Thermal Inactivation D- and Z-Values of *Salmonella* and *Listeria innocua* in Fully Cooked and Vacuum Packaged Chicken Breast Meat during Postcook Heat Treatment

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ABSTRACT Studies were conducted to determine thermal inactivation D- and z-values of *Salmonella* and *Listeria innocua* in fully cooked and vacuum-packaged chicken breast meat. Fully cooked chicken breast meat products that were obtained from three different sources with differing formulations were uniformly inoculated with a cocktail of *Salmonella* (including Senftenberg, Typhimurium, Heidelberg, Mission, Montevideo, and California) or *L. innocua* at approximately 10^7 cfu/g. The inoculated meat samples were vacuum-packaged and then heat-treated at a temperature of 55 to 70 C for 5 to 90 min. After heat treatment, the samples were immediately cooled in an ice-water bath. Survivors of *Salmonella* and *L. innocua* were enumerated for each sample. D- and z-values of *Salmonella* and *L. innocua* were determined for each product and compared among the products. Source and formulation did not cause significant differences in the D- and z-values of *Salmonella* or *L. innocua* among the three fully cooked and vacuum-packaged chicken breast meat products.

(*Key words*: *Salmonella*, *Listeria*, thermal inactivation, postcook, fully cooked chicken)

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INTRODUCTION

The market for fully cooked refrigerated meat and poultry products is rapidly increasing. Consequently, it becomes more and more important to ensure the food safety of these thermally processed retail products. Many of the fully cooked meat or poultry products were recalled due to concern of pathogens (USDA, 2001). Once a cooking process is validated, fully cooked meat or poultry products should be free of pathogens at the end of cooking. However, potential recontamination during postcook handling, prior to packaging, poses a concern. Such an event could introduce pathogens to packaged ready-to-eat meat or poultry products.

In-package pasteurization processes via steam or hot water have been used or considered for use on fully cooked meat and poultry products (Murphy, 2002, unpublished data). Murphy et al. (2001b) demonstrated that postcook pasteurization treatment via steam or hot water could effectively reduce the potential risk of *Salmonella* and *Listeria* in packaged ready-to-eat chicken breast meat products. Thermal pathogen destruction during pasteurization is time and temperature related and is usually described using the time required to cause a one $\log_{10}$ decrease in bacterial numbers at a given temperature (D) and the temperature difference required for the thermal inactivation curve to drop a logarithmic cycle (z). From a known z-value, process lethality (F) for a pathogen during pasteurization treatment can be calculated as

$$F = \int_0^t 10^{(T(t) - T(ref))/z} dt \quad [1]$$

where T(t) is the product temperature at a time t and T(ref) is a reference temperature. To determine the thermal lethality for pathogens in a meat or poultry product that has gone through a pasteurization treatment, the z-values of bacteria in the product should be used. Thermal inactivation D- and z-values of pathogens in different raw meat or poultry products were reported in previous publications (Mazzotta, 2000; Juneja et al., 2001; Murphy et al., 2002). However, no data were generated regarding thermal inactivation kinetic values for pathogens in fully cooked and vacuum-packaged meat or poultry products, and these data are essential in validating postcook pasteurization process.

To increase profitability, most fully cooked and vacuum-packaged commercial meat and poultry products

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Abbreviation Key: ANCOVA = analysis of covariance; TSA = tryptic soy agar.

are value-added or marinated prior to cooking processes. Generally, various food additives are used in marinade formulas. Differing accounts can be found in the literature regarding how product formulations affect the thermal lethality of pathogens (Kotrola and Conner, 1997; Doyle and Mazzotta, 2000). In a review paper, Doyle and Mazzotta (2000) indicated that some food additives, including polyphosphates, hydrogen peroxide, and the lactoperoxidase increased *Salmonella* sensitivity to heat. Also, these food additives could be more effective in culture media than in foods because they might interact with fat and protein, which reduced their availability to bacteria (Doyle and Mazzotta, 2000).

