THERMAL INJURY AND RECOVERY OF *SALMONELLA* ENTERITIDIS IN GROUND CHICKEN WITH TEMPERATURE, PH AND SODIUM CHLORIDE AS CONTROLLING FACTORS

by

LASHAWNDA SHERRE CHAMBLISS

(Under the Direction of Mark Harrison)

ABSTRACT

Cells of *Salmonella* Enteritidis were grown at 25 and 35ºC. The survival and recovery of heat injured (55, 60, and 62.5°C) cells in ground chicken inoculated into tryptic soy broth (TSB) adjusted to various pHs (5.5 and 6.5) and NaCl concentrations (2.0 and 3.5%) was studied. The D values of cells of *S.* Enteritidis grown at 25ºC were 12.99, 1.287 and 0.665 min at 55, 60 and 62.5ºC, respectively. For cells grown at 35ºC the D values were 13.42, 1.555, and 1.255 min at 55, 60 and 62.5ºC. The z-values of 6.48-8.53ºC were obtained depending on heating temperature and plating conditions. The influence of tryptic soy agar (TSA) and double modified lysine agar (DMLIA) on the recovery of injured cells was determined. Recovery was significantly reduced on DMLIA at increased pHs and NaCl concentrations. Higher numbers of cells were recovered in TSB at pH 5.5 and 2.0% NaCl.

INDEX WORDS: *Salmonella* Enteritidis, Thermal injury, Recovery, pH, Sodium chloride, Temperature

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DEDICATION

I would like to dedicate this thesis to my Mother, Ida. Thank you for all your love, support, and encouragement. Thank you for being a great Mother, but most of all thank you for being my best friend. I love you.

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Salmonella is a leading cause of foodborne illness in the United States and continues to be of public health significance because of its ability to withstand harsh environments. Salmonellosis, the disease caused by this organism, is characterized by symptoms such as diarrhea, fever and abdominal pain. Foodborne salmonellae are estimated to cause approximately 1.3 million illnesses, 15,000 hospitalizations and 500 deaths per year in the U.S. (*1*). In an effort to reduce the incidence of this pathogen, the U.S. Department of Agriculture, Food Safety and Inspection Service has implemented a 7 $\log_{10}$ relative reduction in population counts of *Salmonella* for fully and partially cooked poultry products (*13)*.

Microbial growth in foods is controlled primarily by pH, water activity and storage temperature, with additional factors such as preservatives, modified atmosphere packaging or heat treatment also contributing. These factors are usually used in combination because singly they would not be sufficient to control microbial growth (*10*).

Salmonella Enteritidis, a facultative anaerobe, is an etiological agent of typhoid and paratyphoid fever. A recent FoodNet surveillance of foodborne illnesses has shown that the overall incidence of *Salmonella* has decreased by 8% in comparing 1996-1998 to 2004; however, the incidence of *S.* Enteritidis has remained at approximately the same level (*2*). Chicken had not been previously reported as a cause of sporadic cases of *S.* Enteritidis or as a frequent cause of *S.* Enteritidis outbreaks in the U.S. until recently. In 2004, a case-control study in five Foodborne Disease Active Surveillance Network surveillance areas identified chicken consumption as a risk factor for sporadic *S.* Enteritidis infections in the U.S. (*15*).

The control and elimination of *Salmonella* using a heat treatment is a vital method in the safe preparation of many foods. Insufficient processing, cooking and reheating are major causative factors in foodborne illnesses (*13*). An effective thermal process is required to control the potential hazard of *Salmonella* in cooked meat products. An important factor of the heating step is to determine the pathogen's heat resistance. Overestimating the heat resistance will affect product quality, whereas underestimating increases the possibility that the organism will survive the heat treatment or cooking process. The organism studied in this work has been associated with foodborne illness caused by insufficient heating. A variety of foods have been implicated in *Salmonella* infections including meat and poultry (*9*).

There has been some research pertaining to the heat resistance of *Salmonella* in chicken meat and the reported D-values at 55-70ºC range from 30.1 to 0.238 min. These studies indicate that most *Salmonella* serovars do not have an unusual heat resistance, with the exception of *S.* Senftenberg which has been shown to be resistant at unusually high temperatures.

Destruction of microorganisms by heat may deviate from linear declines in log numbers with time. Survival curves may exhibit an initial lag period followed by exponential decline. In some instances a tailing of more persistent bacteria is observed that decline at a slower rate than the majority of the cells (*17*). To detect bacteria in foods, selective agents such as bile salts or novobiocin are added to media. However, these agents may inhibit repair of injured bacteria or may kill them.

The effect of heat on inactivation and destruction of *Salmonella* has been the subject of several investigations, but the effect of sublethal heat or thermal injury has received much less attention. This study seeks to relate this thermal injury to the recovery of *S.* Enteritidis cells in ground chicken breast meat under various NaCl concentrations and pH levels. The findings of

this study will: help to estimate the time required to achieve specific log cycle reductions of *S.* Enteritidis at specified temperatures, help the food industry in determining the influence of salts and pH on the recovery of heat injured *S.* Enteritidis cells, and provide information for the creation of mathematical models to predict the expected time of repair for heat injured *S.* Enteritidis cells.

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

The collaborative work of Salmon and Smith (1885) first described the pathogen *Salmonella*. They isolated a microbe from swine that they named *Bacterium suipestifer*. Similar bacteria were isolated from foodborne intoxication in 1885 by Gaffky and Paak, from meat in 1888 by Gärtner, and from other animal diseases in 1892 by Loeffler. In 1900, Lignieres coined the generic term *Salmonella* to include these organisms (*17*).

Salmonella are gram-negative, non-sporulating, facultatively anaerobic rods, belonging to the family *Enterobacteriaceae*. Although most members of this genus are motile by peritrichous flagella, non-flagellated variants and nonmotile strains resulting from dysfunctional flagella do exist. Salmonellae are chemoorganotrophic, with the ability to metabolize nutrients by both respiration and fermentation (*5*). Some key biochemical characteristics of *Salmonella* include catabolism of D-glucose and other carbohydrates with the production of gas, the ability to grow on citrate as a sole carbon source, decarboxylation of lysine and ornithine, catalase positive and oxidase negative reactivity, the production of hydrogen sulfide and the inability to hydrolyze urea (*5*).

Salmonella are resilient microorganisms that may readily adapt to extreme environmental conditions. The bacteria are mesophilic, with an optimum growth temperature between 35 and 37°C. They have been shown to grow at temperatures ranging from refrigerated storage temperatures of 2 to 4°C to elevated temperatures ≤ 54°C (*5*). The physiological adaptability of *Salmonella* is further established by their ability to survive and grow at pH values ranging from 4.5 to 9.5, with an optimum pH for growth of 6.5 to 7.5 (*5*). High salt concentrations have the

ability to inhibit the growth of bacteria. *Salmonella* are generally inhibited at 3 to 4% sodium chloride (NaCl) (*5*).

CLASSIFICATION

For epidemiological classification, there are three groups of salmonellae. The first group includes those that only infect humans and produce typhoid and paratyphoid fevers. The second group of salmonellae is host adapted serovars, some of which are human pathogens and may be obtained from foods. The third group includes unadapted serovars that are pathogenic for humans and other animals. Most foodborne serovars are unadapted (*12*).

Nomenclature of the *Salmonella* group has progressed through a series of taxonomical schemes based on biochemical and serological characteristics, principles of numerical taxonomy and DNA homology. Early schemes used biochemical reactions as determining factors to separate salmonellae into subgroups. The Kauffmann-White scheme was the first to scientifically classify these microorganisms. Within this scheme, five biochemically defined subgenera (I to V) assign individual serovars species status (*5*).

Currently, all salmonellae have been placed taxonomically into two species, *Salmonella enterica* and *Salmonella bongori* (*5*). The serovars are divided into five subspecies or groups, most of which are classified under Group I (*S. enterica* subsp. *enterica*). The major groups correspond to the following subspecies: Group II (*S. enterica* subsp. *salamae*); Group IIIa (*S. enterica* subsp. *arizonae*); Group IIIb (*S. enterica* subsp. *diarizonae*); Group IV (*S. enterica* subsp. *houtenae*); and Group VI (*S. enterica* subsp. *indica*). The former Group V organisms have been given species status as *S. bongori* (*12*). These species and subspecies are differentiated on biochemical properties. Identification of the various serovars of *Salmonella* is

historically based on the presence of lipopolysaccharide (somatic or O antigen), flagella (H antigen, phase I and II) and capsular (Vi) antigen on the bacterial cell surface as determined by serum agglutination. Each *Salmonella* serogroup has a group-specific O-antigen. Within each O-group, different serovars are differentiated by the combination of O and H antigens that are present (*3*).

RESERVOIRS

Salmonella is prevalent in the natural environment. The primary habitat of the bacteria is the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects; however, they may be found in other parts of the body intermittently (*12*). The ubiquity of *Salmonella* in the environment, combined with intensive husbandry practices and the recycling of offal and inedible raw materials into animal feeds, have enabled the continuance of this pathogen in the food chain. Poultry and eggs are the predominant reservoir of *Salmonella* in the meat industry in many countries, including the U.S. (*5*). Poultry is an important reservoir of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) in humans.

FACTORS AFFECTING GROWTH AND SURVIVAL OF *SALMONELLA*

It is impossible for many raw foods to be produced free from contamination of *Salmonella*; therefore, it is important to minimize the frequency and level of contamination of these raw materials by *Salmonella* from the farm to the retail markets. Within food production processes, a variety of factors, either singly or in combination, can be effective in controlling the survival and growth of *Salmonella* (*4*).

