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Kinetics of *Escherichia coli* Destruction by Microwave Irradiation

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The kinetics of destruction of *Escherichia coli* cells suspended in a solution by microwave irradiation with a microwave oven were studied. During radiation at several powers, the temperature of 0.01 M phosphate buffer (PB), pH 7.0, in a glass beaker increased linearly at a rate of A (degrees Centigrade per second) according to the exposure time. When *E. coli* cells suspended in PB were exposed in the same beaker, the number of viable cells decreased according to the exposure time and the power used. The survival curve was approximated to a set of three linear parts. For each part, a rate constant of destruction (k) and an extrapolated starting temperature (T_0) at several powers were estimated. Thereafter, the relationships between A and k and between A and T_0 were studied. When a flat petri dish was used, the A value of exposed PB was lower and bacterial destruction was inhibited; the survival curve was similar to a curve predicted from the A value by using the relationships between the parameters. As the concentration of salt in the solution increased (from 0 to 1.35 M), the A value decreased and bacterial destruction was more suppressed. No remarkable difference between the destruction profiles for microwave exposure and conventional heating, which had the potential to generate an equal A value, was detected. These results showed that the parameter A of an irradiated solution is essential when kinetics of bacterial destruction by microwave exposure are studied and that the destruction profile can be interpreted mostly by means of thermal effects.

The use of microwave radiation in the cooking and processing of foods has become remarkably popular in recent years. Although many investigators have studied the destruction of microorganisms by microwave irradiation (4, 6, 8), the mechanism of destruction has not been fully clarified (9). It is now thought that the destruction of microorganisms is due mainly to induction heating by microwave exposure (9, 11).

There are, however, two main disadvantages in heating foods with microwaves. Foods cannot be uniformly heated in microwave ovens; the temperature increase of a unit volume in exposed food varies with the position of the unit. High salt concentrations in foods suppress microwave heating, especially in the cores (7). For these reasons, it is possible that the temperatures in some portions of irradiated foods do not become high enough to kill any bacteria present.

Many investigators have already observed the destruction of microorganisms in specific kinds of foods by microwave irradiation (1, 3, 5, 10). However, little is known kinetically about the basic and general relationship between microbial destruction in foods and microwave exposure. We therefore analyzed this relationship.

The spatial pattern of the temperature increase in a food during microwave irradiation varies mainly because of physical factors, including shape, weight, volume, dielectric loss factor, specific heat, conductivity of heat, fluidity (in the case of a liquid), and so on, even under the same exposure conditions. Generally, the thermal pattern of an exposed food is too complex to analyze. Therefore, using the simplest model of bacteria suspended in a liquid, we kinetically studied the destruction by microwave irradiation.

An *Escherichia coli* strain was selected as the test strain because it is a gram-negative rod that commonly contaminates foods.

MATERIALS AND METHODS

Bacterial strain. An *E. coli* strain which was isolated from food and had characteristics of thermal destruction at a constant temperature which represented an average for several *E. coli* isolates in our laboratory was chosen.

Microwave source. Irradiation was performed in a household microwave oven (RE-S650; Sharp Corporation, Osaka, Japan) with a rotating plate (275 mm in diameter) at a speed of 2.46 rpm. The equipment emits rated powers of 100, 200, 300, and 500 W at 2,450 MHz.

Temperature measurement. A 0.01 M phosphate buffer (PB) consisting of monobasic and dibasic sodium phosphates, pH 7.0, was maintained at 20°C in a temperature-controlled water bath (Jr-100; Taitec Corporation, Koshigaya, Japan). A 100-ml portion of the solution in a 200-ml Pyrex glass beaker (86 mm [height] by 63 mm [diameter]) was placed at the center of the rotating plate in the microwave oven and irradiated at several powers for various periods. Immediately thereafter, the solution was thoroughly agitated, and the temperature was measured with a digital thermometer (HL-2; Anritsu Meter Co., Ltd., Tokyo, Japan).

Measurement of the decrease in water volume caused by evaporation. A 100-ml portion of 0.01 M PB in a 200-ml beaker at 20°C was exposed in the oven for various periods. Immediately thereafter, the decrease in weight caused by water evaporation was measured with an electronic digital balance (Libror EB-280; Shimadzu Corporation, Kyoto, Japan).