In a study using finely ground turkey breast meat (3% fat) as base heating medium, Kotrola and Conner (1997) found that some food additives, such as NaCl, sodium lactate, and polyphosphates increased heat resistance of *Escherichia coli* O157:H7. However, the heat resistance of *E. coli* O157:H7 in ground turkey breast meat was not affected by increasing fat content from 3 to 11% (Kotrola and Conner, 1997). In a previous study on different commercial meat products, Murphy et al. (2002) found that thermal inactivation D- and z-values for *Salmonella* and *Listeria innocua* were significant (at $\alpha = 0.05$) among different commercially formulated meat and poultry products, including chicken breast meat, chicken patties, chicken tenders, franks, beef patties, and blended beef and turkey patties. In the above studies, raw meat products were used.

No information could be retrieved from the literature on thermal inactivation D- and z-values of pathogens in fully cooked and vacuum-packaged meat or poultry products. It was also unknown how marinade formulation prior to cooking could affect pathogen thermal lethality in fully cooked and vacuum-packaged meat or poultry products during postcook heat treatment. The objective of this study was to determine thermal inactivation D- and z-values of *Salmonella* and *Listeria* in fully cooked and vacuum-packaged chicken breast meat products. Fully cooked chicken breast meat products from three different sources were evaluated, including (1) plain chicken breast meat that was cooked in a pilot-scale impingement oven, (2) product marinated with phosphate, salt, and water via vacuum tumbler and then cooked in a pilot-scale impingement oven, and (3) fully cooked chicken breast meat products that were commercially formulated, marinated, and cooked.

MATERIALS AND METHODS

Product

Three types of fully cooked chicken breast meat were used in this study, including (1) plain meat product without any additives, (2) product marinated with phosphate and salt prior to cooking, and (3) commercially formulated grilled chicken breast fillets. For plain meat product (1), fresh chicken breast fillets (4 C) were obtained from University of Arkansas Poultry Processing Pilot Plant and cooked in steam (99 C) via an impingement oven[2] to the minimum internal temperature of 71.1 C. For product that was marinated with phosphate and salt (2), fresh chicken breast meat fillets (4 C) were obtained from a commercial poultry processor. The meat was marinated for 60 min via a vacuum tumbler in a solution, containing 0.5% (wt/wt) sodium tripolyphosphate,[3] 2% (wt/wt) sodium salt[4], 70% (wt/wt) water, and 27.5% (wt/wt) ice. Marinated product was then cooked in steam at 99 C in an impingement oven[5] to the minimum internal temperature of 71.1 C. For commercially formulated product (3), chicken breast meat was marinated by injection, formed, and thermally processed by a commercial processor. Detailed product formulation and process information were proprietary to the processor. However, the marinade formulation contained NaCl (4%, wt/wt) and sodium tripolyphosphate (2.4%, wt/wt).

All three products contained about 20.11% protein, 73.64% moisture, and 1.27% fat. For the products (1) and (2), the endpoint internal temperatures of the products during cooking were correlated to cooking time. The correlation of product temperature and cooking time was predetermined via 20 cooking test trials. During the test trials, the internal temperatures of the products were monitored via thermocouple probes in a similar manner as described by Murphy et al. (2001a). The product internal temperatures were correlated to cooking time and verified using a computer simulation program (ThermoPro, Rong Murphy, Thermal Processing and Food Safety Program, University of Arkansas, Fayetteville, AR).

Bacteria

A cocktail of six *Salmonella* serotypes (Senftenberg, Typhimurium, Heidelberg, Mission, Montevideo, and California) were used in this study (Murphy et al., 1999). A nalidixic acid-resistant culture of each serotype was prepared as previously described by Murphy et al. (1999). Subcultures for use as inocula were prepared from the stock culture. A preliminary study was conducted to compare the thermal tolerance of nalidixic acid-resistant culture with the original nonresistance culture and found no significant difference (Murphy, 2001, unpublished data).

