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EFFECT OF ALUMINIUM COOKWARE ON MICROBIAL INACTIVATION DURING PASTEURIZATION OF MILK

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ABSTRACT The effect of aluminium cookware on inactivation of *Escherichia coli* and viable aerobic bacteria (viable aerobes) in milk during pasteurization was studied. Cells of *E. coli* were suspended in commercial milk (homogenized and pasteurized milk) in order to achieve a final concentration of colony-forming units of 10^7 per mL. Fresh raw milk (unhomogenized and non-pasteurized milk) was incubated at 20°C for 48 h to obtain 10^7 microbial cells per mL of viable aerobes. Samples of *E. coli* suspension milk in aluminium cups were immersed in a temperature controlled water bath at 65°C and 67°C for 3 and 2 min, respectively, and incubated milk samples with viable aerobes were treated at 60°C for 30 min. The results were compared with those obtained by using stainless steel cups. Cells that survived were counted after incubation at 37°C for 48 h. Results obtained under the temperature conditions of 65°C and 67°C clearly showed that cells of *E. coli* were eradicated more rapidly in aluminium cups than in stainless steel cups. Furthermore, decimal reduction times (*D-values*) in the aluminium cups were significantly shorter than those in stainless steel cups. For representative viable aerobes that survived in raw milk, there was generally no significant difference between samples in the aluminium and stainless steel cups at 60°C. This study indicates that an aluminium utensil has an inactivation effect on *E. coli* during pasteurization.

Keywords: *E. coli*, Viable aerobic bacteria, Survival cells, Decimal reduction time.

INTRODUCTION Aluminium (Al) and stainless steel have been used widely in cookware, cooking utensils, wrappings and packaging. The use of aluminium cookware can increase the amount of aluminium in food; however, the magnitude of this increase is generally not of practical importance. Aluminium can occur in a number of different forms in water and displays a high reactivity with oxygen at room temperature, and it rapidly reacts with acid and bases to produce metal salts and release hydrogen. Aluminium reacts with organic matter to form complexes such as aluminium chloride ($AlCl_3$), aluminium hydroxide ($Al(OH)_3$) and aluminium oxide (Al_2O_3) (WHO, 1998). According to Verissimo et al. (2006), the leaching of aluminium from cooking pans and food containers is proportional to pH values; the amount of aluminium leached is

increased with decrease in the pH value. Base on these results, the use of aluminium cookware is not recommended for acidic foodstuffs. On the other hand, cooking food in stainless-steel utensils significantly increased its iron content. Although, the increase in iron is small, it is substantial when considering the overall dietary iron intake (Park, 1997). The effect of aluminium on various microorganisms was recently reviewed by Pina and Cervantes (1996). Guida et al. (1992) investigated aluminium toxicity towards *E. coli* and found that growth inhibition was markedly dependent on pH. They 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. Ghernaout et al. (2007) carried out an investigation on electrocoagulation in *E. coli* using three different electrodes. Aluminium electrodes were found to be more efficient than those made from stainless steel and ordinary steel for destruction of *E. coli* cells. Furthermore, Bojic et al. (2001) studied the inactivation of *E. coli* by a microalloyed aluminium-based composite. The composite is material in the form of steel wire plated by microalloyed aluminium. Its effects are based on spontaneous dissolution when in contact with water, with generation of Al (III) and OH-ions, and finally with voluminous insoluble $Al(OH)_3$. The results 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 the treatment. These results also showed that the composite, applied in a semi-flow condition, is a powerful device for inactivation of *E. coli*. The original semi-flow system had no or only a minor influence, indicating that the reduction of *E. coli* cells was definitively the result of the microalloyed aluminium-based composite. Recently, thermal inactivation of *Listeria innocua* in salmon caviar using conventional thermal-death-time glass tubes and that in a novel aluminium tube were tested and compared by Al-Holy (2004). There have been very few reports in the literature on inactivation of microorganisms during heating using made from different types of material cooking utensils. The objective of this study was to determine the inactivating effect of an aluminium utensil on microorganisms during pasteurization in comparison to that of stainless-steel cookware.

