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Effect of Aluminium Cookware on *Escherichia coli* during Pasteurization of Milk

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The effect of aluminium cookware on inactivation of *Escherichia coli* in milk was studied. Samples in aluminium and stainless-steel cups were heated at 60, 63, 65 and 67°C and held at those temperatures for 25, 5, 3 and 2 min, respectively. Results obtained under the temperature of 65 and 67°C clearly showed that cells of *E. coli* were killed more rapidly in aluminium cups than in stainless-steel cups. *D-values* in the aluminium cups were significantly shorter than those in stainless-steel cups, especially at higher temperatures. Samples in aluminium cups revealed a better trend inactivation effect than in stainless-steel cups at 60 and 63°C, however, there were no significant difference. Considering on *z-values*, results which obtained by aluminium cups were slightly lower than that obtained by stainless-steel cups. These results indicate that an aluminium utensil has an inactivating effect on *E. coli* and that rate of inactivation increases as temperature increases.

Keywords: aluminium, *D-value*, *E. coli*, surviving cells, *z-value*

Introduction

Aluminium (Al) has been used in various forms for food such as cookware, cooking utensils, wrappings, cans and packaging materials. It is a light metal with a density of 2.78 $g \cdot cm^{-3}$ and is an excellent conductor of heat and electricity (Pina and Cervantes, 1996). Aluminium leaching and the effect of aluminium on microorganisms have been studied. Preliminary data for aluminium leaching indicate that the use of aluminium cookware, utensils and wrappings may increase the amount of aluminium in food; however, the magnitude of this increase is generally not critical to human health (WHO, 1998). In adults, the average daily intake levels of aluminium from food and drinking water are 5 mg/day and 0.1 mg/liter, respectively. However, average dietary intake levels of aluminium (mg/day) differ geographically: for example, 1.9−2.4 mg/ day in Australia, 4.5 mg/day in Japan, 3.9 mg/day in the United Kingdom, and 7.1−8.2 mg/day in the United States (ii). Aluminium can occur in a number of different forms in water and displays high reactivity with oxygen at room temperature, and it rapidly reacts with acid and bases to produce metal salts and release hydrogen (Pina and Cervantes, 1996). Metal salts produced by these reactions include aluminium chloride ($AlCl_3$), aluminium hydroxide ($Al(OH)_3$), and aluminium oxide (Al_2O_3) (WHO, 1998). The use of aluminium cookware is therefore not recommended for acidic foodstuffs. On the other hand, cooking food in stainless-steel or iron utensils also significantly increases the iron content of food. Although the increase in iron is small, it is substantial when considering the overall dietary iron intake (Park and Brittin, 1997).

The effects of aluminium on microorganisms have been reported in several fields. A study on antibacterial activities in various metal materials showed that aluminium has antibacterial properties (Fukusaki and Hiramatsu, 2007). Aluminium salts are also widely used in water treatment as coagulants to reduce color, turbidity and pathogenic microorganisms and to protect pathogens from chemical disinfection (WHO, 1998; (i)). In microorganisms, aluminium has been shown to interact with bacterial deoxyribonucleic acid (DNA) and activate bacterial genes such as the *E. coli fliC* gene (Scharf *et al.*, 1994; Johnson and Wood, 1990; Guzzo *et al.*, 1991). Furthermore, Guida *et al.* (1991) investigated aluminium toxicity towards *E. coli* and found that growth inhibition was markedly dependent on pH. Their results showed that the growth of *E. coli* in a medium buffered to pH 5.4 was more sensitive to aluminium than was the growth of *E. coli* in media with pH of 6.6−6.8. There has been an accumulation of data in recent years on the toxic effects of aluminium towards various microorganisms. However, there is very

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limited information in the literature about the inactivation of microorganisms during heating treatment using cooking utensils made from different types of material. Therefore, the objective of this study was to determine the inactivating effect of an aluminium utensil on microorganisms during pasteurization by using milk as a representative food.

