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Impacts of Temperature and Salt Concentrations for Thermal Inactivation of Salmonella in Moisture Enhanced Reconstructed Chicken Patties

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Impacts of Temperature and Salt Concentrations for Thermal Inactivation of Salmonella in
Moisture Enhanced Reconstructed Chicken Patties

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at West Virginia University

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Masters of Science in
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ABSTRACT

Impacts of Temperature and Salt Concentrations for Thermal Inactivation of Salmonella in Moisture Enhanced Reconstructed Chicken Patties

Alik Browning

The purpose of this experiment is to determine the thermal kinetic parameters of Salmonella in moisture-enhanced, reconstructed ground chicken patties as affected by temperatures and salt concentrations. Salmonella is responsible for 35% of the foodborne illnesses associated with poultry products (Batz et al., 2011). Nonintact reconstructed chicken meat is mixed with brine solutions containing various salt and polyphosphate concentrations to increase water-holding capacity, decrease cooking losses, improve sensory tasting scores, and maintain the good quality of completed chicken products (Gill et al., 2004). Increasing salt concentrations within meats can increase the thermal inactivation of pathogens. In this study, Salmonella Typhimurium American Type Culture Collection (ATCC) 14028 was used, which is the same strain used in our previous validation studies of antimicrobials on broiler carcasses (Lemonakis et al., 2017). Survival curves for 62°C of 0 and 1% salt samples indicate more heat susceptibility than 3 and 5% with roughly a 1.40 to 1.53 log CFU/g difference. For 66°C, survival curves of 1 and 3% salt samples indicate slightly more heat susceptibility than 0 and 5%, with a 1.21 to 1.66 log CFU/g difference. For 70°C, survival curves of 1 and 3% salt samples indicate more heat susceptibility than seen at other temperatures for 0 and 5%, with 1 and 5% differing from 1 and 3% by 1.20 to 1.23 log CFU/g. For 74°C, heat susceptibility for all salt concentrations (0,1.0,3.0,5.0%) is similar, with 1% and 3% being 0.64 to 0.99 log CFU/g different from 0% and 5%. Based on the results, it can be concluded that the thermal resistance of Salmonella is significantly affected by both temperature and salt concentration. There is evidence to suggest that 0% has the highest amount of resistance among the other concentrations, 1% is the next highest, while both 3 and 5% showed lower, but almost equal z-values. Suggesting that salt concentration above 3% will show a similar or less thermal resistance, and concentrations of 0 and 1% will have equal or more thermal resistance.

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IMPACTS OF TEMPERATURE AND SALT CONCENTRATIONS FOR THERMAL INACTIVATION OF SALMONELLA IN MOISTURE-ENHANCED RECONSTRUCTED CHICKEN PATTIES

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IV. INTRODUCTION

Salmonella is a gram-negative, rod-shaped, non-endospore forming, facultative foodborne pathogen, and the number one pathogen that caused outbreaks for chicken products based on the epidemiology surveillance data in December 2020 (U.S.-CDC, 2020). Salmonella is responsible for 35% of the foodborne illnesses associated with poultry products (Batz et al., 2011). The United States Department of Agriculture-Food Safety and Inspection Service established a new performance standard in 2016 (USDA-FSIS, 2016) that allowed the maximum acceptable positive rate of Salmonella up to 25% in comminuted chicken (325 g sample) and up to 15.4% in chicken parts (4 lb. sample).

Raw chicken carcasses are usually further processed using binding meat pieces with salt and/or phosphate and then followed by grinding for further manufacturing into ground chicken patties, steaks, and roasts. Nonintact reconstructed chicken meat is mixed with brine solutions containing various salt and polyphosphate concentrations to increase water-holding capacity, decrease cooking losses, improve sensory tasting scores, and maintain the good quality of completed chicken products (Gill et al., 2004). A recent sampling showed that Salmonella is present in roughly 36.7 to 83.5% in comminuted chicken products, which indicates a 1.5 to 2.1-fold increase in Salmonella prevalence than other products such as bone-in chicken parts (USDA-FSIS, 2015). The heat generated during processing and possibly translocation of foodborne pathogens from the surface to internal tissues during restructuring could increase the microbial safety risk (Shen et al, 2010).

As recommended by the previous publications, raw chicken is required to cook to an internal target temperature of 74°C for producing a 7-log reduction of Salmonella (NACMCF, 2007). However, studies evaluating chicken breast filets and thermal inactivation observed unexpected heat resistance to Salmonella (WHO, 2010). Factors affecting bacterial heat resistance include the presence of chemical ingredients, product size, cooking method, water activity, fat content, and product pH and salt concentrations (WHO, 2010). Furthermore, Salmonella may survive during the cooking of comminuted chicken manufactured products and be protected by the high salt ingredients, which may cause subsequent illness in consumers, especially if the salt concentrations interfere with the thermal inactivation of the pathogens. The lack of quantitative data relating the impact of salt concentrations on the reduction of Salmonella in nonintact chicken products remains a large, unaddressed problem in food safety guidelines.

Salt has long been used as a preservation technique to maintain freshness and food quality. Salt concentration within foods have led to pathogens being more resistant to higher temperatures for prolonged periods of time. Increasing salt concentrations within meats can increase the thermal inactivation of pathogens. Thermal inactivation is the process of finding the lowest temperature necessary to completely inactivate bacteria.

The purpose of this experiment is to determine the thermal kinetic parameters of Salmonella in moisture-enhanced, reconstructed ground chicken patties as affected by temperatures and salt concentrations.

