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Regan E. Stanley
University of Nebraska - Lincoln, rlee28.rl@gmail.com

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EFFECTS OF SALT AND NITRITE CONCENTRATION ON THE SHELF LIFE OF DELI-STYLE HAM

By

Regan Elise Stanley

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

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Major: Animal Science

Under the Supervision of Professor Gary A. Sullivan

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Effects of salt and nitrite concentration on the shelf life of deli-style ham

Regan Elise Stanley, M.S.

University of Nebraska, 2016

Advisor: Gary A. Sullivan

Deli-style hams were manufactured to measure the effects of salt and nitrite concentration on shelf life and physicochemical characteristics. Three replications of deli-style ham treatments were manufactured in a 3 x 4 factorial arrangement of salt concentration (0.7%, 1.4%, or 2.1%, meat block basis) and nitrite concentration and source (0 ppm, 100 ppm or 200 ppm sodium nitrite, SN, or 100 ppm sodium nitrite equivalent from pre-converted celery juice powder, CP; Veg Stable 506, Florida Food Products). Salt concentration, water activity, cook yield, and texture profile analysis (TPA) were measured on w 0. Color, pH, residual nitrite, and aerobic and anaerobic plate counts (APC and AnPC, respectively) were measured on weeks 0, 2, 4, 6, 8, 10, 12, 14, and 16. A nitrite by salt interaction ($P \leq 0.05$) was found for a*, a/b ratio, ΔE, and hue angle. Treatments without nitrite were less red and more yellow than other treatments. Curing with CP resulted in increased yellowness than SN treatments. An interaction of nitrite and week ($P < 0.001$) was identified for residual nitrite. Residual nitrite values of 0ppm treatments did not change throughout storage, whereas all other treatments declined with increased storage. Excluding interactions above, significant main effects for salt

concentration were identified ($P \leq 0.05$). Treatments with 2.1% salt had lower APC than 0.7% salt ($P=0.033$) and 1.4% salt was similar to both. As nitrite concentration increased, APC was significantly reduced ($P<0.001$) regardless of nitrite source. Overall, 100 ppm CP and SN were only different for a*, b*, a/b ratio, and hue angle. The 100 ppm CP had lower a* values and a/b ratio, but had higher b*, and hue angle values, than 100 ppm SN. This study suggests 200 ppm SN provides greatest shelf life to deli-style ham. Additionally, 0.7% salt resulted in inferior product quality in many traits compared to 1.4% or 2.1% salt and it is therefore suggested to use amounts greater than 0.7% salt when formulating deli-style ham.

Keywords: Salt, Nitrite, Ham, Shelf life.

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1. INTRODUCTION

Today's consumers are becoming increasingly interested in the process of manufacturing the food they eat. They are concerned with the nutritive value of their food, as well as the long-term effect the food that they eat has on their wellness. Additionally, consumers have the idea that there are negative health problems associated with consuming some ingredients such as nitrates and nitrites, and more recently, red and processed meats altogether. Health related issues, such as heart disease and high blood pressure, have been related to high levels of sodium in the American diet, so there is a large push to reduce sodium intake to combat the vast health problems. Reducing salt is known to lower blood pressure, aiding in reduction of health problems.

In response, meat scientists and the meat industry is working diligently to manufacture reduced sodium products, and to slowly reduce sodium in products, which may or may not be labeled as reduced sodium. Due to the functionality of salt, it becomes difficult to remove much of the salt present in these products, and is therefore a challenge for the meat industry as a whole. Salt is necessary for adequate product cohesion and stability of emulsions to provide acceptable meat products. Salt also aids in palatability of the product and improvement of the microbial shelf life of products by shifting microbial populations towards lactic acid producing bacteria.

Another ingredient of high importance and consumer concern is nitrite. Consumers want a clean label product, so natural alternatives must be used to achieve their desire, but we must also provide the same safety as products cured with sodium nitrite. Nitrite is known for its ability to inhibit *Clostridium botulinum*. Typically in natural cured meats, amount of nitrite equivalent present is much lower due to the use of

pre-converted celery juice powder. Celery powder has negative flavor characteristics if added at the same level as sodium nitrite. The combination of lower nitrite and limitations for natural antimicrobials in these products, along with reduced sodium levels may potentially provide a more favorable environment for pathogens to survive when compared to conventionally cured meats. In order to further understand the safety of these products, we must study the impact of reduced sodium and nitrite concentration and source on the shelf life and quality characteristics on these products.

This study compared physiochemical qualities and microbial integrity of deli-style ham produced with salt or nitrite concentration. This study identified how salt and nitrite concentrations affect the quality characteristics of deli-style ham. Additionally, how salt and nitrite concentration affect the growth of natural spoilage flora of sliced deli-style ham was also discovered.

2. REVIEW OF LITERATURE

2.1 History of Meat Preservation and Curing

Since ancient times, meat has been providing a rich source of nutrients for humans, but humans have also known the short life of fresh meat and how easily it can spoil. Freezing, salting, and drying supplemented by fire or dry heat in humid seasons were some methods used early on by humans to preserve meat (Wentworth, 1956). The smoke from the fire would add flavors to the meat. Early development of meat preservation techniques for later consumption was a necessity for survival. Preservation was accomplished in several ways, most of which included the addition or application of salts. Populations of Jewish people used salt from the Dead Sea, and later, Europeans excavated salt mines to use in the preservation of meat (Binkerd & Kolari, 1975). This use of salt helped preserve the meats and if these salts contained saltpeter, they also had the ability to produce the reddish cured meat color, prolonging the action of preventing growth of spoilage microorganisms. Saltpeter (KNO_3), recognized as a contaminant of salt, enhanced the preservative effect of salt, and the salted meat product then had a red color (Honikel, 2008).

Use of saltpeter was recorded as early as 2200 BC, and is thought to be referenced in the bible (Barnum, 2003). In Prussia, settlements were ordered by the King to have a covered shed where compost and vegetables were collected, and men in England were ordered to gather saltpeter present in nitrous soils anywhere they were able to find it. Additionally, under dry conditions, soil from dirt floors of stables, cellars, caverns, or pens could produce from three to five pounds of saltpeter per 100 pounds of soil

(Barnum, 2003). Nitrite, as opposed to nitrate, was identified as the agent responsible for producing the heat stable red color of what is known today as cured meat (Honikel, 2008), and shortly after, Haldane (1901) showed redox reactions occurred in the chemistry of cured meat. Hoagland (1908) confirmed Haldane's work and explained that the color of uncooked meats cured with saltpeter was due to nitrosohaemoglobin, and nitrosohaemochromogen was the color of saltpeter-treated cooked meats (Binkerd & Kolari, 1975). Experiments carried out in the early 1920s led researchers to advocate for the direct addition of sodium nitrite, since the reliance on nitrite formation from nitrate in pickling solution revealed no clear advantages over direct addition (Kerr, 1926). Permission for the direct use of nitrite by a meat processor under Federal inspection was first given on January 19th, 1923 by the USDA's Bureau of Animal Industry (Binkerd & Kolari, 1975). Both salt and sodium nitrite remain important in modern processed meat production.

In 1925, the United States Department of Agriculture (USDA) stated that the levels of ingoing nitrate, nitrite, or the combination of both salts at 0.25 oz. for every 100 pounds of meat was sufficient to provide adequate cured meat pigment at its maximum. (USDA, 1925). Regulatory limits of ingoing nitrite vary by product type and calculations are always based upon weight of the meat. The USDA recognizes sodium nitrite, potassium nitrite, sodium nitrate, and potassium nitrate as curing agents and regulates their usage (USDA, 2016a) however sodium nitrite is the most commonly used curing ingredient. Direct addition of sodium nitrite to comminuted products is limited to 156 ppm (USDA, 1995). Products manufactured with brine added through immersion, massaging, or injection, 200 ppm is the maximum ingoing amount of sodium nitrite

(USDA, 1995). Dry cured products are allowed 625 ppm of ingoing sodium nitrite (USDA, 1995). Nitrate regulations differ, though use is typically limited to products that have extended fermentation and drying periods to allow for a nitrite reserve to aid in bacterial reduction. Dry cured products are limited to 2187 ppm sodium nitrate, and immersion cured products are allowed 700 ppm (USDA, 1995). Bacon regulations are different from others to limit the potential formation of carcinogenic N-nitrosamines during frying (Sen, Seaman, & Miles, 1979). Injected or brine cured bacon products are always produced using 120 ppm nitrite and 550 ppm sodium erythorbate or sodium ascorbate, and the use of nitrate is prohibited (USDA, 2016b). The addition of sodium erythorbate or sodium ascorbate reduces the residual nitrite in the processed meats and has been shown to decrease or inhibit the formation of N-nitrosamines in model systems (Mirvish, Wallcave, Eagen, & Shubik, 1972)

2.2 Chemistry of Nitrate, Nitrite, and Nitric Oxide

An understanding of the transformation of nitrate into nitrite and nitrite into nitric oxide is important for the chemistry behind cured meat characteristics. This understanding began with an early observation of nitrate (NO_3^-) being reduced to nitrite (NO_2^-) by bacteria possessing nitrate reductase activity (Jones, 1933). Certain species of nitrate-reducing bacteria which are commonly studied include species of the following genera: *Staphylococcus, Micrococcus, Aerobacter, Lactobacillus, and Pseudomonas* (Harrison, 1929). The presence of these bacteria were suggested to be found on meat processing equipment, water, and in the meat itself, making the reduction of nitrate to nitrite a likely occurrence (Kerr, 1926). However, in modern meat processing if nitrate

reductase activity is require, a specific strains can be added as a starter culture (Terns, Milkowski, Clause, & Sindelar, 2011).

Nitrate (NO_3^-) is the fully oxidized nitrogen oxide compound. Nitric acid, HNO_3, has a pK_a of -1.6, meaning that when nitrate is dissolved in water, nearly all exists as nitrate anion (Honikel, 2008). In vivo, nitrate has been shown to be a reserve and precursor for nitric oxide and other important nitrogen oxide compounds, though bacterial reduction is necessary for nitrate to have biological activity (Lundberg et al., 2009; Lundberg & Weitzberg, 2010). In meat processing, nitrate must be reduced to nitrite in order meat curing reactions to occur and to develop traditional cured meat characteristics (Terns et al., 2011).

Nitrite is much more reactive when compared to nitrate. Nitrous acid, HNO_2, has a pK_a of 3.3, so when nitrite is dissolved in water, it is found mainly as the nitrite anion, NO_2^-. The nitrite ion, once reduced to act as the nitrosating/nitrosylating agent in cured meats, can occur through several pathways involving endogenous compounds and added ingredients (Honikel, 2004). Reducing compounds such as sodium erythorbate or sodium ascorbate can be added to non-enzymatically reduce nitrite to nitric oxide (Williams, 1988). Temperature, pH, endogenous compounds, and other added ingredients can contribute to the reduction of nitrite to nitric oxide (Cassens, 1997). Two molecules of nitrous acid can form water and the anhydride of nitrous acid, dinitrogen trioxide (N_2O_3) during dissociation. This is the rate-determining step in the production of nitric oxide from nitrite (Pegg & Shahidi, 1997). Residual nitrite, the nitrite remaining in cooked meat products, serves an important role as a reservoir for NO production (Sebranek & Bacus, 2007). Excess residual nitrite can increase the risk of N-

nitrosamine formation (Sebranek & Bacus, 2007). This reservoir may result in reduced discoloration and auto-oxidation in cooked products (Dryden & Birdsall, 1980).

Nitric oxide (NO), was first identified over 200 years ago when early researchers found it readily reacted to form other nitrogen oxide compounds (Gow, 2006). Nitric oxide is a very potent nitrosylating/nitrosating agent in cured meats since it is highly reactive free radical. Depending on the environment, nitric oxide can act as an oxidizing, reducing, or nitrosylating/nitrosating agent (Henry, Ducastel & Guissani, 1997; Wink et al., 2001). As a free radical, nitric oxide can terminate free radical reactions and acts as an important molecule in providing typical cured meat characteristics (Miranda et al., 2000).

2.3 Nitric Oxide and Myoglobin

In the live animal, hemoglobin is the major heme protein found in the animal's body, but after exsanguination and removal of most of the blood, myoglobin becomes the major heme protein in meat (Suman & Joseph, 2013). The porphyrin ring of myoglobin, containing an iron atom bound to four nitrogen atoms, is bound to the globin by a histidine residue, and the remaining heme-iron binding site may be coordinated with a variable ligand (Mancini & Hunt, 2005). Several potential ligands exist, but those most common include oxygen (O_2), carbon monoxide (CO), nitric oxide (NO), carbon monoxide (CO), or water (H_2O). The iron oxidation state, ferrous (Fe^{2+}) or ferric (Fe^{3+}), and which ligand it is bound will determine the color observed from the myoglobin (Quillin, Arduini, Olson, & Phillips, 1993). Packaging, display conditions, and curing

agents can drastically contribute to meat color (Andersen, Bertelsen, Boegh-Soerensen, Shek, & Skibsted, 1988).

Depending on the state of myoglobin during the production of a cured meat product, meat color will change as nitric oxide is formed and bound to myoglobin. When nitrite is first added to meat in an aerobic environment, ferrous myoglobin becomes oxidized to ferric metmyoglobin, changing the color from red to brown, and reducing nitrite to nitric oxide (Skibsted, 2011). Under anaerobic conditions, nitric oxide can bind to the oxidized protein and form an intermediate, nitrosylmetmyoglobin, which can be reduced to nitrosylmyoglobin by a reducing agent such as sodium erythorbate (Dryden & Birdsall, 1980). When the meat product is cooked, the nitrosylmyoglobin will denature and form nitrosylhemochrome (Bonnet, Chandra, Charalambides, Sales, & Scourides, 1980).

2.4 Functional Ingredients and their Effects on Meat Curing

Nitrate and Nitrite

Sodium or potassium salts of nitrate and nitrite are used in curing meats (USDA, 1995). Permission for the direct use of nitrite by a meat processor under Federal inspection was first given on January 19th, 1923 by the USDA's Bureau of Animal Industry (Binkerd & Kolari, 1975). The USDA allowed the use of sodium or potassium nitrite in meat products at levels described by Kerr (1926). Currently, sodium nitrite is used almost exclusively, but nitrate is occasionally used in dry cured and dry or semi-dry products due to their extended curing, drying, or fermentation times (Honikel, 2004; Pearson & Gillett, 1999). Nitrite is required in cured meats to provide cured color,

flavor, aroma, antimicrobial activity, and antioxidant activity, however it must first be reduced to nitric oxide for nitrosation/nitrosylation reactions to occur (Sebranek & Bacus, 2007).

While saltpeter's impact on meat color fixation and preservation is a positive one, Tomhave (1925) warned it must be used in limited quantities, suggesting a need for regulations on preservatives. Strict regulations have been implemented on levels of ingoing nitrate and nitrite for consumer safety of all cured meat products. Direct addition to comminuted products is limited to 156 ppm of sodium nitrite (USDA, 1995). Products manufactured with brine added through emersion, massaging, or injection, 200 ppm of sodium nitrite is the maximum ingoing amount (USDA, 1995). For dry cured products, 625 ppm of ingoing sodium nitrite is allowed (USDA, 1995). Nitrate regulations differ, though use is typically limited to products that have extended fermentation and drying periods to allow for a nitrite reserve to aid in bacterial reduction. Dry cured products are limited to 2187 ppm sodium nitrate, and immersion cured products are allowed 700 ppm (USDA, 1995). Injected or brine cured bacon products are always produced using 120 ppm nitrite and 550 ppm sodium erythorbate or sodium ascorbate, and the use of nitrate is prohibited (USDA, 1995). No true minimum amount of sodium nitrite is required. It is recognized that 40-50 ppm of sodium nitrite is adequate to provide most cured meat characteristics but may not provide the same level of pathogen control (USDA, 1995). However to ensure product safety, the USDA "requires a minimum of 120 ppm of ingoing sodium nitrite to all 'Keep Refrigerated' identified products" unless other processes for preservation are verified and implemented to ensure consumer safety (USDA, 1995).

Salt

Salt is a multifunctional, essential ingredient required in meat processing and is found in all cured meats (Pearson & Gillett, 1999). Data from the National Health and Nutrition Examination Survey (NHANES II) survey in 1976-1980 stated that hot dogs, ham, and lunch meats make up 9.76% of the US diet (Block, Dresser, Hartman, & Carroll, 1985). Since reduction of sodium in the diet is of utmost importance to the government due to the relationship of sodium and hypertension, the meat industry along with others have made efforts to reduce sodium intake over the past half century (Bernstein & Willett, 2010).

Salt can increase moisture content in meat products due to the increased water holding capacity observed at low concentrations of salt however it can have a dehydrating effect when used at high concentrations (Schmidt, Carciofi, & Laurindo, 2009). The effect of myofibrillar protein extraction occurs during processing due to the action of the chloride anion of sodium chloride. The chloride ion increases the negative charges in order to cause repulsion and increase muscle swelling. In order to obtain adequate product quality characteristics such as bind and cohesion, a minimum concentration of 1.4% salt in normal and 1.75% salt in low fat meat products are required to achieve adequate protein extraction and acceptable bind and quality of meat products (Ruusunen & Puolanne, 2005). Sodium chloride is the most common salt used in meat products, but potassium chloride can be used as a substitution at up to 50% of the salt formulation without negative sensory characteristics, which can aid in achieving acceptable meat quality traits in reduced sodium products (Pearson & Gillett, 1999).

