ABSTRACT

Title of Thesis: EFFECT OF THE KASHERING PROCESS ON THE SAFETY AND QUALITY OF MEAT

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The process of making meat kosher, or "kashering," involves soaking the meat, covering it in salt for at least one hour, and several rinses after. This study evaluates the effect this process has on the survivability and thermal resistance of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Newport on fresh chicken and beef, as well as the effect on quality and acceptability of both meats. The process yielded a minor reduction of both pathogens at ~1 log CFU/g. Surviving *Salmonella* from kashered chicken displayed an increase in thermal resistance ($p<0.05$). A sensory analysis panel rated salted chicken and beef higher quality and saltier than not kosher meat ($p<0.05$). The kashering process did change the color of both meats ($p<0.05$), attributable to the significant increase in salt content of the meats ($p<0.05$), but did not affect the texture of the meat ($p>0.05$).

EFFECT OF THE KASHERING PROCESS ON THE SAFETY AND QUALITY OF MEAT

by

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Chapter 1: Introduction

1.1 Prevalence of foodborne illness in meat, problems of pathogen control.

As long as people have been eating food, there have been foodborne illnesses. Most recent estimates from the Center for Disease Control (CDC) estimate 48 million cases of foodborne illness, 128,000 hospitalizations and 3,000 deaths yearly in the United States (CDC, 2018). A CDC study evaluating foodborne illnesses from 1998-2008 estimated 22% of illnesses and 29% of deaths from foodborne pathogens were attributed to illness acquired from meat and poultry products (CDC, 2018). From January-November 2018, there were 7 reported outbreaks from meat and poultry, including an outbreak in ground beef which led to a recall of almost 7 million pounds of ground beef. These 7 outbreaks accounted for 784 reported cases, 245 hospitalizations, and 3 deaths. As of Thanksgiving Day (November 22, 2018), a day in the United States known for consumption of poultry, there were 3 active outbreaks from poultry, all from different strains of *Salmonella* in chicken or turkey. The CDC estimates that for every reported *Salmonella* infection, there are 30 unreported cases. This makes the exact number of cases extremely difficult to assess.

One of the biggest problems with controlling pathogens in meat is the critical kill step, cooking the meat, is left up to consumers. Consumers are often not aware of the cooking temperature recommendations of the USDA, the possible consequences of undercooked meat, or knowingly eat raw or undercooked meat (Lin et al, 205). Most pathogenic bacteria which are found in meat can be traced back to the animal (Martin and Beutin, 2011), and finding pathogenic bacteria on animals bred for

consumption is extremely common and even normal (Foley et al, 2008) (Franchin et al, 2005) (Franco et al, 1995) (Nou et al, 2007). Fresh produce has the highest number of reported cases (CDC, 2018), due to the minimal processing or raw consumption of many types of produce. Meat production involves a higher likeliness of contamination even with hygienic processing of the product (Genigeorsis et al, 1986). Slaughter and evisceration with insufficient sanitation practices raises the possibility of contamination even higher (Genigeorsis et al, 1986). In addition to the high likeliness of having contaminated meat from the animal, as with any food handling, there is also the added possibility of contamination through human contact with the meat, cross-contamination with unclean surfaces, and contaminated water used in the processing (CDC, 2017).

1.2 The kashering process

1.2.1 Method

Almost every type of food has requirements that must be met to be considered kosher. Of all types of food, meat has the strictest requirements to be considered kosher. Only certain animals are considered kosher animals, and even kosher animals require a very specific slaughter, checks to ensure there are no disqualifying deficiencies with the animal, and preparation of the meat. The only acceptable method of slaughter is a very complex and detailed slaughtering method called *shchitah* and may only be performed by a certified person called a *shochet*. After the slaughtering, as the animals are being gutted and skinned and birds are gutted and

defeathered, there must be checks to identify any defects with the animal which would render it not kosher, known as a *treifah.* Parts of the animal which are forbidden to be eaten are then removed. At this point, certain major veins and arteries are removed from meat animals such as cows and sheep, a process known as *nikkur*. After this, almost all commercially sold kosher meat today goes through a process called "kashering" (Hertzmark, 2018).

The kashering process starts when the carcass is soaked in water for a minimum of thirty minutes. The surface of the meat is then completely covered in kosher salt for a minimum of one hour. The salt used for kashering is a flatter, coarse grain salt which is designed to stick to the surface of the meat better than traditional table salt. After the salting, the salt is rinsed off and the meat is washed an additional 2 times (Regenstein et al, 2003). After the meat is kashered, it may be packaged and sold as kosher meat.

1.2.2 History

The processes for making kosher meat have been practiced for over three thousand years and for the most part have not changed. One of the major dietary prohibitions in Jewish law is the prohibition of eating blood. The purpose of kashering, as taught in Jewish theology, is to extract blood from the meat. This is the purpose of the *nikkur* step mentioned above.

In the past several decades, the demand for kosher food has increased dramatically, and many popular brands of foods are kosher, despite the Jewish population of the US being less than 2% of the total population of the United States

(Pew research center, 2013). However, Jews represent the minority of kosher food consumers. Some religious groups rely on kosher foods being acceptable to their dietary restrictions, such as Muslims and Seventh-Day Adventist (Star-K, 2019). Vegetarians sometimes rely on kosher non-meat foods as a guarantee there are no meat byproducts in the foods they purchase. Much of the kosher food market is due to people buying items such as crackers, milk, or coffee, not even knowing that they are kosher (Lindsay, 1998). While kosher meat is still only available at certain stores in areas near certain communities, kosher certification has become desirable for the food production industry.

1.3 Salt in the food industry

1.3.1 Historical

Salt has played a major role in the course of human history. Before the invention of refrigerators, storing food in salt was a primary method of food preservation. The extremely high level of salt during this method of storage inhibits the growth of microorganisms by lowering water activity and increasing salt content to levels beyond bacterial growth capabilities. This is effective for spoilage microorganisms as well as pathogens which present food safety concerns. These uses historically made salt one of the most desired and traded food additives even in ancient times. Although today salt is among the most inexpensive additives, it used to be expensive and was in many cultures traded as a currency itself. Salt was such an integral part of trade and the economies of ancient cultures, the English word “salary”

is rooted in either the latin word “salarium” which was a Roman soldier’s allowance to buy salt, or “salarius” which was revenue coming from the sale of salt. Both are variations of the latin word “sal” meaning salt (Online Etymology Dictionary, 2018).

1.3.2 Current uses

Salt is used commonly in the food industry because of its contribution to the quality of food, enhancing many sensory properties of the food. The ability of salt to enhance flavors of a wide range of food products, along with the many possible uses of salt and being one of the most inexpensive additives available, make salt one of the most widely used additives in the food industry. Salt is a key ingredient in almost all cheeses, cured meat and fish products. It also plays a key role in certain fermented foods such as pickles and sauerkraut. While salt is no longer used as the primary food preservation method, there are antimicrobial properties of salt (Wijnker et al, 2006) and it is often added as a preservative (Silva et al, 2003), as high salt concentrations do slow or inhibit growth of pathogens (Matches and Liston, 1972). However, salt alone is generally inadequate as the only preservation method in ready-to-eat food (Albarracin et al, 2011). High concentrations of salt have been shown to increase water binding capacity of proteins in meat (Albarracin et al, 2011). Addition of salt can have effects on many proteins in meat, such as affecting protein solubility (Machado et al, 2007), and decreasing the activity of many proteases (Armenteros et al, 2009).

Salt has also been shown to increase lipid oxidation in meat (Lin et al, 2015). There have been studies that suggested that salt has no effect on oxidation or even

shows antioxidative activity. Kong et al (2008) reported salmon fillets with 1.5% salt added did not show a significant difference in thiamin loss, lipid oxidation, or fatty acid profile than salmon fillets with no salt added (Kong et al, 2008). Sakai et al (2006) found significantly lower 4-hydroxynonenal (HNE), a major aldehyde formed during lipid peroxidation, in meat samples with higher NaCl content, concluding NaCl may prevent peroxidation (Sakai et al, 2006). Although these works have suggested no oxidative or anti-oxidative properties of salt, most studies indicate salt acts as a pro-oxidant, which may lead to increased rancidity (Mariutti and Bragagolo, 2017). Contrary to Sakai et al's 2006 findings of salt's antioxidative effects, Sakai et al, 2004, found in a similar experiment that the HNE content was higher in pork and beef samples with higher salt contents, concluding from this study that salt may act as a pro-oxidant (Sakai et al, 2004). Overholt et al (2016) measured thiobarbituric acid reactive substances (TBARS) values for sodium chloride salts with 4 different purities and compositions of iron, copper, magnesium, calcium, and manganese on pork patties, showing that salt can induce different rates of lipid oxidation. The results from this study show that all salts used had significantly higher TBARS values than the unsalted control (Overholt et al, 2016). Higher salt concentrations increased peroxide values (PV) and TBARS values in pork (Jin et al, 2012). These are some of the many studies which show higher salt concentrations having a pro-oxidative effect on meat (Lin et al, 2015) (Mariutti and Bragagolo, 2017).