V. LITERATURE REVIEW

I. Foodborne Illness

Foodborne pathogens are responsible for 600 million outbreaks per year worldwide and contribute to 420,000 deaths annually (World Health Organization, 2021). According to the CDC, each year every 1 in 6 Americans become ill due to foodborne illness and roughly 3,000 die annually (CDC, 2022). Foodborne outbreaks are common, few individuals know how to limit their interactions with these pathogens. Although everyone is at risk of getting a foodborne illness, some individuals are considered to be at higher risk of experiencing serious illness and even death. According to the USDA, individuals considered to be at a higher risk are people with auto-immune disorders, older adults, pregnant women, fetuses, young children, and infants (USDA, 2020). Microorganisms are present in foods before purchase, sometimes due to the pre-harvest and pre-slaughter conditions of the foods being purchased. Not all microorganisms cause disease in humans, and only contamination of certain microorganisms can cause foodborne illness. Foods, regardless of preparation technique, can be cross-contaminated with pathogens due to poor personal hygiene, seafood products, liquids excreted from other raw products, raw meat, raw egg products, and simply touching other contaminated foods. One of the most common ways that consumers fail to prevent contamination is through improper heating of foods to kill bacteria. Cooking and preparation techniques in the kitchen are essential for limiting contact with common foodborne pathogens. Bacteria grow or rapidly replicate, in temperatures of 40 to 140 (USDA, 2020). Consumers are recommended to cook raw steak, pork chops, and roast to an internal temperature of 145, measured with a food thermometer, to kill bacteria on the surfaces of the meat. Specific meats like ground beef, pork, lamb, and veal are recommended to be cooked at 160, whereas chicken products require temperatures of at least 165 to ensure the eradication of microorganisms present.

II. Foodborne Bacteria

Various types of disease-causing germs can contaminate foods. According to the CDC, researchers have discovered and identified more than 250 types of foodborne illness causing bacteria (CDC, 2020). But not all foodborne illness is caused by consuming the bacteria itself, it can be caused by ingesting harmful toxins produced by specific types of bacteria. Specific types of bacteria that infect people through toxins and chemicals are *Bacillus Cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli* O157:H7, Hepatitis A, *Listeria monocytogenes*, Norwalk virus, 30 types of *Shigella*, over 2,300 serotypes of *Salmonella*, *Staphylococcus aureus*, and numerous others (Government of the District of Columbia, n.d.). The CDC labels the most common foodborne germs as Norovirus, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, and *Staphylococcus aureus* (CDC, 2020). Certain types of foods are known for their high levels of contamination in comparison to others. Raw foods of animal origin are considered to be the most likely to be contaminated, in comparison to fruits and vegetables. Raw foods like raw or undercooked meat, lightly cooked eggs, unpasteurized milk, and raw shellfish have been found to contain higher amounts of foodborne pathogens (CDC, 2022). Poultry meat has been found to cause the greatest amounts of foodborne illnesses, including poultry meat and eggs. Raw poultry meat is known for being contaminated with *Campylobacter*, along with other common pathogens like *Salmonella*, *Clostridium perfringens*, *Escherichia coli*, and many others (CDC, 2022). Fruits and vegetables are also contributors to food poisoning through pre and post-harvesting practices. They are often contaminated through cross-contamination with harmful germs like *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes*. Dairy products like raw milk, raw milk soft cheeses, and other types of raw milk products have been known to contain *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella*.

III. *Salmonella* Typhimurium

Salmonella enterica serotype Typhi is a common foodborne pathogen known for causing typhoid fever in humans. *Salmonella* is a gram-negative, rod-shaped, flagellated bacterium that is specific to causing infection in people (Ashurst, et al. 2021). At the time of *Salmonella*'s discovery in 1829, Pierre Louis decided to coin the term "typhoid fever," after discovering lesions in the abdominal lymph nodes of patients who had died from what had been called "gastric fever" (Ashurst, et al. 2021). *Salmonella enterica* serotype typhi is contracted through ingesting food or water that has been contaminated by an organism that is sick.

An infectious dose of *Salmonella typhi* is considered to be ingesting anywhere from 1,000 to 1 million organisms or cells but can also depend on the host's immune system (Ashurst, et al. 2021). The pathogen enters the submucosal region of the small intestine by inserting itself into the cystic fibrosis transmembrane conductance regulator or through the M cell (Ashurst, et al. 2021). Once it is attached to the membrane, it causes hypertrophy of the Peyer's patches. People infected with *Salmonella typhi* will present symptoms after a 7 to 14-day asymptomatic period after the initial infection. After the asymptomatic period, people will show flu-like symptoms

followed by a fever, but abdominal symptoms will progressively get worse leading to nausea, vomiting, cramps, constipation, and diarrhea (Ashurst, et al. 2021). Delusion or confusion will follow other symptoms and progressively escalate.

Salmonella typhimurium is known to contaminate a variety of foods including chicken, turkey, beef, pork, eggs, fruits, vegetables, and processed foods (CDC, 2022). But food is not the only way of contamination, Salmonella can be spread through contaminated water from the environment, animals (domestic and non-domestic), and people. Areas often overflowing with people of all ages like zoos, petting farms, fairs, schools, and even daycares have been known to carry Salmonella along with many other harmful germs (CDC, 2022). Salmonella alone causes more illnesses than any other bacteria, according to the CDC (CDC, 2022). Raw chicken is a major source of illness that people come in contact with. Roughly, every 1 in 25 packages of chicken from local and large-scale grocery stores is contaminated with Salmonella (CDC, 2022). Illness from chicken is usually due to undercooking the meat, but sometimes sickness can be caused by juices leaking onto surfaces that will be transferred to other foods later.