Materials and Methods

Preparation of microorganism The microorganism strain used in this work was *Escherichia coli* (ATCC®12435). For preparation of suspended cells of *E. coli*, one loop of frozen stock culture was activated by inoculating into 5 mL of sterile Tryptic Soy Broth (TSB, Merck, Darmstadt, Germany) and aerobically incubating at 37°C for 9 h. Then the inoculums were transferred into 250 mL sterile TSB and incubated with shaking in a temperature-controlled water bath at 37°C for 18 h. Bacterial cells were harvested by centrifugation at 5000 rpm for 10 min at 4°C and the supernatant fluid was discarded. Finally, the pellets were re-suspended in commercial milk (homogenized and pasteurized milk) in order to achieve a final concentration of 10^8 CFU/ mL. The suspended cells were stored in an iced water bath at 4°C until use.

Experimental setting Thermal inactivation treatments were performed in a temperature-controlled water bath with an electrical heater (Yamato, Thermo-mate BF400, Tokyo, Japan) using aluminium and stainless-steel cups of 400 mL in capacity. The aluminium cup was 58 mm in bottom diameter, 80 mm in top diameter and 132 mm in height, and the stainless-steel cup was 55 mm in bottom diameter, 79 mm in top diameter and 135 mm in height. The heat transfer areas of the aluminium and stainless-steel cups were 312 and 307 cm^2, respectively. Death kinetic experiments were performed by pouring milk samples of approximately 250 mL into stainless-steel cups, immersing them in a temperature-controlled water bath, and heating them from 10°C to 60, 63, 65 and 67°C and holding at those temperatures for 25, 5, 3 and 2 min, respectively. During the heating process, each milk sample was stirred by using a glass rod with a thermocouple (*K-type*) to mix it thoroughly. Finally, the results were compared with those obtained by using aluminium cups in the same fashion. Time-temperature profiles were measured every second during heating by a thermocouple to ensure that the temperature was controlled correctly. Measurement in each experimental condition was repeated at least three times.

Enumeration of microorganism One milliliter of sample was taken from the center of each cup at each holding time, and collected samples were immediately cooled in an iced water bath. The numbers of viable colonies of *E. coli* that survived after treatments were then counted in plate count agar (Merck, Darmstadt, Germany) after incubation at 37°C for 48 h.

Calculation of D-values, z-values and statistics In order to estimate the inactivation of microorganisms in liquid food during pasteurization, many researchers have studied thermal inactivation kinetics, which can include *D-values* (decimal reduction time) and *z-values* (temperature - dependency factor in thermal inactivation kinetics) (Yuk *et al.*, 2009). *D-value* is defined as the time necessary for a 90% reduction in the microbial population (Singh and Heldman, 1993) and is calculated from slope of the regression line fitted to the survival plot (Sun *et al.*, 2008). *Z-value* is defined as the temperature in degrees (Celsius or Fahrenheit) required for changing a *D-value* and is determined by plotting the calculated log *D-values* against the corresponding temperatures.

Each value shown is an average of at least three replicate experiments. Statistical comparison analysis of the microbial count and *D-values* was performed at the significance level of 5% using the software MS-Excel 2003 (Microsoft, USA).

Results

Time-temperature profiles measured during heating and holding phases at different temperatures and holding times of each setting temperature are shown in Fig. 1.

In order to eliminate the influence of the temperature difference on death kinetic parameters of microorganism, the time-temperature profile of sample in aluminium cup was adapted to simulate that in stainless-steel cup.

The standard deviations of the temperature difference between the samples and the setting temperatures during the

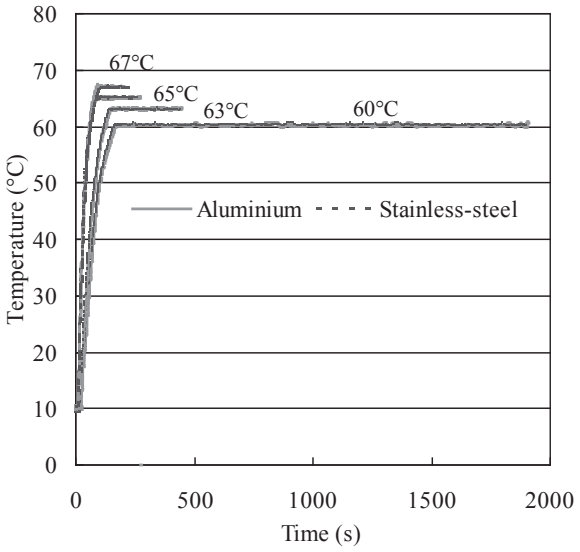

Fig. 1. Temperature history of milk samples with *E. coli* during treatments at 60, 63, 65 and 67°C for 25, 5, 3, and 2 min, respectively, by using aluminium and stainless-steel cups.

holding phase were 0.07−0.11°C for the aluminium cup and 0.08−0.11°C for the stainless-steel cup. The time- temperature profiles of each sample were almost the same in each temperature condition. The data indicate that the sample temperatures were properly controlled at each set temperature.

