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Thermal Lethality of *Salmonella* Senftenberg and *Listeria innocua* on Fully Cooked and Vacuum Packaged Chicken Breast Strips during Hot Water Pasteurization

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ABSTRACT

Seven $\log_{10}$ CFU of *Salmonella* Senftenberg or *Listeria innocua* were surface inoculated on fully cooked chicken breast strips. The inoculated strips (227 or 454 g) were vacuum packaged in 0.2-mm-thick pouches (114 by 114 mm and 241 by 114 mm, respectively). The products were then heat treated in a hot water cooker at 88°C for 0 to 40 min. After heat treatment, *Salmonella* Senftenberg and *L. innocua* survivors were enumerated. Increasing treatment time increased the thermal lethality for *Salmonella* Senftenberg and *L. innocua.* The effect of treatment time interacted with product size. To achieve a 7-$\log_{10}$ reduction for *Salmonella* Senftenberg and *L. innocua,* the 454-g packages needed to be heat treated for 34 min and the 227-g packages needed to be treated for 20 min. Models were developed to correlate treatment time with bacterial survival rate and could be used to predict up to a 7-$\log_{10}$ reduction of *Salmonella* Senftenberg or *L. innocua* for similar products.

Ready-to-eat meat and poultry products should be free of pathogens at the end of a cooking process. However, pathogens could contaminate cooked meat products after cooking before final packaging *(2, 12)*. This contamination most likely is on the product surface *(6)*, and, therefore, it is amenable to surface pasteurization. Although other methods, such as irradiation, are being investigated to reduce the risk of *Listeria monocytogenes* in ready-to-eat meats *(11)*, thermal-based methods can be implemented expeditiously because they do not require regulatory approval. Murphy et al. *(7)* found that postprocess pasteurization using steam at 88°C could eliminate 7 $\log_{10}$ cells of *Salmonella* Senftenberg and *Listeria innocua* on fully cooked and vacuum packaged chicken breast strips. This method was effective when 454 g of product was treated in a continuous cooker for 34 min or a batch cooker for 40 min *(7)*.

The objective of this study was to evaluate the effect of hot water pasteurization on thermal lethality of *Salmonella* Senftenberg and *L. innocua* on the surface of fully cooked chicken strips. Inoculated products were vacuum packaged before heat treatment. Two package sizes of the strips, containing 227 g (114 by 114 mm) or 454 g (241 by 114 mm) were used in this study. The effect of package size on thermal lethality of *Salmonella* Senftenberg and *L. innocua* during hot water treatment was evaluated.

MATERIALS AND METHODS

Products. Fully cooked chicken breast strips were obtained from a commercial processor and held at 4°C. The strips were obtained by slicing freshly cooked chicken breast fillets into 13-mm-wide strips. The products contained 22.6% protein, 3.6% fat, 2.4% carbohydrates, 0.5% sodium salt, and 70.8% water. The pH of the products was approximately 6.5, and water activity was approximately 0.95. Before treatments, the products were sampled and found to have no detectable *Salmonella* or *Listeria* using the Food and Drug Administration methods for detection *(1, 5)*.

Organisms. *Salmonella* Senftenberg and *L. innocua* were used in this study. *Salmonella* Senftenberg was used because of its high resistance to heat *(8)*. *Salmonella* Senftenberg ATCC 43845 was purchased from American Type Culture Collection (Rockville, Md.). A nalidixic acid–resistant culture was prepared as previously described by Murphy et al. *(8, 9)*. *Salmonella* Senftenberg culture was maintained on tryptic soy agar (Difco Laboratories, Sparks, Md.) plus 200 ppm of nalidixic acid sodium salt and transferred monthly. For the stock culture, one loop of the *Salmonella* Senftenberg culture was transferred from the slant into tryptic soy broth (Difco) plus 200 ppm of nalidixic acid sodium salt (Sigma Chemical Co., St. Louis, Mo.) (TSB-N) and incubated at 35°C for 24 h. The stock culture was prepared weekly. The subcultures for use as inocula were prepared in TSB-N as required.