MATERIALS AND METHODS

Preparation of microorganisms The strains of microorganism used in this work were *E. coli* and viable aerobic bacteria. For preparation of suspended cells of *E. coli*, one loop of frozen stock culture was activated by inoculating into 5 mL of sterile Trytic Soy Broth (TSB, Merck, Darmstadt, Germany) and aerobically incubated at 37°C for 9 h. Then the inoculum was transferred into 250 mL sterile TSB and incubated with shaking in a water bath at 37°C for 18 h. Bacterial cells were harvested by centrifugation and the supernatant fluid was discarded. Cells of *E. coli* were then suspended in commercial milk (homogenized and pasteurized milk) in order to achieve a final concentration of colony-forming units of 10^7 per mL. The suspended cells were stored in an iced water bath at 4°C until use. For preparation of viable aerobic bacteria, fresh raw milk (unhomogenized and non-pasteurized milk) was incubated at 20°C for 48 h to obtain approximately 10^7 microbial cells per mL of viable aerobes and stored at 4°C until use.

Experimental setting Thermal inactivation treatments were conventional heating performed in a temperature-controlled water bath with an electrical heater (Yamato, Thermo-mate BF400, Tokyo, Japan) using aluminium and stainless-steel vessels of 400 mL in capacity. The aluminium vessel was 58 mm in bottom diameter, 80 mm in top diameter and 132 mm in height, and the stainless-steel vessel was 55 mm in bottom

diameter, 78.5 mm in top diameter and 135 mm in height. The heat transfer areas of the aluminium and stainless-steel vessels were 312 and 307 cm^2, respectively. The aluminium cups with suspended *E. coli* milk were immersed in a temperature-controlled water bath at 65ºC and 67ºC for 3 and 2 min, respectively. The incubated milk with viable aerobes bacteria was treated at 60ºC for 30 min. The results were compared with those obtained by using stainless-steel cups in the same fashion. The temperature history of samples was observed during the experiments.

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

Calculation of *D-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 decimal reduction times (*D-values)* and *Z-values*. (Yuk et al., 2009) *D-values* were determined by linear regression from the expression of Singh and Heldman (1993):

$$\log N = \log N_0 - t / D, \quad (1)$$

where N is the count value at time t and N_0 is the initial count value. The number of survivors in the samples exposed to heat treatment for defined time intervals was determined by the plate count (Pereira et al., 2007). To evaluate the significance of difference found in the kinetic parameters determined in the aluminium and stainless-steel cups, statistical comparison analysis was carried out using software (MS-Excel 2003, 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

Escherichia coli

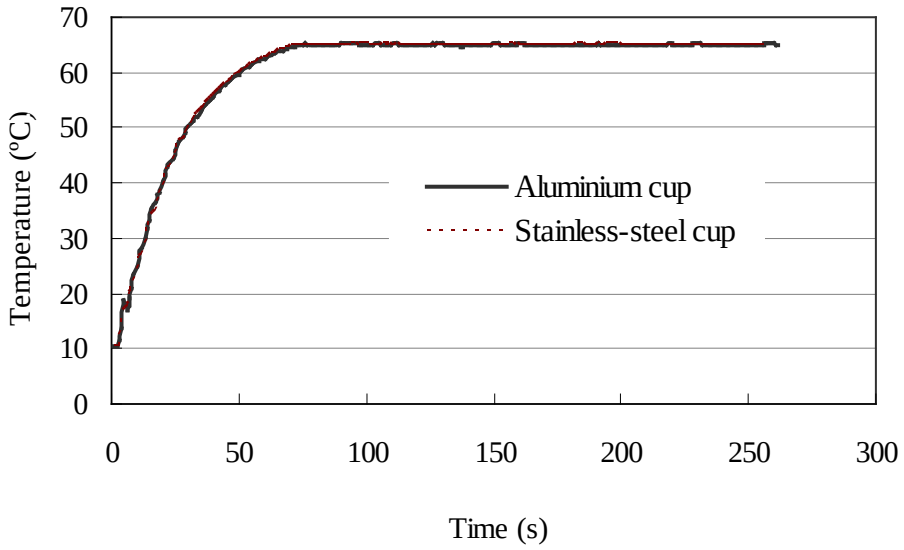

Figure 1. Thermal history of milk samples with *E. coli* during treatment at 65ºC for 3 min by using aluminium and stainless-steel cups.