The flavor salt introduces to meat is one that is desired by consumers. Reducing sodium in meat reduces the flavor opportunities available for human perception. The addition of salt to foods increases the rate of salivation, increasing the juiciness to improve overall eating experience (Neyraud, Prinz & Dransfield, 2003). Less salivation resulting from low sodium products may explain the bland taste found in these products. Salt is also a flavor enhancer since it reduces perceived bitter taste, therefore improving the taste of sweet and sour components of food (Keast, Breslin, & Beauchamp, 2001). While the amount of salt amount used in products varies widely, salt is considered self-limiting since products with excess salt become too salty and are no longer palatable (Martin, 2001). Adequate amounts of salt to achieve enough protein extraction is essential in processed meat products.

Salt also aids in antimicrobial activity in processed meats due to its ability to reduce water activity and increase ionic strength in meat products. While salt lowers the water activity of a product, other functions of salt are necessary to fully explain the preservative effect observed (Jay, 2000; Sperber & Peck, 1983). Though varying osmotolerance exists among bacteria, salt can be used as a hurdle in bacterial inhibition when combined with other functional ingredients like sodium nitrite, and other methods such as vacuum packaging (Doyle & Glass, 2010). One example, *Staphylococcus aureus*, is able to grow when greater than 20% salt is present, however *Campylobacter spp.* are much more sensitive to salt and grow best at only 0.5% salt (Doyle & Glass, 2010). Because of this, reduced salt products typically result in reduced shelf life due to bacterial spoilage.

Nitrite and salt function synergistically to improve cured meat color, flavor, aroma, and antimicrobial characteristics. The chloride ion is responsible for increasing the rate of nitric oxide formation during curing (Sebranek & Fox, 1991). Products that are more acidic further accelerate these reactions. Additionally, N-nitrosamine formation may be reduced by adding 0.5% or greater concentrations of salt to meat products (Theiler, Sato, Aspelund, & Miller, 1981).

Sweeteners

Several sweeteners are used in meat products, and each has a different impact on product color, flavor, and microbial growth. The most common sweeteners used in the meat industry are sugar, brown sugar, dextrose, and corn syrup (Martin, 2001). Sweeteners are commonly added to meat products to balance the potentially harsh flavor of salt (Townsend & Olson, 1987), but can also be added as an energy source for fermentation, or to increase surface browning in products when desired (Pearson & Gillett, 1999). Other sweeteners such as maple syrup, molasses, and honey can be used to impart specific flavor profiles and aromas (Pearson & Gillett, 1999). Sugars can lower the water activity of products and provide antibacterial effects, however in meat products low enough concentrations are added, so this is not usually a practical application for reduction of water activity (Pearson & Gillett, 1999).

2.5 Cured Meat Characteristics

Cured Meat Color

The pink color of cured meats is a very distinct indicator of cured versus uncured meats, and is the most understood reaction of meat curing. Raw meat is primarily found

as one of three colors depending on the myoglobin state and bound ligand. Deoxymyoglobin is present when iron is in the ferrous state (Fe^{2+}), nothing is bound to the ligand, and meat is purplish red (Mancini & Hunt, 2005). Oxymyoglobin is the state in which the iron is in the ferrous (Fe^{2+}) state, oxygen (O_2) is bound to the ligand, and the color is bright red (Mancini & Hunt, 2005). Finally, metmyoglobin is present when the iron is in the ferric (Fe^{3+}) state, nothing is bound to the ligand, and the meat is brown in color (Mancini & Hunt, 2005). During thermal processing, these three states all denature to hemochromagen, which is brown in color (Reith & Szakaly, 1967). The color chemistry observed in fresh meat changes with the addition on nitrite and its subsequent reduction to nitric oxide. The nitrosylation of myoglobin and subsequent cooking results in a stable, pink cured meat pigment, nitrosyl hemochromagen (Honikel, 2008). Cured meat color is much more stable than that of oxymyoglobin (Dryden & Birdsall. 1980), which partially contributes to the longer shelf life of cured meat products than for fresh meats. Following thermal processing to an internal temperature of at least 150°F, the globin protein is denatured, forming stable nitrosylhemochromagen color (Fox, 1966; Hornsey, 1956). Though many cured meat products are treated with ingoing sodium nitrite levels of 120-200 ppm, satisfactory and stable color development can occur at ingoing nitrite concentrations as low as 40 ppm (Froelich, Gullett, & Usborne, 1983). Exposure to oxygen and fluorescent lighting results in cured color fading, giving a brownish-gray color, though sufficient residual nitrite can slow this process (Andersen et al., 1988).

Cured Meat Flavor

Cured meat reactions for flavor and aroma are not fully understood, but it has been proposed that they are primarily related to the limited formation of oxidation products. One study had an untrained panel rate ham samples containing 50 ppm and 150 ppm sodium nitrite and hams were equally desirable and more desirable than ham lacking nitrite (Froelich et al., 1983). They also noted that a trained panel found that greater levels of salt and nitrite led to a more intense cured meat flavor, suggesting the salt may enhance cured meat flavor (Froelich et al., 1983). Volatile compounds are responsible for much of the flavor of foods. Differences in volatile compound production have been observed for cured and uncured pork (Ramarathnam, Rubin & Diosady, 1993). Uncured pork had 60 components identified, and cured pork had 34 components. Of these, 13 were detected only in aroma concentrate of cured pork (Ramarathnam et al., 1993), and in part may be responsible for cured meat flavor.

Components in the Aroma of Cured Pork

- 2-Methyl-3-hexanone
- 2,3,5-trimethyl-hexane
- 4-ethyl-1-methylhexane
- 2,2,5,5-tetramethylhexane
- 2,2,4-trimethylheptane
- 2-methylcyclopentanol
- 2-butyl-2-octenal
- hexadecane
- 4-nonylphenol
- 1,3-dihydro-2*H*-imidazo[4,4-*b*]pyridine-2-one
- 4-ethyl-2,6-dimethylpyridine
- (*E*)-5-octadecene
- methyl 11,14-eicosadienoate

(Ramarathnam et al., 1993)

Antimicrobial Properties

Increased antimicrobial activity in cured meats is observed with as amount of nitrite is increased in processed meats due to more residual nitrite remaining in these products (Myers et al., 2013). Nitrite has the unique ability to hinder outgrowth of *Clostridium botulinum* spores, which has been the primary pathogen of investigation when studying nitrite's antimicrobial impact (Christiansen et al., 1974). Many antimicrobials are available for use in meat products, but none have come close to the effectiveness, affordability, safety, and practicality offered by nitrite (Pierson & Smoot, 1982). Salt, as an example of a traditional ingredient in meat preservation, may inhibit outgrowth of spores from anaerobes, but only at very high amounts in which the product would not be palatable (Duncan & Foster, 1968).

Listeria monocytogenes has been of concern in ready-to-eat cured meats due to its ability to grow at refrigerated temperatures and in high salt concentrations (Swaminathan, 2001), across a pH range of 4.7-9.2. This pathogen is responsible for listeriosis, which can cause abortions in pregnant women and mortality in infants and immunocompromised individuals (Larsson, Cronberg, & Winblad, 1979). In a study by Myers et al. (2013), hams were inoculated with *L. monocytogenes* and then subjected to high hydrostatic pressure (HHP) and nitrite at varying levels and concentrations. Hams with 200 ppm sodium nitrite and 0 or 400 MPa HHP had less growth of *Listeria monocytogenes* compared to hams made without sodium nitrite, 50, or 100 ppm nitrite derived from natural sources, and exposed to 600 MPa HHP. Nitrite source did not impact bacterial growth, however exclusion of nitrate or nitrite allowed for greater bacterial growth (Myers et al., 2013). In a cured meat model system, similar results were

reported that samples with 150 or 200 ppm ingoing sodium nitrite had less *Listeria monocytogenes* growth than those manufactured with 0, 50, or 100 ppm of ingoing sodium nitrite (Xi, Sullivan, Jackson, Zhou, & Sebranek., 2011). Many factors impact the antimicrobial activity of nitrite and affect the product safety and shelf life. Tompkin (2005) identified the following factors:

1. pH of the product during abuse
2. Injection level
3. Residual nitrite at point of abuse and the rate of depletion during abuse
4. Amount of viable botulinal spores and vegetative cells at the time of abuse
5. Temperature of abuse
6. Concentration of ascorbate or isoascorbate
7. Concentration of “available” iron in the product
8. Type of meat and other formulation ingredients
9. The thermal process applied to the product
10. The growth of competitive flora
11. The concentration and type of phosphate may play a role

Antioxidant Properties

Cured meats are known for having an increased oxidative stability, and this contributes to the longer shelf life achieved with cured meats when compared to cooked, uncured meat products. Cured meats are not characterized with warmed-over flavor that is normally associated with re-heated, uncured meats due to the inhibition of lipid oxidation (Skibsted, 2011). One way of ensuring oxidative stability is to add a reducing agent to the product formulation. Reducing agents like sodium ascorbate are found in cured meats and act synergistically with sodium nitrite in order to deter oxidation of the meat product (Yun, Shahidi, Rubin, & Diosady, 1987). Nitric oxide stabilizes the heme iron and reduces lipid oxidation and the prooxidant activity of the iron is limited (Bergamaschi, 2009). Sato and Hegarty (1971) showed that the addition of as little as 50 ppm of sodium nitrite effectively reduces lipid oxidation products by nearly 65 percent.

2.6 Microbial Shelf Life of Processed Meats

Shelf life of cured meat products ranges widely, depending on the product. Shelf life is usually defined by the number and type of initial microorganisms, as well as the growth rate and amount of growth present at given times throughout shelf life (Borch, Kant-Muermans, & Blixt, 1996). This is impacted largely by the growth of Lactic acid bacteria (LAB), which the product is exposed to by several post-thermal processing opportunities, including uncleaned surface reservoirs, worker's hands, peeling process of products with inedible casings, and slicing (Dykes, Cloete, & von Holy, 1991). Even with this exposure, the number of lactic acid bacteria is generally very low, however they still dominate the microbial flora in a vacuum package, ultimately leading to spoilage of the meat product (Blickstad & Molin, 1983). Part of the reason LAB dominate is due to the inhibition of aerobic spoilage bacteria from growing, since the product is pulled under vacuum. Vacuum packaging provides an anaerobic environment, which may be too high in salt concentration for other flora to grow (Egan, 1983). Additionally, vacuum packaging cured meats provides conditions which favor growth of psychotrophic LAB since they are tolerant to the atmosphere, low pH values, and presence of curing salts. Pseudomonads typically found in uncured, cooked deli meats are usually controlled by curing salts (von Holy, Cloete, & Holzapfel, 1990). Since LAB is able to grow at relatively high salt concentrations and lower pH values, they flourish and prevent the growth of gram-negative aerobes such as pseudomonads (Egan, 1983).

Most alternatively cured meat products have use by/sell by dates significantly shorter than conventionally cured meats. Because of this, it is desired to find a way to

alternatively cure meats and increase shelf life to meet that of its conventionally cured counterparts. With high pressure processing (HPP), aerobic counts were found to remain below detectable level for up to 8 weeks, and contained less than 2 logs of LAB growth at week 12 of refrigerated storage (Pietrasik, Gaudette, & Johnston, 2016). Products are considered spoiled once they achieve greater than 7 logs of growth. At this point, they tend to have rancid, sour aroma and off-flavors (Borch et al., 1996). While there is variation among species, *B. thermosphacta, Carnobacterium* spp., *Enterobacteriaceae, Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp., and *Sh. putrefaciens* are common spoilage organisms present in the spoilage of refrigerated pork (Dainty & Mackey, 1992).

2.7 Health Impacts of Nitrite and Nitric Oxide

Nitric oxide formation from nitrite is known for promoting physiological well-being within the human body, and is produced in human saliva (Lundberg, Weitzberg, & Gladwin, 2008). The NO molecule can perform many physiologically important functions:

1. Promotes cardiovascular health
2. Maintains nervous system signaling
3. Destroys pathogenic and cancerous cells
4. Regulates mucosal blood flow
5. Produces mucus
6. Prohibits platelet activity

(Milkowski, Garg, Coughlin, & Bryan, 2010; Lundberg et al., 2008; Lundberg & Govoni, 2004).

One reason NO is so effective is due to its ability to move rapidly from endothelial cells to its targeted muscle cells, making it a very effective messenger (Wells, 2000). Nitrate is concentrated in human saliva and bacterial reduction occurs in the oral cavity

(Tannenbaum, Sinskey, Weisman, & Bishop, 1974). Due to the pH of the stomach, gastric juices readily form and absorb nitric oxide from nitrate. Dietary nitrite and nitrate have been shown to provide nitric oxide homeostasis in animals deficient in nitric oxide synthase (Bryan, Calvert, Gundewar, & Lefer, 2008; Carlstrom et al., 2010). Approximately 80 percent of ingested nitrate in the average diet comes from vegetables, and water provides about 10-15 percent of daily nitrite intake, though this may be higher in countries with an unregulated water supply (Archer, 2002; Lundberg et al., 2008).

Even with the positive health impacts that are being identified, health concern exist related to the production of N-nitrosamines under conditions of high heat when frying bacon, which has been shown to be carcinogenic (Martin, 2001). Multiple studies have reported that the presence of N-nitrosopyrrolidine (NYPR), a common N-nitrosamine, is about twice as high in fried-out bacon fat than the fried bacon itself (Canas, Havery, Joe, & Fazio, 1986; Fazio, White, Dusold, & Howard, 1973; Fiddler et al., 1974). Fortunately, several options exist as reducing agents that effectively reduce the nitrosamine formation in cured meat products (Gray & Dugan, 1975). Some of these are ascorbate, glutathione, alpha-tocopherols, and tertiary butyhydroquinone (TBHQ) (Mirvish et al., 1972). As nitrite is decreased and ascorbate levels increased in bacon curing mixtures, nitrosamine presence is also lessened (Scanlan, 1983). Furthermore to combat this issue, the USDA modified regulations in bacon to require 120 ppm of sodium nitrite and 550 ppm of sodium erythorbate to reduce the likelihood of N-nitrosamine formation.

2.8 Salt Reduction in Meat

Americans currently consume an excess of salt in their diets, which has been linked to hypertension, risk of stroke, and premature death from cardiovascular diseases (Ruusunen & Puolanne, 2005). As salt from sodium chloride increases in the diet, blood pressure also increases (Sacks et al., 2001; Johnson, Nguyen, & Davis, 2001). In order to combat these health risks, sodium intake must be reduced nationally. Diets rich in potassium chloride can aid in reducing this risk due to its association with reducing blood pressure when used in place of sodium chloride (Sacks et al., 2001).

One challenge with this necessary dietary reduction is that salt has been viewed as a food preservative that aids in human health since it kills or limits growth of foodborne pathogens and spoilage organisms (Doyle & Glass, 2010). We must find a way to reduce sodium in processed foods while maintaining product safety. The efforts to reduce salt must be balanced with the original purpose of preventing growth of pathogenic and spoilage organisms, while maintaining quality characteristics (Doyle & Glass, 2010).

Methods have been developed in the meat industry that can help us achieve these quality characteristics. Solubilization of proteins to enhance to binding of protein and fat is one main function of salt, but there are currently no compounds sufficient to completely substitute sodium chloride in food. Protein extraction and hydration can still be achieved with new technologies such as blends of KCl and NaCl (Charlton, MacGregor, Vorster, Levitt, & Steyn, 2007). Additionally, adequate safety must be achieved with reduced sodium products, but are limited since there is less control by salt. The hurdle method can be used to achieve product safety in a reduced sodium meat

product. Salt reduces water activity in foods, acting as a critical hurdle in growth of pathogens and spoilage organisms, but other hurdles such as pH, antimicrobials or preservatives, packaging, and storage methods may be used to overcome this (Fulladosa, Serra, Gou, & Arnau, 2009). Care must be taken not to reduce sodium so much so that products not longer have acceptable quality or shelf life.

2.9 Alternative Meat Curing

Though cured meat products are made with sodium nitrate and sodium nitrite and have a high product safety and shelf life, consumers are demanding products made without conventional curing agents and want products with clean labels, meaning no ingredients they do not recognize as household items (McDonnell, Glass, & Sindelar, 2013). The demand for these products began in the late 1960s when nitrosamine formation was discovered to be present in products during cooking, or *in vivo* after consumption, triggering distrust of conventional curing methods since nitrosamines were found to be carcinogenic (Cassens, 1990). In order to meet this demand, products are now being made with naturally occurring forms of nitrate, such as celery juice powder, which was used since the 1990s (Sebranek & Bacus, 2007; Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). Processors add celery juice powder or other ingredients high in nitrate, and a nitrate reducing starter culture, to produce nitrite in order to naturally cure meat products (Terns et al., 2011). Products that are alternatively cured have been shown to have similar sensory characteristics as traditionally cured meats (Sindelar, Cordray, Sebranek, Love, & Ahn, 2007), but may have a slightly more yellow color due to the use of celery and cherry powders (Redfield and Sullivan, 2015). Recently, manufactures

have begun to pre-convert the celery juice with a bacterial reduction of nitrate prior to the drying process, which provides a natural ingredient already containing nitrite. This allows meat processors to increase production and eliminate the need for a bacterial reduction step in their thermal process (Sebranek et al., 2012). Additionally, there are no regulations on ingoing amount of nitrite from celery juice powder though commonly of 100 ppm equivalent of sodium nitrite (Redfield & Sullivan, 2015) is used. This level has been shown by researchers to be used effectively without any negative flavor characteristics.