L. innocua M1 was developed as a heat resistance model for *L. monocytogenes (3, 4)*. *L. innocua* M1 was originally obtained from Dr. P. M. Foegeding (Department of Food Science, North Carolina State University, Raleigh, N.C.) via Dr. M. G. Johnson (Department of Food Science, University of Arkansas, Fayetteville, Ark.). The *Listeria* culture was resistant to 50 ppm of rifampicin and 250 ppm of streptomycin. The lyophilized culture was revived in tryptic soy broth plus 0.6% yeast extract, 50 ppm of rifampicin, and 250 ppm of streptomycin (TSBYE-R-S) for 24 h at 35°C. *L. innocua* culture was maintained on TSBYE-R-S slant and transferred monthly. For the stock culture, one loop of the *L. innocua* culture was transferred from the slant into TSBYE-R-S and incubated at 35°C for 24 h. The stock culture was prepared weekly. The subcultures for use as inocula were prepared in TSBYE-R-S as required.

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TABLE 1. *Type III tests for the effect of hot water treatment time and package sizes (227 g versus 454 g) on the survivors of* Salmonella *Senftenberg and* Listeria innocua *on fully cooked and vacuum packaged chicken breast strips*

Culture	Effect	df	χ^2	*P*
Salmonella Senftenberg	Package size	1	3.23	0.0722
	(treatment time)^2	1	207.60	<0.0001
	(treatment time)^2 × package size	1	88.84	<0.0001
Listeria innocua	Package size	1	1.77	0.1838
	(treatment time)^2	1	226.70	<0.0001
	(treatment time)^2 × package size	1	91.84	<0.0001

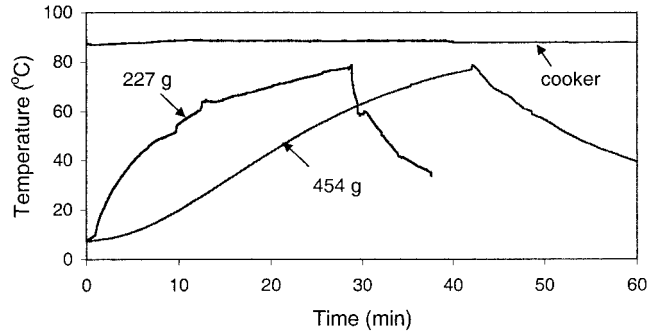

FIGURE 1. *A thermal profile for the cooker and the packages that contained 272 or 454 g of fully cooked chicken breast strips.*

Inoculation. In this study, a 24-h culture was prepared at 35°C in TSB-N for *Salmonella* Senftenberg and in TSBYE-R-S for *L. innocua.* This resulted in approximately 10^9 CFU/ml of *Salmonella* or *Listeria.*

A total of 454 g of product and 10 ml of a 24-h culture of *Salmonella* Senftenberg or *L. innocua* were placed in a sterile bowl and mixed for 5 min using a sterile speculum (Fisher Scientific Co., Itasca, Ill.). The excess water was allowed to drip off. The inoculated product was held at 4°C for 60 min to allow the cells to attach on to the product surfaces. The surface-inoculated strips were placed in a 0.2-mm-thick gas/moisture barrier pouch (241 by 114 mm for 454-g products or 114 by 114 mm for 227-g products, Tyson Foods, Inc., Springdale, Ark.). The pouches were sealed with a vacuum packaging machine (Komet, Stuttgart-W, Kornbergstr 27–29, Germany) at a vacuum of 1.0 bar.

Processing. The packaged products were processed in a hot water cooker (Magni Whirl, Blue M Electric Company, Blue Island, Ill.) at 88°C for 5 to 30 min at a sampling interval of 0.5 to 4 min for 227-g packages or for 10 to 40 min at a sampling interval of 0.5 to 3 min for 454-g packages. Treatment time was determined by preliminary tests to provide up to a 7-$\log_{10}$ cell reduction for *Salmonella* Senftenberg and *L. innocua.*

During heat treatment, cookers and meat temperatures were monitored every 1 s by a data acquisition system (CR23X Micrologger, Campbell Scientific, Inc., Logan, Utah) via thermocouple probes (type K). Five probes were sealed between meat pieces at the pouch center in two of six packages processed in each trial as one experimental unit. The lowest temperature recorded by the probes (among 10 probes in 2 packages) was used as the product temperature at the pouch center. After thermal treatment, the products were immediately submerged in an ice-water bath. The cooling time for the products to reach a temperature below 23°C was 8 to 9 min (for 227-g packages) and 16 to 18 min (for 454-g packages).