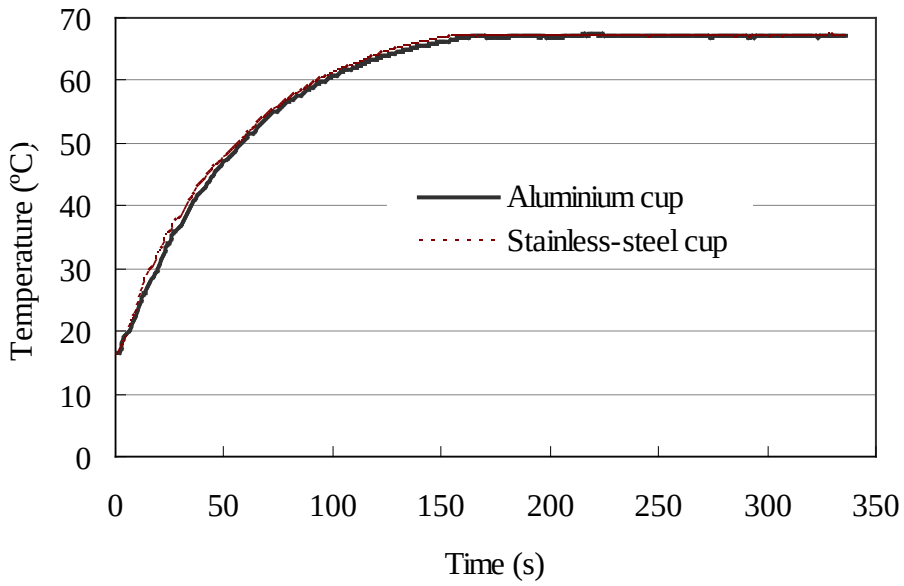

Figure 2. Thermal history of milk samples with *E. coli* during treatment at 67ºC for 2 min by using aluminium and stainless-steel cups.

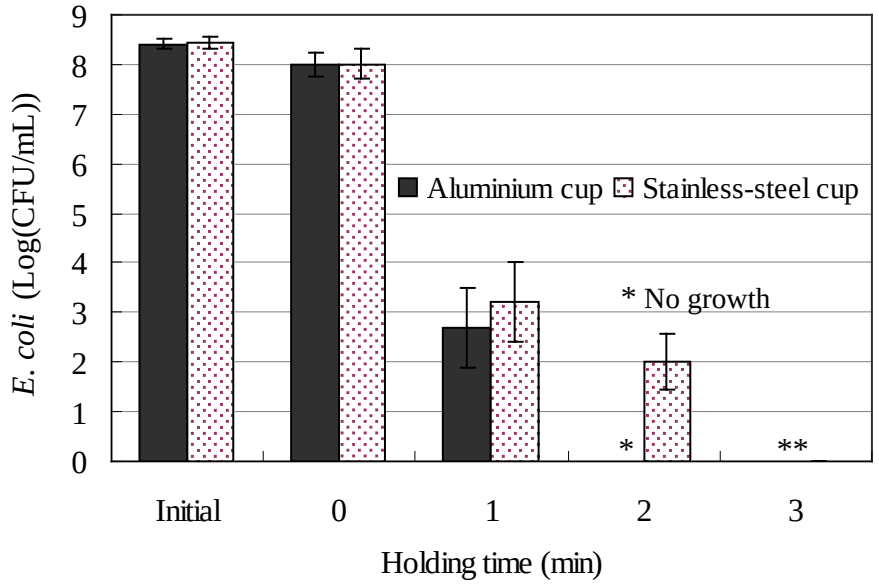

Figure 3. Survival of *E. coli* in milk during treatment at 65°C for 3 min by using aluminium and stainless-steel cups.