Temperature is a major factor that affects the growth and survival of microorganisms. The sensitivity of *Salmonella* to heat depends on the conditions to which the organism has been exposed to prior to heat treatment and the components of the medium or food matrix in which the organism is heated. In conditions of low water activity, heat resistance increases; however in conditions of low pH, heat resistance is reduced. The heat resistance of *Salmonella* can also be increased by heating to sublethal temperatures prior to exposure to lethal temperatures. A similar effect occurs when salmonellae are heated gradually to lethal temperatures. This may be due, in part, to the rapid production of heat shock proteins, which may offer a higher heat resistance to the organism (*4*).

Other physico-chemical properties can also contribute to the control and growth of *Salmonella* that may be present in food. pH is an important growth limiting parameter. Alkaline pH conditions enhance thermal destruction of salmonellae while more acidic conditions enhance resistance. Solute concentrations may also affect the growth of *Salmonella*. They effectively lower water activities, partially dehydrate cells and increase heat resistance (*9*).

SALMONELLOSIS

There are over 2,400 serovars of *Salmonella* and all are regarded as human pathogens (*21*). For foodborne salmonellosis, an individual generally has to consume about 10^5 to 10^6 cells; however, there are some virulent strains where ingestion of fewer cells can cause the disease (*21*). After ingestion of *Salmonella* cells, the pathogens invade the mucosa of the small intestine, proliferate in the epithelial cells, and produce a toxin resulting in an inflammatory reaction and fluid accumulation in the intestine. The ability of the pathogens to invade and damage the cells is attributed to the production of a thermostable cytotoxic factor. Once inside

the epithelial cells, the pathogens multiply and produce a thermolabile enterotoxin that is directly related to the secretion of fluid and electrolytes. Production of the enterotoxin is directly related to the growth rate of the pathogens (*21*). Following ingestion of the pathogen, symptoms appear within 8 to 42 h, generally in 24 to 36 h. The symptoms usually last about 2 to 3 days, but in certain people can last for an extended period of time (*4*). Illnesses caused by *Salmonella* serovars range from gastroenteritis to enteric (typhoid) fever and septicemia and chronic pathological conditions resulting from the disease (*4*).

An important factor that contributes to salmonellosis is inadequate time-temperature exposure during thermal processing or cooking procedures in food service establishments or homes (*13*). *S.* Enteritidis is a leading cause of foodborne salmonellosis. A person infected with the *S.* Enteritidis bacterium usually has fever, abdominal cramps, and diarrhea beginning 12 to 72 hours after consuming a contaminated food or beverage. The illness usually lasts 4 to 7 days, and most people recover without antibiotic treatment. However, the diarrhea can be severe, and the person may require hospitalization (*1*). Foodborne salmonellae are estimated to cause approximately 1.3 million illnesses, 15,000 hospitalizations and 500 deaths per year in the United States (*1*).

SOURCES OF *SALMONELLA* CONTAMINATION IN POULTRY

Salmonella can be isolated from many different stages of poultry production from the hatchery to the processing plant. A significant source of flock contamination is the presence of *Salmonella* in houses prior to placement of the chickens within these facilities (*16*). A common problem and source of *Salmonella* contamination is rodent infestation in flock houses (*8, 20*). Rodents may play an important part in introducing *Salmonella* to the poultry environment, but

there are other possible sources of *Salmonella* to consider. *S.* Enteritidis has been found in litter-beetles and centipedes on poultry farms (*6*). Wild birds are another potential source for introducing *Salmonella* into the poultry environment. Animal feed has long been considered an important source for *Salmonella* contamination on the poultry farm as well (*7*). Increased *Salmonella* transmission has been observed in poultry during transport, and the transport crates themselves have been identified as an important source for contamination (*22, 23*). There is also evidence that poultry can become contaminated at the plant while the carcasses are being processed (*11*).

SALMONELLA ENTERITIDIS

S. Enteritidis was first isolated in Germany in 1888 by A. Gärtner, following an outbreak caused by consumption of meat from an infected cow. Until about 1970-80, *S.* Enteritidis was isolated at a medium to low frequency from humans and animals in most countries (*24*). An international pandemic of *S.* Enteritidis infection began in the mid 1980s. The root cause of the pandemic was the spread of infection in chickens, and the consequent human *S.* Enteritidis food poisoning came from contaminated chicken carcasses and eggs, as well as intact hens' eggs (*24*). Since the mid 1980s, *S.* Enteritidis has remained among the top two *Salmonella* serovars involved in foodborne infections; however, the incidence of *Salmonella* infections overall has decreased. In comparing *Salmonella* surveillance of 1996-1998 to 2004, the incidence of *Salmonella* infections decreased overall by 8% (*2*). Although *Salmonella* incidence decreased overall in 2004, the incidence of *S.* Enteritidis did not (*2*). *S.* Enteritidis poses a direct and indirect risk to public health. The direct risk arises from contaminated contents of eggs used for

human consumption. The indirect source is associated with the ability of the microorganism to be transmitted vertically from infected breeder flocks to their offspring.

OUTBREAKS AND INFECTIONS

Foods of animal origin have been associated with large numbers of salmonellosis outbreaks. These include beef, chicken, turkey, pork, eggs, milk, and products made from them. Salmonellae have also been isolated from foods of plant origin and seafood (*21*). In addition, many different types of foods have been linked to sporadic cases and outbreaks. These foods were contaminated either directly with fecal matter from carriers and eaten raw or improperly cooked, or indirectly from contamination following adequate heat treatment. Cross-contamination at home and food service establishments are the major sites of contamination of heated foods with *Salmonella*. In 2004, FoodNet cases were part of 239 nationally reported foodborne disease outbreaks; 138 (58%) of these outbreaks were associated with restaurants. An etiology was reported in 152 (64%) outbreaks. The most common etiologies were norovirus (57%) and *Salmonella* (18%) (*2*).

Although there are over 2,400 serovars of *Salmonella*, only a small portion of them have been frequently associated with foodborne illnesses. In 2004, FoodNet surveillance identified 6,464 laboratory diagnosed cases of *Salmonella* infections. Of the 5,942 (92%) *Salmonella* isolates serotyped, five serotypes accounted for 56% of infections, as follows: Typhimurium, 1,170 (20%); Enteritidis, 865 (15%); Newport, 585 (10%); Javiana, 406 (7%); and Heidelberg, 304 (5%) (*2*). *Salmonella* Typhimurium has been associated in the U.S. as the major causative agent of foodborne salmonellosis. However, from 1996 to 2004 the incidence of *S.* Typhimurium has decreased significantly (*2*). One contributing factor to the decline in

foodborne infections caused by this pathogen is a change in the industry and regulatory approach to meat and poultry safety. Beginning in 1996, the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) began implementing the Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) systems regulations in the meat and poultry slaughter and processing plants. The decline in the incidence of *Salmonella* Typhimurium infections in humans may be related to changes in meat processing as evidenced by a decline in the prevalence of *Salmonella* isolated from FSIS regulated meat and poultry products reported by USDA. However, substantial declines have not occurred in the incidence of infection caused by other major *Salmonella* serotypes, including Enteritidis, Newport, and Heidelberg. The incidence of *S.* Javiana infections increased markedly in 2004, in part because of a multistate outbreak associated with Roma tomatoes (*2*).

THERMAL INJURY OF *SALMONELLA*

Heat treatments used by the food processing industry and the cooking of foods at home are usually effective in killing *Salmonella*; however, sometimes there are salmonellae that survive food processing techniques (*9*). For example, microwave cooking does not reliably destroy salmonellae on chickens and turkeys, even though the recommended internal temperature has been reached. In addition, eggs fried sunny sides up have been found to still contain viable salmonellae. This survival of cooking methods may be due to changes in the characteristics of the food that affect the thermal tolerance of salmonellae (*9*).

The determination of thermal injury of bacterial cells is usually achieved by exposing the cells to a variety of temperatures and determining the rate of survival during heating by viable plate counts (*25*). The two parameters used to describe the thermal injury of bacterial cells are

the D value and z value. These are obtained from the logarithmic portion on a thermal survival curve. The D value or decimal reduction time represents the time required, at a particular temperature, to reduce the surviving number of organisms by 90% or 1 log (*25*). The z value is the temperature required to change the D value by l log (*25*). Reactions that have small z values are highly temperature dependent.

There have been some heating studies done on *Salmonella* in food products including eggs, milk and dairy products, poultry and other meats, chocolate, wheat flour, corn flour and corn-soy milk blends, shellfish, coconut, pecans, and alfalfa seeds (*9*). However, most studies have been performed with nutrient medium broth. Due to the highly complex structure and composition of meat, heating studies of microorganisms within meat matrices may differ from those in liquid media (*19*). The heat resistance of pathogens in meat is influenced by meat species, muscle type, pH, fat content, and other environmental factors (*19*). There is little published information regarding pathogen thermal inactivation in real meat products, especially chicken (*18*).

RECOVERY

One of the most important characteristics of injured bacterial cells is the ability to repair the injury and exhibit normal cell function. Heating of bacterial cells at sublethal temperatures kills some of the population and may injure others. Depending on the environmental conditions of these injured cells after the heat treatment, they may survive to grow and multiply or they may die.

Following exposure to sublethal temperatures, a bacterial population contains three physiologically different subpopulations: the uninjured cells that are capable of growth and

multiplication in selective and non-selective media, reversibly injured cells that are capable of multiplication in non-selective media, and irreversibly injured cells which are incapable of multiplication under any conditions (*26*). Reversibly injured cells will usually lose their viability upon prolonged exposure to selective medium; however, in a non-selective environment, they will usually be capable of repairing their injury. In general, nutritionally rich media allow a relatively rapid repair for a high proportion of injured cells. Cells with little injury will repair under a wider range of conditions (*26*).

