

EFFECTS OF VARIOUS SALT PURITY LEVELS ON LIPID OXIDATION AND SENSORY
CHARACTERISTICS OF GROUND TURKEY AND PORK

BY

KELSEY NICOLE BESS

THESIS

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Professor John Killefer

Abstract

Salt use in meat products is changing. Consumers desire sea salt which may also contain trace metals and the government is demanding a reduction in sodium. Therefore a need exists to understand how varying impurity levels in salt affect meat quality. This study evaluated the effects of various salt preparations on lipid oxidation, sensory characteristics, protein extractability, and bind strength of ground turkey and pork. This study was a completely randomized design with 5 treatment groups and 6 replications in 2 species. Ground, turkey and pork meat was formulated into one hundred and fifty gram patties with sodium chloride (1%) containing varying amounts of metal impurities (copper, iron, and manganese). Samples were randomly assigned to frozen storage periods of 0, 3, 6, and 9 weeks. After storage, samples were packaged in PVC overwrap and stored under retail display for 5 days. Samples were evaluated for proximate analysis to ensure the fat content was similar for all of the starting material. Thiobarbituric acid reactive substances (TBARS) were determined on raw and cooked samples to evaluate lipid oxidation. A trained six member sensory panel evaluated the samples at each storage period for saltiness, off flavor, and oxidized odor. Break strength was conducted using a Texture Analyzer and compared with salt soluble proteins (increasing salt concentrations) to evaluate protein extractability characteristics. Statistical analyses were conducted using the MIXED procedure of SAS within repeated measures over time where appropriate. No significant differences were observed among the salt treatments for raw and cooked TBARS when the control group was removed ($P>0.05$). Sensory panelists detected increased levels of off flavor and oxidized odor over the entire storage duration. Less force was required to break the patties from the control group when compared with the salt treatments ($P<0.05$). As salt concentration increased salt-soluble protein extraction increased, but there was no effect of salt type. Overall,

no meaningful statistical differences among the various salt treatments were observed for all of the parameters evaluated for turkey and pork. Salt at a 1% inclusion rate containing varying levels of copper, iron, and manganese impurities in ground turkey thigh meat and ground pork served as a prooxidant. However, if a meat processor uses a 1% inclusion rate of salt in turkey and pork regardless of impurities included, it is unlikely that differences in shelf life or protein functionality would be observed.

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Chapter I

REVIEW OF THE LITERATURE

Americans consume more than the daily recommended amount of sodium. The recommended daily intake of sodium provided by the *2005 Dietary Guidelines for Americans* for people over the age of 2 years is a maximum of 2,300 mg. However, the typical American consumes approximately two times that amount (Henney et al. 2010). This increased salt intake has been linked to health concerns such as hypertension and cardiovascular disease. Therefore, in April 2010, the Institute of Medicine mandated that sodium be reduced in food (FDA Issues Statement on IOM Sodium Report) . Walmart stores have made a commitment to provide customers with processed foods containing 25% less sodium by 2015 (Walmart Plans Healthier Foods at Lower Prices). Sodium reduction is especially difficult for the meat industry as processed meats such as ham, bacon, sausage, frankfurters, and bologna may include high levels of sodium. Salt provides functionality characteristics and enhances flavor in meat products. Using substitutes for sodium can negatively impact these properties. Also, salt is relatively inexpensive and alternatives to replace salt will likely increase overall costs for food processors. Thus, meat processors are searching for ways to produce premium products with reduced levels of sodium. Further complicating the issue is the increasing consumer demand for all things “natural”. This has led to increased use of sea salt which consumers perceive as healthier or more natural. Little, though, is known about the impact of heavy metal or other impurities in sea salt and their effect on further processed meat products. This literature review will discuss the role of sodium chloride and its impact on meat quality, in addition to discussing sodium reduction and its effect on the meat industry as a whole, and examine the current literature regarding the use of impure salts similar to sea salts in meat products.

Functions of Salt in the Meat Industry

Salt (sodium chloride) is used to improve texture, enhance the flavor, and extend the shelf life of meat products that include ham, bacon, sausage, frankfurters, and bologna. Salt is commonly found in meat products at a 2% inclusion level (Offer and Trinick 1983). However, frankfurters and bologna typically include salt at inclusion levels greater than 2% (Smith and Hui 2004). Matulis et al. (1995) found that lowering salt levels to less than 1.3% in frankfurters resulted in incomplete protein extraction and allowed water to escape. Protein extraction with the use of salt is important in the meat industry to obtain desirable textural properties. Salt changes the ionic strength and allows the proteins to be exposed within a meat batter. The hydrophilic ends of the protein bind to water, whereas the hydrophobic ends of the protein bind with fat stabilizing an emulsion. The concept of fat being encapsulated by solubilized proteins is important for emulsion stability in meat batter matrices, where the solubilized proteins swell up with free water (Lan et al. 1993).

Bind strength, water-holding capacity, break strength, and cook loss are influenced by the amount of salt-soluble proteins present and affect the overall texture of a product. Foegeding (1987) reported that positive effects of texture and water-holding capacity of processed meat are attributed to myofibrillar proteins within the matrix of a meat batter. Myofibrillar proteins, which include myosin and actin, are two of the major proteins that are extracted from muscle tissue by salt. Binding strength of a meat product is increased with salt soluble proteins (Swift and Ellis 1956; Mandigo et al. 1972, Acton 1972, Rhee et al. 1983). Break strength is measured by breaking meat products. If it takes more force to break a meat patty, then there is more bind within the product. Thus, data from break strength evaluation provides information in regards to the amount of bind strength between the meat particles and fat within a product (Herrero et al.

2008). Bind strength is important in uniformity and slicability of meat products like bologna. If bind strength is weakened, particles within meat products crumble, which is detrimental in further processing. In addition to bind strength, water-holding capacity and cook loss affect texture. If water-holding capacity is reduced and cook loss is increased in meat products, an undesirable texture is created. Meat is dry and overall palatability is reduced. Gelabert et al. (2003) demonstrated that water-holding capacity increased with salt inclusion, while cook loss is reduced in meat when salt is added (Huffman et al. 1981).

In addition to textural properties, salt is also used as a flavor precursor in meat products and for extending shelf life. The amount of water activity within a meat product impacts microbial growth. Lower water activity extends the shelf life by reducing microbial growth. Marsh (1983) found that water activity in fermented meat products is lowered with the use of salt. Overall, flavor and functional properties of meat determine if consumers will accept the product (Bourne 1978; Herrero et al. 2008).

Issues Surrounding Salt Use in the Meat Industry

Although salt has functionality purposes in the meat industry, it can also be detrimental to meat products by accelerating lipid oxidation, which is undesirable in food products and leads to oxidative rancidity. Furthermore, accumulation of lipid peroxides in the diet has been linked with certain human diseases such as atherosclerosis (Kanazawa and Ashida 1998). Lipid oxidation is an auto-catalytic reaction involving free radical formation. Lipid oxidation consists of three stages that include initiation, propagation, and termination within the phospholipid bilayer of the muscle tissue. Initiation of the process occurs when a methylene hydrogen atom is removed from the double bond on the unsaturated fatty acid. Free radicals are generated from the

unsaturated fatty acids as a result. The fatty acid free radical connects with a molecule of oxygen to create a peroxyradical during propagation (Damodaran et al. 2008). Hydroperoxides are formed during the primary change of lipid oxidation (Coxon 1987). Termination of free radical formation occurs when there is a combination of two radicals to form a nonradical species (Damodaran et al. 2008). Secondary products such as aldehydes, ketones, and alcohols result when primary products are broken down with accelerated oxidation. These secondary products are responsible for the production of off flavors and off odors. (Ahn et al. 1993b). Meat products containing a higher degree of polyunsaturated fatty acids are more susceptible to lipid oxidation than products containing saturated fatty acids (Dawson and Gartner 1983) because free radical formation increases with the degree of unsaturation. Exposure to oxygen, grinding during processing, and transition metals such as iron and copper enable the primary radicals to form and accelerate oxidation (Asgar et al. 1988; Kanner and Rosenthal 1992). Light and increased temperatures can accelerate the process as well, as cooked meat is known to oxidize faster than raw meat (Rhee et al. 1983) .

