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MODEL OF INACTIVATION OF *CAMPYLOBACTER JEJUNI*

IN SCALDING OF CHICKEN PROCESS*

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Abstract

The purpose of this study was to develop a model of inactivation of *Campylobacter jejuni* in industrial scalding of chickens.   Models can be used as a guide for broiler slaughterhouse operations for reducing levels of *C. jejuni* contamination on broiler carcasses.   Mean concentrations of *C. jeuni* in terms of colony farming units (CFU) in scald tank water and in carcass rinse solution after scalding were 2.90±0.07 and 3.86±0.11 LogCFU/ml, respectively.   Scald tank water temperature, flow rate, pH, and total solids in scalding process water were 54.15±0.2°C, 172.0±8.4 L/min, 8.0±0.01, and 2,565±114.3 mg/L, respectively.   Inactivation models were developed by using literature data for inactivating kinetics, of *Campylobacter* and the Arrhenius equation. Results of the inactivion models of scalding process indicated that high temperature and short time (less than 2 minutes) of scalding process were effective in reducing the number of viable cells.   The model fits the experimental data well and the values of the estimated parameters provide insight for this process.   The model can be used for process design and potential process modifications.

1. INTRODUCTION

Chicken meat is reported as a source of human campylobacteriosis because of the high frequencies of detection of *Campylobacter* in fresh chicken meat, especially, *C. jejuni/ coli*

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(Saengthongpinit *et al*., 2005). It was reported that 28% of bacterial pathogens isolated from the cases of acute bacterial dysentery from 623 cases in children aged one month to twelve years were *Campylobacter* positive (in that 80% *C. jejuni*, 20% *C. coli*) (Bodhidatta *et al*., 2002). The risks of campylobacteriosis from chicken meat consumption are food handling and consumption of undercooked problematic chickens.

This research models the scalding process, which is the first process step to reduce the amount of pathogenic bacteria on carcasses (Russell, 2003). From our previous studies of sensitivity analysis, risk factors associated with broiler carcass contamination with *Campylobacter* spp. included pH and temperature of scalding water, and numbers of *Campylobacter* in scald tank water (Osiriphun *et al.,* 2011). Therefore, the scalding process was suggested as a critical control point (CCP) in the HACCP plan for broiler slaughterhouses. Because of the high rates of broilers entering into the scalder, which could be as high as 200/min, bacterial cross contamination from one carcass to others is possible. The goal of this study was to develop a model of inactivation to describe the relationship between operating variables and thermal inactivation rates for *C. jejuni* on chicken meat.

The chickens with feathers were attached to a moving line that passed through the scalding tank following a path 37.1 m in length in a tank 9.275 m long, 0.835 m wide, and 0.81 m high.   There was approximately 10 cm between chickens and 200 chickens per minute.   The scale water flow rate leaving was 172 L/min, and the water volume was about 5900 L.   The chicken line had 3 U turns and there were 4 parallel rows of chickens.   The hot scald water entered near the location where the chickens exited from the tank.   There were no baffles in the tank.   There were 371 chickens in the tank, and the residence time was 1.85 minutes for each chicken.

2. Materials and methods

2.1 Data acquisition.

All samples were collected from the slaughterhouse nearby Bangkok, Thailand, during 2006-2008. The appropriated samples (carcass and water) and parameters (pH, temperature, and total solids) at

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scalding were collected and analyzed for the factors that affected the number of viable *C. jejuni*.

2.1.1 Carcass Sampling

Chicken carcass samples (n=20) were collected after the scalding stage. Carcass rinse samples were collected for measurement by using the NACMCF procedure (NACMCF, 2007). Briefly, the carcass was put into a plastic bag (30 cm×60 cm) and one hundred (100) milliliters of Butterfield's Peptone Water (BPW) was added. The carcass was rinsed with a rocking motion for one minute. Then the carcass was removed. The remaining fluid was kept in an icebox and sent to the laboratory for the quantitative analysis of *C. jejuni.*

2.1.2 Water Sampling

Approximately 500 ml of scalding water were collected in sterile food storage plastic bags. All samples were placed in an insulated box filled with flakes ice and sent to *RADAL* laboratory within 2 hours for microbiological analysis and physical analysis (Total solids). During the time of sample collection, pH (n=20), and temperature of scalding water samples were also analyzed.