Bacterial enumeration. After the products were cooled to below 23°C, the pouches were wiped with 70% ethanol and then a 25-mm slot was aseptically cut in one end of the pouch. Fifty milliliters of sterile peptone solution (0.1%) was pipetted into the pouch and mixed with the products by hand through the package for 2 min. Thereafter, serial dilutions were surface plated onto selective agar medium (TSB-N for *Salmonella* Senftenberg and TSBYE-R-S for *L. innocua*) and overlaid with tryptic soy agar to allow resuscitation and enumeration of heat-injured cells. The plates were incubated at 35°C for 24 h, counted, returned to the incubator, and recounted the next day until the viable counts did not increase further. The plates were kept for 1 week before they were discarded. To detect low levels of *Salmonella* Senftenberg and *L. innocua,* at each treatment, 300 ml of sterile tryptic soy broth plus 0.6% yeast extract were mixed with each package of the product and incubated at 35°C in a shaker at 600 rpm for 24 h. After the incubation, the solution was plated to check for the growth of *Salmonella* Senftenberg and *L. innocua. Salmonella* and *Listeria* were confirmed according to Andrews et al. *(1)* and Hitchins *(5)*, respectively. Noninoculated control studies were also conducted following the same procedure described herein.

Statistical analysis. In each trial, six packages were processed and analyzed as one experimental unit. Three replicated experiments were conducted at each treatment condition. Bacterial counts were performed on all packages.

Surviving rates, $\ln(N/N_0)$, of each organism were analyzed using log-linear models. For *Salmonella* Senftenberg, for example, since $\ln(N/N_0) = \ln(N) - \ln(N_0)$, $\ln(N)$ was modeled using $\ln(N_0)$ as an offset variable in SAS procedure GENMOD, where N was the cell count at a certain treatment time and N_0 was the initial cell count. The counts for surviving organisms were assumed to have been sampled from negative binomial distributions. The final cell count, N, could be considered as coming from a binomial distribution (number of surviving cells, N, of N_0 initial bacterial cells). Because of the large initial cell counts ($\sim 10^7$) and the (cell

TABLE 2. *Parameter estimates of the negative binomial model for* Salmonella *Senftenberg counts (CFU/g[1]) on fully cooked and vacuum packaged chicken breast strips*[a]

Parameter	df	Estimate	SE	95% confidence interval	χ^2	$P > \chi^2$
τ	1	1.0408	0.5317	−0.0012 to 2.0828	3.83	0.0503
γ	1	−1.1745	0.6642	−2.4762 to 0.1273	3.13	0.0770
β	1	−0.0144	0.0007	−0.0158 to −0.0129	388.78	<0.0001
η	1	−0.0216	0.0016	−0.0248 to −0.0184	175.95	<0.0001

[a] $\ln(N/N_0) = \tau + \gamma\delta_{i2} + \beta(\text{time})^2 + \eta(\text{time})^2\delta_{i2}$, where $i = 1$ for the packages with a mass of 454 g, $i = 2$ for the packages with a mass of 227 g, $\delta_{i2} = 0$ when $i = 1$ and $\delta_{i2} = 1$ when $i = 2$. Deviance/*df* = 1.1304.