TBARS (Thiobarbituric acid reactive substances) and sensory characteristics

Lipid oxidation is evaluated to assess food quality and is associated with sensory characteristics such as off flavor that are produced from the decomposition of hydroperoxides. There are various assays to measure lipid oxidation. The peroxide value determination method is used to quantify hydroperoxides. Secondary products can be measured by the TBA test (Tarladgis et al. 1960; Witte et al. 1970) or by hexanal values (Shahidi and Pegg 1994). The TBA Test is the most common method used to measure lipid oxidation and is also referred to as the TBARS (thiobarbituric acid reactive substances) method. This assay measures the pink (red) chromophore that is formed by the reaction of 2-Thiobarbituric Acid (TBA) with secondary

products, such as malondialdehyde, by using spectrophotometry (Sørensen and Jørgensen 1996). TBARS values are reported as milligrams of malondialdehyde equivalents per milligram of tissue or samples and have been correlated with off flavor scores (Nolan et al. 1989). There are two main methods for conducting TBARS which include the extraction method and the distillation method. The extraction method has higher and more accurate yields than the distillation method in non-cured meat products, as well as in ground chicken, pork, beef, veal, and lamb (Sørensen and Jørgensen 1996; Wang et al. 1997).

Salt as a prooxidant

Salt at varying levels has been proven to be a prooxidant in meat products. Anderson and Skibsted (1991) found that salt acted as a prooxidant at a 1% inclusion level in pork patties. In another study conducted by King and Bosch (1990), sodium chloride at a 2% inclusion level was more prooxidant compared with potassium chloride (2%) in turkey patties, even when the levels of copper and iron were held constant. There are many postulations as to how sodium chloride acts as a prooxidant. Kanner and Rosenthal (1992) argue that sodium chloride acts as a prooxidant by displacing the iron ions with sodium in the heme pigments of the muscle tissue, whereas others recognize the chloride ion acting upon the lipid as the source (Ellis et al. 1968). The metal impurities, particularly iron, within salt are also thought to cause lipid oxidation (Chang and Watts 1950; Denisov and Emanuel 1960; Salih 1986b). Tichivangana and Morrissey (1985) discovered that iron was more prooxidant than copper and cobalt in fish, turkey, chicken, pork, beef, and lamb. Salih et al. (1989) agree with these results, as they found that when different salt varieties at a 2% inclusion level were used with added metal contaminants that included copper, iron, and magnesium in turkey breast and thigh meat mixtures, the combination of sodium chloride with copper and iron was the most prooxidant. Rock salt, with no added

levels of copper and iron, was no more of a prooxidant than pure salt. However, when 50 ppm of iron and 50 ppm of copper was added to pure salt, the sodium chloride with the added iron was found to be more prooxidant than the sodium chloride with the same amount of copper added (Salih et al. 1989). Farouk et al. (1991) investigated the effects of salt in combination with added iron after storage time and found that TBARS in ground beef were significantly increased by iron. Further investigation, however, is needed to evaluate the effects of sodium chloride with varying levels of trace metals such as iron and copper.

Lipid oxidation: Other factors

Even though salt acts as a prooxidant, storage, species, and muscle type influences TBARS values as well. As storage time is increased, TBARS values will increase. Huffman et al. (1981) evaluated restructured pork chops constructed from hams and Boston butts under frozen storage (-15 ° C) for 0 days and 30 days. At 0 days of storage the TBARS values were 0.18 mg malonaldehyde (MDA)/ kg sample, whereas the TBARS values at 30 days were 0.26 mg MDA/kg sample. In another study conducted by Rhee et al. (1983), ground beef samples were stored for 30 days and 60 days in frozen storage at -20 °C. The TBARS values for 30 days after frozen storage were reported as 2.46 mg MDA/kg tissue and 2.58 mg MDA/kg at 60 days (Rhee et al. 1983).

In addition to storage time, species affects the rate of oxidation. Different species contain different levels of polyunsaturated fatty acids. Tichivangana and Morrissey (1985) found that species containing more polyunsaturated fatty acids have higher TBARS values. Fish oxidized quicker than turkey, chicken, pork, beef, and lamb, where lamb was the least oxidized. Within species, muscle type impacts oxidation. Turkey breast meat oxidizes in a slower manner than

thigh meat, as indicated by higher TBARS values in thigh meat (Salih 1986a; Botsoglou et al. 2003). This is attributed to the fact that breast meat contains less fat than thigh meat (Salih et al. 1989).

Packaging and the use of antioxidants can delay the onset of the oxidation reaction when salt is included in processed products. The type of packaging used for a product is dependent upon how quickly the product will be used. Vacuum packaging, modified atmosphere packaging with the use of nitrogen or carbon dioxide gases, and polyvinylchloride overwrap are often used to minimize lipid oxidation in meat products. Overwrapping and modified atmosphere packaging are used for products undergoing retail display, whereas vacuum packaging is used for meat products that are going to be stored for extended periods of time. Nolan et al. (1989) found that cooked pork and turkey was less oxidized when stored in vacuum packaging compared to storage in modified atmospheric packaging (carbon dioxide or nitrogen), or exposed to the air. Cooked turkey patties with added iron, hemoglobin, salt, or with a combination of those had lower TBARS values when they were vacuum packaged compared to loose, oxygen-permeable packaging (Ahn et al. 1993a). Matlock et al. (1984) observed similar results in precooked frozen pork sausage patties stored over 8 weeks.

In addition to packaging, antioxidants are used in the food industry to interrupt the free radical mechanism involved in lipid oxidation before the process is catalyzed. Some antioxidants used in the meat industry include alpha-tocopherol, herbal extracts and oils. Chen et al. (1984) report that TBARS values of beef with salt (2%) and added alpha-tocopherol were higher than the unsalted control groups after 2 days of storage, yet were significantly lower than the TBARS values of beef with only salt (2%). This implies that the salt was prooxidant, but the alpha-tocopherol slowed the process of lipid oxidation when salt was included. Although alpha-

tocopherol serves as an antioxidant when salt is used in food products, research has been conducted on grape seed, oregano, and rosemary extract to evaluate their effectiveness as an antioxidant in meat. Rojas and Brewer (2007) found that TBARS values and off flavor scores were most improved when grape seed extract was used in combination with sodium chloride in beef and pork patties compared to oregano or rosemary.

Antioxidants can also be added to the animal's diet to delay the onset of rancidity in the meat products that will be obtained from them. Botsoglou et al. (2003) found that turkeys fed with alpha-tocopherol acetate and oregano oil resulted in less lipid oxidation compared to turkeys that were not provided with antioxidants. They determined the combination of oregano oil and alpha-tocopherol provided the best protection against oxidation. Another study conducted by Wen et al. (1996) is in agreement with these results, as it was determined that incorporating alpha-tocopherol in turkey diets lowered TBARS values in raw and cooked samples that had been stored in refrigeration and during frozen storage. Adding higher levels of alpha-tocopherol can further prevent oxidative rancidity from occurring in turkeys. Higgins et al. (1999) discovered that turkeys fed with 600 mg alpha-tocopherol/kg feed for 21 weeks prior to harvest were less oxidized compared to meat from turkeys fed with lower levels of alpha-tocopherol. Also, TBARS values were higher for the samples containing meat from the turkeys fed 600 mg alpha-tocopherol/kg and 1 % salt, confirming previous evidence that salt acts as a prooxidant.