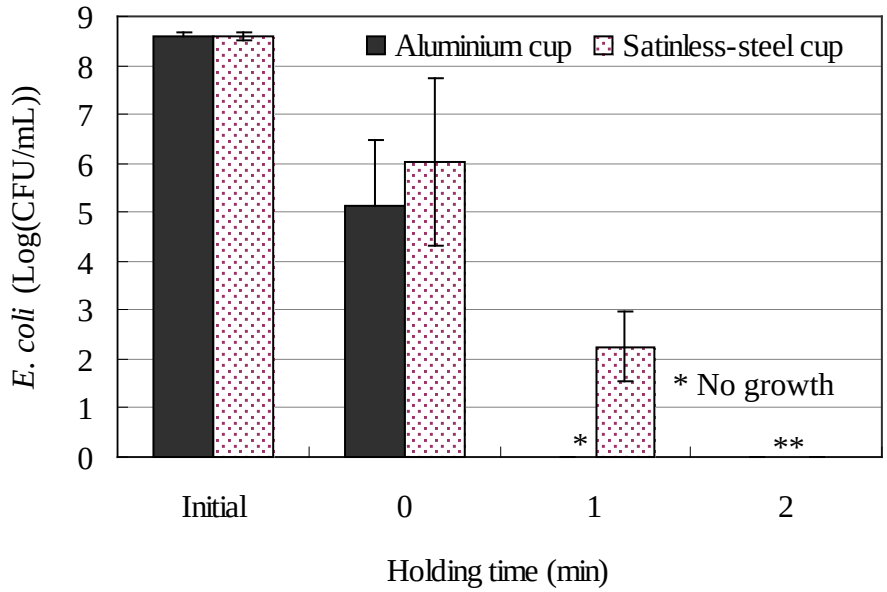

Figure 4. Survival of *E. coli* in milk during treatment at 67°C for 2 min by using aluminium and stainless-steel cups.

Table 1. *D-values* for *E. coli* at 65°C and 67°C.

Treatment	Description	D65 (min)	D67 (min)
Aluminium cup	Mean ± S.D	$0.33^{a} \pm 0.01$	$0.23^{a} \pm 0.00$
Stainless-steel cup	Mean ± S.D	$0.45^{b} \pm 0.03$	$0.33^{b} \pm 0.05$

a, b : *D-values* with different letters within column are significantly different at a level of 5%.

In Figures 1 and 2, temperature-time profiles measured at the center of each cup during

heating and holding phases are shown. A close coincidence of the temperature profiles was a necessary condition for evaluating the inactivity effects. The standard deviations of the temperature difference between the samples and the setting temperature (65ºC) during the holding phase were 0.08ºC for the stainless-steel cup and 0.11ºC for the aluminium cup.

Results obtained under the temperature conditions of 65 ºC and 67ºC clearly showed that cells of *E. coli* were eradicated more rapidly in aluminium cups than in stainless-steel cups as indicated in Figures 3 and 4. The *D-values* at 65ºC and 67ºC for the aluminium cups were 0.33 and 0.23 min, respectively, and those for the stainless-steel cups were 0.45 and 0.33 min, respectively (Table 1). The decimal reduction time (*D-value*s) was significantly shorter in the aluminium cups than in the stainless-steel cups ($P<0.05$).

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 using the conventional thermal-death-time glass tube and a novel aluminum tube, which showed that *D-values* were shorter in the aluminum tubes than in the glass tubes.

On the other hand, an indirect effect is an effect resulting from microorganisms in contact with oxidants generated during heating treatment. Based on the observation of aluminium leaching from the surface of aluminium vessels, this reaction can be proposed in terms of a general reaction (Ghernaout et al., 2007):

$$Al(s) + 5H_2O \longrightarrow O_2(g) + (7/2)H_2(g) + Al(OH)_3(s) \qquad (2)$$

However, Sadiq et al. (2009) studied antimicrobial sensitivity of *E. coli* to alumina nanoparticles and found that alumina nanoparticles have a mild inhibitory effect on growth of *E. coli*, only at very high concentrations. Furthermore, Guida et al. (1992) reported that the inhibitory effect aluminium toxicity on growth of *E. coli* was markedly dependent on pH.