In addition to measurable effects high amounts of salt have on meat, there are significant health problems associated with consumption of excess salt. Excess sodium increases the risk of high blood pressure, stroke, and cardiovascular disease,

the leading cause of death in the United States (CDC, 2016). The recommended upper limit of sodium intake for adults is 2,300 mg per day, and the estimated average intake for adult females is over 3,000 mg per day and about 4,500 mg per day for adult males (USDA, 2015). About 90% of children eat more sodium than recommended and has contributed to over 10% of all children in the United States having elevated blood pressure (CDC, 2018). As health issues exacerbated by excess sodium intake increase over the last several decades, there has been more incorporation of salt alternatives, such as potassium chloride, to reduce the sodium content in foodstuffs. Most salt producers also sell reduced-sodium salt, which incorporates potassium chloride with sodium chloride and can be found at almost any supermarket. Approximately 71% of sodium Americans consume is from processed foods or restaurant foods (CDC, 2018). This has led to the food industry doing much research into using salt alternatives in products which normally have high salt content, such as cured products (Alino et al, 2009), and effects using potassium chloride may have on the product (Gheizari and Motamedi, 2010).

1.4 Effects of kosher meat processing

1.4.1 Removal of blood

As mentioned above, the underlying purpose of the kashering process is to extract blood from the meat, as eating blood is forbidden by Jewish law. Kotula and Helbacka found there was a higher amount of blood throughout chicken carcasses which were slaughtered by kosher method (Kotula and Helbacka, 1965), although this

study did not account for the salting process. There is little scientific research evaluating the efficacy of the kashering process in extracting blood from meat. Television show producer Jigal Krant looked at the effectiveness of the kashering process at removing the blood from the meat with researchers from the University of Utrecht, and found that while some moisture and blood may have been removed from the surface, the interior of the meat was completely unaffected by the salting and there was no difference with the amount of blood in the blood vessels in the interior the meat tissue (Krant, 2015).

1.4.2 Effects of kosher processing on meat

Researchers have been investigating the efficacy of the kashering process for both safety and quality for many decades. Hajmeer, et. al (1999), and Shin et al (2013) both examined commercial kosher meat processing facilities and concluded that salting does have the potential to achieve a microbial reduction in *Salmonella* and *E. coli* in a commercial plant. Shin et al (2013) found a 1.4 log CFU/mL reduction of *E. coli* on salted chicken, and a 2.3 CFU/mL with chilling after salting (Shin et al, 2013). Hajmeer et al (1999) found in 80% of brisket samples salting reduced APC by 0.11 log CFU/mL. Only 3 of the samples originally tested positive for *E. coli*, averaging 0.09 log CFU/mL reduction after salting. In Hajmeer et al's study, 4 brisket samples tested positive for *Salmonella,* and after salting all 4 samples tested negative for *Salmonella,* indicating a reduction of *Salmonella,* however the authors did acknowledge the sample size was too small to make firm conclusions. Since both of these studies were done in commercial plants which are not as well-

controlled and do not employ aseptic technique, cross-contamination, employee contamination, and variability due to specific worker practices are possibilities which could have skewed the results of their studies (Hajmeer et al, 1999) (Shin et al, 2013). In some cases, the meat which is only required to be salted for one hour, was salted for a longer time due to employees being occupied with other duties or taking breaks.

There have been many laboratory studies performed to test the efficacy of the kashering process on the safety of meat. Many of these studies found the kashering process produced a significant reduction of pathogens. Oscar (2008) reported that the salting step is a significant reduction step in the prevalence of *Salmonella* on chicken skin (Oscar, 2008). However, even at initial concentrations of *Salmonella* on chicken skin as low as 0.4 log CFU/cm^2 of skin, the salting did not eliminate the *Salmonella* from all samples. At higher concentrations of *Salmonella* above 2.5 log CFU/cm^2, prevalence was not significantly diminished. While the findings at low concentrations of *Salmonella* were significant, few researchers would conclude that this result is enough to consider the process an effective kill-step. As prevalence of pathogenic microorganisms has been established, effective pathogen controls are still required. Zuckerman and Abraham (2002) found after defeathering, chicken carcasses showed a prevalence of *Listeria monocytogenes* of 0-14% immediately after defeathering, and by the end of the line the prevalence was 15-86%. Juven and Rogol (1985) found a 70-85% prevalence of *Campylobacter jejuni* and *C. coli* on chicken carcasses in a kosher processing facility at the soaking step, and the water in the soaking tanks tested positive for 12 different serogroups of *C. jejuni*. Holzer et al (2003) reported over 1 log reduction on spoilage microorganisms aerobic plate counts (Holzer et al,

2003). The prevalence of pathogens in retail poultry was found by Uyttendaele et al (1999) to be 36.5% for *Salmonella* spp., 28.5% for *Campylobacter* spp., and 38.2% for *L. monocytogenes* (Uyttendaele et al, 1999). These studies noted that prevalence of pathogens and serotypes varied from flock to flock, and chicken raised, processed, and sold in different areas may have different prevalence levels.

There are several studies that examined factors affecting the kashering process, such as the effect salt has on microbes. Wijnker et. al (2005) showed that salt can exhibit antimicrobial properties by lowering a_w (Wijnker et al, 2005). A 25% salt spray was shown to produce a 1 log reduction of *E. coli*, but not *Staphylococcus aureus* on beef brisket (Hajmeer et al, 2004). Changcheng et al (2016) reported that higher salt concentration in salmon roe increases thermal resistance in *Listeria monocytogenes* (Changcheng et al, 2016). Millman et al (2013) reported a different problem. They reported a significantly higher prevalence of antibiotic resistance in kosher chicken (Millman et al, 2013). According to their study, over 75% of bacterial isolates from kosher chicken showed antibiotic resistance to at least one of twelve antibiotics tested, as opposed to 55-60% of isolates from conventional, organic, and raised without antibiotics (RWA) chicken showing resistance to at least 1 antibiotic. Almost 40% of the kosher chicken isolates were resistant to at least 5 of the 12 antibiotics tested, whereas no other group had above 10% of isolates resistant to at least 5 antibiotics. On average, isolates from kosher chicken were resistant to 2-3 times the number of test antibiotics. These findings are in an area not generally associated with kosher meat, and the authors were not able to present a scientific reasoning as to why the kosher chicken showed higher prevalence of antibiotic resistance.

Table 1. Reduction and inhibition of growth of *Escherichia coli* and *Salmonella*.

Study	Description	Results
Hajmeer et al, 1999	Reduction of *E. coli* and *Salmonella* on beef briskets from a kosher commercial processing plant.	*E. coli:* 0.09 log reduction of after kosher salting. *Salmonella*, not enumerated: 4 carcasses positive for *Salmonella* pre-salting, 0 positive post-salting.
Shin et al, 2013	Reduction of *E. coli* and *Salmonella* from kosher salting and chilling.	*E. coli*: 1.4 log reduction from salting, 2.3 log reduction from salting and chilling. *Salmonella*: no significant results.
Oscar, 2008	Persistence of *Salmonella* on chicken skin after salting and rinsing.	When inoculated with 0.5 log CFU/cm^2 *Salmonella*, persistence dropped from 93% to 21% of samples positive after rinsing, salting, and rinsing again.
Holzer et al, 2003	Aerobic Plate Count from beef strip loins day of kosher salting and after 14 days storage.	Kosher salted samples had 1.42 log reduction due to salting on day 0 and 1.26 log less than controls on day 14.
Wijnker et al, 2005	Survival of *E. coli* and *Salmonella* at a_w 0.85 after inoculation on sheep casings.	*E. coli*: 0.41 log reduction per day. *Salmonella:* 0.34 log reduction per day.

Hajmeer et al, 2004	Reduction of *E. coli* after 25% NaCl spray on beef briskets.	*E. coli*: NaCl treatment, water treatment, and acidified sodium chlorite treatment produced between 0.6 and 0.81 log reduction.
How et al, 2013	Inhibition of *E. coli* at different salt concentrations.	*E. coli* grown in 7% NaCl had reduced OD of 0.093, 9% NaCl had almost complete inhibition of growth with OD of 0.004.
Stollework et al, 2012	Survival of *Salmonella* in salted (NaCl) and NaCl free (KCl) ham.	*Salmonella*: NaCl yielded 1 log reduction after 112 days storage. KCl yielded equal results.
Doyle and Glass, 2010	Slowed generation times of *E. coli* and *Salmonella* at higher salt concentrations.	*E. coli* has a generation time of 6 hours at 0.5% NaCl and a generation time of 14 hours at 4.5% NaCl *Salmonella* has a generation time of 9 hours at 0.5% NaCl and a generation time of 18 hours at 4.5% NaCl.
Matches and Liston, 1973	Incubation growth rate of *Salmonella* at different NaCl concentrations.	*Salmonella*: At 8˚C, 2% NaCl had 1 log growth after 24 days, 4% NaCl had 2 log reduction after 24 days. At 12˚C, 4% NaCl had an OD of 0.25 lower than control after 10 days. 6% NaCl showed an OD of 0.6 lower than control after 10 days.

