Time and temperature on *E. Coli* survival during hot water treatment of spoons

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Abstract

Escherichia coli is an indicator of microbial contamination in food production. It can cause several health problems, such as, bloody diarrhea, abdominal pain and fever. To avoid the foodborne illness, it is a normal practice in Thai cafeterias, food courts and canteens for consumers to immerse their spoons into hot water. Unfortunately, there was no any information regarding temperature and time to inactivate *E. coli* attached on spoons. The objective of this study was to determine the sufficient time and temperature to destroy *E. coli* attached on spoons. It was revealed that a temperature at either 90ºC for at least 10 s or at 100ºC for at least 5 s could completely inactivate *E. coli*. Water temperature and time that consumers used for immersing spoons into a water bath were also observed. It was found that the temperature of the hot water was approximately 70-80ºC and the time for dipping spoons collected from 100 people was 3-7 s. Considering the survival curve of *E. coli* during heating, it indicated that this condition could not completely inactivate *E. coli* attached on their spoons.

Keywords: dipping time, *Escherichia coli*, heat resistance, hot water treatment, spoon

1. Introduction

Nowadays, hot water treatment has been widely used in most cafeterias, canteens, and food courts located in Thailand. A customer practically immerses their spoons into hot water with an intention of inactivating foodborne pathogenic microorganisms, which may contaminate due to lack of hygiene. The water temperature and time used for dipping spoons varies greatly and it may not be sufficient to inactivate foodborne pathogen leading to illness.

Escherichia coli is considered as a foodborne pathogen of primary concern. It is usually used for a sanitation indicator in food production. This bacterium is mostly found in the lower intestine of ruminants including feces or stool of humans or animals (Moghadam et al., 2013).

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It can also spread to soil, water, air, raw fruits and vegetables. Although several strains of *E. coli* are harmless to human, some others can cause adverse effects on human health such as diarrhea, severe anemia, vomiting and chronic inflammation of intestine (Heijnen and Medema, 2006; Khan and Cash, 2013).

Since *E. coli* is involved in food safety and quality, there have been several reports studying about the method and condition for inactivation of *E. coli* in a variety of foods. For example, grilling ground beef at 71°C for 2.7 min resulted in destruction of *E. coli* (Rhree et al., 2003). *E. coli* in milk was inactivated when heating at 55 and 60°C for 180 and 30 min were applied, respectively. (Usajewicz and Nalepa, 2006). Gurtler et al. (2011) also reported that pasteurization of strawberry juice by pulsed electric field at 55°C for 1 s could reduce 3.79 log CFU/mL of *E. coli*. Nevertheless, no information relating to temperature and time used to destroy *E. coli* attached on spoons has been published. To reduce a risk of *E. coli* infection, there is a need to determine temperature and time to inactivate *E. coli*. The objective of this research was to investigate the effects of water temperature and dipping time on survival of *E. coli* inoculated on spoons undergoing hot water treatment.

2. Materials and Methods

2.1 Bacterial culture and culture media

E. coli DMST 4212 obtained from the Division of Biotechnology, Faculty of Science, Maejo University was streaked onto plate count agar (PCA). The cells were incubated at 37°C for 24-48 h and kept at 4°C until the time of experiment. Prior to each experiment one loopful of the stock culture was transferred into 5 mL of nutrient broth (Lab M, Salford, England). After incubation at 37°C for 18-24 h, a tube of *E. coli* culture was inoculated into Erlenmeyer flask containing 500 mL of nutrient broth. The flask was then shaken continuously via the use of an incubating shaker at 150 rpm at 37°C for 24 h to obtain the bacterial cells in their stationary phase.

2.2 Heat Treatment

A sterilized spoon was dipped into 500 mL of the *E. coli* suspension for 5 min. The spoon was then left in a laminar flow cabinet (Holten, HB 2472, Burladingen, Germany) at room temperature for 15 min until dry. The inoculated spoon was soaked in hot water at temperature of

60, 70, 80, 90 and 100°C for 5, 10, 15 and 20 s. The initial and survival numbers of *E. coli* were determined by Swab test. Swabs were placed into 10 mL of Ringer's solution (6.5 g/L NaCl, 0.42 g/l KCl, 0.25 g/L $CaCl_2$ and 0.05 g/l $NaHCO_3$). After vortexing for 30 sec, serial ten-fold dilutions in sterile saline dilution (0.85% NaCl) were prepared and plated onto PCA using pour plate technique. Plates were incubated at 37°C for 18-24 h. Colony forming units (CFU) were counted. The experiments were carried out with three replications. The ratio of survival was calculated using Eq.

