Thermal inactivation of *Salmonella* Enteritidis on chicken skin previously exposed to acidified Sodium chlorite or tri-sodium phosphate

K. Karuppasamy¹ · Ajit S. Yadav¹ · Gaurav K. Saxena¹

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Abstract Thermal inactivation of normal and starved cells of *Salmonella* Enteritidis on chicken skin previously exposed to different concentrations of acidified sodium chlorite (ASC) or tri-sodium phosphate (TSP) was investigated. Inoculated skin was pretreated with different concentration of ASC or TSP, packaged in bags, and then immersed in a circulating water bath at 60 to 68 °C. The recovery medium was Hektoen enteric agar. *D*-values, determined by linear regression, for normal cells on chicken skin, were 2.79, 1.17 and 0.53 min whereas *D*-values for starved cells were 4.15, 1.83 and 0.66 at 60, 64 and 68 °C, respectively. *z*-values for normal cells were 3.54 and for starved cells were 2.29. Pretreatment of *Salmonella* Enteritidis cells with 0 to 200 ppm of ASC or 0 to 1.0 % TSP resulted in lower *D*-values at all temperatures. Sensory results indicated no significance differences for control and treatments. Thus, results of this study indicated that pretreatment of chicken skin with ASC or TSP increased sensitivity of *Salmonella* Enteritidis to heat without affecting organoleptic quality of chicken meat.

Keywords *Salmonella* Enteritidis · Dressed chicken skin · Acidified sodium chlorite · Trisodium phosphate · Thermal inactivation · Food safety

Introduction

Salmonella infection causes significant health and economic burden worldwide (Kubota et al. 2008; CDC 2010). Foodborne salmonellosis is most often associated with consumption of undercooked poultry meat and eggs. Incidence of *Salmonella* in raw poultry carcasses has been reported as 30 to 50 %, and the number of *Salmonella* ranges from 1 to 30 CFU/carcass (Waldroup 1996). Among the serovars of *Salmonella*, *S. Typhimurium* and *S. Enteritidis* are the top serovars implicated in cases of human salmonellosis with poultry products serving as important sources of these serovars (Messens et al. 2007; O'Regan et al. 2008). The consumption of raw or undercooked poultry or cross-contamination of other foods in the home while preparing raw poultry meat, are seen as an important causes of foodborne disease due to *Salmonella* (ACMSF 2005; Adak et al. 2005; Linam and Gerber 2007; Kabir 2010; Freitas et al. 2010). Therefore, it is desirable that birds being sent to poultry processing plants should have low levels of *Salmonella* contamination.

The significance of birds that carry *Salmonella* into the processing plant is not only carcass contamination but also cross-contamination of the processing plant environment and other carcasses (Erol 1999; Nayak et al. 2003; Nde et al. 2007). Bacteria that survive processing plant decontamination regimes with antimicrobial agents can become stress hardened and become harder to kill (Sampathkumar et al. 2004; Braoudaki and Hilton 2004; Alonso-Hernando et al. 2009). Therefore, it is logical to target primary intervention at the farm level through use of biosecurity measures. However, such methods to improve the biosecurity of poultry birds and thus prevent contamination of poultry with *Salmonella* spp. are a difficult task and also costly (Gibbens et al. 2001). Therefore, in combination with the approaches being applied at farm level, other treatments are needed

✉ Ajit S. Yadav
asinghcari@gmail.com

K. Karuppasamy
drayankaruppasamy@gmail.com

Gaurav K. Saxena
gauravksaxena2008@gmail.com

¹ Food Microbiology Laboratory, Post Harvest Technology Division, ICAR-Central Avian Research Institute, Izatnagar 243122, UP, India

during processing that can reduce the contamination of such hardy bacteria.