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CHAPTER 3

THERMAL INJURY AND RECOVERY OF *SALMONELLA* ENTERITIDIS IN GROUND CHICKEN WITH TEMPERATURE, PH AND SODIUM CHLORIDE AS CONTROLLING FACTORS[1]

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ABSTRACT

Cells of *Salmonella* Enteritidis were grown at 25 and 35ºC. The survival and recovery of heat injured (55, 60, and 62.5°C) cells in ground chicken inoculated into tryptic soy broth (TSB) adjusted to various pHs (5.5 and 6.5) and NaCl concentrations (2.0 and 3.5%) was studied. The D values of cells of *S.* Enteritidis grown at 25ºC were 12.99, 1.287 and 0.665 min at 55, 60 and 62.5ºC, respectively. For cells grown at 35ºC the D values were 13.42, 1.555, and 1.255 min at 55, 60 and 62.5ºC. The z-values of 6.48-8.53ºC were obtained depending on heating temperature and plating conditions. The influence of tryptic soy agar (TSA) and double modified lysine agar (DMLIA) on the recovery of injured cells was determined. Recovery was significantly reduced on DMLIA at increased pHs and NaCl concentrations. Higher numbers of cells were recovered in TSB at pH 5.5 and 2.0% NaCl.

INTRODUCTION

Salmonella is a foodborne pathogen that occurs in different sectors of the agricultural and food processing industries. The common occurrence of *Salmonella* in the natural environment, combined with intensive husbandry practices, has favored the continued prominence of this human bacterial pathogen in the global food chain. Of the many sectors within the meat industry, poultry and eggs remain a predominant reservoir of *Salmonella* in many countries (*3*).

Factors which lead to outbreaks of foodborne illness, including salmonellosis, are insufficient time and temperature exposure during thermal processing and inadequate reheating of food products to kill foodborne pathogens (6*, 7*). Various heat treatments utilized by the food processing industry and the cooking of foods at home are generally effective at destroying vegetative, foodborne pathogenic bacteria. However, occasionally there are salmonellae that survive food processing techniques (*5*). An effective thermal process is essential to control the potential hazard of *Salmonella* in cooked food products. An essential part of the heating stage is to determine the target pathogen's heat resistance (*8*) and its ability to recover from thermal injury. There are several factors that affect heat resistance in food products, including temperature pre-, post- and during heat treatment, food composition, pH of the food matrix, water activity of the food, species variation, and the presence of competitive microflora.

The ability of salmonellae to survive in food products has been the subject of several investigations (6*, 8, 9, 16-18*), but the relationships of pH, NaCl concentration and plating medium on the recovery of *Salmonella* Enteritidis in ground chicken have not been thoroughly examined. This investigation had a three-fold purpose: (i) to determine any differences in the effects of selective and non-selective media on the growth and survival of *S.* Enteritidis cells

thermally injured in ground chicken breast meat; (ii) to assess the thermal inactivation of *S.* Enteritidis in ground chicken breast meat by determining D- and z-values; and (iii) to determine the effects of pH and NaCl concentration on the recovery of sublethally injured *S.* Enteritidis cells in ground chicken breast meat.

MATERIALS AND METHODS

Bacterial strain. *Salmonella* Enteritidis (ATCC 13076) was purchased from the American Type Culture Collection (Rockville, MD) and used in this study. The strain pellet was rehydrated with 0.5 ml of brain heart infusion (BHI: pH 7.4) broth. A 10 µl loop of the rehydrated pellet was surface plated onto blood agar plates and incubated for 24 h at 35 ± 1ºC. The strain was preserved by freezing the hydrated culture at -20 ± 2ºC. Isolated colonies from the blood agar plates were inoculated onto two tryptic soy agar (TSA: trypticase, phytone, NaCl, pH 7.3) slants, which were incubated for 24 h at 35 ± 1ºC. One of the TSA slants was used as the working culture and the other used exclusively for propagation of the culture. The slants were maintained at 4 ± 2ºC and the propagation culture was transferred to new slants on a monthly basis.

Products. Raw boneless, skinless lean chicken breasts were obtained from a local retail supermarket. The chicken breasts contained approximately 1-4% fat. The chicken breasts were ground using a meat grinder (Waring Consumer Product, East Windsor, NJ). The ground chicken breast meat was placed into Whirl-Pak® filter bags (Nasco, Fort Atkinson, WI) (25 g/bag) and frozen at -20 ± 2ºC. A total of 216 samples were used in this study. Random samples were tested to verify the absence of indigenous *Salmonella* by enrichment in buffered peptone water (BPW: peptone, NaCl, dibasic sodium phosphate, monobasic potassium phosphate, pH

7.2), followed by 20-24 h incubation at 35 ± 1ºC (*1*). After incubation, the samples were tested using a BAX® PCR system, following manufacturer's instructions (Dupont Qualicon, Wilmington, DE).

Preparation of test cultures. To propagate the cultures, a 10 µl loop of the working slant culture was transferred to a 10 ml tube of BHI broth and incubated for 24 h at the appropriate experimental growth temperature (25 or 35ºC). These cultures were not used in the heating studies to eliminate the presence of freeze damaged cells. A transfer of 0.1 ml of the incubated cultures were made to a 10 ml tube of BHI and incubated for 24 h at the appropriate experimental growth temperature (25 or 35ºC). The inocula for use in heating studies were prepared by transferring 0.1 ml of the culture free of freeze damaged cells to 250 ml of BHI in a flask that was incubated for 24 h at the appropriate experimental growth temperature (25 or 35ºC). On the day of the experiment, the test cultures were pipetted into 50 ml centrifuge tubes and centrifuged for 15 min at 2,990 × *g* in a Sorvall RC-5B Refrigerated Superspeed Centrifuge (Dupont Instruments, Newtown, CT). Following centrifugation, the supernatants were discarded from each tube and the pellets were each suspended in 2 ml of 0.1% peptone water (PW). The suspensions were combined in a sterile conical vial to obtain the inocula with a target level of 8-9 $\log_{10}$ CFU/ml prior to inoculation of the ground chicken breasts. The population densities of the inocula were enumerated after diluting in 0.1% PW by spread plating, in duplicate, onto TSA plates that were incubated at 35 ± 1ºC for 48 h.

Sample preparation and inoculation. The inoculum (0.1 ml) was added to 25 g thawed ground chicken breasts in Whirl-Pak® filter bags. Each inoculated sample of meat was pummeled with a Stomacher Lab-Blender 400 (Cooke Laboratory Products, Alexandria, VA) for 2 min, to ensure even distribution of *S.* Enteritidis throughout the samples. Filter bags containing

ground chicken breasts samples inoculated only with 0.1 ml of 0.1% sterile PW were used as negative controls. After pummeling, the samples were formed into uniform sizes (8 × 0.5 cm) using a circular template. The contact surfaces of the filter bags were pressed firmly against the meat sample within the filter bag, creating a close fit that prevented air pockets within the filter bag.

Experimental design. To assess the interactions of growth temperature, heating temperature, sodium chloride (NaCl) concentration and pH on the thermal injury and recovery of *S.* Enteritidis in ground chicken breasts, a randomized design with the controlling factors of temperature and media was used. Levels of the factors studied were as follows: growth temperature, 25 and 35ºC; heating temperatures, 55, 60, and 62.5ºC; NaCl concentration, 2.0 and 3.5%; and pH, 5.5 and 6.5.

Twenty-four different design points of the above factors were studied (Table 1). For each experimental combination three replicates were performed. A replicate consisted of one sample subjected to the appropriate treatment.

Heating protocol. To ensure a consistent starting temperature was used in each heating study, the samples were placed at 4 ± 2ºC for 1 h. Afterwards, the bags were placed in a basket and fully submerged in a water bath. The heating studies were carried out in a temperature controlled circulating water bath (Fisher Scientific, Pittsburgh, PA) stabilized at 55, 60 or 62.5 ± 0.2ºC. The temperature was continuously monitored by thermocouples inserted at the center of two uninoculated samples. The thermocouple readings were measured and recorded using a Leeds and Northrup Speedomax Recorder (Pittsburgh, PA). Bags for each replicate were removed at predetermined time intervals and placed directly into an ice water bath.

Enumeration of surviving bacteria. Cooled heat treated samples were combined with 50 ml of the appropriate tryptic soy broth (TSB with either 2.0% NaCl at pH 5.5; 2.0% NaCl at pH 6.5; 3.5% NaCl at pH 5.5; or 3.5% NaCl at pH 6.5) within 40 min of heat treatment for determination of surviving bacteria. The samples were pummeled with a Stomacher Lab-Blender 400 (Cooke Laboratory Products, Alexandria, VA) for 2 min. Decimal serial dilutions were prepared in 0.1% PW and appropriate dilutions (0.1 ml) were surface plated, in duplicate, onto non-selective TSA and selective double modified lysine iron agar (DMLIA: modified lysine iron agar, 15 mg sodium novobiocin per liter, distilled water, pH 6.7) plates. Samples not inoculated with *S.* Enteritidis were plated as controls. Also, 0.1 ml aliquots of undiluted suspensions were surface plated, where necessary. All plates were incubated at 35 ± 1ºC for at least 48 h prior to counting colonies.

Recovery of thermally injured bacteria. The pummeled samples containing the appropriate experimental TSB medium were incubated aerobically at 35± 1ºC. Aliquots were taken at predetermined time intervals (0, 6, 12-14, 16-18, and 24 h) from each sample. The quantification of recovery of heat injured cells was performed only on cells at the time interval at each heat treatment in which the lowest detectable numbers of *S.* Enteritidis were present on both DMLIA and TSA. This occurred at 45 min at 55ºC and 8 min at both 60 and 62.5ºC. These cells were diluted into 0.1% PW and appropriate dilutions (0.1 ml) were surface plated, in duplicate, onto TSA and DMLIA plates. Also, 0.1 ml aliquots of undiluted suspensions were surface plated, where necessary. All plates were incubated at 35 ± 1ºC for at least 48 h prior to counting colonies.