IV. Poultry Meat Industry

In the United States, the poultry industry is considered the world's largest producer and second-largest exporter of poultry and eggs (USDA, 2022). Consumption of poultry meat such as broilers, chicken, and turkey, is considered to be the highest in comparison to beef, pork, and other red meats than anywhere else in the world (USDA, 2022). The United States alone is considered to export nearly 18% of total poultry produced to other countries while exportation relies heavily on an influx in currency and other economic growths due to importing (USDA, 2022). According to the USDA, Foreign Agricultural Service, the global poultry meat production for 2022 is projected to output nearly 100.8 million tons, while exports increased by 1% to 13.4 million tons (USCB, 2022).

V. Introduction to Poultry Meat Processing

Since World War II, the United States poultry industry has quadrupled and has developed highly efficient forms of production systems (Paul & Regenstein, 2018). Poultry processing is considered the preparation of meat from various types of fowl for consumption by humans, according to Paul and Regenstein. Poultry is a desirable food for consumers for various reasons but the most sought-after is the amount of consumable animal protein. Due to consumers, chicken and turkey are considered to be the most common types of poultry, however, there are various types of poultry meats being produced like ducks, geese, pigeons, quails, etc. (Paul & Regenstein, 2018).

The poultry meat processing begins with the classification of birds. Birds for poultry production are grown for a select amount of time until they reach the desired weight for slaughter. Certain breeds of birds can be considered full-grown within 5 to 6 weeks of age, like Rock Cornish hens

(Paul & Regenstein, 2018). But other common types of birds like broilers or fryers are considered to be full-grown and ready for consumption at close to 7 weeks of age (Paul & Regenstein, 2018).

When birds have reached the desired weight and age, also known as "harvesting time", slaughtering procedures begin. The first step in the slaughtering process is the birds are no longer given food or water for their digestive tracts to empty, this allows for lower chances of contamination with harmful bacteria (Paul & Regenstein, 2018). Then the desired birds are caught and put into plastic wooden transport cages prepared for the slaughterhouse. They are then transported in trucks containing sets of fans to keep constant ventilation of the cages. Once received by the slaughter facility, they are removed from the cages and placed in continuously moving shackles that restrain both of their legs (Paul & Regenstein, 2018). This transfer of the birds is commonly conducted in a dark space only containing a red light to keep the birds calm. Stress caused by the slaughter process can have negative effects on the final quality of the meat so keeping the birds calm is a priority (Paul & Regenstein, 2018).

Slaughtering begins shortly after to maintain the integrity of the meat. The birds are then stunned by putting their heads through a water bath that conducts an electric current (Paul & Regenstein, 2018). The stunning process puts the birds in an unconscious state and then they are killed by hand or with a machine that contains a rotary knife specifically to cut the jugular veins and carotid arteries (Paul & Regenstein, 2018). After the initial killing, birds are required to bleed for a fixed amount of time depending on their species and size, for example, it is normally 1 and a half minutes for broilers (Paul & Regenstein, 2018). The birds are then sent through scalding tanks that contain hot water, this allows the skin to soften and makes defeathering easier. The temperature of the water bath is closely monitored for desired skin colors. If retention of yellow skin is desired then the water bath is set to 50C or 122F to ensure a soft scald (Paul & Regenstein, 2018). If a white bird is desired then hotter temperatures will be achieved, temperatures reaching scalding, which removes the yellow pellicle (Paul & Regenstein, 2018). Once the desired scalding has been achieved, the defeathering of the birds begins. The carcasses will go through a feather-plucking machine that is equipped with rubber "finger-like" appendages that are used to beat the feathers off (Paul & Regenstein, 2018). The birds will go through a set of machines each specifically optimized at removing different sets of feathers, and then they are sent through a machine to singe off any remaining feathers. The feathers and blood are not disposed of but are carefully collected to be used in blood meal, feather meal, and used for down-stream processing (Paul & Regenstein, 2018). Next, the heads and legs of the carcasses must be removed. The heads of the birds are sent through a channel where they are pulled off by specialized machinery, and the legs are removed with a rotary knife. Lastly, the carcasses are hung by their hock and are prepared for evisceration on the eviscerating shackle line (Paul & Regenstein, 2018).

The next steps within the process involve eviscerating and inspecting the bird carcasses. The oil glands within the carcasses, specifically found at the tail, are removed, and the vents are opened

to remove the remaining organs (Paul & Regenstein, 2018). The evisceration process can be done by hand or with specialized machinery that is capable of processing roughly 70 birds per minute (Paul & Regenstein, 2018). To keep contamination low, the machines are cleaned with a chlorine solution after every bird (Paul & Regenstein, 2018). After, the inspection begins. Inspection procedures vary from country to country, but in the United States, all carcasses are evaluated by inspectors from the USDA. Upon inspection, parts of the birds that are rejected are thrown into containers labeling them as "inedibles" where they will later be dyed to prevent mixing with edible parts (Paul & Regenstein, 2018).

Following the inspection process, the carcasses are cleaned further, the viscera along with the edible offal are removed and sent to their respective locations depending on edibility (Paul & Regenstein, 2018). Internal organs such as the heart, stomach, and liver are considered to be edible and will be processed separately (Paul & Regenstein, 2018). The lungs and kidneys are removed separately with a vacuum pipe. Then the final inspection process can begin and this is followed by a thorough washing of the carcasses (Paul & Regenstein, 2018).