Inactivation effects of *E. coli* in the different material containers at different temperatures were shown in Figs. 2 to 5. In these figures, the log numbers of the survival of *E. coli* at each temperature are plotted against time. 'Initial' is the log numbers of *E. coli* in the sample without treatment, '0' is the time point when the temperature reached each set temperature (start of holding time at each set temperature), and the following data such as 5 and 10 are the holding times at each set temperature.

When the sample temperature reached the set temperature at 0 min, the numbers of surviving cells of *E. coli* were almost the same in the samples in the aluminium and stainless-steel cups with the only exception at 67°C. Although during increasing phases of 65 and 67°C, the temperature histories were in the same curve. In order to increase temperature from 65 to 67°C, it took time around 18 s. Because of the different period of the increasing time and high temperature at 67°C which reached a killing point of *E. coli*, the survived cells of *E. coli* in aluminium cup were less than that in stainless-steel at 0 min.

At the end of the holding phase, results obtained under temperature conditions of 65 and 67°C clearly showed that cells of *E. coli* were killed more rapidly in aluminium cups than in stainless-steel cups as indicated in Figs. 4 and 5.

In Figs. 2 and 3, although the inactivation effect at 60 and 63°C showed no significant difference between the aluminium and stainless-steel cups, the sample in aluminium cups revealed a better trend in the inactivation effect on *E. coli*

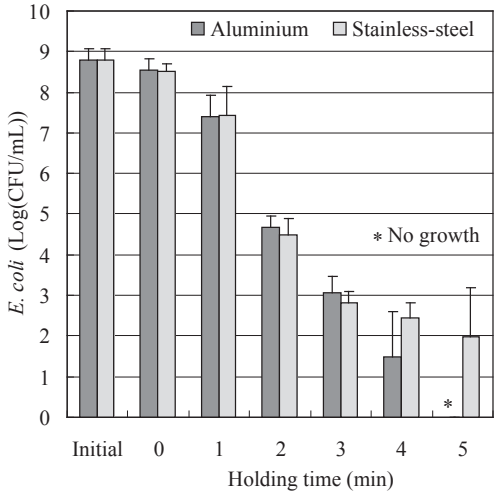

Fig. 3. Survival of *E. coli* in milk during treatment at 63°C for 5 min by using aluminium and stainless-steel cups.

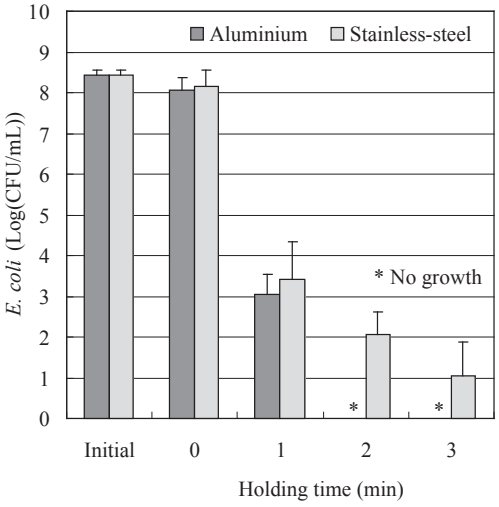

Fig. 4. Survival of *E. coli* in milk during treatment at 65°C for 3 min by using aluminium and stainless-steel cups.