Post-evisceration is the stage during poultry processing at which pathogen intervention strategies involving thermal or chemical treatments can be applied for decontamination. Among pathogen intervention strategies for produce and poultry some involve use of antimicrobial chemicals in rinses or washes; however, efficacy of most chemical intervention treatments is reduced by presence of organic matter. Thermal treatment is another effective means of reducing pathogens, however, thermal inactivation of poultry carcasses at very high temperature can cause damage to outer epidermal layers of broiler skin and subsequent deterioration in carcass appearance, sensory attributes and quality of meat. Moreover, use of higher chemical concentration or high temperature for decontamination of carcasses increase the thermotolerance and pH resistance of bacterial pathogen (Sampathkumar et al. 2004).

Antimicrobials like acidified sodium chlorite (ASC) and tri-sodium phosphate (TSP) have been used as preservatives but also have food processing applications (Ricke 2003). The main advantage of ASC is the ability to maintain antimicrobial efficacy even in the presence of extraneous organic matter (Cherrington et al. 1992) unlike chlorine (Ricke et al. 2005). Combination of thermal treatment and antimicrobial treatments are, therefore, needed that are practical, cost-effective and safe to use while maintaining the organoleptic attributes of poultry meat. However, many studies have been carried out on thermal inactivation of *Salmonella* with or without antimicrobial on ground chicken meat, poultry meat and poultry products (Murphy et al. 2000, 2004; Mazzotta 2000; Juneja et al. 2001; Osaili et al. 2006; Juneja 2007; Bucher 2008), but there is no study available on the thermal inactivation of *Salmonella* on dressed chicken skin surface using antimicrobials. Because many countries consume dressed poultry meat, so it is necessary to assess the effect of thermal inactivation of *Salmonella* on dressed chicken skin. Hence, this unique study was designed to develop thermal death time regimes in combination with antimicrobials against normal and starved cells of *S. Enteritidis* on dressed chicken skin.

Materials and methods

Chemicals

Acidified sodium chlorite was prepared with sodium chlorite (80 %, molecular weight 90.44; CAS 7758-19-2) and Citric acid monohydrate (≥ 99.7 , molecular weight 210.14; CAS 5949-29-1) procured from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Tri-sodium phosphate (98 %, molecular weight 380.13; CAS 10101-89-0) was procured from Fisher Scientific India Private Limited (Mumbai, India) as tri-sodium orthophosphate.

Organisms

A culture of *Salmonella Enteritidis* (E-2478), obtained from National *Salmonella* Centre, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India, was used in this study. This strain, maintained as frozen (-70°C) stocks in brain heart infusion broth (BHI; Difco, Detroit, MI) supplemented with 10 % (v/v) glycerol (HIMEDIA), were obtained from our laboratory culture collection. To maintain viability, individual stock cultures were maintained on brain heart infusion agar (BHI; Difco, Detroit, MI) slants at 4°C with monthly transfers.

Culture preparation

To propagate the cultures, vials were partially thawed at room temperature and 0.1 ml of the thawed culture was transferred to 10 ml Luria Bertani broth (LB; Difco) and incubated for 24 h at 37°C . Two consecutive 24 h transfers were made using 0.1 ml inocula. These cultures were maintained in LB for 2 weeks at 4°C .

Normal cells

A day before the experiment, inocula for conducting heating studies were prepared by transferring 1 ml of culture to LB broth (100 ml) in 250 ml flasks and incubating aerobically for 18 h at 37°C , to provide late stationary phase cells. On the day of the experiment, culture was applied on chicken skin samples to give a final concentration of 8 to $9 \log_{10}$ CFU/skin.

Starved cells

For starved cells, 2 days before the experiment, inocula for conducting heating studies were prepared by transferring 1 ml of culture to LB broth (100 ml) in 250 ml flasks and incubating aerobically for 18 h at 37°C , to provide late stationary phase cells. A day before the experiment, cultures were centrifuged ($5000\times g$, 15 min, 4°C), the pellet was washed twice in phosphate buffer saline (PBS), re-suspended in phosphate buffer saline to a target level of 8 to $9 \log_{10}$ CFU/ml, and then incubated for 24 h at 37°C . On the day of the experiment, the culture was applied on chicken skin samples to give a final concentration of 8 to $9 \log_{10}$ CFU.