The same *L. innocua* M1 strain as that used by Murphy et al. (1999) was used in this study. *Listeria innocua* M1 has been used as a biological indicator for *L. monocytogenes* (Foegeding and Stanley, 1991; Fairchild and Foegeding, 1993). Lyophilized culture was revived in tryptic soy broth[6] plus 0.6% yeast extract[7] for 24 h at 35 C, plated on tryptic soy agar (TSA) plus 0.6% yeast extract, and incubated for 24 h at 35 C. The *Listeria* culture was resistant to 50 ppm rifampicin and 250 ppm streptomycin. A study by Walsh et al. (2001) indicated antibiotic-resistant

[2]Model 102, Stein-DSI, Sandusky, OH.
[3]George's Inc., Springdale, AR.
[4]George's Inc., Springdale, AR.
[5]Model 102, Stein-DSI, Sandusky, OH.
[6]Becton Dickinson and Company, Sparks, MD.
[7]Becton Dickinson and Company, Sparks, MD.

Listeria did not respond differently to thermal treatments in meat substrate from the original strain.

Heat Treatment

Fully cooked chicken breast meat was ground in a sterile Cuisinart™ food processor[8] and inoculated with *Salmonella* or *L. innocua*. An actively growing culture of *Salmonella* or *L. innocua* (10 mL) was used per 500 g of fully cooked chicken. The culture was individually maintained and grown overnight in tryptic soy broth plus 200 ppm nalidixic acid sodium salt[9] for *Salmonella* or tryptic soy broth plus 0.6% yeast extract, 50 ppm rifampicin[10], and 250 ppm streptomycin[11] for *L. innocua*. The six *Salmonella* cultures were mixed to form a cocktail just prior to the inoculation.

The inoculated samples were kept at 4 C for 30 min to allow attachment of the bacterial cells to the meat tissues. Samples of 25 g were then placed in a 152 mm wide × 254 mm long × 0.0508 mm thick gas/moisture barrier pouch[12] and sealed under 1 bar vacuum. The sealed samples were rolled flat into a thin layer with a rolling pin. The flatted samples filled all of the space in the pouch and had a thickness of approximately 0.5 mm.

The sample pouches were placed flat inside a stainless steel wire rack and immediately submerged in a circulated water bath that was maintained at 55, 57.5, 60, 62.5, 65, 67.5, or 70 C for 5 s to 90 min depending on the treatment temperature. In each trial, two inoculated and unheated controls were prepared to determine the initial inoculation values. After heat treatment, the samples were immediately placed in an ice-water bath. During heating and cooling, time temperature history was monitored via a thermocouple probe (40 gauge, type E) that was sealed in the pouch. Within 20 s of treatments, meat sample temperatures increased from 20 C to within 0.5 C of the heating-bath temperature. Eight seconds in the subsequent ice bath caused the meat sample temperature to fall below 20 C.

Enumeration

After cooling, pouches were blotted dry with paper towels, wiped with 75% ethanol, and a corner of the pouch was cut in 2.5 cm slot. Peptone[13] (0.1%; 60 mL) was slowly pipetted into the pouch. The sample solution mixture was manipulated by hand, transferred into a nylon mesh-lined bag, and blended in a Stomacher[14] for 2 min. Serial dilutions were plated in duplicate. *Salmonella* was plated on TSA overlaid with TSA containing 200 ppm of nalidixic acid sodium salt. *Listeria* was plated on TSA overlaid with TSA containing 0.6% yeast extract, 50 ppm of rifampicin, and 250 ppm of streptomycin. Inoculated unheated samples and uninoculated heated samples were included as controls.

All plates were incubated at 35 C and colonies were counted daily. Plates were returned to the incubator and recounted every day for 7 d until viable counts did not increase further.

Survival Models

For each product, the survivors, $\log_{10}(N)$, of *Salmonella* or *Listeria* were plotted against heating times at each temperature. The following linear primary model (Murphy et al., 2000) was used to model the thermal destruction of *Salmonella* and *Listeria* and to determine the decimal reduction time (D).

$$\text{Log}_{10}(N) = \text{Log}_{10}(N_0) \quad [t \le t_L] \tag{2}$$

$$\text{Log}_{10}(N) = \text{Log}_{10}(N_0) + s\,(t - t_L) \quad [t > t_L] \tag{3}$$

where N was colony-forming unit (cfu) per gram at time t, N_0 was cfu/g of inoculated unheated samples, s = slope of the survival curve, t = time (min), and t_L = duration of lag period (min).

