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The Effect of Salt Reduction on the Microbial Composition and Quality Characteristics of Sliced Roast Beef and Turkey

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THE EFFECT OF SALT REDUCTION ON THE MICROBIAL COMPOSITION AND QUALITY CHARACTERISTICS OF SLICED ROAST BEEF AND TURKEY BREAST

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THE EFFECT OF SALT REDUCTION ON THE MICROBIAL COMPOSITION AND QUALITY CHARACTERISTICS OF SLICED ROAST BEEF AND TURKEY BREAST

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This study analyzed the effects of salt reduction on microbiological composition and quality characteristics of deli-style turkey breast and roast beef. Turkey breast and roast beef were manufactured with four different salt concentrations: 1.0%, 1.5%, 2.0%, and 2.5% on a meat block basis in addition to sugar, phosphate, and water. Samples were cooked, chilled overnight, sliced, and packaged. On the day of slicing, samples were evaluated for water activity, cooking yield, proximate composition and percent salt. Samples were evaluated throughout 18w of refrigerated storage for pH, texture profile analysis, aerobic plate count (APC), and anaerobic plate count. Bacterial communities were analyzed by 16S rRNA gene sequencing. Beef with 2.5% salt had the lowest APC. Aerobic plate count increased until week 6 and then reached a plateau. Family *Pseudomonadaceae* was the dominant flora on all samples. Relative abundance of *Pseudomonadaceae* was 46.4% of all sequences in beef samples and 36.0% in turkey samples after slicing, and increased at week 2 for the remainder of storage time. Salt reduction negatively impacted cooking yield and increased water activity. Beef samples had a lower pH than turkey, and 1.5% salt had a lower pH than all other salt concentrations. Decreasing salt concentration increased hardness. In beef, gumminess and

cohesiveness increased as salt decreased. In turkey samples, the greatest gumminess and cohesiveness was observed with 1.5% salt while turkey with 2.5% salt had the least gumminess and cohesiveness. Beef samples with 1.0% salt had the greatest chewiness, followed by beef with 1.5% salt. Turkey samples with 2.5%, 2.0%, and 1.0% had the lowest chewiness values. Salt reduction decreased springiness, and turkey samples had lower values than beef. These results show that bacterial population dynamics of cooked deli meat may be more dependent on initial load than salt concentration. Furthermore, reducing salt alters the textural properties of cooked deli meats, changes the proximate composition of cooked meat products, and reduces cooking yields.

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INTRODUCTION

Excess sodium intake can lead to hypertension, which increases the chances of cardiovascular disease, heart attack, and stroke. High blood pressure is estimated to contribute to 49% of all coronary heart disease incidences and 62% of all stroke patients (Kochanek, Murphy, Xu, & Statistics, 2015). In response to high sodium intake, blood pressure increases to prevent salt and fluid accumulation by increasing total excretion of salt and water, and in cases of salt-sensitive hypertension, patients excrete less sodium in the urine, causing an increased expansion of plasma volume, increasing cardiac output and systemic vascular resistance (Karppanen & Mervaala, 2006). Americans consume nearly double the recommended daily sodium intake (USDA & HHS, 2010).

Salt is the most widely used ingredient in processed meats, and is essential to not only sensory attributes and safety of processed meats, but increases bind, texture, water holding capacity, cooking yield, and microbial shelf life of meat products (Desmond, 2006; Ruusunen & Puolanne, 2005). Furthermore, salt increases the shelf life of meat products by drawing water out of cells of both the meat and bacteria, causing a bacteriostatic effect (Albarracín, Sánchez, Grau, & Barat, 2011). Besides reducing bacterial growth overall, salt may also cause a shift in the microbial flora toward more salt tolerant bacteria, such as lactic acid bacteria, that are slower growing less detrimental to product quality and shelf life (Blickstad & Molin, 1983), further emphasizing the importance of salt as a preservative.

Previous work from our laboratory has shown that reducing salt in deli meat had a significant negative impact on flavor, texture, palatability, and cooking loss (Schroeder, 2013). Furthermore, it is well documented that the addition of salt in meat products will

help shift the microbial flora away from pseudomonads toward lactic acid bacteria (Blickstad & Molin, 1983; Miller, Liu, & Mcmullen, 2015; Ruusunen & Puolanne, 2005) which are known to be less detrimental to product quality and increase the time before spoilage (Vermeiren, Devlieghere, & Debevere, 2004). However, a threshold of salt concentration required to cause this shift is unknown, and high resolution population dynamics of cooked meat products on a species basis has rarely been evaluated. Benson et al. (2014) recently took a similar approach as the current study of profiling the spoilage microbiota of fresh pork sausage, and were able to identify obvious differences in microbial successions that otherwise would have been unnoticed using only standard plating methods to monitor bacterial growth. The current study aims to identify similar patterns in cooked roast beef and turkey breast.

The objective of this research was to identify microbiological and physiochemical changes in deli-style roast beef and turkey breast with varying salt concentrations. More specifically, this study aimed to complete the following objectives:

1. Identify changes in bacterial populations associated with varying salt concentrations in products similar to popular retail deli meats;
2. Determine the differences in processing and quality characteristics of products with reduced salt;
3. Recognize any changes to products effecting profitability from a processing perspective, such as cooking yield and shelf life.

The long term goal of this research is to lower sodium content in meat products to reduce the negative health effects typically associated with processed meats, while still

maintaining the current level of quality, sensory properties, safety, and shelf life. As health effects associated with certain meats and meat products increases, it is the responsibility of meat processors and the meat industry to strive to produce the most wholesome, healthy product possible, without sacrificing quality or safety. This research could provide the basis for development of strategies to reduce salt in processed meat products and achieve the highest value products from a quality, shelf life, and wholesomeness standpoint.

REVIEW OF LITERATURE

Introduction

Salt is one of the oldest known food preservatives, especially in meat products. Salt is essential to nearly all biological function, but is also effective as a preservative, a flavor enhancer, and also imparts its own flavor in food (Toussaint-Samat, 1992). The use of salt as a preservative can be documented as far back as the fifth to eighth century B.C. (Pearson & Gillett, 1999). Salt helps control the microbiological composition of modern processed meat products as well as quality, sensory, textural, and yield attributes. Salt has multiple functional properties in meat products: it increases binding properties of proteins which both increases water holding capacity and improves textural properties, it adds salty taste to products and serves as a flavor enhancer, and it can have bacteriostatic effects and aid in controlling the microbial flora of meat (Desmond, 2006). If salt is removed or reduced, each of these properties must be addressed individually either through the use of additional functional ingredients or altered processing techniques.

Role of Salt in Processed Meats

Salt is a unique, multi-functional ingredient in meat processing which has many distinct functions beyond flavor and preservative capacity. Salt increases the extraction of myofibrillar proteins, influencing bind, texture, and water holding capacity (Desmond, 2006). A simple reduction of salt is difficult because each of these functions must be addressed individually with a multitude of ingredients or processes.

Flavor

Saltiness is one of the basic tastes perceived by the tongue, and sodium chloride has the saltiest flavor of all sodium compounds (Doyle & Glass, 2010). According to McCaughey (2007), sodium is essential for the normal physiological function of humans, and salt taste is one of the processes developed through selective pressure in order to maintain sodium levels in the body. Because of this, only sodium or lithium containing compounds taste primarily salty to humans. While other minerals, such as potassium and calcium, can have a salty component to their taste, these can have other bitter or metallic components as well (McCaughey, 2007). On taste receptor cells, a sodium specific transduction mechanism involving epithelial sodium channels (ENaCs) is responsible for the exclusivity of sodium as a stimulus for salt taste (Chandrashekar et al., 2010). There are two types of ENaCs: One is more sodium specific, is activated at low sodium levels, and is responsible for the pleasantness of salty taste. The other is activated by multiple cations and at high sodium concentrations, and is believed to be responsible for the distaste of other cations or excessive sodium concentration (Liem, Miremadi, & Keast, 2011). This explains why salt flavor is nearly impossible to replace without the use of sodium and why common salt replacers such as potassium chloride have a similar but still off-flavor.

Because of the unique interactions of sodium and taste receptor cells, salt flavor is one of the most difficult functions to replace in reduced sodium meat products. The salt content in bacon, ham, and sausage typically ranges from 1.5 to 3.0%, while dry cured or semi-dry products are greater (Desmond, 2006). While salty taste itself is an important attribute to processed meats, salt can also act as a flavor enhancer, increasing

characteristic meat flavor (Ruusunen, Sarkka-Tirkkonen, & Puolanne, 1999). Salt concentrations below 1.5% can have a negative effect on consumer acceptability (Tobin, O'Sullivan, Hamill, & Kerry, 2012a). In ham, cured meat flavor intensity is effected by both nitrite and salt, but the greatest effects are seen by salt (Froehlich, Gullet, & Usborne, 1983). Sensory properties of turkey breast muscle were significantly improved when muscle was tumbled with salt and phosphates (Froning & Sackett, 1985) Even a moderate 20-25% salt reduction in salami and bacon negatively affected sensory properties (Aaslyng, Vestergaard, & Koch, 2014). Although fat is largely responsible for characteristic meat flavor, it has been reported that frankfurters were most acceptable to consumers with lower fat and higher salt such as 10 to 15% fat and 2.5-3.0% salt (Tobin et al., 2012a). This shows that salt content increases consumer acceptability and flavor intensity across a wide array of meat products, and it is difficult to replace that flavor in reduced salt products.

Consumers easily notice change in taste due to sodium reduction in meat products, therefore the best way to reduce sodium is for the food industry to gradually reduce salt in products (Ruusunen & Puolanne, 2005). In a normal dining setting, a nearly 50% reduction in sodium causes a noticeable difference in saltiness, but not necessarily a decrease in liking the food (Lucas, Riddell, Liem, Whitelock, & Keast, 2011). However, that is not to say that consumers' preference for salty food cannot be adjusted over time. Bertino and others (1982) reported that after a five month period of targeting a 50% reduction in dietary sodium intake, subjects' preference for salty foods was decreased. After the five month period, the rated intensity of salt in solid food increased and the salt concentration that produced the most acceptable food decreased

(Bertino et al., 1982). This supports the idea that reducing sodium over time may help to reduce consumers craving for salt, and create a consumer base more receptive to lower sodium and less salty products.

Water Holding

There are multiple theories on the exact mechanism of salt and its effects on water binding in meat. Ruusunen & Puolanne (2005) summarize the most probable theory that the chloride ion of salt binds more strongly to meat proteins, causing an increase in negative charges. These negative charges cause an increase in repulsion between the proteins producing muscle swelling, limited by the cross bridges between myofilaments. With the increased space between proteins, additional water is allowed to interact with the polar side chains of amino acids and bind to the surface of proteins via ion-dipole interactions. Once water molecules are then bound to polar portions of proteins, dipole-dipole interactions attract additional layers of water molecules. In addition, non-polar side chains push water molecules forming an arch like structure around themselves. These two forces combined create tension that forms water in a near-solid structure to not only increase the amount of water held within the protein, but to bind water more tightly throughout cooking or processing (Ruusunen & Puolanne, 2005).

It is well documented that salt aids in cooking yield and water holding abilities in meat products. In beef sausage, increasing salt from 1.0% to 2.5% led to an increase in cooking yield to such an extent that the effects of added polysaccharide gums was diminished (Xiong, Noel, & Moody, 1999). Tobin and others (2012b) found that cooking loss was negatively correlated to salt concentration in beef patties, where lower salt

concentration resulted in higher cooking loss. Similar results were observed in frankfurters (Tobin et al., 2012a; Horita et al., 2014). Likewise, both cooking loss and expressible moisture were improved with sodium addition in white chicken meat (Ros-Polski et al., 2015). These results support the theory of salt increasing protein solubility and increasing water retention.

Extraction of Myofibrillar Protein

In addition to water retention, protein solubility also directly effects bind, texture, slicing ability, and slice stability in processed meats. There is a general consensus that increasing salt concentration increases myofibrillar protein extraction and product cohesion, however salt concentrations in meat products are limited by taste to between 2-3% (Tobin et al., 2012a, 2012b). Because of this, salt is combined with other ingredients or processes to optimize protein extraction and adhesion. Bombrun and others (2014) reported that the stress required to break two pieces of pork leg bound by a tumbling exudate gel increased significantly when tumbled with salt compared to tumbling without salt. This phenomenon of binding two meat pieces or multiple smaller pieces together with the use of salt can be attributed to protein extraction. As described by Desmond (2006), when salt is added to meat products, salt-solubilized myofibrillar proteins form a sticky exudate on the surface of meat products. This exudate then forms a matrix of heat coagulated proteins, entrapping water and binding pieces to one another (Desmond, 2006). In low fat beef sausages, 1% added salt produced a relatively weak bind and 2.5% formulation was harder, more cohesive, and less deformable according to instrumental texture profile analysis (Xiong et al., 1999). Likewise, emulsified products rely on protein extraction to form the correct bind and texture. According to Aberle, Forrest,

Gerrard, & Mills (2012), myofibrillar protein solubilization and swelling takes place increasing the batter's ability to bind water, but at the same time fat droplets are reduced in size and must be held in the batter. Hydrophobic portions of the solubilized proteins will interact with and surround fat droplets, while hydrophobic portions of protein will interact with water and other proteins. This heat coagulable gel is then capable of suspending the fat droplets in the protein and water matrix and holding both fat and water throughout cooking (Aberle et al., 2012). Using scanning electron microscopy, Horita et al. (2014) found that frankfurters formulated with 1% salt had an open and spongy structure compared to 2% salt. The decrease in salt concentration caused a decrease in myofibrillar protein extraction, lowering the water holding capacity and gel strength (Horita et al., 2014). In a study by Hand and others (1987), emulsion stability of frankfurters was reduced as salt decreased as measured by the amount of fat, gel, and proteinaceous solid loss. Thus, with reduced salt meat products, myofibrillar protein extraction decreases leading to a reduction in water holding capacity, protein gelation, and emulsion stability.

Preservative Effects of Salt

To use salt by itself as a preservative would require a much greater salt addition than seen in most modern meat products. When used in combination with other hurdles or processing steps, however, salt is essential to extending shelf life and microbial safety of products. Salt works as a preservative mainly by lowering water activity and drawing water from cells of both the food and bacteria (Albarracín et al., 2011). When combined

with cooking, reduced pH, or antimicrobials, salt is an essential preservative agent. Whiting and others (1984) found that reducing salt to 1.5% resulted in a more rapid growth of the natural flora of frankfurters. A study by Madril and Sofos (1985) found that reducing sodium from 2.5% salt to 1.25% salt reduced the preservative capacity of comminuted meat products greatly. With 2.5% salt, inoculating samples with *C. sporogenes* spores reduced shelf life by nearly 4 days while the preservative capacity of products with 1.25% salt was reduced to such an extent that inoculation did not change the already short shelf life of the reduced salt products (Madril & Sofos, 1985).

In addition to an overall bacteriostatic effect of salt, it can also cause a slight shift in the microbial flora toward slower growing, gram positive bacteria species that are less detrimental to product quality and require more time to reach spoilage levels. Miller et al. (2015) reported that reduced sodium products on the retail market had a higher bacterial diversity than their standard sodium counterparts. Salt addition can slow or even halt the growth salt-sensitive microorganisms *Pseudomonas* spp. and *Enterobacteriaceae,* and the microflora will shift to more salt tolerant lactic acid bacteria (Blickstad & Molin, 1983). In fact, according to Ruusunen and Puolanne (2005), simple salt reduction in fermented meat products cannot be made because of the lower water activity required to control the microbial flora and ensure lactic acid bacteria dominate.

In addition to its own preservative effects, salt can be combined with other ingredients to achieve even greater microbial control. This application can be referred to as hurdle technology, and combines multiple parameters to further control microbial growth beyond the ability of any one of the parameters by itself (Leistner, 2000). Salt can be an essential ingredient in many different hurdle combinations or applications.

Blickstad and Molin (1983) reported that curing pork loins reinforced the inhibitory effect of CO_2 storage and increased shelf life of cured pork loins. According to Duranton and others (2012), simultaneous use of salt and moderate high pressure processing (HPP) resulted in an bacterial inactivation rate even greater than salt or high pressure alone. Similar interactions between salt and nitrite have been observed (Desmond, 2006). Sodium and potassium lactates, which are the salt forms of lactic acid, are effective at slowing microbiological growth in ground meat products, and when used in combination with salt were even more effective than lactate alone at delaying the onset of spoilage (Sallam & Samejima, 2004; Tan & Shelef, 2002). While salt used at typical concentrations may not control biological growth completely, it is an effective hurdle when used in combination with other ingredients or processes.

Additional Sources of Sodium in Meat

While salt is responsible for the majority of sodium added to meat products, many other ingredients also contribute sodium. Table 1 shows common meat ingredients and their sodium contribution when used at typical levels. Although most of these sodium compounds contribute a relatively small amount of sodium to meat product in comparison to salt, they are still a small source of sodium and should be accounted for when sodium reduction is desired. Many of these compounds are available in either the sodium or potassium form, therefore the effects of different mixtures of sodium and potassium compounds should be examined to determine their effects on the quality, sensory, and microbiological characteristics of reduced sodium meat products.

Table 1. Sodium contribution of different sodium containing compounds commonly added to meat products (adapted from Doyle & Glass, 2010)

Sodium Compound	Typical Inclusion	%Na	mg of Na/100g food
Chloride (Salt)	1.5 – 2.0%	39.34	590 – 790
Diacetate	0.1 – 0.4%	16.18	16 – 65
Lactate	1.5 – 3.0%	20.51	310 – 620
Nitrite	0.01%	33.32	4
Acid Pyrophosphate	0.35%	20.72	100
Tripolyphosphate	0.35%	31.24	160
Pyrophosphate	0.35%	34.57	170
Hexametaphosphate	0.35%	22.55	110

Sodium nitrite is one of the most commonly used and oldest known sodium compounds used in meat. Around the 19th century, saltpeter (KNO_3) was recognized as a contaminant of salt that increased preservative capacity and gave the meat a unique red color. Since then, it has been discovered that nitrate (NO_3) is converted by microorganisms to nitrite (NO_2), which converts to nitrous acid (HNO_2) or nitric oxide (NO) to react with myoglobin and perform the act of "curing" (Honikel, 2008).