Measurement of rate constants of thermal destruction. The bacterial strain was shaken in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 36°C for 16 to 16.5 h.

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TABLE 1. Rate constants of *E. coli* destruction at various temperatures

Temp (°C)	Rate constant (1/s)
56	6.55×10^{-3}
58	1.70×10^{-2}
60	4.70×10^{-2}
62	1.01×10^{-1}
64	2.69×10^{-1}

Cultured cells (stationary phase, about 10^{10} CFU/ml) were diluted 1:100 with 0.01 M PB. A 4.5-ml portion of PB in a capped test tube (100 mm [height] by 10 mm [diameter]) was placed in the water bath at a given temperature. Forty-five microliters of the cell suspension was injected into the test tube (10^6 CFU/ml), and the tube was incubated for a defined period. The suspension was then cooled in an ice bath, and after serial dilution, 1 ml of the sample was mixed with plate count agar (Eiken Chemical Co., Ltd., Tokyo, Japan) in duplicate. After incubation at 35°C for 48 h, the number of viable cells in the sample (N) was measured. As a control, the viable-cell count of an untreated cell suspension (N_0) was estimated. The difference between the logarithm of a viable-cell count of a test sample and that of the control, that is, the survival rate ($\log N - \log N_0$), was plotted against the exposure time (t) to yield the survival curve. From the survival curve, the slope of the decline was determined by the least-squares method. By using the value of the slope, the rate constant of bacterial destruction (k) was calculated by the following equation:

$$-dN/dt = kN \quad (1)$$

Irradiation of bacteria. Cells cultured as described above were diluted 1:10,000 with 0.01 M PB or other solutions. The cell suspension (10^6 CFU/ml) was maintained at 20°C in the water bath. A 100-ml portion of the suspension in a 200-ml Pyrex beaker was placed at the center of the rotating plate in the oven and then exposed at several powers for various periods. Immediately after exposure, the suspension was thoroughly agitated and a sample was removed from it. The viable-cell count of the sample was measured as described above. The volume loss of the cell suspension during irradiation was compensated for by using the evaporation data (see Fig. 3 and equations 2 to 4), on the assumption that the percent loss of volume in the sample would be equal to that of the weight. The kinetics of the destruction were also analyzed as described above.

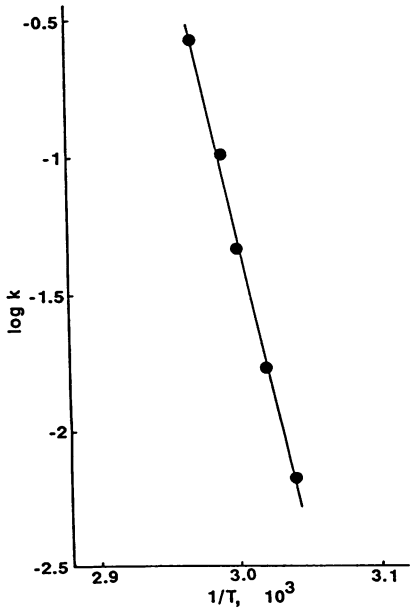

FIG. 1. Arrhenius plot of thermal destruction of *E. coli*. Results shown in Table 1 were used for the plot. The coefficient of correlation was 0.998.

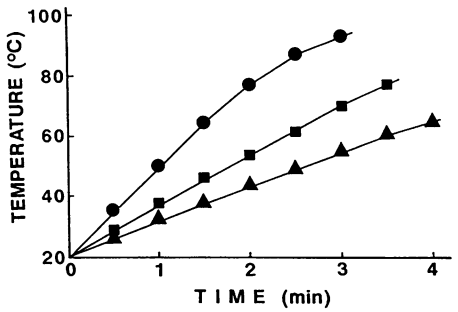

FIG. 2. Increase in temperature of PB caused by microwave exposure. PB was irradiated at 100 (▲), 200 (■), and 300 (●) W.