Another area of interest researchers have is on the quality effects of kashering on meat. Since salting is the central part of the kashering process, most research has been related to salt content in meat and affect salt has on meat. Angel et al (1988) found significant differences in salt concentration in different parts of the chicken, and amount of salt in the meat was affected by how much salt was used in the salting process and how long the meat was salted for (Angel et al, 1985). In this study, the researchers found the back and neck and the skin had higher salt contents after salting than the breasts and thighs. The thighs and skins had significantly higher salt contents with higher amounts of salt for the salting step during the kashering process. Longer times also showed higher salt content, however the most significant changes in salt content was after 1.5 hours and was generally not significantly higher after the required 1 hour of salting. This was consistent with what Powers and Mast (1980) who found that chicken skin retains more salt than the meat and has a much higher sodium content than unsalted meat. Powers and Mast (1980) found that 1-hour salting was enough time for a significant increase in salt content. Salted meat has also shown to exhibit some discoloration post salting. Holzer et al (2003) found significant discoloration of salted meat after 14 days of storage (Holzer et al, 2003). The increase in salt content has also led to increased lipid oxidation in salted meat (Gheizari and Motamedi, 2010) (Mariutti and Bragagnolo, 2017). At low salt concentrations of solutions, protein solubility rises due to the “salting in” effect, as demonstrated by Inyang and Iduh (1996) who showed an increase in protein solubility of sesame proteins up to 1 M of NaCl. At higher concentrations of salt, protein solubility decreases due to “salting out” effect, as demonstrated by Trevino et al (2008) who

found higher levels of NaCl above 1 M decreases both crystalline and amorphous solubility values for egg white lysozymes. While the salting in and out effects were studied, Machado et al (2007) asserted pH and type of salt affects protein solubility much more than concentration of salt. This study found the biggest increase in protein solubility in more basic solutions. The types of salt used in the study made a difference and found NaCl led to the lower solubility than Na_2SO_4 or $(NH_4)_2SO_4$. The concentration, 0.05-0.5 M NaCl had the smallest effect on protein solubility of the 3 salts studied (Machado et al, 2007). A review by Albarracin et al (2011) asserts higher salt contents in food can slow or interrupt microbial processes because of the osmotic effect of salt and can reduce nutritional value in certain high salt foods (Albarracin et al, 2011). Despite most of these studies finding significant drawbacks to having higher salt content in meat, some taste panels showed a significant preference for salted meat, rating higher on tenderness, overall acceptability, and taste. Other taste panels showed no significant preference for salted or unsalted meat ((Powers and Mast, 1980) (Mast and MacNeil, 1983). Neither of these studies showed any preference of unsalted meat over salted meat.

1.5 Stress response

Bacterial stress response is a heavily studied area in microbiology. There are many factors which contribute to certain bacteria gaining resistance to certain stresses. Salt is the main stressor applied to pathogens in the kashering process and has been shown to induce stress response in many microorganisms. Cheville et al (1996) showed a *rpoS* mutant of *E. coli* O157:H7 showed much higher susceptibility

to high salt environments, concluding *rpoS* could be an important factor in salt tolerance (Cheville et al, 1996). Munro et al (1995) found RpoS mutants for both *E. coli* and *Salmonella* did not exhibit much resistance to seawater because of the salinity (Munro et al, 1995). Many studies have shown *rpoS* to be an important factor in *E. coli* O157:H7's famously efficient acid resistance (Arnold and Kaspar, 1995) (Hengge-Aronis, 1993). Other studies have shown heat shock proteins (HSPs) to be involved in resistance to a high salt environment (Wang et al, 2004) (Kilstrup et al, 1997) (Sugino et al, 1999). In bacteria, the DnaK (bacterial HSP70), is upregulated during stresses (Lindquist and Craig, 1988) (Sugino et al, 1999).

Many factors which contribute to *E. coli* stress response also are factors in *Salmonella* stress response. Many studies have shown just like in *E. coli*, *rpoS* influence acid resistance in *Salmonella* (Kusumoto et al, 2012) (Rowbury, 1995) (Lee et al, 1995). Kang et al (2018) found exposing *Salmonella* Enteritidis to acid and salt stressors with subsequent refrigeration can increase heat resistance and showed an increase in *rpoH*, *dnaK*, and *groEL* genes, indicating these genes influence the raise in themal resistance (Kang et al, 2018). Di Pasqua et al (2013) also found increased DnaK and GroEL protein in *Salmonella* Thompson in response to salt stress. (Di Pasqua et al, 2013). Yuan et al (2012) found lactate and acetate salts also has the ability increase heat resistance in *Salmonella* Typhimurium (Yuan et al, 2012). In addition to sigma factors, other stress responses in *Salmonella* can be controlled by regulators such as phosphor-relay-based two component systems and transcriptional regulators (Spector and Kenyon, 2012).

Volker et al (1992) found inducing a mild heat shock to *Bacillus subtilis* provided a cross-protection against otherwise lethal salt stresses and identified the stress proteins expressed to be analogs of DnaK found in *E. coli* (Volker et al, 1992). However, inducing the *B. subtilis* to mild salt stress was not as effective at increasing thermal resistance. Serrano (1996) reported the *nhaA* gene for the Na^+-H^+ antiporter is an essential gene for salt resistance in *E. coli* (Serrano, 1996). Christian and Waltho (1961) found a positive correlation between potassium content in non-halophilic bacteria and salt tolerance (Christian and Waltho, 1961). In plants, H_2O_2 and nitric oxide has been shown to play a role in increasing salt and thermal resistance (Gong et al, 2001) (Uchida et al, 2002).

These studies indicate the step in the kashering process which can have the greatest effect on pathogens is the salting step. Stress response is expected with exposure to high amounts of salt and can act to protect the pathogens from other mild stresses such as heat.

1.6 Not kosher processing

The majority of the meat production in the United States does not follow the laws of kashering, and the processes do differ slightly (Schuchman, 2016). Most chickens that will be sold as not kosher chickens are stunned before slaughter, so they are unconscious for the actual slaughter (Farouk, 2013). After they are slaughtered, they are let to bleed out for a short time. After they have been bled, not kosher birds are scalded in hot water, at approximately 50-60˚C for about 2 minutes to loosen the feathers and allow for easier plucking (Yang et al, 2001). This time and temperature

can vary with different species of bird, and if there are any skin color requirements for the processed bird. Following the scalding of the chickens they are defeathered, eviscerated, inspected, and chilled. Two of these practices are forbidden by Jewish law and would render any chicken slaughtered this way not kosher. Stunning the animal before slaughter is never done with kosher chicken and would render the chicken not kosher. Scalding chickens before they are salted is also forbidden according to Jewish law, and kosher chicken is sent directly to defeathering (Hertzmark, 2018).

Previous studies have shown mixed results of how the scalding effects the safety of the chicken. Yang et al (2000) reported almost a 2-log reduction of *Salmonella typhimurium* after 5 minutes of scalding at 60˚C, and over a 2-log reduction of *Campylobacter jejuni* over the first minute and a tail over the last 4 minutes. This study was run with a scalding time of about 5 minutes, however scalding times in the industry are rarely that long at high temperatures (Yang et al, 2001). In the first minute of scalding, even at 60˚C, the reduction of *Salmonella* was minimal (Yang et al, 2000). A minimal reduction of *Salmonella* on the skin from the scalding which is pre-evisceration, leads to a conclusion there is much less effect, even no effect on the reduction of *Salmonella* in the chicken which is not on the skin surface. Other studies which have looked at the microtopography of chicken skin at various scalding temperatures have found a higher ability for *Salmonella* Typhimurium and *Campylobacter jejuni* to attach to the surface of chicken skin which had be scalded at higher temperatures (Slavik et al, 1995) (Kim et al, 1993).

While scalding the chickens cause some reduction of pathogens, the process is not validated to achieve a reduction of pathogens prevalent in chicken. The purpose of the scalding is to loosen the feathers to allow for defeathering, and the temperature is not hot enough nor is the chicken submerged long enough to achieve a large reduction on the skin. It should also be noted that the scalding is done before the evisceration. The evisceration step can be a critical step for contamination of chicken (Martin and Beutin, 2011), and may have an even greater risk for not kosher birds which have been scalded at high temperatures.

Most meat producers also give an antimicrobial spray or wash with peracetic acid or chlorine, and this is done with both kosher and not kosher meat producers, then the carcasses are always chilled after slaughter (Hertzmark, 2018).

1.7 Research objectives

While the microstatic potential of salt is well-known, the salting during the kashering process is only for one hour, much shorter than any time used for preservation. This study has two focuses, the first of which is to evaluate the safety implications of the kashering process. For these safety evaluations, the objectives of this study are to:

- Determine the effectiveness of the kashering process as a food safety processing technique for microbial reduction. We hypothesized that there will be some reduction in bacterial population during the kashering process, but not enough to be considered an effective kill step.