2.2 Water temperature, pH and Total solids measurements

Water temperature of scald tank samples was monitored and recorded by using thermocouples of a 10-channel temperature data logger (ellab a-s CMC 281, Copenhagen). Signals from thermocouples attached to different spots of the scalder were sent to the recorder and printed out at every minute. Portable waterproof pH meter (HANNA HI 98128; H) was used to determine pH of scalding water at various times. Total solids concentrations in scalding water were analyzed by using procedure of APHA (APHA, 1999).

2.3 Microbiological analysis

2.3.1 MPN Methods for *Campylobacter*

SimPlate™ (BioControl Systems) for *Campylobacter* was used to detect and enumerate *C. jejuni* and other thermophilic *Campylobacter*. The samples were analyzed following the manufacturer's instructions. The culture medium was dissolved in 100 ml of sterile distilled water and distributed in sterile test tubes (9.0 mL per tube). One milliliter of the sample or the dilution was added to each tube,

and the mixture was transferred to the SimPlate™ tray. The inverted plates were incubated in the dark for 48-52 hours at 42°C in a microaerophilic condition (5% O_2, 10% CO_2, and 85% N_2). The red wells are presumptive positive for *Campylobacter,* and the number of red wells that do not fluoresce blue by holding UV light (366 nm wavelength) approximately 5 cm (2 inches) above the SimPlate device was observed to confirm quantification of thermophilic *Campylobacter*. The Most Probable Number of Colony Forming Units (MPN of CFU) was determined using the manufacturer chart.

2.3.2 Plating media method for *C. jejuni*

Serial dilutions of the rinse were made in Phosphate Buffer Saline (PBS), and *Campylobacter* were enumerated by plating in duplicate onto the surface of mCCDA agar (Oxoid Ltd., Hampshire, England). Four samples of 0.25 mL of the dilution fluid were spread on the surface of 4 plates with a sterile glass hockey stick. Plates were incubated at 42 °C for 48 hours in a microaerophillic environment (5%O_2, 10%CO_2, and 85%N_2). Colony forming units characteristic of *Campylobacter* were counted from all 4 plates to get numbers/ milliliter.

2.3.3 Isolation and identification of *C. jejuni*

The colony grown on mCCDA plates originated from positive and negative wells of simplate method for *Campylobacter* and colonies from direct plating on mCCDA were picked up for *C. jejuni* identification. Then, the presumptive positive isolates were confirmed as *C. jejuni* by biochemical tests. The microscopic examination to observe high motility and spiral rod morphology was also used. Growth was observed under microaerophilic conditions at 42°C. Catalase and oxidase production, motility, and hippurate hydrolysis tests were also made. Strains were stored at −40 °C in Brain Heart Infusion (BHI) broth (Oxoid, Hampshire, England) containing 20% glycerol.

3. Data Analysis

Before proceeding to the main analysis, it is important to report the sources of inactivation kinetic data selected for use in this study. From the data of Doyle and Roman (1981), D-values of *C. jejuni* at temperatures from 48°C to 55°C were used.   The data of Ugwuanyi *et al*. (1999) at 55°C and 60°C

were also used.

3.1 Governing Equations

The flow of chickens through the scald water can be modeled using a plug flow model. For a single chicken,

$$\frac{dC}{d\tau} = -(k_{cw} + I_c)C \qquad (1)$$

with initial condition $C=C_o$ at $\tau=0$ at the entrance of the scalder. Here C is the number of *Campylobacter* on the carcass, τ is residence time in the scald water, k_{cw} is the rate constant for transfer of *Campylobacter* from a carcass to the scald water, and I_c is the inactivation rate constant on the carcass. Integration of Equation (1) leads to

$$C = C_o e^{-(k_{cw}+I_c)\tau} \qquad (2)$$

or

$$\ln \frac{C}{C_o} = -(k_{cw} + I_c)\tau \qquad (3)$$

In the water phase, the steady state mass balance for *Campylobacter* is

$$F_c(C_o - C)\frac{k_{cw}}{k_{cw}+I_c} - F_w W - I_w W V_w = 0 \qquad (4)$$

where F_c is the flow rate in number of chickens per minute entering and leaving the scalder, V_w is the volume of water in the scald tank, F_w is the volumetric flow rate of the scald water leaving the scald tank, W is the concentration in the water phase of live *Campylobacter* in the scald tank, and I_w is the inactivation rate constant for *Campylobacter* in the water phase. The complete mixing model is used to model the water phase.