In modern meat products, sodium nitrite is typically added to increase control of these products and help in the curing process. There are four main functions of nitrite in processed meats: (1) to stabilize the color of lean tissues, (2) to contribute to the characteristic flavor of cured meat, (3) to inhibit growth of a number of pathogenic and spoilage microorganisms, and (4) to retard development of rancidity (Pearson & Gillett, 1999). The ability of sodium nitrite to inhibit the growth of *Clostridium botulinum*, a spore-forming organism that produces a deadly toxin, warrants the continued use of nitrite in cured meat products (Jay, 2000). Sodium nitrite is limited by the USDA Food Safety and Inspection Service to 200ppm ingoing for immersion or pumped products,

156ppm for comminuted products, and 120ppm for bacon (USDA, 1995), which is why the sodium contribution from nitrite is minimal.

Lactate and diacetate salts are common ingredients in fully cooked, ready-to-eat (RTE) as an antimicrobial agent to reduce growth of organisms introduced to the product after cooking, during slicing or packaging. Sodium lactate is a humectant and flavor enhancer that contributes to water holding capacity and cooking yields in meat and poultry, and has been shown to be effective in limiting the growth of *Listeria monocytogenes*, without negatively effecting product pH or sensory characteristics (Chen & Shelef, 1992). Sodium diacetate is a flavoring agent and antimicrobial that is effective in controlling the growth of *L. monocytogenes* in meat products (Schlyter, Glass, Loeffelholz, Degnan, & Luchansky, 1993). Under the USDA-FSIS Compliance Guideline on controlling *Listeria* in RTE meats, lactates and diacetates are considered an "antimicrobial agent or process" to qualify for Alternative 1 or 2 which require less frequent sampling than without the use of an antimicrobial (USDA, 2014). Although sodium lactate contributes a significant amount of salt to processed meat products, using a mixture of potassium lactate and sodium diacetate can allow up to a 40% salt reduction in cooked meat products while maintaining or even increasing product shelf life (Devlieghere, Vermeiren, Bontenbal, Lamers, & Debevere, 2009).

Sodium phosphates are added to meat products to increase protein solubility, improve cooking yield and product texture (Madril & Sofos, 1985). Polyphosphates have the unique ability to cleave the actomyosin bond in muscle cells, causing muscle swelling, increased tenderness, greater water holding capacity, and improved protein solubility. Furthermore, alkaline phosphates raise the pH of meat products, increasing

the net negative charge on proteins resulting in more charged groups available for water binding (Aberle et al., 2012). Some alkaline phosphates, however, can cause soap production in meat products containing fat, therefore it is best to use blends of phosphates that produce a more neutral pH (Pearson & Gillett, 1999). With this combination of functions, we can use lower concentrations of sodium phosphate than we would of salt, decreasing sodium input from functional ingredients. If further sodium reduction is desired, potassium phosphates can also be used in place of sodium phosphates with the same functional advantages (Ruusunen, 2002).

Sodium and Human Health

Excess sodium intake can be detrimental to human health, including high blood pressure and increased risk of heart attack or stroke (Karppanen & Mervaala, 2006). The mechanisms of how salt causes these diseases are not fully understood. It has been suggested that, in response to high salt intake, people with salt-sensitive hypertension excrete less sodium in the urine than do salt resistant individuals (Doyle & Glass, 2010). Furthermore, in response to high salt intake, blood pressure increases to prevent salt and fluid accumulation by increasing total excretion of salt and water. This expansion of plasma volume causes an increase in cardiac output, and a sustained increase in systemic vascular resistance (Karppanen & Mervaala, 2006).

Processed meats receive a large amount of blame for health problems related to excess sodium intake. Meat naturally contains less than 100mg/100g of Sodium, therefore the majority of sodium is added the product in the form of salt, or sodium

chloride. Processed meats account for roughly 10% of total sodium intake in the United States population, while cold cuts alone account for 4.5% (U.S. Department of Agriculture, 2010). The current United States Department of Agriculture (USDA) dietary guidelines recommend consuming no more than 2,300mg of sodium per day, and further reducing to 1,500mg among people who are 51 and older, African American, or have hypertension, diabetes, or chronic kidney disease (U.S. Department of Agriculture, 2010). Furthermore, the World Health Organization recommends a reduction to less than 2g sodium per day to reduce blood pressure and risk of cardiovascular disease, stroke, and coronary heart disease in adults (World Health Organization, 2012). According to the USDA, Americans consume an average of 3,400mg sodium daily (U.S. Department of Agriculture, 2010).

Although processed meats have been blamed traditionally as contributors to the sodium epidemic, they can still be included in a healthy, satisfying diet. The Dietary Approaches to Stop Hypertension (DASH) diet, suggests restricting red meat to lower blood pressure, but gives little clear justification besides a strategy to reduce saturated fat intake (Vogt et al., 1999). Nowson and others (2009), reported that a low sodium DASH diet including lean red meat on most days of the week was effective in reducing blood pressure in postmenopausal women. It is also widely known that meat provides a wide variety of essential amino acids, long chain *n*-3 fatty acids, iron, zinc, selenium, vitamin D, and vitamin B12, many of which are more bioavailable in meat than in alternative sources (Williamson, Foster, Stanner, & Buttriss, 2005). In order to maintain processed meats as a choice to meet these nutritional needs, low sodium alternatives should be explored.

Sodium Reduction in Processed Meats

A simplistic approach to lowering sodium intake from meat products would be to remove some or all of the added salt, however with the multiple functions salt plays in processed meats, it is difficult to produce similar quality products with a simple salt reduction. In order to successfully lower sodium addition significantly, the multiple functions of salt must be replaced with either added ingredients or modified processing techniques.

Besides flavor, the sodium ion itself is not necessarily required for most of the functions of salt, therefore salt substitutes such as potassium chloride (KCl) can replace many of the functions of salt. Their inclusion, however, is limited by taste (Desmond, 2006). A study by Horita and others (2014) showed that emulsion stability of franks, as determined by percent total liquid and fat released, was maintained by replacing up to 50% Salt with KCl, while both a 50% CaCl substitution and simply reducing sodium by 50% resulted in a decrease in emulsion stability. In this same study, sensory attributes and overall acceptance by consumers was reduced in 50% KCl substitution, while CaCl substitution was equal to the full salt control (Horita et al., 2014). Therefore, KCl can replace the functionality of salt but can produce off flavors, while CaCl has little impact on flavor but reduces protein solubility and emulsion stability. Sea salt is another low sodium alternative used in meat products. In a study by Pietrasik and Gaudette (2014), sea salts containing 60% less sodium had no detrimental effect on water binding and texture, nor did they effect consumer acceptability attributes. In a similar study, bind strength of hams did not differ between 45% or 60% reduced sodium sea salt and the control formulation (Pietrasik & Gaudette, 2014b). This shows that low sodium salt

alternatives can be used to produce products of similar quality as their full sodium counterparts.

In some products, altered processing techniques can improve the quality and texture of products when reduced sodium products are desired. Preblending involves grinding and mixing part of the meat with all or most of the salt and cure, and then added to the rest of the meat block to create desired product composition. This process allows for the creation of a greater salt concentration to maximize protein solubility, and then precise control over product composition once blended with the remaining amount of meat to the desired salt and cure concentration (Pearson & Gillett, 1999). Puolanne & Terrell (1983) reported that sausages made with a 2, 3, or 4% preblend had greater water binding capacity and less released fat than unsalted preblends, indicating that the preblending process allowed for a greater release of functional proteins. Tumbling and massaging are two processes used commonly in sectioned and formed products, and greatly aid in the process of protein extraction. Tumbling involves a large stainless steel drum lined with baffles. As the tumbler rotates, the product is lifted and dropped, or tumbled, causing the protein matrix to accumulate on the surface and the meat to become pliable. This allows greater salt and cure absorption and increases protein extraction (Pearson & Gillett, 1999). Tumbling of boneless, cured hams improved external appearance, color, sliceability, taste and aroma, and yield, and tumbling 18 hours intermittently resulted in even greater product quality improvement than did 3 hour continuous tumbling (Krause, Ockerman, Krol, Moerman, & Plimpton, 1978). This shows that tumbling of products aids in protein extraction and product quality, and also supports the idea that intermittent tumbling and resting time is also essential to the

tumbling process (Pearson & Gillett, 1999). Massaging works similarly to tumbling, but is more of a gentle stirring or agitation of the meat, similar to mixing but designed for large chunks of meat (Pearson & Gillett, 1999). Massaging of low fat bologna formulations increased Instron TPA values as well reduced the amount of purge loss, indicating that massaging is also a viable option for increasing water retention and product bind.

Similar to protein solubilization, the antimicrobial functions of salt are not specific to the Na^+ ion, but rather come from the ionic strength of Salt. Therefore, in theory, we can replace salt with another substitute and achieve similar antimicrobial function, as long as the ionic strength is similar. When calculated on a molar basis, KCl had an equivalent antimicrobial effect of Salt on the growth of *A. hydrophila*, *E. sakazakii*, *S. Flexneri*, *Y. enterocolitica*, and three strains of *S. aureus* (Bidlas & Lambert, 2008). Likewise, mesophilic aerobic counts and salt tolerant flora counts were unaffected by substitution of 50% KCl or 45% blend of KCl, CaCl, and $MgCl_2$ compared to purely Salt (Blesa et al., 2008). Thus, salt substitutions have little to no effect on the microbial composition of meat products compared to straight salt.

Microbial Spoilage of Meat

From a microbiological standpoint, shelf life is defined as the storage time until spoilage (Borch, Kant-Muermans, & Blixt, 1996). Microbial spoilage can be either a determined amount of bacterial growth, or noticeable off-odors or flavors due to bacteria or their by-products. With all types of meat and meat products, refrigeration is the main

method of preservation, selecting for slower growing psychrophilic organisms. In cooked meats, the cooking process eliminates nearly all microorganisms with the exception of spore-formers. Therefore, any spoilage organisms on cooked meat products are from post-lethality contamination (Borch et al., 1996).

According to Borch and others (1996) the dominate flora of cooked sliced meat products is a mixture of *Bacillus* spp., *Micrococcus* spp., and *Lactobacillus* spp., while *Pseudomonas* spp. can increase up to 10^5 cfu/g. If spoilage of fresh meat is dominated by *Pseudomonas* spp., high growth rate limits the shelf life of products to a matter of days (Davies, 2003). If we can slow *Pseudomonas* spp. growth, such as in vacuum packaged meats, lactic acid bacteria (LAB) will dominate and shelf life may be increased to up to 10-12 weeks (Egan, 1983). Egan (1983) also notes that the presence of a flora of psychrotrophic LAB on vacuum packaged chilled meats ensures that shelf life is maximal. The addition of salt to meat products can slow or inhibit the growth of salt sensitive *Pseudomonas* spp., shifting the population towards LAB and extending shelf life (Blickstad & Molin, 1983). In fact, certain strains of LAB can even be added to cooked meat products for use as a protective culture to slow spoilage (Vermeiren et al., 2004). Therefore, any steps that can be taken to shift the flora of cooked meat products away from gram negative *Pseudomonas* spp. and toward gram positive LAB may increase shelf life. Since salt addition is one such method, it is important to know how reducing salt concentration may effect this shift.

Conclusion and Further Questions

Data is currently limited on how varying salt concentrations effect the growth of different populations of microorganisms. The general consensus is that as we increase salt, bacterial growth will decrease and shelf life is improved (Madril & Sofos, 1985; Whiting et al., 1984). Furthermore, adding salt to meat products causes a shift toward the more salt tolerant lactic acid bacteria (Blickstad & Molin, 1983; Ruusunen & Puolanne, 2005). However, it has not been documented exactly how varying salt concentrations within typical inclusion levels (1.0-2.5%) alter the microbiota of cooked meats over time. Benson and others (2014) used deep pyrosequencing of 16S rRNA gene amplicons to determine the effects of lactate and diacetate on the microbial successions of refrigerated fresh pork sausage. The purpose of this study is to use similar advanced next generation sequencing methods to identify population shifts of cooked turkey and roast beef deli slices from day 0 after processing to week 18. Likewise, we will observe total aerobic and anaerobic plate counts in order to quantify growth of different microorganisms. Quality parameters including pH, objective color, and texture profile analysis will also be observed over time, as well as water activity and percent salt the day after processing. Our results should shed some light on how reducing salt content effects microbial spoilage and shelf life of cooked deli meats.

Materials and Methods

Deli Meat Processing

For three independent replications, boneless beef top round (*semimembranosus*) were attained from the Loeffel Meat Laboratory (University of Nebraska, Lincoln, NE) and boneless, skinless, turkey breast (*pectoralis*) were purchased from a wholesale

distributor and delivered to the Loeffel Meat Laboratory for processing. All meat was stored frozen until use, and tempered at 2°C for approximately 3-4 days prior to processing. Turkey and beef muscles were trimmed of external fat and connective tissue, and separately ground through a 12.7 mm plate using a Hobart Meat Grinder (Model #4734; Hobart MFG. Co., Troy, OH). Within a specie, meat was then mixed by hand to ensure a homogeneous mixture between treatments prior to batching into 9.1kg batches.

Within each replication, two species (beef [B] and turkey [T]) and four different formulations were used according to salt concentrations based on meat block: 1.0% NaCl, 1.5% NaCl, 2.0% NaCl, and 2.5% NaCl. A brine for 25% extension was formulated and added to water to achieve each of the target salt concentrations above, 1.0% sugar, and 0.35% sodium phosphate (Brifisol 85 Instant, Bk Giulini, Ladenburg, Germany) on a meat block basis. Ground meat and brine were added to a vacuum tumbler (Model DVTS R2-250; Daniels Food Equipment, Parkers Prairie, MN) and were then tumbled under vacuum (66.7 kPa) at 4°C for 90 minutes. Each treatment batch was stuffed using a vacuum stuffer (Vemag Robot 1000 DC; Reiser, Canton MA) into 90mm x 24" pre-stuck fibrous casings (Kalle; Gurnee, IL) and casings were pulled (50psi) and clipped using a Tipper Clipper (Model PR465L; Tipper Tie, Inc., Apex, NC). Chubs within treatment were hung on a smokehouse stick, weighed, and thermally processed in an Alkar smokehouse (Alkar-RapidPak Inc., Lodi, WI) to an internal temperature of 71°C, followed by a 30 minute cold water shower. Products were chilled overnight. Replications were produced on three separate days.

The day after processing, turkey and roast beef rolls were weighed, casing removed, and sliced (Bizerba Model SE12; Bizerba, Balingen, Germany) into 2mm slices

for microbiological sampling and 13mm slices for quality and texture analysis. One slice from each of the two chubs per treatment was then placed into each 3mil std barrier nylon/PE vacuum pouch, vacuum sealed (Multivac Model C500; Multivac Inc., Kansas City, MO), and placed in a covered plastic lug and stored at 1-2°C until sampling. Water activity and salt content were measured on the day of slicing, and shelf life parameters were measured every two weeks starting on the day of slicing until 18w shelf life.

Microbial Analysis

Microbial analysis was performed by sampling from a package of two slices per treatment starting on the day of slicing (week 0) and continuing every two weeks until week 18. Samples were aseptically transferred from the vacuum pouch into a WhirlPak bag (Nasco, Fort Atkinson, WI), combined with 50ml of BBL Peptone water (Becton, Dickinson and Company, Franklin Lakes, NJ) and placed in a bag blender (bioMerieux Inc., Durham, NC) for 3 minutes to homogenize sample. Two 2ml samples were collected from sample and stored at -20°C until DNA extraction. If necessary, serial tenfold dilutions were made prior to plating. An Eddy Jet spiral plater (IUL, S.A., Barcelona, Spain) was used to plate 50µl of sample on Brain, Heart, Infusion agar (Thermo Fisher Scientific, Waltham, MA) and incubated at 37°C, and for 48h. Colonies were then counted manually according to EddyJet owner's manual method. Bacterial counts were converted to $\log_{10}$ colony forming units (CFU)/gram of sample.

Analysis of Bacterial Communities

Bacterial community analysis was performed using the MiSeq Illumina Sequencing Platform as outlined by Kozich, Westcott, Baxter, Highlander, & Schloss

(2013). Microbial DNA extraction was performed using a modified version of the Epicentre QuickExtract DNA extraction protocol. A 1ml sample was centrifuged at 10,000xG for 10 minutes at 20°C, supernatant was removed, and 500µl of QuickExtract solution (Epicentre, Madison, WI) was added to the pellet. Sample was then vortexed, incubated at 65°C for 10 minutes, vortexed again, and incubated at 98°C for 2 minutes.

The V4 region of the 16S rRNA gene was amplified using barcoded primers in a 20µl volume. A PCR reaction contained 0.625 Units of Terra PCR Direct Polymerase (Clontech Laboratories Inc., Mountain View, CA), 1X reaction buffer, 160 µmol dNTPs (Clontech Laboratories Inc., Mountain View, CA), 250 nmol of each primer, and 4 µl of sample extract. The following cycle was used for PCR amplification of the 16S rRNA gene using a Veriti 96 well thermocycler (Thermofisher Scientific, Walther, MA):

98°C	2 min	
98°C	30 sec	30 cycles
58°C	30 sec	
68°C	45 sec	
68°C	4 min	

PCR products were then normalized using an Invitrogen Sequal Prep Normalization Kit (Thermofisher Scientific, Walther, MA) and following manufacturer's directions for binding, washing, and elution steps to yield ~5ng DNA per well. Barcoded PCR products were pooled and gel purified using the Pippin Prep system (Sage Science, Inc., Beverly, MA). Final concentration was determined using quantitative PCR (qPCR),

and DNA were sequenced using Illumina MiSeq platform (Illumina, Inc., San Diego, CA).