After being washed thoroughly, the carcasses are chilled below 4C or 40F using water chilling and air chilling (Paul & Regenstein, 2018). Water chilling is a process commonly used in North America that involves a countercurrent flow of chilled water that is intended to lower the temperature of the carcasses for chilling. The carcasses are then transported into a chiller, which is considered a large tank that is designed to move the carcasses through in a specific amount of time (Paul & Regenstein, 2018). Two chillers are used in conjunction with one another to minimize the risk of cross-contamination. The chilling process is used to limit the chances of bacterial cross-contamination by heavily diluting them in the tanks to prevent recontamination.

Lastly, raw poultry products are sent to be packaged for sale. Fresh poultry is then cut or sliced accordingly and placed on foam trays that are covered with a plastic film (Paul & Regenstein, 2018). The meat rests on top of an absorbent pad used to catch liquids that will be released from the meat. It is recommended that fresh poultry be used within 14 to 21 days post-slaughter and should not be stored in the refrigerator for more than 3 days (Paul & Regenstein, 2018).

VI. Food Safety Practices Used In The Poultry Meat Industry

Food contamination does not begin solely within the kitchen of the common consumer, but oftentimes it begins during processing. Cross-contamination poses a serious threat to the well-being of consumers along with the company's ability to prevent foodborne contamination within the facility (Food Safety Audits, 2011). As a way to keep contamination as low as possible, government agencies like the USDA and FDA conduct food safety audits (Food Safety Audits, 2011). Food safety audits are a way to conduct quality control while evaluating the processing practices and technologies of the plants producing and processing (Food Safety Audits, 2011). Plants can complete audits themselves to help maintain process control but they can also use it as a means of marketing tool. According to Ollinger et al., at least 90% of poultry and cattle

slaughter along with ready-to-eat food products produced comes from audited plants (Ollinger et al., 2011). They also state that more than one-half of all plants focused on poultry slaughter production were audited (Ollinger et al., 2011). This study also found that plants that hire customers or plant-hired auditors have been found to have higher levels of food safety protocol in comparison to plants that do not hire auditors (Ollinger et al., 2011). Larger plants along with plants owned by multiplant firms were found to have higher levels of food safety technology being incorporated in comparison to other plants being evaluated (Ollinger et al., 2011).

Although the poultry industry focuses heavily on maintaining the cleanliness and safety of their plants, not all microorganisms are detected throughout the process of conducting audits. The poultry industry emphasizes reducing infection or contamination within the plants themselves but also through pre-harvest techniques used by small corporations.

VII. Introduction to Antimicrobial and Antibiotic Resistance

Antimicrobial resistance is considered a global public health issue within the United States. Various types of antibiotics and antimicrobial products have been used widely across the United States poultry industry for years to prevent contamination of potential outbreaks. Nearly 80% of all antimicrobials produced in the US are applied to animal production (Tabler et al., 2021). Extensive use of antimicrobials within the animal food production industry can lead to microbial resistance. Antibiotic resistance occurs due to the overuse of antimicrobials that are meant to prevent disease and treat infections. The poultry industry is well aware of the issue of antimicrobial resistance and conducts ongoing research to target safe and effective alternatives for preventing the growth of microorganisms. The FDA has reported that in the past 25 years there has been a significant decrease in the amount of antibiotic use by the poultry industry (Tabler et al., 2021). According to the USDA, 20 to 52% of broiler companies used antibiotics for production purposes instead of disease control, but similar reports found a long-term decline in the overall amount of antibiotic use within broiler production (USDA, 2011).

In a more recent study conducted by the FDA, it was found that antimicrobial use intended for food-producing animals was down by 33% between the years 2016 and 2017 (FDA, 2017). The greatest reason for the recent decline in antibiotic use is due to the demand by consumers for NAE ("no antibiotics ever") poultry products. NAE systems accounted for nearly 58% of all US production in 2019, bringing a seven-point increase from the previous year (FDA, 2019). The demands have persisted regardless of the increase in price due to NAE products and the majority of consumers are not motivated to pay the cost of products that do not contain antibiotics. Broiler industries have decided to meet consumer demands and have been making efforts to reduce antibiotics and become NAE producers. Consumers growing interested in understanding the process of sustainable food production and caring about how their food is raised, this has made processors more motivated to meet consumer needs.

VIII. Applications of Antimicrobial and Antibiotic Resistance in Real-World Situations

A large concern for growers, researchers, and the poultry industry is the idea of poultry litter management. Poultry litter is considered to be bedding material such as shavings, sawdust, and rice hulls, combined with manure, wasted feed, and a collection of feathers (Tabler et al., 2021). Poultry litter management is a growing concern because 20,000 broilers will produce roughly 150 tons of litter per year (Tabler et al., 2021). Areas of large poultry production are produced in small geographic areas causing large volumes of litter to be produced, causing a possible source of antibiotic and antimicrobial resistance (Tabler et al., 2021). Roughly 14 million tons of poultry litter is produced annually by US broilers (Tabler et al., 2021). Soil containing poultry litter is considered to be a nonpoint source for antibiotics because the antibiotics that enter the surface are transferred to ground waters through runoff and leaching (Tabler et al., 2021). Nearly 30 to 80% of veterinary-administered antibiotics are excreted through manure and urine, therefore being placed back into the soil (Tabler et al., 2021). This leads to the growing concern that poultry litter will transport antibiotic and antimicrobial-resistant microorganisms.