If a product is made without the direct addition of nitrate or nitrite, including indirect addition to achieve cured meat characteristics, the product must be labeled as "Uncured" in a font style similar to that of the product name listed on the package, and must contain the statement "Not Preserved – Keep Refrigerated Below 40°F at all times", unless other conditions exist which make the product safe (Code of Federal Regulations [CFR], 2013). These conditions could be met by pH, water activity, or thermal processing thresholds, which can be met to provide additional product safety (CFR, 2013). Meat and poultry products may be labeled as "natural" if no artificial ingredients are included and the product has not been treated with greater than minimal processing (USDA, 2005). Demand for alternatively cured meat and poultry products that identify as "natural" has grown recently, possibly due to consumer's misconception that conventionally cured products present more health hazards than alternatively cured products (Sebranek et al., 2012). Due to the high demand, alternatively cured "natural" meat and poultry products have experienced rapid growth in commerce due to consumer willingness to pay a premium for seemingly healthier food (Nath, 2012). While the

growth of alternatively cured products varies, one brand of natural ham has experienced a 16 percent increase in annual sales since its commercial release (Nunes, 2011).

While alternatively cured products are safe, they do not have the same shelf life as conventionally cured products (Sebranek & Bacus, 2007). Much work has been conducted to try to find a substitute for nitrite, however no single natural ingredient has been discovered that can replace all functions of nitrite (Pegg & Shahidi, 2000). In addition to sodium nitrite not being allowed in alternatively cured "natural" meat products, antimicrobials, sodium phosphates, ascorbate, and erythorbate do not meet the USDA definition for minimally processed. Natural forms of other non-meat ingredients may be used, such as natural flavorings and cherry powder, high in ascorbic acid, as a substitute for sodium ascorbate or sodium erythorbate (Sebranek & Bacus, 2007).

Concerns with Alternative Curing

Concerns about product safety and quality of alternatively cured meat products have been proposed, since lower amounts of ingoing nitrite are used than those in conventionally cured meat (Krause, Sebranek, Rust, & Mendonca, 2011). Due to the limitation of 100 ppm celery juice powder equivalent to ingoing sodium nitrite based on quality (Redfield & Sullivan, 2015), less safety is achieved when compared to conventionally cured products which often contain from 120 ppm to 200 ppm ingoing sodium nitrite. Additionally, certain antimicrobials are excluded in natural or organic alternatively cured meats, therefore control of pathogenic bacteria within a product may be weakened (Sullivan et al., 2012). The USDA states that 120 ppm of ingoing nitrite is a necessary minimum concentration to provide control of pathogens in processed meats,

but concentrations at this level are difficult to achieve in alternatively cured meat products, and would still be labeled as “uncured” (USDA, 1995) due to the lack of a recognized curing agent. Because of this, alternatively cured meats may allow for pathogens, including *Clostridium botulinum* and *Clostridium perfringens* to grow in these products (Jackson, Sullivan, Kulchaiywawat, Sebranek, & Dickson, 2011; Sebranek & Bacus, 2007). Furthermore, the prohibition of use of several antimicrobials in natural or organic alternatively cured meat products can further limit pathogen control in these products. While some of the cured meat characteristics of alternatively cured products are very similar to those of conventionally cured meats, safety is of the greatest concern.

The concern of carcinogenic N-nitrosamines is another concern in alternatively cured meats due to the lack of reducing compounds added to “natural” products (De Mey, De Maere, Paelinck, & Fraeye, 2015). Since consumers desire alternatively cured meats due to perceived health benefits, and desire products which avoid the risk of consumption of nitrosamines, it is ironic that alternatively cured meats may be a higher risk than conventionally cured meats. Variable rates of nitrite formation are observed when the nitrate source with starter culture method is used, and this can lead to abnormally high levels of residual nitrite in alternatively cured products (Sebranek & Bacus, 2007). Parthasarathy and Bryan (2012) also stated that products that lack ascorbic acid and erythorbic acid might have enhanced formation of nitrosamines, which may apply to alternatively cured meats.

2.10 Summary

Nitrite is a multi-functional ingredient in cured meat products that is highly regulated due to the potential toxicity risks associated with nitrite. However due to relatively low levels of nitrite used in meat, toxicity is not a concern in commercially produced products. Recently, with the development of pre-converted celery juice powders, alternative curing methods are gaining popularity due to perceived health benefits and clean labels. Cherry powder is also gaining popularity and since it is high in ascorbic acid, it can be used as a natural alternative to sodium erythorbate.

Salt is another major multi-functional ingredient in cured meat products. It is essential for protein extraction, increases water holding capacity, control of microbial populations and increased shelf life, and the desired salty flavor of cured meat products. Furthermore, NaCl is the most common salt used in meat products, however Americans currently intake too much sodium in the diet. In order to aid in the national movement to reduce sodium in the diet, steps must be taken to create products that maintain safety and quality of cured meats, while reducing sodium. While efforts are being made to reduce salt among all foods, cured meats are an avenue that has been able to use innovation and technology to achieve this goal. Since salt is vital in providing product safety and extended shelf life, as well as quality indicative of cured meats, work must be done in order to find formulation and processing techniques which can be used to reduce salt while still achieving expected standards for all products.

Research has been done on sodium reduction in meats, alternative curing methods, shelf life of conventionally cured meats, and quality, however an opportunity remains to conduct research comparing sodium reduction and nitrite source at different

concentrations to determine the effects on shelf life and quality. Comparing conventionally and alternatively cured meat products at varying levels of salt reduction is vital for determining the safety and acceptability of these products as the industry works to lower sodium in consumer diets.

3. MATERIALS AND METHODS

3.1 Treatments and Product Formulations of Hams

Twelve ham treatments, arranged in a 3 x 4 factorial arrangement. Treatments included 3 salt concentrations, 0.7%, 1.4%, and 2.1% sodium chloride on a meat block basis, and 4 ingoing nitrite treatments (0 ppm of sodium nitrite, 100 ppm sodium nitrite, 200 ppm sodium nitrite, and 100 sodium nitrite equivalent from pre-converted celery juice powder). Ham treatments were manufactured to evaluate the effect of salt and curing method on the physicochemical and microbiological qualities of deli-style ham. Pre-converted celery juice powder (natural nitrite; VegStable 506, Florida Food Products, Eustis, FL; CP) was used as an alternative curing agent, and sodium nitrite curing salt (6.25% sodium nitrite, 93.75% sodium chloride; SN) was used as a conventional curing agent. Sodium chloride content was measured in the pre-converted celery juice powder to allow for formulation adjustments. All product formulations were based on a 11.34 kg meat block, and the total weight of non-meat ingredients was 25% of the meat block. All treatments contained 1% sugar (w/w), 0.35% sodium phosphate (w/w; Brifisol® 85 Instant, BK Giulini Corporation, Semi Valley, CA). Sodium chloride, adjusted for sodium chloride in the curing agents, was added to achieve 0.7%, 1.4%, 2.1% sodium chloride (w/w). Sodium erythorbate (495 ppm) or cherry juice powder to achieve 440 ppm ascorbic acid (VegStable 506, Florida Food Products, Eustis, FL) were added as reducing agents. Sodium nitrite or pre-converted celery juice powder were added to achieve desired ingoing sodium nitrite concentrations. Water was added to achieve the 25% extension. Full product formulations can be found in Table 1. Three independent replications were manufactured.

3.2 Deli-Style Ham Manufacture

Hams were manufactured at the Loeffel Meat Laboratory using pork inside ham muscles (IMPS 402F, USDA, 2014) following the formulations in Table 1. Ham muscles were obtained from Hormel Foods® (Fremont, NE) and frozen prior to use to certify uniformity of raw materials. Ham muscles were tempered for approximately 48 hours at -1°C and then coarse ground through a plate with 12.70 mm holes, and fine ground through a plate with 4.75 mm holes and weighed into twelve, 11.34 kg batches. Brine was manufactured with all non-meat ingredients and mixed with fine ground ham for three minutes in a double action mixer (Model 100DA70, Leland Southwest, Fort Worth, TX, USA). Meat batter was stuffed into 6M x 107 cm pre-stuck, fibrous casings (Kalle, Wiesbaden, Germany) and clipped. Two logs of equal length were made for each treatment. The logs were hung on a smoke stick on a smokehouse truck, and were thermally processed according to Table 5.2 to an internal temperature of 68.3°C to meet Appendix A regulations (USDA, 1999a). Ham logs were chilled overnight at 3°C to meet FSIS Appendix B regulations (USDA, 1999b) for cooling heat-treated, cured pork products (0.7% NaCl 100 SN, 1.4% NaCl 100 SN, 2.1% NaCl 100 SN, 0.7% NaCl 200 SN, 1.4% NaCl 200 SN, and 2.1% NaCl 200 SN) and heat-treated, uncured pork products (0.7% NaCl 0 ppm, 1.4% NaCl 0 ppm, 2.1% NaCl 0 ppm, 0.7% NaCl 100 CP, 1.4% NaCl 100 CP, and 2.1% NaCl 100 CP).

After chilling overnight, the fibrous casings were removed and sliced into13 mm and 2 mm thick slices, for physicochemical and microbial analyses, respectively, from each log within a treatment (SE 12D manual slicer; Bizerba, Piscataway, NJ). Two slices

(one from each log) of one thickness were placed side-by-side in a 3 mil, 10”x12” vacuum bag (Ultravac Solutions, LLC, Kansas City, MO), vacuum sealed (Model #C500, Sepp Haggenmuller GmbH and Co. KG, Wolfertschwenden, Germany), and stored at 0°C in a covered white lug until analysis. Fourteen 13mm and fourteen 2mm slice packages per treatment were prepared. Week 0 was the day of slicing.

3.3 Physicochemical Analyses

On the appropriate day of analysis, one package of 13 mm slices per treatment was opened and samples were evaluated for objective color and samples for TPA were removed. The remaining sample was then homogenized for 30 seconds using a food processor (Handy Chopper; Black & Decker, Shelton, CT) to be used for subsequent analysis. Water activity (a_w), salt concentration, texture profile analysis (TPA), and proximate analysis were performed on w 0. Color, residual nitrite, and pH were tested every two weeks throughout the 16-week study (w 0, 2, 4, 6, 8, 10, 12, 14, and 16).

3.3.1 Objective Color

Objective color was measured in L*, a*, and b* values with a colorimeter (Chroma Meter CR-400; Konica Minolta Sensing Americas, Inc., Ramsey, NJ) using a 2° standard observer and a D65 illuminate with an 8 mm aperture. The calibration plate was read through saran wrap (Polyvinyl chloride film) since samples were covered with saran wrap to measure color of each sample. The color of six locations characterized by a consistent color on the two slices was measured, and the six measurements were averaged to obtain values for each treatment. Color was measured on w 0, 2, 4, 6, 8, 10, 12, 14,

and 16. After color was measured, sample slices were finely chopped in a food processor (Handy Chopper, Black and Decker, Shelton, CT) for approximately 30 s for subsequent physicochemical laboratory analyses.

3.3.2 Water Activity

A water activity (a_w) meter (Aqualab 4TE water activity meter, Decagon Devices, Inc., Pullman, WA) was calibrated using a set of standards with a_w values of 0.984 and 0.760 (Decagon Devices, Inc., Pullman, WA). Ground meat was packed into disposable sample cups (Decagon Devices, Inc., Pullman, WA) so the bottom of the cup was covered, but containers were not more than half full for each sample. Samples were read using the water activity meter in duplicate. Measurements were obtained only on w 0.

3.3.3 Salt Concentration

The procedure used followed the directions written by Sebranek, Lonergan, King-Brink, Larson, and Beermann (2001). Ground sample (10 g) and 90 ml of double distilled, deionized boiling water (DDD water) were added to a 150 ml plastic beaker. The meat mixture was stirred with a metal stir rod for 30 seconds, left to rest for 60 seconds, and stirred once more for 30 seconds. A Whatman No. 1 filter paper (GE Healthcare UK Ltd., Buckinghamshire, UK) was folded into a cone shape and placed into the beaker. Once the solution had filtered, a Quantab high chloride range titration strip (Hach Company, Loveland, CO) was set in the filter so the end was submerged in the filtrate. When the indicator bar turned blue, the chloride concentration was measured and

converted to sodium chloride concentration adjusted for dilution. Measurements were conducted in duplicate and only on w 0.

3.3.4 pH

For each treatment, 10 g of ground meat and 90 ml DDD water was added to a 150 ml plastic beaker. A magnetic stir bar was placed in the beaker. A stir plate (Thermolyne® Cimarec®-top stirring hotplate; Barnstead Thermolyne, Dubuque, IA) was used and the sample cup containing the stir bar was placed on the stir plate to allow the sample to be continuously stirred throughout measurement. Sample pH was read from the stirring sample with a pH meter (Orion 410Aplus; ThermoFisher Scientific, Waltham, MA), which was calibrated with a set of standards of pH 4.01, 7.00, and 10.01 (Orion 910104, 910107, and 910110, respectively, ThermoScientific, Waltham, MA). Measurements were conducted in duplicate on w 0, 2, 4, 6, 8, 10, 12, 14, and 16.

3.3.5 Residual Nitrite

Residual nitrite was measured using methods adapted from AOAC 973.31 methods (AOAC, 1990a). Production of the reagents, nitrite standard solutions, and standard curve for this assay are described in Appendix 1. Five grams of ground meat was measured into a 150 ml plastic beaker (Nalgene, Rochester, NY). Next, 70 ml DDD boiling water was added to the beaker. The solution was swirled and poured through a funnel into a 500ml volumetric flask. An additional 250 ml of boiling DDD water was used to transfer the rest of the meat from the plastic beaker into the flask and rinse the funnel and flask neck. The flask was then corked with a rubber stopper. The flasks were

placed in 87°C water baths for 2 hours. Every 30 minutes, the flasks were swirled and pressure relieved from the flasks. Flasks were removed from the water baths and stored at 3°C for 2 hours until the solutions had cooled to room temperature.

Upon reaching room temperature, DDD water was added to the flasks to bring each sample of solution to volume. The flasks were then inverted approximately 5 times to ensure a homogenous solution, and then approximately 50 ml of solution was poured through a Whatman No. 1 filter paper cone (GE Healthcare UK Ltd., Buckinghamshire, UK) into 150 ml plastic beakers (Nalgene, Rochester, NY). Then, 4 ml of filtrate was added to a test tube (Pyrex Borosilicate Glass Disposable Culture Tubes 18x150mm, Corning, Inc., Corning, NY) for each of the samples, in duplicate. Sulfanilamide solution (0.5 g sulfanilamide dissolved in 150 ml 15% v/v glacial acetic acid), 0.22 ml, was added to each test tube, mixed using a vortex mixer (Pulsing Vortex Mixer, Fisher Scientific, Pittsburg, PA) for 3 s. After 5 min, 0.22 ml N-(1-napthyl) ethylenediamine dihydrochloride (NED) solution (0.2 g NED dissolved in 150 ml 15% v/v glacial acetic acid) was added to each test tube, mixed using a vortex mixer (Pulsing Vortex Mixer, Fisher Scientific, Pittsburg, PA) for 3s. Samples were allowed to set for 15 min to allow for the azo dye development. A blank solution of 4.5 ml DDD water, 0.25 ml sulfanilamide, and 0.25 ml NED was prepared, and was measured at 540 nm using a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) with a sipper flow cell attachment. Sample solutions were then measured at 540 nm with the spectrophotometer and DDD water was flushed between the sets of uncured, SN and CP treatments. The linear formula obtained from the standard curve was used to determine residual nitrite concentration from absorbance (A_{540}) values. Measurements were made

in quadruplicate (two flasks per treatment and two test tubes per flask) on w 0, 2, 4, 6, 8, 10, 12, 14, and 16.

3.3.6 Proximate Analysis

Proximate composition was determined using powdered meat sample. For fat, 2 g of sample was weighed onto filter paper, folded, and paper clipped for analysis using the Soxhlet Method (AOAC, 1990b). Moisture and ash (AOAC, 1990c) were analyzed using a LECO Gravimetric Analyzer, which was loaded with 1 g of the powdered sample. Protein (AOAC, 1990d) was calculated using the LECO FP-528 foil method using 0.20 g of powdered meat sample weighed into foil. Measurements were made in duplicate on w 0.

3.3.7 Texture Profile Analysis

A 4.0cm x 4.0cm square was cut out of each of the two 13mm sample slices. Texture profile analysis was measured using an Instron Universal Testing Machine (Instron Model 1123; Instron Worldwide, Norwood, MA) and with a 2,500kg load cell with a 140 mm plate. Each sample was compressed with a head speed of 30mm/min to to 75% of its original thickness two times to obtain values for hardness, springiness, cohesiveness, gumminess, and chewiness characteristics. This procedure followed protocol according to Bourne (1978) and samples were analyzed in duplicate. Measurements were made on w 0.

3.4 Microbiological Analyses

On the appropriate day analysis samples were evaluated for anaerobic plate count and aerobic plate count. Each sample was prepared using a sterile environment by transferring each of the two 2mm slices into a 4 oz Whirl-Pak bag (B01062WA Whirl-Pak bag, Nasco, Ft. Atkinson, WI), which was labeled for each treatment. Samples were then weighed on a scale tared for the weight of the Whirl-Pak bag, and weights were recorded. Peptone water (50 ml ;BBL Buffered Peptone Water; Becton, Dickinson, and Company, Sparks, MD) was added to each bag, closed, and homogenized using a paddle blender (AES Laboratoire Stomacher; AES Laboratoire, Bruz, France) for 3 minutes to prepare the solution for analysis.