Sequences were sorted and analyzed according to the pipeline described by Anderson, Schneider, Erickson, Macdonald, and Fernando (2016). Reads from Illumina sequencing were filtered initially to remove reads not matching the following criteria: (i) having a complete forward primer and barcode; (ii) no ambiguous bases ('N'); (iii) sequence length between 200 nt and 500nt; (iv) average quality score ≥ 20. Additional quality filtering steps were applied to remove sequences with two or more errors in the barcode, more than one error in the forward primer, or more than two errors in the reverse primer using QIIME (ver. 1.9.1; Caporaso et al., 2010). Furthermore, mothur (ver. 1.36.1; Schloss et al., 2009) was used to remove sequences <245 and >275 bp. Sequences were clustered into OTUs based on 97% similarity according to the UPARSE pipeline with both *de novo* and reference based chimera removal of nonsingleton reads (Edgar, 2013).

Representative sequences for each OTU were assigned taxonomy with the UCLUST consensus taxonomy assigner (QIIME default) method with Greengenes database release 119 (23) as reference sequences. Only a portion of the OTUs could be classified at the genus level (41.7% of OTUs, 95.5% of the reads), however a majority of the reads were classified at family level (70.9% of OTUs, 99.2% of the reads). Therefore, results are discussed at family level.

Cooking Yield, Water Activity, and Salt Content

Cooking yield was calculated by weighing each treatment batch (2 chubs/stick) prior to going in the smokehouse, and again after overnight stabilization prior to slicing. Cooking yield was calculated as cooked weight as a percentage of raw weight [cooked weight/raw weight] x 100. Samples used for water activity and salt concentration were homogenized using a food processor (Black & Decker Handy Chopper, Black & Decker Inc., Baltimore, MD). Water activity was measured the day of slicing using an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA) according to manufacturer's specifications. Ground samples were placed in Aqualab water activity cups (Decagon Devices, Inc., Pullman, WA) with approximately enough sample to cover the entire bottom of the cup. Salt concentration was measured the day of slicing according to Sebranek, Lonergan, King-Brink, Larson, & Beermann (2001) by adding 90ml of boiling water to 10g of ground sample, stirring, and straining water to measure using Quantab high range chloride titration strips (Hach Company, Loveland, CO).

Texture Profile Analysis (TPA)

Texture profile analysis was measured every two weeks throughout storage by cutting 13mm slice into a 4.0cm x 4.0cm square, and measured using a 2,500 kg load cell on an Instron (Model number 1123; Instron Worldwide, Norwood, MA) with a 140mm plate. Each slice was compressed with a head speed of 30 mm/min to 75% of its original thickness two times, and the characteristics of hardness, springiness, cohesiveness, gumminess, and chewiness according to Bourne (1978). Hardness was measured as the maximum force during the first cycle of compression. Springiness was calculated as the amount of elastic recovery of the sample, as a ratio of the two times of compression from start to finish. Cohesiveness was calculated as a ratio of the area under both compression

curves. Gumminess was calculated as hardness x cohesiveness. Chewiness was calculated as gumminess x springiness.

Proximate Composition

Moisture, fat, protein, and ash of pulverized raw meat samples were determined. Two g of pulverized tissue prepared in duplicate were used to quantify moisture and ash using a LECO thermogravimetric analyzer (LECO Corporation, St. Joseph, MI). Total fat was determined as outlined by AOAC (1990) using the Soxhlet extraction procedure. Protein was measured using a LECO Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, MI).

Objective Color

Objective color (L^*, a^*, b^*) of deli slices was measured using a colorimeter (Chroma Meter CR-400; Konica Minolta Sensing Americas, Inc., Ramsey, NJ) using a 2° standard observer and a D65 illuminant. The calibration plate and samples were read through Saran plastic wrap to keep from contaminating the colorimeter lens. Six readings were taken from the two 13mm slices within a treatment for each storage time and averaged for color values. Measures of ΔE, change in color, were calculated using the following formula: $\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$ with ΔE calculated for the difference between week 0 and week of observation (Minolta Co., n.d.)

pH

Cooked meat pH was measured every two weeks throughout storage in duplicate using an Orion 410A+ pH meter (Thermo Electron Corporation, Waltham, MA) on a mixture of 10g of cooked meat sample in 90ml of double distilled water.

Statistical Analysis

Water activity, proximate composition, salt, and cooking yield were analyzed as a 4 x 2 factorial (Salt concentration x meat species) using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Objective color, pH, TPAs, and bacterial growth were analyzed as a 4 x 2 x 10 factorial (Salt x meat species x storage time) using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA). For relative abundance of bacterial taxa, weeks 0-14 were analyzed. Relative abundance data were analyzed as a 4 x 2 x 8 factorial (salt x meat species x storage time) using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA). All means were separated using the LSMEANS function with PDIFF option and Tukey's adjustment for least significant difference (LSD) at $P < 0.05$.

In order to identify bacterial communities characterizing differences between treatments, an algorithm for high-dimensional biomarker discovery was used. To perform this analysis, LDA Effect Size (LEfSE; Segata et al., 2011) was used to identify differences in the bacterial community related to salt concentration over storage time. Beef and turkey samples were analyzed independently using LEfSE.

LITERATURE CITED

Aaslyng, M. D., Vestergaard, C., & Koch, A. G. (2014). The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science*, *96*(1), 47–55.

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2012). *Principles of Meat Science* (5th ed.). Dubuque, IA: Kendall Hunt Publishing.

Albarracín, W., Sánchez, I. C., Grau, R., & Barat, J. M. (2011). Salt in food processing; usage and reduction: a review. *International Journal of Food Science & Technology*, *46*(7), 1329–1336.

AOAC. (1990). *AOAC: Official Methods of Analysis* (15th ed.). Arlington, VA: Association of Official Analytical Chemists.

Anderson, C.L., Schneider, C.J., Erickson, G.E., MacDonald, J.C., Fernando, S.C. (2016). Rumen bacterial communities can be acclimated faster to high concentrate diets than currently implemented feedlot programs. *Journal of Applied Microbiology*, *120,* 588-599.

Benson, A. K., David, J. R. D., Gilbreth, S. E., Smith, G., Nietfeldt, J., Legge, R., Kim, J., Sinha, R., Duncan, C. E., Junjie, M., Singh, I. (2014). Microbial successions are associated with changes in chemical profiles of a model refrigerated fresh pork sausage during an 80-day shelf life study. *Applied and Environmental Microbiology, 79* (17), 5112-5120.

Bertino, M., Beauchamp, G. K., & Engelman, K. (1982). Longterm reduction in dietary sodium alters the taste of salt. *American Journal of Clinical Nutrition*, *36*, 1134–1144.

Bidlas, E., & Lambert, R. J. W. (2008). Comparing the antimicrobial effectiveness of NaCl and KCl with a view to salt/sodium replacement. *International Journal of Food Microbiology*, *124*(1), 98–102.

Blesa, E., Aliño, M., Barat, J. M., Grau, R., Toldrá, F., & Pagán, M. J. (2008). Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. *Meat Science*, *78*(1-2), 135–142.

Blickstad, E., & Molin, G. (1983). Carbon-Dioxide as a controller of the spoilage flora of pork, with special reference to temperature and sodium-chloride. *Journal of Food Protection*, *46*(9), 756–766.

Bombrun, L., Gatellier, P., Carlier, M., & Kondjoyan, A. (2014). The effects of low salt concentrations on the mechanism of adhesion between two pieces of pork semimembranosus muscle following tumbling and cooking. *Meat Science*, *96*(1), 5–13.

Borch, E., Kant-Muermans, M. L., & Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology*, *33*(1), 103–120.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology*, *32*, 62-66.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. a, Hummler, E., Ryba, N. J. P., & Zuker, C. S. (2010). The cells and peripheral representation of sodium taste in mice. *Nature*, *464*(7286), 297–301.

Chen, N., & Shelef, L. A. (1992). Relationship between water activity, salts of lactic acid, and growth of Listeria monocytogenes in a meat model system. *Journal of Food Protection*, *55*(8), 574–578.

Davies, A. R. (2003). Modified atmospheres and vacuum packaging. In N. J. Russell & G. Gould (Eds.), *Food Preservatives SE - 11* (pp. 218–239). Springer US.

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science*, *74*(1), 188–196.

Devlieghere, F., Vermeiren, L., Bontenbal, E., Lamers, P. P., & Debevere, J. (2009). Reducing salt intake from meat products by combined use of lactate and diacetate salts without affecting microbial stability. *International Journal of Food Science and Technology*, *44*(2), 337–341.

Doyle, M. E., & Glass, K. A. (2010). Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. *Comprehensive Reviews in Food Science and Food Safety*, *9*(1), 44–56.

Duranton, F., Guillou, S., Simonin, H., Chéret, R., & De Lamballerie, M. (2012). Combined use of high pressure and salt or sodium nitrite to control the growth of endogenous microflora in raw pork meat. *Innovative Food Science and Emerging Technologies*, *16*, 373–380.

Edgar, R.C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods 10*, 996-998.

Egan, A. F. (1983). Lactic acid bacteria of meat and meat products. *Antonie van Leeuwenhoek*, *49*(3), 327–336.

Froehlich, D. A., Gullet, E. A., & Usborne, W. R. (1983). Effect of nitrite and salt on the color, flavor and overall acceptability of ham. *Journal of Food Science*, *48*(1), 152–154.

Froning, G. W., & Sackett, B. (1985). Effect of salt and phosphates during tumbling of turkey breast muscle on meat characteristics. *Poultry Science*, *64*(7), 1328–1333.

USDA. (2014). FSIS Compilance Guideline: Controlling Listeria Monocytogenes in Post-lethaly Exposed Ready-to-Eat Meat and Poultry Products. Available online: http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/compliance-guides-index#Listeria

Hand, L. W., Hollingsworth, C. A., Calkins, C. R., & Mandigo, R. W. (1987). Effects of Preblending, Reduced Fat and Salt Levels on Frankfurter Characteristics. *Journal of Food Science*, *52*(5), 1149–1151.

Honikel, K.O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, *78*(1-2), 68–76.

Horita, C. N., Messias, V. C., Morgano, M. A., Hayakawa, F. M., & Pollonio, M. A. R. (2014). Textural, microstructural and sensory properties of reduced sodium frankfurter sausages containing mechanically deboned poultry meat and blends of chloride salts. *Food Research International*, *66*(0), 29–35.

Jay, J. M. (2000). *Modern Food Microbiology* (6th ed.). Gaithersburg, MD: Aspen Publishers.

Karppanen, H., & Mervaala, E. (2006). Sodium Intake and Hypertension. *Progress in Cardiovascular Diseases*, *49*(2), 59–75.

Kochanek, K. D., Murphy, S. L., Xu, J., & Statistics, V. (2015). Deaths : Final Data for 2011, *National Vital Statistics Reports 63*(3), 1-17.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120.

Krause, R. J., Ockerman, H. W., Krol, B., Moerman, P. C., & Plimpton, R. F. (1978). Influence of tumbling, tumbling time, trim and sodium tripolyphosphate on quality and yield of cured hams. *Journal of Food Science*, *43*(3), 853–855.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, *55*(1-3), 181–186.

Liem, D. G., Miremadi, F., & Keast, R. (2011). Reducing Sodium in Foods: The Effect on Flavor. *Nutrients*, *3*(12), 694–711.

Lucas, L., Riddell, L., Liem, G., Whitelock, S., & Keast, R. (2011). The influence of sodium on liking and consumption of salty food. *Journal of Food Science*, *76*(1), 72–76.

Madril, M., & Sofos, J. (1985). Antimicrobial and functional effects of six polyphosphates in reduced NaCl comminuted meat products. *Lebensmittel-Wissenschaft Technologie*, *18*(5), 316–322.

McCaughey, S. (2007). Dietary salt and flavor: mechanisms of taste perception and physiological controls. In *Reducing Salt in Foods: Practical Strategies* (pp. 77–98).

Miller, P., Liu, X., & Mcmullen, L. M. (2015). Microbiota of regular sodium and sodium-reduced ready-to-eat meat products obtained from the retail market. *Canadian Journal of Microbiology*, *154*, 150–154.

Minolta Co. (n.d.). Precise color communication: color conrol from perception to instrumentation.

Nowson, C. A., Wattanapenpaiboon, N., & Pachett, A. (2009). Low-sodium Dietary Approaches to Stop Hypertension-type diet including lean red meat lowers blood pressure in postmenopausal women. *Nutrition Research (New York, N.Y.)*, *29*(1), 8–18.

Pearson, A. M., & Gillett, T. A. (1999). *Processed Meats* (Third ed.). Gaithersburg, MD: Aspen Publishers.

Pietrasik, Z., & Gaudette, N. J. (2014a). The impact of salt replacers and flavor enhancer on the processing characteristics and consumer acceptance of restructured cooked hams. *Journal of the Science of Food and Agriculture*, *96*(3), 1165–1170.

Pietrasik, Z., & Gaudette, N. J. (2014b). The impact of salt replacers and flavor enhancer on the processing characteristics and consumer acceptance of restructured cooked hams. *Meat Science*, *96*(3), 1165–1170.

Puolanne, E. J., & Terrell, R. N. (1983). Effects of Salt Levels in Prerigor Blends and Cooked Sausages on Water Binding, Released Fat and pH. *Journal of Food Science*, *48*(4), 1022–1024.

Ros-Polski, V., Koutchma, T., Xue, J., Defelice, C., & Balamurugan, S. (2015). Effects of high hydrostatic pressure processing parameters and NaCl concentration on the physical properties, texture and quality of white chicken meat. *Innovative Food Science & Emerging Technologies*, *30*, 31–42.

Ruusunen, M. (2002). Sodium reduction in cooked meat products by using commercial potassium phosphate mixtures. *Agricultural and Food Science in Finland*, *11*(August 2001), 199–207.

Ruusunen, M., & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science*, *70*(3), 531–541.

Ruusunen, M., Sarkka-Tirkkonen, M., & Puolanne, E. (1999). The effect of salt reduction on taste pleasantness in cooked “bologna type” sausages. *Journal of Sensory Studies*, *14*(2), 263–270.

Sallam, K. I., & Samejima, K. (2004). Microbiological and chemical quality of ground beef treated with sodium lactate and sodium chloride during refrigerated storage. *LWT - Food Science and Technology*, *37*(8), 865–871.

Schlyter, J. H., Glass, K. A., Loeffelholz, J., Degnan, A. J., & Luchansky, J. B. (1993). The effects of diacetate with nitrite, lactate, or pediocin on the viability of Listeria monocytegenes in turkey slurries. *International Journal of Food Microbiology*, *19*(4), 271–281.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J.,Weber, C.F. (2009). Introducing mothur: open-source, platform independent, community-supported software for describing and comparing microbial communities. *Applied Environmental Microbiology 75*, 7537-7541.

Schroeder, D. J. (2013). *Effect of Vacuum Tumbling Time , Salt Level , and Phosphate Alternatives on Processing Characteristics of Natural Deli-style Turkey Breast.* M.S. Thesis, University of Nebraska-Lincoln, Lincoln, NE.

Sebranek, J., Lonergan, S., King-Brink, M., Larson, E., & Beermann, D. (2001). *Meat science and processing* (3rd ed.). Zenda, WI: Peerage Press.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60.

Tan, W., & Shelef, L. a. (2002). Effects of sodium chloride and lactates on chemical and microbiological changes in refrigerated and frozen fresh ground pork. *Meat Science*, *62*(1), 27–32.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., & Kerry, J. P. (2012a). Effect of varying salt and fat levels on the sensory and physiochemical quality of frankfurters. *Meat Science*, *92*(4), 659–666.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., & Kerry, J. P. (2012b). Effect of varying salt and fat levels on the sensory quality of beef patties. *Meat Science*, *91*(4), 460–465.

Toussaint-Samat, M. (1992). *History of Food*. Barnes and Noble. New York.

U.S. Department of Agriculture. (2010). Dietary Guidelines 2010.

USDA. (1995). *Processing Inspectors' Calculations Handbook*. Washington, DC.

USDA, & HHS. (2010). *Dietary Guidelines for Americans, 2010*. Washington, DC.

Vermeiren, L., Devlieghere, F., & Debevere, J. (2004). Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology*, *96*(2), 149–164.

Vogt, T. M., Appel, L., Obarzanek, E., Moore, T. J., Vollmer, W. M., Svetkey, L., Sacks, F.M., Bray, G.A., Cutler, J.A., Windhauser, M.M. Lin, P.H., Karanja, N. M. (1999). Dietary Approaches to Stop Hypertension: Rationale, design, and methods. *Journal of the American Dietetic Association*, *99*(8), S12–S18.

Whiting, R., Benedict, R., Kunsch, C., & Woyochik, J. (1984). Effect of sodium chloride levels in frankfurters on the growth of Clostridium sporogenes and Staphylococcus aureus. *Journal of Food Science*, *49*(2), 351–355.

Williamson, C. S., Foster, R. K., Stanner, S. a, & Buttriss, J. L. (2005). Red Meat in the Diet. *BNF Nutrition Bulletin*, *30*(4), 323–355.

World Health Organization. (2012). Guideline: Sodium intake for adults and children, 1–56. Availale online: http://www.who.int/nutrition/publications/guidelines/sodium_intake_printversion.pdf

Xiong, Y. L., Noel, D. C., & Moody, W. G. (1999). Textural and sensory properties of low-fat beef sausages with added water and polysaccharides as affected by pH and salt. *Journal of Food Science*, *64*(3), 550–554.