IX. Implementing Antibiotic Resistance in Foodborne Bacteria

A common type of antibiotic used in bacteria is Nalidixic acid, it is a synthetic quinolone that is an antibacterial agent that is used to treat lower urinary tract infections due to gram-negative bacteria (NCBI, 2022). Bacteria that are affected by Nalidixic acid are the majority of E. Coli, Enterobacter, Klebsiella, and Proteus species (NCBI, 2022). Adding this antibiotic to the medium before incubation ensures that no other living pathogen will be able to survive inside the media, isolating your desired pathogen for testing. Allowing bacteria to incubate while exposed to the antibiotic, Nalidixic acid, causes the bacteria to then be antibiotic resistant, therefore allowing counting respective colonies to be free of cross-contamination (NCBI, 2022).

X. Sodium Chloride

Sodium chloride is considered to be an essential nutrient that is used in modern healthcare, manufacturing plastics, environmental efforts to maintain safe driving conditions, cooking, and preservation techniques (American Chemistry Council, 2022). Within the medical and health field, sodium chloride is used in intravenous sodium chloride solutions to be able to give patients water and stop any dehydration that may have been an issue (American Chemistry Council, 2022). Sodium chloride is essential when maintaining the balance of electrolytes in fluids that a person consumes (American Chemistry Council, 2022). During manufacturing, large amounts of sodium chloride are used to make numerous products like plastics, papers, soaps, common bleach, chlorine, rubber, glass, and even detergents (American Chemistry Council, 2022). In environmental circumstances, sodium chloride is used on roadways and sidewalks to alleviate issues of ice on transport surfaces (American Chemistry Council, 2022). The salt used for roadways and sidewalks is in the form of rock salt, which is the same type of salt consumed in everyday meals (American Chemistry Council, 2022). In terms of preservation and cooking, sodium chloride has been used for thousands of years for adding flavor and preserve the shelf-

life of foods (American Chemistry Council, 2022). As a preservative, it is commonly used to prevent the growth of spoilage bacteria, preventing spoilage, and helps keep "ready-to-eat foods" safe for longer periods (American Chemistry Council, 2022).

XI. Salt Concentrations in Foods

Sodium has many uses within the food industry including preservation, thickening, helping retain moisture, and taste, along with curing various types of meats. Numerous types of salt, or sodium, are used within foods; for example, sodium bicarbonate, sodium nitrate, monosodium glutamate, and sodium benzoate. Although these types of sodium are included in foods they are not represented by the total amount of sodium within food on the nutrition facts on food labels. Even though certain foods may not taste salty, they may contain high levels of sodium which has significant health risks. Foods that do not taste salty but contain high levels of salt include cereals, sweets, and various types of bread (FDA, 2022).

High levels of sodium have been found to cause serious health conditions, such as an increase in high blood pressure that can cause hypertension. Naturally, salt attracts water so a high sodium diet will pull water from the bloodstream, causing an increased volume in blood and raising blood pressure (FDA, 2022). Hypertension is classified as a condition that causes the overall blood pressure to remain elevated over a prolonged period, causing strain on the heart along with other substantial organs like the kidneys, brain, and eyes (FDA, 2022). Blood pressure has been found to increase with age so limiting sodium intake to a healthy amount is crucial for health. According to the FDA, roughly 40% of Americans consume sodium through foods like deli meats, pizza, burritos, tacos, various types of snacks, poultry products, burgers, eggs, and many more (FDA, 2022). Even though restaurant foods are a high source of sodium for people, Americans consume nearly 70% of their daily sodium intake from processed foods and restaurant foods (CDC, 2021).

Recommended amounts of salt vary heavily depending on the individual and pre-existing health conditions. It is recommended to consume less than 2,300 milligrams of salt a day for people above the age of 14 (The Nutrition Source, 2021). Unprocessed foods like fruits, vegetables, whole grains, meats, dairy, and even nuts are known to contain low amounts of sodium (The Nutrition Source, 2021). Salt does not only affect people, it also changes the chemical composition of food. By adding salt to food, it triggers a chemical process known as osmosis which causes the flow of water to migrate from an area of low concentration to an area of high salt concentration (Quimica das Coisas, 2011). Ultimately, salt will remove water from food, therefore inhibiting the growth of microorganisms. The microorganisms that are in foods that are affected by salt concentrations are the bacteria that cause food to decompose and produce toxins, like foodborne pathogens. These types of bacteria cannot survive when exposed to high osmotic pressure, the result of osmosis (Quimica das Coisas, 2011). This explains why salt has been used for decades as a preservation technique for extending the shelf life of certain foods.

XII. Introduction to the Effects of Salt on Bacteria

For decades salt has been used as a preservation technique, minimizing spoilage and extending the shelf life of common foods. Salt has been added to inhibit the growth and survival of microorganisms that contribute to spoilage. Although common technology and recent packing protocols have limited the growth and survival of microorganisms, salt remains a cost-effective means of limiting microorganisms in foods while extending the shelf life. Salt does not inhibit the growth of all microorganisms but eradicates the issues of undesirable pathogens while allowing desired microorganisms to flourish. Various types of bacteria are used in the fermentation process and these types of bacteria can grow and preserve meats while enhancing their flavor.