Salt Reduction and the Meat Industry

Increased sodium intake is associated with several health concerns centered around the cardiovascular system. High blood pressure (hypertension) and cardiovascular risks have been

associated with increased levels of salt intake (Stamler et al. 1989; He et al. 2002). Sacks et al. (2001) indicated that lowering salt in the diet will reduce hypertension and reduce the occurrence of other cardiovascular complications. Strazzullo et al. (2009) conducted a meta-analysis on the relationship between the amount of salt consumed and the risk of stroke or cardiovascular disease and found that there was a significantly higher risk for stroke or cardiovascular disease with higher salt intake. Also, a 5g reduction in daily salt intake is associated with a reduced risk of stroke by 23% and a reduced risk of cardiovascular disease by 17%. If the global population reduced their salt intake by 5g daily, 250,000 deaths related to stroke and 3,000,000 deaths from cardiovascular disease could potentially be prevented annually (Strazzullo et al. 2009).

As cardiovascular risks have been associated with increased salt intake, the meat industry has been challenged to find alternative ways to formulate products with reduced sodium. Studies focused on lowering sodium content and or replacing sodium chloride with other salts such as magnesium chloride, calcium chloride, or potassium chloride have indicated that sensory, shelf life, and functionality properties of meat were altered. Sensory panelists detected bitterness when 30% of sodium chloride was replaced with potassium chloride or when sodium chloride was replaced with magnesium chloride in fermented sausage. (Terrell and Olson 1981; Gou et al. 1996). When 50% of sodium chloride was replaced with potassium chloride in dry-cured pork loins, sensory panelists detected increased bitterness (Gou et al. 1996). Terrell and Olson (1981) evaluated the effect of replacing sodium chloride with potassium chloride and magnesium chloride on microbial growth in ground pork and found that *Lactobacillus* counts were increased when sodium was lowered or replaced with other salts. Reducing sodium chloride to less than 2% in beef-pork frankfurters resulted in a decrease of texture and flavor (Sofos 1983). However, acceptable sensory scores and bind characteristics were obtained when sodium chloride was

reduced and replaced with potassium chloride in cooked hams, but not when sodium chloride was reduced and substituted with magnesium chloride (Frye et al. 1986). King and Bosch (1990) observed that turkey patties containing more than 1% of potassium chloride were more rancid than patties with sodium chloride or any combination of sodium chloride and potassium chloride.

In addition to using replacements for sodium chloride such as potassium or magnesium salts, other alternatives such as phosphates may be used in combination with these salts to alleviate undesirable functionality properties, shelf life, and sensory characteristics. Matlock et al. (1984) conducted a study in pork sausage patties after frozen storage and found that phosphates reduced cook loss. Reduction of sodium chloride with added phosphates improved the overall quality of the meat, as TBARS values over extended storage when any salt inclusion level was used were decreased. Off flavor scores, however, were higher in samples containing phosphates. It was concluded that the minimum level of sodium chloride needed for precooked sausage patties with added phosphates during frozen storage was 1% in order to maintain desired functional and sensory characteristics. Olson (1982) claims that sodium chloride should only be reduced in meat products by 25% to prevent problems associated with shelf-life, flavor, and texture.

Objectives

Overall, sodium chloride acts as a prooxidant. Trace metals such as iron and copper in salt can also be detrimental to meat quality and cause rancidity. It would be beneficial to determine if varying purity levels within salt alter the rate of lipid oxidation over extended storage. Also, there is pressure from the government to reduce sodium chloride levels in further

processed products. If less sodium chloride can be used to extract proteins and increase bind strength, meat processors could ultimately reduce the amount of total sodium used in products.

The objectives of this study were:

- 1.) Determine how salts with varying levels of impurities impact lipid oxidation and sensory characteristics in turkey and pork
- 2.) Investigate how salts with varying purity levels affect protein extractability characteristics and bind strength in turkey and pork

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Chapter II

EFFECTS OF VARIOUS SALT PURITY LEVELS ON LIPID OXIDATION AND SENSORY CHARACTERISTICS OF GROUND TURKEY AND PORK

Abstract

Salt use in meat products is changing. Consumers desire sea salt which may also contain trace metals and the government is demanding a reduction in sodium. Therefore a need exists to understand how varying impurity levels in salt affect meat quality. This study evaluated the effects of various salt preparations on lipid oxidation, sensory characteristics, protein extractability, and bind strength of ground turkey and pork. This study was a completely randomized design with 5 treatment groups and 6 replications in 2 species. Ground, turkey and pork meat was formulated into one hundred and fifty gram patties with sodium chloride (1%) containing varying amounts of metal impurities (copper, iron, and manganese). Samples were randomly assigned to frozen storage periods of 0, 3, 6, and 9 weeks. After storage, samples were packaged in PVC overwrap and stored under retail display for 5 days. Samples were evaluated for proximate analysis to ensure the fat content was similar for all of the starting material. Thiobarbituric acid reactive substances (TBARS) were determined on raw and cooked samples to evaluate lipid oxidation. A trained six member sensory panel evaluated the samples at each storage period for saltiness, off flavor, and oxidized odor. Break strength was conducted using a Texture Analyzer and compared with salt soluble proteins (increasing salt concentrations) to evaluate protein extractability characteristics. Statistical analyses were conducted using the MIXED procedure of SAS within repeated measures over time where appropriate. No significant differences were observed among the salt treatments for raw and cooked TBARS when the

control group was removed ($P>0.05$). Sensory panelists detected increased levels of off flavor and oxidized odor over the entire storage duration. Less force was required to break the patties from the control group when compared with the salt treatments ($P<0.05$). As salt concentration increased salt-soluble protein extraction increased, but there was no effect of salt type. Overall, no meaningful statistical differences among the various salt treatments were observed for all of the parameters evaluated for turkey and pork. Salt at a 1% inclusion rate containing varying levels of copper, iron, and manganese impurities in ground turkey thigh meat and ground pork served as a prooxidant. However, if a meat processor uses a 1% inclusion rate of salt in turkey and pork regardless of impurities included, it is unlikely that differences in shelf life or protein functionality would be observed.

Introduction

Salt is used in the meat industry for further processing purposes including reducing microbiological growth, enhancing flavor, improving texture and forming stable emulsions (Terrell 1983). Despite the fact that salt improves functionality of processed meat products, it can also be detrimental to meat quality by increasing lipid oxidation (Huffman et al. 1981) leading to oxidative rancidity thus decreasing shelf life of meat products (Gray 1978; Morrissey et al. 1994). When oxygen interacts with fat, hydroperoxide formation occurs (Gray 1978) and as a result, secondary products are formed that cause undesirable sensory characteristics such as off-odors and off-flavors (Ladikos and Lougovois 1990; St. Angelo et al. 1996).

The rate and extent of fat oxidation is affected by several factors. First, salt purity plays a role. Sodium chloride alone acts as a prooxidant, and metal contaminants within salt mixtures like copper and iron can also contribute to lipid oxidation (Denisov and Emanuel 1960; Labuza and Dugan 1971; Love and Pearson 1971; Ladikos and Lougovois 1990; St. Angelo et al. 1996).

Sodium chloride leads to more oxidation when compared with potassium chloride when iron and copper are held constant (King and Bosch 1990). Iron is more of a prooxidant compared with copper (Salih et al. 1989). Species can also significantly impact lipid oxidation. First, fatty acid composition plays an important role in oxidative rancidity, where unsaturated fatty acids are more prone to lipid oxidation (Allen and Foegeding 1981) than saturated fats. Turkey meat contains a high degree of unsaturated fatty acids, making it more susceptible to lipid oxidation than lamb, beef, and pork (Tichivangana and Morrissey 1985). Distillers dried grains with solubles (DDGS), commonly found in pig diets, increases unsaturated fatty acids in pork (Boler et al. 2009). Secondly, the level of antioxidants present in meat counteracts oxidation and often varies between species. Tocopherol serves as an antioxidant preventing free radical formation during lipid oxidation (Botsoglou et al. 2003). Turkey meat is more susceptible to lipid oxidation when turkeys are not fed a vitamin E supplemented diet because they store little α -tocopherol in the lipid bilayer of their cell membranes (Sklan et al. 1982; Wen et al. 1997). Other factors such as total lipid content and preparation such as grinding can further dispose meat to oxidation (King and Bosch 1990; Galvin et al. 1997). Therefore, ground turkey thigh meat was used in Experiment 1 as a system that strongly promotes lipid oxidation compared to pork in Experiment 2, which is more saturated in fat content than turkey (Tichivangana and Morrissey 1985). The objective of these two experiments was to determine if impurities such as copper, iron, and manganese in different salts would impact the rate of lipid oxidation and textural characteristics in turkey meat and ground pork. It was expected that salts with increased iron and copper contamination would accelerate oxidation.