MANUSCRIPT

The effect of salt reduction on the microbial composition and quality characteristics of sliced roast beef and turkey breast

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ABSTRACT

This study analyzed the effects of salt reduction on microbiological composition and quality characteristics of deli-style turkey breast and roast beef. Turkey breast and roast beef were manufactured with salt concentrations of 1.0%, 1.5%, 2.0%, and 2.5% on a meat block basis in addition to sugar, phosphate, and water. Samples were cooked, chilled overnight, sliced, and packaged. On the day of slicing, samples were evaluated for water activity, cooking yield, proximate composition and percent salt. Samples were evaluated throughout 18w of refrigerated storage for pH, texture profile analysis, and aerobic and anaerobic plate counts. Bacterial communities were analyzed by sequencing of 16S rRNA from the V4 region. Beef with 2.5% salt had the lowest APC. Family *Pseudomonadaceae* was dominant on all samples throughout storage time. Salt reduction negatively impacted cooking yield and reduced water activity. Decreasing salt concentration increased hardness. Salt reduction decreased springiness. These results show that bacterial population dynamics of cooked deli meat may be more dependent on initial load than salt concentration. Furthermore, reducing salt alters the textural properties of cooked deli meats and reduces cooking yields.

Keywords: Salt, turkey, beef, microbiota, quality

INTRODUCTION

Excess sodium intake has been a health concern for many years, however pressure to further reduce sodium in the diet has recently surfaced and increased the demand for lower sodium foods. Recommendations from public health agencies for consumers to reduce sodium intake as well as producers to decrease added sodium in processed foods

has been a driving factor behind this demand. Excess sodium intake can be detrimental to human health and can be a contributor to high blood pressure and increased risk for heart disease (Karppanen & Mervaala, 2006). According to the latest United States Department of Agriculture (USDA) dietary guidelines, Americans consume an average of 3,400mg of sodium daily, compared to the recommended 2,300mg per day. People who are 51 and older, African American, or have hypertension, diabetes, or chronic kidney disease are recommended to further reduce sodium intake to 1,500mg daily (USDA & HHS, 2010).

Salt plays an important role in processing and preservation of further processed meat products. Fresh meat naturally contains very little sodium, therefore the majority of sodium associated with meats is added in the form of sodium chloride (NaCl) and other sodium containing ingredients. Roughly 77% of dietary sodium comes from salt added during processing, while salt added at the table and during home cooking contribute 6.2% and 5.1%, respectively (Mattes RD, 1991). Processed meats account for roughly 10% of total sodium intake in the United States; the subcategory of cold cuts account for 4.5% (USDA & HHS, 2010). Most processed meat products contain between 1.5 and 2.5% added salt, and salt is limited by taste to a maximum of 2-3% (Tobin, O'Sullivan, Hamill, & Kerry, 2012, 2013). The Dietary Approaches to Stop Hypertension (DASH) suggests restricting red meat, but gives little clear justification (Vogt et al., 1999). However, a low sodium Dash-type diet including red meat can be successful in reducing blood pressure (Nowson, Wattanapenpaiboon, & Pachett, 2009). With the obvious health advantages of consuming meat, reduced sodium processed meat products should be investigated in

order to continue providing a convenient way of including meat in a balanced diet without increasing the risks of excess sodium consumption.

The importance of salt in processed meat products has been well documented. Salt is a multi-functional ingredient that helps alter the texture, flavor, bind, water holding capacity, and microbial growth processed meats. In meat products, sodium reduction is not as simple as just removing or reducing salt; each one of its functions needs to be addressed individually using additional ingredients or processing techniques. The most intuitive function of salt is the flavor imparted on products from the sodium ion. Simply reducing salt in meat products can negatively affect sensory properties and consumer acceptability (Aaslyng, Vestergaard, & Koch, 2014; Tobin et al., 2012). Reducing sodium intake over an extended period of time can lead consumers to change their taste preference toward less salty foods (Bertino, Beauchamp, & Engelman, 1982). Thus, consumers easily notice the change in taste due to sodium reduction, so reduction should be carried out slowly over time. Salty taste also enhances the perception of meat flavor (Bertino, et al., 1982). Besides taste, salt is also essential to the processing characteristics of meat products. Added salt extracts protein, increasing muscle to muscle bind, as well as water and fat binding (Ruusunen & Puolanne, 2005). These functions can somewhat be replicated with less added sodium, however it requires the use of multiple ingredients or processing techniques in order to replace each of the functions of salt.

One of the main and certainly oldest functions of salt is preservation. The main preservative action of salt is lowering water activity and drawing out water from cells of both the food and bacteria (Doyle & Glass, 2010). In the concentrations typically used in meat products, salt is not the sole mode of preservation, however it is effective when used

in combination with other preservative actions such as cooking, packaging, reduced pH, or other antimicrobial ingredients (Leistner, 2000). Still, a reduction in salt causes a more rapid growth of the natural flora of cooked meats resulting in a shortened shelf life (Whiting, Benedict, Kunsch, & Woyochik, 1984). Besides an overall reduction in bacterial growth, salt addition will also cause a shift from gram-positive *Pseudomonas* and *Enterobacteriaceae* toward more salt tolerant lactic acid bacteria (LAB; Blickstad & Molin, 1983). Lactic acid bacteria results in odors and off-flavors that are less offensive and take longer to spoil than products dominated by *Pseudomonas* (Egan, 1983). In fact, it has been proposed that certain strains of LAB can actually be added to cooked meat products for use as a protective culture to slow spoilage (Vermeiren, Devlieghere, & Debevere, 2004). Shifting the microbial flora of meat products toward LAB an effective way to maximize shelf life, and salt addition is one strategy to accomplish this shift.

With growing concerns about sodium intake and associated health issues and with the demand for reduced sodium products on the rise, the meat industry must address these concerns by making reduced sodium processed meat products with equivalent quality, safety, and shelf life of their full-sodium counterparts. It has been widely reported that simple salt reduction causes a decrease in shelf life of meat products due the increased growth of spoilage organisms. However, it has not been shown exactly how spoilage populations change in processed meat products depending on added salt within the range of near-typical salt inclusion. The aim of this study is to use advanced DNA sequencing to map microbial population shifts over time in deli turkey breast and roast beef formulated with different salt concentrations to understand how the reduction of salt effects the shelf life and quality characteristics of cooked deli meat products.

MATERIALS AND METHODS

Deli Meat Processing

For three independent replications, boneless beef top round (*semimembranosus*) were attained from the Loeffel Meat Laboratory (University of Nebraska, Lincoln, NE) and boneless, skinless, turkey breast (*pectoralis*) were purchased from a wholesale distributor and delivered to the Loeffel Meat Laboratory for processing. All meat was stored frozen until use, and tempered at 2°C for approximately 3-4 days prior to processing. Turkey and beef muscles were trimmed of external fat and connective tissue, and separately ground through a 12.7 mm plate using a Hobart Meat Grinder (Model #4734; Hobart MFG. Co., Troy, OH). Within a specie, meat was then mixed by hand to ensure a homogeneous mixture between treatments prior to batching into 9.1kg batches.

Within each replication, two species (beef [B] and turkey [T]) and four different formulations were used according to salt concentrations based on meat block: 1.0% NaCl, 1.5% NaCl, 2.0% NaCl, and 2.5% NaCl. A brine for 25% extension was formulated and added to water to achieve each of the target salt concentrations above, 1.0% sugar, and 0.35% sodium phosphate (Brifisol 85 Instant, Bk Giulini, Ladenburg, Germany) on a meat block basis. Ground meat and brine were added to a vacuum tumbler (Model DVTS R2-250; Daniels Food Equipment, Parkers Prairie, MN) and were then tumbled under vacuum (66.7 kPa) at 4°C for 90 minutes. Each treatment batch was stuffed using a vacuum stuffer (Vemag Robot 1000 DC; Reiser, Canton MA) into 90mm x 24” pre-stuck fibrous casings (Kalle; Gurnee, IL) and casings were pulled (50psi) and clipped using a Tipper Clipper (Model PR465L; Tipper Tie, Inc., Apex, NC). Chubs within

treatment were hung on a smokehouse stick, weighed, and thermally processed in an Alkar smokehouse (Alkar-RapidPak Inc., Lodi, WI) to an internal temperature of 71°C, followed by a 30 minute cold water shower. Products were chilled overnight. Replications were produced on three separate days.

The day after processing, turkey and roast beef rolls were weighed, casing removed, and sliced (Bizerba Model SE12; Bizerba, Balingen, Germany) into 2mm slices for microbiological sampling and 13mm slices for quality and texture analysis. One slice from each of the two chubs per treatment was then placed into each 3mil std barrier nylon/PE vacuum pouch, vacuum sealed (Multivac Model C500; Multivac Inc., Kansas City, MO), and placed in a covered plastic lug and stored at 1-2°C until sampling. Water activity and salt content were measured on the day of slicing, and shelf life parameters were measured every two weeks starting on the day of slicing through 18 weeks of storage.

Microbial Analysis

Microbial analysis was performed by sampling from a package of two slices per treatment starting on the day of slicing (week 0) and continuing every two weeks until week 18. Samples were aseptically transferred from the vacuum pouch into a WhirlPak bag (Nasco, Fort Atkinson, WI), combined with 50ml of BBL Peptone water (Becton, Dickinson and Company, Franklin Lakes, NJ) and placed in a bag blender (bioMerieux Inc., Durham, NC) for 3 minutes to homogenize sample. Two 2ml samples were collected from sample and stored at -20°C until DNA extraction. If necessary, serial tenfold dilutions were made prior to plating. An Eddy Jet spiral plater (IUL, S.A.,

Barcelona, Spain) was used to plate 50µl of sample on Brain, Heart, Infusion agar (Thermo Fisher Scientific, Waltham, MA) and incubated at 37°C, and for 48h. Colonies were then counted manually according to EddyJet owner's manual method. Bacterial counts were converted to $\log_{10}$ colony forming units (CFU)/gram of sample.

Analysis of Bacterial Communities

Bacterial community analysis was performed using the MiSeq Illumina Sequencing Platform as outlined by Kozich, Westcott, Baxter, Highlander, & Schloss (2013). Microbial DNA extraction was performed using a modified version of the Epicentre QuickExtract DNA extraction protocol. A 1ml sample was centrifuged at 10,000xG for 10 minutes at 20°C, supernatant was removed, and 500µl of QuickExtract solution (Epicentre, Madison, WI) was added to the pellet, and incubated at 65°C for 10 minutes followed by 98°C for 2 minutes.

The V4 region of the 16S rRNA gene was amplified using barcoded primers in a 20µl volume. A PCR reaction contained 0.625 Units of Terra PCR Direct Polymerase (Clontech Laboratories Inc., Mountain View, CA), 1X reaction buffer, 160 µmol dNTPs (Clontech Laboratories Inc., Mountain View, CA), 250 nmol of each primer, and 4 µl of sample extract. Reactions were subjected to the following cycle using a Veriti 96 well thermocycler (Thermofisher Scientific, Walther, MA): an initial denaturation of 98°C for 2 minutes; 30 cycles of 98°C for 30 seconds, 58°C for 30 seconds, and 68°C for 45 seconds; and a final extension of 68°C for 4 minutes.

PCR products were normalized using an Invitrogen Sequal Prep Normalization Kit (Thermofisher Scientific, Walther, MA) and following manufacturer's directions for

binding, washing, and elution steps to yield ~5ng DNA per well. Barcoded PCR products were then pooled and gel purified using the Pippin Prep system (Sage Science, Inc., Beverly, MA). Final concentration was determined using quantitative PCR (qPCR), and DNA were then sequenced by synthesis using the Illumina MiSeq platform (Illumina, Inc., San Diego, CA).

Sequences were sorted and analyzed according to the pipeline described by Anderson, Schneider, Erickson, Macdonald, and Fernando (2016). Raw reads from Illumina sequencing were filtered using QIIME (ver. 1.9.1; Caporaso, et al., 2010). Mothur (ver. 1.36.1; Schloss et al., 2009) was used to remove sequences that were not between 245 and 275 bp. Sequences were clustered into OTUs based on 97% similarity according to the UPARSE pipeline (Edgar, 2013)

Representative sequences for each OTU were assigned taxonomy with the UCLUST consensus taxonomy assigner (QIIME default) method with Greengenes database release 119 (23) as reference sequences. Only a portion of the OTUs could be classified at the genus level (41.7% of OTUs, 95.5% of the reads), however a majority of the reads were classified at family level (70.9% of OTUs, 99.2% of the reads). Therefore, results are discussed at family level.

Cooking Yield, Water Activity, and Salt Content

Cooking yield was calculated as cooked weight as a percentage of raw weight [cooked weight/raw weight] x 100. Samples used for water activity and salt concentration were homogenized using a food processor (Black & Decker Handy Chopper, Black & Decker Inc., Baltimore, MD). Water activity was measured the day of

slicing using an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA) according to manufacturer's specifications. Salt concentration was measured the day of slicing according to Sebranek, Lonergan, King-Brink, Larson, & Beermann (2001) by adding 90ml of boiling water to 10g of ground sample, stirring, and straining water to measure using Quantab high range chloride titration strips (Hach Company, Loveland, CO).

Texture Profile Analysis (TPA)

Texture profile analysis was measured every two weeks throughout storage by cutting 13mm slice into a 4.0cm x 4.0cm square, and measured using a 2,500 kg load cell on an Instron International Testing Machine (Model number 1123; Instron Worldwide, Norwood, MA) with a 140mm plate. Each slice was compressed with a head speed of 30 mm/min to 75% of its original thickness two times, and the characteristics of hardness, springiness, cohesiveness, gumminess, and chewiness were determined according to Bourne (1978).

Proximate Composition

Moisture, fat, protein, and ash of pulverized raw meat samples were determined. Two g of pulverized tissue prepared in duplicate were used to quantify moisture and ash using a LECO thermogravimetric analyzer (LECO Corporation, St. Joseph, MI). Total fat was determined as outlined by AOAC (1990) using the Soxhlet extraction procedure. Protein was measured using a LECO Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, MI).

Objective Color

Objective color (*L**, *a**, *b**) of deli slices was measured using a colorimeter (Chroma Meter CR-400; Konica Minolta Sensing Americas, Inc., Ramsey, NJ) using a 2° standard observer and a D65 illuminant. The calibration plate and samples were read through Saran plastic wrap to keep from contaminating the colorimeter lens. Six readings were taken from the two 13mm slices within a treatment for each storage time and averaged for color values. Measures of ΔE, change in color, were calculated using the following formula: $\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$ with ΔE calculated for the difference between week 0 and week of observation (Minolta Co., n.d.)

pH

Cooked meat pH was measured every two weeks throughout storage in duplicate using an Orion 410A+ pH meter (Thermo Electron Corporation, Waltham, MA) on a mixture of 10g of cooked meat sample in 90ml of double distilled water.

Statistical Analysis

Water activity, proximate composition, salt, and cooking yield were analyzed as a 4 x 2 factorial arrangement (Salt concentration x meat species) using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Objective color, pH, TPAs, and microbial growth were analyzed as a 4 x 2 x 10 factorial (Salt x meat species x storage time) using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA). For relative abundance of microbial taxa, weeks 0 to 14 were analyzed. Relative abundance data were analyzed as a 4 x 2 x 8 factorial (salt x meat species x storage time) using the PROC GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA). All

means were separated using the LSMEANS function with PDIFF option and Tukey's adjustment for least significant difference (LSD) at $P < 0.05$.

In order to identify bacterial communities characterizing differences between treatments, an algorithm for high-dimensional biomarker discovery was used. To perform this analysis, LDA Effect Size (LEfSE; Segata et al., 2011) was used to identify differences in the microbial community related to salt concentration over storage time. Beef and turkey samples were analyzed independently using LEfSE.

RESULTS

Data is presented for main effect of meat species, salt concentration, and storage time (Table 1, Table 2, and Table 3, respectively). When significant interactions ($P \leq 0.05$) were identified for meat species by salt concentration or meat species by storage time, data is reported in Table 4 and Table 5, respectively.

Microbial Analysis

The preservative effect of salt can be attributed to a reduction in water activity and drawing water out of bacterial cells. As salt increases, water activity decreases, slowing bacterial growth. A meat species by salt concentration interaction ($P = 0.009$) and a storage time effect ($P < 0.001$) were seen for aerobic plate count. No other significant interactions ($P > 0.062$) were identified. Mean initial aerobic count (week 0) across all treatments was 1.27 log CFU/g, and increased with time, until week 6, when growth plateaued between 6.72 and 7.56 log CFU/g (Table 1). For the meat species by salt interaction, B/2.5% had the lowest mean aerobic plate count at 5.29 log CFU/g, but

was only significantly less than treatments B/2.0%, B/1.5%, B/1.0%, and T/2.5% (Table 4).

For anaerobic counts, there were no significant interactions ($P > 0.263$). Meat species ($P = 0.010$), salt concentration ($P = 0.035$), and storage time ($P < 0.001$) main effects were observed for anaerobic plate counts (AnPC). Turkey samples had higher anaerobic counts at 2.50 log CFU/g, while beef mean AnPC were 1.80 log CFU/g (Table 2). Anaerobic growth generally increased over time, with week 16 having the highest mean count at 3.69 log CFU/g and week 2 having the lowest count at 0.15 log CFU/g (Table 1). For salt concentration (Table 3), although there was a main effect, there were no significant differences between treatments once the Tukey adjustment for mean separation was applied. The main effect mean for AnPC for all salt concentrations ranged between 2.83 and 1.86 log CFU/g.

Bacterial Community Analysis

For bacterial community analysis, beef and turkey samples were analyzed separately as two independent 7 x 4 factorial arrangements with storage time ceasing at 14w rather than 18w.

There were a total of 1901 operational taxonomic units (OTUs) identified in this study. Operational taxonomic units were first grouped by family. Families that were identified in at least 50% of the 192 total samples (Table 5) were analyzed for treatment and storage time effects and interactions. There were 475 OTUs that could not be identified to the family level. Once grouped by family, relative abundance (RA) was

calculated as proportion of bacterial family relative to total number of sequences recovered for each sample.