- Determine the level of injury the kashering process has on pathogenic bacteria on the meat. Salt is a known and widely used stressor, so we hypothesized that salting will cause an increase in bacterial injury.
- Determine ability of bacteria to recover after the kashering process for 2 hours at room temperature and refrigeration temperature. We hypothesized there will be some bacterial recovery at room temperature, and less recovery at refrigeration temperature.
- Determine if there is any effect on the thermal resistance of bacteria on meat which has been subjected to the kashering process. As it is known many stresses increase thermal resistance in many pathogens, we hypothesized the kashering process will cause a rise in thermal resistance.

The second focus of this study is to determine if the salt used during the kashering process contributes to increased quality of the meat over non-salted product which have not undergone the kashering process. For the quality evaluations, the objectives were to:

- Determine if the average person was able to perceive any difference in overall quality and saltiness between kosher, salted meat and not kosher, non-salted meat. We hypothesized the kosher samples will be perceived as both better and saltier than not kosher samples.
- Determine the effect the kashering process had on the color and texture of the meat. We hypothesized the salting will affect the both the color and texture of the meat.

Determine how much the kashering process affects the salt content of meat. We hypothesized there will be significantly higher salt content in kosher meat than not kosher meat.

Chapter 2: Methods and Materials

2.1 Effect of the kashering process on the safety of kosher meat

2.1.1 Bacterial cultures

A stock culture of shiga toxin negative *Escherichia coli* O157:H7 (ATCC #700728, Manassas, VA, USA) was streaked onto TSA and incubated at 37˚C for 24 h. For each experiment using *E. coli*, a single colony of *E. coli* was gathered by a loop and inoculated in 10 mL of tryptic soy broth (TSB) (Bacto, Becton Dickinson, Sparks, MD), and inoculated at 37˚C for 24 h before the experiment. *Salmonella enterica* serovar Newport was cultured from a single colony from a stock culture on a blood agar plate being taken by loop and inoculated into 10 mL of TSB and inoculated at 37˚C for 24 hours. Before the experiments, the cultures were centrifuged at 7,830 rpm at 20˚C for 10 minutes by an Eppendorf 5430 R centrifuge (Hamburg, Germany). The culture was then diluted to approximately 7 log CFU/mL.

2.1.2 Measurement of bacterial death, injury, and recovery

To determine death and recovery within 2 hours of being kashered, 10 mL cultures were grown and diluted by 2-log to 7-log CFU/mL. Boneless chicken breast and beef shoulder chuck steak were purchased from a local grocery store. Pieces of chicken and beef each weighing 3 ± 0.2 g, approximately 2 cm^3, were soaked in the diluted bacterial culture to allow attachment to the surface of the meat. After a 30-minute inoculation period, each piece meat was allowed to dry for 30 minutes before

the kashering process began. Each piece of meat was soaked in deionized (DI) water for 30 minutes for the initial soak. The meat was then transferred to a surface and the surface of the meat was covered in dry Morton® Kosher salt and salted for 1 hour. After the salting was complete, the meat was washed by dipping in 3 different reservoirs of DI water to remove the salt. After this process was complete, samples of kashered meat were held at room temperature for 2 hours, and other samples were held at 4˚C for 2 hours. Samples were taken before the first 30-minute soak in water, after the 30 minute soak, after the 1 hour salting, after 1 post salting wash, after 3 post-salting washes, after 1 hour holding at both 4˚C and room temperature, and after 2 hours holding at both 4˚C and room temperature. For each sample, the 3 g piece of meat was placed in a stomacher bag with 27mL of 0.1% peptone water. The chicken pieces were stomached for 2 minutes and the beef was stomached for 3 minutes, then was serially diluted and plated on tryptic soy agar (TSA) (Difco, Becton Dickinson, Sparks, MD), incubated at 37˚C for 24 hours, and enumerated. Data was collected independently in triplicate.

To determine injury and recovery within 2 hours of being kashered, the same procedure as the reduction experiment above was followed, with the addition of plating the serial dilutions on selective media as well as non-selective TSA. Non-selective plating media used for both *E. coli* and *Salmonella* was TSA (Difco, Becton Dickinson, Sparks, MD). Selective plating media used for *E. coli* was MacConkey agar (Difco, Becton Dickinson, Sparks, MD). Selective plating media used for *Salmonella* was Xylose-Lysine-Tergitol 4 (XLT4) (Millipore, Billerica, MA) with the

addition of the XLT4 supplement (Millipore, Billerica, MA). To calculate % injury, the following formula was used:

$$\%\ injury = \frac{CFU/mL\ on\ nonselective\ media - CFU/mL\ on\ selective\ media}{CFU/mL\ on\ non-selective\ media}$$

Data was collected independently in triplicate.

2.1.3 Determination of thermal resistance of bacteria after the kashering process

2.1.3.1 Bacterial cultures and kashering treatment

Stationary phase cultures of *E. coli* O157:H7 and *Salmonella enterica* serovar Newport were grown in TSB for 24 hours and used directly in the thermal resistance experiment. For the experimental cultures, each pathogen was inoculated on chicken and beef samples in the same method as discussed in section 2.1.3. After the third post-salting wash, the samples were placed in a stomacher machine and the chicken samples were stomached for 2 minutes and the beef samples were stomached for 3 minutes. Serial dilutions were plated on TSA and grown for 24 hours at 37˚C for 24 hours. A single colony of each surviving pathogen on each type of meat was loop inoculated into 10 mL of TSB and grown for 24 hours at 37˚C. After 24 hours, the culture was centrifuged at 7830 rpm for 10 minutes then resuspended in 20 mL of new TSB.

2.1.3.2 Determination of thermal resistance

The thermal resistance experiment was performed using a COIL-100 Immersed coil apparatus (Sherwood Instruments, Lynnfield, MA). The immersed coil apparatus holds about 10 mL of solution and was set to disperse 400 μL of solution at each designated time point. Experiments for both *E. coli* and *Salmonella* was run at 57˚C for 15 minutes. Untreated *E. coli* and *Salmonella* was run through the immersed coil machine and samples were taken at 0 min, 3 min, 6 min, 9 min, 12 min, and 15 min. Plating for the thermal resistance experiment was done with an EddyJet 2 Spiral plater (IUL Instruments, Barcelona, Spain). The plater was set to spiral plate 50 μL per plate. Each sample was serially diluted and plated by the spiral plater, allowed to incubate for 24 hours at 37˚C, then enumerated using a Flash and Go plate reader (IUL Instruments, Barcelona, Spain). This was run with 1) Untreated *E. coli* control, 2) *E. coli* which had been inoculated on chicken and survived the kashering process, 3) *E. coli* which had been inoculated on beef and survived kashering, 4) Untreated *Salmonella* control, 5) *Salmonella* which had been inoculated on chicken and survived kashering, and 6) *Salmonella* which had been inoculated on beef and survived kashering. Data was collected independently in triplicate.

2.2 Effect of the kashering process on the quality of kosher meat

2.2.1 Sensory analysis of kosher and not kosher meat

A sensory analysis panel of 33 untrained participants was used to quantify the perceived quality and saltiness of kosher and not kosher meat by average consumers.

The meat used for the texture and color experiments was the same meat as used in the safety experiments. The sensory analysis and salt content experiments included commercially prepared kosher meat which was purchased from a local kosher grocery store. Boneless chicken breast was purchased for the kosher chicken samples, and shoulder chuck was purchased for the kosher beef samples. For the salting of the meat, Morton coarse kosher salt was used. Each participant was given 3 samples of chicken breast and 3 samples of beef, chuck steak one at a time. One sample was commercially prepared not kosher (not kosher), one was store bough kosher (kosher), and the third was commercially prepared not kosher and salted within 6 hours of the experiment (salted). Chicken was baked with a small amount of black pepper until the internal temperature reached 165˚F, and the beef was pan-grilled with canola oil and a small amount of black pepper until the internal temperature reach 145˚F. For each sample, participants were asked to rate 6 quality characteristics of the meat on a 10-point hedonic scale. the 6 quality characteristics were overall quality, flavor, texture, aroma, saltiness, and sweetness. After all of the samples were rated, a second questionnaire gathering overall opinions and impressions of the meat samples was collected. Sensory analysis experiment was submitted to the University of Maryland, College Park Institutional Review Board (IRB), project number 1220434-1, and was determined to be exempt from IRB review.

2.2.2 Color of meat through the kashering process

Color analysis was run with chicken and beef to determine the effect on the color of the meat. The kashering process was done with tap water for the soak and

post-salting rinse, and Morton Kosher Salt was used for the salting step. A Hunterlab Color flex spectrophotometer (Hunter Associate Laboratory, Reston, VA) was used for all meat samples to detect color changes. The color of each type meat was measured in Lab values and converted to RGB color values to visualize the total affect the kashering process has on the meat. After the kashering process was complete, the meat was held in a refrigerator for 24 hours and then rinsed. One piece of meat was run through this entire process and was considered 1 sample. Color samples were taken 1) before the initial soak, 2) after salting, 3) after the 3rd rinse, 4) after 24 h in a refrigerator, and 5) after 24 h in a refrigerator with rinsing under a sink. Total color change, ΔE, was calculated using the equation:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Change in each color value was calculated by subtracting each L, a, or b value from the control value on the same sample. Data was collected independently in triplicate.