3.2 Inactivation Model

In the scald tank, the rate of inactivation depends on temperature.

$$I = I_0 e^{-E/RT} \qquad (5)$$

where *E* is the activation energy. Heat inactivation of *C.jejuni* can be explained by the following model (Wang *et al.,* 1979):

$$\frac{dN}{dt} = -IN \tag{6}$$

where N is concentration of viable organisms in number/ml, I is specific death rate constant in minute^{-1}, and t is time in minutes. Then, integrating the equation when $N=N_0$ and $t=t_0=0$

$$\ln N = \ln N_0 - It \tag{7}$$

To calculate the Decimal reduction (D) of *C. jejuni* by the equation for $N_0/N=10$,

$$\log_{10}\frac{N_0}{N} = \log_{10} 10 = 1 = \frac{t}{D} \tag{8}$$

where $D=$ time for reduction by a factor of 10 and D has units of minutes.

Since $\ln 10=2.303$, the I in Equation 7 is related to D as follows:

$$ln10 = 2.303 \log_{10}10$$

$$I = 2.303/D \tag{9}$$

The activation energy E is estimated using

$$\ln I = \ln I_0 + (-E/RT) \tag{10}$$

where

$$Slope = -E/R \tag{11}$$

where I = specific death rate constant in minute^{-1}, R= Universal gas constant in cal/mole °K (1.987 cal/mole °K), T= absolute temperature in °K.

4. RESULTS AND DISCUSSION

4.1 Results from scalding operations

One set of experimental results is presented in Table 1. The values from microbiological analysis are larger for the carcass rinse sample, in which the carcass was subjected to a rocking motion for one minute to obtain a representative sample (NACMCF, 2007). With this method approximately 50% of the *Campylobacter* are found in the 100 ml solution based on the data in which the process is repeated on the same carcass (Jorgensen et al., 2002). At 54.15 °C the experimental values in CFU are 7244/ml for the carcass solution and 794/ml for the scald water. These results show

that the transfer rate for *Campylobacter* from the carcass to the scald water is small compared to values that would be needed for very rapid exchange between the carcass and water. The slow release of organisms from the broiler carcass may occur because *C. jejuni* are capable of adhesion onto the feathers and poultry carcass. It is very difficult to wash some organisms from the chickens because they may be located in crevices and feather follicles of the chicken skin. The cells in the feather follicles have been reported to float in entrapped water, even after the skin has been rinsed quite thoroughly (Jang et al., 2007). Similarly, adhesion occurs on the fascia or loose connective tissue that is under the skin and covers muscle (ICMSF, 2005). Thermal inactivation of *Campylobacter* on the chicken accounts for the larger fraction of the reduction of live cells according to the model results shown below.

The second set of experimental data was collected at an average temperature of 61.08 ± 0.05 ºC (Osiriphun, 2009). For this set of data the average values for the *Campylobacter* on the chickens after scalding and in the scald water are $\log_{10}CFU = 2.93 \pm 0.07$ and $\log_{10}CFU = 1.39 \pm 0.16$, respectively. These values correspond to 851 CFU/ml of rinse solution for the *Campylobacter* on the chicken and 24.5 CFU/ml in the scald water. At this higher temperature, *Campylobacter* that are unprotected in the scald water are inactivated very rapidly. The ratio of viable cells in the scald water to *Campylobacter* in the rinse solution is smaller for the higher temperature. The same values were used for scald water flow rate, production rate for the chickens, volume of scald water, and residence time of the chickens in the scald water for this set of data.

4.2 Estimated values of parameters

The parameter value for I_w was estimated from the data in Table 2. The values of the parameters, k_{cw} and I_c were estimated from the slaughterhouse data and the data in Table 2. For 54.15 ºC, the data in Table 1 was used together with the production rate of 200 chickens/minute, a residence time of 1.85 minutes for each chicken, 5900 L of water in the tank, and a water outflow of 172 L/min. The measured value of *Campylobacter*/ml in the carcass solution was multiplied by 100 because 100 ml of solution was used and by a factor of 2 to estimate the number of *Campylobacter* on each carcass.