Comparison of microwave irradiation with conventional heating. The conditions of both experiments described below were optimized so that both had the potential to generate almost the same value of parameter A (rate of temperature increase) (0.076°C/s). A cell suspension (10^6 CFU/ml) in a water bath at 20°C was prepared. A 300-ml portion of the suspension in a 300-ml Pyrex beaker was irradiated at 100 W for a defined period and then cooled. For conventional heating, 2.5 liters of water was poured at 20°C into a plastic container equipped with a heater (Jr-100). A cell suspension in a capped test tube, as described above, was placed in the water. After temperature equilibration at 20°C, the temperature of the water was increased to about 90°C. The sample was treated for a certain period and then cooled. The temperature of the cell suspension was monitored with a digital thermometer. Viable-cell counts in samples from both experiments were determined for five (or four) plates. The difference ($\log N - \log N_0$) between the treatments in the survival rate was statistically analyzed for variance with the F test and then for the mean with the t test.

RESULTS

Thermal destruction characteristic of the strain. Thermal destruction of *E. coli* at various constant temperatures was studied. The rate constant of thermal destruction at each

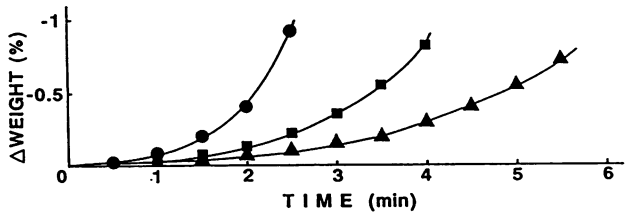

FIG. 3. Evaporation of water caused by microwave radiation. PB was exposed at 100 (▲), 200 (■), and 300 (●) W. Immediately after exposure, the decrease in the weight of PB was measured.

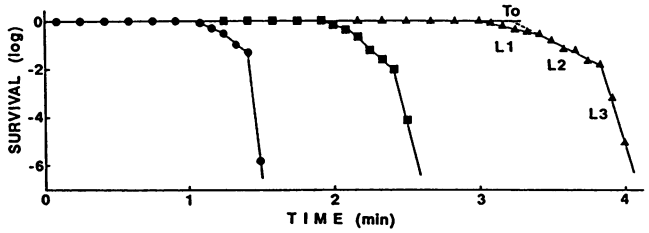

FIG. 4. Destruction of *E. coli* in PB by microwave exposure. The bacterial suspension was irradiated at 100 (▲), 200 (■), and 300 (●) W. The linear parts (L1, L2, and L3) at 100 W are indicated as an example. An example of T_0 is shown at the second part (L2) of the 100-W exposure.

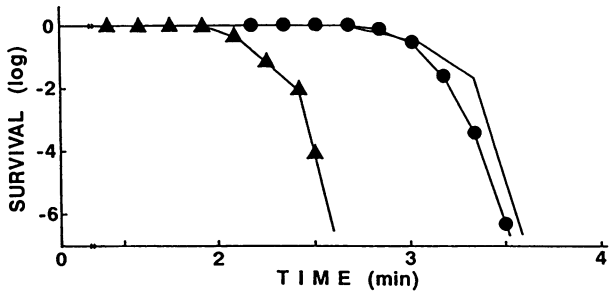

FIG. 6. Effect of the container shape on bacterial destruction. A bacterial suspension in a petri dish (17 mm [height] by 109 mm [diameter]) was exposed to 200 W. Symbols: ●, petri dish; ▲, control (200-ml beaker); ——, a line predicted from the A value (0.22°C/s).

temperature is shown in Table 1. An Arrhenius plot (2) was generated from these data (Fig. 1). From the slope of the plotted line, the activation energy of thermal destruction of the organism was calculated as 4.3×10^5 J/mol.

Temperature increase of PB. When PB in a glass beaker was irradiated at 20°C, the temperature increased linearly up to about 65 to 70°C, according to the exposure time (Fig. 2). From the slope of the plotted line, the rate of temperature increase of PB, A, was calculated as 0.20, 0.29, and 0.50°C/s at 100, 200, and 300 W, respectively.