Materials and Methods

Raw Materials

Approximately 200 kg of frozen, boneless and skinless turkey thigh meat was obtained from a commercial poultry plant and stored frozen (-29 °C) at the University of Illinois Meat Science Lab until it was ready for use. After storage, meat was allowed to thaw for 3 d at 4°C prior to formulation. Approximately 180 kg of boneless pork butt was obtained from a commercial pork plant and stored at (4 °C) in vacuum packaged bags at the University of Illinois Meat Science Lab until formulation occurred. Four different salts (NaCl) were obtained from Morton Salt (Chicago, IL). The treatment groups included a control group (no salt added) and 4 different salt varieties with differing metal impurity levels of copper, iron, and manganese (Table 1).

Formulation, Packaging, and Storage

Experiment 1(turkey) and Experiment 2 (pork) were each a completely randomized design with five treatment groups (no salt and 4 salt varieties) and 6 replications for a total of 30 independent experimental units for each species. The two experiments were conducted independently but using the same salt formulations which were stored in airtight containers at ambient temperature. Meat was ground using an industrial meat grinder with a 1.3 cm plate (4152 Series Meat Grinder, Hobart Corporation, Troy, OH, U.S.A.) and thoroughly mixed. Turkey thigh meat was reground through a 0.48 cm plate and boneless pork butt was reground through a 0.32 cm plate. After the meat was ground, it was allotted into 5 kg batches, which served as the experimental units. A 454 g sample was collected from each experimental unit for determination of proximate composition and salt-soluble protein analysis. Experimental units were weighed to a target of 4.5 kg and further chopped in an industrial bowl chopper. Salt was

blended with the ground meat at a 1% inclusion by mixing the sample for 4 revolutions in the bowl chopper. Patties (150 g) were made with a manual patty maker (Patty Moulding Machine, MH-120, Manica USA, St. Louis, MO., U.S.A.). Patty paper was placed between each patty before being placed in boxes. A total of 560 patties for turkey and pork were created from each of the 30 experimental units. Patties from each experimental unit were stored for 0, 3, 6, or 9 weeks prior to evaluation. The patties designated for 0 weeks of storage were immediately packaged in styrofoam trays (Bush Brothers Inc., Urbana, IL) with polyvinylchloride (PVC) overwrap film (oxygen transmission rate = 11,627.9 cc/m²/day; moisture vapor transmission rate = 170.5 gm/m²/day) and stored at refrigerated temperatures (4 °C) for 5 days in simulated retail display. The remaining patties were randomly assigned to boxes without an oxygen barrier for the appropriate storage duration (3, 6, or 9 weeks). After frozen storage at -29 °C, samples were placed in Styrofoam trays, overwrapped with PVC film, and placed in simulated retail display for 5 days.

The fat and moisture content of each experimental unit was determined using the method described by Novakofski et al. (1989). Moisture content was determined by drying samples in an oven at 110 °C for 48 hours. After drying, samples were washed in an azeotropic mixture of chloroform: methanol to determine the amount of extractable lipid present.

Thiobarbituric Acid Reactive Substances (TBARS)

One patty per experimental unit for each display period was evaluated for raw or cooked turkey and pork TBARS (n=240). On the first day of the 5 day display period, raw TBARS were conducted. Cooked TBARS were conducted on the fifth day of the simulated retail display period after samples were cooked at 191 °C for 16 minutes in an oven (South Bend Convection

Oven, Model V-15, South Bend, IN, USA). Cook loss was determined by recording the weight of the patty before cooking and immediately after cooking.

TBARS were evaluated using a modified version of the procedure described by Leick et al. (2010) with a 96-well plate in a plate reader (Synergy HT Multi-Mode Microplate Reader, Bio-Tek, Winooski, VT, U.S.A.) reading at 530 nm to determine malonaldehyde (MDA) content. The standard curve was extended (0-22.5 mg MDA/mL) to include higher levels of oxidation. TBARS values were expressed as mg MDA/kg tissue.

Break Strength

Two patties from each experimental unit and each storage period were evaluated (n=240) for turkey and pork using a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) to determine the amount of force required to break the patty in half. Patties were cooked in an oven similar to the procedure used for cooked TBARS evaluation. Patties were cooled at room temperature for an hour and then broken by the Texture Analyzer using a modified version of the protocol as described by Souza et al. (2010). Continuous force was applied directly in the middle of each patty at 3.33 mm/sec with a crossbar (10mm diameter), at a platform gap of 3.2 cm, and travel distance of 70 mm. Break strength values were expressed as kg force.

Salt-soluble Proteins

Extractability characteristics of sarcoplasmic and myofibrillar proteins using different salt concentrations of each salt variety were evaluated using the procedure described by Boler et al. (2011). One hundred and twenty turkey and pork samples were analyzed for salt-soluble proteins. Extraction buffer was prepared by mixing 0.01M 2-[N-Morpholino]ethanesulfonic acid (MES) in ultrapure water. This extraction master mix was used to make each extraction buffer,

which contained increasing salt concentrations of 0.5% (0.09M), 1.5% (0.26M), 2.5% (0.43M) and 3.5% (0.6M) salt by volume. The pH of the extraction buffer for the pork salt-soluble proteins was adjusted to a pH of 6.0 according to Lan et al. (1993). Samples were quantified with a BCA Protein Assay Kit (Pierce Protein Research Products, Rockford, IL) using a plate reader reading at 562 nm. Amount of soluble proteins were calculated using a second order polynomial quadratic equation. Salt-soluble proteins were expressed as a percentage of wet tissue weight.

Sensory Evaluation

Sensory evaluation for saltiness, off flavor, and oxidized odor detection was conducted with a 6-member trained panel. There were 2 sensory evaluation sessions, each on the same day, for each storage period. During each evaluation, panelists were provided one sample per treatment and every treatment was represented in a session. A total of 80 patties for turkey and pork were used for sensory evaluation over the 4 different storage periods. The patties were cooked similar to those for cooked TBARS and break strength. Pieces of cooked patties were placed in 2 oz. plastic cups with a lid. Panelists were provided scorecards with a 15 cm unstructured line scale, where 0 cm indicated no salt flavor, off flavor, or oxidized odor was present. A score of 15 cm indicated the sample was extremely intense for each of these characteristics.

Statistical Analysis

Statistical analyses for raw and cooked TBARS, saltiness, off flavor, oxidized odor, and break strength were conducted using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as repeated measures over time. Fixed effects in the model included salt variety, storage duration, and their interaction. Fat content of the raw materials was included in the pork model as a covariate to account for significant variation ($P < 0.05$) of fat content in the meat blocks used

among treatments to determine raw and cooked TBARS. Data were analyzed using an unstructured covariance matrix based upon a goodness fit analysis using Akaike's information criterion to minimize variance. For salt-soluble proteins, salt variety, concentration, and the interaction between variety and concentration were included in model as fixed effects. Predetermined single degree of freedom contrast statements were used to compare the no salt treatment group to the average of all salt treatment groups for TBARS, saltiness, off flavor, oxidized odor, break strength and salt-soluble proteins. Initial analysis indicated that the magnitude of differences was greater between the no salt group and the average of the salt groups. Therefore, a new model was written and means separation was conducted among the salt varieties. If the effect of treatment was not significant when the control group was removed, it was concluded that there were no differences for the trait among the salt-included treatments. If treatment effect was significant, means were separated with the pdiff option of Proc Mixed.