In the modern food safety industry, the multiple hurdles concept is used as a means of controlling microbial growth while increasing overall shelf stability. Hurdles are considered to be types of prevention that can be used in combination with one another. Examples of hurdles include temperature during processing, storage conditions, pH, redox potential, along with other additives (Institute of Medicine, 2010). No one hurdle has been shown to make a product considered "safe" but when used in combination a desirable and safe product can be achieved. Salt is considered to be one of the many additives that can be used as a hurdle for food safety purposes.

Salt limits the water activity within foods and therefore limits the water available to microorganisms to use when growing and causing chemical reactions. The reason salt can decrease water activity is because of the sodium and chloride ions associate with water molecules in foods (Institute of Medicine, 2010). Another result of adding salt to food is an osmotic shock. Osmotic shock is when the water from the cell is removed causing death or retarded growth (Institute of Medicine, 2010). Research has also shown that salt can limit oxygen solubility along with interfering with cellular enzymes and even force cells to use energy to remove salt from the cells (Institute of Medicine, 2010).

XIII. Sodium Tripolyphosphate

Sodium tripolyphosphate, also known as sodium triphosphate, has many uses inside and outside of the food industry (Independent Chemical Co., 2019). It is characterized as a colorless salt that is often used in food preservation and as a water softener for commercial detergents (Independent Chemical Co., 2019). In cleaning products, it is used to improve the overall ability of common detergents to penetrate the internal fibers of clothes and other materials, leading to a better wash (Independent Chemical Co., 2019). It also helps with foaming capabilities along with its ability to be a pH buffer so therefore it doubles as a water softener (Independent Chemical Co., 2019). As a food additive, it is known for helping retain moisture, along with preserving the natural color of meat and fish while also improving the overall texture of the meats (Independent Chemical Co., 2019). It is also recognized as an emulsifying agent to help prevent meats from

becoming greasy and breaking apart during the cooking process (Independent Chemical Co., 2019). Mostly, sodium tripolyphosphate is used just in the meat and dairy processing industry (Independent Chemical Co., 2019).

IXX. Thermal Inactivation

XX. Introduction to Thermal Inactivation

Thermal inactivation is defined as finding the lowest temperature necessary to completely inactivate a pathogen. Thermal inactivation processes include temperature and determining the amount of time required at a specific temperature before the pathogen is inactivated. Thermal inactivation uses heat processing techniques to ensure sterilization. Naturally, bacteria thrive in warm temperatures specifically ranging from 40°- 140°F, and this temperature period is referred to as the "danger zone". Naturally, poultry contains Salmonella which can be killed by simply cooking the meat to 165°F or higher. Current standards require a 6-log to 7-log reduction for *Listeria monocytogenes*, a 6-log reduction for Salmonella in poultry, and a 12-log reduction for *Clostridium botulinum* in canned goods (Membraé, et al. 2007). Heat processing is considered to be one of the most important preservation methods even after hundreds of years of use (Smelt & Brul, 2014). Heat processing has aimed to alleviate preserved foods of pathogens and bacteria that can lead to food-borne diseases or spoilage (Smelt & Brul, 2014). Traditional canning methods, which include heat processing techniques, focus on sterilization or commercial sterilization to destroy all spores and bacteria (Smelt & Brul, 2014).

XXI. Heat Resistance

Microorganisms are considered to be far more resistant to dry heat in comparison to wet heat (Smelt & Brul, 2014). Dry heat specifically is relevant in situations like disinfecting devices for surgery, whereas food microbiology mainly focuses on wet heat resistance (Smelt & Brul, 2014). During dry heat treatments, microorganisms are inactivated by oxidation; while in wet heat inactivation, protein denaturation and damage to the membrane play a large role in inactivation (Smelt & Brul, 2014).

Consumer demand for fresh food products has caused milder heat processes to ensure the safety of foods (Smelt & Brul, 2014). This has caused heat resistance of spores from cold-growing spore formers along with their vegetative cells (Smelt & Brul, 2014). Heat resistance begins as early as cultivating the growing cells, the newly growing cells are more sensitive to heat but can be incubated at high temperatures during growth to ensure higher heat resistance and rigidity of the cell membrane (Smelt & Brul, 2014). Also, stationary phase cells are found to be more heat resistant because they are exposed to stressful environmental conditions (Smelt & Brul, 2014). Still, various other factors play a role in creating heat resistance in bacteria; for example, the composition of culture medium pre and post-incubation, the time and temperature of incubation,

pH of the medium, water activity, and the density of suspension after incubation (Hansen & Riemann, 1963).

VI. MATERIALS AND METHODS

Bacteria strains

In this study, *Salmonella* Typhimurium American Type Culture Collection (ATCC) 14028 was used, which is the same strain used in our previous validation studies of antimicrobials on broiler carcasses (Lemonakis et al., 2017). Individual strains of *Salmonella* were stored in the frozen culture at -80°C freezer and activated by streak-plating onto Tryptic soy agar with 100 ppm of nalidixic acid (TSA-NaL) (Hardy Diagnostics, MD, USA) followed by incubating at 35 C for 24 h to obtain the single colonies of *Salmonella*.

Preparation of bacterial inoculum

Two single colonies from the TSA-NaL (*Salmonella*) agars were picked up by a sterilized plastic loop and transferred into 10 ml of TSB-NaL followed by incubating at 35 C for 24 h. The fresh 24 h culture broth was then washed in 0.1% buffered peptone water (BPW, Hardy Diagnostics) by centrifuging for 15 min in a micro-centrifuge (VWR Symphony 4417, VWR International, Radnor, PA, 5,000 × g) and then resuspending in 10 ml of 0.1% BPW. The two 10 ml of *Salmonella* strains were mixed with 100-fold serial dilution in 0.1% BPW to determine the concentration of the inoculum (~8.0 log CFU/ml).