Decrease in water caused by evaporation. A small amount of water evaporated from PB during microwave irradiation (Fig. 3). The relationship between the percent decrease of water weight, ΔW, and the exposure time, t (seconds), at 100, 200, and 300 W was exponentially approximated by the following equations, with correlation coefficients of 0.998, 0.997, and 0.998, respectively.

$$\Delta W = -0.0166 \exp(0.0118t) \text{ (100 W)} \quad (2)$$

$$\Delta W = -0.0215 \exp(0.0153t) \text{ (200 W)} \quad (3)$$

$$\Delta W = -0.0140 \exp(0.0282t) \text{ (300 W)} \quad (4)$$

Destruction of *E. coli* suspended in PB. The number of *E. coli* cells suspended in PB decreased according to the exposure time and power (Fig. 4). The survival curve was approximated to a set of three linear parts (L1, L2, and L3) at each power.

Let k denote a rate constant of bacterial destruction at each linear part. Let T_0 denote the extrapolated temperature (degrees Centigrade) at the point of intersection of the horizontal line of $\Delta(\log N) = 0$ and an extrapolated line of the linear part. Relationships between A and T_0 and between A and k were studied for each linear part at different powers. As the A value increased, that of k for each part increased and reached the maximum (Fig. 5A). The opposite phenomenon was observed for T_0 (Fig. 5B). For L3 at 300 W, the bacterial destruction occurred too fast to allow precise estimation of the values of the parameters.

Effects of container shape. When PB (100 ml) in a flat petri dish was irradiated at 200 W, the temperature increased linearly (data not shown). The value of A was 0.22°C/s, which was lower than that of the control (beaker). Bacterial destruction in PB in the petri dish was suppressed compared with that of the control (Fig. 6).

A survival curve was predicted from the value of A by using the relationships between the parameters shown in Fig. 5 (Fig. 6). The predicted curve was similar to the experimental one.

Effect of pH of PB on bacterial destruction. When cells were irradiated in PB of various pHs from 5 to 9, more cells were destroyed (Fig. 7). No differences in the value of A among these solutions were detected.

Effects of salt and sucrose on temperature increase and bacterial destruction. (i) **Sodium chloride.** As the sodium

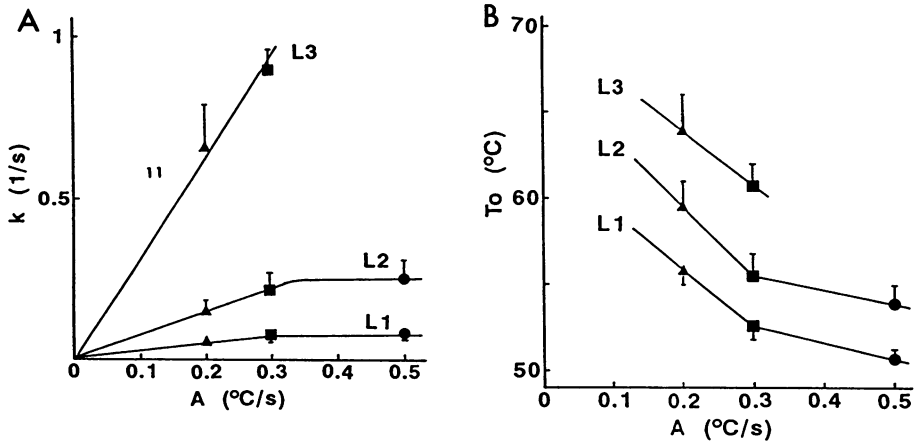

FIG. 5. (A) Relationship between parameters A and k for each linear part. By using the results in Fig. 4, k values for the linear parts at 100 (▲), 200 (■), and 300 (●) W were calculated. Data represent the means ± standard deviations (bars) of six experiments. (B) Relationship between parameters A and T_0 for each linear part. By using the results in Fig. 4, extrapolated T_0 values for the linear parts at 100 (▲), 200 (■), and 300 (●) W were estimated. Data represent the means ± standard deviations (bars) of six experiments.