(1)

$$\text{Ratio of survival} = 100 - \left[\frac{\log N_0 - \log N_t}{\log N_0} \times 100\right] \quad (1)$$

where N_0 = initial numbers of *E. coli* (CFU/piece)

N_t = numbers of *E. coli* at time *t* (CFU/piece)

The experiment data were also fitted to the Weibull model (Peleg, 2000) as shown in Eq. (2).

$$\log S = -bt^n \quad (2)$$

where Log *S* = the log of the survival ratio of the microorganisms at time *t*

b and *n* = the scale and shape parameters of the Weibull model, respectively

2.3 Survey of consumer's behavior on dipping time at Maejo University cafeteria

Temperature of water in a water bath within Maejo University cafeteria was measured every hour from 10 am to 3 pm. Time that 100 consumers used for dipping spoons into the water bath was also investigated.

2.4 Statistical analysis

The experiments were designed with completely randomized design (CRD). The data were analyzed and presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests. Values were considered significant at a confidence level of 95%.

3. Results and Discussion

3.1 Heat resistance of *E. coli*

Fig. 1 shows the survival curves of *E. coli* attached on spoons subjected to hot water at different temperatures and times. The initial number of *E. coli* on spoon was approximately 8 log CFU/piece. In all cases the survival of *E. coli* decreased when heating time was longer. Higher heating temperatures resulted in faster reduction of *E. coli*. This is because higher thermal energy at higher heating temperature could more produce damage important cell components, mainly cell protein and genetic materials resulting in loss of cell proper function (Yadav et al., 2009; Phungamngoen et al., 2011). It was also observed that for heating at 60-80°C, the survival cells first decreased and almost remained constant after 5 s of heating. However, in the case of heating at 90 and 100°C the survival of *E. coli* sharply decreased at the initial period of heating and was completely destroyed after 10 and 5 s, respectively.

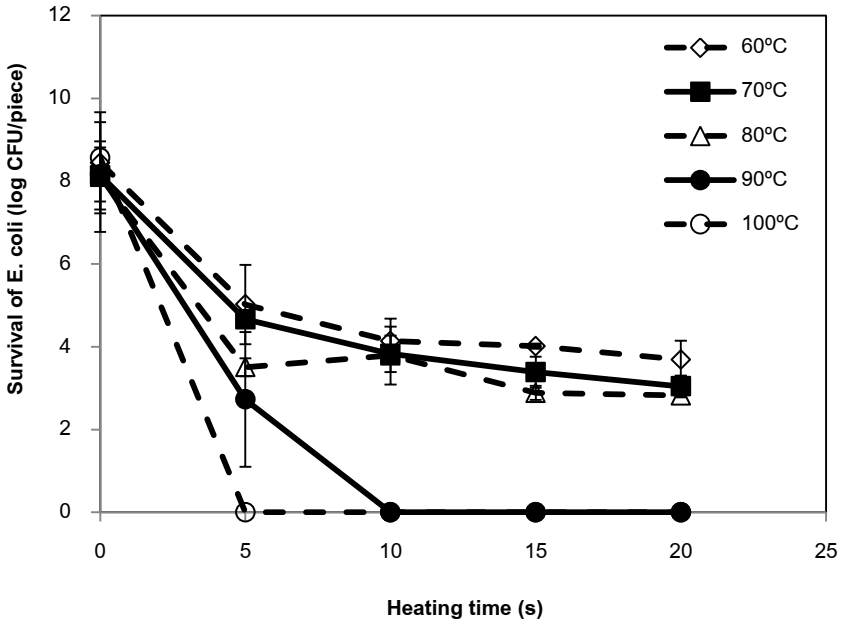

Figure 1. Survival curves of *E. coli* attached on spoons subjected to hot water at different temperatures and times.

The heat resistance of *E. coli* was predicted by following the Weibull model (Fig. 2). Excellent fits were obtained with high correlation coefficients (R^2 = 0.99). The calculated parameters of the model are presented in Table 1. The *n* values obtained for all heating conditions were less than 1, indicating different heat resistance among individual cells.