TABLE 3. *Parameter estimates of the negative binomial model for* Listeria innocua *counts (CFU/g[1]) on fully cooked and vacuum packaged chicken breast strips*[a]

Parameter	df	Estimate	SE	95% confidence interval	χ^2	$P > \chi^2$
τ	1	0.6137	0.4420	−0.2527 to 1.4800	1.93	0.1650
γ	1	−0.8098	0.6102	−2.0057 to 0.3861	1.76	0.1845
β	1	−0.0152	0.0006	−0.0164 to −0.0139	589.43	<0.0001
η	1	−0.0225	0.0018	−0.0261 to −0.0190	154.84	<0.0001

[a] $\ln(N/N_0) = \tau + \gamma\delta_{i2} + \beta(\text{time})^2 + \eta(\text{time})^2\delta_{i2}$, where $i = 1$ for the packages with a mass of 454 g, $i = 2$ for the packages with a mass of 227 g, $\delta_{i2} = 0$ when $i = 1$ and $\delta_{i2} = 1$ when $i = 2$. Deviance/df = 1.0755.

count) data overdispersion ($10^7 - 0$), a negative binomial distribution was used. Negative binomial distribution accounts for large initial cell counts and the overdispersion *(10).* Since 454-g packages and 227-g packages were inherently paired by design, they were specified as repeated measures (using the generalized estimating equation approach) in GENMOD. The interactions between treatment time and product size were also tested. The type III statistical analysis was conducted to determine the significance difference of treatment time and product size on survivors of *Salmonella* Senftenberg and *L. innocua.* The data analysis was conducted using SAS version 8.1 (copyright 1999–2000, SAS Institute, Inc., Cary, N.C.).

The following model was used to express the surviving rate, $\ln(N/N_0)$, of *Salmonella* Senftenberg or *L. innocua* on the chicken breast strips as a function of treatment time:

$$\ln(N/N_0) = \tau + \gamma\delta_{i2} + \phi(\text{time}) + \lambda(\text{time})\delta_{i2} + \beta(\text{time})^2 + \eta(\text{time})^2\delta_{i2} \quad (1)$$

where $i = 1$ for the packages with a mass of 454 g, $i = 2$ for the packages with a mass of 227 g, $\delta_{i2} = 0$ when $i = 1$, and $\delta_{i2} = 1$ when $i = 2$. For the 454-g packages, this model reduces to:

$$\ln(N/N_0) = \tau + \phi(\text{time}) + \beta(\text{time})^2 \quad (2)$$

and for 227-g products, we can write the model as:

$$\ln(N/N_0) = (\tau + \gamma) + (\phi + \lambda)(\text{time})\delta_{i2} + (\beta + \eta)(\text{time})^2 \quad (3)$$

The parameters λ and η represent the difference in the effect of a unit change in treatment time and (treatment time)^2, respectively, on 227-g packages versus 454-g packages. A test of whether λ or η is significantly different from 0 at $\alpha = 0.05$ will indicate if there is a significant interaction between treatment time and product size or (treatment time)^2 and product size, respectively. A measure of lack of fit for this model is the deviance statistic. This statistic has an associated degrees of freedom, and it follows an approximate chi-square distribution. An assessment of lack of fit can be obtained by calculated deviance/df, where deviance/df equal to 1 indicates a perfect fit.

TABLE 4. *Predicted models for the surviving rate, ln(*N/N_0*), of* Salmonella *Senftenberg and* Listeria innocua *on fully cooked and vacuum packaged chicken breast strips*[a]

Culture	227-g packages	454-g packages
Salmonella Senftenberg	-0.1337 to $0.036t^2$	1.0408 to $0.0144t^2$
L. innocua	-0.1961 to $0.0377t^2$	0.6137 to $0.0152t^2$

[a] *t,* heating time in min.

RESULTS AND DISCUSSION

Thermal profile. Figure 1 shows the cooker temperature and the product temperature at the pouch center for 227-g and 454-g packages, respectively. In 227-g packages, the product temperature at the pouch center reached 70°C in about 20 min, which was about 40% faster than that in the 454-g packages. The thermal profile for 454-g packages was similar to that in a continuous steam cooker at 88°C *(7).* A treatment time of 34 min was needed for the 454-g packages to reach 70°C.

Thermal lethality for *Salmonella* Senftenberg and *L. innocua*. A negative binomial regression model was fit to the surviving counts, with the natural log of the initial counts as the offset variable, and the deviance criteria indicated that the model provided a good fit (deviance/*df* = 1.1304 and 1.0755 for *Salmonella* Senftenberg and *L. innocua,* respectively). The linear terms (ϕ and λ) in equations 2 and 3 were nonsignificant at $\alpha = 0.05$ and therefore were removed in the final models.

