the inactivation experiment.

Raw milk was centrifuged (3000 rpm, 30 min, 1500 g of relative centrifugal force) at 4°C to obtain low-fat milk. The low-fat milk was used for the protein denaturation experiment (Fig. 1).

Conductivity of raw milk and commercial whole sterilized milk was 0.39 S/m, while that of low-fat milk was 0.42 S/m.

**Experimental equipment** The ohmic heating equipment consisted of a wide-band function generator (FG-143, NF Electronic Instruments, Kanagawa, Japan), a precision power amplifier (4510, NF Electronic Instruments), two digital multimeters (SC-303A GP-IB unit, Voac 7411; Iwatsu, Tokyo, Japan), a data logger (Thermodac model 5020A, Eto Denki, Tokyo, Japan), a personal computer, and a heating unit as illustrated in Fig. 2. The heating unit consisted of titanium (Ti) square electrodes in contact with the sample and thermocouples (k-type) inserted at the center of an acryl plastic vessel of 350 mL in capacity. The distance between the two electrodes was 30 mm, and the size of each electrode in con-

tact with the milk sample was 100×85 mm. The alternating current wave adapted to the ohmic heating system was measured with a digital multimeter. Time and temperature data were collected in the data logger linked to the personal computer. Conventional heating as a reference was performed in a water bath using an aluminum vessel with a 300-mL capacity and an electric heater (Thermo-Mate BF400, Yamato, Tokyo, Japan). The aluminum vessel is 51 mm in bottom diameter, 74 mm in top diameter and 117 mm in height. The heat transfer area of the aluminum vessel is 203 cm<sup>2</sup> during conventional heating.

**Treatment procedures for ohmic heating and conventional heating** The experimental vessel for ohmic heating or conventional heating was filled with 250 mL of milk sample. The initial temperature of the sample was kept at 20°C. The sample was heated to a set temperature by ohmic heating or by conventional heating. Conventional heating was performed in a water bath controlled at a temperature that was 1.5°C higher than the set temperature. Ohmic heating was

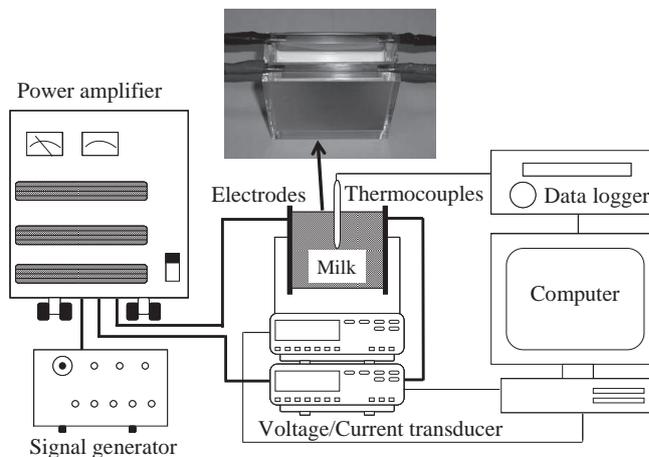


Fig. 2. Schematic diagram of the experimental equipment.