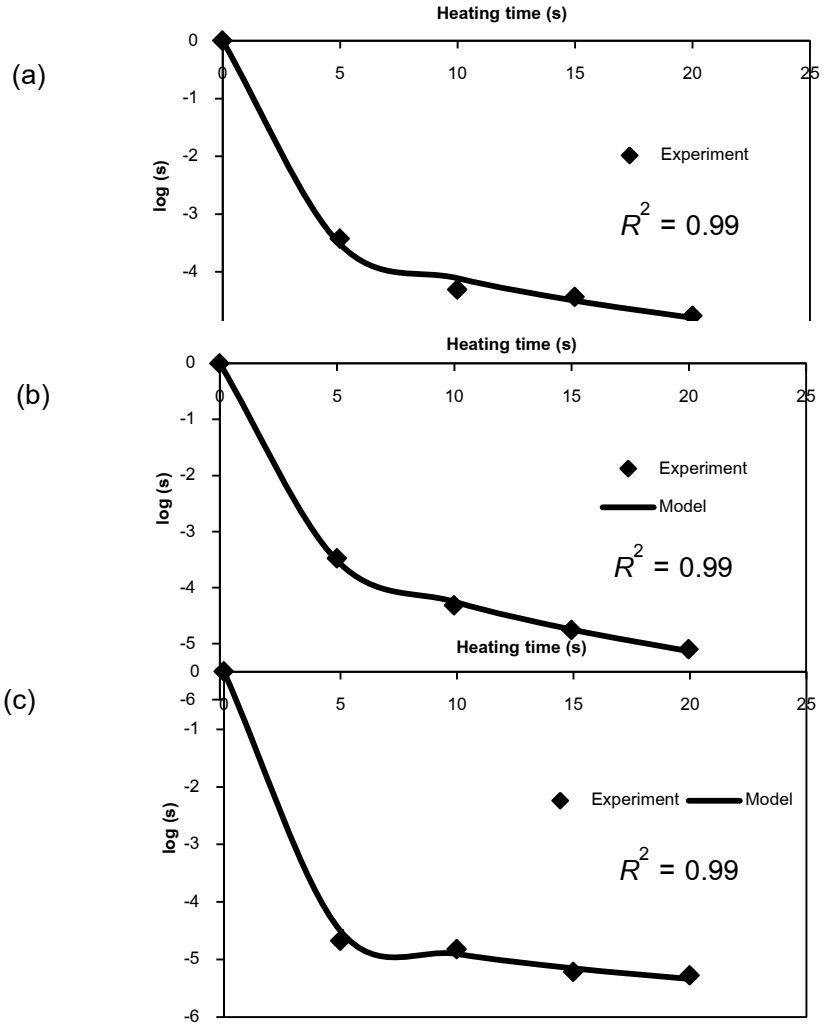

Figure 2. Comparison between the log of survival ratio obtained from experiment and model during heating at (a) 60°C, (b) 70 °C and (c) 80°C.

Table 1. Heat resistant parameters of *E. coli* attached on spoons.

Heating temperature (ºC)	*b*	*n*
60	2.460	0.223
70	2.271	0.272
80	3.696	0.123
90	N/A	N/A
100	N/A	N/A

*N/A = not applicable.

The survival ratios at different heating temperatures and times are presented in Table 2. It was clearly seen that there was no survival of *E. coli* on spoons when heating 90ºC for 10 s and 100 °C for 5 s.

3.2 Survey of consumer's behavior on dipping time at Maejo University cafeteria

For consumer's behavior survey, it was observed that consumers took 3-7 s for immersing spoons into hot water. The results showed that the average water temperature was 70-80°C. At high temperature (>55°C) most of the colon-aerogenes groups are usually diminished, but certain number of bacteria and virus persist. This indicated that there was a risk that consumers could be infected if their spoons were contaminated with *E. coli* since the water temperature and dipping time were not sufficient for *E. coli* destruction.

4. Conclusion

E. coli attached on spoons could be completely inactivated by immersing the spoons into hot water at either 90°C for 10 s or 100°C for 5 s. The results, which were taken from the consumer's behavior survey and measuring water temperature in the water bath within Maejo University cafeteria, revealed that consumers immersed their spoons into hot water at 70-80°C for 3-7 s. Based on the survival curve of *E. coli* during heating, this implied that their hot water treatment conditions could not completely destroy numbers of *E. coli* attached on spoons.

Table 2. Survival ratio of *E. coli* undergoing different heating temperatures and times.

Heating temperature (ºC)	Time (s)	Survival ratio*
60	5	59.5 ± 1.0[a]
	10	48.2 ± 0.5[b]
	15	47.5 ± 0.1[b]
	20	43.7 ± 0.5[c]
70	5	57.2 ± 0.1[a]
	10	41.6 ± 0.4[c]
	15	39.5 ± 0.4[c]
	20	37.3 ± 0.3[c]
80	5	46.7 ± 0.5[b]
	10	40.4 ± 0.4[c]
	15	35.6 ± 0.2[c]
	20	34.8 ± 0.0[c]
90	5	39.0 ± 1.1[c]
	10	0.0 ± 0.0[d]
	15	0.0 ± 0.0[d]
	20	0.0 ± 0.0[d]
100	5	0.0 ± 0.0[d]
	10	0.0 ± 0.0[d]
	15	0.0 ± 0.0[d]
	20	0.0 ± 0.0[d]

*Values in the same column with different superscripts mean that the values are significantly different ($p<0.05$).

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