Manufacturing of chicken patties and inoculation and cooking

Frozen boneless chicken breasts were purchased from Young & Stout, Inc., Bridgeport, West Virginia. The frozen chicken meat was thawed overnight at 4 C before the experiment. On the day of the experiment, the thawed meat was manually cut into small slices with knives and distributed into 500 g batches. Fresh 500 g of chicken breast was ground, inoculated with Nalidixic-acid (NaL-200 ppm) resistant *Salmonella* Typhimurium, followed by adding NaCl (0,1.0, 3.0, and 5.0%) plus Na-tripolyphosphate (0.5%) solutions to achieve pump rates of 8%. Cooking of non-intact chicken patties. Samples were then weighed in 10 g portions and added to filtered food bags, vacuum packaged, and stored at 4C for 42 hours before heating. Heating begins in a circulated water bath set at 62, 66, 70, and 74 C from 0-180 seconds respectively. Counts of the pathogenic cells were analyzed on tryptic soy agars plus NaL-200 ppm.

Microbiological analyses

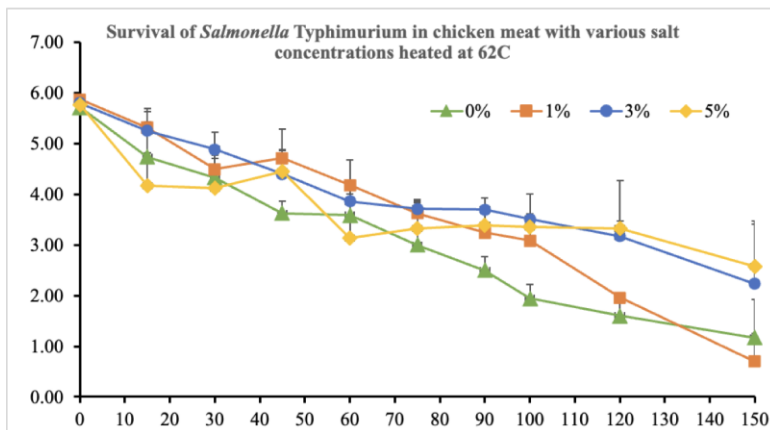
After cooking, chicken samples were immediately placed in a WhirlPak® filter bag (19×30 cm, Nasco, Modesto, CA, U.S.A) containing 10 mL of refrigerated TSB plus 0.1% sodium pyruvate (Fisher Scientific, Fair Lawn, NY, U.S.A) for enumeration of bacteria populations including heat injured cells. The sample bags with chicken meat were homogenized in a blender (Microbiology

International, Frederick, MD, U.S.A) for 2 min. The liquid solution from the filtered side of the sample bags was serially diluted in 9.0 or 9.9 ml of 0.1% BPW. One-tenth mL of this solution was spread-plated onto TSA-NaL agars. After spread-plating, each agar was incubated at 35 C for 48 h to manually count the colonies. All bacterial cell counts were transformed to log₁₀CFU/g with the detection limit of 0.3 log₁₀CFU/g.

Statistical analysis

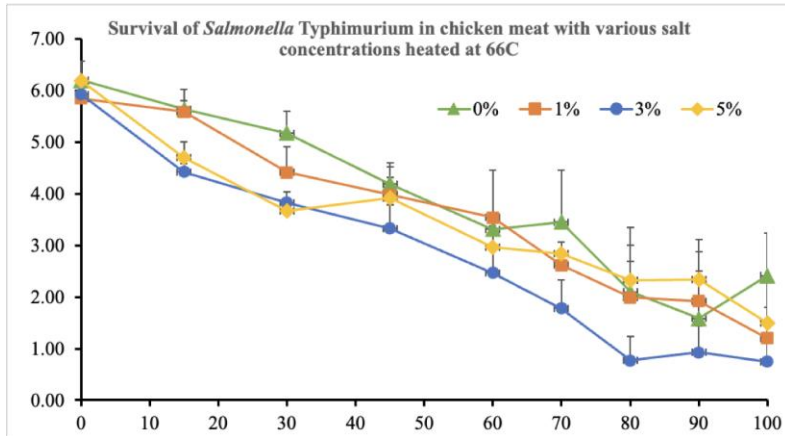
The whole experiments were conducted using 2 replicates with 4 chicken patties (10 g per sample unit) Survival and reduction data of the two bacterial cells were first analyzed using the SAS mixed model procedure (version 9.2, SAS Institute, Cary, NC) with individual factors and interactions between them. After that, the average thermal kinetic parameters of each cooking treatment were calculated using the United States Department of Agriculture (USDA)-Global-Fit software according to the procedures described in Huang Huang (2017). The differences in each comparison were determined by Tukey's HSD with the significance level at $\alpha=0.05$.