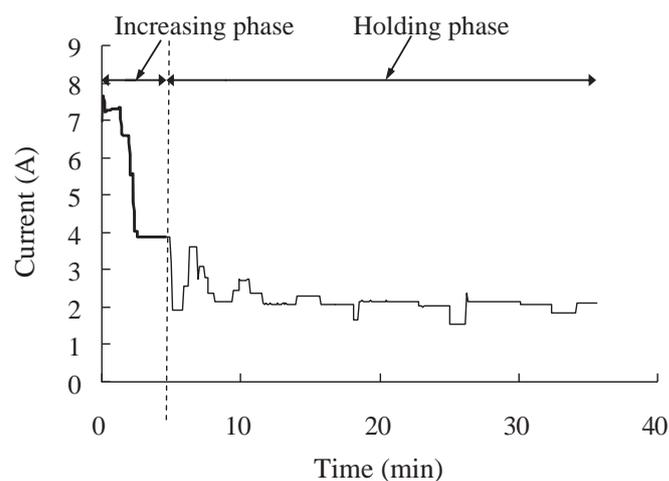
achieved by an alternating current of 20 kHz. It took about 5 min for the sample center temperature to reach the set temperature and then the sample was held at the set temperature for a specified period. In all experiments, care was taken to standardize the time-temperature histories of the samples for the two treatments. To achieve identical thermal histories in addition to obtaining a smooth run, the current was controlled artificially during ohmic heating. To obtain a uniform temperature, the samples were stirred with a glass rod by hand while heating in both treatments. The conditions used in the sterilization experiments are shown in Table 1. The changes in current during the temperature rising phase and the temperature holding phase at a set temperature of 70°C and frequency of 20 kHz for the *S. thermophilus* inactivation experiment are shown in Fig. 3. In order to achieve identical thermal histories of ohmic heating and conventional heating, the current was controlled during the rising and holding phases of ohmic heating in the range of 7.3 to 2.0 A (voltage in the range of 70 to 12 V), and the average currents were 5.33 A (31.50 V) and 2.16 A (10.51 V) during the rising and holding phases, respectively.

**Enumeration of microorganisms** One-milliliter samples for microbiological analysis were taken at regular intervals (sampling time shown in Table 1) and were immediately cooled in an ice-water bath. Viable aerobe counts were determined by the plate method using plate count agar (Merck, Darmstadt, Germany). Plates were incubated at 37°C for 48 h, and numbers of colonies were counted. *S. thermophilus* 2646 counts were determined by the same method as that used for viable aerobes using M17 broth (Merck) mixed with agar. Plates were incubated at 37°C for 72 h, and numbers of colonies were counted.

**Analysis of protein denaturation** Heat treatment affects the sensory, biophysical and nutritional properties of milk. The main events occurring upon heating are, successively, protein denaturation, early and advanced Maillard reaction, and lactose isomerization. The FAST (fluorescence of

advanced Maillard products (AMP) and soluble tryptophan (Trp)) method is a rapid and sensitive fluorimetric method to evaluate the effect of heat treatment on commercial milk. It is based on the simultaneous determination of protein denaturation by Trp fluorescence (290 nm/340 nm: maximum excitation and emission wavelengths of tryptophan) and formation of fluorescent advanced Maillard products (350 nm/440 nm) in the milk fraction soluble at pH 4.6. In this study, the FAST method was used to determine protein denaturation in pasteurized milk in order to evaluate the effect of ohmic heating on quality of milk (Birlouez-Aragon *et al.*, 1998).

Initial temperature of raw milk was 10°C. It took about 5 min for 250-mL samples (raw milk) to be heated to set temperatures of 40, 50, 60, 65, 70, 75 and 80°C, and the samples were kept at each temperature for 30 min under ohmic heating and conventional heating conditions. Ten-milliliter samples for protein denaturation were taken after 30 min and were immediately cooled in an ice water bath. The heating procedure was the same as that in the inactiva-



**Fig. 3.** Changes in current during temperature increasing phase and temperature holding phase by ohmic heating at a set temperature of 70°C for the *S. thermophilus* inactivation experiment.

**Table 1.** Conditions of sterilization experiments.

Microorganism	Set temperature (°C)	Holding time (min)	Sampling time (min)	Average current (I) and voltage (V) during increasing (I) and holding (H) phases			
				I <sub>I</sub> (A)	I <sub>H</sub> (A)	V <sub>I</sub> (V)	V <sub>H</sub> (V)
Viable aerobes	57	30	5	3.91	1.91	26.99	11.18
	60	30	5	4.98	1.82	30.11	9.11
	72	5	5	5.19	2.17	32.05	10.59
<i>S. thermophilus</i>	70	30	10	5.33	2.16	31.50	10.51
	75	15	5	5.91	2.55	35.10	11.15
	80	1	0.5	5.47	2.88	31.20	13.99