Calculation of D-values and z-values. Data from the thermal destruction studies was analyzed by analysis of covariance using Proc GLM (SAS Institute, Cary, NC) to calculate D-

values (time to inactivate 90% of the population) for each heating temperature. These data were compared using contrasts to compare the slopes of the individual $\log_{10}$ colony forming units (CFU) versus time regressions. The z-values were calculated by regressing $\log_{10}$ D-values versus their corresponding heating temperatures.

Determination of lethal rates. The lethal rates (log-unit reductions per minute) were determined as the reciprocal of the D-value.

Statistical analyses. The bacterial counts were transformed to $\log_{10}$ counts and analyzed by analysis of variance (ANOVA) using SigmaStat (Systat Software, Point Richmond, CA) to determine if there were significant differences among treatments. Also, t-tests were performed to determine significant differences between treatments using SigmaStat (Systat Software, Point Richmond, CA).

RESULTS

Survival of *S*. Enteritidis in chicken breast meat

BAX® analyses of uninoculated samples revealed that *Salmonella* was not initially present in the meat samples used in this study. The initial inoculum range of 8 to 9 $\log_{10}$ (CFU/ml) was obtained in all the trials.

S. Enteritidis cells heated at 55, 60 and 62.5 ± 0.2ºC exhibited nonlinear declines in surviving cells with time. The numbers of survivors were determined by plating heated cells onto selective and non-selective media. Survivor curves were constructed by plotting $\log_{10}$ CFU/ml against heating time (Fig. 1). The curves differed in slope, depending upon the growth temperature. As heating temperature increased from 55 to 62.5ºC, survival of *S.* Enteritidis cells decreased (Fig. 1). Survivor curves at 55ºC exhibited an initial shoulder before any noticeable

decrease in salmonellae populations occurred in the meat. In contrast, at 60 and 62.5ºC, the survivor curves showed a rapid decrease in the microbial population (Fig. 1). It was observed that cells that were grown at 35ºC prior to the heat treatments exhibited a greater heat resistance than cells grown at 25ºC. Survivor curves exhibited a tailing effect; however, tailing was most pronounced at higher temperatures. For example, at 62.5ºC tailing occurs once the cells reach approximately 2 $\log_{10}$ CFU/ml (Fig 1c).

After heat treatment at 55ºC for 90 min, the viable population of *S.* Enteritidis cells grown at 35ºC, as determined by its ability to grow on non-selective media (TSA), decreased from 8 $\log_{10}$ to 3 $\log_{10}$ colony forming units (CFU)/ml (Fig 1a). After 45 min, the uninjured cell population, as determined by its ability to grow on selective media (DMLIA), decreased from 8 $\log_{10}$ to 3 $\log_{10}$ CFU/ml and colonies were not detected at later time intervals. The populations of *S.* Enteritidis cells grown at 25ºC exhibited a more rapid decrease than that of the cells grown at 35ºC. After heat treatment at 55ºC for 90 min, the viable population decreased from 8 $\log_{10}$ to 1 $\log_{10}$ CFU/ml. After 30 min, the uninjured cell population decreased from 8 $\log_{10}$ to 1 $\log_{10}$ CFU/ml and colonies were not detected at later time intervals, as determined by plating onto DMLIA.

At 60ºC, the viable population of *S.* Enteritidis cells grown at both 25 and 35ºC decreased from 8 $\log_{10}$ to 3 $\log_{10}$ CFU/ml within 8 minutes (Fig 1b). Within this time interval, the uninjured cell population of cells grown at both 25 and 35ºC also decreased from 8 $\log_{10}$ to 3 $\log_{10}$ CFU/ml. A heat treatment of 62.5 ± 0.2ºC decreased the viable populations, as well as the uninjured populations, of cells grown at 25 and 35ºC from 8 $\log_{10}$ to 2 $\log_{10}$ CFU/ml within 8 minutes (Fig 1c).

Thermal inactivation of *S.* Enteritidis in ground chicken breast meat

Surviving populations of *S.* Enteritidis cells in ground chicken breast meat were determined and the D-values calculated. The average D values for *S.* Enteritidis in ground chicken meat (Table 2) were calculated from analysis of covariance using Proc GLM (SAS Institute, Cary, NC) for each heating temperature. The D values decreased with increasing temperature. For D values at 55, 60 and 62.5ºC there was not a statistically significant difference between plating medium or growth temperature of the cells prior to heat treatments ($p = 0.240$).

When *S.* Enteritidis cells were heated in ground chicken breast meat, thermal resistance (D values in min) at 55ºC was significantly higher ($p = 0.004$) than at 60 and 62.5ºC. However, the D values at 60 and 62.5ºC for each growth temperature were not found to be statistically different ($p = 0.100$). At 55ºC the D values of *S.* Enteritidis cells, grown at 25ºC, in ground chicken breast meat and enumerated onto TSA were 12.99, 1.287 and 0.665 min at 55, 60 and 62.5ºC, respectively. On DMLIA the D values of the cells grown at 25ºC were 12.27, 0.913 and 0.353 min at 55, 60 and 62.5ºC, respectively. For *S.* Enteritidis cells grown at 35ºC and plated onto TSA, the D values were 13.42, 1.555 and 1.255 min at 55, 60 and 62.5ºC. From DMLIA plates, the calculated D values of *S.* Enteritidis cells grown at 35ºC were 13.35, 0.935 and 0.951 min at 55, 60 and 62.5ºC, respectively.

The z values were calculated by regression of $\log_{10}$ D values against temperature. The z values for *S.* Enteritidis enumerated on TSA and DMLIA are given in Table 3 and were similar for both plating conditions. There was not found a statistically significant effect on z values due to plating medium or growth temperature of the cells prior to heat treatments ($p = 0.333$). The z values of *S.* Enteritidis cells grown at 25ºC were 7.97 and 8.53ºC when plated onto TSA and DMLIA with correlation coefficients of 0.65 and 0.86, respectively. For cells grown at 35ºC, the

z values were 8.04 and 6.48ºC when plated onto TSA and DMLIA with correlation coefficients of 0.99 and 0.91, respectively.

Recovery of *S.* Enteritidis in ground chicken breast meat

Recovery of *S.* Enteritidis was performed on cells subjected to 55ºC for 45 min, 60ºC for 8 min and 62.5ºC for 8 min. NaCl was employed as a selective agent and the effect of NaCl concentrations (2.0 and 3.5%) in the recovery medium (TSB) was investigated (Fig. 2-7). Following incubation at 35ºC in TSB, heat treated cells were plated onto DMLIA and TSA. There was a substantial decrease in the number of cells recovered on DMLIA. The recovery of heat injured cells was inhibited by increasing the level of NaCl in the recovery medium. It was observed at 55 and 62.5ºC that with 3.5% NaCl added to the recovery medium, there was a marked decrease in the growth rate of the heated organisms and a longer time was required for colonies plated on DMLIA to recover from the heat stress. At 60ºC, there was not a statistically significant difference in the rate of recovery of heat injured *S.* Enteritidis cells at the various NaCl concentrations and pH levels of the recovery medium for cells grown at either 25ºC ($p = 0.13$) or 35ºC ($p = 0.15$) (Fig. 3 and 4).

Another selective agent that was utilized in the recovery medium was pH (5.5 and 6.5). There was an observed increase in the rate of recovery of heat injured cells at pH 5.5 (Fig. 2-7). The time of recovery was greatest for colonies plated onto non-selective media for both pH 5.5 and pH 6.5. It was observed that cells that were grown at 35ºC prior to the heat treatments exhibited a faster rate of recovery in the recovery medium at each NaCl concentration and pH combination.

DISCUSSION

Heat treatment is one of the most common methods used in food processing to destroy or inactivate microorganisms. The higher the treatment temperature or the longer the treatment time, the higher the efficiency of the process becomes. However, heat treatments do not elicit the same degree of injury to all cells within a population. Some cells may die; others may survive and recover from the thermal injury. The ability of heat injured salmonellae to repair sublethal damage in different environments is of great significance to the food industry and consumers. Sublethally injured cells exhibit a longer lag phase than uninjured bacteria. Consequently, there is an increased time for the organisms to attain a critical level in foods (*7*). Recently, chicken consumption has been identified as a risk factor for sporadic illness caused by *S.* Enteritidis (*10*). If the conditions that inhibit growth and survival of this organism in chicken products are identified, they may be utilized by processors and consumers to reduce the incidence of foodborne illness. Therefore, the present study examined the sublethal injury and the rate of recovery of *S.* Enteritidis cells in ground chicken breast meat.

Exposure to temperatures above the range for normal cell growth leads to progressive loss of bacterial viability. A degree of protection against the lethal effects of a higher temperature or an acquired thermotolerance is achieved, when bacteria are grown slightly above their normal growth range (*3*). Acquired thermotolerance begins during the brief incubation period at elevated but nonlethal temperature as a result of heat shock response. Heat shock proteins mediate recovery to stress induced damage (*3*). Heat shock effects can be a significant factor in bulk foods heated slowly and in foods receiving a marginal heat treatment (*6*). The tailing effect that was observed in the present study may be due to an adaptive survival mechanism brought on by physiological and biochemical changes in the heat injured *S.*

Enteritidis cells. Within the population of cells, there were inevitably varying degrees of heat resistance due to differences in stages of the microbial life cycle. Cells in stationary phase are more resistant than those in lag or exponential phase (*10*). The heterogeneity of heat resistances within the population probably caused the nonlinearity of the survivor curves at each heating temperature.

In addition, some components present in foods can protect bacterial cells from thermal injury by stabilizing membranes or other cellular structures (*6*). It is possible that the level of fat in the ground chicken meat affected the heat resistance of the *S.* Enteritidis cells; however a study by Juneja and others (*10*) revealed that fat levels less than nine percent did not give cells a significantly higher heat resistance. In the present study the chicken breasts contained between one to four percent fat. In chicken products, enhanced survival of *S.* Enteritidis cells may be of great concern if the resistance is sustained by the altered cells.