3.4.1 Anaerobic Plate Count

Plates were prepared by pouring approximately 10 ml Brain Heart Infusion Agar (Brain Heart Infusion Agar; Oxoid, Basingstoke, Hampshire, England) into a 10 cm sterile petri dish (Sterile 100mmx15mm Polystyrene Petri Dish, Fisher Scientific, Waltham, MA), and allowed to set. Plates were stored at 2°C until use. Two ml of sample solution was placed in a test tube (12x75mm Pyrex Borosilicate Glass Disposable Culture Tubes, Corning, Inc., Corning, NY), and serial dilutions were performed to the necessary dilution, up to 1:100 depending on microbial growth. The solution of the necessary dilution was plated using a spiral plater (Eddy Jet Spiral Plater; IUL Instruments, Barcelona, Spain) onto each petri dish, in duplicate. Plates were covered, inverted, and placed in an anaerobic chamber (BD GasPak EZ Large Insulation Container; Becton, Dickinson, and Company, Sparks, MD) with three oxygen absorbent

packs (BD GasPak EZ Anaerobe Container System with Indicator; Becton, Dickinson, and Company, Sparks, MD). Plates were incubated at 38°C for 48 hours. Plates were counted at 24 and 48 h; oxygen absorbent packs were replaced at 24 h. Anaerobic plate count was measured on w 0, 2, 4, 6, 8, 10, 12, 14, and 16.

3.4.2 Aerobic Plate Count

Plates were prepared as described above. Two ml of sample solution was placed in a test tube (12x75mm Pyrex Borosilicate Glass Disposable Culture Tubes, Corning, Inc., Corning, NY), and serial dilutions were performed to the necessary dilution, up to 1:10,000 depending on microbial growth. The solution of the necessary dilution was plated using a spiral plater (Eddy Jet Spiral Plater; IUL Instruments, Barcelona, Spain) onto each petri dish, in duplicate. Lids were placed on the plates, plates were inverted, and incubated at 37°C for 48 hours. Plates were counted at 24 and 48 h. Aerobic plate count was measured on w 0, 2, 4, 6, 8, 10, 12, 14, and 16.

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5. Effects of salt and nitrite concentration on the shelf life of deli-style ham

R.E. Stanley, C.G. Bower, S.C. Fernando and G.A. Sullivan[1]

Department of Animal Science, University of Nebraska, Lincoln 68583-0908

A contribution of the University of Nebraska Agriculture Research Division, Lincoln, NE 68583

[1]Correspondence: [A213 Animal Science; Telephone (402) 472-6430; Fax (402) 472-6362]
Email address: gary.sullivan@unl.edu (Gary A. Sullivan)

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5.1 Abstract

Deli-style hams were manufactured to measure the effects of salt and nitrite concentration on shelf life and physicochemical characteristics. Three replications of deli-style ham treatments were manufactured in a 3 x 4 factorial arrangement of salt concentration (0.7%, 1.4%, or 2.1%, meat block basis) and nitrite concentration and source (0 ppm, 100 ppm or 200 ppm sodium nitrite, SN, or 100 ppm sodium nitrite equivalent from pre-converted celery juice powder, CP; Veg Stable 506, Florida Food Products). In addition to salt and nitrite, all treatments contained 1% sugar, 0.35% sodium phosphate (Brifisol 85 Instant, BK Giulini Corporation), and either 495 ppm sodium erythorbate or 440 ppm of ascorbic acid from cherry powder (Veg Stable 515, Florida Food Products) with the balance as water to achieve a 25% extension. A salt by nitrite interaction ($P \leq 0.05$) was found for all color characteristics except for L* and b*. Overall, cured treatments were more red than uncured. This interaction was also observed for texture profile analysis (TPA) hardness and gumminess, and aerobic plate count (APC). In cured treatments, APC decreased as salt increased, however in all 0 SN, APC did not change dependent on salt. The treatments with 200 SN had the lowest APC, but were not different from 2.1% salt and 100 CP, 1.4% salt and 100 SN, or 2.1% salt and 100 SN. A nitrite by week interaction was identified for residual nitrite (RN) and APC. As storage continued, RN decreased and APC increased ($P \leq 0.05$). As nitrite increased, APC decreased, and 0 SN treatments had the most growth throughout the sampling period. Main effects for salt concentration were identified for all traits ($P \leq 0.05$) not involved in the above interactions, except anaerobic plate count, %fat, and %protein. For these as salt increased, L*, b*, a_w, AnPC, and TPA cohesiveness and chewiness characteristics decreased, whereas cooking yield, pH, RN, moisture, ash, and TPA

springiness increased. This study suggests 200 ppm SN provides the greatest shelf life to deli-style ham, and 0.7% salt results in inferior product quality and shortest shelf life.

Keywords: Salt, Nitrite, Ham, Shelf life.

5.2 Introduction

In early history, humans used a few main methods for preserving their meat, one of which was salting (Wentworth, 1956). Impurities in these salts, salt peter (KNO_3), enhanced the preservative effect of salt and gave the salted meat a red color when cooked (Honikel, 2008). Later on, nitrite was confirmed to be the agent responsible for producing the heat stable red color of cured meat (Honikel, 2008). Regulatory limits were implemented to provide safety with using nitrite in meat products (USDA, 1925). Though cured meat products made with sodium nitrite have high product safety and shelf life, a subsection of consumers are demanding products made without conventional curing agents and want products with clean labels, meaning only ingredients consumers recognize as household items (McDonnell, Glass & Sindelar, 2013). These consumers associate negative health effects with consuming common meat processing ingredients (Bernstein et al., 2015).

Cured meats have a characteristic stable pink cured meat color and flavor in addition to decreased oxidation and reduced risk of certain pathogens (Borch, Kant-Muermans, & Blixt, 1996). In order to manufacture processed meats with characteristics associated with cured meats, nitrogen oxide compounds, typically sodium nitrite, must be added. However to meet the growing consumer preference for products without conventional meat curing ingredients, processors began using ingredients, such as celery juice powder, that are naturally high in nitrate (Sebranek & Bacus, 2007). These ingredients, in combination with nitrate reducing bacteria, allow for processed meats with cured meat characteristics to be manufactured without the direct addition of sodium nitrite (Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). There are no regulations on the amount of celery juice powder that can be added, but based on supplier recommendations it is common to have up to the equivalent of

100 ppm of sodium nitrite added. In alternatively cured products, these usage levels provide typical cured meat characteristics (Terns, Milkowski, Claus, & Sindelar, 2011; Myers et al., 2013; Redfield & Sullivan, 2015; & Pietrasik, Gaudette, & Johnston, 2016) but ingoing sodium nitrite is lower than the USDA regulatory limit. These products may be more susceptible to pathogen growth (Borch et al., 1996; McDonnell, Glass, & Sindelar, 2013) but little research has been reported on the effect of amount or source of nitrite on spoilage microorganisms.

High sodium consumption has been associated with hypertension and associated health issues (Lichtenstein et al., 2006). Currently, about 2.3 to 4.3 g/day is consumed in the diet, but only 1.5 to 2.5 g/day is recommended (ICRG, 1988). As a result, there has been a push to reduce sodium intake to combat the vast health problems. One prominent method of dietary sodium reduction, salt intake, is known to lower blood pressure (Lichtenstein et al., 2006). On average, consumers in the U.S. have diets that contain 9.76% hot dogs, ham, and lunch meats (Block, Dresser, Hartman, & Carroll, 1985). This amounts to approximately 21.84% of sodium in the diet (Block et al., 1985) contributing to excess sodium in the American diet. In response, meat scientists and the processed meats industry have worked diligently to reduce sodium products in processed meat products. However, due to the multiple functionality of salt, it can be difficult to simply reduce salt. Salt is necessary for adequate protein extraction and increases the moisture binding capability of meat. A minimum concentration of 1.4% salt in typical and 1.75% salt in low fat meat products are required to achieve acceptable bind and quality of meat products (Ruusunen & Puolanne, 2005). Salt aids in palatability of the product as well as improves shelf life of products by shifting microbial populations towards lactic acid producing bacteria, and away from

Pseudomonads bacteria. While certain microorganisms, such as *Staphylococcus aureus*, are able to grow when greater than 20% salt is present, species such as *Campylobacter spp.* are much more sensitive to salt and grow best at only 0.5% salt (Doyle & Glass, 2010).

Since salt and sodium nitrite are multifunctional ingredients, reduction or removal of these ingredients can result in dramatic impacts on the quality and shelf life of processed meats. The objective of this study is to determine the impact of salt concentration, and nitrite concentration and source on the shelf life and quality characteristics of deli-style ham.

5.3 Materials and Methods

5.3.1 Treatments and Product Formulations of Hams

To evaluate the effect of ingoing salt concentration, and nitrite concentration and source on the physicochemical characteristics and microbial outgrowth of deli-style ham, 12 treatments in a 3 (salt) by 4 (nitrite) factorial arrangement were manufactured. Ingoing salt concentrations of 0.7%, 1.4%, and 2.1% sodium chloride (meat block basis) and ingoing nitrite treatments of 0 ppm, 100 ppm, or 200 ppm of sodium nitrite, or equivalent to 100 ppm sodium nitrite from pre-converted celery juice powder, CP (VegStable 506, Florida Food Products, Eustis, FL; CP) were evaluated. CP was quantified to contain 21,696.7 ppm equivalent to sodium nitrite. Sodium chloride content was measured in the CP to allow for formulation adjustments. Product formulations were based on an 11.34 kg meat block, and the total weight of non-meat ingredients was 25% of the meat block. All treatments contained 1% sugar (w/w) and 0.35% sodium phosphate (w/w; Brifisol® 85 Instant, BK Giulini Corporation, Semi Valley, CA). Sodium chloride, adjusted for sodium chloride in the curing agents, was added to achieve 0.7%, 1.4%, 2.1% sodium chloride, meat block basis.

Sodium erythorbate (495 ppm) or cherry juice powder to achieve 440 ppm ascorbic acid (VegStable 506, Florida Food Products, Eustis, FL) were added as reducing agents. Sodium nitrite or pre-converted celery juice powder were added to achieve desired ingoing sodium nitrite concentrations. Water was added to create the balance of the brine. Full product formulations can be found in Table 1.

5.3.2 Deli-Style Ham Manufacture

Hams were manufactured at the Loeffel Meat Laboratory using pork inside ham muscles (IMPS 402F, USDA, 2014) following the formulations in Table 1. Ham muscles were obtained from Hormel Foods® (Fremont, NE), vacuum packaged, and placed in frozen storage at -20°C prior to use. Ham muscles were tempered for approximately 48 hours at -1°C and then coarse ground through a plate with 12.70 mm holes, and fine ground through a plate with 4.75 mm holes and weighed into 11.34 kg batches. Brine was manufactured with all non-meat ingredients and mixed with ground ham for three minutes in a double action mixer (Model 100DA70, Leland Southwest, Fort Worth, TX, USA). Meat batter was stuffed into 6M x 107 cm pre-stuck, fibrous casings (Kalle, Wiesbaden, Germany) and clipped; two logs of ham were made for each treatment. The logs were hung on a smoke stick on a smokehouse truck, and were thermally processed in a smokehouse (Alkar-Rapid Pak, Lodi, WI) according to Table 2 to an internal temperature of 68.3°C to meet Appendix A regulations (USDA, 1999). Ham logs were chilled overnight to 3°C to meet FSIS Appendix B stabilization regulations (USDA, 1999) for cooling heat-treated, cured pork products (0.7% NaCl 100 SN, 1.4% NaCl 100 SN, 2.1% NaCl 100 SN, 0.7% NaCl 200 SN, 1.4% NaCl 200 SN, and 2.1% NaCl 200 SN) and cooked, uncured pork products (0.7% NaCl 0 ppm, 1.4%

NaCl 0 ppm, 2.1% NaCl 0 ppm, 0.7% NaCl 100 CP, 1.4% NaCl 100 CP, and 2.1% NaCl 100 CP). The weights of ham logs were measured before cooking and after chilling to calculated cooking yield.

Casings were removed and hams sliced into13 mm and 2 mm thick slices, for physicochemical and microbiological analyses, respectively, were taken from each log within a treatment (SE 12D manual slicer; Bizerba, Piscataway, NJ). Two slices (one from each log) of one thickness were placed side-by-side in a 3 mil, 10"x12" vacuum bag (Ultravac Solutions, LLC, Kansas City, MO), vacuum sealed (Model #C500, Sepp Haggenmuller GmbH and Co. KG, Wolfertschwenden, Germany), and stored at 3°C in a covered white lug until analysis. Fourteen 13mm and fourteen 2mm slice packages per treatment were prepared. Three independent replications were manufactured. Day of slicing was considered w 0.

5.3.3 Physicochemical Analyses

Objective Color

Objective color, L*, a*, and b* values were measured with a colorimeter (Chroma Meter CR-400; Konica Minolta Sensing Americas, Inc., Ramsey, NJ) using a 2° standard observer and a D65 illuminate, with an 8 mm aperture. Each of the samples and the calibration plate were covered with polyvinyl chloride film (Bakers & Chefs Food Service Film, Sam's West, INC. Bentonville, AR) prior to measuring color or calibration. Color was measured at six locations across two slices and the measurements were averaged to determine the color characteristics for each treatment within replication. Additionally, a/b ratio, hue angle, saturation index, and delta E were calculated according to the protocols

given by the AMSA Meat Color Measurement Guidelines (Hunt & King, 2012). Color was measured on w 0, 2, 4, 6, 8, 10, 12, 14, and 16. After color was measured, sample slices were finely chopped in a food processor (Handy Chopper, Black and Decker, Shelton, CT) for approximately 30 s for subsequent physicochemical laboratory analyses.

Water Activity

Water activity was measured according to AquaLab's protocol for the AquaLab 4TE water activity meter (Decagon Devices, Inc., Pullman, WA). Two measurements were taken per treatment. Measurements were conducted in duplicate on w 0.

Salt Concentration

Salt concentration was measured using the procedure found in Sebranek, Lonergan, King-Brink, Larson, and Beermann (2001) using Quantab high chloride range titration strips (Hach Company, Loveland, CO). Measurements were conducted in duplicate and only on w 0.

pH

For each treatment, 10 g of ground meat and 90 ml double distilled deionized (DDD) water was added to a 150 ml plastic beaker. A magnetic stir bar was placed in the beaker and placed on a stir plate (Thermolyne® Cimarec®-top stirring hotplate; Barnstead Thermolyne, Dubuque, IA) to keep the solution in continuous motion while the pH was measured with a pH meter (Orion 410Aplus; ThermoFisher Scientific, Waltham, MA). The meter was calibrated with a set of standards of pH 4.01, 7.00, and 10.01 (Orion 910104, 910107, and

910110, respectively, ThermoScientific, Waltham, MA). Measurements were conducted in duplicate on w 0, 2, 4, 6, 8, 10, 12, 14, and 16.

Residual Nitrite

Residual nitrite was measured using methods adapted from AOAC 973.31 methods (AOAC, 1990) with modifications described in Redfield and Sullivan (2015). Five grams of ground meat and approximately 350 ml boiling DDD water were added to a 500ml volumetric flask and corked with a rubber stopper. The flasks were placed in 87°C water baths for 2 hours. Every 30 minutes, the flasks were swirled and pressure relieved from the flasks. Flasks were removed from the water baths and stored at room temperature for 2 hours until the solutions had cooled.

Upon reaching room temperature, DDD water was added to the flasks to bring each solution to 500 ml volume. The flasks were then inverted to ensure a homogenous solution, and solution was filtered through a Whatman No. 1 filter paper cone (GE Healthcare UK Ltd., Buckinghamshire, UK). Then, 4 ml of filtrate was added to a test tube (Pyrex Borosilicate Glass Disposable Culture Tubes 18x150mm, Corning, Inc., Corning, NY) for each of the samples. Sulfanilamide solution (0.5 g sulfanilamide dissolved in 150 ml 15% v/v glacial acetic acid), 0.22 ml, was added to each test tube, mixed using a vortex mixer (Pulsing Vortex Mixer, Fisher Scientific, Pittsburg, PA) for 3 s. After 5 min, 0.22 ml N-(1-napthyl) ethylenediamine dihydrochloride (NED) solution (0.2 g NED dissolved in 150 ml 15% v/v glacial acetic acid) was added to each test tube, mixed using a vortex mixer for 3s. Samples were allowed to set for 15 min to allow for the azo dye development. A blank solution of 4.5 ml DDD water, 0.25 ml sulfanilamide, and 0.25 ml NED was prepared, and

was measured at 540 nm using a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) with a sipper flow cell. A standard curve was prepared by adding 0, 10, 20, 30, and 40 ml of working solution containing 1 ppm sodium nitrite was added to a 50 ml volumetric flask and 2.5 ml sulfanilamide and 2.5 ml NED was added according to the steps above. Flasks were filled to volume with DDD water, yielding 0, 0.2, 0.4, 0.6, and 0.8 ppm. Standards and sample solutions were then measured at 540 nm with the spectrophotometer. The linear formula obtained from the standard curve was used to determine residual nitrite concentration from absorbance (A_{540}) values. Measurements were made in quadruplicate (two flasks per treatment and two test tubes per flask) on w 0, 2, 4, 6, 8, 10, 12, 14, and 16.