D- and z-values

Triplicate thermal inactivation trials were performed at each temperature. The D-values for *Salmonella* and *Listeria* at each temperature were calculated by taking the negative inverse of the relevant s-value (Murphy et al., 2000). The z-values were determined as the negative inverse slope of the $\log_{10}$D vs. temperature plot.

Statistical Analyses

To test if D-values of *Salmonella* or *L. innocua* were equal at each temperature for the three products, assuming N_0 was the count of *Salmonella* or *L. innocua* at time = 0 and N was the count at time = t, for each temperature, an

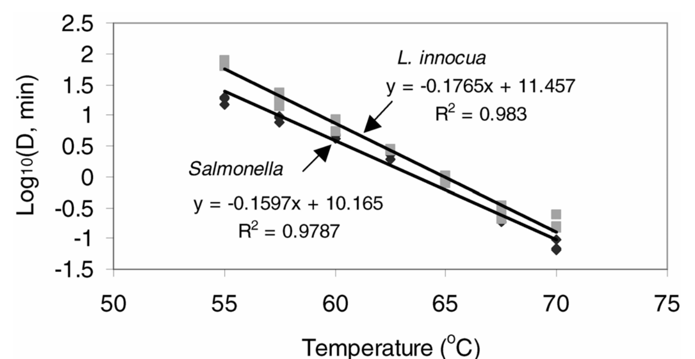

FIGURE 1. Log_{10}(D, min) of *Salmonella* and *Listeria innocua* vs. treatment temperature for fully cooked chicken breast products.

[8]Model CFP 5A, Robot-Coupe, Stamford, CT.
[9]Sigma Chemical Co., St. Louis, MO.
[10]Sigma Chemical Co., St. Louis, MO.
[11]Sigma Chemical Co., St. Louis, MO.
[12]Tilia, Inc., San Francisco, CA.
[13]Becton Dickinson and Company, Sparks, MD.
[14]Lab Blender 400, Tekmar Co., Cincinnati, OH.

TABLE 1. The P values from the Analysis of Covariance for the effect of heating time and product on $\log_{10}(N/N_0)$ of *Salmonella* and *Listeria innocua* in fully cooked and vacuum-packaged chicken breast meat

Culture	Temperature (°C)	Source	*P*-value
Salmonella	55	Product	0.8449
		Time (min)	<0.0001
		Time × product	0.0881
	57.5	Product	0.4783
		Time (min)	<0.0001
		Time × product	0.0543
	60	Product	0.4030
		Time (min)	<0.0001
		Time × product	0.3756
	62.5	Proudct	0.1595
		Time (min)	<0.0001
		Time × product	0.4082
	65	Product	0.0972
		Time (min)	<0.0001
		Time × product	0.8848
	67.5	Product	0.5715
		Time (min)	<0.0001
		Time × product	0.7763
	70	Product	0.9575
		Time (min)	<0.0001
		Time × product	0.3185
L. innocua	55	Product	0.4468
		Time (min)	0.0002
		Time × product	0.9892
	57.5	Product	0.2450
		Time (min)	<0.0001
		Time × product	0.2950
	60	Product	0.5498
		Time (min)	<0.0001
		Time × product	0.2745
	62.5	Product	0.4662
		Time (min)	<0.001
		Time × product	0.9598
	65	Product	0.0852
		Time (min)	<0.0001
		Time × product	0.5308
	67.5	Product	0.9325
		Time (min)	<0.0001
		Time × product	0.3089
	70	Product	0.7718
		Time (min)	<0.0001
		Time × product	0.1539

Analysis of Covariance (ANCOVA) was performed where $\log_{10}(N/N_0)$ was the response, treatment was the effect of product formulations, and time was the covariate. The interaction between product formulation and time was also included in the test.