Culture enumeration

Population concentrations in each cell suspension (normal and starved) were enumerated by spiral plating using PlateMaster (HIMEDIA, Mumbai, India), in duplicate, on Hektoen enteric agar (HEA; Difco) plates. All plates were incubated for 24 h at 37°C , prior to counting colonies.

Chicken skin collection

Chicken breast skins (5×5 cm) were collected from poultry processing plant within 10 min after being removed. The pieces were selected only if there was no visible damage on the skin surface. The skin samples were brought to the laboratory under aseptic conditions. To eliminate the contaminated microorganisms present in the samples, skin samples were treated with ultra-violet radiation for 30 min on a clean bench in biosafety cabinet (Labconco Purifier Class II Biosafety Cabinet).

Sample preparation and inoculation

Pre-calibrated standard strain of *Salmonella* Enteritidis was inoculated on the chicken skin samples to give a final concentration of 8 to 9 log₁₀ CFU. Skin samples were left undisturbed at room temperature for 30 min to allow bacterial attachment (Conner and Biligli 1994). To apply antimicrobials, skin samples were dipped in targeted concentrations of antimicrobial solutions for 30 s.

Thermal inactivation and bacterial enumeration

Thermal inactivation studies were carried out in a temperature-controlled water bath stabilized at 60, 64 and 68 °C. After thermal treatment, bags for each replicate were removed at predetermined time intervals and placed into an ice-water bath until analysis (approximately within 30 min). For determination of the number of surviving bacteria, skin samples were transferred to a new filter bag containing 20 ml PBS (pH 7.4) and pummeled for 2 min with a BagMixer 400 (Interscience, St. Nom, France). One ml of inocula from each bag was serially diluted with sterile 0.1 % peptone water and appropriate dilutions were surface plated onto agar dishes containing HEA using a spiral plater. In addition, where necessary, 0.1 and 1.0 ml of undiluted suspension were surface plated. All plates were incubated at 37 °C for at least 48 h prior to counting colonies. Two independent experiments were done for each temperature and antimicrobial combination. To determine estimates of the lethality kinetics, an average CFU/skin of two platings of each sampling point were used for each replicate experiment. To determine D-values, an average CFU/skin of four platings of each sampling point was used for each replicate experiment performed in duplicate ($N=4$; $n=2$ replicates per trial).

Calculation of D-values and z-values

The D-values for each temperature were determined by plotting the log₁₀ number of survivors against time using Excel Software (Microsoft Corporation, Seattle, WA). D-values were calculated by linear regression, with five values in the

straight-line portion of the plots descending more than 5 log₁₀ cycles. The z-values (the temperature in °C required to change the D-value by 1 log₁₀ scale) were calculated by linear regression of mean log₁₀ D-values versus their corresponding heating temperatures using Excel Software as described by Juneja et al. (2012).

Sensory evaluation of chicken with skin

Sensory evaluation was carried out after 7D treatment of dressed carcass. After pre-dipping in antimicrobials, carcasses were subjected to 60 °C for 7D thermal treatment as suggested by Canadian Food Inspection Agency (CFIA 2010) for foods containing poultry. After simple cooking only, sensory quality was evaluated by a panel consisting of six semi-trained members using an 8 point descriptive scale (Keeton 1983) for sensory attributes including color and appearance, flavor, sourness, juiciness and overall acceptability.

Statistical analysis

Heat resistance data were analyzed by analysis of variance (ANOVA) using IBM SPSS to determine if there were statistically significant differences among treatments. The Duncan test was used to determine significant differences ($P<0.05$) among means.