Results and Discussion

Proximate Analysis and Cook Loss

Raw materials for each turkey experimental unit were sourced from a single master blend of turkey thigh meat. Proximate composition was determined on each experimental unit prior to salt inclusion to ensure any differences for TBARS were not due to differences in fat levels between treatment groups. Samples contained an average of 7.74% fat and an average of 73.92% moisture (Table 2). Fat ($P>0.37$) and moisture ($P>0.50$) content were not different between treatments. Similarly, ground pork was blended into a single master blend and the raw materials for each experimental unit were collected. Proximate analysis was conducted and it was determined that the samples contained an average of 21.63% fat and 62.28% moisture (Table 2). In contrast to turkey, fat content did vary between treatments. Therefore, fat content was used as

a covariate in further analyses of pork. Salt inclusion reduced cook loss ($P < 0.01$) by approximately 10% when compared to the control in both turkey and pork. However, there were no differences in cook loss among the salt-included treatment groups for either species ($P > 0.29$). This is not surprising, as salt is known to improve water holding capacity (Terrell 1983), and therefore reduce cook loss.

TBARS

As expected, TBARS values of raw patties increased as storage time increased ($P < 0.01$, Figure 1A,C) for both turkey and pork. In turkey, the control treatment group had lower raw TBARS values ($P < 0.01$) when compared to the average of all the salt-included treatment groups at each evaluated time point, however, there were no differences between salt-included treatments. This was also true for pork at 3, 6 and 9 weeks of storage. Interestingly, TBARS values of control pork did not increase with storage. The magnitude of the difference between the control and salt included groups increased with storage time. For turkey, the difference between control and the average of salt-included treatments was 0.83 mg /kg at 0 and 3 weeks but increased to an average difference of 2.11 mg /kg in weeks 6 and 9. For pork, the average TBARS of the salt-included groups at 0 and 3 weeks was 0.055 mg/kg higher compared with control but increased to 0.26 mg/kg at 6 and 9 weeks. The increase in the magnitude of difference between the control and salt-included treatment groups suggests salt increased the rate of lipid oxidation in raw meat.

Results were more mixed in cooked meat. As storage time increased, cooked TBARS values also increased in cooked turkey ($P < 0.01$, Figure 1B). There were no differences in cooked TBARS values among any of the salt-included treatment groups ($P > 0.05$). The average of the salt-included treatment groups had numerically higher cooked TBARS values when

compared to the control for all weeks tested and was significantly higher at weeks 0 ($P<0.01$) and 6 ($P=0.02$). The magnitude of the difference between the control and the average of the salt treatments for cooked TBARS values at week 0 was 1.88 mg MDA/kg, 0.46 at week 3, 1.00 mg MDA/kg at 6 weeks, and 0.33 mg MDA/kg at 9 weeks of storage. Therefore, an acceleration of oxidation with salt inclusion as observed in raw TBARS was not apparent in cooked TBARS values for turkey.

As storage time increased, cooked TBARS values also increased ($P=0.05$) in pork (Figure 1D). At week 0, the average TBARS level of the salt treatments was increased ($P=0.01$) compared with the control. However, the salt treatments were not different from one another ($P=0.68$). The magnitude of difference between the control and the average of salt treatments at week 0 was 0.14 mg MDA/kg, 0.17 mg MDA/kg at week 3, 0.06 mg MDA/kg at week 6, and 0.06 mg MDA/kg for week 9. As with turkey, the increased rate of lipid oxidation observed with salt inclusion in raw pork was not observed in cooked pork.

Lipid oxidation was lower in raw meat samples compared to cooked samples in agreement with previous reports (King and Earl 1988; Salih et al. 1989) and was expected because cooking promotes oxidation (Love and Pearson 1971). Cooking disrupts cell membranes within muscle and releases non-heme iron from myoglobin, and exposes lipids to oxygen (Sato and Hegarty 1971; Igene et al. 1985). Compared to pork, TBARS values for raw and cooked turkey samples were quite high. However, turkey fat has a high degree of unsaturation (Tichivangana and Morrissey 1985) and thigh meat contains twice the amount of fat than breast meat (Salih et al. 1989). The lack of differences between the salt-included treatment groups in each species, however, was unexpected, as previous studies have reported that heavy metals like copper and iron serve as prooxidants. Given the results of previous research, salt variety A would

be expected to be the least prooxidant because it contained the least copper and iron, whereas salt variety D had the highest level of iron compared to the other salts and would be expected to be the most prooxidant. This, however, was not the case. It is possible that the turkey used in Experiment 1 oxidized too rapidly and may have masked any detectable differences among the different salt treatments, but using pork with overall lower levels of oxidation did not alter this result. Even so, these results are similar to Salih et al. (1989) who did not observe differences in oxidation between rock and pure salt used in turkey. Rock salt contained 37.43 ppm iron and 1.19 ppm copper, which are much higher contaminant levels compared to the levels within salt variety D (Table 1).

Sensory Analysis

For turkey, panelists were able to detect differences between the salt-included and control groups for saltiness but outliers prevented control samples from being included in the evaluation of saltiness for turkey (Figure 2A). In pork, saltiness was increased by the inclusion of salt in samples at each storage time point ($P < 0.05$, Figure 2B). However, in each species, no differences were detected among the salt-included treatments (turkey, $P=0.85$; pork, $P=0.15$). Saltiness level was not affected by storage time ($P=0.06$) and saltiness levels detected by the panelists for the salt-included treatment groups did not change over storage for either species.

The turkey control had higher off flavor scores ($P=0.01$, Figure 2C) when compared to the average of the salt treatments at week 0 (Figure 2), however, there were no differences ($P>0.05$) between any treatments for weeks 3, 6, or 9. There was no effect of treatment for off-flavor of pork. Off flavor scores increased over the entire storage period for both species but did not interact with salt treatment. Therefore, off flavors were detected, but salt did not alter the rate of the development of off flavors.

Similar to off flavor, oxidized odor increased with storage time for all treatment groups in both species (Figure 2E,F). When compared to the average of the turkey salt-included treatment groups, the turkey control group had higher oxidized odor scores ($P=0.04$) at week 6 but was not different than the salt-included groups at other weeks. Similarly, the pork control group exhibited higher oxidized odor values ($P=0.01$) than the average of the pork salt treatments at week 6. There were no differences between salt varieties for oxidized odor for either species.

All salt treatments were similar to each other; therefore, there is no clear pattern of oxidized odor development related to contaminant content. Off flavor and oxidized odor increased over storage time, which is in agreement with TBARS analysis in Experiment 1 and Experiment 2, along with previous results in pork and poultry (Marusich et al. 1975; Poste et al. 1986; Nolan et al. 1989). Oxidation causes hydroperoxides, aldehydes, and ketones to accumulate (Allen and Foegeding 1981; Ladikos and Lougovois 1990; Gray and Monahan 1992) leading to the development of off-odors and flavors.

Break Strength and Salt-soluble Proteins

In each species, the control group required less force ($P<0.05$) to break cooked patties regardless of storage time when compared to the average of the salt varieties (Figure 3). When the control group was removed, the treatment effect was still significant ($P\leq 0.03$), yet the interaction between the treatment and duration of storage was not significant ($P\geq 0.17$). In turkey, salt variety D had the most bind strength (Figure 4A), as it took more force to break the patties. Varieties A and B were intermediate and salt variety C had the least bind strength. Salt variety A (Figure 4B) required the most force to break the patties in pork compared to the other salt varieties and was significantly different ($P<0.05$) from the other salt varieties. In contrast to

turkey, salt variety D had the least amount of break strength in pork. Thus, there is no clear pattern among the salt varieties for break strength in turkey and pork.