It is feasible to compare the thermal inactivation data obtained in this study with those in the published literature on the heat resistance of *Salmonella* serovars. When comparing the results in this study with those reported from other studies, it should be kept in mind that meat species, muscle type, pH, fat content, and other environmental factors including the method of enumeration may influence the bacterial heat resistance. Also, certain strains of *Salmonella* are less resistant and are less tolerant to changes in temperature (*19*). The thermal inactivation data in this study were inconsistent with those reported elsewhere (*9, 17*), which may be attributed to the above factors. The D values obtained in this study were lower than published values. However, the media conditions for enumeration of bacteria were different in the current study. One of the aims of this study was to analyze the effect of NaCl and pH on the recovery of heat injured *S.* Enteritidis cells in ground chicken breast meat; thus, TSB supplemented with NaCl

(2.0% or 3.5%) and adjusted to pH 5.5 or 6.5 was combined with the heat treated samples and subsequently plated onto selective and non-selective media. Previous studies have enumerated surviving bacteria in sterile peptone water following heat treatments. In addition, the current study used a single strain of *Salmonella*; whereas, other studies in the published literature conducted thermal inactivation of *Salmonella* cocktails in chicken products (*9, 17*). *Salmonella* Enteritidis was used as the target pathogen in this study due to its recent recognition as a risk factor for sporadic infection through chicken consumption (*10*).

In comparing the results of this study with previous literature, Juneja and others (*9*) tested an eight strain *Salmonella* cocktail in chicken (7% fat) at 58-65ºC and obtained D values of 7.08 min at 58ºC, 5.20 min at 60ºC, and 1.36 min at 62.5ºC. They reported a z value of 5.86ºC for *S.* Enteritidis phage type 13A and a z value of 6.46ºC for *S.* Enteritidis phage type 4. Using these estimates of D values, and assuming 0 lag times, the times needed to obtain a 7 $\log_{10}$ relative reduction would be about 49.56 min at 58ºC, 36.4 min at 60ºC, and 9.52 min at 62.5ºC. In a study by Murphy and others (*17*), D values in chicken breast meat of 30.1 min, 5.88 min, and 2.51 min were obtained at 55, 60, 62.5ºC, respectively. Using these estimates of D values, and assuming 0 lag times, the times needed to obtain a 7 $\log_{10}$ relative reduction would be about 210.7 min at 55ºC, 41.16 min at 60ºC, and 17.57 min at 62.5ºC. In the present study, D values of 12.99, 1.287 and 0.665 min at 55, 60 and 62.5ºC, respectively, were obtained for *S.* Enteritidis cells grown at 25ºC. D values of 13.42, 1.555 and 1.255 min at 55, 60 and 62.5ºC were obtained for cells grown at 35ºC. Using these D values, the times needed to obtain a 7 $\log_{10}$ relative reduction of *S.* Enteritidis cells grown at 25ºC would be about 90.93 min at 55ºC, 9.01 min at 60ºC, and 4.66 min at 62.5ºC. For cells grown at 35ºC, the times needed to obtain a 7 $\log_{10}$ relative reduction would be 93.94 min at 55ºC, 10.89 min at 60ºC, and 8.79 min at 62.5ºC.

The D values obtained in previous studies using chicken meat have some variation. This may be due to the highly complicated structure and composition of chicken meat and differences in strains used. Because of the use of a single strain and the difference in testing media, it was expected that D- and z-values in this study would be lower than previous published data.

Growth temperature affects lipid biosynthesis, composition of membranes, and protein synthesis, and thereby influences the ability of *Salmonella* to withstand thermal inactivation (*6*) and to recover from sublethal heat injury. In this study *S.* Enteritidis cells were grown at 25 and 35ºC. It was found that when grown at 35ºC *S.* Enteritidis had a greater heat resistance and injured cells recovered at a faster rate, compared to cells grown at 25ºC. Cells grown at higher temperatures are more heat resistant than those grown at lower temperatures (*6*), presumably because of differences in gene expression and membrane fluidity.

A population of bacteria after a heat treatment contains three physiologically different types of cells: the uninjured cells, the reversibly injured cells and the irreversibly injured cells. The proportion of injured cells depends on different factors, such as the target species, the nature and intensity of the heat treatment, and the selectivity of the selective medium. The extent of injury in a bacterial population can be determined by plating samples separately on a selective and a nonselective medium. Selective compounds that are typically used in selective media include surface active compounds such as bile salts, toxic chemicals, dyes, NaCl, antibiotics, organic and inorganic acids, some fatty acids, some food preservatives, and some antimicrobial enzymes (*21*). The selective medium used in this study was DMLIA, which contains novobiocin, bile salts, lactose, and sucrose to enhance the selectivity and differentiation capacity of the medium (*19*). In addition, the recovery medium (TSB) contained various concentrations of NaCl and enhanced the selectivity of the plating medium. It has been reported that the

addition of NaCl to the recovery media decreased the heat resistance of some microorganisms probably by impairing heat damage repair mechanisms. The high degree of selectivity in the plating and recovery media probably resulted in lower numbers of colonies present on the DMLIA plates after heat treatments and a decreased rate of recovery of heat injured *S.* Enteritidis cells. The cells plated onto DMLIA were not able to reach 8-9 $\log_{10}$ CFU/ml within the 24 h recovery period, compared to cells plated on TSA. Injured cells usually lose their viability with prolonged exposure to selective media. However, in a non-selective environment, they will usually be capable of repairing their injury (*21*).

In comparing the effects of media on survival and growth of microorganisms with previous literature, Brashears and others (*2*) found that *Escherichia* coli 0157:H7 and *Salmonella* subjected to stress conditions exhibited significant reductions in populations when plating was done on non-selective media. Injured *E.* coli 0157:H7 cells were not recovered on recovery or selective media compared with TSA. The findings of the present study seem to agree with this previous study. The heat stress that was placed on *S.* Enteritidis cells in ground chicken reduced the populations by at least 5 $\log_{10}$ CFU/ml. Injured cells were not able to recover on the selective media of DMLIA. These findings are also in agreement with a study by Kobayashi and others (*12*) in which the count determined by the plating assay with TSA, a non-selective medium, was nearly 1,000-fold higher than that determined by the assay with a selective medium.

The rate at which a population of injured cells undergoes repair will vary with incubation temperature, pH, and salt concentration of the medium. In general, nutritionally rich media allow a relative rapid repair for a high proportion of injured cells. Cells with very little injury are less demanding and repair may occur under a wider range of conditions than cells with a high degree of injury (*21*). In the present study, *S.* Enteritidis cells were able to repair heat injury with

non-selective (TSA) enumeration agar. However, at increased pH levels and NaCl concentrations, the rate of recovery was slower. These findings agree with an investigation by Chawla and others (*4*) in which repair of injured *L.* monocytogenes occurred slower at higher NaCl concentrations and at higher pH values.

The present study found that the addition of NaCl to the recovery medium negatively impacted the ability of *S.* Enteritidis cells to recover from heat damage at 55 and 62.5ºC. These results are in agreement with those of other authors who also found that increasing NaCl in the recovery medium inhibited the recovery of heat injured cells (*13, 14*, *20*).

In a previous study by McKay and Peters (*15*) the effect of NaCl concentration and pH on the growth of *S.* Typhimurium was assessed. It was found that increasing the NaCl concentration and decreasing the pH had little effect on colony growth. This was true in the present study for cells that underwent a heat treatment at 60ºC for 8 min. There was not a statistically significant difference found between the various NaCl concentrations and pH values of the recovery media.

A good understanding of sublethal heat injury and recovery is important. The growth conditions of the *S.* Enteritidis cells prior to heat treatment influence the survival of the organisms during and after the process. The presence of sublethally injured cells after a heat treatment may lead to an underestimation of the numbers of survivors, particularly if selective media are used. Since sublethal injury leads to a higher sensitivity of *S.* Enteritidis survivors to stress conditions in ground chicken breast meat after a heat treatment, from a practical point of view, the combined processes of increased NaCl concentrations and elevated pH levels to chicken products would prove valuable in inhibiting the growth and repair of these organisms for food processors.

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CHAPTER 4

CONCLUSIONS

CONCLUSIONS

The results of the present study can be used to predict the time required at the specified temperatures to achieve 7 $\log_{10}$ reductions of *Salmonella* Enteritidis when heated in ground chicken. Based on the D values determined in this study, contaminated ground chicken should be heated to an internal temperature of 60.0ºC for at least 9.01 min. D values from this study determined for ground chicken will assist food processors in designing acceptance limits on critical control points that ensure safety against *S.* Enteritidis in cooked chicken meat.

The results of the recovery experiments suggest growth temperature and enumeration agar affect the heat resistance of *S.* Enteritidis cells in ground chicken. Cells grown at higher temperatures have a greater heat resistance to sublethal heat treatments. It was found that the use of selective media to enumerate the survivors resulted lower numbers of colonies. This may lead to an underestimation of the numbers of survivors of a heat treatment of *S.* Enteritidis cells in ground chicken. In addition, the results suggest that the NaCl concentration and pH of the environment of injured *S.* Enteritidis cells after a heat treatment affect the ability of these cells to recover. At higher NaCl concentrations and pH levels, heat injured *S.* Enteritidis cells require longer periods of time to recover from the damage. Since sublethal injury leads to a higher sensitivity of *S.* Enteritidis survivors to stress conditions in ground chicken breast meat after a heat treatment, from a practical point of view, the combined processes of increased NaCl concentrations and elevated pH levels to chicken products would prove valuable in inhibiting the growth and repair of these organisms for food processors.

Table 1. Randomized design used to assess the effects of interactions of growth temperature and heating temperature on the heat resistance of *Salmonella* Enteritidis in ground chicken breast meat and to investigate the effects of NaCl concentrations and pH levels on the recovery of heat injured *S.* Enteritidis cells.