2.2.3 Texture of meat through the kashering process

Another attribute of meat that consumers may consider is texture of meat. To measure texture changes, a TA.XT2i Texture Analyzer (Texture Technologies and Stable Micro Systems, Hamilton, MA) was used for all meat samples. The texture analyzer was set to move toward the sample at 2 mm/sec, compress the sample at 1 mm/sec until it had compressed the meat sample for 15 seconds, and retract from the sample at 10 mm/sec. Measurements taken were in grams of force applied to compress and hold compression of each meat sample. Texture analyses were run for

the same samples and at the same time points as the color analysis in section 2.2.2. Data was collected independently in triplicate.

2.2.4 Salt content through the kashering process

To assess salt concentration in kosher and not kosher meats, 3 g samples of chicken and beef were subjected to the kashering process, and samples from each step was taken an analyzed. To avoid including the salt present in tap water, DI water was used for the soaking and post-salting steps. Morton Kosher Salt was used for the salting. An ExStik II Conductivity/TDS/Salinity meter (Extech Instruments, Nashua, NH) was used on the salinity measurement setting to measure salt concentration of the meat samples in parts per million or parts per thousand. Each sample was placed in a stomacher bag with 27 mL of DI water, then stomached for 5 minutes. To filter out the meat particles, the solution was then filtered through a Whatman 70 mm filter paper with a 23 μm pore size, and filtrate was subsequently analyzed for salinity by the ExStik II salinity meter. This was repeated with commercially prepared kosher meat, commercially prepared not kosher meat, and commercially prepared not kosher meat which was subjected to the kashering process in the lab. Data was collected independently in triplicate.

2.3 Statistical Analysis

Statistical analysis for all microbial reduction and injury experiments for both chicken and beef and both *E. coli* and *Salmonella* was conducted with the SAS studio University edition version 9.4 (SAS Institute, Cary, NC). For reduction and injury

experiments, significance due to salting was determined by a t-test comparing unsalted controls and samples after the salting step. To determine recovery after salting, one-way ANOVA tables were generated, and all ANOVAs which showed significance had the groups compared with Dunnett's post-ANOVA analysis method using the salted samples as the comparison control. ANOVA and Tukey's post-ANOVA analysis method was used with the sensory analysis to determine significant difference with the meat samples, determine changes in color and texture of both meats, and determine differences in salt content for the meat samples from the store and throughout the kashering process. For change in thermal resistance, the t-test data analysis function in Microsoft Excel was used (Microsoft, Redmond, WA) to compare each salted sample against the unsalted control. Significance levels were set at a p-value below 0.05 for all analyses.

Chapter 3: Results

3.1 Reduction, injury, and thermal inactivation of pathogens

3.1.1 Microbial inactivation of E. coli *and* Salmonella

3.1.1.1 Microbial inactivation of E. coli *and* Salmonella *on Chicken*

The reduction of *E. coli* and *Salmonella* throughout the kashering process and 2 hours post kashering at room temperature and refrigeration temperature when inoculated on chicken is shown in figure 1A. For *E. coli* inoculated on chicken, there was a significant 1.23±0.58 log reduction from the unsalted control to the salted samples ($P<0.05$). Reduction of *E. coli* after the salting at room temperature and refrigeration were not significant ($P>0.05$). *Salmonella* which was inoculated on chicken showed a significant 0.73±0.17 log reduction between the unsalted control and the salted samples ($P<0.05$). As with *E. coli*, the reduction exhibited during the post salting points at room temperature and refrigeration temperature were not significant ($P>0.05$).

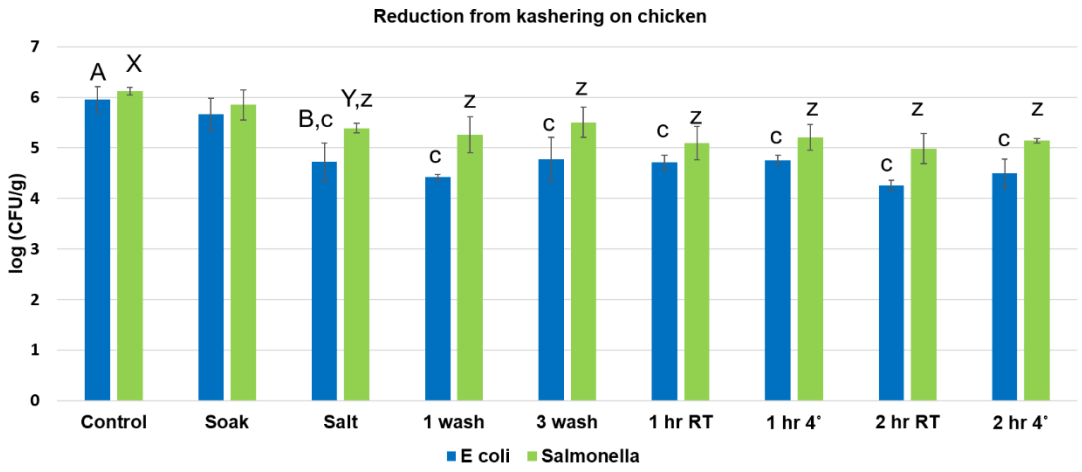
3.1.1.2 Microbial inactivation of E. coli *and* Salmonella *on Beef*

The reduction of *E. coli* and *Salmonella* throughout the kashering process and 2 hours post kashering at room temperature and refrigeration temperature when inoculated on beef is shown in figure 1B. For *E. coli* which was inoculated on beef, there was a significant 0.84±0.22 log reduction from the unsalted control to the salted samples ($P<0.05$). Reduction of *E. coli* after the salting at room temperature and refrigeration were not significant ($P>0.05$). *Salmonella* which had been inoculated on

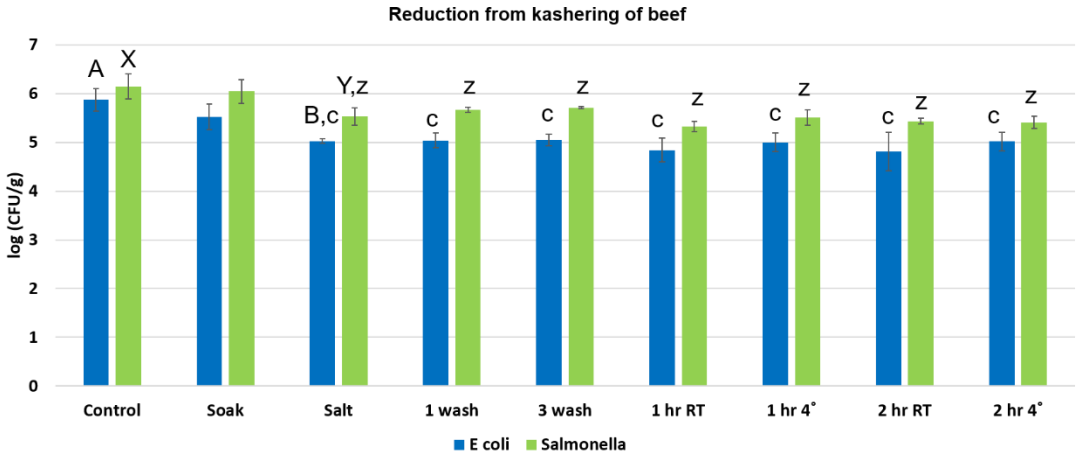
beef showed a significant 0.62±0.24 log reduction from the unsalted control and the salted samples ($P<0.05$). The reduction exhibited post salting at room temperature and refrigeration temperature were not significant ($P>0.05$).

Figure 1. Reduction of *Escherichia coli* and *Salmonella* throughout the kashering process and 2 hours post kashering at room temperature and refrigeration temperature when inoculated on A) chicken and B) beef.

A.



B.