Adding salt to meat extracts sarcoplasmic and myofibrillar proteins at increasing levels as salt concentration increased. Increasing levels of each salt were analyzed to determine if the pattern of extraction differed between salt varieties. Results for turkey and pork were quite similar for this trait. The salt-included treatment groups had higher values ($P < 0.05$) at all salt concentrations when compared to the control in both species (Figure 5). Although salt was not added to the control group, water-soluble sarcoplasmic proteins (Scopes 1970) were likely extracted in the control buffer resulting in the low level of apparent salt-soluble proteins. However, there were no differences ($P > 0.65$) in protein extractability among any of the salt-included treatment groups for either species. Therefore, each salt variety resulted in a similar pattern of protein extraction in both species tested. It was expected that differences would be detected among the salt varieties due to the presence of contaminants, as sodium chloride acts to change the ionic strength by altering the charges on proteins in meat matrices resulting in a more stable emulsion. Calcium chloride has been shown to decrease emulsion stability, whereas iron chloride increases emulsion stability in broiler meat when used in combination with sodium chloride (Whiting and Richards 1978).

When salt is added to a meat product, proteins are extracted and texture is improved (Terrell 1983; Foegeding 1987). In this study, an increase in break strength of the patties with added salt and increased protein extraction were observed. This is a desirable function of salt in ground meat products. However, as there were no meaningful differences between salt varieties among turkey or pork; it did not appear that metal contamination of salt at these levels impacts salt's extraction and binding capabilities.

Conclusions

Lipid oxidation was expected to be higher for turkey and pork patties containing salts with higher levels of copper and iron contaminants. However, no meaningful differences were detected among the salt treatments for TBARS, sensory characteristics, break strength, or salt-soluble proteins for either species. Turkey was very prone to lipid oxidation, as indicated by very high TBARS values at early storage times, and therefore may have masked the differences from start to finish for the various salt treatments. As a result, pork was chosen as the model in Experiment 2 because it contains a less degree of unsaturation than turkey (Tichivangana and Morrissey 1985) and it was hypothesized that pork would allow differences to be detected among the different salts. The lack of difference between salts may also be attributed to the small amounts of trace metals present within the sodium chloride compared with that of raw materials. Turkey thigh meat contains approximately 20 ug/g of total iron (Guidi et al. 2006), which is ten times more than the iron content used in salt variety D as a contaminant. The amount of iron in the triceps brachii of the pig is approximately 13 ug/g (Schricker et al. 1982). However, there were no meaningful differences among the salt varieties in pork. Additionally, the salts used in both experiments had a variety of copper, iron, and manganese levels without a clear pattern of increasing contamination. Therefore, to determine the role of single contaminants in oxidation, these levels would need to be precisely controlled. Although there were minor differences detected between salt treatments in break strength, they are likely too small to be detected by the average consumer. Regardless of the metal impurities within the salt, salt increased TBARS in raw and cooked samples, increased salt-soluble protein concentrations, increased binding strength of patties and but had minimal effect on sensory characteristics.

Salt at a 1% inclusion rate containing these levels of copper, iron, and manganese impurities in ground, turkey thigh meat and ground pork served as a prooxidant. There were no meaningful differences among the different salt varieties. If a meat processor uses a 1% inclusion rate of salt with minimal levels of metal impurities or high levels of metal impurities, it is unlikely that differences in shelf life or protein functionality would be observed.

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Tables and Figures

Table 1. Metal contamination levels (ppm) of each salt variety

Treatment	Total Copper	Total Iron	Total Manganese
No Salt	---	---	---
Salt A	0.005	0.118	0.100
Salt B	0.048	0.232	1.300
Salt C	0.044	0.721	0.300
Salt D	0.019	1.995	1.700

Table 2. Fat and moisture percentage of batches prior to salt addition

	Turkey		Pork	
	Fat	Moisture	Fat	Moisture
No Salt	8.1	73.7	21.7	62.3
Salt A	7.5	74.0	22.4	61.7
Salt B	7.5	74.2	22.0	61.8
Salt C	7.7	73.9	21.4	62.5
Salt D	7.9	73.8	20.6	63.0
SEM	0.002	0.002	0.4	0.3
P-value of Trt	0.4	0.5	0.1	0.1

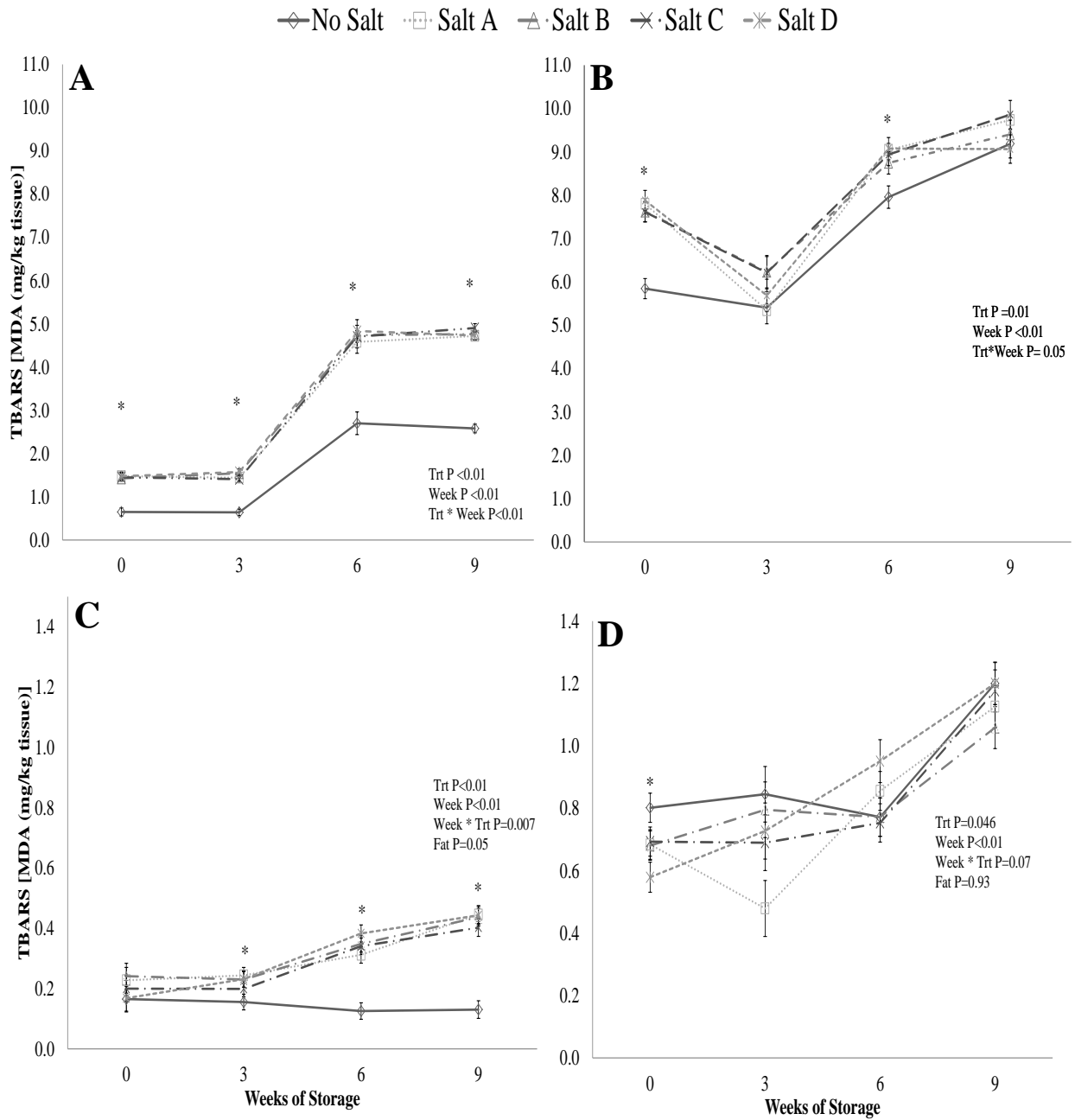


Figure 1. TBARS values (mg MDA/kg muscle) of raw ground turkey patties (A), cooked ground turkey patties (B), raw ground pork patties (C), and cooked ground pork patties (D) stored for 9 weeks. Asterisks indicate a difference ($P < 0.05$) between “no salt” treatment and the mean of all salt treatments as determined by a single degree of freedom contrast statement.