There were no significant salt by storage time interactions for any of the families analyzed in beef samples ($P > 0.05$). In beef samples, *Pseudomonadaceae* was the most prevalent family identified, present in all beef samples. Figure 1 shows relative abundances of various families throughout storage time. There was a storage time effect ($P < 0.001$) for *Pseudomonadaceae* in beef samples (Table 6), where *Pseudomonadaceae* had a RA of 46.42% at week 0, increased at week 2, and remained between 82.67% and 97.98% for the remainder of storage time. *Prevotellaceae, Oxalobacteraceae, Veillonellaceae, Lachnospiraceae, Paraprevotellaceae, Ruminococcaceae, and Flavobacteriaceae*, all showed a storage time effect ($P < 0.001$; Table 6), where each family had the greatest relative abundance at week 0, and then declined at week 2 for the reminder of storage time. There was also a main effect of storage time on *Lactobacillaceae* and *Succinivibrionaceae* RA ($P = 0.023$ and $P = 0.041$, respectively), however after separation of means using Tukey's adjustment, there were no statistical differences ($P > 0.05$) between any weeks throughout storage time. None of the beef samples showed a significant salt effect on family RA ($P > 0.05$; Table 7).

In turkey samples, there was a salt concentration by storage time interaction for *Lactobacillaceae* ($P = 0.036$). At each storage time, there were no significant differences in RA of *Lactobacillaceae*, with the exception of week 10, where 2.0% salt had a greater RA than the remaining salt concentrations at week 10 (Figure 3). *Listeriaceae* showed a significant salt concentration main effect ($P = 0.048$; Table 9) overall, however after separation of means using Tukey's adjustment, there were no differences between any of

the salt concentrations. Storage time main effects for family RA are shown in table 7 and figure 2. Similar to beef samples, *Pseudomonadaceae* was the most prevalent family identified in turkey samples, present in 100% of all turkey samples. There was a main effect of week on *Pseudomonadaceae* ($P < 0.001$), where RA was lowest at week 0, and increased at week 2 for the remainder of storage time. A main effect for storage time on *Oxalobacteraceae* RA was observed ($P < 0.001$). *Oxalobacteraceae* had the greatest RA at week 0, and declined at week 2 for the remainder of storage time, however week 4 was statistically similar to week 0 RA. A main effect of storage time was observed for *Prevotellaceae, Veillonellaceae, Lachnospiraceae, Paraprevotellaceae, Succinivibrionaceae, and Ruminococcaceae* ($P < 0.001$) where RA of each family was the greatest at week 0, and declined at week 2 for the reminder of storage time.

The LEfSe algorithm was used to identify differences in relative abundance of OTUs related to salt concentration and storage time. After LEfSe analysis, there were no reported significantly discriminative features for either beef or turkey samples. This indicates that there were no specific OTUs that were statistically different according to salt concentration over storage time.

Cooking yield

Salt aids in myofibrillar protein extraction, increases water holding capacity and produces a greater ability to retain water throughout the cooking process (Ruusunen & Puolanne, 2005). Our results were consistent with this; in general, cooking yield increased as salt concentration increased. A meat species by salt concentration interaction was observed for cooking yield ($P < 0.001$; Table 4). Cooking yield

decreased as salt concentration decreased, with the exception of treatments T/1.5% and T/1.0%, which had similar yields. Treatments B/2.5% and T/2.5% had the highest cooking yield at 90.60% and 90.47% respectively. In beef, cooking yield was significantly reduced by reducing salt to 1.0% (B/1.0%), while turkey samples at the same concentration were not negatively affected.

Texture Profile Analysis

A meat species by salt interaction was observed for hardness ($P < 0.001$), gumminess ($P < 0.001$), cohesiveness ($P = 0.002$), and chewiness ($P < 0.001$; Table 4). For hardness, beef samples became harder as salt decreased. Turkey samples showed a similar trend, however treatments T/1.0% and T/1.5% were similar to B/2.5% and B/2.0%. For gumminess, treatment B/1.0% was the greatest, followed by B/1.5%, and then treatments B/2.0%, T/1.0%, T/1.5%, and T/2.0%. Treatments B/2.5% and T/2.5% were the lowest on gumminess values. For cohesiveness, treatments B/2.5%, B/2.0%, and T/2.5% had the lowest values. For chewiness, treatment B/1.0% was the greatest, followed by B/1.5%, while B/2.0%, B/2.5% were the lowest. All of the turkey samples similar to B/2.5% and B/2.0% for chewiness. For springiness, there was a salt effect ($P < 0.001$) and a meat species effect ($P = 0.001$). As salt decreased, springiness also decreased (Table 3). Beef samples had greater springiness values than did turkey (Table 2). There was a storage time effect on hardness ($P < 0.001$), gumminess ($P < 0.001$), and chewiness ($P = 0.046$; Table 1). Samples became less hard from week 0 until week 8, and then increased beginning at week 10 through the end of storage. Similar results were observed in gumminess, where samples decreased in gumminess from week 0 to week 8, and then increased starting at week 10 throughout the remaining storage. For chewiness,

although there was a significant main effect of storage time ($P = 0.046$), there were no differences between any treatments once the Tukey's LSD adjustment was applied.

Proximate Composition

Moisture, fat, ash, and protein were measured on week 0 for each sample. There were no meat species by salt interactions for any proximate composition traits measured ($P > 0.05$). There was a meat species effect on percent fat ($P < 0.001$) and percent moisture ($P < 0.001$). Turkey samples had a mean fat percentage of 0.71%, while beef samples had a mean fat percentage of 3.05% (Table 2). Inversely, turkey samples had a mean moisture percentage of 75.10%, while beef were lower at 72.61%. Salt concentration had an effect on moisture ($P = 0.026$), ash ($P = 0.021$), and protein ($P = 0.001$; Table 3). Samples with 2.0% added salt had the highest percent moisture with 74.61%, while samples with 1.0% salt had the lowest percent moisture at 72.67%. Mean ash percent generally decreased from 2.56% in the 2.5% salt samples to 1.92% in the 1.0% samples, however only the highest and lowest salt treatments were statistically different. Protein was inversely related to moisture and ash, where 2.5% salt had the least percent protein at 20.13%, and increased as salt inclusion decreased to 22.36% in the 1.0% salt samples, however only 2.5% salt was significantly different from 1.5% and 1.0% salt treatments.

Salt concentration and Water Activity

Formulated salt concentrations were calculated on a meat block basis, where measured salt concentrations were on the finished product. There were no significant interactions ($P < 0.05$) for water activity nor measured salt concentration. As expected,

the measured salt concentration significantly increased (P < 0.001) with each increase in formulated salt (Table 3). Water activity had an inverse relationship to salt concentration ($P < 0.001$), with 2.5% formulated treatments having the lowest water activity at 0.9795, and 1.0% treatments having the greatest water activity at 0.9863 (Table 3).

Instrumental Color

The total change in color of a sample compared to initial sampling on week 0 was calculated as ΔE. There was a meat species x storage time interaction for ΔE ($P < 0.001$), but no salt effects were observed ($P = 0.126$). For turkey samples, ΔE increased with storage time, whereas beef samples had ΔE values that were similar across all weeks of measure (Table 10). For the measure of lightness (L^*), there were no storage time by meat species or storage time by salt interactions, however there was a storage time main effect ($P = 0.001$). Week 4 samples were the lightest, while weeks 10, 12, 16, and 18 were the darkest but only statistically different from week 4 samples (Table 1). Although these were statistically different, this small difference, ranging from 69.17 to 70.18, is likely of little practical importance. There was a meat species by storage time interaction (Table 10) for both a^* (redness; $P = 0.016$) and b^* (yellowness; $P < 0.001$). All turkey samples were less red than the beef samples at each time point. Beef samples were the most red at week 0, but only greater than weeks 4 and 10. Turkey generally increased in b* over time and were more yellow than beef at all time points except week 0. There was also a meat species by salt interaction for L^* ($P < 0.001$) and a^* ($P = 0.035$; Table 4). All turkey samples were lighter than beef samples. Within turkey, treatment T/1.0% was the lightest, treatment T/1.5% intermediate, and treatments T/2.0% and T/2.5% were the darkest. Treatments B/1.0% and B/1.5% were lighter than B/2.0% and B/2.5%. Both

turkey and beef generally decreased in redness as salt increased with turkey being less red than beef. Salt concentration affected *b** values ($P < 0.001$), treatment 4 were the most yellow, followed by treatment 3. Treatments 1 and 2 were the least yellow.

pH

There were no interactions of time, meat species or salt for pH measurement. There was a main effect of time ($P < 0.001$), meat species ($P < 0.001$), and salt ($P = 0.003$) on pH. Mean pH over all treatments was 6.09 at week 0, and increased to a maximum of 6.23 at week 4, where pH then gradually decreased to 5.82 at week 18 however only week 18 was statistically different form week 0 (Table 1). Beef samples had a lower pH (6.04) than turkey (6.14; Table 2). 1.5% salt had a lower pH than all other treatments which were similar (Table 3).

DISCUSSION

Microbiological growth and population

Spoilage of meat products is typically somewhat subjective, being identified as gross discoloration, strong off-odors, or the development of slime (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Using strictly bacterial growth as an indicator of spoilage is not the most consistent method of determining spoilage, however a total plate count above 10^7 cfu/cm^2 is commonly used as an indicator of spoilage (Borch, Kant-Muermans, & Blixt, 1996; H. Korkeala, Lindroth, Ahvenainen, & Alanko, 1987). In this study, samples, regardless of meat species or salt concentration, reached 7 log cfu/g at 10 weeks, indicating between 8 and 10 weeks of shelf life, according to total plate count.

For aerobic plate count, there was not an obvious pattern of growth affected by salt or meat species. There was a trend of increasing counts until around week 10, followed by a plateau through the end of testing.

Although there were no time interactions observed for bacterial growth, there was a meat species by salt interaction where beef containing the highest level of salt (2.5%) slightly suppressed aerobic growth throughout shelf life. This could be attributed to the slightly lower pH seen in the beef samples in combination with the increased salt content, placing more hurdles to improve microbial stability (Leistner, 2000). For anaerobic plate count, turkey samples had increased growth compared to beef, most likely due to a more favorable pH of turkey meat (Duffy, Vanderlinde, & Grau, 1994; Gibson, Bratchell, & Roberts, 1988).

Results from this study showed that under these processing conditions, microbiota from family *Pseudomonadaceae* were dominant within the initial load, and continued to dominate spoilage flora. Most vegetative cells are eliminated during the cooking process, therefore spoilage microbiota is determined by post-lethality recontamination (Borch et al., 1996). Nearly half of all OTUs identified in both beef and turkey samples belonged to the family *Pseudomonadaceae* on week 0, therefore we can conclude that under the current processing conditions, *Pseudomonadaceae* was the most prevalent family of spoilage organism present, whether from slicers, tabletops, or packaging equipment. The spoilage flora of cooked, sliced meat products typically consists of *Bacillus* spp., *Micrococcus* spp., and *Lactobacillus* spp., however *Pseudomonas* spp. may also grow rapidly (Borch et al., 1996). Pseudomonads do not have significant mechanisms of inhibiting and out-competing other organisms in meat products, such as lactic acid and

other metabolites produced by lactic acid bacteria (Ame & Brashears, 2002). The advantage that pseudomonads possess to compete in meat spoilage flora is the rate of growth, which is more rapid than most other common spoilage organisms (Gill & Newton, 1977). This rapid growth rate could explain how in the current study, an initial load with a large abundance of pseudomonads resulted in spoilage flora being dominated by family *Pseudomonadaceae* throughout storage time. Other studies have concluded that the use of salt and vacuum packaging typically selects for LAB (H. J. Korkeala & Björkroth, 1997), however in the current study, large numbers of *Pseudomonadaceae* were present initially allowing *Pseudomonadaceae* to dominate throughout shelf life.

Quality and Processing Characteristics

Salt is essential for the quality and processing characteristics of processed meats, especially with regards to texture, water holding capacity, and bind. The extraction of myofibrillar proteins greatly contributes to these aspects, and salt is the main ingredient responsible for myofibrillar protein extraction. Ruusunen & Puolanne (2005) summarize the theory of protein extraction that chloride ions from salt bind strongly to meat proteins, increasing negative charges and repulsion between proteins, producing muscle swelling. With this increased space between proteins, additional water is allowed to interact with the polar side chains of amino acids, increasing water holding ability throughout processing and cooking (Ruusunen & Puolanne, 2005). Furthermore, addition of salt to meat extracts salt-soluble myofibrillar proteins, forming a matrix of heat coagulable proteins which will bind additional water as well as bind pieces of meat together after cooking (Desmond, 2006).

It is expected that decreased salt concentration would result in less solubilization of myofibrillar proteins, reduced product bind and water holding capacity. Our results showed that in general, cooking yield decreased as salt concentration decreased. In our samples, beef with 1.0% added salt was more negatively affected than turkey at the same concentration. This could be attributable to fiber type, considering turkey breast has a much greater abundance of white fibers compared to beef (Xiong, 1994). It has been reported that when placed in solutions of similar salt and phosphate concentration, proteins were readily extracted from muscles with predominately white or fast twitch fibers, while muscles containing primarily slow twitch or red fibers resisted extraction and required greater salt concentration to extract myofibrils (Parsons & Knight, 1990; Starr, Almond, & Offer, 1985). This could explain why at low salt concentrations, the turkey samples had less decrease in yield, indicative of greater protein extraction and increased water holding ability in turkey compared to beef.

Water activity is defined as the ratio of vapor pressure of water in a food to the vapor pressure of pure water (M. P. Doyle, Beuchat, & Montville, 2001). In general, food products with a lower water content will be less perishable than high moisture products, however differences in perishability may be seen between two products of similar water content. Thus, the term water activity was developed to more accurately predict the amount of water in a food available for biological function (Scott, 1957). Water associates with non-aqueous constituents at varying intensities depending on the amount of solutes in relation to water content of a food. Therefore, the more a solute or ingredient interacts with water, the more water activity will be reduced (Fennema, 1996). In our samples, water activity decreased as salt concentration increased, which is in

agreement with these theories of water activity. According to Raoult's law for "ideal" solutions, an increase in solute concentration reduces water activity (M. P. Doyle et al., 2001).

Laboratory analysis of salt in the finished product was directly related to ingoing salt concentration of our formulas. The treatments of 1.0%, 1.5%, 2.0%, and 2.5% were based on a meat block basis, while the product was extended with 25% added ingredients. Therefore, the total salt concentrations on a formulation basis were 0.8%, 1.2%, 1.6%, and 2.0%, respectively. The measured salt concentration (0.77%, 1.09%, 1.43%, and 1.71%, respectively) in the finished cooked product were slightly lower than we would have expected, the samples follow the same near linear pattern as our formulations. This may be in part due to inherent error in the use of Quantab titration strips but nonetheless verify the differences in formulated salt amount.

Objective color was measured using CIE $L^*a^*b^*$ which is a measure of light to dark (L^*), red to green (a^*), and blue to yellow (b^*). Total color change in a sample compared to week 0 was calculated as ΔE. For L^* values, a significant storage time effect was identified, however there were minimal differences among the different time points and may have limited visual differences. Similarly, there was a meat species x storage time interaction for shelf a^*, but only the differences between meat species would likely be noticed visually; differences within meat species over time were statistically different, but likely have little practical importance. Turkey samples throughout shelf life were less red in color, which would be expected as turkey breast muscles contain a much less myoglobin than beef which is inherent with greater amounts of type I muscle fibers (Xiong, 1994). For b^*, which measures a scale of yellow to blue, turkey samples

generally increased throughout shelf life and had greater *b** values at each time point when compared to beef. These differences between meat species can also be attributed to fiber type, as greater concentrations of white Type I fibers may give turkey a somewhat more yellow color when compared to beef. Furthermore, samples generally increased over time, which would agree with our understanding of myoglobin oxidation during storage of cooked meat, in which red color fades and turns to brown, causing a slight increase in *b** values (Mancini & Hunt, 2005). Furthermore, the meat species x salt interaction for both *L** and *a** in this study indicated that an increase in salt content generally decreased both *L** and *a** in beef and turkey samples. Beef samples were darker and more red than turkey. The differences between meat species can be attributed to fiber type as previously mentioned. Devatkal & Naveena (2010) reported that the addition of salt to ground beef caused a decrease in lightness and redness, similar to our findings of increased salt concentration causing a decrease in lightness and redness. Salt is a pro-oxidant in meat products that tends to oxidize myoglobin, turning it to a brown color, and also works to solubilize or denature myoglobin, destabilizing meat color (King (née Turner) & Whyte, 2006; Lytras, Geileskey, King, & Ledward, 1999). Thus, as salt concentration is increased, an increase in myoglobin oxidation and denaturation would be expected, explaining the decrease in red color as well as a slight darkening effect.

For pH measurements, a trend of decreasing pH throughout shelf life was observed. This is to be expected during the storage of meat products due to the growth of lactic acid bacteria (LAB) and their by-products (Huis In't Veld, 1996). Although these microbiological results showed that the flora was not dominated by LAB, multiple LAB species were identified in our samples. Meat species also effected pH, where turkey

samples were slightly greater than beef samples. These pH values are similar to previous studies in sliced turkey breast (Redfield & Sullivan, 2015) and roast beef (Pietrasik & Shand, 2004) and the addition of alkaline phosphates to samples explains the slight increase seen in the finished products compared to what we would expect in fresh meat samples (Puolanne, Ruusunen, & Vainionpää, 2001). Salt concentration affected pH, where 1.5% samples had a lower pH than 1.0%, 2.0%, and 2.5% regardless of meat species or shelf life. Samples with a lower pH may be the result of increased growth of LAB and production of lactic acid (Gram et al., 2002; Huis In't Veld, 1996; Nychas et al., 2008). These results show a similar trend, where 1.5% salt had the lowest pH and the most LAB growth, however from our population data this salt effect on LAB growth was not statistically significant ($p = 0.165$).