VII. Results



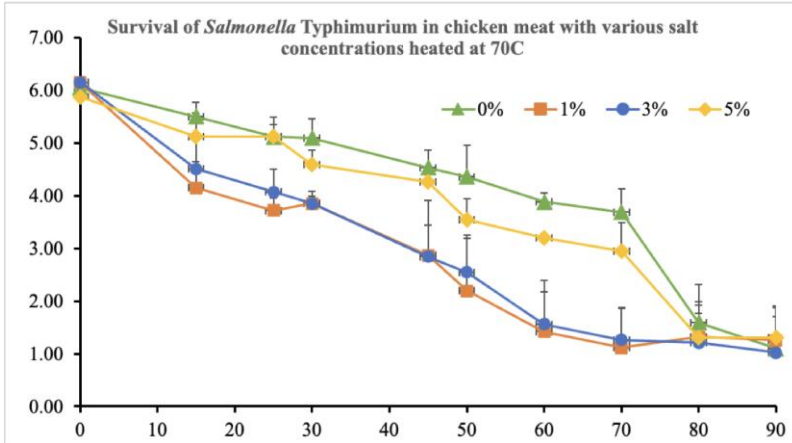
For 62 C, the initial Salmonella population was 5.70 to 5.87 log CFU/g. After heating for 150 seconds, they decreased to 0.70 to 2.57 log CFU/g. Survival curves of 0 and 1% salt samples indicate more heat susceptibility than 3 and 5% with roughly a 1.40 to 1.53 log CFU/g difference. Results are shown in Figure 1.

Figure 1. Survival of *Salmonella Typhimurium* in chicken meat with various salt concentrations heated at 62 C.



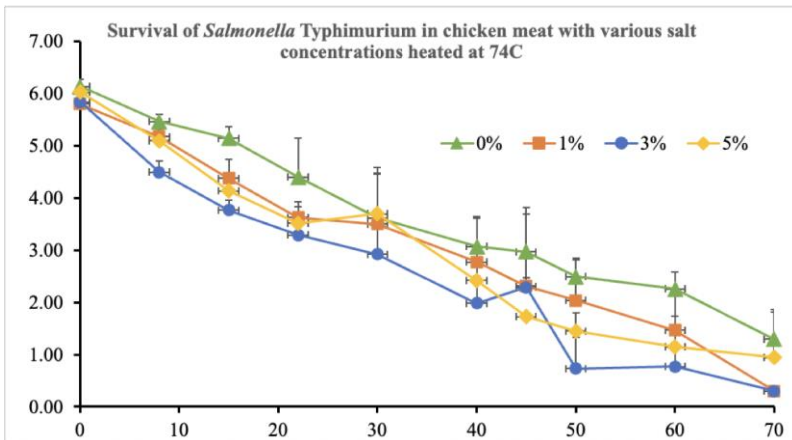
For 66 C, the initial Salmonella population was 5.84 to 6.19 log CFU/g. After heating for 100 seconds, they decreased to 0.75 to 2.41 log CFU/g. Survival curves of 1 and 3% salt samples indicate slightly more heat susceptibility than 0 and 5%, with a 1.21 to 1.66 log CFU/g difference. Results are shown in Figure 2.

Figure 2. Survival of *Salmonella Typhimurium* in chicken meat with various salt concentrations heated at 66 C.



For 70 C, the initial Salmonella population was 5.87 to 6.14 log CFU/g. After heating for 90 seconds, they decreased to 1.02 to 1.30 log CFU/g. Survival curves of 1 and 3% salt samples indicate more heat susceptibility than seen at other temperatures for 0 and 5%, with 1 and 5% differing from 1 and 3% by 1.20 to 1.23 log CFU/g. Results are shown in Figure 3.

Figure 3. Survival of *Salmonella Typhimurium* in chicken meat with various salt concentrations heated at 70 C.



For 74 C, the initial Salmonella population was 5.79 to 6.13 log CFU/g. After heating for 70 seconds, the population decreased to 0.30 to 1.29 log CFU/g. According to survival curves, heat susceptibility for all salt concentrations (0,1,0,3,0,5.0%) is roughly uniform, with 1% and 3% being 0.64 to 0.99 log CFU/g different from 0% and 5%.

Figure 4. Survival of Salmonella Typhimurium in chicken meat with various salt concentrations heated at 74 C

As shown in Table 1, the D-values of 62C for all tested samples from 0 to 5% increased from 23.3 to 27.4 s. D-values of 66, 70, and 74C all decreased from 13 to 21.1 s of 0% salt to 6.16 to 9.89 when the salt concentrations increased to 3%, but increased back to 7.95 to 15.5 s when extra salt of 5% were added.

Table 1 USDA-IPMP results (D-values and z-values)

D-values (s)	62C	66C	70C	74C	z-value (°C)
0% salt	23.3	21.1	20.3	13.0	52.2
1% salt	25.2	17.5	12.2	9.86	30.2
3% salt	26.9	9.89	9.23	6.16	24.1
5% salt	27.4	15.5	15.0	7.95	25.2

VIII. Discussion

Based on the results, it can be concluded that the thermal resistance of Salmonella is significantly affected by both temperature and salt concentration. There is evidence to suggest that 0% has the highest amount of resistance among the other concentrations, due to its z-value of 52.2 C, while 1% showed the next highest amount, 30.2 C, of temperature resistance. Both 3 and 5% showed lower, but almost equal z-values. Suggesting that salt concentration above 3% will show a similar or less thermal resistance, and concentrations of 0 and 1% will have equal or more thermal resistance. Suggests in future research to evaluate the fine line in temperature where thermal resistance is at its highest for 0 and 1%, and when 3 and 5% resistance is considered its lowest. This study is helpful to the poultry meat industry when developing proper thermal processes to eliminate Salmonella from moisture-enhanced (MH) chicken products.

IX. Conclusions

Overall, results will show that the thermal resistance of Salmonella is significantly affected by temperature along with salt concentrations. This will be useful for the poultry meat industry to develop proper thermal processes to eliminate Salmonella in moisture-enhanced (MH) chicken products.

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