Texture Profile Analysis

A 4.0cm x 4.0cm square was cut out of each of the two 13mm sample slices. Texture profile analysis was measured using a 2,500 kg load cell on an Instron Universal Testing Machine (Instron Model 1123; Instron Worldwide, Norwood, MA) with a 140 mm plate. Each sample was compressed with a head speed of 30 mm/min to 75% of its thickness, twice, to obtain values for hardness, springiness, cohesiveness, gumminess, and chewiness characteristics. This procedure and calculation of measurements followed protocol according to Bourne (1978) and measurements were made on w 0.

Microbiological Analyses

On the appropriate day of analysis, samples were evaluated for anaerobic plate count and aerobic plate count. Each sample was prepared using a sterile environment by transferring each of the two 2mm slices into a 4 oz Whirl-Pak bag (B01062WA Whirl-Pak bag, Nasco, Ft. Atkinson, WI) and weighed. To each sample, 50 ml peptone water (BBL Buffered Peptone Water; Becton, Dickinson, and Company, Sparks, MD) was added and homogenized for 3 minutes using a laboratory paddle blender (AES Laboratoire Stomacher; AES Laboratoire, Bruz, France). Two ml of the appropriate serial dilution (up to 1:10,000, dependent on microbial growth) solution was plated using a spiral plater (Eddy Jet Spiral Plater; IUL Instruments, Barcelona, Spain) onto a 10 cm sterile petri dish (Sterile 100mmx15mm Polystyrene Petri Dish, Fisher Scientific, Waltham, MA) with brain heart infusion agar (Brain Heart Infusion Agar; Oxoid, Basington, Hampshire, England). Solutions were plated in quadruplicate for two plates to be incubated anaerobically (BD GasPak EZ Large Insulation Container; Becton, Dickinson, and Company, Sparks, MD) with three oxygen absorbent packs (BD GasPak EZ Anaerobe Container System with Indicator; Becton, Dickinson, and Company, Sparks, MD) and two plates aerobically. Plates were incubated at 38°C for 48 hours and counted at 24 and 48 h. Aerobic plate counts (APC) and anaerobic plate counts (AnPC) were measured on w 0, 2, 4, 6, 8, 10, 12, 14, and 16.

5.3.4 Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) through the PROC GLIMMIX procedure in SAS 9.2 (Cary, NC). Physicochemical data were analyzed according to a factorial arrangement (3 salt concentrations x 4 nitrite concentration and

source combinations) for traits measured only on d 0. For traits measured over time, data were analyzed using factorial arrangement (3 salt concentrations x 4 nitrite concentration and source combinations x 9 storage times). Storage time was considered a repeated measure and evaluated using an unstructured covariance structure. Significant differences ($P \leq 0.05$) between means from main effects or interactions were separated with LSMEANS functions of SAS. Tukey's adjustment was applied to all comparisons for means separation.

5.4 Results

5.4.1 Objective color

No significant salt by nitrite concentration interaction was observed for L*, or b* ($P \geq$ 0.08). Treatment main effects of salt and nitrite on L* and b* can be found in table 5.3. Salt concentration ($P < 0.01$), nitrite concentration ($P < 0.01$), and week of storage ($P = 0.01$) impacted L* values. As salt concentration increased in product formulations, L* decreased (Table 5.3). Additionally, treatments with 0 SN had the greatest L* values, 200 SN treatments had the lowest L* values, and both 100 ppm nitrite treatments were intermediate. As nitrite increased in ham samples, darker color was observed. Ham was the darkest on Week 0 with no differences among all other weeks (Table 5.4). A significant salt concentration by nitrite concentration interaction ($P < 0.01$) was observed for a* showing all 0 SN treatments had lower values than all cured treatments, regardless of salt concentration (Figure 5.1). No significant week of storage effects were observed in a* values ($P = 0.23$). Yellowness (b*) values were impacted by salt ($P < 0.01$), nitrite ($P < 0.01$), and week ($P <$ 0.01). For each increase in salt concentration in ham samples, b* values decreased. Yellowness was highest in ham formulations without nitrite, and was higher in formulations

containing CP when compared to either 100 or 200 SN treatments (Table 5.3). Although a significant storage time effect was observed for b* values, the treatment means, ranging from 7.11 to 7.47, are likely of little practical importance.

There was a salt by nitrite concentration *(P < 0.01)* interaction observed for ΔE (Figure 5.2). The 2.1% salt and 100 CP treatment had a greater ΔE than all treatments except 0 SN at 0.7% and 2.1% salt and all remaining treatments were similar. No significant week of storage effect for ΔE occurred ($P = 0.09$). A salt by nitrite concentration ($P < 0.01$) interaction was observed for a/b ratio (Figure 5.3). Values increased as salt concentration increased in all treatments except for O SN. Additionally, treatments with 0 SN had the lowest a/b ratios overall. Week of storage had an effect on a/b ratio ($P = 0.01$). Weeks 14 and 16 differed from week 6, but all other time points were similar for a/b ratio (Table 5.4). All 0 SN treatments had lower a/b ratios than all other treatments. The salt by nitrite concentration ($P < 0.01$) interaction affected hue angle (Figure 5.4) showing the highest hue angle values for treatments with 0 SN, and hue angles decreased as salt concentration increased in all cured treatments. Hue angle was impacted by week of storage ($P < 0.05$), and decreased over time, however the only time points which were different were week 6, which was higher than weeks 14 and 16. For saturation index, a salt by nitrite concentration interaction ($P < 0.01$) was observed (Figure 5.5). Saturation index was lowest in 0 SN treatments. Within a given salt concentration, all other nitrite concentrations were similar with the exception of 100 CP at 0.7% salt, which was greater than either 100 SN or 200 SN with 0.7% salt. Saturation index was impacted by week ($P < 0.05$). Overall, saturation was different between weeks 8 and 12, but all other comparisons were similar (Table 5.4).

5.4.2 Water Activity, Cooking Yield, Measured Salt Concentration, and Proximate Composition

No salt by nitrite concentration interaction was observed for water activity, salt, cooking yield, or proximate analysis ($P \geq 0.65$). Water activity (a_w) was affected by salt ($P < 0.01$) and nitrite ($P < 0.01$) concentrations. As expected, water activity decreased as salt concentration increased in formulations. Similar effects were observed with nitrite, where water activity was lower in formulations containing more than 0 ppm nitrite, regardless of source (Table 5.5). Salt concentration was impacted by the amount of ingoing salt based on the formulation ($P < 0.01$). As ingoing salt increased, measured salt concentration increased (Table 5.5). Salt concentration did not vary among nitrite treatments ($P = 0.68$). Salt concentration impacted cooking yield ($P < 0.01$), increasing as salt increased (Table 5.5). Yield was unaffected by nitrite concentration and source ($P = 0.55$). Salt concentration impacted moisture ($P < 0.01$) and ash ($P < 0.01$) content, but nitrite concentration impacted only ash ($P < 0.01$) content. As salt increased in the formulation, moisture and ash content both increased (Table 5.5). Products cured with SN had higher ash values than 0 ppm nitrite or CP treatments. Fat and protein content were unaffected by both salt and nitrite ($P \geq 0.05$).

5.4.3 Texture Profile Analysis

A significant salt by nitrite concentration interaction was observed for hardness and gumminess ($P \leq 0.02$), but not for cohesiveness, springiness, or chewiness ($P > 0.08$). Hardness (Figure 5.6) decreased as salt concentration increased, but was highest in the treatment with 0.7% salt and 100 CP. This treatment was only similar to 0.7% salt and 200 SN. Gumminess (Figure 5.7) had a very similar trend to hardness, decreasing as salt

increased, with 0.7% salt and 100 CP having the highest value, only similar to 0.7% salt and 200 SN. Cohesiveness, springiness, and chewiness were significantly affected by the amount of salt present in the formulation ($P \leq 0.05$; Table 5.7). Cohesiveness and chewiness significantly decreased as each salt concentration increased ($P < 0.01$). Springiness was increased with 2.1% salt, but was not different between 0.7% and 1.4% salt ($P < 0.01$). Cohesiveness ($P = 0.92$), springiness ($P = 0.48$), and chewiness ($P = 0.10$) were not impacted by nitrite (Table 5.7).

5.4.4 pH

No significant salt by nitrite concentration interaction or nitrite by storage time interaction occurred ($P \geq 0.05$). Salt concentration, nitrite concentration, and week of storage impacted pH ($P < 0.01$). Treatments with 2.1% salt had the highest pH values, with no difference between 0.7% salt or 1.4% salt (Table 5.5). Treatments with 200 ppm SN had a greater pH than all other treatments. Storage time impacted pH. Weeks 0, 6, 12, 14, and 16 were similar with the lowest pH values, weeks 2, 4, 8, and 10 had the highest values and were similar, and weeks 6, 10, and 14 were similar (Table 5.6).

5.4.5 Residual Nitrite

A nitrite concentration by storage week ($P < 0.01$) interaction was identified (Figure 5.8). As storage time increased, residual nitrite decreased in all nitrite formulations except 0 SN as no nitrite was added initally. Residual nitrite concentration was similar over storage time for 100ppm SN and 100 ppm CP. Residual nitrite concentrations were greater in

treatments with 2.1% salt than 0.7% salt where 1.4% salt treatments were similar to both ($P < 0.05$; Table 5.5).

5.4.6 Microbiological Analyses

Aerobic and anaerobic plate counts were measured throughout the 16-week period in all ham treatments. A significant salt by nitrite concentration interaction was observed for APC ($P < 0.01$). Treatments with 0 SN had higher plate counts than all cured treatments. Within each of the cured treatments, APC decreased as salt is increased, but this is not the case with 0 SN treatments, where there was no significant differences between salt concentrations. Additionally, a nitrite by week interaction was observed for APC ($P < 0.01$). Formulations with 0 SN had higher counts from weeks 2 through 16 compared to all cured treatments (Figure 5.9). Additionally, 100 SN and 100 CP were similar throughout storage.

Anaerobic plate counts fluctuated throughout the study. No significant interactions were observed for AnPC ($P > 0.05$). Anaerobic plate counts were affected by nitrite ($P < 0.01$) and week ($P < 0.01$) but not salt concentration ($P = 0.19$). Hams containing 0 ppm nitrite had AnPC greater than 100 or 200 SN treatments, and those with 200 ppm SN had lower AnPC than 0 ppm and 100 CP treatments. No differences were identified between 100 SN and 100 CP treatments. For AnPC, weeks 6, 12, 14, and 16 were greater than week 0 and weeks 14 and 16 were greater than week 2.

5.5 Discussion

Salt concentration, nitrite concentration, and week of storage impacted L* values. L* values were the lightest in 0 SN treatments, darkest in 200 SN treatments, and 100 ppm

treatments were intermediate regardless of source. Similar results in deli-hams were found by Myers and others in 2013. Miller, Bower, Redfield, and Sullivan (2015) also found similar results in all-beef frankfurters where cured products had darker values than 0 SN frankfurters. L* values were higher only on d 0 than all other time points, but the values ranged from 73.39 to 72.32, which is likely of little practical importance. Contrasting these findings, L* values were observed in a ham study that measured color over 90 days of shelf life, where their L* values were lowest on d 0 and were higher at all other timepoints (Sindelar, Cordray, Sebranek, Love, & Uhn, 2007). Similarly, Terns, Milkowski, Rankin, and Sindelar (2011) observed the lowest L* values on d 0 of their study evaluating cured, emulsified cooked sausages, and L* values significantly ($P < 0.05$) increased throughout their 84-day storage period. A significant salt by nitrite interaction for a* values showed that all uncured treatments had lower values than any cured treatments. This is due to the absence of cured meat color since no nitrite of any kind or amount was added to these products, resulting in a less red visual appearance. All cured treatments had similar a* values since an adequate amount of nitrite, regardless of source, was added to achieve cured color formation. A study evaluated hams manufactured with different sources and concentrations of nitrite and observed a* values which were comparable to this study. All treatments containing greater than or equal to 50 ppm nitrite, regardless of source, had higher a* values than treatments containing no nitrite or unconverted vegetable juice powder (Myers et al., 2013). Although many cured meat products are treated with ingoing sodium nitrite levels of 120-200 ppm, satisfactory and stable color development can occur at levels as low as 40 ppm (Froelich, Gullett, & Usborne, 1983).

Treatments cured with CP had higher b* values, indicative of a more yellow product. This data agrees with that reported by Redfield and Sullivan (2015), who showed that turkey products cured with CP, regardless of concentration, had higher b* values than those cured with SN at the same concentrations. Furthermore, Miller and others (2015) reported higher b* values in the internal color of all-beef frankfurters. Myers and others (2013) reported higher b* values in sliced hams with natural nitrite sources compared to treatments with sodium nitrite. This may be explained by the color of celery powder and cherry powder used as the curing and reducing compounds. Treatments with no added nitrite had the highest b* values in this study, which also occurred in the study by Miller and others (2015), as well as Myers and others (2013), likely due to the lack of cured meat color, resulting in a lighter brownish pink cooked product.

For calculated color values, significant ($P \leq 0.05$) salt by nitrite interactions were observed for a/b ratio, hue angle, and saturation index. These values are calculated using measured L*, a*, and b* values and the differences reported affect these calculations. Regardless of salt, a/b ratios are lower in all 0 ppm treatments than other treatments. This is likely due to the above values found for a* and b*, since the higher b* values would decrease the overall ratio for those treatments. For all treatments with added nitrite, a/b ratio increased as salt increased. The a/b ratio was lower for 100 CP treatments than 100 SN or 200 SN within each salt concentration likely due to the greater b* values in these treatments. In order to identify color differences based on tristimulus colorimetry data, it is important to identify hue and chroma (McGuire, 1992). Hue angle was highest in 0 SN treatments regardless of salt concentration but in all other nitrite concentrations, hue angle decreased as salt increased. This is indicative 0 ppm products having greater b* values and lower a* values as they did

not contain nitrite. As a* and b* are both included in calculating hue angle, this difference is to be expected.

Saturation index decreased as salt concentration increased within each nitrite treatment group however 100 CP and 0.7% salt had a higher saturation than all other treatments except for 200 SN and 0.7% salt. All treatments with 0 SN had lower saturation index values than all other treatments, indicative of a less intense red meat color. L* and b* values decreased as ingoing salt concentration increased, since products with 2.1% salt resulted in lighter, more intense red products than those with lower salt concentrations.

Treatments with 0 ppm SN had lower a_w values ($P \leq 0.05$) than treatments with all other nitrite concentrations. Additionally, salt concentration impacted a_w values. As measured salt increased, a_w values decreased, which is not surprising since more water is bound as salt is added. Redfield and Sullivan (2015) reported differences in a_w values due to nitrite, but data were not shown. Salt measurements were expectedly impacted by ingoing salt concentration, but not by nitrite. Redfield and Sullivan (2015) also observed no difference in salt due to nitrite, with similar values for salt concentration. As salt concentration increased, measured salt concentration increased ($P \leq 0.05$). Formulations were adjusted for salt contained in the curing agent so the lack of significant nitrite concentration effect is to be expected. Salt impacted product yield ($P \leq 0.05$) where yield increased as salt concentration increased. This is due to the increased protein extraction and water holding capacity associated with salt in processed meats (Offer & Knight, 1988). Nitrite did not affect yield of products, which would be expected since this does not affect the water holding capacity. Similar results were found in a study in ground, cooked, and sliced ham (Krause, Sebranek, Rust, & Mendonca, 2011).

In this study, pH was significantly ($P \leq 0.05$) impacted by salt concentration, nitrite concentration, and week of storage. Products with 2.1% salt had higher pH values than others, and products with 200 SN had higher pH values than others. Furthermore, pH decreased over storage time beginning at w 2. These values ranged from 6.32 to 6.21. This may be explained by the production of lactic acid by spoilage bacteria present as products reached the end of their shelf life. One study supported these findings in cured meat products, stating that the pH value doesn't necessarily restrict microbial growth on the product, but pH will decrease during storage due to growth of *Lactobacillus* spp. (Borch et al., 1996).

Residual nitrite (RN) was impacted by a nitrite by week interaction ($P < 0.01$), where RN decreased throughout storage time in all treatments except 0 SN. This is due to the lack of opportunity for 0 SN treatments to decrease. Several studies, including one by Xi, Sullivan, Jackson, Zhou, and Sebranek (2012) had data showing depletion in residual nitrite values over 49 days of storage in frankfurters. Dethmers, Rock, Fazio, & Johnston (1975) found similar depleting RN values over time in raw emulsion thuringer sausages.

For proximate data, fat and protein were not affected by salt or nitrite concentration. Salt impacted moisture of ham samples. Hams with 0.7% salt had lower ($P \leq 0.05$) moisture than other treatments, and ash was lowest in 0.7% salt and increased ($P \leq 0.05$) as salt concentration increased. A few studies reported no change in proximate composition, however both studies contained formulations that either had no variation in ingoing salt concentration (Terns et al., 2011), or had a small variation in salt in their brine (11% versus 9.8%; Sindelar et al., 2007). It can be concluded that salt impacts proximate composition due to the increase in water holding capacity of the raw material and retention during cooking.