Results from this study indicate that ingoing salt concentrations within a typical inclusion range effect processing characteristics such as cooking yield and products texture. Furthermore, under the conditions of this study, high initial concentrations of pseudomonads can lead to microbial flora dominated by pseudomonads, regardless of salt concentration. The methods used in this study may be applied to various processed meat products to further evaluate how salt or antimicrobial ingredients my alter spoilage flora.

LITERATURE CITED

Aaslyng, M. D., Vestergaard, C., & Koch, A. G. (2014). The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science*, *96*(1), 47–55.

Ame, A., & Brashears, M. M. (2002). Competitive Inhibition of Listeria monocytogenes in Ready-to-Eat Meat Products by Lactic Acid Bacteria. *Journal of Food Protection*, *65*(2), 316–325.

AOAC. (1990). *AOAC: Official Methods of Analysis* (15th ed.). Arlington, VA: Association of Official Analytical Chemists.

Bertino, M., Beauchamp, G. K., & Engelman, K. (1982). Longterm reduction in dietary sodium alters the taste of salt. *American Journal of Clinical Nutrition*, *36*, 1134–1144.

Blickstad, E., & Molin, G. (1983). Carbon-Dioxide as a controller of the spoilage flora of pork, with special reference to temperature and sodium-chloride. *Journal of Food Protection*, *46*(9), 756–766.

Borch, E., Kant-Muermans, M. L., & Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology*, *33*(1), 103–120.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology*, *32*, 62-66.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. a, Hummler, E., Ryba, N. J. P., & Zuker, C. S. (2010). The cells and peripheral representation of sodium taste in mice. *Nature*, *464*(7286), 297–301.

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science*, *74*(1), 188–196.

Devatkal, S. K., & Naveena, B. M. (2010). Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. *Meat Science*, *85*(2), 306–311.

Doyle, M. E., & Glass, K. A. (2010). Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. *Comprehensive Reviews in Food Science and Food Safety*, *9*(1), 44–56.

Doyle, M. P., Beuchat, L. R., & Montville, T. J. (2001). *Food Microbiology: Fundamentals and Frontiers*. Washington, DC: American Society for Microbiology.

Duffy, L. L., Vanderlinde, P. B., & Grau, F. H. (1994). Special Issue Predictive Modelling Growth of Listeria monocytogenes on vacuum-packed cooked meats: effects of pH, aw, nitrite and ascorbate. *International Journal of Food Microbiology*, *23*(3), 377–390.

Edgar, R.C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods 10*, 996-998.

Egan, A. F. (1983). Lactic acid bacteria of meat and meat products. *Antonie van Leeuwenhoek*, *49*(3), 327–336.

Fennema, O. R. (1996). *Food Chemistry* (3rd ed.). Madison, WI: Marcel Dekker, Inc.

Gibson, A. M., Bratchell, N., & Roberts, T. A. (1988). Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *International Journal of Food Microbiology*, *6*(2), 155–178.

Gill, C. O., & Newton, K. G. (1977). The Development of Aerobic Spoilage Flora on Meat Stored at Chill Temperatures. *Journal of Applied Bacteriology*, *43*(2), 189–195.

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., & Givskov, M. (2002). Food spoilage--interactions between food spoilage bacteria. *International Journal of Food Microbiology*, *78*(1-2), 79–97.

Huis In't Veld, J. H. (1996). Microbial and biochemical spoilage of foods: An overview. *International Journal of Food Microbiology*, *33*(1), 1–18.

Karppanen, H., & Mervaala, E. (2006). Sodium Intake and Hypertension. *Progress in Cardiovascular Diseases*, *49*(2), 59–75.

King (née Turner), N. J., & Whyte, R. (2006). Does It Look Cooked? A Review of Factors That Influence Cooked Meat Color. *Journal of Food Science*, *71*(4), R31–R40.

Korkeala, H. J., & Björkroth, K. J. (1997). Microbiological Spoilage and Contamination of Vacuum-Packaged Cooked Sausages, *60*(6), 724–731.

Korkeala, H., Lindroth, S., Ahvenainen, R., & Alanko, T. (1987). Interrelationship between microbial numbers and other parameters in the spoilage of vacuum-packed cooked ring sausages. *International Journal of Food Microbiology*, *5*(4), 311–321.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, *55*(1-3), 181–186.

Lytras, G. N., Geileskey, A., King, R. D., & Ledward, D. A. (1999). Effect of muscle type, salt and pH on cooked meat haemoprotein formation in lamb and beef. *Meat Science*, *52*(2), 189–194.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science*, *71*(1), 100–121.

Mattes RD, D. D. (1991). Relative Contributions of Dietary Sodium Sources. *Journal of the American College of Nutrition*, *10*(4), 383–393.

Minolta Co. (n.d.). Precise color communication: color conrol from perception to instrumentation.

Nowson, C. A., Wattanapenpaiboon, N., & Pachett, A. (2009). Low-sodium Dietary Approaches to Stop Hypertension-type diet including lean red meat lowers blood pressure in postmenopausal women. *Nutrition Research (New York, N.Y.)*, *29*(1), 8–18.

Nychas, G.-J. E., Skandamis, P. N., Tassou, C. C., & Koutsoumanis, K. P. (2008). Meat spoilage during distribution. *Meat Science*, *78*(1-2), 77–89.

Parsons, N., & Knight, P. (1990). Origin of variable extraction of myosin from myofibrils treated with salt and pyrophosphate. *Journal of the Science of Food and Agriculture*, *51*(1), 71–90.

Pietrasik, Z., & Shand, P. J. (2004). Effect of blade tenderization and tumbling time on the processing characteristics and tenderness of injected cooked roast beef. *Meat Science*, *66*(4), 871–879.

Puolanne, E. J., Ruusunen, M. H., & Vainionpää, J. I. (2001). Combined effects of NaCl and raw meat pH on water-holding in cooked sausage with and without added phosphate. *Meat Science*, *58*(1), 1–7.

Redfield, A. L., & Sullivan, G. A. (2015). Effects of conventional and alternative curing methods on processed turkey quality traits. *Poultry Science*, *94* (12), 3005-3014.

Ruusunen, M., & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science*, *70*(3), 531–541.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J.,Weber, C.F. (2009). Introducing mothur: open-source, platform independent, community-supported software for describing and comparing microbial communities. *Applied Environmental Microbiology 75*, 7537-7541.

Scott, W. J. (1957). Water relations of food spoilage microorganisms. *Advances in Food Research*, *7*, 83–127.

Sebranek, J., Lonergan, S., King-Brink, M., Larson, E., & Beermann, D. (2001). *Meat science and processing* (3rd ed.). Zenda, WI: Peerage Press.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology, 12*(6), 1-18.

Starr, R., Almond, R., & Offer, G. (1985). Location of C-protein, H-protein and X-protein in rabbit skeletal muscle fibre types. *Journal of Muscle Research & Cell Motility*, *6*(2), 227–256.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., & Kerry, J. P. (2012). Effect of varying salt and fat levels on the sensory and physiochemical quality of frankfurters. *Meat Science*, *92*(4), 659–666.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., & Kerry, J. P. (2013). The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. *Meat Science, 93*(2), 145–152.

USDA, & HHS. (2010). *Dietary Guidelines for Americans, 2010*. Washington, DC.

Vermeiren, L., Devlieghere, F., & Debevere, J. (2004). Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology*, *96*(2), 149–164.

Vogt, T. M., Appel, L., Obarzanek, E., Moore, T. J., Vollmer, W. M., Svetkey, L., Sacks, F.M., Bray, G.A., Cutler, J.A., Windhauser, M.M. Lin, P.H., Karanja, N. M. (1999). Dietary Approaches to Stop Hypertension: Rationale, design, and methods. *Journal of the American Dietetic Association*, *99*(8), S12–S18.

Whiting, R., Benedict, R., Kunsch, C., & Woyochik, J. (1984). Effect of sodium chloride levels in frankfurters on the growth of Clostridium sporogenes and Staphylococcus aureus. *Journal of Food Science, 49*(2), 351–355.

Xiong, Y. L. (1994). Myofibrillar Protein from Different Muscle Fiber Types : Implications of Biochemical and Functional Properties in Meat Processing.*Critical Reviews in Food Science and Nutrition*, *34(3)*, 293-320.

TABLES AND FIGURES

Table 1. Least square means for main effects of storage time for aerobic plate count (APC), anaerobic plate count (AnPC), pH, L*, a*, b*, ΔE, hardness, gumminess, cohesiveness, springiness, and chewiness.

	Storage time (weeks)											
Trait	0	2	4	6	8	10	12	14	16	18	*P*-value	SEM
APC	1.27^{d}	3.39^{c}	5.30^{b}	6.98^{a}	6.72^{a}	7.31^{a}	7.31^{a}	7.37^{a}	7.16^{a}	7.57^{a}	<0.001	0.19
AnPC	0.32^{cd}	0.15^{d}	1.71^{bcd}	2.17^{abc}	1.69^{bcd}	3.20^{ab}	3.48^{ab}	2.86^{ab}	3.69^{a}	2.22^{abc}	<0.001	0.42
pH	6.09^{abcd}	6.20^{ab}	6.23^{a}	6.20^{ab}	6.16^{abc}	6.09^{abcd}	6.06^{bcd}	6.03^{cd}	5.99^{d}	5.82^{e}	<0.001	0.03
L*	69.46^{ab}	69.39^{ab}	70.18^{a}	69.38^{ab}	69.25^{ab}	69.80^{b}	69.92^{b}	69.46^{ab}	69.20^{b}	69.17^{b}	0.001	0.21
[1]a*	6.42	6.23	6.14	6.41	6.28	6.17	6.16	6.28	6.16	6.18	0.024	0.07
[1]b*	8.72	9.06	9.15	9.52	9.88	9.79	10.00	10.24	10.26	10.48	<0.001	0.12
[1]ΔE	0.00	1.28	1.57	1.66	1.68	2.00	2.00	2.33	2.02	2.02	<0.001	0.15
Hardness	1586^{ab}	1466^{bcd}	1418^{cd}	1388^{d}	1382^{d}	1558^{abc}	1560^{abc}	1563^{abc}	1559^{abc}	1658^{a}	<0.001	36.07
Gumminess	499.99^{a}	443.45^{ab}	444.06^{ab}	420.73^{b}	421.62^{b}	471.40^{ab}	457.38^{ab}	476.11^{ab}	472.64^{ab}	501.35^{a}	<0.001	15.29
Cohesiveness	0.314^{a}	0.301^{a}	0.313^{a}	0.303^{a}	0.304^{a}	0.302^{a}	0.300^{a}	0.304^{a}	0.302^{a}	0.301^{a}	0.082	0.004
Springiness	0.362^{a}	0.376^{a}	0.348^{a}	0.369^{a}	0.374^{a}	0.364^{a}	0.341^{a}	0.363^{a}	0.361^{a}	0.346^{a}	0.136	0.01
Chewiness	180.39^{a}	166.88^{a}	153.06^{a}	154.04^{a}	157.37^{a}	169.60^{a}	154.51^{a}	171.00^{a}	169.93^{a}	171.76^{a}	0.046	7.95

[a-d]Means in the same row lacking a common superscript are significantly different.

[1]Indicates a significant ($p < 0.05$) meat species*storage time interaction for the trait

Table 2. Least square means for main effects of meat species (turkey or beef) for aerobic plate count (APC), anaerobic plate count (AnPC), pH, cooking yield, water activity (aW), salt concentration, *L**, a*, b*, ΔE, fat, moisture, protein, ash, hardness, gumminess, cohesiveness, springiness, and chewiness.

	Meat Species			
Trait	Beef	Turkey	*P*-value	SEM
[1]APC	6.01	6.07	0.638	0.09
AnPC	1.80[b]	2.50[a]	0.01	0.19
pH	6.04[b]	6.14[a]	<0.001	0.02
[1]Cooking yield (%)	83.74	84.97	0.004	0.26
aW	0.9828	0.9828	0.989	0.0004
Salt (%)	1.22	1.28	0.091	0.02
[1]L*	59.04	79.60	<0.001	0.09
[1,2]a*	8.28	4.21	<0.001	0.03
[2]b*	8.82	10.60	<0.001	0.05
[2]ΔE	7.54	1.78	0.0134	0.07
Fat (%)	3.05[a]	0.71[b]	<0.001	0.32
Moisture (%)	72.61[b]	75.10[a]	<0.001	0.31
Protein (%)	20.95	21.61	0.052	0.22
Ash (%)	2.30	2.22	0.549	0.10
[1]Hardness	1622	1406	<0.001	13.82
[1]Gumminess	489.61	432.14	<0.001	5.84
[1]Cohesiveness	0.300	0.309	<0.001	0.002
Springiness	0.3503[b]	0.3705[a]	0.001	0.004
[1]Chewiness	180.17	149.54	<0.001	3.046

[a,b] Means in the same row lacking a common superscript are significantly different.
[1]Indicates a significant ($p < 0.05$) salt*meat species interaction for the trait
[2]Indicates a significant ($p < 0.05$) meat species*storage time interaction for the trait

Table 3. Least square means for main effects of ingoing salt concentration for aerobic plate count (APC), anaerobic plate count (AnPC), pH, cooking yield, water activity (aW), salt concentration, *L**, a*, b*, ΔE, fat, moisture, protein, ash, hardness, gumminess, cohesiveness, springiness, and chewiness.

	Ingoing Salt Concentration					
Trait	1.00%	1.50%	2.00%	2.50%	*P*-value	SEM
[1]APC	6.17	6.12	6.15	5.71	0.026	0.12
AnPC	2.83[a]	2.04[a]	1.87[a]	1.86[a]	0.032	0.27
pH	6.11[a]	6.02[b]	6.10[a]	6.12[a]	0.003	0.02
[1]Cooking yield (%)	76.45	83.12	87.33	90.54	<0.001	0.37
aW	0.9863[a]	0.9835[b]	0.9820[bc]	0.9795[c]	<0.001	0.0004
Salt	0.77[d]	1.09[c]	1.43[b]	1.71[a]	<0.001	0.03
[1]L*	70.70	69.57	68.53	68.48	<.0001	0.13
[1]a*	6.47	6.39	6.12	5.99	<.0001	0.04
*b**	10.28[a]	9.72[b]	9.42[c]	9.41[c]	<.0001	0.08
ΔE	1.75	1.47	1.76	1.63	0.1262	0.10
Fat (%)	2.41	2.09	1.50	1.52	0.434	0.64
Moisture (%)	72.67[b]	73.69[ab]	74.61[a]	74.46[ab]	0.026	0.44
Protein (%)	22.36[a]	21.52[a]	21.12[ab]	20.13[b]	0.001	0.31
Ash (%)	1.92[b]	2.12[ab]	2.45[ab]	2.56[a]	0.021	0.14
[1]Hardness	1707	1556	1426	1368	<0.001	19.54
[1]Gumminess	528.29	487.94	428.28	398.48	<0.001	8.26
[1]Cohesiveness	0.309	0.313	0.300	0.295	<0.001	0.002
Springiness	0.3436[c]	0.3514[bc]	0.3663[ab]	0.3802[a]	<0.001	0.006
[1]Chewiness	181.45	170.87	156.32	150.77	<0.001	4.31

[a-d] Means in the same row lacking a common superscript are significantly different.
[1]Indicates a significant ($p < 0.05$) salt*meat species interaction for the trait

Table 4. Least square means for meat species*salt concentration interaction for aerobic plate count (APC), cooking yield, L*, a*, hardness, gumminess, cohesiveness, and chewiness.

	Meat Species * Salt concentration									
Trait	B 1.0%	B 1.5%	B 2.0%	B 2.5%	T 1.0%	T 1.5%	T 2.0%	T 2.5%	*P*-value	SEM
APC	6.26^{a}	6.23^{a}	6.25^{a}	5.29^{b}	6.07^{ab}	6.01^{ab}	6.05^{ab}	6.14^{a}	0.009	0.17
Cooking yield (%)	72.79^{e}	84.43^{c}	87.16^{b}	90.60^{a}	80.10^{d}	81.82^{d}	87.50^{b}	90.47^{a}	<0.001	0.52
*L**	60.02^{d}	59.40^{d}	58.18^{e}	58.57^{e}	81.39^{a}	79.74^{b}	78.88^{c}	78.39^{c}	<0.001	0.19
*a**	8.61^{a}	8.34^{ab}	8.13^{bc}	8.03^{c}	4.34^{de}	4.43^{d}	4.11^{ef}	3.95^{f}	0.035	0.06
Hardness	1917.01^{a}	1664.91^{b}	1483.07^{cd}	1423.97^{cde}	1496.04^{c}	1447.65^{cd}	1368.71^{de}	1311.68^{e}	<0.001	27.63
Gumminess	600.13^{a}	513.37^{b}	434.84^{cde}	410.10^{de}	457.45^{cd}	462.52^{c}	421.72^{cde}	386.86^{e}	<0.001	11.68
Cohesiveness	0.3131^{ab}	0.3080^{ab}	0.2929^{cd}	0.2877^{d}	0.3052^{abc}	0.3189^{a}	0.3077^{ab}	0.3021^{bcd}	0.002	0.003
Chewiness	213.17^{a}	182.43^{b}	164.73^{bc}	160.36^{bc}	149.74^{c}	159.31^{bc}	147.92^{c}	141.19^{c}	<0.001	6.09

$^{a-d}$Means in the same row lacking a common superscript are significantly different.

Table 5. Prevalence of bacterial families† based on total percentage of all samples in which each family was identified.

Family	Prevalence
Pseudomonadaceae	100.00%
Moraxellaceae	86.98%
Prevotellaceae	80.21%
Oxalobacteraceae	63.02%
Listeriaceae	62.50%
Enterobacteriaceae	60.94%
Veillonellaceae	58.85%
Lachnospiraceae	56.25%
Lactobacillaceae	55.73%
Paraprevotellaceae	53.65%
Succinivibrionaceae	52.60%
Ruminococcaceae	50.52%
Flavobacteriaceae	50.00%
Carnobacteriaceae	50.00%

†Families which were present in ≥50% of all samples were analyzed for salt concentration and storage time main effects and interactions.