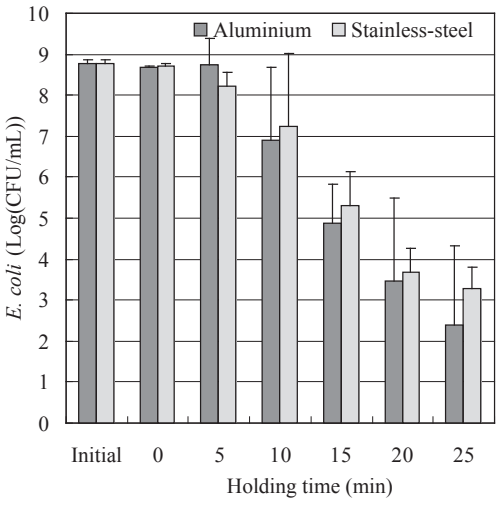

Fig. 2. Survival of *E. coli* in milk during treatment at 60°C for 25 min by using aluminium and stainless-steel cups.

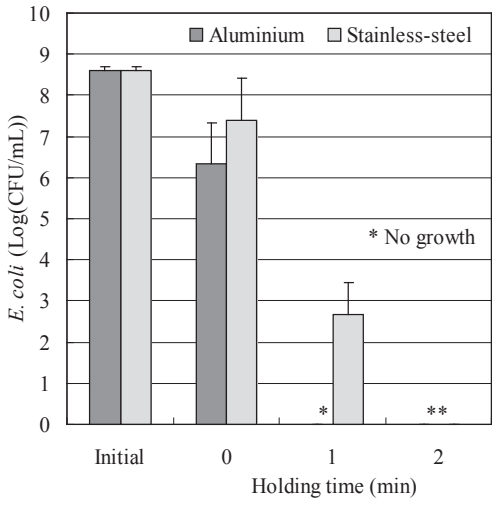

Fig. 5. Survival of *E. coli* in milk during treatment at 67°C for 2 min by using aluminium and stainless-steel cups.

Table 1. *D-values* and *z-values* of suspended *E. coli* in milk by using aluminium and stainless-steel cups.

Heating apparatus	*D-value* ± SD (min)				*z-value* (°C)
	60°C	63°C	65°C	67°C	
Aluminium	$2.84^{a} \pm 0.67$	$0.51^{a} \pm 0.09$	$0.25^{a} \pm 0.01$	$0.18^{a} \pm 0.03$	4.67
Stainless-steel	$3.52^{a} \pm 0.56$	$0.69^{a} \pm 0.07$	$0.43^{b} \pm 0.09$	$0.32^{b} \pm 0.08$	5.35

a, b : *D-values* letters within a column are significantly different below the 5% of error level.

than in the stainless-steel cups.

To estimate the inactivation of microorganisms in food during pasteurization, many researchers have studied thermal inactivation kinetics which can include *D-values* and *z-values*. *D-values* were obtained from log-linear portions of the survival curves and the come-up time by linear regression (Yuk *et al.*, 2009). Results for death kinetic parameters of *E. coli*, in terms of *D-values* and *z-values*, are summarized in Table 1. The results showed that *D-values* were significantly shorter in the aluminium cups than in the stainless-steel cups ($P < 0.05$), especially at high temperatures. *D-values* at 65 and 67°C for the aluminium cups were 0.25 and 0.18 min, respectively, and those for the stainless-steel cups were 0.43 and 0.32 min, respectively. On the other hand, there were no significant differences in *D-values* between the aluminium cup (2.84 and 0.51 min) and stainless-steel cup (3.52 and 0.69 min) during treatment at 60 and 63°C ($P > 0.05$).

The death kinetics of *E. coli* was explained in a term of *z-value*. *Z-values* were calculated by linear regression of log *D-values* versus temperatures, commonly referred to as thermal death time curves. The *z-value* obtained by the aluminium cup ($z = 4.67$°C) was slightly lower than that obtained by the stainless-steel cup ($z = 5.35$°C).

Discussion

In this study, there were significant differences of *D-values* for *E. coli* between in aluminium cups and stainless-steel cups during treatments at 65 and 67°C ($p < 0.05$). Even though there were no significant differences during treatments at 60 and 63°C, the calculated *D-values* were generally lower in the aluminium cups than in the stainless-steel cups.

Furthermore, *z-values* for the aluminium cups was lower than that obtained for cells treated in the stainless-steel cups, indicating that samples in the aluminium cups had higher inactivation of *E. coli* than did samples in the stainless-steel cups.