The values of C_o and $(k_{ew}+I_c)$ were adjusted to give a good fit of the experimental data.   Results are shown in Tables 3 and 4 for inlet values of 1.0×10^8 and 1.1×10^8 *Campylobacter* per chicken, respectively.   The available data did not include measured values of *Campylobacter* per chicken for the chickens prior to scalding; however, data taken at another time are shown in Table 5.   The model provides a good fit of the data for these two values of *Campylobacter* per chicken entering the scalding process.   For $C_o=1.0 \times 10^8$, the estimated value of I_c that satisfies the model for the first set of data corresponds to a temperature of 52.69 ºC, which is less than 1.5 ºC below 54.15 ºC.   The temperature of the carcass is expected to be slightly less than the scald water temperature.   For the value of $C_o=1.1 \times 10^8$ *Campylobacter* per carcass for the entering chickens in Table 4, the second set of data gives an estimate of I_c that is only slightly less than I_w, which means that the temperature at the carcass surface is very close to the scald water temperature of 61.08 ºC.   Thus, the estimated values of 10^8 *Campylobacter* per carcass and 1.1×10^8 *Campylobacter* per carcass both provide a reasonable fit to the model for the measured experimental data and the available inactivation kinetic data.   These values are in reasonable agreement with the data in Table 5, especially if the set one data in Table 5 are multiplied by 2 to provide an estimate of the number of *Campylobacter* on each chicken, which includes the *Campylobacter* in the rinse solution and the *Campylobacter* that are still associated with the chicken.   The average value of 60,800,000 from Table 5 multiplied by 2 is 121,600,000 *Campylobacter* per chicken, which is slightly larger than the values of C_o that give a reasonable fit to the data.   Since some of the original rinse solution remains with the chicken when the set two data are collected, the estimated value of 121,600,000 *Campylobacter* per chicken may be too large.

The model can be used to investigate potential process changes in the mean residence time, scald water flow rate, scald water temperature, and feed rate of the chickens.   For example increasing the residence time by making the tank larger by 50% to $\tau=2.775$ minutes and 8850 liters of scald water results in values of 868 *Campylobacter*/ml of carcass solution at 54.15 ºC and 34.93 *Campylobacter*/ml in the scald water at 61.08 ºC.   At 54.15ºC, this value of 868 is about 12% of the value for the earlier residence time of 1.85 minutes.   At 61.08 ºC, the value of 34.93 is about 4% of

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851.   Thus a 50% increase in the residence time of the chickens should result in a product after scalding with a much lower number of *Campylobacter* present on the chicken surfaces.   Since the scalding step occurs before evisceration, inactivation of all *Campylobacter* during scalding is not a process operation goal.

4.3 Estimation of activation energy

The data of Doyle and Roma (1981) presented in Table 2 was used to estimate the activation energy shown in Equation (5).   The value found for the slope (-E/R) from a plot of ln I vs 1/T was used to estimate the activation energy E.   The estimated value of 70 k_{cal}/g mole is similar to other values of activation energy for thermal death kinetics (Wang et al., 1979).

4.4 Temperature for scalding chickens

Results are presented for two average operating temperatures in this work, 54.15ºC and 61.08ºC. Good results were obtained at both temperatures. Significant reduction in both *Salmonella* and *Campylobacter* has also been recorded by others at scald temperatures of 58-60 °C (Notermans and Kampelmacher, 1975; Notermans *et al*., 1977; Oosterom *et al*., 1983; Wempe *et al*., 1983; Mead, 2005). *C. jejuni* is sensitive to thermal conditions; it has been destroyed at temperatures between 55-60 ^{O}C for times between 0.21-2.5 minutes (Omar, 2005).   The data from our work shows that we can reduce *C. jejuni* in scald tank water by using a higher temperature of 61 °C scald tank water. Based on the work of Osiriphun (2009), the work described in this paper, and the literature cited above, the most appropriate temperature for scald tank water to reduce *C. jejuni* is between 55-.60 °C.