Results and discussion

In the present study, starved and normal cells of *Salmonella* Enteritidis were used to assess the change in thermal resistance of starved cells of *Salmonella* Enteritidis which revealed that starved cells of *Salmonella* Enteritidis showed higher degree of thermal resistance as compared to normal cells. Some previous studies also suggested that bacteria exhibits hardiness in response to harsh environmental conditions, such as a temperature change, high salinity, or nutrient deprivation (Roszak et al. 1984; Smith et al. 1994; Ravel et al. 1995). Acidified sodium chlorite (ASC) and Trisodium phosphate (TSP) are responsible for the reduction in salmonellae recovery (Bourassa et al. 2005; Sexton et al. 2007). El-Gazzar and Marth (1992) reported that heat resistance of *Salmonella* varies among different *Salmonella* serotypes and serotype Senftenberg is the most heat resistant. After simulating conditions that occur in the food industry, Murphy et al. (1999) reported D-values of 0.286 min at 67.5 °C and 0.176 min at 70 °C in ground chicken breast meat during thermal processing. Chambliss et al. (2006) reported that for ground chicken meat, the time needed to obtain a 7 log₁₀ reduction was 86.7 min at 55 °C; 52.2 min at 60 °C; and 39.9 min at 62.5 °C. The higher D-values of bacterial population also depends upon use of heterologous population of

Salmonella strains, differences in experimental conditions, such as meat animal species, muscle type, pH, fat content, and other compositional and environmental factors including the method of enumeration used (Waldroup 1996; Juneja et al. 2012).

In this study, we assessed the effect of ASC or TSP treatment on dressed chicken skin at 60–68 °C for thermal inactivation of normal and starved cells of *Salmonella* Enteritidis, separately. We found that, pre-dipping in ASC

or TSP resulted in lower D-values (thermal resistance) of *Salmonella* Enteritidis and its effective destruction on chicken skin surface as compared to thermal treatment alone. The $\log_{10}$ populations of surviving *Salmonella* Enteritidis cells are plotted against time at each test temperature with corresponding antimicrobial concentration (Fig. 1). To visualize inactivation trends at 60, 64 and 68 °C, data are expressed as the $\log_{10}$ ratio of bacterial count at time t (N) and initial count (N_0).

Fig. 1 Destruction of *Salmonella* on dressed chicken skin at 60, 64 and 68 °C. The data are expressed as $\log_{10}$ of the ratio of the bacterial count at time t (N) and initial count (N_0)

Effect of antimicrobials on D-values

In ASC and TSP experiments of this study, D-values of normal cells of *Salmonella* Enteritidis without antimicrobial treatment on dressed chicken skin were 2.80 and 2.79 min, 1.15 and 1.19 min, 0.53 and 0.53 min at 60, 64, 68 °C, respectively. Similarly, Juneja et al. (2012) also reported D-values of a cocktail of 8 *Salmonella* strains in ground chicken meat as 17.45, 2.89, 0.75 and 0.29 min at 50, 60, 65 and 71 °C, respectively. Pre-dipping in 100 and 200 ppm ASC resulted in decreased D-values as 1.61 and 0.76 min, 0.47 and 0.28 min, 0.29 and 0.12 min at 60, 64, 68 °C, respectively. Further, 0.5 and 1.0 % TSP decreased the D-values as 1.63 and 0.69 min, 0.63 and 0.37 min, 0.36 and 0.20 min at 60, 64, 68 °C, respectively (Table 1). Similarly, D-values of starved cells of *Salmonella* Enteritidis on dressed chicken skin without antimicrobial treatment, in ASC and TSP experiments were 4.11 and 4.19 min, 1.82 and 1.84 min, 0.67 and 0.64 min at 60, 64, 68 °C, respectively. Pre-dipping in 100 and 200 ppm ASC resulted in decreased D-values as 2.92 and 1.42 min, 1.41 and 0.67 min, 0.35 and 0.20 min at 60, 64, 68 °C, respectively. Further 0.5 and 1.0 % TSP

decreased the D-values as 2.67 and 1.74 min, 1.41 and 0.66 min, 0.41 and 0.22 min at 60, 64, 68 °C, respectively (Table 2).