3.1.2 Injury of E. coli *and* Salmonella

3.1.2.1 Injury of E. coli *and* Salmonella *on chicken*

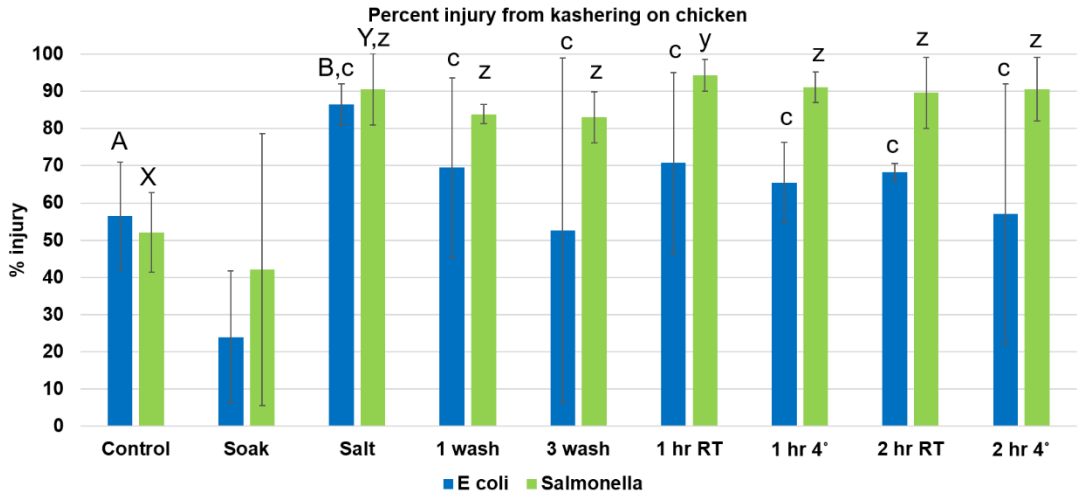
The injury of *E. coli* and *Salmonella* throughout the kashering process and 2 hours post kashering at room temperature and refrigeration temperature when inoculated on chicken is shown in Figure 2A. For *E. coli* which was inoculated on beef, there was a significant 29.9±18.6% rise in injury from the unsalted control to the salted samples ($P<0.05$). Recovery of injured *E. coli* during after the salting at room temperature and refrigeration were not significant ($P>0.05$). *Salmonella* which had been inoculated on chicken showed a significant 38.5±17.5% rise in injury from the unsalted control and the salted samples ($P<0.05$). Recovery of injured cells exhibited post salting at room temperature and refrigeration temperature were not significant ($P>0.05$).

3.1.2.2. Injury of E. coli *and* Salmonella *on beef*

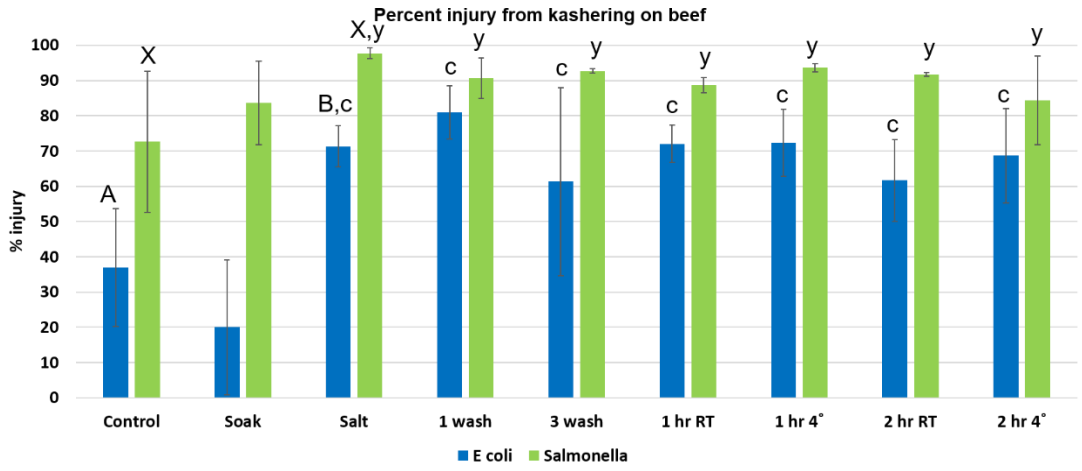
The injury of *E. coli* and *Salmonella* throughout the kashering process and 2 hours post kashering at room temperature and refrigeration temperature when inoculated on beef is shown in Figure 2B. For *E. coli* which was inoculated on beef, there was a significant 34.3±21.5% rise in injury from the unsalted control to the salted samples ($P<0.05$). Recovery of injured *E. coli* after the salting at room temperature and refrigeration were not significant ($P>0.05$). *Salmonella* which had been inoculated on beef showed a 25.0±20.5% rise in injury from the unsalted control and the salted samples, however this was found to be not significant ($P>0.05$). Recovery of injured cells exhibited post salting at room temperature and refrigeration temperature were not significant ($P>0.05$).

Figure 2. Percent injury of *Escherichia coli* and *Salmonella* throughout the kashering process and 2 hours post kashering at room temperature and refrigeration temperature when inoculated on A) chicken and B) beef.

A.



B.

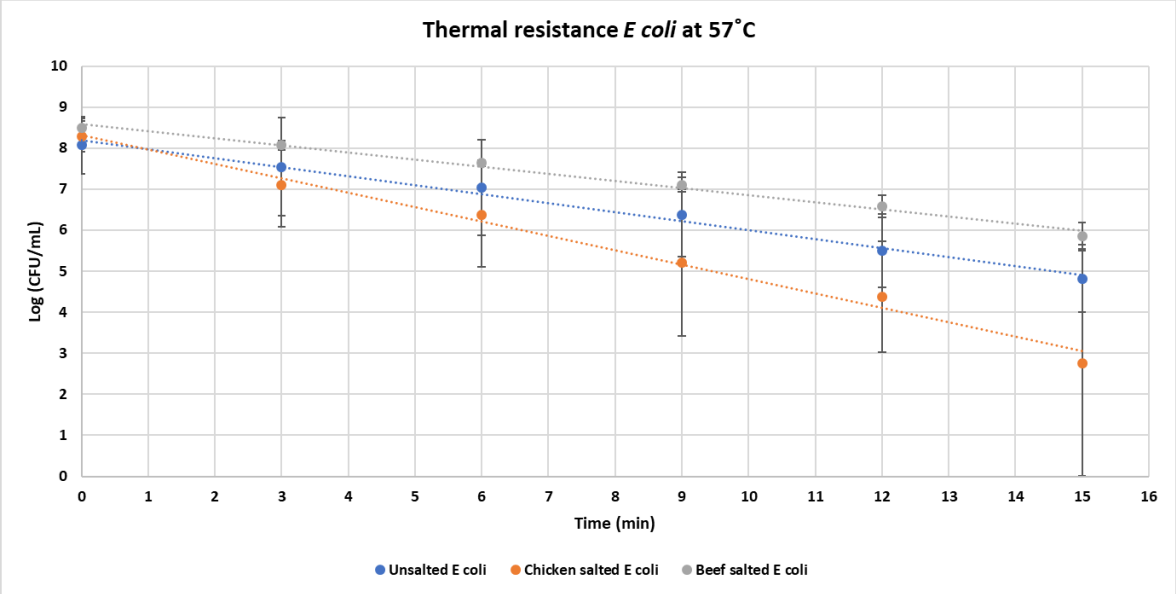


3.1.3 Thermal inactivation of E. coli *and* Salmonella

Thermal inactivation kinetics of untreated *E. coli* and *E. coli* recovered from kashered chicken and beef are shown in Figure 3A. The corresponding D-values at 57˚C are shown in Table 2. Neither the *E. coli* which had been salted on chicken nor the *E. coli* which had been salted on beef showed a significant change in D-value ($P>0.05$). Thermal inactivation kinetics of native *Salmonella* and *Salmonella* recovered from kashered chicken and beef are shown in Figure 3B. While the rise in D-value at 57˚C indicating a rise in thermal resistance was not significant on the *Salmonella* which had been salted on beef, the rise in thermal resistance for *Salmonella* which had been salted on chicken was significant ($P<0.05$).

Figure 3. Reduction of A) *Escherichia coli* and B) *Salmonella* at 57˚C over 15 minutes.

A.



B.

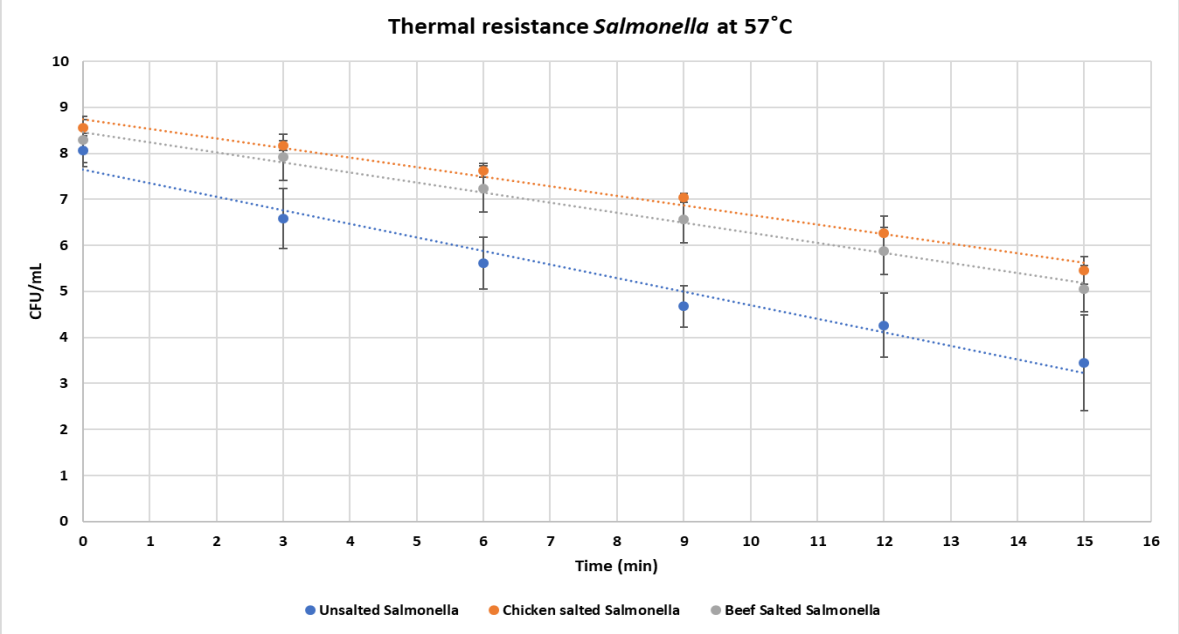


Table 2. D-values for *Escherichia coli* and *Salmonella* at 57˚C.