Design	Thermal Injury Conditions[a]		Recovery Conditions[b]	
	Growth temp (ºC)	Heating temp (ºC)	%NaCl	pH[c]
1	35	55.0	2.0	5.5
2	35	55.0	2.0	6.5
3	35	55.0	3.5	5.5
4	35	55.0	3.5	6.5
5	25	55.0	2.0	5.5
6	25	55.0	2.0	6.5
7	25	55.0	3.5	5.5
8	25	55.0	3.5	6.5
9	35	60.0	2.0	5.5
10	35	60.0	2.0	6.5
11	35	60.0	3.5	5.5
12	35	60.0	3.5	6.5
13	25	60.0	2.0	5.5
14	25	60.0	2.0	6.5
15	25	60.0	3.5	5.5
16	25	60.0	3.5	6.5
17	35	62.5	2.0	5.5
18	35	62.5	2.0	6.5
19	35	62.5	3.5	5.5
20	35	62.5	3.5	6.5
21	25	62.5	2.0	5.5
22	25	62.5	2.0	6.5
23	25	62.5	3.5	5.5
24	25	62.5	3.5	6.5

[a] *S.* Enteritidis cells were grown at experimental temperatures of 25 or 35ºC and underwent heat treatments at 55, 60 and 62.5ºC in a circulating water bath.

[b] Heat injured cells subjected to 55, 60 and 62.5ºC were recovered in tryptic soy broth (TSB) with various NaCl concentrations (2.0 and 3.5%) and pH levels (5.5 and 6.5).

[c] TSB adjusted to pH 5.5 or 6.5 using 1N hydrochloric acid (HCl)

Table 2. Heat resistance (expressed as D-values in min)[a] for *Salmonella* Enteritidis in ground chicken breast meat heated at 55-62.5ºC and subsequently plated onto selective and non-selective media

Plating medium[b]	Temp[c] (ºC)	Growth temp[d] (ºC)	Ground Chicken Breast Meat		
			D (min)	(r^2)[e]	lethal rate[f]
TSA	55.0	25	12.99 ± 0.75	0.88	0.08
	55.0	35	13.42 ± 0.46	0.89	0.07
	60.0-62.5	25	0.976 ± 0.31	0.75	1.02
	60.0-62.5	35	1.405 ± 0.15	0.72	0.71
DMLIA	55.0	25	12.27 ± 1.38	0.63	0.08
	55.0	35	13.35 ± 1.27	0.53	0.07
	60.0-62.5	25	0.633 ± 0.28	0.76	1.58
	60.0-62.5	35	0.943 ± 0.01	0.69	1.06

[a] D-values were calculated by analysis using Proc GLM (SAS Software) of three replicate experiments and are expressed as mean ± standard error.

[b] Survivors of thermal treatments were plated onto both non-selective (TSA: tryptic soy agar) and selective (DMLIA: double modified lysine iron agar) agar.

[c] Samples of ground chicken breast meat were heated at 55, 60, or 62.5ºC

[d] Two different growth temperatures (25 or 35ºC) were used to provide the inocula used to inoculate the ground chicken breast meat samples

[e] Correlation coefficients

[f] Lethal rate (log-unit reductions per minute) were determined as the reciprocal of the D-value

Table 3. Heat resistance expressed as z-values[a] in ºC for *Salmonella* Enteritidis in ground chicken breast meat heated at 55-62.5ºC and subsequently plated onto selective and non-selective media

Plating medium[b]	Growth temp[c] (ºC)	z-value (ºC)	(r^2)[d]
TSA	25	7.97 ± 2.75	0.65
	35	6.48 ± 1.82	0.91
DMLIA	25	8.53 ± 3.15	0.86
	35	8.04 ± 2.80	0.99

[a] z-values were estimated by computing the linear regression of mean $\log_{10}$ D-values versus their corresponding heating temperatures and are expressed as mean ± standard error

[b] Survivors of thermal treatments were plated onto both non-selective (TSA: tryptic soy agar) and selective (DMLIA: double modified lysine iron agar) agar

[c] Two different growth temperatures (25 or 35ºC) were used to provide the inocula used to inoculate the ground chicken breast meat samples prior to heat treatment

[d] Correlation coefficients

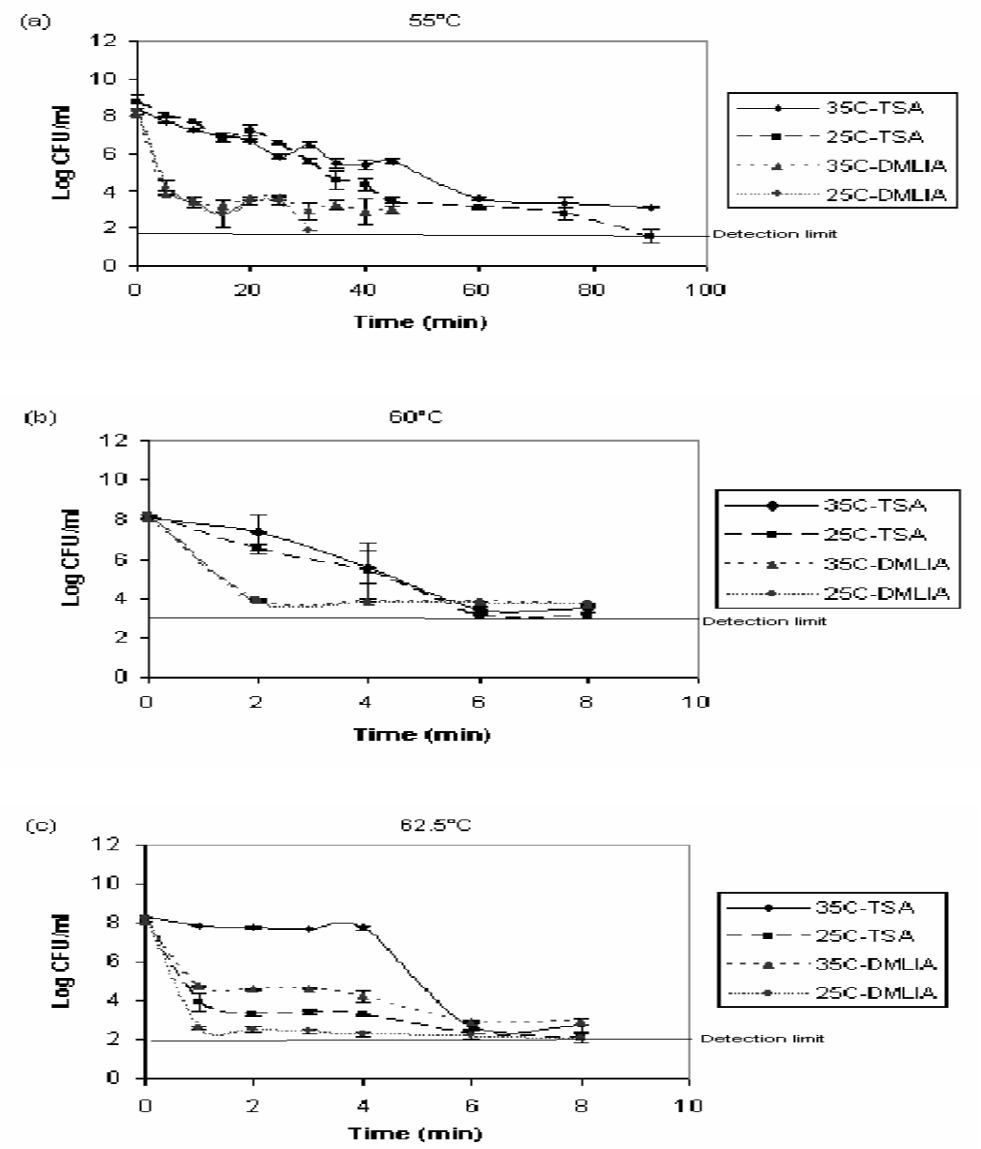

Figure 1. Thermal injury of *Salmonella* Enteritidis cells, grown at 25 and 35ºC and enumerated onto tryptic soy agar (TSA) and double modified lysine iron agar (DMLIA), after heat treatment at: (a) 55.0ºC; (b) 60.0ºC; and (c) 62.5ºC. The lag times were as follows: 2.50 min at 55ºC; 2.15 min at 60ºC; and 1.50 min at 62.5ºC.