Texture profile analysis had significant salt by nitrite concentration interactions for hardness and gumminess traits ($P \leq 0.05$). Hardness values decreased as salt increased, and the treatment with 100 CP and 0.7% salt was higher than all treatments except for 200 SN with 0.7% salt. A similar effect was seen with gumminess. Main effects affected by salt were observed for cohesiveness, springiness, and chewiness ($P \leq 0.05$). Springiness increased as salt increased, which may be explained by a firmer ham surface. Additionally, cohesiveness and chewiness values decreased as salt increased. This is contradictory of the study on low-fat beef sausage by Xiong, Noel, and Moody (1999), where they observed cohesiveness and chewiness values that increased as salt increased, however their study also included various polysaccharides and pH changes as treatments in these sausages.

Significant salt by nitrite concentration and nitrite concentration by week of storage interactions were observed for aerobic plate counts ($P \leq 0.05$). Aerobic plate counts were highest in 0 SN treatments, and decreased as salt increased within each nitrite treatment group. This was expected since microorganisms do not grow as rapidly in cured meat products due to the antimicrobial effects of salt and nitrite. Salt works as an antimicrobial in processed meats due to its ability to reduce water activity and increase ionic strength in meat products. While salt lowers the water activity of a product, other functions are still necessary to fully explain the preservative effect (Jay, 2000; Sperber & Peck, 1983). Nitrite and salt function synergistically because the chloride ion of salt is responsible for increasing the rate of nitric oxide formation during curing (Sebranek & Fox, 1991). Additionally, since salt has a great impact on which microorganisms grow, it would be expected to observe less growth in products with higher salt levels (Doyle & Glass, 2010).

As storage time increased, APCs increased as expected. All ham treatments started at less than 2 logs cfu/g of growth, and 0 SN treatments surpassed 7 log cfu/g of growth at week 8. All other treatments did not surpass 7 log CFU/g by week 16 of storage. No treatments containing 100 ppm or 200 ppm nitrite were considered spoiled (≥7 log cfu/g) at the end of the 16-week study, and 200 SN products had the lowest plate counts when the study ended. Samelis, Kakouri, and Rementzis (2000) had results that agree with this study, showing increasing plate counts for lactic acid bacteria grew as shelf life was carried to 30 d.

Nitrite concentration and week of storage significantly impacted ($P \leq 0.05$) anaerobic plate counts. Anaerobic plate counts were highest in 0 SN treatments and lowest in 200 SN treatments, which is expected due the impact nitrite has on growth of bacteria. Additionally, plate counts increased overall as shelf life continued, indicative of continued growth, which was expected.

This study suggests using ingoing concentrations of nitrite at maximum levels allowed, regardless of source, in order to attain the longest shelf life possible for products. Additionally, products formulated with 0.7% salt had shortcomings with shelf life and poor physicochemical traits.

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Table 5.1: Deli-Style Ham formulations

Treatment		Ham (kg)	Water (%)	[4]Salt (%)	Sugar (%)	Sodium nitrite curing salt[1] (g)	CP[2] (%)	Sodium Erythorbate (ppm)	Cherry Powder[3] (g)	Sodium Phosphate (%)
Salt (%)	**Sodium Nitrite (ppm) and Source**									
0.7	**0 SN**	11.34	22.90	0.7	1.0	0	0	495	0	0.35
1.4	**0 SN**	11.34	22.20	1.4	1.0	0	0	495	0	0.35
2.1	**0 SN**	11.34	21.50	2.1	1.0	0	0	495	0	0.35
0.7	**100 SN**	11.34	22.89	0.55	1.0	18.14	0	495	0	0.35
1.4	**100 SN**	11.34	22.19	1.25	1.0	18.14	0	495	0	0.35
2.1	**100 SN**	11.34	21.49	1.96	1.0	18.14	0	495	0	0.35
0.7	**200 SN**	11.34	22.87	0.41	1.0	36.29	0	495	0	0.35
1.4	**200 SN**	11.34	22.17	1.11	1.0	36.29	0	495	0	0.35
2.1	**200 SN**	11.34	21.47	1.81	1.0	36.29	0	495	0	0.35
0.7	**100 CP**	11.34	22.49	0.65	1.0	52.26	2.17	0	4.99	0.35
1.4	**100 CP**	11.34	21.79	1.35	1.0	52.26	2.17	0	4.99	0.35
2.1	**100 CP**	11.34	21.07	2.05	1.0	52.26	2.17	0	4.99	0.35

[1]6.25% nitrite curing salt added to achieve 0 ppm, 100 ppm, or 200 ppm of sodium nitrite.
[2]CP=Celery Juice Powder (VegStable 506, Florida Food Products) added to achieve equivalent to 100 ppm sodium nitrite based upon 21,696.7 ppm laboratory quantification.
[3]Cherry powder (VegStable Cherry 515, Florida Food Products) added to achieve 440 ppm ascorbic acid.
[4]Salt was formulated to account for salt from curing salt or celery powder.

Table 5.2: Deli-Style Ham Thermal Processing Cycle

Step	Dry Bulb set point (°C)	Wet Bulb set point (°C)	Time (Min)	Internal Temp (°C)
1	54.4	37.8	60	
2	58.3	51.7	45	
3	64.4	57.2	45	
4	72.8	65.6	45	
5	79.4	71.1	45	
6*	79.4	76.7	5	68.3
7	15.6 (cold shower)	0	30	

*Step 6 cooking continued for the greater of 5 minutes or time until internal temperature reached 68.3°C.

Table 5.3: Least square means for main effects of nitrite concentration and source (0, 100 ppm sodium nitrite, SN, 100 ppm sodium nitrite equivalent from celery juice powder, CP, or 200 ppm SN) and ingoing salt concentration (0.7, 1.4, or 2.1%) for reflective color measurements.

	Trait						
Ingoing Salt Concentration (%)	**L***	**a***	**b***	**a/b ratio**	**HA§**	**SI$**	**ΔE€**
0.7	74.81[a]	9.04	7.96[a]	1.19	41.81	12.26	1.24
1.4	72.39[b]	9.04	7.24[b]	1.32	39.23	11.79	1.27
2.1	70.55[b]	8.84	6.68[c]	1.42	37.92	11.34	1.54
P-value	<0.01	<0.01‡	<0.01	<0.01‡	<0.01	<0.01‡	<0.01‡
SEM[2]	0.07	0.05	0.04	0.01	0.23	0.04	0.06
Ingoing Nitrite Concentration (ppm) and Source	**L***	**a***	**b***	**a/b ratio**	**HA§**	**SI$**	**ΔE€**
0 SN	73.69[x]	5.54	9.24[x]	0.61	58.98	10.80	1.43
100 SN	72.38[y]	10.32	6.35[z]	1.64	31.56	12.12	1.27
100 CP	72.32[y]	9.90	7.35[y]	1.36	36.52	12.34	1.44
200 SN	71.94[z]	10.15	6.24[z]	1.64	31.55	11.92	1.28
P-value	<0.01	<0.01‡	<0.01	<0.01‡	<0.01	<0.01‡	0.14‡
SEM[2]	0.08	0.05	0.05	0.01	0.27	0.05	0.07

[1]Commision Internationale de l'Eclairage (CIE) L*, a*, b*, in which L* indicates lightness on a scale of 0(black) to 100(colorless), a* indicates redness (+a*) or greenness (-a*), and b* indicates yellowness (+b*) or blueness (-b*).

[2]SEM=standard error of the means for deli-style ham.

[a-c;x-z] Within each main effect, means in the same column with different superscripts are significantly different ($P \leq 0.05$).

‡Indicates a significant ($P \leq 0.05$) nitrite by salt concentration interaction for the trait.

§HA= hue angle: indicative of how similar to a color (ie, red, blue, green, yellow) of the deli-style ham.

$SI= saturation index: indicative of how vivid the color of deli-style ham.

€ΔE= delta E, a single value showing color differences over time. Calculated as $\Delta E^*_{ab}=\sqrt{[(L_2-L_1)^2+(a_2-a_1)^2+(b_2-b_1)^2]}$.

Table 5.4: Least square means for main effects of time for reflective color measurements.

	Trait						
Storage Time (Weeks)	**L***	**a***	**b***	**a/b ratio**	**HA§**	**SI$**	**ΔE€**
0	73.39^{a}	9.23	7.22^{ab}	1.34^{ab}	38.73^{ab}	11.90^{ab}	-
2	72.32^{b}	8.96	7.37^{abc}	1.30^{ab}	40.08^{ab}	11.86^{ab}	1.37
4	72.32^{b}	9.01	7.40^{ab}	1.30^{ab}	40.01^{ab}	11.91^{ab}	1.36
6	72.79^{b}	9.07	7.11^{c}	1.36^{a}	38.65^{b}	11.74^{ab}	1.09
8	72.49^{b}	8.97	7.47^{a}	1.29^{ab}	40.22^{ab}	11.93^{a}	1.33
10	72.52^{b}	9.01	7.23^{abc}	1.33^{ab}	39.24^{ab}	11.77^{ab}	1.28
12	72.32^{b}	8.87	7.17^{bc}	1.32^{ab}	39.54^{ab}	11.63^{b}	1.48
14	72.73^{b}	8.81	7.38^{abc}	1.28^{b}	40.37^{a}	11.72^{ab}	1.40
16	72.37^{b}	8.85	7.31^{abc}	1.29^{b}	40.04^{ab}	11.70^{ab}	1.51
P-value	0.01	0.23	< 0.01	0.01	0.03	0.02	0.09
SEM[2]	0.11	0.08	0.07	0.02	0.38	0.07	0.10

[1]Commision Internationale de l'Eclairage (CIE) L*, a*, b*, in which L* indicates lightness on a scale of 0(black) to 100(colorless), a* indicates redness(+a*) or greenness (-a*), and b* indicates yellowness (+b*) or blueness (-b*).

[2]SEM=standard error of the means for deli-style ham.

[a-c]Means in the same column with different superscripts are indicative of significantly different values ($P \leq 0.05$).

[§]HA= hue angle: indicative of how similar to a color (ie, red, blue, green, yellow) of the deli-style ham.

[$]SI= saturation index: indicative of how vivid the color of deli-style ham.

[€]ΔE= delta E, a single value showing color differences comparing w 0 to all other timepoints. Calculated as $\Delta E^*_{ab}=\sqrt{[(L_2-L_1)^2+(a_2-a_1)^2+(b_2-b_1)^2]}$.

Table 5.5: Least square means for main effects of nitrite and salt concentrations for a_w, salt, yield, pH, residual nitrite, and proximate composition.

	Trait										
Ingoing Salt Concentration (%)	**a_w[1]**	**Salt %**	**Cooking Yield %**	**pH**	**RN[3]**	**Fat %**	**Moisture %**	**Ash %**	**Protein %**	**APC[4]**	**AnPC[5]**
0.7	0.989^{a}	0.63^{c}	84.32^{b}	6.26^{b}	29.02^{b}	3.51	74.71^{b}	1.74^{c}	18.20	4.54	1.76
1.4	0.987^{b}	0.98^{b}	91.87^{a}	6.26^{b}	32.35^{ab}	3.49	75.77^{a}	2.18^{b}	18.11	4.26	1.49
2.1	0.984^{c}	1.41^{a}	92.54^{a}	6.28^{a}	34.07^{a}	3.77	75.43^{a}	2.66^{a}	18.99	3.94	1.28
P-value	< 0.01	< 0.01	< 0.01	< 0.01	0.02	0.53	< 0.01	< 0.01	0.09	< 0.01‡	0.19
SEM[2]	0.01	0.02	0.53	0.01	1.31	0.19	0.19	0.03	0.29	0.12	0.19
Ingoing Nitrite Concentration (ppm) and Source	**a_w[1]**	**Salt %**	**Cooking Yield %**	**pH**	**RN[3]**	**Fat %**	**Moisture %**	**Ash %**	**Protein %**	**APC[4]**	**AnPC[5]**
0 SN	0.988^{x}	1.03	89.36	6.25^{y}	1.50^{z}	3.63	75.62	2.14^{x}	18.45	6.11	2.19^{x}
100 SN	0.986^{y}	1.01	90.18	6.26^{y}	28.93^{y}	3.99	75.22	2.26^{x}	18.59	3.76	1.12^{yz}
100 CP	0.986^{y}	1.01	88.98	6.24^{y}	32.33^{y}	3.28	75.36	2.13^{x}	18.25	4.21	1.91^{xy}
200 SN	0.986^{y}	1.00	89.80	6.31^{x}	64.49^{x}	3.46	75.01	2.24^{x}	18.45	2.91	0.82^{z}
P-value	< 0.01	0.68	0.55	< 0.01	< 0.01	0.17	0.26	0.02	0.91	< 0.01‡	< 0.01
SEM[2]	0.01	0.02	0.61	0.01	1.51	0.22	0.21	0.03	0.34	0.14	0.22

[1]a_w=water activity
[2]SEM=standard error of the means for deli-style ham.
[3]RN=Residual nitrite
[4]APC=aerobic plate count (log cfu/g).
[5]AnPC=anaerobic plate count (log cfu/g).
‡Indicates a significant ($P \leq 0.05$) nitrite by salt concentration interaction for the trait.
$^{a-c;x-z}$ Within each main effect, means in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 5.6: Least square means for main effects of time for pH, residual nitrite, aerobic plate count, and anaerobic plate count. *P*-values are indicative of a significance ($P \leq 0.05$) of storage time effect on each trait.

	Trait			
Storage Time (Weeks)	**pH**	**RN[1]**	**APC[3]**	**AnPC[4]**
0	6.22^{c}	46.92^{ab}	0.99	0.23^{d}
2	6.32^{a}	56.85^{a}	2.68	0.66^{cd}
4	6.32^{a}	42.87^{bc}	3.86	1.36^{abcd}
6	6.25^{bc}	32.81^{cd}	4.40	2.00^{abc}
8	6.32^{a}	30.48^{d}	4.48	1.04^{bcd}
10	6.29^{ab}	24.22^{de}	4.81	1.38^{abcd}
12	6.22^{c}	20.04^{e}	5.46	1.72^{abc}
14	6.24^{bc}	17.56^{e}	5.77	2.78^{a}
16	6.21^{c}	14.54^{e}	5.77	2.42^{ab}
P-value	< 0.01	< 0.01	< 0.01‡	< 0.01
SEM[2]	0.01	2.27	0.21	0.32

[1]RN=Residual Nitrite

[2]SEM=standard error of the means for deli-style ham.

[3]APC=aerobic plate count (log cfu/g)

[4]AnPC=anaerobic plate count (log cfu/g)

‡Indicates a significant ($P \leq 0.05$) nitrite concentration by week of storage interaction for the trait.

$^{a-e}$ Means in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 5.7: Least square means for main effects of salt and nitrite concentration for texture profile analysis measures.

	TPA[1] Trait				
Ingoing Salt Concentration %	**Hardness**	**Gumminess**	**Cohesiveness**	**Springiness**	**Chewiness**
0.7	1713.50	620.90	0.362[a]	0.388[b]	243.23[a]
1.4	1479.39	497.09	0.336[b]	0.397[b]	198.39[b]
2.1	1324.20	382.02	0.288[c]	0.441[a]	167.66[c]
P-value	< 0.01‡	< 0.01‡	< 0.01	< 0.01	< 0.01
SEM[2]	23.14	11.30	0.01	0.01	8.13
Ingoing Nitrite Concentration (ppm) and Source	**Hardness**	**Gumminess**	**Cohesiveness**	**Springiness**	**Chewiness**
0 SN	1412.69	470.15	0.330	0.393	184.06
100 SN	1499.51	496.88	0.330	0.412	202.70
100 CP	1581.10	525.02	0.326	0.418	218.40
200 SN	1529.49	507.96	0.328	0.411	207.22
P-value	< 0.01‡	0.05‡	0.92	0.48	0.10
SEM[2]	26.72	13.05	0.01	0.01	9.39

[1]TPA=Texture Profile Analysis measures

[2]SEM=standard error of the means for deli-style ham.

‡Indicates a significant ($P \leq 0.05$) nitrite by salt concentration interaction for the trait.

[a-c;x-z] Within each main effect, means in the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 5.8: Least square means for nitrite concentration by week of storage interaction effects for aerobic plate count (P = 0.02), SEM[5]=0.43.

	Ingoing Nitrite Concentration (ppm) and Source			
Week of Storage	**0 SN[1]**	**100 SN[2]**	**100 CP[3]**	**200 SN[4]**
0	1.36^{ij}	0.63^{j}	0.78^{j}	1.21^{ij}
2	4.84^{bcdefg}	2.21^{hij}	2.82^{ghij}	0.84^{ij}
4	5.89^{abcd}	3.13^{efghi}	3.75^{defgh}	2.66^{ghij}
6	6.53^{abc}	3.94^{defgh}	4.17^{defgh}	2.95^{fghij}
8	7.06^{ab}	4.22^{cdefgh}	4.14^{defgh}	2.50^{ghij}
10	6.96^{ab}	4.55^{cdefgh}	4.82^{bcdefg}	2.92^{ghij}
12	7.40^{a}	4.60^{cdefgh}	5.42^{abcde}	4.52^{cdefgh}
14	7.37^{a}	5.30^{abcdef}	5.84^{abcd}	4.56^{cdefgh}
16	7.53^{a}	5.37^{abcde}	6.19^{abcd}	3.99^{defgh}

[1]Treatments with 0 Nitrite added.
[2]Treatments with 100 ppm Sodium nitrite added.
[3]Treatments with 100 ppm equivalent of Sodium nitrite added as Celery Powder.
[4]Treatments with 200 ppm Sodium nitrite added.
[5]SEM= standard error of the means for deli-style ham.
$^{a-j}$ Means within the same column with different superscripts are significantly different ($P \leq 0.05$).