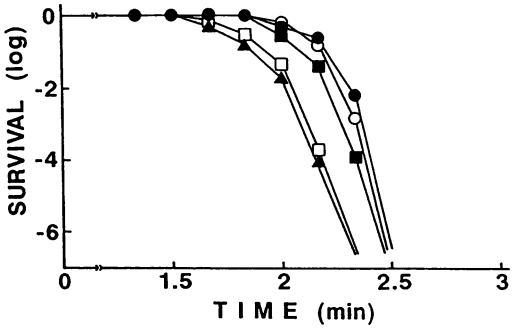

FIG. 7. Effect of pH of PB on bacterial destruction. Bacterial suspensions at various pHs were exposed to 200 W. Symbols: ●, pH 5; ○, pH 6; ■, pH 7; □, pH 8; ▲, pH 9.

chloride concentration increased (from 0 to 1.35 M), the estimated value of parameter A of the exposed solution decreased: the A values were 0.28, 0.28, 0.27, and 0.25°C/s in 0, 0.15, 0.45, and 1.35 M salt solutions, respectively. The higher the salt concentration was, the fewer the cells that were destroyed by microwave irradiation (Fig. 8). No significant differences in parameter A and bacterial destruction between 0 M salt solution (distilled water) and the control (0.01 M PB) were observed.

(ii) Sucrose. No differences in temperature parameter A and bacterial destruction were detected in sucrose solutions (0 to 1.0 M) (data not shown).

Comparison of microwave irradiation with conventional heating. The difference between the destruction profiles of *E. coli* with microwave exposure and conventional heating was studied (Fig. 9). The two treatments had the potential to increase the temperature of the cell suspension at the same A value (0.076°C/s). The survival curve for microwave irradiation was similar to that for conventional heating.

DISCUSSION

For a unit volume of bacterial suspension, the temperature (T) and bacterial survival rate (S) after a given microwave exposure are considered to be functions of $T(x, y, z, t)$ and $S(x, y, z, t)$, where x, y, and z are spatial coordinates of the unit in the suspension and t is the exposure time. When the suspension is agitated to homogeneity immediately after the exposure, T and S are considered to be simply functions of $T(t)$ and $S(t)$. We analyzed the uniform suspension.

The quantity of microwave radiation absorbed in PB was affected by the location of the beaker on the rotating plate.

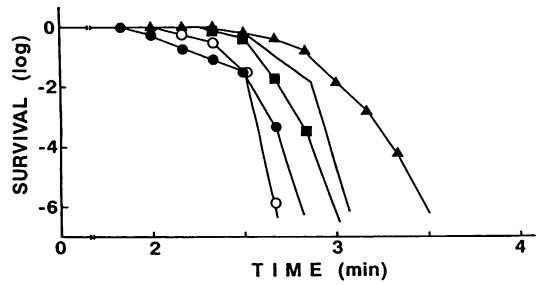

FIG. 8. Effect of salt on bacterial destruction. Bacterial suspensions at various salt concentrations were irradiated at 200 W. Symbols: ○, 0 M; ●, 0.15 M; ■, 0.45 M; ▲, 1.35 M; ——, a line predicted from the A value at a 1.35 M salt solution (0.25°C/s).

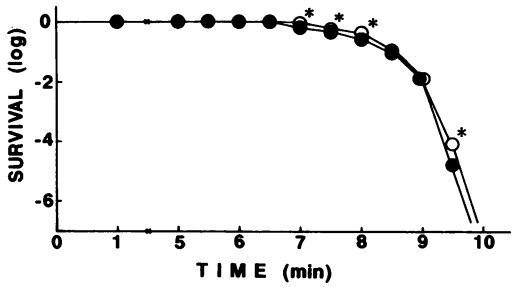

FIG. 9. Microwave irradiation compared with conventional heating with regard to the bacterial destruction profile. The two treatments had the potential to increase the temperature of the cell suspension at the same A value (0.076°C/s). Symbols: ●, microwave exposure; ○, conventional heating. Asterisks show significant differences ($P < 0.01$) at each exposure time. The standard deviation at each time is not shown because the value is too small to be represented in the figure.

When the PB was located at the edge of the plate, the A value was 93% of that of PB at the center of the plate, for each power. This percentage would correspond to the amount of microwave energy absorbed in PB located at the edge, compared with that absorbed in PB at the center. Also, it is thought that the quantity of microwave energy absorbed in PB kept in the petri dish would be smaller (76%) than that absorbed in the beaker, judging from the A values.