Figure 2.

◆ No Salt □ Salt A △ Salt B × Salt C * Salt D

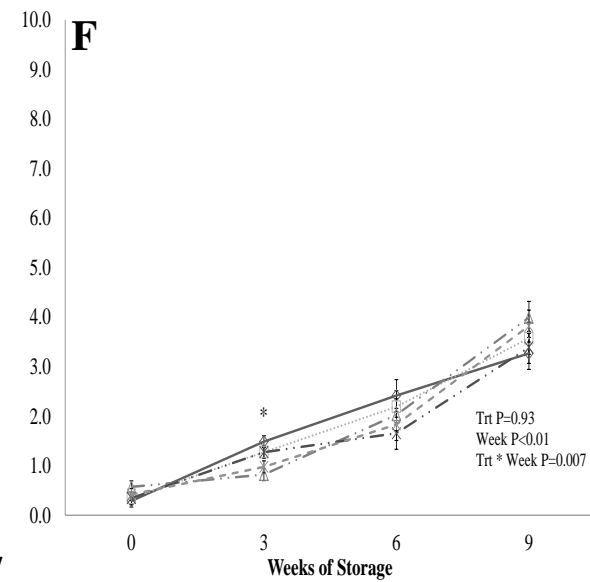
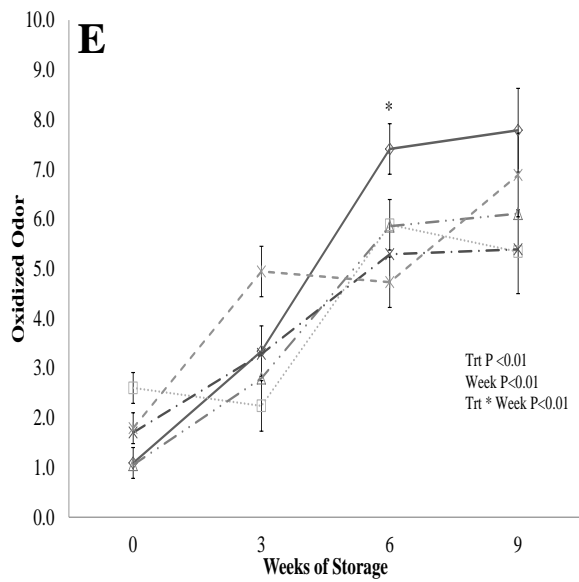
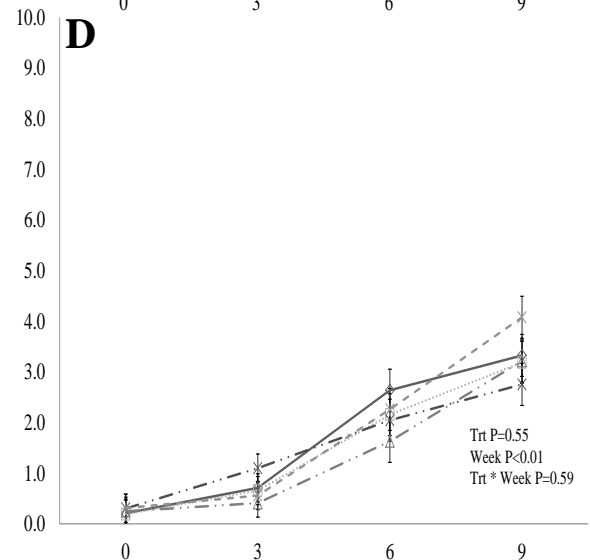
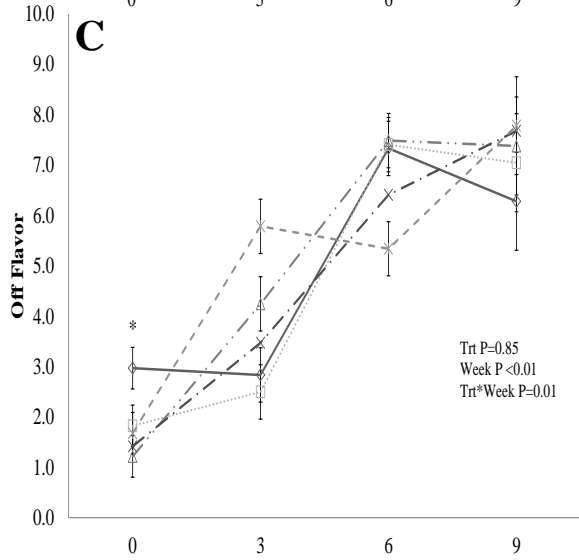
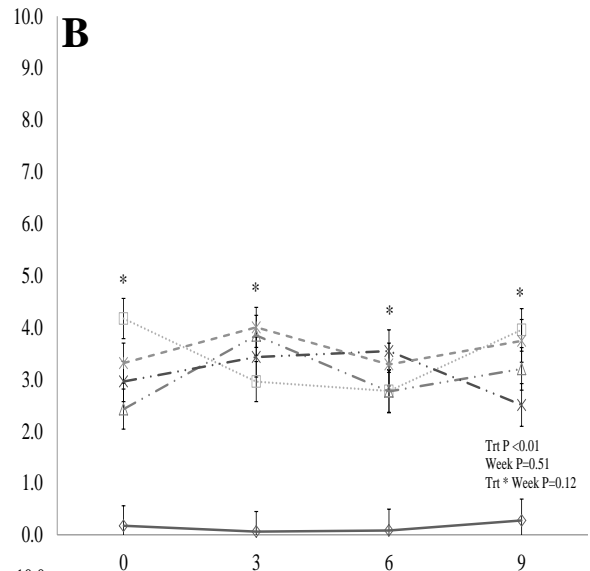
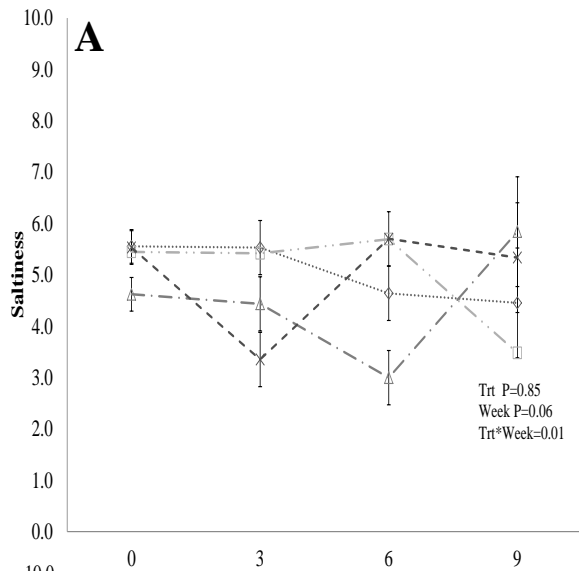


Figure 2. Sensory panel ratings of cooked ground turkey and pork patties stored for 9 weeks. Saltiness (turkey A, pork B) off flavor (turkey C, pork D), and oxidized odor (turkey E, pork F) were rated on a 15cm unstructured line scale where 0 indicated no detection for each trait and 15 indicated an intense presence. Asterisks indicate a difference ($P < 0.05$) between “no salt” treatment and the mean of all salt treatments as determined by a single degree of freedom contrast statement.