Table 5.9: Least square means for nitrite concentration by week of storage interaction effects for residual nitrite ($P < 0.01$), [5]SEM=4.54.

	Ingoing Nitrite Concentration (ppm) and Source			
Week of Storage	**0 SN[1]**	**100 SN[2]**	**100 CP[3]**	**200 SN[4]**
0	1.6^{n}	$41.8^{defghij}$	49.5^{cdefg}	94.9^{a}
2	1.6^{n}	57.6^{cde}	67.0^{bc}	101.2^{a}
4	1.5^{n}	$39.5^{efghijk}$	46.3^{cdefgh}	84.2^{ab}
6	1.1^{n}	$28.1^{fghijklm}$	$35.5^{efghijklm}$	66.6^{bcd}
8	0.7^{n}	$31.0^{fghijklm}$	$25.5^{ghijklmn}$	64.76^{bcd}
10	1.3^{n}	$23.0^{hijklmn}$	21.1^{ijklmn}	51.5^{cdef}
12	1.8^{n}	15.3^{klmn}	17.0^{jklmn}	$46.1^{cdefghi}$
14	1.9^{n}	13.3^{lmn}	17.0^{jklmn}	12.2^{mn}
16	2.1^{n}	10.8^{mn}	12.2^{mn}	$33.1^{efghijklm}$

[1]Treatments with 0 Nitrite added.
[2]Treatments with 100 ppm Sodium nitrite added.
[3]Treatments with 100 ppm equivalent of Sodium nitrite added from Celery Powder.
[4]Treatments with 200 ppm Sodium nitrite added.
[5]SEM= standard error of the means for deli-style ham.
[a-m] Means within the table with different superscripts are significantly different ($P \leq 0.05$).

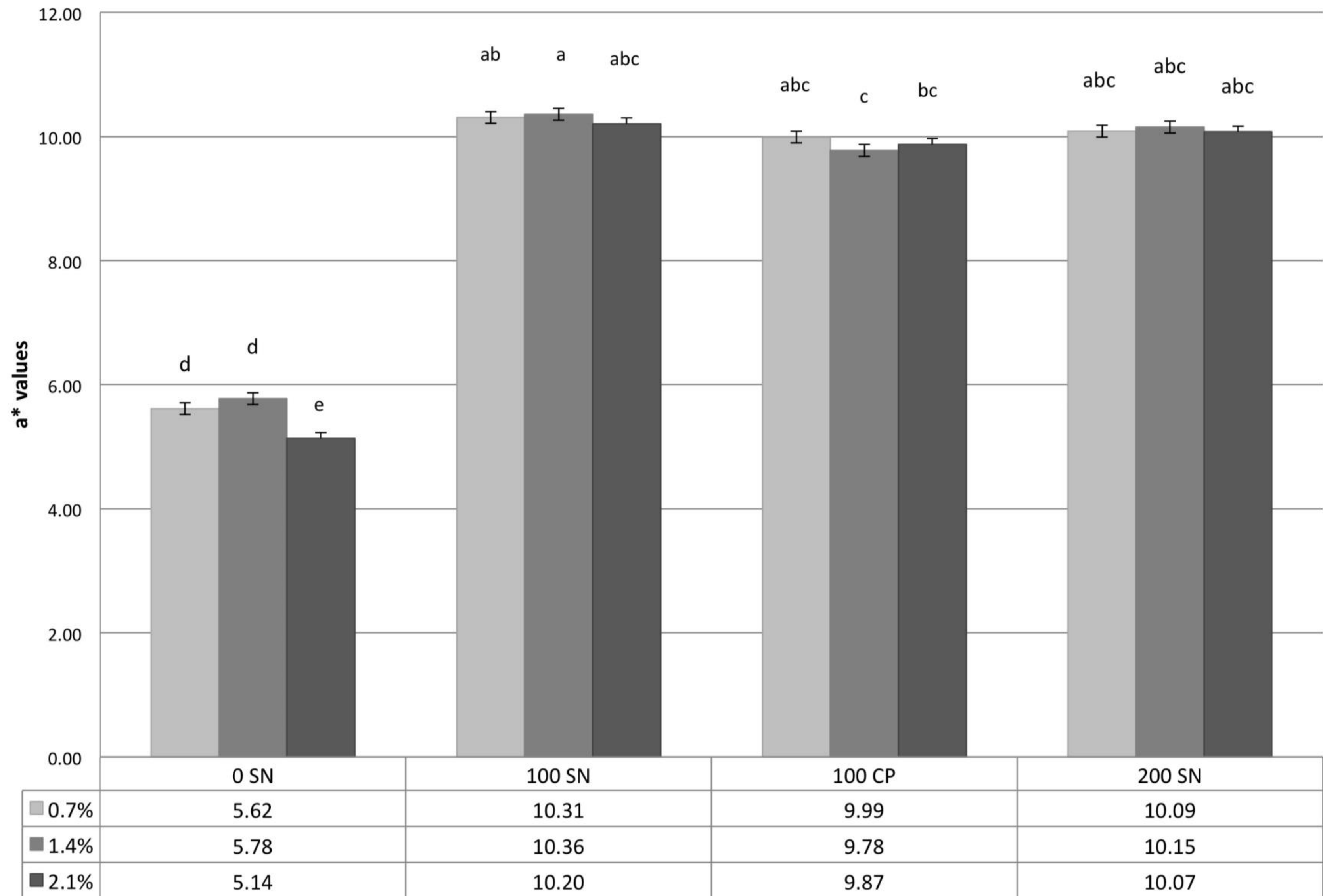


Figure 5.1: Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P < 0.01$) on a* values for 0.7, 1.4, and 2.1% salt and 0 SN (sodium nitrite), 100 SN, 100 CP (100 ppm equivalent to sodium nitrite of celery powder), and 200 SN products. Bars with different superscripts ($^{a-e}$) are significantly ($P \leq 0.05$) different.

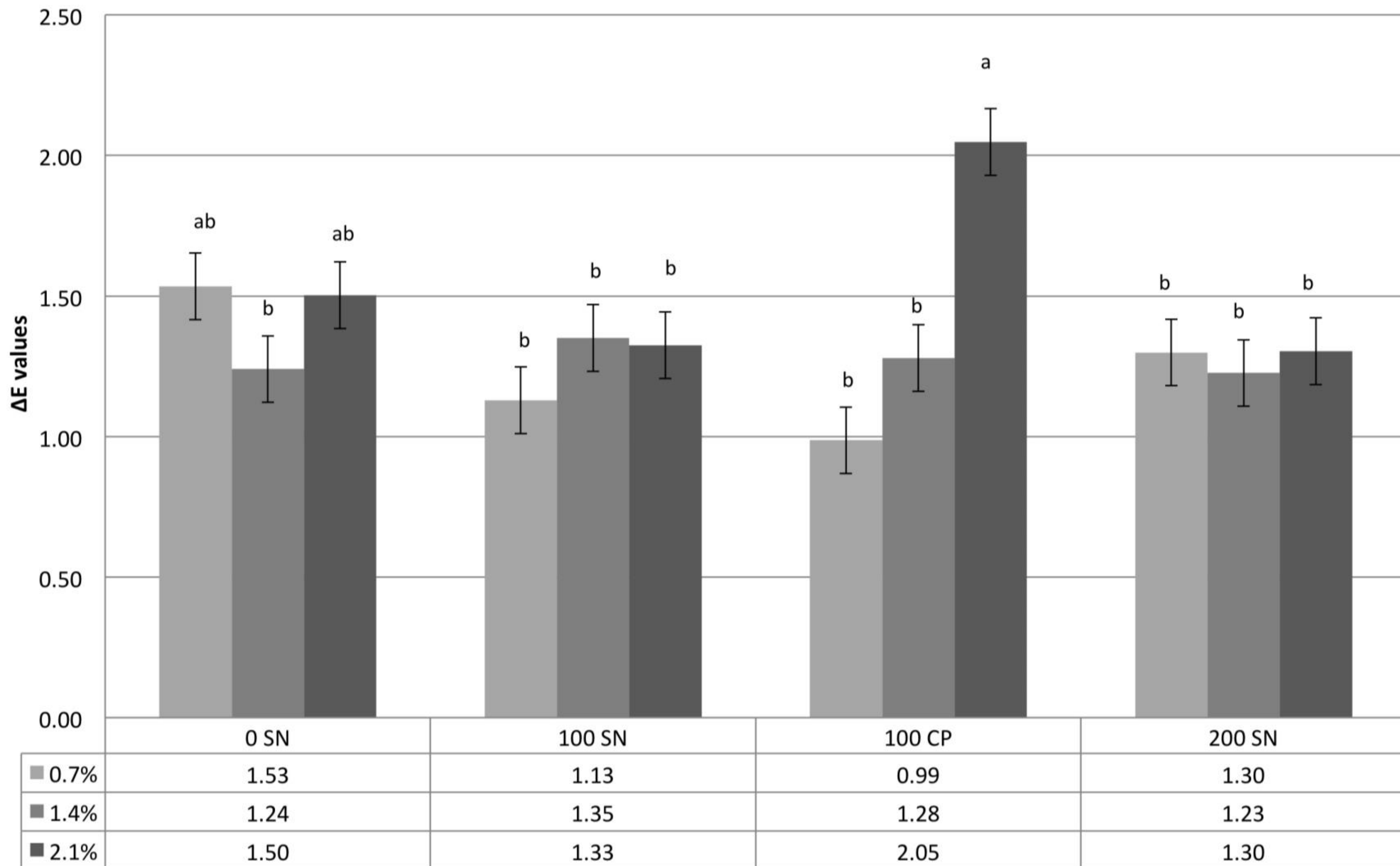
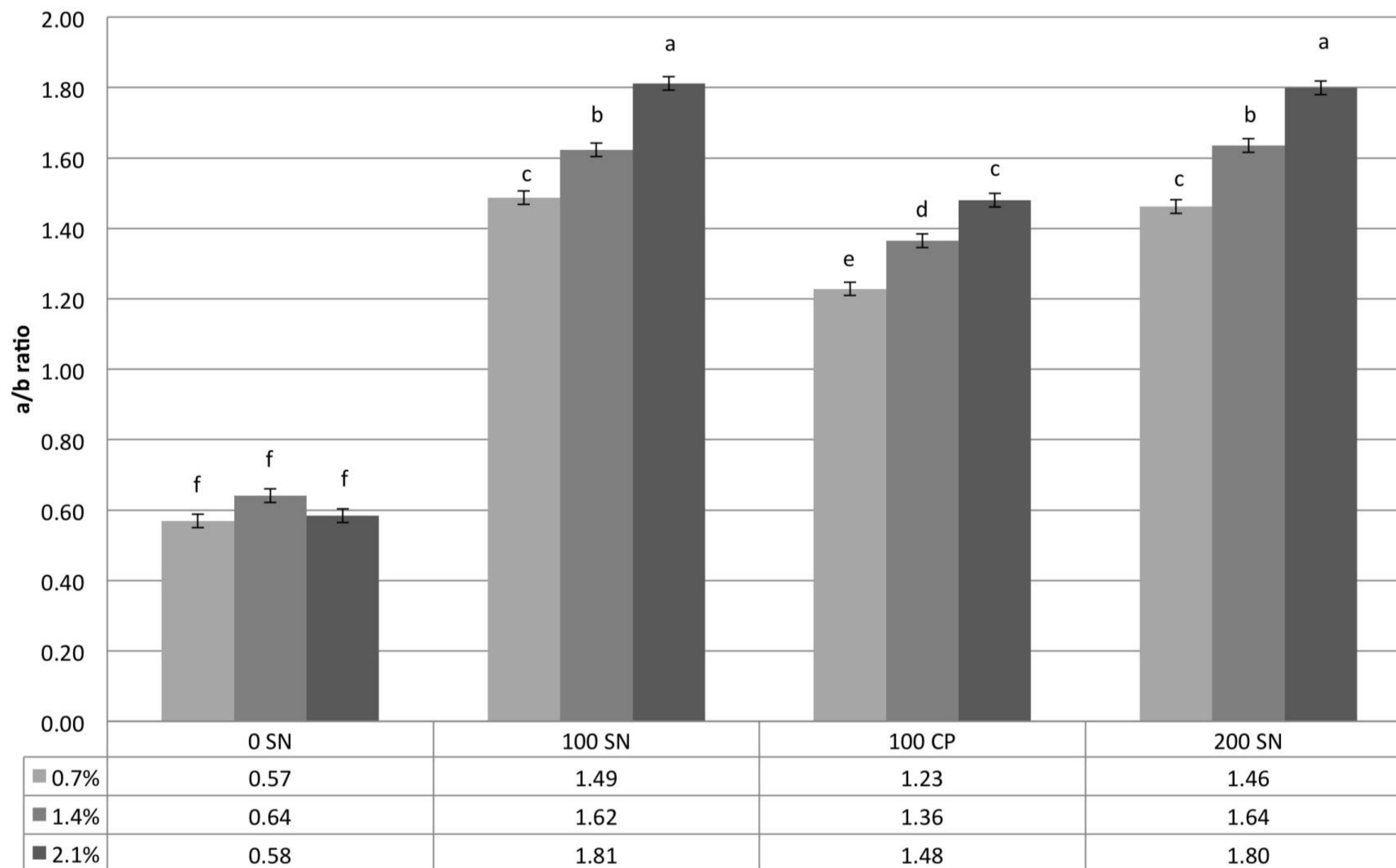


Figure 5.2 Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P <$ 0.01) on ΔE[1] values for 0.7, 1.4, and 2.1% salt and 0 SN (sodium nitrite), 100 SN, 100 CP (100 ppm equivalent to sodium nitrite of celery powder), and 200 SN products. Bars with different superscripts ($^{a-b}$) are significantly ($P \leq 0.05$) different. [1]ΔE is a single number that represents the distance between two colors.

[1] ΔE= delta E, a single value showing color differences between w 0 and all other time points. Calculated as $\Delta E^*_{ab}=\sqrt{[(L_2-L_1)^2+(a_2-a_1)^2+(b_2-b_1)^2]}$.



	0 SN	100 SN	100 CP	200 SN
0.7%	0.57	1.49	1.23	1.46
1.4%	0.64	1.62	1.36	1.64
2.1%	0.58	1.81	1.48	1.80

Figure 5.3: Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P <$ 0.01) on a/b ratios[1] for 0.7, 1.4, and 2.1% salt and 0 SN, 100 SN, 100 CP, and 200 SN products. Bars with different superscripts ($^{a\text{-}f}$) are significantly ($P \leq 0.05$) different.