tion experiment. The conditions of the protein denaturation experiment are shown in Table 2. The milk sample was acidified by adding 9 volumes of acetate buffer (0.1 M, pH 4.6) and then centrifuged at 4000 g for 10 min at 4°C (KH-180, Kubota, Tokyo, Japan). The supernatant was diluted 10 times in distilled water. Fluorescence spectra of tryptophan and advanced Maillard products of the diluted supernatant were measured on a fluorescence spectrophotometer (F-4500, Hitachi, Tokyo, Japan) with excitation and emission monochromators at 290 nm/340 nm and then at 350 nm/440 nm, respectively. In this study, Trp fluorescence was standardized by using a bovine serum albumin (BSA) solution at 1 mg/1 L in acetate buffer. The amount of denatured protein was expressed as the FAST index from  $F_{AMP}$  and  $F_{Trp}$ . The FAST index was defined as follows:

$$\text{FAST index} = 100 \times F_{AMP} / F_{Trp},$$

where  $F_{AMP}$  is the fluorescence of AMP (cps) and  $F_{Trp}$  is Trp concentration of the milk sample standardized by BSA (fluorescence of milk sample/fluorescence of BSA, mg/L). A higher value of FAST index (100 cps.L.mg<sup>-1</sup>) indicates a higher degree of denaturation of protein in the milk sample.

**Statistical analysis** Presented plate count data are average values obtained from five independent trials, each of these values being obtained from duplicated samples. Decimal reduction time (D value) was calculated from the slope of the regression line fitted to the survival plot. To evaluate whether the differences found in the microbial counts and D values determined under the two treatments were significant or not, analysis of variance was performed at a significance level of 5% (MS-Excel 2003, Microsoft, Redmond, USA).

## Results and Discussion

**Comparison of the inactivation effects of ohmic heating and conventional heating** A comparison of the thermal his-

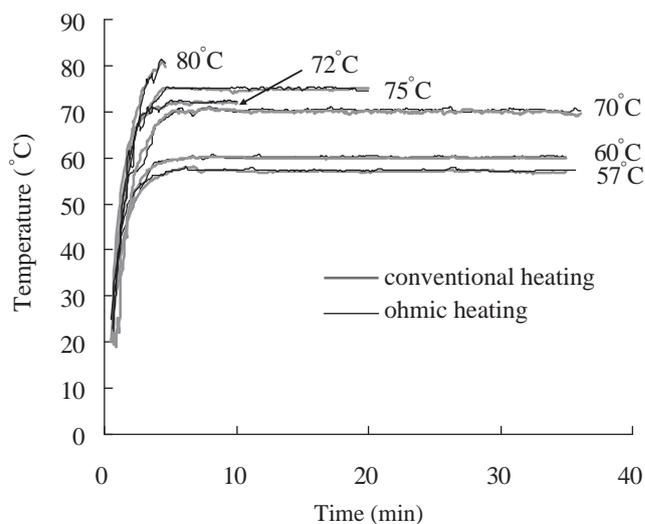
**Table 2.** Conditions of protein denaturation analysis.

Set temperature (°C)	Average current (I) and voltage (V) during increasing (I) and holding (H) phases			
	I <sub>I</sub> (A)	I <sub>H</sub> (A)	V <sub>I</sub> (V)	V <sub>H</sub> (V)
40	2.84	0.77	33.50	7.85
50	3.51	1.07	31.79	10.06
60	4.82	1.32	46.20	10.46
65	5.40	1.60	48.26	11.00
70	5.59	1.73	48.77	11.55
75	5.57	1.97	47.82	11.84
80	6.07	2.00	52.15	12.58