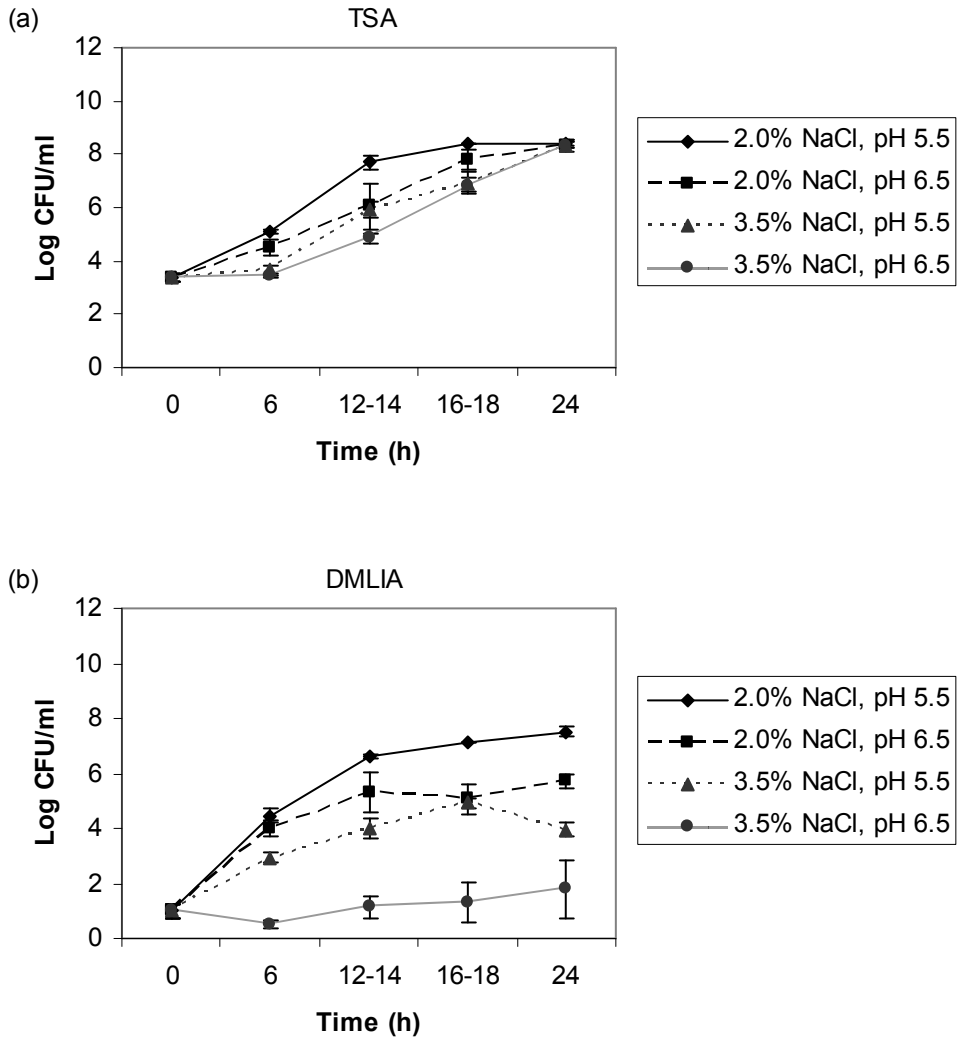

Figure 2. Recovery of *Salmonella* Enteritidis cells grown at 25ºC, injured in ground chicken at 55.0ºC, recovered in tryptic soy broth (TSB) with various NaCl concentrations and pH levels, and enumerated onto (a) tryptic soy agar (TSA) and (b) double modified lysine iron agar (DMLIA)

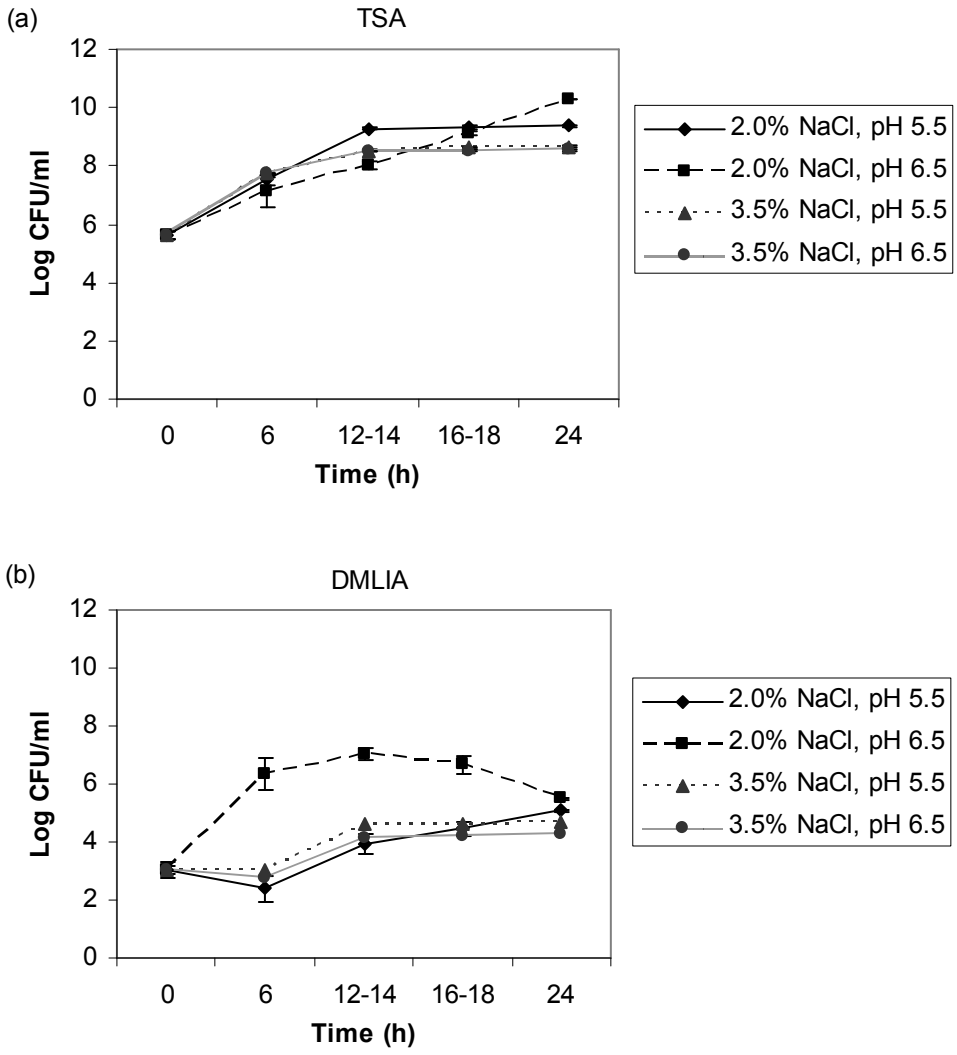

Figure 3. Recovery of *Salmonella* Enteritidis cells grown at 35ºC, injured in ground chicken at 55.0ºC, recovered in tryptic soy broth (TSB) with various NaCl concentrations and pH levels, and enumerated onto (a) tryptic soy agar (TSA) and (b) double modified lysine iron agar (DMLIA)

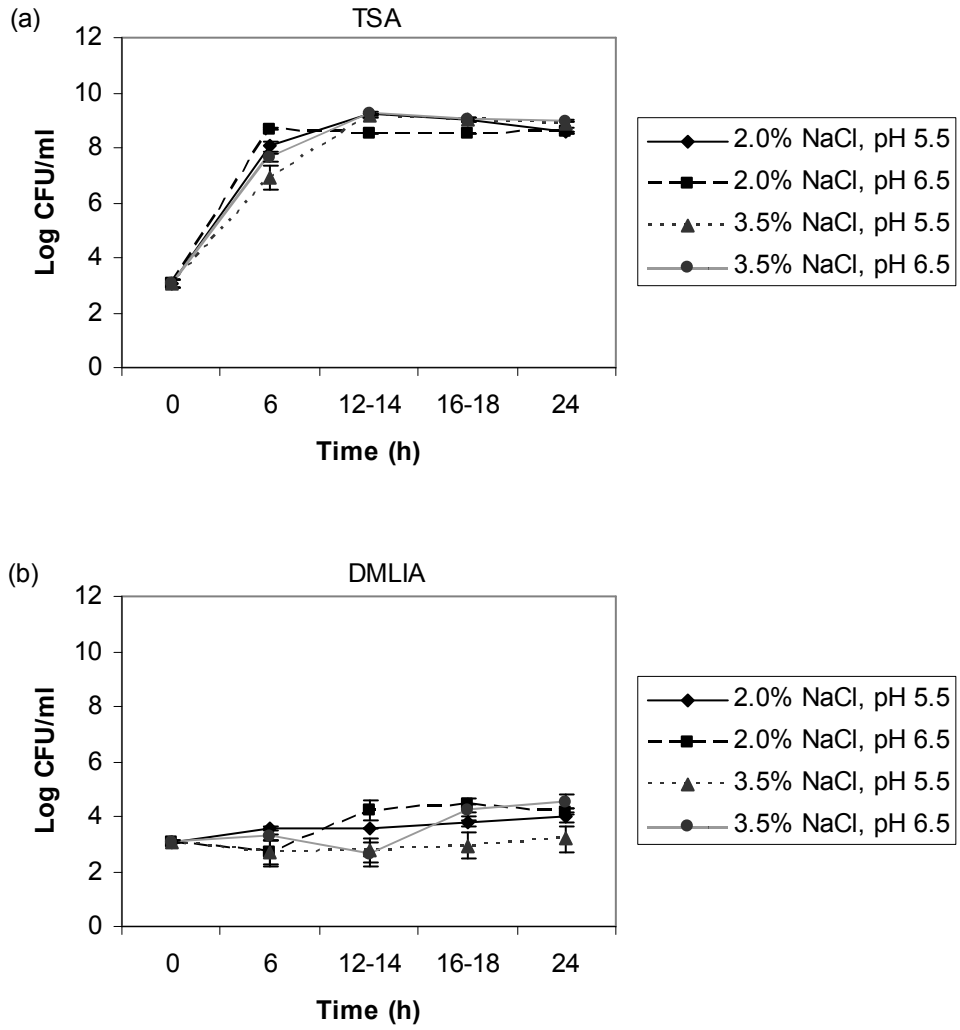

Figure 4. Recovery of *Salmonella* Enteritidis cells grown at 25ºC, injured in ground chicken at 60.0ºC, recovered in tryptic soy broth (TSB) with various NaCl concentrations and pH levels, and enumerated onto (a) tryptic soy agar (TSA) and (b) double modified lysine iron agar (DMLIA)