In present study, D-values of starved *Salmonella* Enteritidis cells were relatively higher than normal cells at each temperature. The higher D-values of starved cells might be due to bacterial stress adaptation, stress adaptive response, or stress hardening (Yousef and Courtney 2003) due to starvation stress, which render the pathogen more resistant to subsequent heat treatment (Juneja et al. 2013).

Effect of antimicrobials on z-values

The z-value represents the change in heating temperature necessary to change the D-value by 90 %. To calculate z-values, thermal-death-time curves were plotted using $\log_{10}$ D-values at 60 to 68 °C, with or without antimicrobials (Fig. 2a, b, c, and d). The z-values (in °C) for normal *Salmonella* Enteritidis cells, increased from 3.52 to 6.06 °C and 12.50 °C with pre-dipping in 100 and 200 ppm ASC, respectively (Fig. 2a). With pre-dipping in 0.5 and 1.0 % TSP, z-values increased from

Table 1 Heat resistance (expressed as D-values) for *Salmonella* Enteritidis on chicken skin with 100 or 200 ppm ASC or 0.5 or 1.0 % TSP at 60 to 68 °C with normal cells

Temp (°C)	Heat+ Additive	Linear Regression	
		D-value (min) ^a	(R ²) ^b
60	Control	2.80±0.03 ^c	0.91
	100 ppm ASC	1.61±0.02 ^b	0.96
	200 ppm ASC	0.76±0.01 ^a	0.98
	Control	2.79±0.05 ^c	0.96
	0.5 % TSP	1.63±0.04 ^b	0.95
	1 % TSP	0.69±0.01 ^a	0.96
64	Control	1.15±0.01 ^c	0.98
	100 ppm ASC	0.47±0.00 ^b	0.98
	200 ppm ASC	0.28±0.00 ^a	0.89
	Control	1.19±0.01 ^c	0.98
	0.5 % TSP	0.63±0.01 ^b	0.97
	1 % TSP	0.37±0.00 ^a	0.92
68	Control	0.53±0.01 ^c	0.99
	100 ppm ASC	0.29±0.01 ^b	0.94
	200 ppm ASC	0.12±0.00 ^a	0.97
	Control	0.53±0.01 ^c	0.96
	0.5 % TSP	0.36±0.01 ^b	0.98
	1 % TSP	0.20±0.00 ^a	0.98

^a D-values are the mean±standard deviation of two replicates and were obtained by linear regression using Excel. Means within a treatment combination followed by different letters are significantly different ($P<0.05$)

^b Coefficient of determination

Table 2 Heat resistance (expressed as D-values) for *Salmonella* Enteritidis on chicken skin with 100 or 200 ppm ASC or 0.5 % or 1.0 % TSP at 60 to 68 °C with starved cells

Temp (°C)	Heat+ Additive	Linear Regression	
		D-value (min) ^a	(R ²) ^b
60	Control	4.11±0.07 ^c	0.97
	100 ppm ASC	2.92±0.09 ^b	0.95
	200 ppm ASC	1.42±0.03 ^a	0.99
	Control	4.19±0.08 ^c	0.96
	0.5 % TSP	2.67±0.02 ^b	0.98
	1 % TSP	1.74±0.04 ^a	0.92
64	Control	1.82±0.01 ^c	0.99
	100 ppm ASC	1.41±0.03 ^b	0.94
	200 ppm ASC	0.67±0.02 ^a	0.96
	Control	1.84±0.03 ^c	0.99
	0.5 % TSP	1.41±0.02 ^b	0.95
	1 % TSP	0.66±0.01 ^a	0.98
68	Control	0.67±0.01 ^c	0.98
	100 ppm ASC	0.35±0.00 ^b	0.98
	200 ppm ASC	0.20±0.00 ^a	0.99
	Control	0.64±0.02 ^c	0.98
	0.5 % TSP	0.41±0.00 ^b	0.94
	1 % TSP	0.22±0.00 ^a	0.99