5. CONCLUSION

A model for inactivation of *C. jejuni* on poultry carcasses and in scald water was developed and used with data from an operating plant to estimate the parameters in the model.   The results show that most of the *Campylobacter* are inactivated while on the chickens.   For the two temperatures investigated, a larger fraction of the *Campylobacter* were inactivated on the carcasses at the higher temperature.   The model was used to predict the impact of increasing the mean residence time of the

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chickens in the scald tank by 50% by increasing the scald water volume by 50%. The predicted number of *Campylobacter* on the carcasses after scalding was reduced to about 12% and 4% compared to the measured experimental values for 54.15 °C and 11.08%, respectively.

6. ACKNOWLEDGMENTS

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Campylobacter jejuni and *Salmonella* Typhimurium in poultry scalding and chilling. *Journal of Food Science*, 67(5), 1836-1843.

Table 1. Summary of physical, chemical and microbiological analyses of scalding water and broiler carcass samples at scalding step.

Physical and chemical analysis of scalding water*				Microbiological analysis*	
Temperature (°C)	Flow rate (liters/min.)	pH	Total solids (mg/liter)	*C. jejuni* (LogCFU/ml)	
				SW	SC
54.15±0.21	172.0±8.40	8.0±0.01	2,565±114.32	2.90±0.07	3.86±0.11
N=20	N=20	N=20	N=20	20/20**	20/20**

Remark:
* =Mean±Standard error,
* * = total number of samples/number of positive samples, SW=scald tank water, SC=scalded carcass
Data from Osiriphun (2009).

Table 2. Average values of parameter (*lnI*) at temperatures 48, 50, 53, 55, 60° 75, 80, and 85 °C.*

Temperature	48°C	50°C	53°C	55°C	60°C	75 °C	80 °C	85 °C
lnI	-1.50	-0.691	0.307	0.929	1.177	1.488	1.314	1.780
				0.844		2.542	1.902	2.156
						1.795	2.449	2.701
Average *lnI*	-1.50	-0.691	0.307	0.8865	1.177	1.942	1.888	2.212
1/T (ºK)	0.00312	0.00310	0.00307	0.00305	0.00300	0.00287	0.00283	0.00279

* Data from Doyle and Roman (1981), Ugwuanyi et al. (1999), and Whyte et al. (2003).

Table 3. Values of the parameters T, I_w, I_c, k_{cw}, $k_{cw}/(k_{cw}+I_c)$, and C/C_o for two sets of poultry slaughterhouse data for $C_o=10^8$ *Campylobacter* per carcass.

T (°C)	I_w (min^{-1})	I_c (min^{-1})	k_{cw} (min^{-1})	$\dfrac{k_{cw}}{k_{cw}+I_c}$	$\dfrac{C}{C_o}$
54.15	1.896	1.242	1.047	0.4575	0.01449
61.08	3.455	3.36	0.0869	0.0252	0.001702

Table 4. Values of the parameters T, I_w, I_c, k_{cw}, $k_{cw}/(k_{cw}+I_c)$, and C/C_o for two sets of poultry slaughterhouse data for C_o=1.1x10^8 *Campylobacter* per carcass.

T (ºC)	I_w (min^{-1})	I_c (min^{-1})	k_{cw} (min^{-1})	$\dfrac{k_{cw}}{k_{cw}+I_c}$	$\dfrac{C}{C_o}$
54.15	1.896	1.368	0.972	0.4154	0.01317
61.08	3.455	3.418	0.0802	0.0229	0.001547

Table 5. Numbers of *Campylobacter* in 100 ml of buffered peptone water solution following rocking for 30 times in 1 minute for each chicken after slaughter and prior to entering the scalding operation. Set two data were obtained by repeating the experiment on the same chicken using a new 100 ml solution.

Set one		Set Two	
$\log_{10}$ CFU	Millions of CFU	$\log_{10}$ CFU	Millions of CFU
7.7	50.1	7.4	25.1
7.7	50.1	7.5	31.6
7.8	63.1	7.4	25.1
7.7	50.1	7.5	31.6
7.8	63.1	7.4	25.1
7.9	79.4	7.4	25.1
7.8	63.1	7.5	31.6
7.8	63.1	7.4	25.1
7.8	63.1	7.5	31.6
7.8	63.1	7.5	31.6