	D-value (min)
E. coli-unsalted	4.83±0.29
E. coli-salted on chicken	3.50±1.50
E. coli-salted on beef	5.67±0.58
Salmonella-unsalted	3.89±0.76 **a**
Salmonella-salted on chicken	5.16±0.28 **b**
Salmonella-salted on beef	4.77±0.76 **ab**

3.2 Quality characteristics of kosher meat

3.2.1 Sensory analysis of kosher and not kosher meat

Six quality characteristics of the meat were rated, but only 2 were of interest. The characteristics of interest which were looked at were overall quality and saltiness of the meat. For the overall quality, the kosher chicken sample had the highest response for highest overall quality, while the not kosher sample had the lowest average response. The kosher chicken responses were significantly higher than the not kosher chicken ($P<0.05$) and was not significantly higher than the salted chicken ($P>0.05$). The salted chicken was not significantly higher than the not kosher chicken. The salted beef was rated significantly higher in overall quality than both the kosher and not kosher pieces of beef. The kosher and not kosher sample responses did not show a significant difference from each other. Responses for overall quality are shown in table 3.

The other characteristic of interest was saltiness. The salted chicken was rated significantly saltier than either kosher or not kosher chicken (P<.0001). The not kosher chicken had the lowest average but was not significantly lower than the kosher chicken. The saltiness rating for the beef showed the same results. The salted beef had a significantly higher rating for saltiness than either of the other beef samples (P<.0001). The not kosher beef had the lowest average but was not significantly lower than the kosher beef. Responses for saltiness are shown in table 3.

Table 3. Sensory analysis responses for overall quality and saltiness of chicken and beef samples.

	Quality	Saltiness
Not Kosher Chicken	5.55±1.82 **a**	3.42±2.11 **x**
Kosher Chicken	6.67±1.89 **b**	4.58±1.84 **x**
Salted Chicken	6.45±1.92 **ab**	7.00±2.06 **y**
Not Kosher Beef	5.94±1.60 **a**	3.18±1.67 **x**
Kosher Beef	5.15±1.56 **a**	3.64±1.85 **x**
Salted Beef	7.09±1.31 **b**	6.97±1.59 **y**

3.2.2 Color of meat through the kashering process

Chicken did undergo a small but significant color change. Changes is L and a-values are shown in table 4A. The drop in L value indicates the salting process made the chicken significantly darker, and lower a-values indicate the chicken was significantly greener, if only slightly. Changes in the b value were not significant ($P>0.05$). Beef also underwent a significant color change. Changes in color for beef are shown in table 4B. The L value only showed a significant change due to the washing step, getting lighter. The a and b values show significant increases due to the salting step, getting more red and yellow. The L and b values also showed a significant change during the 24-hour period held in the refrigerator, getting lighter and more blue while sitting in the refrigerator. It was noted that the meat did show some browning due to oxidation in the refrigerator, which likely accounted for the color changed during the 24-hour period in the refrigerator.

Table 4. Changes in color for A) chicken and B) beef at different stages in the kashering process, as well as 24 hours in refrigeration and after subsequent rinsing.

A.

	ΔL	Δa	Δb	ΔE
Control	0 **a**	0 **a**	0	0 **a**
Salted	5.8±1.8 **b**	-1.96±.4 **b**	-0.12±1.8	6.39±1.7 **b**
Washed	4.61±3.9 **b**	-1.02±1.2 **ab**	1.86±5.0	7.60±2.0 **b**
24 hr	5.61±1.2 **b**	-0.94±.9 **a**	-0.65±1.2	5.83±1.3 **b**
24 hr + rinse	5.24±2.0 **b**	-0.41±.9 **ab**	-0.31±1.5	5.45±1.6 **b**

B.

	ΔL	Δa	Δb	ΔE
Control	0 **a**	0 **a**	0 **a**	0 **a**
Salted	2.8±2.2 **ac**	8.99±1.6 **b**	8.23±1.2 **b**	12.59±2.3 **b**
Washed	-0.34±1.9 **b**	10.15±2.0 **b**	8.6±2.3 **b**	13.44±2.7 **b**
24 hr	5.02±1.9 **c**	7.37±2.1 **b**	3.8±1.4 **c**	9.83±2.5 **b**
24hr + rinse	4.06±3.0 **c**	7.25±0.7 **b**	4.4±2.0 **c**	9.75±1.9 **b**

3.2.3 Texture of meat through the kashering process

The results for the texture analysis are shown in table 5. These results were inconsistent. For the chicken, even though all samples showed higher values for all step after the salting, meaning the meat is firmer and needs more pressure to compress the chicken, the high standard deviations lead the results to be not statistically significant. The beef texture results were more inconsistent than the chicken, and even though changes were observed throughout the process, there was no significant change in texture measured. Since the ANOVA table generated by SAS did not show a significant difference with any groups, a post-ANOVA analysis was not run.

Table 5. Texture of A) chicken and B) beef through the kashering process, measured in force (g) needed to compress meat by 2mm.

A.

	Sample 1	Sample 2	Sample 3	Average	Standard deviation
Control	47.6	89.3	52	63.0	22.9
Salted	89.2	113.2	173.9	125.4	43.7
Washed	78.1	114.1	178	123.4	50.6
24 hr	85.8	109.1	116	103.6	15.8
24+wash	70.7	112.8	145.4	109.6	37.5

B.

	Sample 1	Sample 2	Sample 3	Average	Standard deviation
Control	96.2	42	193.3	110.5	76.7
Salted	76.5	67.1	103.9	82.5	19.1
Washed	55.1	69.3	77.2	67.2	11.2
24 hr	88.7	176	183.1	149.3	52.6
24+wash	78.5	157.3	312.2	182.7	118.9

3.2.4 Salt content through the kashering process

The measured salt concentrations for the commercially prepared not kosher meat, commercially prepared kosher meat, and commercially prepared not kosher

meat which I brought through the kashering process are shown in table 6. For both the chicken and beef, there was not a significant difference with the kosher and not kosher samples, but there was a significantly higher level of salt in the salted samples. As both the chicken and beef went through the kashering process, a significant increase in salt content was observed after the salting step. The step with the highest level measured was just after the first was post-salting. after the second and third washes, there was a significantly lower amount of salt than after the first wash, but still a significantly higher amount of salt than in the pre-salted samples.

Table 6. Salt concentration of chicken and beef at different stages of the kashering process in parts per million (ppm).

	Chicken	Beef
Control (Not kosher)	539±195 **a**	691±26 **a**
Soaked	647±97 **a**	427±15 **a**
1 wash	9353±1345 **b**	9567±1056 **b**
2 wash	6303±1114 **c**	7110±1142 **c**
3 wash	6380±1081 **c**	6910±474 **c**
Commercially prepared	832±264 **a**	648±125 **a**

Chapter 4: Discussion

4.1 Effect of the kashering process on the safety of meat

Many studies have shown salt to have the potential as an effective antimicrobial. Of the several steps in the kashering process, the salting step has the greatest effect, and is the only step which consistently provided significant change in reduction, injury, as well as color changes in both chicken and beef. While the reduction is consistent and significant, the reduction does not come close to the reduction needed to be considered an effective kill step. However, *Salmonella* which had been salted on chicken did show an increase in thermal resistance, which may negate the benefit of having a small reduction in microbial load on the meat. The level of reduction found in this study is consistent with Hajmeer et al (2004), Holzer et al (2004), and other studies which have looked at reduction of pathogens due to salting. The findings of Shin et al (2013) and Hajmeer et al (1999) indicate pathogens inoculated on beef had a higher resistance to pathogens inoculated on chicken. As salt lowers the a_w of meat for the duration of the salting, one possible explanation as to why *Salmonella* appears to be more resistant to the salting than *E. coli* is the minimum a_w in which each bacterium can grow. *E. coli* has a minimum a_w for growth of about 0.95, while *Salmonella* has a slightly lower minimum a_w at around 0.93 (Stringer and Pin, 2005), making *Salmonella* slightly more halotolerant than *E. coli* to withstand a greater drop in a_w. The one-hour salting period is a mild stress applied on the pathogens, which leads to some reduction, however is not enough to be considered an extreme stress which would have provided a higher reduction.