Table 6. Least square means for main effects of storage time on proportions of bacterial families in beef samples relative to total number of sequences recovered from beef samples.

	Storage Time (Weeks)									
Family	0	2	4	6	8	10	12	14	*P*-Value	SEM
Pseudomonadaceae	46.42[b]	82.67[a]	92.90[a]	97.98[a]	98.70[a]	90.42[a]	96.42[a]	97.67[a]	< 0.001	4.84
Moraxellaceae	3.33	2.44	1.20	1.26	0.56	6.81	1.85	0.55	0.544	2.23
Prevotellaceae	8.86[a]	2.08[b]	0.50[b]	0.11[b]	<0.01[b]	0.25[b]	0.01[b]	<0.01[b]	< 0.001	0.91
Oxalobacteraceae	5.25[a]	1.51[b]	0.17[b]	0.01[b]	<0.01[b]	<0.01[b]	0.12[b]	<0.01[b]	< 0.001	0.73
Listeriaceae	0.27	0.01	0.14	<0.01	0.02	<0.01	0.22	0.10	0.169	0.09
Enterobacteriaceae	0.45	0.17	0.02	<0.01	0.39	1.62	1.03	0.52	0.585	0.62
Veillonellaceae	2.28	0.73	0.12	0.01	<0.01	0.22	<0.01	<0.01	< 0.001	0.25
Lachnospiraceae	1.54[a]	0.35[b]	0.09[b]	0.01[b]	<0.01[b]	0.08[b]	<0.01[b]	<0.01[b]	< 0.001	0.15
Lactobacillaceae	0.08	0.07	0.10	0.03	<0.01	<0.01	<0.01	<0.01	0.023[†]	0.03
Paraprevotellaceae	1.38[a]	0.40[b]	0.07[b]	0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	< 0.001	0.15
Succinivibrionaceae	3.00	0.42	0.09	0.01	<0.01	0.08	<0.01	0.08	0.041[†]	0.69
Carnobacteriaceae	0.11	0.04	0.14	0.01	0.01	0.04	0.08	0.77	0.536	0.27
Ruminococcaceae	1.11[a]	0.37[b]	0.07[b]	0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	< 0.001	0.11
Flavobacteriaceae	3.93[a]	0.59[b]	0.03[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.001	0.62

[a,b] Means within the same trait lacking a common superscript are significantly different.

[†]Indicates significant overall P value, but no significant differences between treatments after Tukey's LSD adjustment.

Table 7. Least square means for main effects of salt concentration on proportions of bacterial families in beef samples relative to total number of sequences recovered from beef samples.

	Ingoing Salt Concentration					
Family	1.0%	1.5%	2.0%	2.5%	*P*-value	SEM
Pseudomonadaceae	91.74	88.46	89.78	81.61	0.185	3.41
Moraxellaceae	1.09	1.91	0.59	5.42	0.138	1.58
Prevotellaceae	0.96	0.90	1.74	2.30	0.371	0.65
Oxalobacteraceae	0.61	0.71	1.03	1.18	0.848	0.52
Listeriaceae	0.18	0.08	0.03	0.09	0.395	0.06
Enterobacteriaceae	0.61	1.17	0.12	0.20	0.309	0.44
Veillonellaceae	0.29	0.23	0.60	0.57	0.319	0.17
Lachnospiraceae	0.13	0.26	0.23	0.42	0.311	0.11
Lactobacillaceae	0.02	0.04	0.04	0.05	0.806	0.02
Paraprevotellaceae	0.26	0.16	0.21	0.30	0.805	0.10
Succinivibrionaceae	0.20	0.18	1.04	0.42	0.565	0.49
Carnobacteriaceae	0.44	0.13	0.01	0.02	0.350	0.19
Ruminococcaceae	0.15	0.17	0.13	0.32	0.315	0.08
Flavobacteriaceae	0.22	0.47	0.90	0.68	0.724	0.44

Table 8. Least square means for main effects of storage time on proportions of bacterial families in turkey samples relative to total number of sequences recovered from turkey samples.

	Storage Time (Weeks)									
Family	0	2	4	6	8	10	12	14	*P*-Value	SEM
Pseudomonadaceae	35.954[b]	88.18[a]	96.41[b]	99.09[b]	99.77[b]	95.82[b]	92.36[b]	97.21[b]	< 0.001	4.42
Moraxellaceae	1.15	0.75	0.71	0.32	0.06	1.03	0.94	1.91	0.525	0.59
Prevotellaceae	12.29[a]	1.94[b]	0.42[b]	0.03[b]	0.01[b]	0.03[b]	0.02[b]	0.02[b]	< 0.001	1.41
Oxalobacteraceae	1.73[a]	0.11[b]	0.45[ab]	0.03[b]	0.01[b]	0.08[b]	0.05[b]	0.01[b]	0.001	0.30
Listeriaceae	0.15	0.14	0.01	0.14	0.04	<0.01	5.56	0.24	0.381	1.85
Enterobacteriaceae	0.75	0.06	0.03	<0.01	<0.01	2.51	0.10	0.05	0.455	0.88
Veillonellaceae	2.47[a]	0.39[b]	0.13[b]	0.01[b]	<0.01[b]	0.01[b]	0.01[b]	<0.01[b]	< 0.001	0.25
Lachnospiraceae	2.72[a]	0.47[b]	0.09[b]	<0.01[b]	<0.01[b]	0.01[b]	<0.01[b]	<0.01[b]	< 0.001	0.36
Lactobacillaceae	0.30	0.22	0.08	0.12	0.03	0.41	0.29	0.07	0.392	0.13
Paraprevotellaceae	1.27[a]	0.37[b]	0.09[b]	0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	< 0.001	0.17
Succinivibrionaceae	1.56[a]	0.21[b]	0.28[b]	<0.01[b]	<0.01[b]	0.01[b]	<0.01[b]	0.01[b]	< 0.001	0.21
Carnobacteriaceae	0.05	0.05	0.05	0.11	0.01	0.04	0.42	0.29	0.085	0.11
Ruminococcaceae	1.22[a]	0.34[b]	0.05[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	<0.01[b]	< 0.001	0.17
Flavobacteriaceae	0.39	0.05	0.78	0.02	0.01	<0.01	0.01	0.01	0.206	0.24

[a,b] Means within the same trait lacking a common superscript are significantly different.

Table 9. Least square means for main effects of salt concentration on proportions of bacterial families in turkey samples relative to total number of sequences recovered from turkey samples.

	Ingoing Salt Concentration					
Family	1.0%	1.5%	2.0%	2.5%	*P*-value	SEM
Pseudomonadaceae	91.85	88.82	86.56	85.17	0.460	3.12
Moraxellaceae	0.70	0.88	0.81	1.04	0.952	0.42
Prevotellaceae	1.21	1.58	1.83	2.75	0.731	0.99
Oxalobacteraceae	0.51	0.15	0.24	0.34	0.657	0.30
Listeriaceae	0.16	0.25	2.65	0.08	0.048†	1.31
Enterobacteriaceae	0.12	0.16	0.11	1.37	0.404	0.62
Veillonellaceae	0.31	0.28	0.46	0.46	0.850	0.18
Lachnospiraceae	0.27	0.32	0.38	0.68	0.144	0.36
Lactobacillaceae	0.10	0.29	0.30	0.07	0.161	0.09
Paraprevotellaceae	0.17	0.14	0.18	0.38	0.490	0.12
Succinivibrionaceae	0.17	0.15	0.34	0.25	0.821	0.15
Carnobacteriaceae	0.30	0.04	0.05	0.12	0.072	0.08
Ruminococcaceae	0.11	0.14	0.20	0.35	0.476	0.12
Flavobacteriaceae	0.10	0.04	0.11	0.38	0.470	0.17

†Indicates significant overall P value, but no significant differences between treatments after Tukey's LSD adjustment.

Table 10. Least square means for meat species*storage time interaction for a*, b*, and ΔE.

		Storage time (weeks)											
Trait	Species	0	2	4	6	8	10	12	14	16	18	*P* value	SEM
*a**	Beef	8.64^{a}	8.36^{ab}	8.08^{b}	8.43^{ab}	8.16^{ab}	8.05^{b}	8.19^{ab}	8.36^{ab}	8.22^{ab}	8.29^{ab}	0.016	0.10
	Turkey	4.20^{c}	4.11^{c}	4.20^{c}	4.40^{c}	4.39^{c}	4.28^{c}	4.13^{c}	4.19^{c}	4.11^{c}	4.07^{c}		
*b**	Beef	8.33^{g}	8.57^{fg}	8.33^{g}	8.90^{efg}	8.99^{efg}	8.75^{efg}	8.85^{efg}	9.09^{efg}	9.04^{efg}	9.33^{cdef}	<0.001	0.17
	Turkey	9.12^{defg}	9.54^{cde}	9.97^{bcd}	10.15^{bc}	10.77^{ab}	10.82^{ab}	11.14^{a}	11.39^{a}	11.48^{a}	11.62^{a}		
ΔE	Beef	0.00^{e}	1.50^{bcd}	1.58^{abcd}	1.96^{abcd}	1.53^{abcd}	1.77^{abcd}	1.88^{abcd}	2.18^{abc}	1.53^{abcd}	1.43^{bcd}	<0.001	0.22
	Turkey	0.00^{e}	1.05^{ed}	1.56^{abcd}	1.37^{cd}	1.83^{abcd}	2.22^{abc}	2.11^{abcd}	2.47^{ab}	2.52^{ab}	2.61^{a}		

[a-g]Means within the same trait lacking a common superscript are significantly different.

Figure 1. Relative abundance of various microbial families throughout storage time in beef samples.

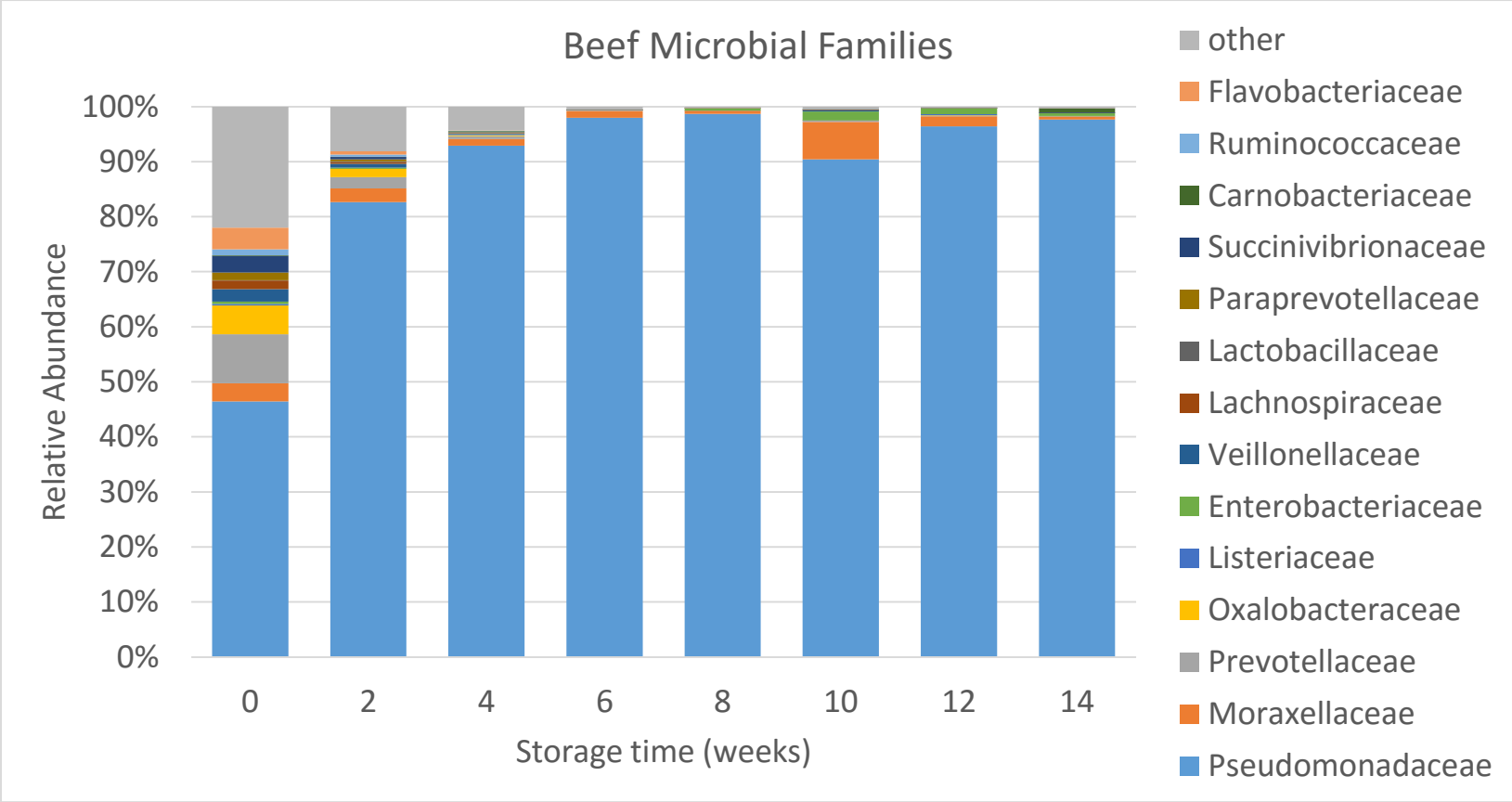
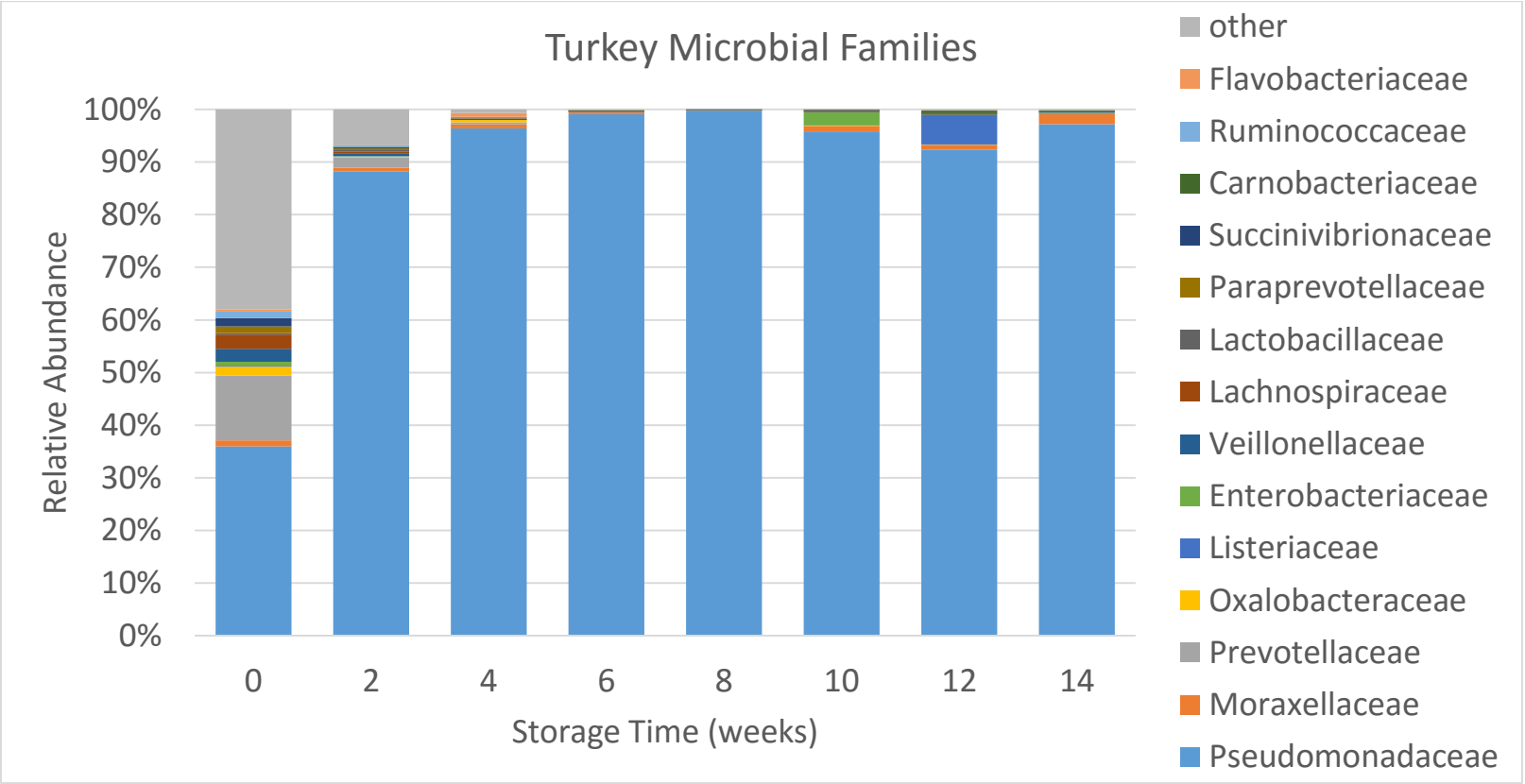


Figure 2. Relative abundance of various microbial families throughout storage time in turkey samples.