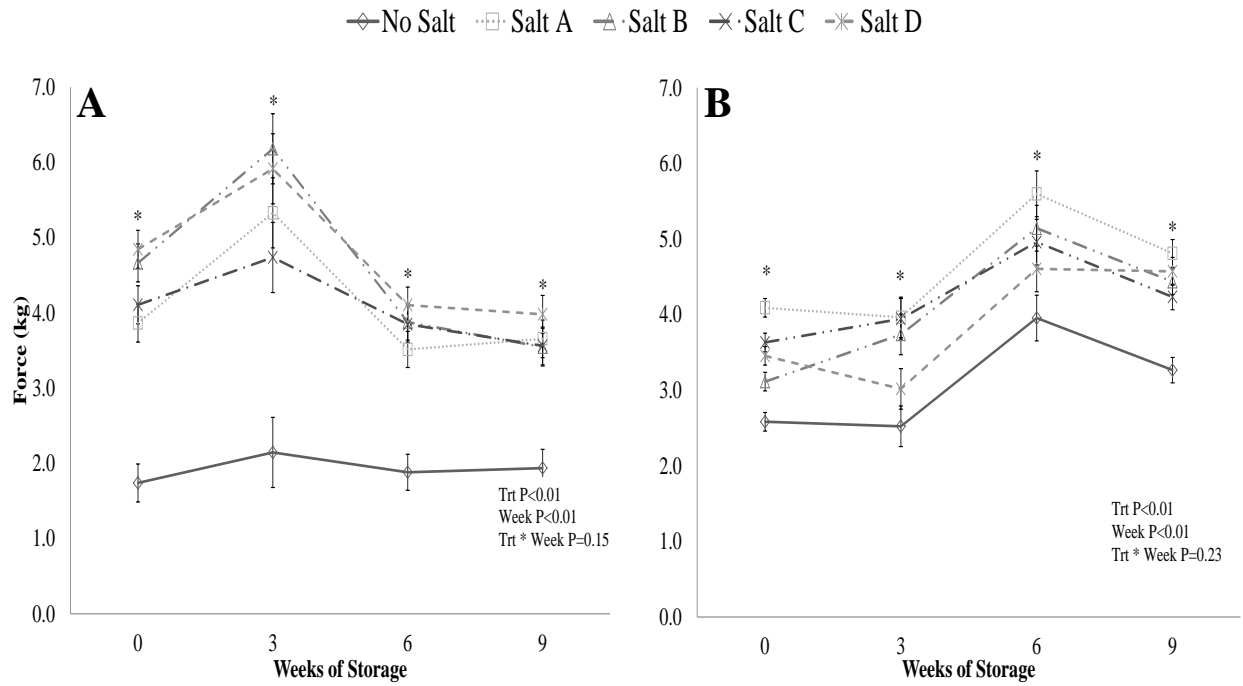


Figure 3. Force required to break cooked ground turkey patties (A) and cooked ground pork patties (B) stored up to 9 weeks. Asterisks indicate a difference ($P < 0.05$) between “no salt” treatment and the mean of all salt treatments as determined by a single degree of freedom contrast statement.

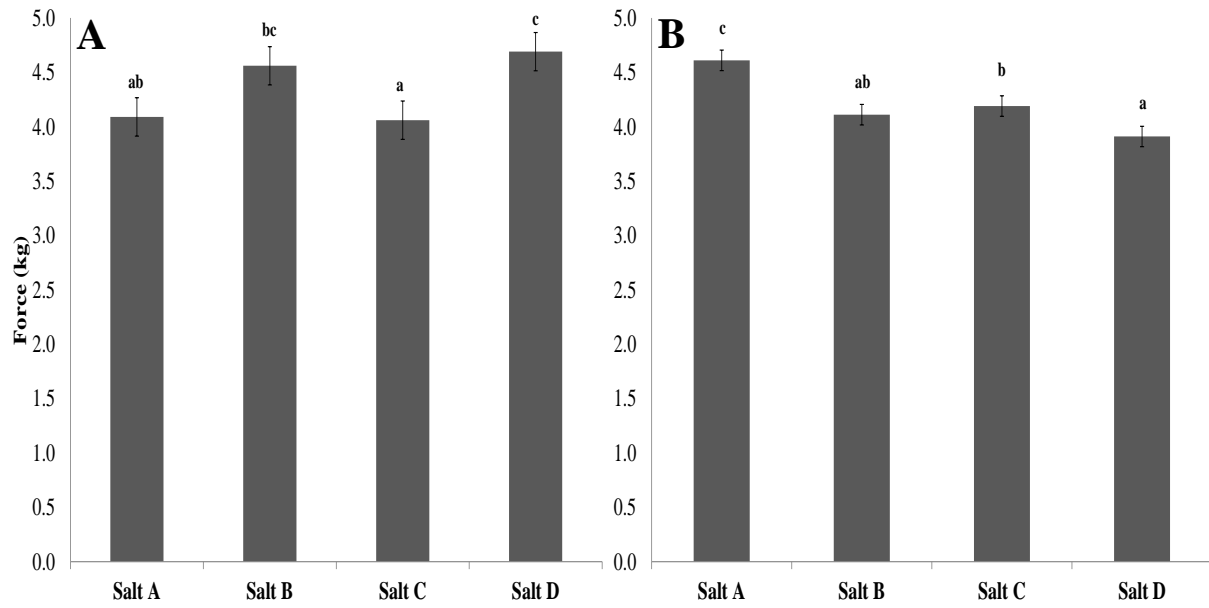


Figure 4. Least Square Means of Break Strength expressed in kg as the amount of force needed to break cooked turkey (A) and pork (B) patties. Least Square Means with the same superscript are not different ($P < 0.05$).

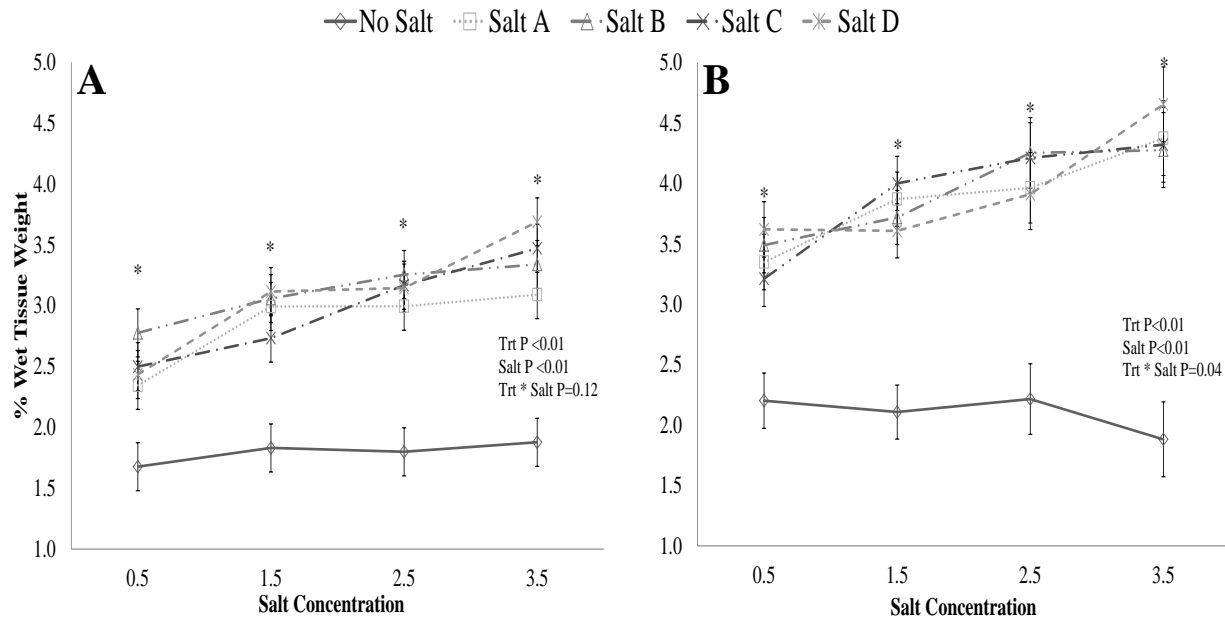


Figure 5. Salt-soluble proteins of raw ground turkey (A) and raw ground pork (B) (% of wet tissue weight) extracted in increasing salt concentrations. Asterisks indicate a difference ($P < 0.05$) between “no salt” treatment and the mean of all salt treatments as determined by a single degree of freedom contrast statement.