To test whether the z-value of *Salmonella* or *L. innocua* was equal, $\log_{10}(D)$ was the response, treatment was the effect of product formulations, and temperature was the covariate. The interaction between product formulation and temperature was also tested. The D-values of *Salmonella* and *L. innocua* were obtained at each treatment temperature for each product (plain, marinated with phosphate and salt, or commercially formulated). Letting D_S and D_L represent the decimal reduction time for *Salmonella* and *Listeria*, respectively, and the T represent the temperature, the $\log_{10}(D_S)$ or $\log_{10}(D_L)$ would be the response, product formulation would be the explanatory variable, and T would be the covariate. The data were fitted to the following linear regression model:

for *Salmonella*:

$$\log_{10}(D_S)_i = a + b\,\delta_{i2} + c\,\delta_{i3} + f\,(temp) + g\,(temp)\delta_{i2} + h\,(temp)\delta_{i3} \quad [4]$$

for *L. innocua*:

$$\log_{10}(D_L)_i = a + b\,\delta_{i2} + c\,\delta_{i3} + f\,(temp) + g\,(temp)\delta_{i2} + h\,(temp)\delta_{i3} \quad [5]$$

where i = 1 and 3 corresponds to fully cooked chicken breast meat that was commercially formulated, plain, and marinated with phosphate and salt, respectively, and $\delta_{ij} = \begin{cases} 1 \text{ if } i = j \\ 0 \text{ otherwise} \end{cases}$, where a, b, c, f, g, and h were the parameters to be determined.

Using SAS,[15] the Analysis of Covariance Type III SS (sum of square) tests were conducted to determine the effect of product formulation and heating temperatures

[15]Release 8.1, copyright 1999–2000, SAS Institute, Inc., Cary, NC.

TABLE 2. The *P* values from the Analysis of Covariance Type III SS test for the effect of temperature and product on the z-values of *Salmonella* and *Listeria innocua* in fully cooked and vacuum-packaged chicken breast meat[1]

Culture	Parameter	*P*
Salmonella	Temperature	<0.0001
	Product formulation	0.4364
	Temperature × product formulation	0.4360
L. innocua	Temperature	<0.0001
	Product formulation	0.6110
	Temperature × product formulation	0.5188

[1]R^2 was 0.9810 for *Salmonella* and 0.9875 for *L. innocua*.

on z-values. Paired comparisons were also conducted to determine the significant differences of the z-values between each pair of the products. The comparisons were also conducted using SAS.

RESULTS AND DISCUSSION

At each heating temperature, no difference in time and temperature history was observed among the three products. The analysis for the effect of heating time and product formulation on $\log_{10}(N/N_0)$ of *Salmonella* or *L. innocua* included a constant (intercept) term, a heating time (linear) term, and the effect of product on each term. The negative reverse of the linear term (slope) was the D-value for *Salmonella* or *L. innocua* at each corresponding temperature. Table 1 gives the result from ANCOVA analysis for $\log_{10}(N/N_0)$ of *Salmonella* and *L. innocua* at a heating temperature of 55 to 70 C. All of the *P* values for heating time were less than 0.0002, indicating that at temperatures 55 to 70 C, the heating time significantly (at $\alpha = 0.05$) affected the survivors, $\log_{10}(N/N_0)$, of *Salmonella* and *L. innocua*. The *P* values for all of the terms between time and product were greater than $\alpha = 0.05$, indicating that product formulation had no significant effect on bacterial numbers at $\alpha = 0.05$.