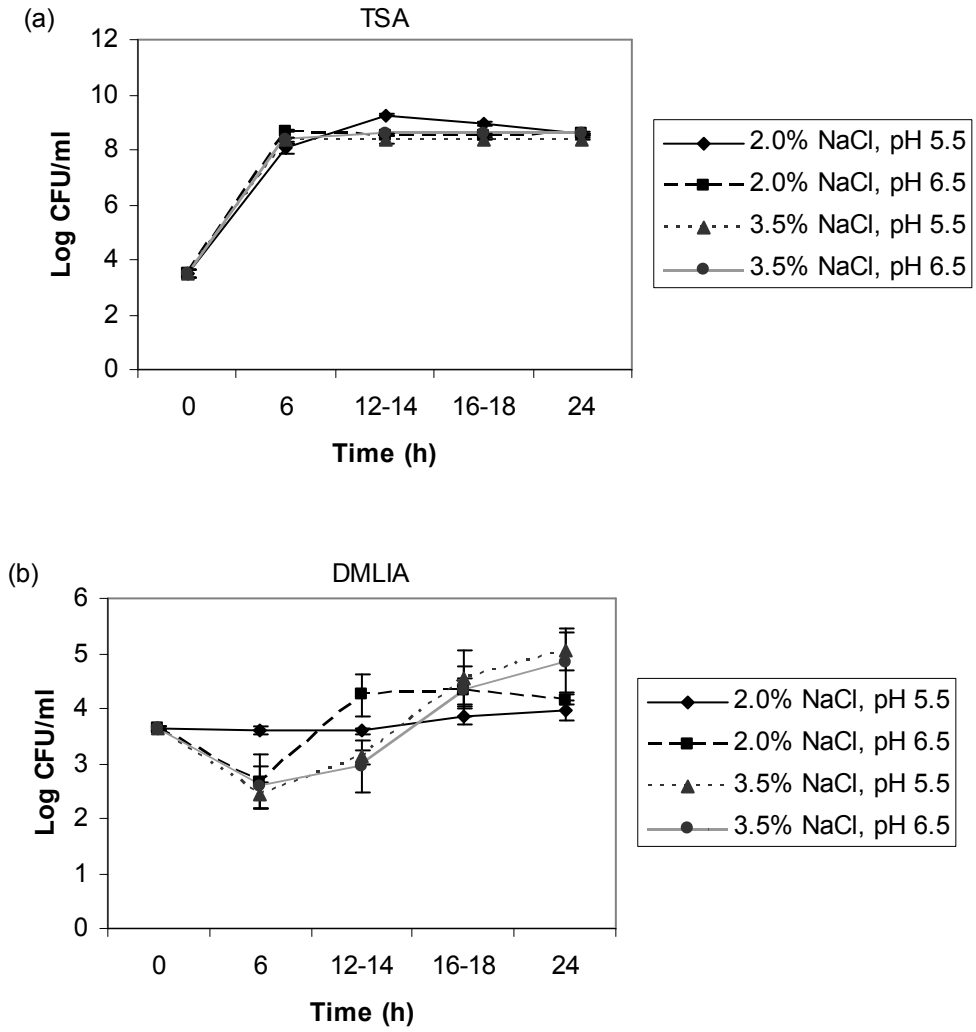

Figure 5. Recovery of *Salmonella* Enteritidis cells grown at 35ºC, injured in ground chicken at 60.0ºC, recovered in tryptic soy broth (TSB) with various NaCl concentrations and pH levels, and enumerated onto (a) tryptic soy agar (TSA) and (b) double modified lysine iron agar (DMLIA)

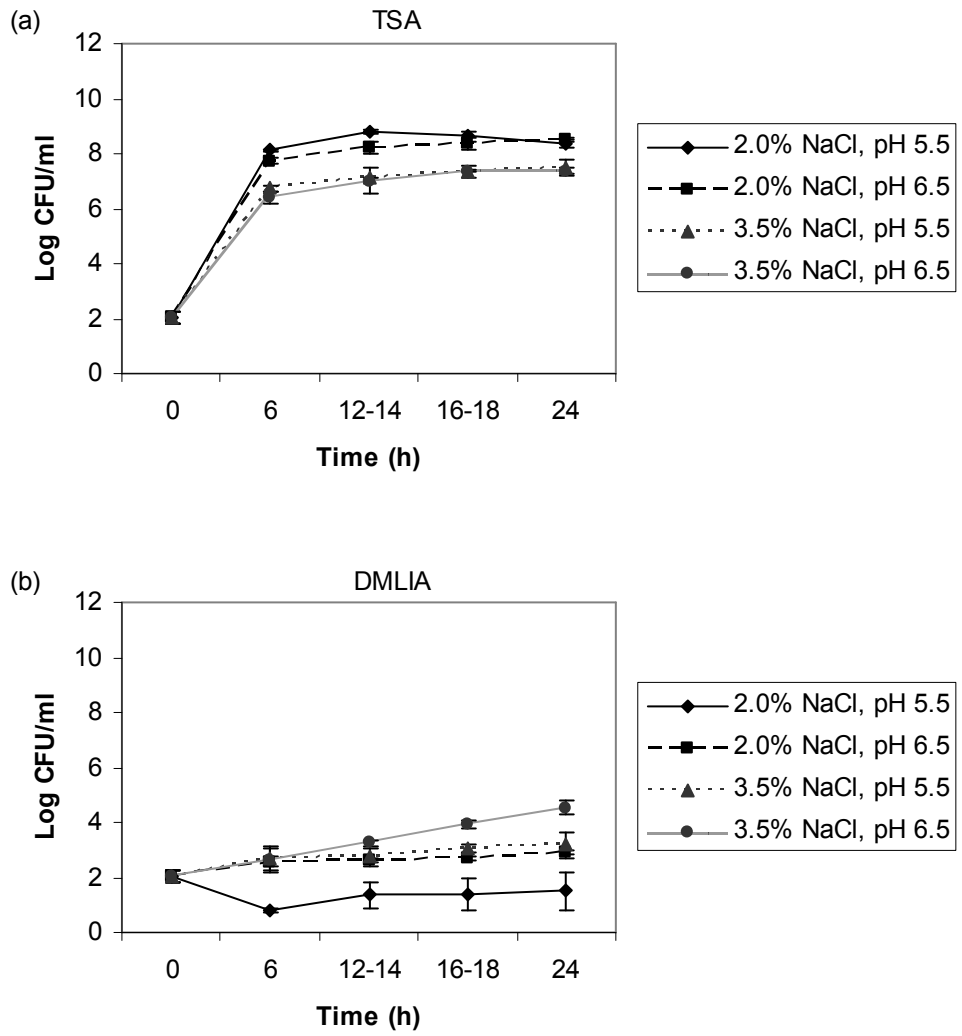

Figure 6. Recovery of *Salmonella* Enteritidis cells grown at 25ºC, injured in ground chicken at 62.5ºC, recovered in tryptic soy broth (TSB) with various NaCl concentrations and pH levels, and enumerated onto (a) tryptic soy agar (TSA) and (b) double modified lysine iron agar (DMLIA)

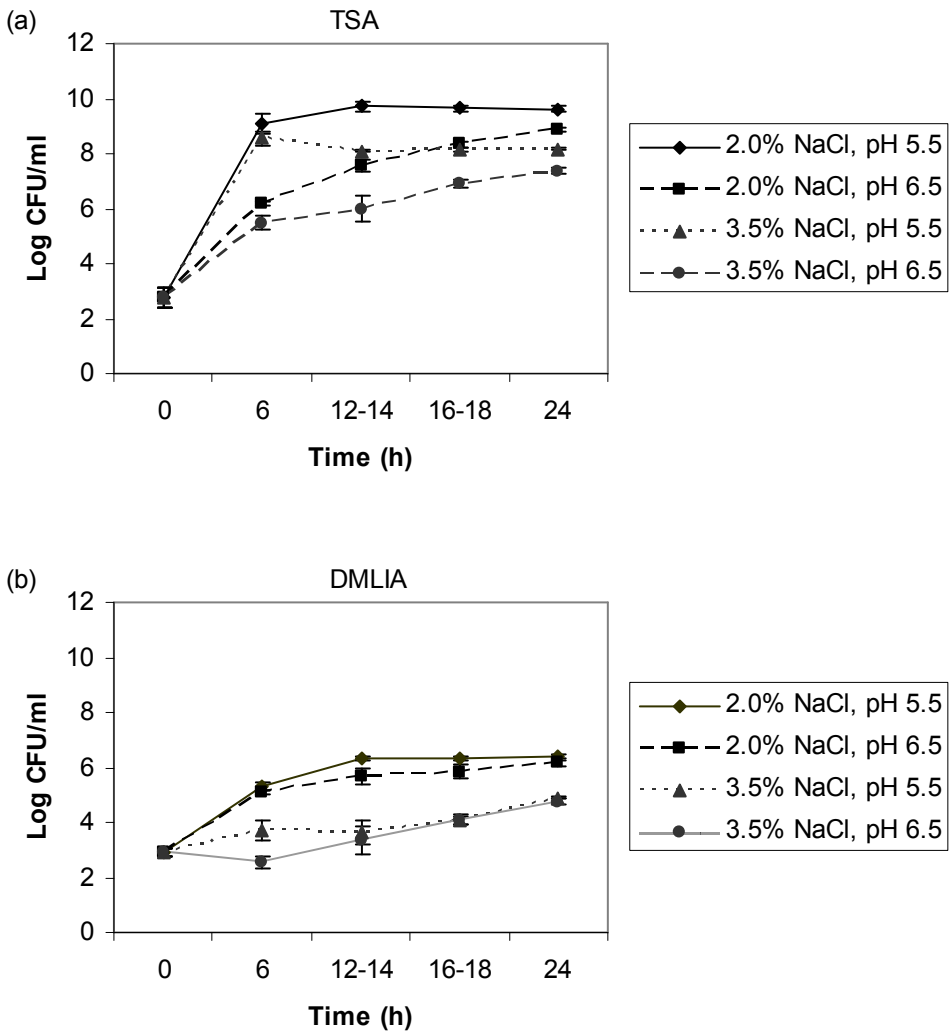

Figure 7. Recovery of *S.* Enteritidis cells grown at 35ºC, injured in ground chicken at 62.5ºC, recovered in tryptic soy broth (TSB) with various NaCl concentrations and pH levels, and enumerated onto (a) tryptic soy agar (TSA) and (b) double modified lysine iron agar (DMLIA)