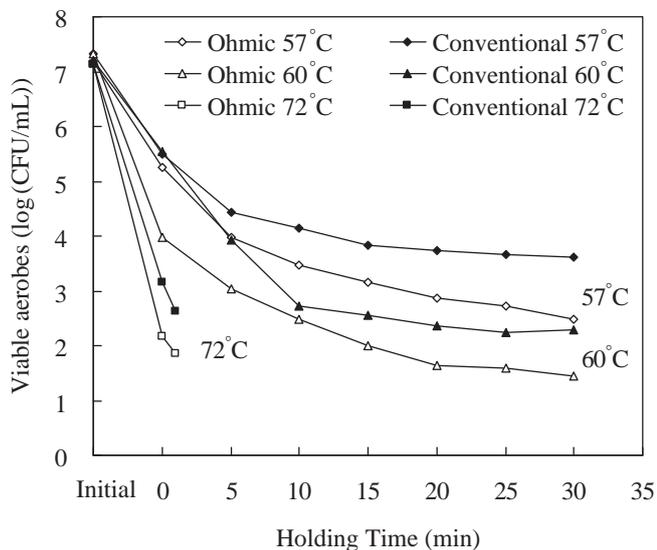
stories of samples subjected to ohmic and conventional heating is shown in Fig. 4. To ensure that the temperatures were correctly controlled, the difference between the temperature at the center of the sample and the set temperature was monitored. In the holding phase in all experiments, standard deviations of the differences between sample temperatures were 0.19-0.21°C for ohmic heating and 0.11-0.18°C for conventional heating. The data indicate that the sample temperatures were properly controlled at each set temperature.

Survival curves for viable aerobes and *S. thermophilus* 2646 in milk during ohmic heating and conventional heating at different temperatures are shown in Figs. 5 and 6, respectively. In these figures, the horizontal axis indicates sampling time, 'Initial' is the time point before samples were treated by ohmic heating or conventional heating, '0' is the time point when the temperature reached the set temperature (start of holding time at a set temperature), and the following data such as 5 and 10 are the holding times at each set temperature. For all experiments, the initial counts of viable aerobes and the initial counts of *S. thermophilus* were almost the same. When the temperature reached the set temperature, microbial counts had already been reduced and the reductions in microbial counts in ohmic heating were greater than those in conventional heating. At the end of the holding phase, microbial counts in ohmic heating were ca. 1 log (CFU/mL) lower than those in conventional heating. Moreover, for both viable aerobes and *S. thermophilus*, microbial counts were significantly different ( $P < 0.05$ ) between the two treatments at each temperature.

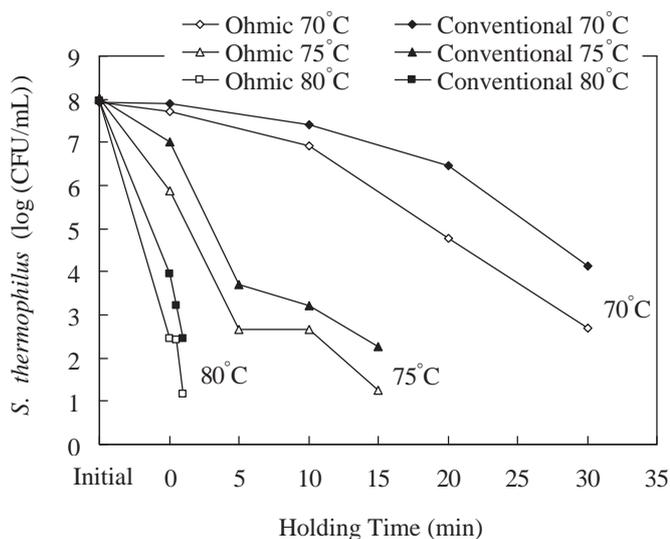
The D values of viable aerobes and *S. thermophilus* subjected to ohmic heating and conventional heating at different temperatures are shown in Tables 3 and 4, respectively. The D values of viable aerobes were significantly different



**Fig. 4.** Comparison of temperature histories for ohmic heating and conventional heating at each set temperature.



**Fig. 5.** Survival of viable aerobes at different holding temperatures by ohmic heating and conventional heating.



**Fig. 6.** Survival of *S. thermophilus* at different holding temperatures by ohmic heating and conventional heating.

( $P < 0.05$ ) between the two treatments. The D values of *S. thermophilus* under the conditions of ohmic heating and conventional heating were significantly different ( $P < 0.05$ ) at temperatures of 70 and 80°C. The D value of *S. thermophilus* in ohmic heating at 75°C was lower than that in conventional heating. These results clearly show that ohmic heating causes a higher microbial death rate than conventional heating does.