Viable aerobic bacteria

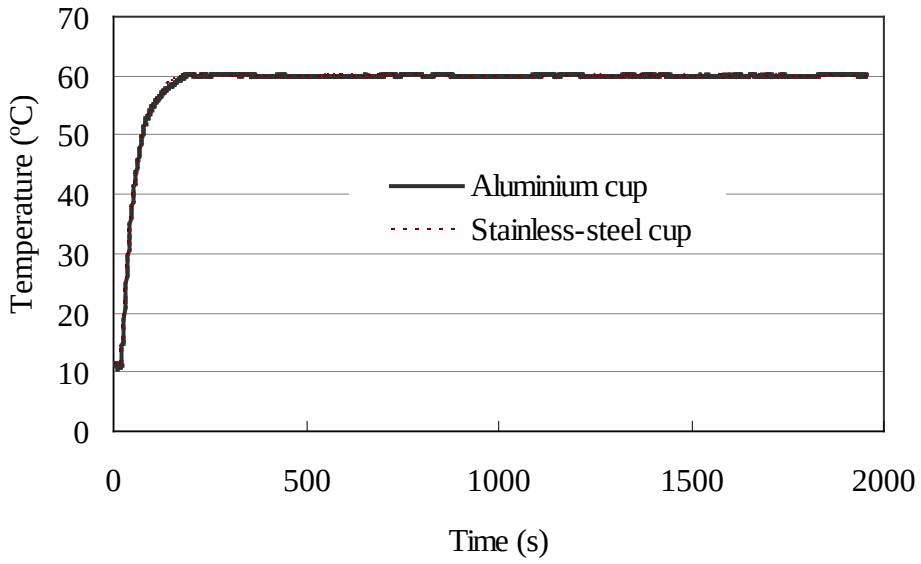

Figure 5. Thermal history of incubated milk with viable aerobic bacteria during treatment

at 60°C, 30 min by using aluminium and stainless-steel cups.

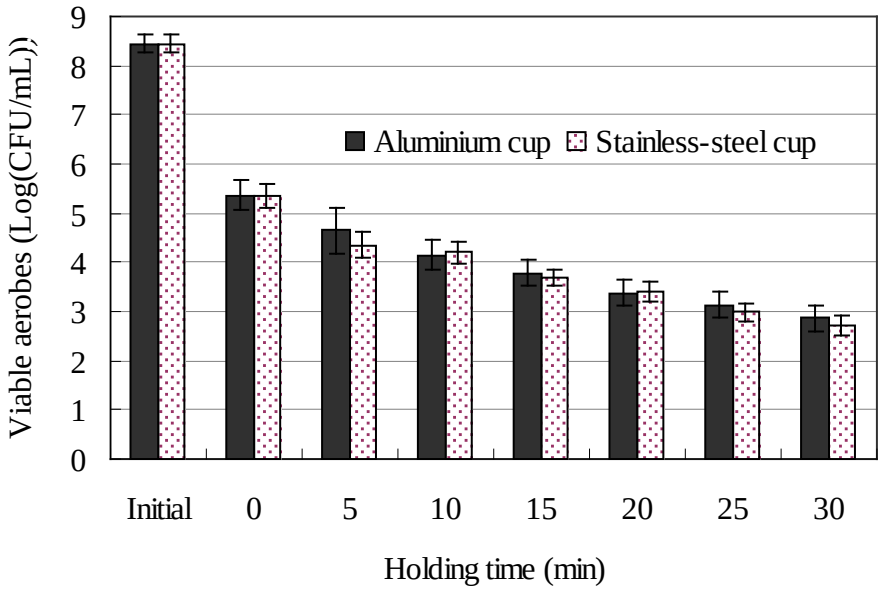

Figure 6. Survival of aerobic bacteria during treatment at 60°C for 30 min by using aluminium and stainless-steel cups.

A close coincidence of temperature-time profiles measured at the center of each cup during heating and holding phases is shown in Figure 5. For representative viable aerobic bacteria that survived in raw milk after treatment at 60°C for 30 min, the results of thermal inactivation are summarized in Figure 6. The *D-values* at 60°C were 2.50 min for the aluminium cup and 2.46 min for the stainless-steel cup (Table 2). There was generally no significant difference between samples in the aluminium and stainless-steel cups at 60°C.

Table 2. *D-values* for viable aerobic bacteria at 60°C.

Treatment	Description	D60 (min)
Aluminium cup	Mean ± S.D.	2.50 ± 0.08
Stainless-steel cup	Mean ± S.D.	2.46 ± 0.04

CONCLUSION For cells of *E. coli* in milk, at the same degree of inactivation temperature, the time required for reducing cells of *E. coli* in aluminium cups was shorter than that in stainless-steel cups, with a significant level *($P<0.05$).* In conclusion, this result indicates that an aluminium utensil has an inactivation effect on *E. coli* during heating treatment. However, no such effect on viable bacteria in raw milk was observed.

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