^a D-values are the mean±standard deviation of two replicates and were obtained by linear regression using Excel. Means within a treatment combination followed by different letters are significantly different ($P<0.05$)

^b Coefficient of determination

3.55 to 6.30 °C and 16.33, respectively (Fig. 2b). For starved *Salmonella* Enteritidis cells, *z*-values increased from 2.33 to 3.11 °C and 6.56 °C with pre-dipping in 100 ppm and 200 ppm ASC, respectively (Fig. 2c). With pre-dipping in

Fig. 2 a–d. Thermal-death-time curves (*Z*-values) for normal and starved *Salmonella* Enteritidis cells over the temperature range of 60 to 68 °C. The means of *D*-values were used to determine the *Z*-values. *D*-values were obtained from enumerated survivors on the recovery medium. *D*-values calculated by linear regression; ^aControl ASC, ^b100ppm ASC, ^c200ppm ASC (Normal Cells), ^dControl TSP, ^e0.5 % TSP, ^f1.0 % TSP (Normal Cells), ^gControl ASC, ^h100ppm ASC, ⁱ200ppm ASC (Starved Cells), ^jControl TSP, ^k0.5 % TSP, ^l1.0 % TSP (Starved Cells)

Table 3 Evaluation of sensory attributes of chicken meat after application of antimicrobials and thermal treatment

Groups	Color and appearance	Flavor	Sourness	Juiciness	Overall acceptability
Control	7.29±0.18	6.71±0.18	5.71±0.52	6.58±0.30	7.00±0.22
ASC 100 ppm	7.43±0.20	6.86±0.14	5.29±0.61	7.14±0.14	7.14±0.26
ASC 200 ppm	7.29±0.18	7.00±0.00	4.86±0.51	6.86±0.26	6.86±0.26
TSP 0.5 %	7.14±0.14	6.71±0.18	5.43±0.61	6.86±0.14	6.71±0.18
TSP 1.0 %	7.00±0.38	6.86±0.26	5.14±0.59	6.71±0.29	6.43±0.30

All values are the mean±standard deviation

There were no differences ($P>0.05$) among groups for any sensory attribute, evaluated by a panel consisting of six semi-trained members using an 8 point descriptive scale

0.5 and 1.0 % TSP, z -values increased from 2.25 to 3.54 °C and 5.26 °C (Fig. 2d).

These results indicated that in response to pretreatment with antimicrobials, a larger change in temperature is required to change the D -value by one log-scale during thermal inactivation. The higher z -values with antimicrobials indicated that the thermal inactivation of *Salmonella* Enteritidis was less temperature dependent in the presence of antimicrobial (Juneja et al. 2012). These results suggest that z -values calculated under one set of time-temperature formulation combination are unique, and cannot be applied to another set of food formulation.

Sensory evaluation

After simple cooking (steam), five different sensory attributes were analyzed to assess acceptability of chicken carcasses pretreated with ASC or TSP. Color, appearance, flavor, and juiciness showed no significant difference between treated groups and the control group (Table 3). However, sourness of chicken ranged from 4.86 to 5.71. The 200 ppm ASC group showed lower score, when compared to control and other ASC and TSP groups. For all groups, overall acceptability was not different and ranged from 6.43 to 7.00.

Conclusions

Use of antimicrobials with thermal treatment is a better strategy for the decontamination of dressed chicken, if applied just before the thermal treatment. It was found that the pre-dipping in antimicrobials before thermal treatment resulted in an effective destruction of *Salmonella* Enteritidis on dressed chicken skin as indicated by lower D -values than thermal treatment alone. Environmental stresses render the pathogens more resistant to subsequent heterogeneous stresses. In present study, starved cells of *Salmonella* Enteritidis showed higher degree of thermal resistance (D -values) during thermal inactivation at all the selected temperatures. This study on thermal inactivation of *Salmonella* Enteritidis on dressed chicken skin using antimicrobials can be helpful for the food processors to design effective decontamination regimes for *Salmonella* for animal meat and meat products with enhanced food safety and consumer acceptance.

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