Ohmic heating of food products involves the passage of an alternating current through them, thus generating internal heat as a result of electrical resistance. The electric fields as well as the heat generated by ohmic heating may facilitate sterilizing effects. In this study, microbial counts and calculated decimal reduction time (D value) resulting from ohmic heating were significantly lower than those resulting

**Table 3.** D values for viable aerobes at 57, 60 and 72°C.

Treatment	Description	D <sub>57</sub> (min)	D <sub>60</sub> (min)	D <sub>72</sub> (min)
Conventional	Mean	11.25 <sup>a</sup>	9.39 <sup>a</sup>	0.44 <sup>a</sup>
	S.D.	1.45	0.85	0.00
Ohmic	Mean	8.64 <sup>a</sup>	6.18 <sup>a</sup>	0.38 <sup>a</sup>
	S.D.	1.08	0.44	0.00

<sup>a</sup>significance level of 5%

**Table 4.** D values for *S. thermophilus* at 70, 75 and 80°C.

Treatment	Description	D <sub>70</sub> (min)	D <sub>75</sub> (min)	D <sub>80</sub> (min)
Conventional	Mean	7.54 <sup>a</sup>	3.30	0.20 <sup>a</sup>
	S.D.	0.37	0.42	0.03
Ohmic	Mean	6.59 <sup>a</sup>	3.09	0.16 <sup>a</sup>
	S.D.	0.35	0.55	0.03

<sup>a</sup>significance level of 5%

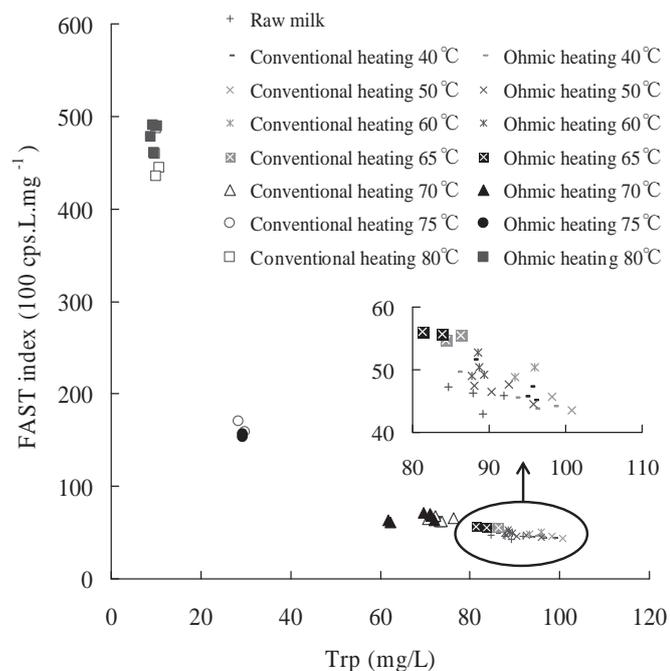
from conventional heating under identical temperature history conditions. The results indicated that ohmic heating had a thermal-lethal effect and an additional nonthermal-lethal effect on viable aerobes and *S. thermophilus* 2646. Previous studies showed that there was a significant effect of alternating current on microbial lethality at sublethal temperatures (Pareilleux and Sicard, 1970; Shimada and Shimahara, 1981, 1982, 1983, 1985a, 1985b). The results of the present experiment and the results of those previous studies suggested that the additional nonthermal-lethal effect on microorganisms during ohmic heating is caused by electric current.