Table 1 lists the type III statistical analysis for *Salmonella* Senftenberg and *L. innocua.* The results showed that the treatment time significantly affected the survivors of *Salmonella* Senftenberg and *L. innocua* and the effect of treatment time interacted with product size. Tables 2 and 3 list the parameter estimate of the obtained models for *Salmonella* Senftenberg and *L. innocua,* respectively. From equations 2 and 3, the predicted models for the surviving rate, $\ln(N/N_0)$, of *Salmonella* Senftenberg and *L. innocua* in 227-g or 454-g packages are calculated and given in

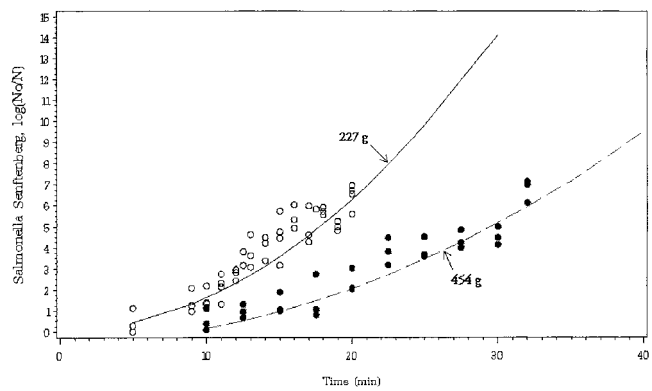

FIGURE 2. *Experimental values and the model predictions for the log reduction, log(*N_0/N*), of* Salmonella *Senftenberg on fully cooked and vacuum packaged chicken breast strips.*

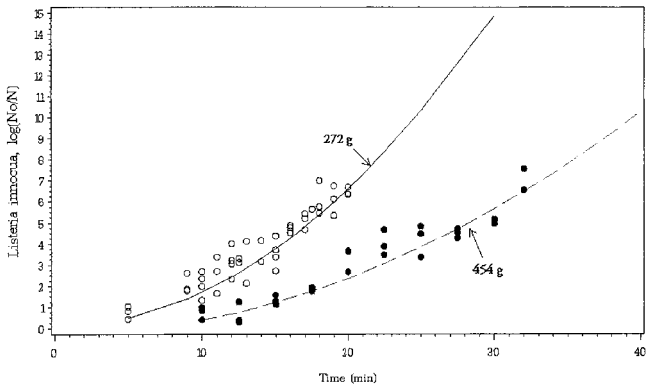

FIGURE 3. *Experimental values and the model predictions for the log reduction, log(N_0/N), of* Listeria innocua *on fully cooked and vacuum packaged chicken breast strips.*

Table 4. The data in which the survivors were not detected were excluded in the model prediction.

Figures 2 and 3 show the log reduction, $\log_{10}(N_0/N)$, of *Salmonella* Senftenberg and *L. innocua* in packaged chicken breast strips. The plot in Figures 2 and 3 indicated that as time increased, $\log_{10}(N_0/N)$ also increased. The packages with a mass of 227 g had a significantly higher $\log_{10}(N_0/N)$ than the packages with a mass of 454 g. To achieve a 7-$\log_{10}$ cell reduction for *Salmonella* Senftenberg or *L. innocua,* the 227-g packages would need to be treated in 88°C hot water for about 20 min, and the 454-g packages would need to be treated for 34 min. The result from this study for 454-g packages was similar to that obtained by Murphy et al. *(7)* for a similar product and package size that was heat treated in a continuous steam cooker at 88°C. Note that the results from this study should not be extrapolated outside the testing range.

CONCLUSIONS

This information is useful for commercial processors to evaluate postcook pasteurization processes to treat fully cooked and vacuum packaged chicken products. These results show that both *Salmonella* Senftenberg and *L. innocua* can be eliminated from the surface of the postprocess contaminated chicken products by a hot water pasteurization step after packaging. The reduction of *Salmonella* Senftenberg or *L. innocua* was affected by treatment time and packaging size.

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