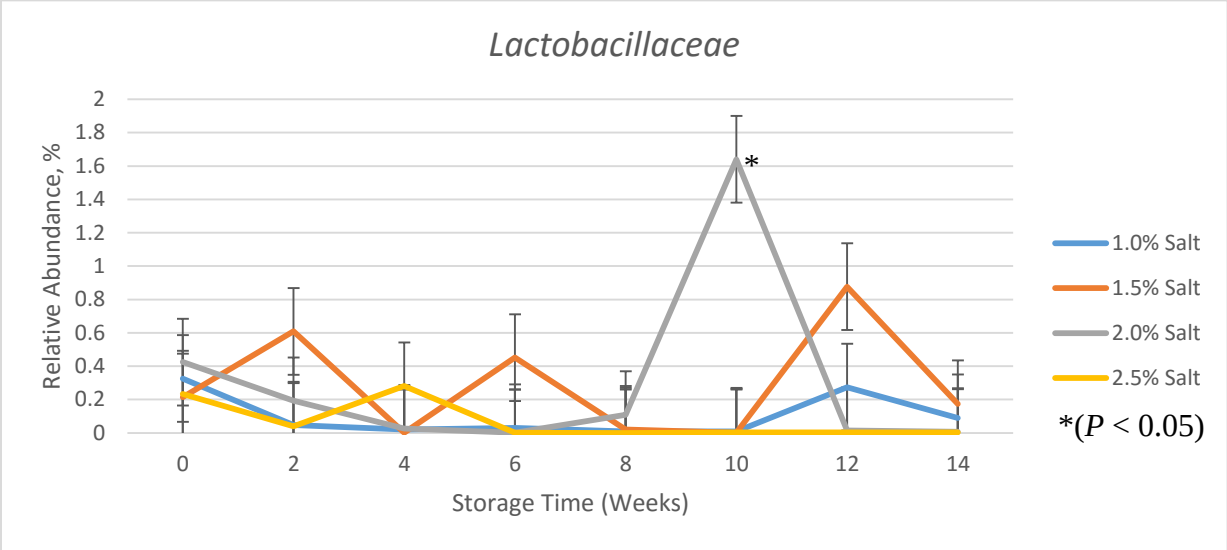


Figure 3. Salt concentration by storage time interaction of family *Lactobacillaceae* relative abundance.

RECCOMENDATIONS FOR FUTURE RESEARCH

There are still many questions to be addressed for this research. Results from this study indicated that if a high initial load of *Pseudomonadaceae* are present, then salt concentration has little effect on bacterial populations throughout shelf life. This may not be the case under different circumstances where other bacteria are dominant initially. In future studies similar to this, it would be valuable to sample the processing environment in which cooked products are handles to assess bacterial population of the environment. Furthermore, since PCR does not differentiate between vegetative and inactivated bacteria, it may be valuable to perform a study to determine the amount of background DNA from inactivated cells that may be amplified and identified in the sequencing process. With this innovate strategy to observe microbial population dynamics, the spoilage patterns of multiple meat products utilizing many antimicrobial methods may be evaluated. Furthermore, knowledge may be gained in fermented meat products such as summer sausage and salami, so that we may understand how various ingredients alter fermentative populations. Furthermore, this strategy may be applied to cured meat products to determine how sodium nitrite alters spoilage populations, and also if various sources of nitrite effect growth, such as celery powder.

The method of spoilage by different organisms varies, and using these methods alongside indicators of spoilage such as souring, slime formation, or rancidity could broaden our knowledge of how spoilage organisms deteriorate food quality. If correlations are present between a certain species of bacteria and spoilage indicator, methods may be developed to limit the growth of species that are the most detrimental to product quality.

Further studies should be performed that observe how the population dynamics of cured products alter based on cured vs. uncured, naturally cured, and reduced salt cured products. Furthermore, the effects of salt substitutes, such as potassium chloride, should be observed on how they affect growth. Finally, since fermented products typically have greater salt inclusion than deli meat, the effects of salt or sodium reduction on the fermentation process should also be observed.

APPENDICES

1. TURKEY FORMULATIONS

Product Name:	Turkey Breast			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Turkey	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.6625**	**2568.465**	**22.65%**	**18.12%**
Non-Meat Ingredients	**0.5**	**226.796**	**2.00%**	**1.60%**
Salt	0.25	113.398	1.00%	0.80%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11606.29**		

Product Name:	Turkey Breast			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Turkey	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.5375**	**2511.766**	**22.15%**	**17.72%**
Non-Meat Ingredients	**0.625**	**283.495**	**2.50%**	**2.00%**
Salt	0.375	170.097	1.50%	1.20%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11662.98**		

Product Name:	Turkey Breast			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Turkey	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.4125**	**2455.067**	**21.65%**	**17.32%**
Non-Meat Ingredients	**0.75**	**340.194**	**3.00%**	**2.40%**
Salt	0.5	226.796	2.00%	1.60%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11719.68**		

Product Name:	Turkey Breast			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Turkey	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.2875**	**2398.368**	**21.15%**	**16.92%**
Non-Meat Ingredients	**0.875**	**396.893**	**3.50%**	**2.80%**
Salt	0.625	283.495	2.50%	2.00%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11776.38**		

2. BEEF FORMULATIONS

Product Name:	Roast Beef			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Beef	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.6625**	**2568.465**	**22.65%**	**18.12%**
Non-Meat Ingredients	**0.5**	**226.796**	**2.00%**	**1.60%**
Salt	0.25	113.398	1.00%	0.80%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11606.29**		

Product Name:	Roast Beef			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Beef	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.5375**	**2511.766**	**22.15%**	**17.72%**
Non-Meat Ingredients	**0.625**	**283.495**	**2.50%**	**2.00%**
Salt	0.375	170.097	1.50%	1.20%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11662.98**		

Product Name:	Roast Beef			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Beef	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.4125**	**2455.067**	**21.65%**	**17.32%**
Non-Meat Ingredients	**0.75**	**340.194**	**3.00%**	**2.40%**
Salt	0.5	226.796	2.00%	1.60%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11719.68**		

Product Name:	Roast Beef			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Beef	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.2875**	**2398.368**	**21.15%**	**16.92%**
Non-Meat Ingredients	**0.875**	**396.893**	**3.50%**	**2.80%**
Salt	0.625	283.495	2.50%	2.00%
Sugar	0.25	113.398	1.00%	0.80%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
	0	0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
		0	0.00%	0.00%
Restricted Ingredients:	0.0875	39.6893	0.35%	0.28%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0	0	0.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11776.38**		

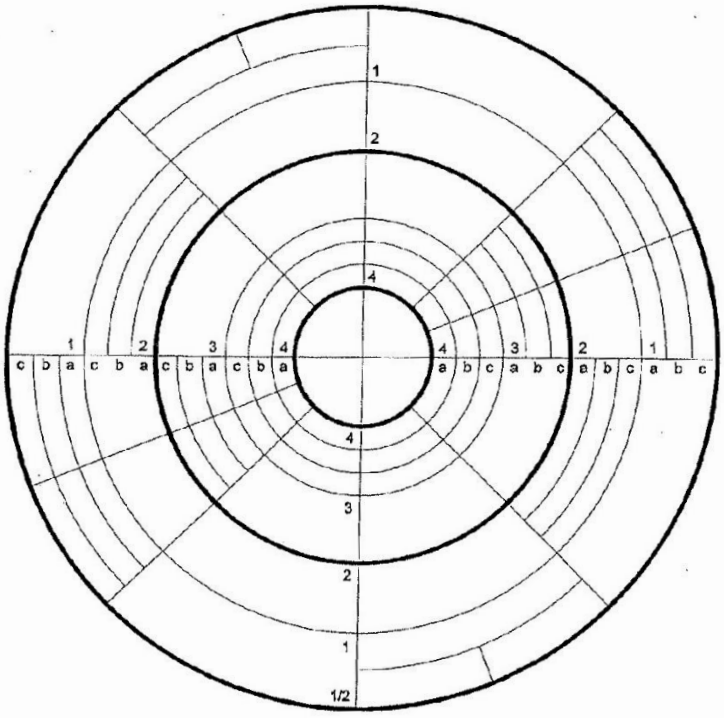
3. COOKING CYCLE

STEP	TIME	DRY BULB	WET BULB	NOTES
1	45 MIN	160°F	0°F	STEAM COOK
2	30 MIN	170°F	170°F	STEAM COOK
3	TO 165°F I.T.	180°F	180°F	STEAM COOK
4	30 MIN	N/A	N/A	COLD SHOWER

4. EDDYJET ENUMERATION METHOD

6. SPREADING DISTRIBUTIONS

The operator can choose between several spreading distributions. The grid used for all distribution to separate regions is the following.



When the 10 cm Petri dish is used the spreading is confined in 6 regions: 4a , 4b, 4c, 3a, 3b, 3c.

In every region there are different amounts of sample according to the distribution program. Always in the exponential spiral distribution the most diluted region correspond to the most external (3c).

Table of Volume (µl) for Eddyjet spiral spreadings

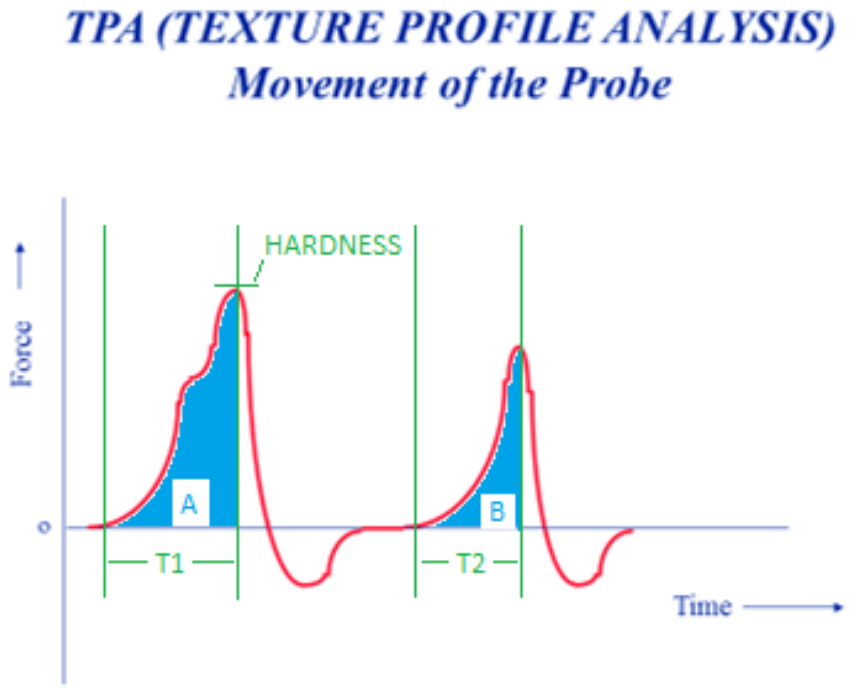
Region(s)	Volume spread within region (µl)
3c	2.56
3c+3b	6.44
3c+3b+3a	12.08
3+4c	20.36
3+4c+4b	32.32
3+4c+4b+4a	50.00

Plates were divided into sections of either $1/8^{ths}$ or $1/4^{ths}$ and two sections opposite from one another were counted manually, and calculated to CFU per plate (50µl) using the volume table above, ¼ or ½ factor (plate division), and dilution factor (if applicable).

From CFU per plate (50µl), counts were transformed to log CFU/g using the following equation:

$$\log \text{CFU/g} = (\text{CFU}/50\mu\text{l}) * 20 * 50 / \text{sample weight}$$

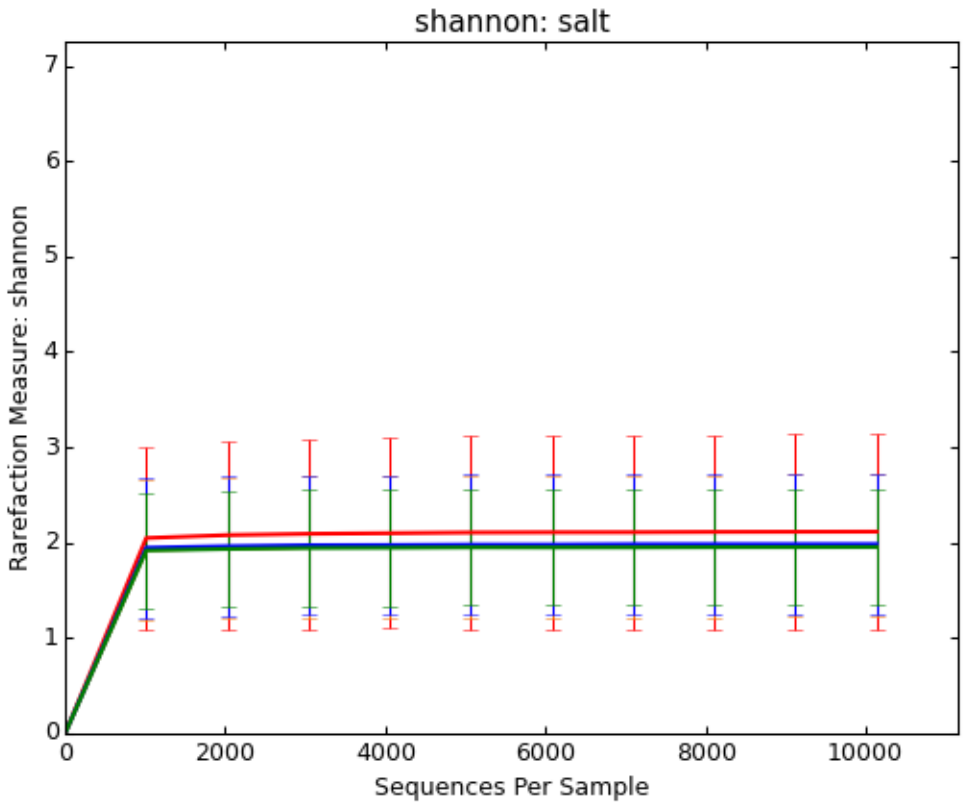
5. TPA MEASUREMENTS



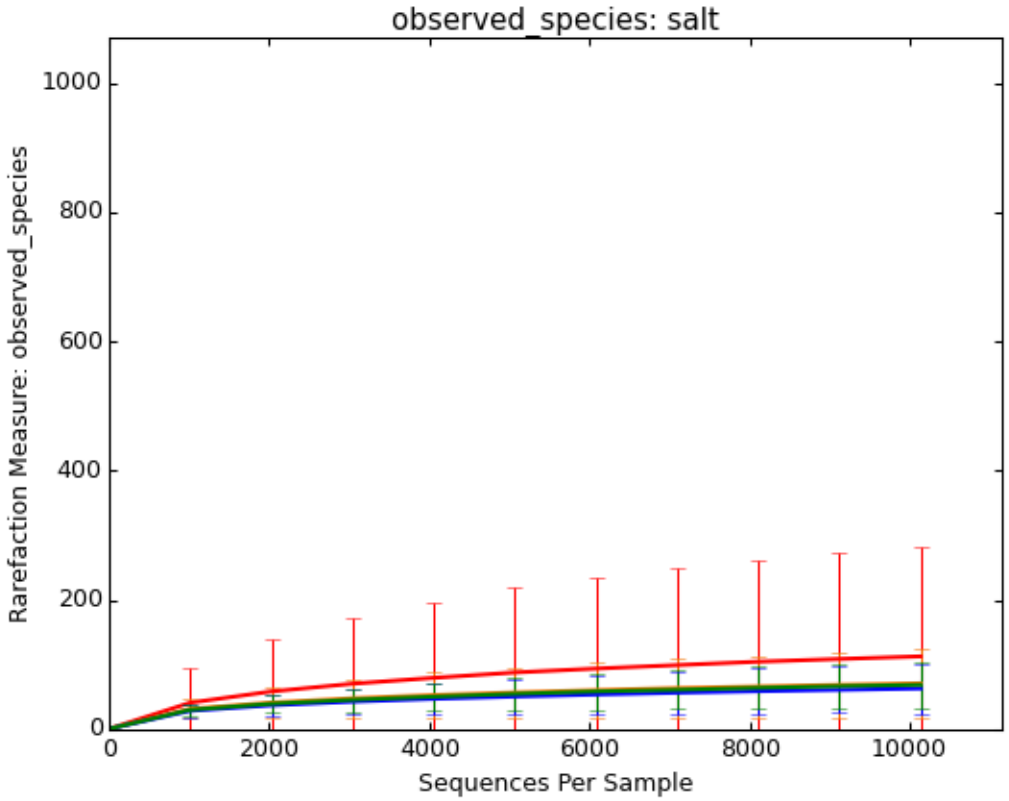
COHESIVENESS = AREA B / AREA A
GUMMINESS = HARDNESS * COHESIVENESS
SPRINGINESS = T1 / T2
CHEWINESS = GUMMINESS * SPRINGINESS

6. ALPHA DIVERSITY PLOTS

Shannon diversity estimates of 16S rRNA gene sequences from varying salt concentrations (green=1.0%, orange=1.5%, blue=2.0%, red=2.5%).



Rarefication measures of 16S rRNA gene sequences from varying salt concentrations (green=1.0%, orange=1.5%, blue=2.0%, red=2.5%).



7. TABLE OF P VALUES

Effect	APC	AnPC	delta E	pH	L*	a*	b*	Hardness	Gumminess	Cohesiveness	Springiness	Chewiness
spc[1]	0.638	0.01	0.0134	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0009	0.001	<.0001
salt[2]	0.026	0.032	0.1262	0.0026	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
spc*salt	0.004	0.263	0.225	0.2055	0.0005	0.035	0.2019	<.0001	<.0001	0.0022	0.708	0.0004
week[3]	<.0001	<.0001	<.0001	<.0001	0.0012	0.0243	<.0001	<.0001	<.0001	0.0818	0.1363	0.0462
spc*week	0.87	0.444	0.0009	0.0719	0.2091	0.0159	<.0001	0.9069	0.7123	0.6198	0.8953	0.8025
salt*week	0.063	0.569	0.9499	0.7847	0.9856	0.8081	0.9998	0.9901	0.879	0.9936	1.0000	0.9837
spc*salt*week	0.548	0.52	0.9827	0.8835	0.9988	0.9415	0.9879	0.942	0.8051	0.9994	0.9995	0.8772

[1]meat species
[2]salt concentration
[3]week of storage time

Effect	Cooking Yield	Water Activity	Measured Salt
spc	0.004	0.989	0.0906
level	<.0001	<.0001	<.0001
spc*level	<.0001	0.4	0.33

[1]meat species
[2]salt concentration