In Equations 4 and 5, $\log_{10}(D)$ of *Salmonella* or *L. innocua* was a linear function of treatment temperature. The negative inverse of the linear term (slope) for $\log_{10}(D)$ vs temperature was the z-value. Table 2 gives the results from ANCOVA Type III analysis that was conducted to determine the effect of the three products on the z-values of *Salmonella* or *L. innocua*. Treatment temperature affected the z-values of *Salmonella* and *L. innocua* ($P < 0.05$). However, there was no significant difference in the z-value of *Salmonella* and *L. innocua* among the three products. The product did not interact with treatment temperature ($P > 0.05$). Paired comparison tests were also conducted to determine whether the z-values of *Salmonella* or *L. innocua* between each two pairs of products were significantly different (Table 3). The z-values of *Salmonella* or *L. innocua* were not different between any two pairs of the products.

Since the D- and z-values of *Salmonella* or *L. innocua* were not significantly different among the three fully cooked chicken breast meat products, the data set for all three products was combined at each treatment temperature and time. At each heating temperature, linear regressions were performed for Equations 1 and 2 to obtain the plot of the survivors, $\log_{10}(N)$, of *Salmonella* or *L. innocua* vs. heating time. The D-values of *Salmonella* and *L. innocua* at each treatment temperature were obtained from these plots and are presented in Table 4. This resulted in 90 observations at each treatment temperature, and R^2 was greater than 0.86 for all of the regressions. The D-value of *Salmonella* ranged from 24.071 ± 1.852 min at 55 C to 0.097 ± 0.034 min at 70 C, and the D-value of *L. innocua* was from 56.169 ± 4.016 min at 55 C to 0.126 ± 0.038 min at 70 C. From the D-values at each temperature, linear regression was conducted for $\log_{10}(D)$ vs temperature and is shown in Figure 1.

The z-value was obtained from the slope of $\log_{10}(D)$ vs temperature plot and was 6.262 C for *Salmonella* and 5.666 C for *L. innocua*. The R^2 of the regression was greater than 0.98. Since no reports were found on D- and z-values of *Salmonella* or *L. innocua* in fully cooked chicken breast meat, it is difficult to compare this study with previous publications. In general, the D- and z-values of *Salmonella* and *L. innocua* from this study were in the same magnitude as those in cooking raw chicken meat products reported by Murphy et al. (2000, 2002).

TABLE 3. Paired comparison for the z-value of *Salmonella* and *Listeria* among different products

Culture	Formulate_i	Formulate_j	*P*-value for testing z of Formulate_i = z of Formulate_j
Salmonella	Plain	PS[1]	0.9049
	Plain	Commercial	0.2475
	PS[1]	Commercial	0.2964
L. innocua	Plain	PS[1]	0.9699
	Plain	Commercial	0.3357
	PS[1]	Commercial	0.3180

[1]Fully cooked chicken breast meat marinated with phosphate and salt prior to cooking.

TABLE 4. D-values of *Salmonella* and *Listeria innocua* in fully cooked chicken breast meat products (N = 90 per temperature)

Temperature (C)	*Salmonella*			*Listeria innocua*		
	D (min)	SD	R^2	D (min)	SD	R^2
55	24.071	1.852	0.902	56.169	4.016	0.896
57.5	9.600	1.556	0.920	20.355	1.031	0.883
60	3.828	0.750	0.907	7.362	0.833	0.923
62.5	1.527	0.299	0.893	2.665	0.424	0.921
65	0.609	0.135	0.890	0.965	0.185	0.871
67.5	0.243	0.027	0.862	0.349	0.028	0.912
70	0.097	0.034	0.876	0.126	0.038	0.888

In the current study, the products tested were fully cooked, had similar compositions, and differed only on source, marinade formulations, and thermal processing methods. Therefore, the addition of food additives such as NaCl (<4%) and polyphosphate (<2.4%) may not affect the thermal inactivation of *Salmonella* or *L. innocua* in fully cooked commercial chicken products during postcook pasteurization.

The results from this study will be useful in validating postcook pasteurization processes for fully cooked chicken breast meat products. No significant difference was observed in thermal inactivation D- and z-values of *Salmonella* or *L. innocua* between the three chicken breast meat products with different sources and formulations. The D- and z-values obtained from this study should be useful in determining the process lethality of *Salmonella* or *L. innocua* during commercial postcook pasteurization of similar products.

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