The inactivation effect of electric current depends on the energy (current and time) passing through the medium and on the time during which the cells are left standing in the medium after electric treatment (Pareilleux and Sicard, 1970; Shimada and Shimahara, 1981). Moreover, microbial inactivation caused by ohmic heating was shown to be related to electrical voltage or frequency (Yoon *et al.*, 2002). Palaniappan *et al.* (1992) found no difference between the effects of ohmic and conventional heating treatments with identical thermal histories on the death kinetics of yeast at currents ranging from 0.5 to 1.0 A at 60 Hz. They found that mild electrical pretreatment reduced the requirement of heat for subsequent inactivation of *E. coli* at certain temperatures. Their results are not in agreement with the results of this study. It is thought that a significant inactivation effect of ohmic heating on microorganisms was obtained due to the higher current and frequency used in this study (2.3 to 7 A

and 20 kHz) than those used in the experiments of Palaniappan *et al.* (1992). Therefore, the additional lethal effect of ohmic heating might be due to the electrical current and frequency applied during ohmic heating. The effect of electric current at high electric field strength and high frequency on inactivation of microorganisms should be further investigated.

Although several studies have been conducted on the mechanism of inactivation by electric current, no conclusion has yet been reached. Shimada and Shimahara (1982, 1983) noted that hydrogen peroxide is formed electrolytically in a phosphate buffer solution exposed to an alternating current (AC). Moreover, it was found that surviving fractions of resting *E. coli* B are related to the amount of hydrogen peroxide. They also reported that exposure to AC causes an increase in the magnitudes of negative surface charge of *E. coli* cells and change in some physiological properties (Shimada and Shimahara, 1985a, 1985b). On the other hand, Yoon *et al.* (2002) reported that microbial inactivation caused by ohmic heating was due to the electroporation of cell membranes by the electric current. Although the precise killing activities of ohmic heating still remain to be elucidated, the results obtained in this study suggest that ohmic heating has not only a thermal-lethal effect but also a nonthermal-lethal effect due to the electric current on microorganisms.

**Analysis of protein denaturation** The FAST indexes for raw milk and sterilized milk treated at different temperatures by ohmic heating and conventional heating are shown in Fig. 7. When the set temperature was below 60°C, the FAST in-



**Fig. 7.** FAST indexes of raw milk and sterilized milk subjected to both treatments at different temperatures.

dex and the Trp (non-denaturated protein) value of raw milk were almost the same as those of samples heated by both treatments. With an increase in temperature from 60 to 80°C, the FAST index increased, but the Trp value decreased during both treatments. The results suggest that no protein denaturation occurred at temperatures below 60°C, while protein denaturation increased with an increase in temperature from 60 to 80°C during both treatments as previous works showed (Birlouez-Aragon *et al.*, 1998). However, there was no significant difference between FAST indexes or degrees of protein denaturation in ohmic heating and conventional heating at all temperatures ( $p < 0.05$ ). Protein denaturation during ohmic heating was primarily due to the thermal effect. There was no difference between pH of raw milk and that of pasteurized milk. Moreover, no difference in pH was found in samples treated by the two heating methods at all holding temperatures. Therefore, it was concluded that electrical current had no additional effect on protein denaturation.

## Conclusion

Our results indicated that there were significant differences between ohmic heating and conventional heating in death rates of aerobes and death rates of *S. thermophilus* 2646. These results suggest that the lethal effect of electricity is significant compared to that of heat. No difference was found between protein denaturation during ohmic heating and conventional heating. It is therefore clear that the undesirable thermal effect on microbial inactivation of milk can be reduced by ohmic heating. However, it is necessary to elucidate the mechanism of the nonthermal effect of electricity on microorganisms.

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## References

- Birlouez-Aragon, I., Nicolas, M., Meyais, A., Marchond, N., Grenier, J. and Calvo, D. (1998). A rapid fluorimetric method to estimate the heat treatment of liquid milk. *Int. Dairy J.*, **8**, 771-777.
- Cho, H.-Y., Yousef, A. E. and Sastry, S. K. (1999). Kinetics of inactivation of *Bacillus subtilis* spores by continuous or intermittent ohmic and conventional heating. *Biotechnol. Bioeng.*, **62**, 368-372.
- Guillou, S. and El Murr, N. (2002). Inactivation of *Saccharomyces cerevisiae* in solution by low-amperage electric treatment. *J.*