Some studies have shown pathogens inoculated on beef exhibit higher stressor resistance than pathogens inoculated on chicken. This may be due to the different fat composition of chicken and beef. Different parts of animal have very different fat compositions. Chicken breasts, as used in this experiment, has an average fat content around 7%, and other parts of chickens can have fat contents ranging from 7-16% total fat (USDA, 2011). Chuck steak, as used in this experiment, has a fat content about 21%, and other cuts of steak can vary from below 8-24% total fat (USDA, 2011). The amount of fat in a particular animal can also vary greatly depending on age, species, diet, health, and living conditions of the animal (Leventhal, 2018). Many studies have shown meats with higher fat have shown greater resistance to stressors. Juneja and Eblen (2000) measured heat inactivation of eight strains of *Salmonella* Typhimurium in beef samples of varying fat content. They found greatest heat resistance in the *Salmonella* inoculated on samples with the highest fat content at 24%, and lowest resistance on the beef with the lowest fat content at 7% (Juneja and Eblen, 2000). Because fat in beef is unevenly distributed, this can lead to unequal moisture content throughout the surface of the meat. Meat surfaces with little fat may not induce resistance in pathogens on the low-fat surface, while surfaces with high amounts of fat or surfaces of the fat itself may induce resistance to cells on the high fat surface. While this study did not find pathogens inoculated on beef significantly more resistant than when inoculated on chicken, the reduction was slightly less on the high fat beef, which would be consistent with other studies. Running the experiment with a larger sample size may have increased the significance in reduction between chicken and beef.

Possible explanations of the rise in thermal resistance in response to the salting stress as observed with *Salmonella* on chicken, may be due to upregulation of stress response proteins discussed in section 1.5 which have been shown to increase thermal resistance in response to some stressors. A reason why the increase in thermal resistance may not have been observed in the other samples is the time between the salting and the thermal inactivation kinetics experiment. After kashering, the bacteria were grown for 24 hours in optimal conditions, then inoculated in broth for a further 24 hours. This experiment was run with cultures which were 2 generations removed from the actual kashering process, and the bacteria which may have upregulated stress response proteins may have reverted to non-stress conditions before the thermal resistance experiment was run.

The reduction of pathogens presents one possible safety benefit for kosher chicken, and there may be another benefit for kosher chicken in the lack of scalding. Kosher chicken cannot be scalded as not kosher chicken is. The scalding does not produce much microbial reduction even on the surface of the meat (Yang et al, 2000), and can change the microtopography of chicken skin to allow higher bacterial attachment (Slavik et al, 1995) (Kim et al, 1993).

4.2 Effect of the kashering process on the quality of meat

The sensory analysis panel rated kosher chicken significantly better than not kosher chicken, and salted beef the best for overall quality. For saltiness, the salted samples rated significantly higher in saltiness. The kosher and not kosher chicken and beef samples did not significantly differ in terms of saltiness. This may be because in

most major processing facilities, meat in packaged directly out of the post-salting washes and sits in small pools of residual water inside the packaging until the package in shipped and opened at the retail store. Sitting in pools of water for this amount of time may have an affect extracting some of the salt which was up taken by the meat during the salting step. Another possible explanation for the discrepancy between kosher meat and salted meat is the size of meat during the salting. In most commercial kosher processing facilities, meat is kashered in very large pieces, either whole chickens or very large pieces of beef. Since the only pieces salted were the chicken breast and chuck steak cuts used in the experiment, the surface area to volume of meat ratio was much smaller. The smaller percentage of meat surface area which is salted could contribute to a larger uptake of salt. These two reasons are also likely explanations for salt content measurements being significantly higher is the salted samples, while not significantly higher in samples bought from stores, and is consistent with the findings of the salt content decreasing in the latter post-salting washes.

The kashering process does affect the color of meat, however even the color changes that were significant were minimal. The largest change for chicken was less than 6 points for the L value, indicating the meat was darker due to salting. A 6-point difference for the L value is a perceptible change by the untrained eye in a side-by-side comparison, but this difference is not likely to be perceived in a grocery store refrigerator when looking at a shelf full of chicken packages. The beef showed a larger color change due to salting, both the a and b values changing more than 8 and 9 points, respectively. This is perceptible, and because the change for the a value is

more towards the “red” side of the scale, consumers may want to choose the more red meat sample because meat that is more red is usually perceived as fresher. This could possibly affect peoples’ decisions while the meat is fresh, but the increased salt content which can lead to faster lipid oxidation could lower the shelf life (Mariutti and Bragagolo, 2017) and make kosher meat less desirable. While the texture did show major changes throughout the process, for both chicken and beef, the salting did not have a significant change overall on the texture of meat and was also affected by washing and not just salting. These results indicate the kashering process does not likely affect the texture of meat nearly as much as color.

Chapter 5: Conclusion

For both *E. coli* O157:H7 and *Salmonella enterica* serovar Newport and both types of meat, chicken and beef, the koshering process produced a statistically significant ($p<0.05$) but marginal inactivation (~ 1 log CFU/g). The salting caused a significant rise in injury for both pathogens and both types of meat ($p<0.05$). *Salmonella* that had survived koshering on chicken showed a significant increase in thermal resistance ($p<0.05$), but no other samples did ($p>0.05$). While this study found significant reductions due to salting for all samples, none of the reductions observed were close to the amount of reduction necessary to be considered an effective kill step. The most critical step in ensuring safe meat is still the cooking step. Consumers of both not kosher and kosher meat should ensure their meat is cooked to temperatures advised by the USDA to avoid foodborne illness.

The koshering process produced significant changes in meat for most quality aspects evaluated. Kosher chicken was rated significantly higher by consumers than not kosher chicken for overall quality ($p<0.05$), but not significantly higher than salted chicken ($p>0.05$), and salted chicken was rated the saltiest of the chicken samples ($p<0.05$). Salted beef was significantly rated the highest for both overall quality and saltiness ($p<0.05$). The koshering process produced a significant color change for both chicken and beef ($p<0.05$), and significantly increased the salt content of chicken and beef ($p<0.05$). There were changes in texture for both chicken and beef observed throughout the koshering process, but these were not significant ($p>0.05$).

Chapter 6: Future studies

A further study could be run examining whether bacterial stress response proteins are exhibited immediately after the salting, and if these proteins are still found several days after salting. As most meat is consumed at least 1-2 days post salting, whether these proteins are expressed several days post salting or not may affect the safety of the meat.

A different aspect of kosher meat which may impact the safety of the meat is the exclusion of *treifos* in kosher meat production. After slaughter, the animals are checked to determine if there are any exclusionary blemishes, one example being a hole in the animal's lungs. If found, any blemishes would render the animal *treif* and cannot be considered kosher. While beyond the scope of this study, studies could be run to examine if some of these blemishes which render animals *treif* could have negative health impacts on consumers who consume *treif* animals. An aspect of the quality of kosher versus not kosher meat which could be looked at is the methods of making premium aged meat. Because of the salting process and higher salt content in kosher meat immediately post salting, there may be a difference in the aging process and microorganisms used for best aged meat products.

Appendices

A1. Dark meat chicken

A preliminary experiment was run on chicken dark meat from a chicken thigh, to test if the different composition of dark meat would produce a different microbial reduction than white meat. The same procedure for growing bacterial cultures was used as in described in section 2.1.2, and inoculation and kashering was done almost as described in section 2.1.3. The differences are the selective media, MacConkey and XLT4 agars were not used to determine level of injury, and the meat was not held an extra 2 hours at room temperature and refrigeration temperature. Otherwise, the procedure was the same as described in section 2.1.3. The one preliminary experiment run showed consistent results with the results for white meat. *E. coli* showed a 1.11 log reduction on dark meat, very close to the average 1.18 log reduction showed on the white meat. *Salmonella* showed a 0.63 log reduction on dark meat, almost identical to the average 0.62 log reduction on white meat.

A2. Freshly slaughtered chicken

Another preliminary experiment was run on freshly slaughtered white meat chicken, to test if the not kosher processing affected the meat to make bacteria more resistant or susceptible to kashering. Freshly slaughtered chicken was obtained from a local farm, and the meat was confirmed to have no processing of any kind, besides butchering, before the experiment was run. The chicken was not scalded, and was defeathered, eviscerated, and butchered by hand. The procedures used for the bacterial culture preparation, inoculation, and experiment for the freshly slaughtered

chicken were the same as the procedure used for dark meat, as outlined in Appendix A1. *E. coli* showed a 0.83 log reduction, which is lower than the average commercially prepared white meat chicken reduction of 1.18 log. However, this difference was not determined to be large enough to pursue further studies with unprocessed freshly slaughtered chicken. *Salmonella* showed a 0.90 log reduction, which is higher than the commercially prepared white meat chicken average reduction of 0.62 log. While it is interesting that *Salmonella* exhibited a greater susceptibility to kashering than *E. coli*, which is not consistent with the other results, the data from this experiment did not differ enough from the previously run commercially prepared chicken breast experiments to pursue further. A future study could be run to determine if this preliminary result is due to random variability, or if contaminated freshly slaughtered unprocessed chicken does react differently than commercially prepared chicken.

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