Aluminium is a metal with a thermal conductivity (at 25°C) of 250 W/(m·°C), and that of stainless steel is 16 W/(m·°C) (iii). Aluminium is an excellent conductor of heat compared with metals such as stainless steel. Therefore many cooking vessels are manufactured from aluminium, which provides very quick heat distribution. A rapid heat transfer in an aluminium container is a potential effect inducing greater inactivation of microorganism.

As Fig. 1 indicates, we attempted to control the time-temperature profiles of samples in the same temperature condition of the aluminium cup and the stainless-steel cup. In order to eliminate the influence of the temperature difference on death kinetic parameters of *E. coli*, the time-temperature profile of sample in the aluminium cup was adapted to simulate that in the stainless-steel cup.

However, during heating treatments the inside surface temperature of the aluminium cup might be higher than that of stainless-steel cup, even though milk sample temperatures were the same as the setting temperature. Generally, heat was diffused from the water bath through the surface of the aluminium and stainless-steel cups and then transferred into the milk sample. It is likely that when the suspended cells of *E. coli* came into contact with the inside surface of the aluminium cup, the cells might be rapidly killed, especially at a high temperature.

Our results are consistent with the results of a study by Al-Holy *et al.* (2004) on thermal inactivation of *Listeria innocua* in salmon caviar showing that the come-up time and *D-values* were shorter in aluminium tubes than in glass tubes. The effect of heat transfer on thermal inactivation kinetics of *E. coli* in solid food by a tube method was studied by Chung *et al.* (2007), and they showed that slow heat transfer resulted in higher survival of *E. coli*.

This was a study to compare the effect of aluminium and stainless-steel cups on microbial inactivation under high setting temperatures within short time treatments. Many researchers have studied the aluminium reaction in microorganisms; however, there were no comparisons in the aluminium reaction on microorganism under the lethal temperature treatments. A study on antibacterial activities in various metal materials at room temperature by Fukuzaki and Hiramatsu (2007) showed that aluminium has antibacterial properties. Aluminium reaction can be explained as the same reaction as

that occurring in aluminium electrodes described by Ghernaout *et al.* (2008):

$$Al(s) + 5H_2O \rightarrow O_2(g) + (7/2)H_2(g) + Al(OH)_3(s) \quad (1)$$

The results of a study by Bojic *et al.* (2001) on inactivation of *E. coli* by using a microalloyed aluminium-based composite showed that the number of *E. coli* cells was reduced by about one log10 count every 10 min, with complete inactivation as the outcome of treatment. Its effects are based on spontaneous dissolution in contact with water, with generation of Al(III) and OH-ions, and finally voluminous insoluble $Al(OH)_3$. The results of a study by Yamamoto *et al.* (1964) showing that bacteriophages are rapidly inactivated when a suspension of bacteriophages comes into contact with the aluminium alloy surface. Several studies on the effects of aluminium on microorganisms indicated that the toxic effect of aluminium on bacteria might be connected to the interference of the metal with the heme biosynthetic pathway (Scharf *et al.*, 1994). A previous study suggested that the antibacterial mechanism of aluminium by competition with iron and magnesium and binding to deoxyribonucleic acid (DNA), membranes or cell walls is responsible for the main toxic effect of aluminium on microbes (Pina and Cervantes, 1996). For example, aluminium binds almost 10^7 times more tightly to adenosine tri-phosphate (ATP) than magnesium does, indicating that aluminium concentrations lower than nanomolar concentrations are sufficient to compete with magnesium at millimolar concentrations (Macdonald and Martin, 1988; Pina and Cervantes, 1996).

Our results indicated that aluminium had an antimicrobial property, and the rate of inactivation increased as temperature increased. A high thermal conductivity of aluminium and a toxic effect of aluminium may cause the antimicrobial property of aluminium. This finding of inactivation properties of aluminium can be available for designing pasteurization equipment, cooking utensils and antibacterial containers or packages in food industries.

Conclusion

Decimal reduction time (*D-values*) of *E. coli* in aluminium cups was significantly shorter than that in stainless-steel cups during pasteurization of milk. The inactivation effect of *E. coli* in aluminium cups increased as pasteurization temperature increased. These results indicate that aluminium utensils have an inactivating effect on *E. coli*.

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