- Appl. Microbiol.*, **92**, 860-865.
- Lebovka, N. I., Praporscic, I., Ghnimi, S. and Vorobiev, E. (2005). Dose electroporation occur during the ohmic heating of food? *J. Food Sci.*, **70**, 308-311.
- Leizerson, S. and Shimoni, E. (2005). Stability and sensory shelf life of orange juice pasteurized by continuous ohmic heating. *J. Agric. Food Chem.*, **53**, 4012-4018.
- Ozkan, N., Ho, I. and Farid, M. (2004). Combined ohmic and plate heating of hamburger patties: quality of cooked patties. *J. Food Eng.*, **63**, 141-145.
- Palaniappan, S., Sastry, S. K. and Richter, E. R. (1992). Effects of electroconductive heat treatment and electrical pretreatment on thermal death kinetics of selected microorganisms. *Biotechnol. Bioeng.*, **39**, 225-232.
- Pareilleux, A. and Sicard, N. (1970). Lethal effects of electric current on *Escherichia coli*. *Appl. Microbiol.*, **19**, 421-424.
- Piette, G., Buteau, M. L., Halleux, D. E., Chiu, L., Raymond, Y., Ramaswamy, H. S. and Dostie, M. (2004). Ohmic cooking of processed meats and its effects on product quality. *J. Food Sci.*, **69**, 71-78.
- Praporscic, I., Lebovka, N. I., Ghnimi, S. and Vorobiev, E. (2006). Ohmically heated, enhanced expression of juice from apple and potato tissues. *Biosystems Eng.*, **93**, 199-204.
- Sastry, S. K. (2003). Ohmic heating. In "Encyclopedia of Agricultural, Food, and Biological Engineering" ed. by Heldman, D. R., Marcel Dekker, New York, 707-711.
- Shimada, K. and Shimahara, K. (1981). Factors affecting the surviving fractions of resting *Escherichia coli* B and K-12 cells exposed to alternating current. *Agric. Biol. Chem.*, **45**, 1589-1595.
- Shimada, K. and Shimahara, K. (1982). Responsibility of hydrogen peroxide for the lethality of resting *Escherichia coli* B cells anaerobically exposed to an alternating current in phosphate buffer solution. *Agric. Biol. Chem.*, **46**, 1329-1337.
- Shimada, K. and Shimahara, K. (1983). Sublethal injury to resting *Escherichia coli* B cells aerobically exposed to alternating current. *Agric. Biol. Chem.*, **47**, 129-131.
- Shimada, K. and Shimahara, K. (1985a). Changes in surface charge, respiratory rate and stainability with crystal violet of resting *Escherichia coli* B cells anaerobically exposed to an alternating current. *Agric. Biol. Chem.*, **49**, 405-411.
- Shimada, K. and Shimahara, K. (1985b). Leakage of cellular contents and morphological changes in resting *Escherichia coli* B cells exposed to an alternating current. *Agric. Biol. Chem.*, **49**, 3605-3607.
- Shirsat, N., Brunton, N. P., Lyng, J. G., Mckenna, B. and Scannell, A. (2004). Texture, colour and sensory evaluation of a conventionally and ohmically cooked meat emulsion batter. *J. Sci. Food Agric.*, **84**, 1861-1870.
- Tsong, T. Y. (1991). Electroporation of cell-membranes. *Biophys. J.*, **60**, 297-306.
- Uemura, K. and Isobe, S. (2002). Developing a new apparatus for inactivating *Escherichia coli* in saline water with high electric field AC. *J. Food Eng.*, **53**, 203-207.
- Uemura, K. and Isobe, S. (2003). Developing a new apparatus for inactivating *Bacillus subtilis* spore in orange juice with a high electric field AC under pressurized conditions. *J. Food Eng.*, **56**, 325-329.
- Vikram, V. B., Ramesh, M. N. and Prapulla, S. G. (2005). Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. *J. Food Eng.*, **69**, 31-40.
- Yoon, S. W., Lee, C. Y. J., Kim, K. M. and Lee, C. H. (2002). Leakage of cellular material from *Saccharomyces cerevisiae* by ohmic heating. *J. Microbiol. Biotechnol.*, **12**, 183-188.