The main result of this study, that the profile of destruction of bacteria by microwave irradiation was approximated to a set of three linear parts, may be difficult to understand. This result can be clearly observed when the controlled conditions of low power, for example, 100 W, and short exposure intervals (every 5 s) are used. With high power or a long interval, the survival curve is trivial, with a steep descent, which has been previously reported by other investigators (9). The trivial curve is hard to analyze kinetically.

Generally, when bacterial destruction is a first-order reaction, its kinetics are expressed as follows:

$$\ln(N/N_0) = -\int_0^t k(t)dt \qquad (5)$$

where $k(t)$ is a function of time t. In a constant temperature field, k is constant, as shown in Table 1. Equation 5 can be changed to equation 1 in this case. In an increasing temperature field caused by microwave irradiation, the destruction profile was approximated to a set of linear parts in our study. This means that k is constant during the exposure period corresponding to each linear part at a given microwave power. Therefore, in this case, equation 5 is expressed as follows:

$$\ln(N/N_0) = 0 \qquad (0 < t < t_1)$$

$$= -\int_{t_1}^{t} k_1 dt \qquad (t_1 \le t < t_2)$$

$$= -\left(\int_{t_1}^{t_2} k_1 dt + \int_{t_2}^{t} k_2 dt\right) \qquad (t_2 \le t < t_3)$$

$$= -\left(\int_{t_1}^{t_2} k_1 dt + \int_{t_2}^{t_3} k_2 dt + \int_{t_3}^{t} k_3 dt\right) \quad (t_3 \le t)$$

where t_i and k_i are an initial time and a rate constant of the destruction for the linear part i (i = 1, 2, and 3) of the

survival curve, respectively. In a preliminary study, a similar destruction profile for microwave-irradiated *Staphylococcus aureus* was observed. The reason for this phenomenon is not understood. Kinetics of inactivation (or denaturation) of a given substance by microwave irradiation may help to solve the problem.

No significant difference in bacterial destruction was detected when A values of solutions were almost the same, as seen for the sucrose solutions. As the A value of a solution increased, the destruction was accelerated, as seen in Fig. 4 and 8. These results indicate the importance of parameter A in the kinetics of destruction of bacteria by microwave exposure.

Furthermore, relationships between A and k and between A and T_0 were found in this study (Fig. 5). By using these relationships, the bacterial survival curve can be predicted, to a certain extent, from the A value of a given solution. With the flat petri dish, the experimental curve was similar to the predicted one, despite exposure in different-shape containers (Fig. 6). This result confirms the existence of relationships between the parameters.

It is still not clear whether a nonthermal killing effect occurs when bacteria are exposed to microwaves (9). The thermal parameter A was essential for analyzing the destruction kinetics. When microwave treatment was compared with conventional heating, the two destruction profiles were almost the same. These results showed that bacterial destruction by microwave irradiation could be explained as thermal inactivation. Moreover, a nonthermal killing effect such as ionization would not occur in microwave irradiation because of the low energy calculated from its frequency, compared with that of X rays.

Small differences between the destruction patterns of the treatments were found in the initial and final parts (Fig. 9). These results are hard to interpret. It is not understood whether this difference is due to the nonthermal effect of microwaves. Another factor(s), such as the spatial temperature profile of the cell suspension during microwave exposure, might be involved.

Variation in the destruction profiles for PB at various pHs and for salt solutions seen in Fig. 7 and 8 was demonstrated in this study. In a preliminary study, a similar variation in salt solution at a constant temperature was observed for the test strain. Thus, the variation is thought to be related to nonthermal interactions between cells and the surrounding atmosphere during microwave exposure. The fact that the experimental curve had a longer tail than the predicted curve at a high salt concentration (1.35 M) (Fig. 8) also appears to be related to this interaction.

E. coli organisms were suspended in PB, salt, and sucrose solutions in this study, but the ingredients of common liquid foods, such as soup, potage, and milk, etc., are much more complex. The thermal characteristics of such foods and the interactions with bacteria during microwave treatment may vary among foods. Basic studies of destruction of bacteria in liquid and solid foods by microwave irradiation will be needed. Also, the profiles of destruction of other kinds of bacteria should be studied in the future.

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