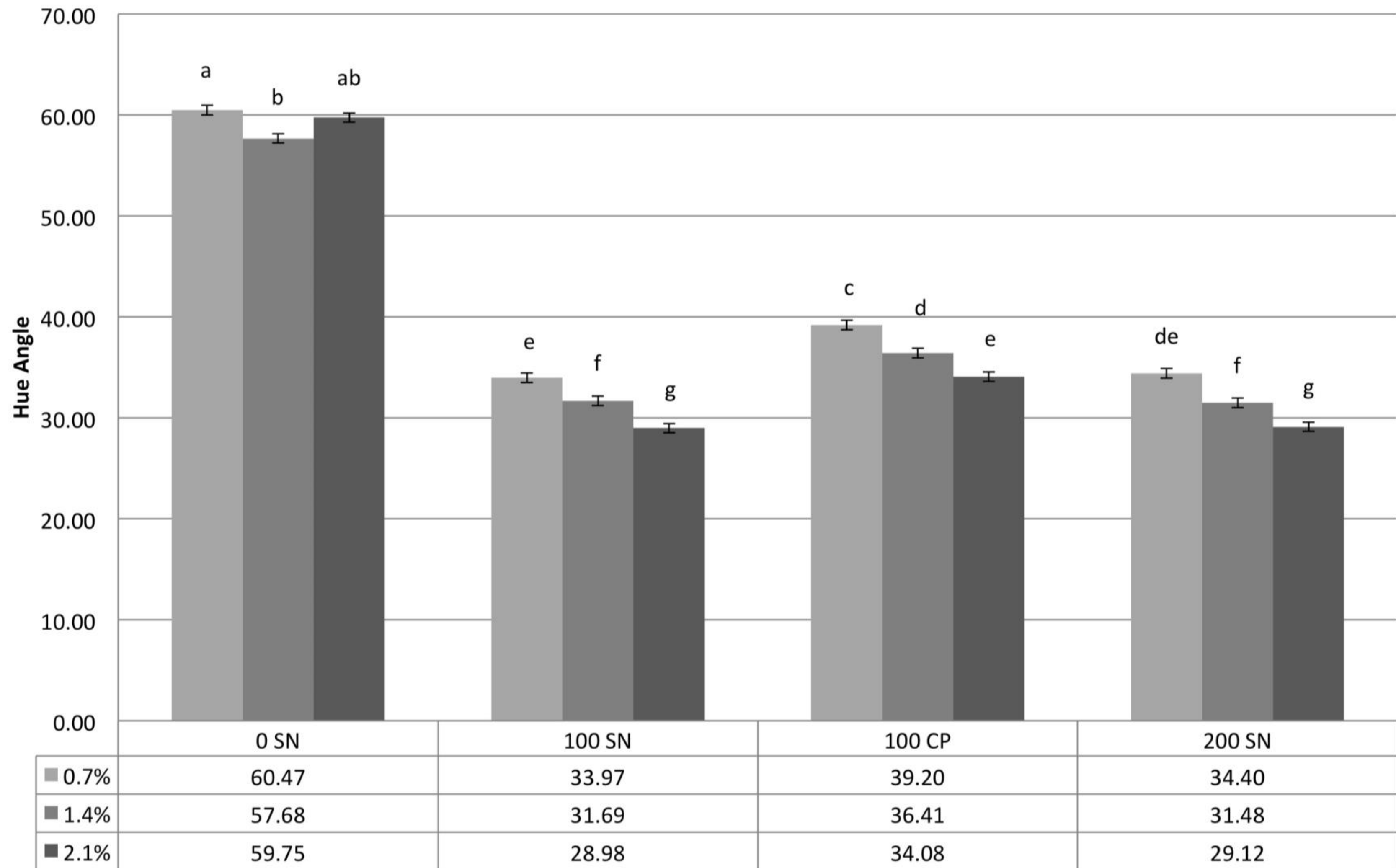
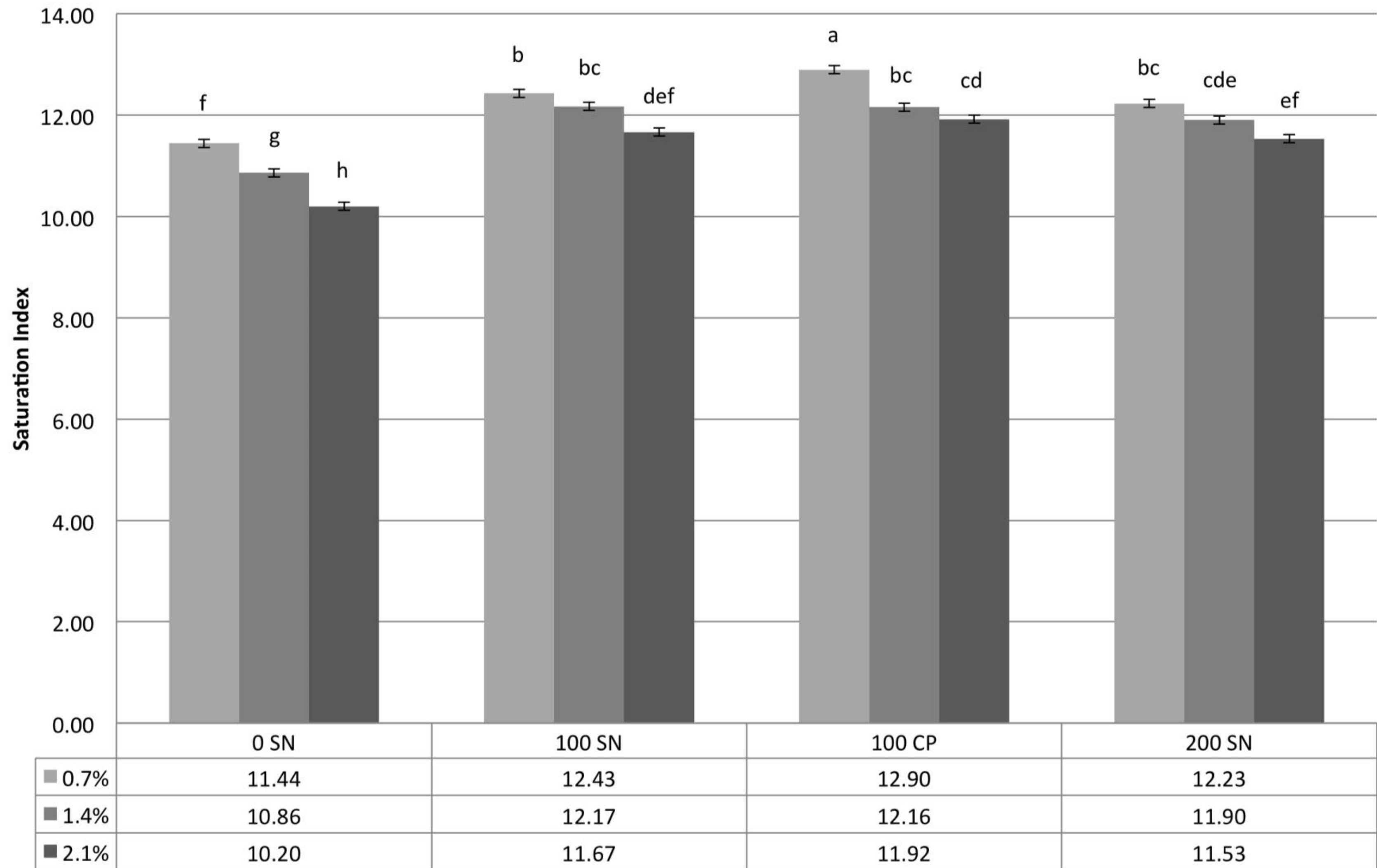
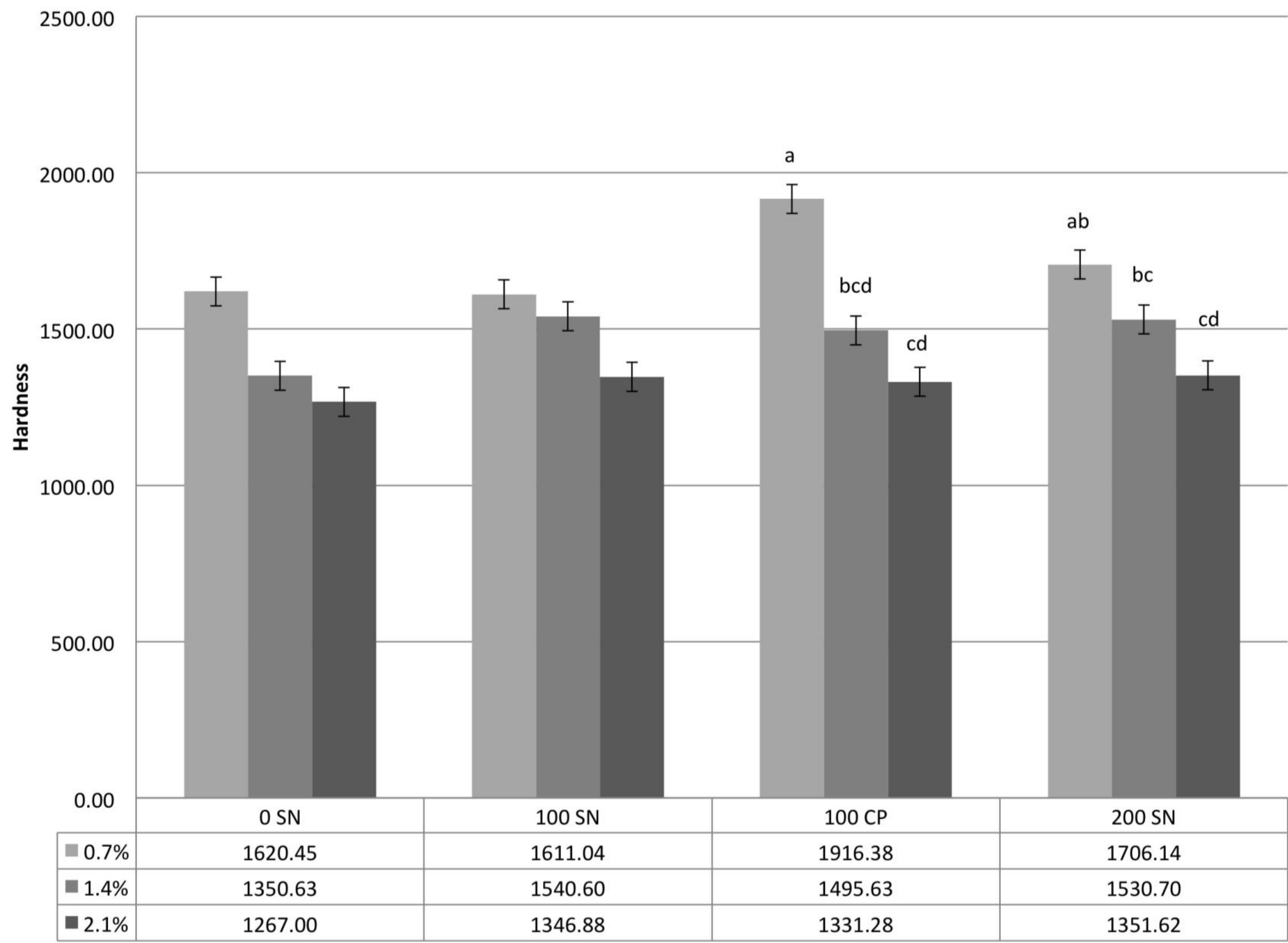


Figure 5.4: Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P <$ 0.01) on hue angle[1] for 0.7, 1.4, and 2.1% salt and 0 SN, 100 SN, 100 CP, and 200 SN products. Bars with different superscripts ($^{a\text{-}g}$) are significantly ($P \leq 0.05$) different.



	0 SN	100 SN	100 CP	200 SN
0.7%	11.44	12.43	12.90	12.23
1.4%	10.86	12.17	12.16	11.90
2.1%	10.20	11.67	11.92	11.53

Figure 5.5: Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P <$ 0.01) on saturation index[1] for 0.7, 1.4, and 2.1% salt and 0 SN, 100 SN, 100 CP, and 200 SN products. Bars with different superscripts ([a-g]) are significantly ($P \leq 0.05$) different.



	0 SN	100 SN	100 CP	200 SN
0.7%	1620.45	1611.04	1916.38	1706.14
1.4%	1350.63	1540.60	1495.63	1530.70
2.1%	1267.00	1346.88	1331.28	1351.62

Figure 5.6: Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P <$ 0.01) on hardness[1] for 0.7, 1.4, and 2.1% salt and 0 SN, 100 SN, 100 CP, and 200 SN products. Bars with different superscripts ([a-b]) are significantly ($P \leq 0.05$) different.

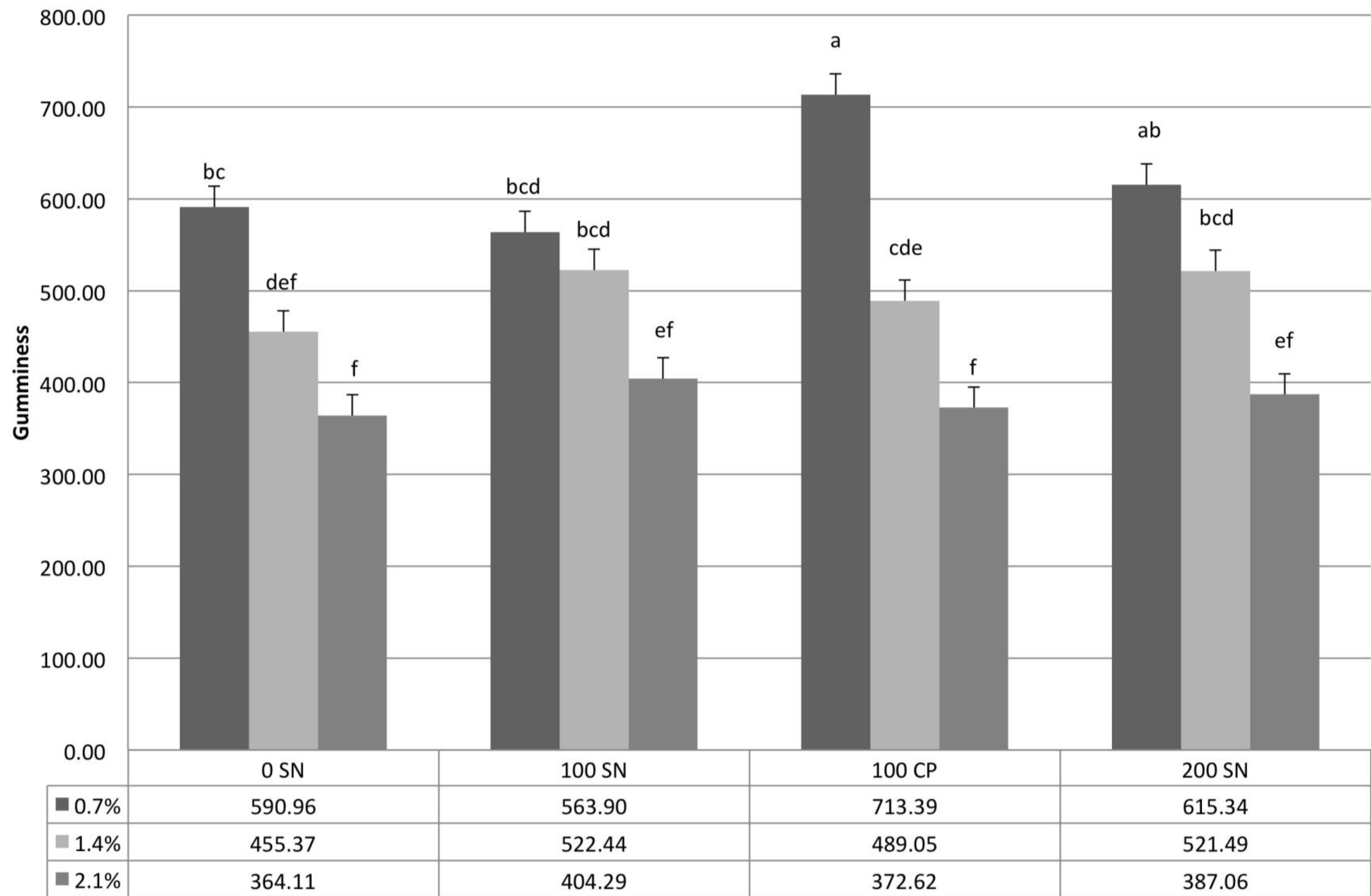
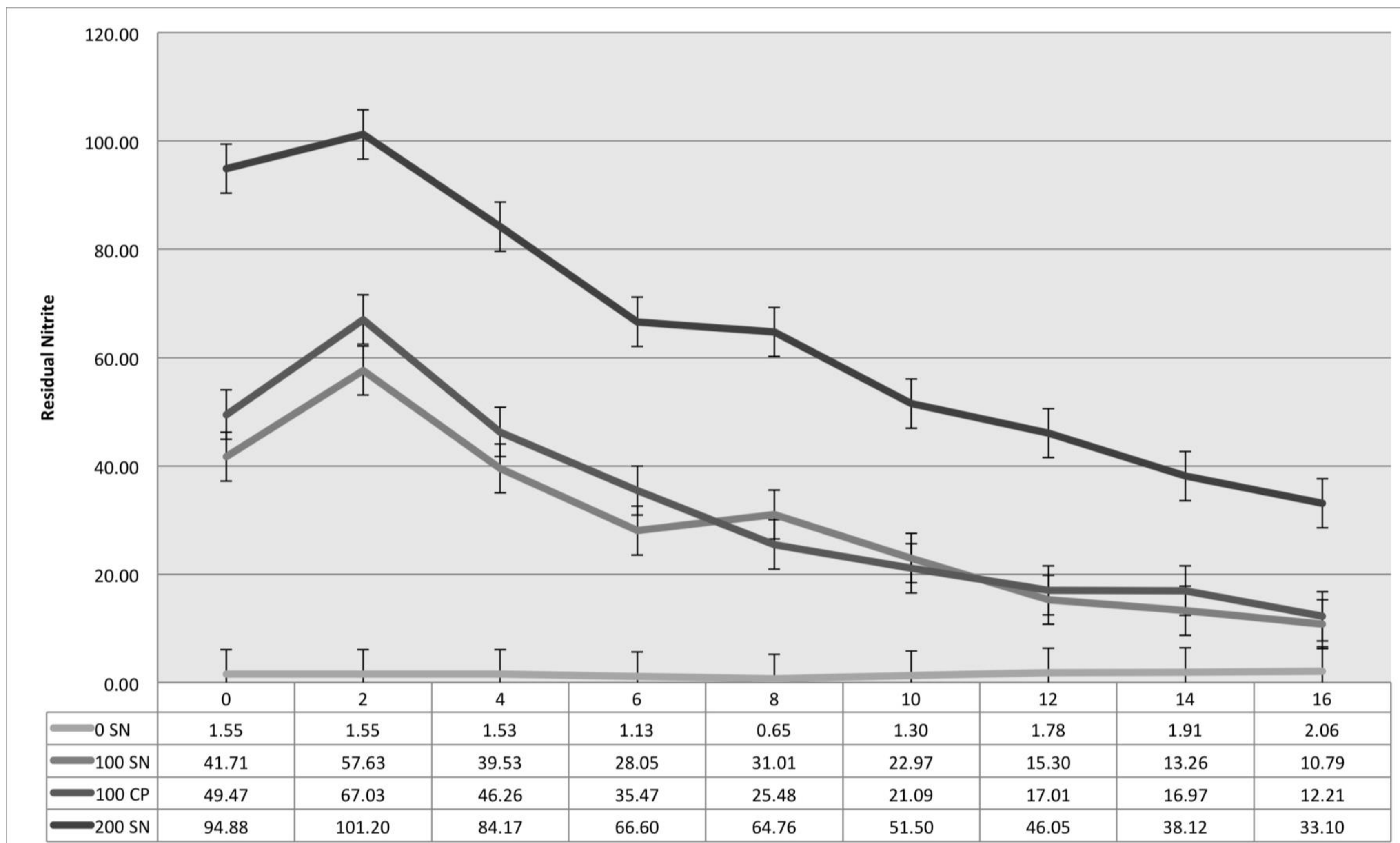
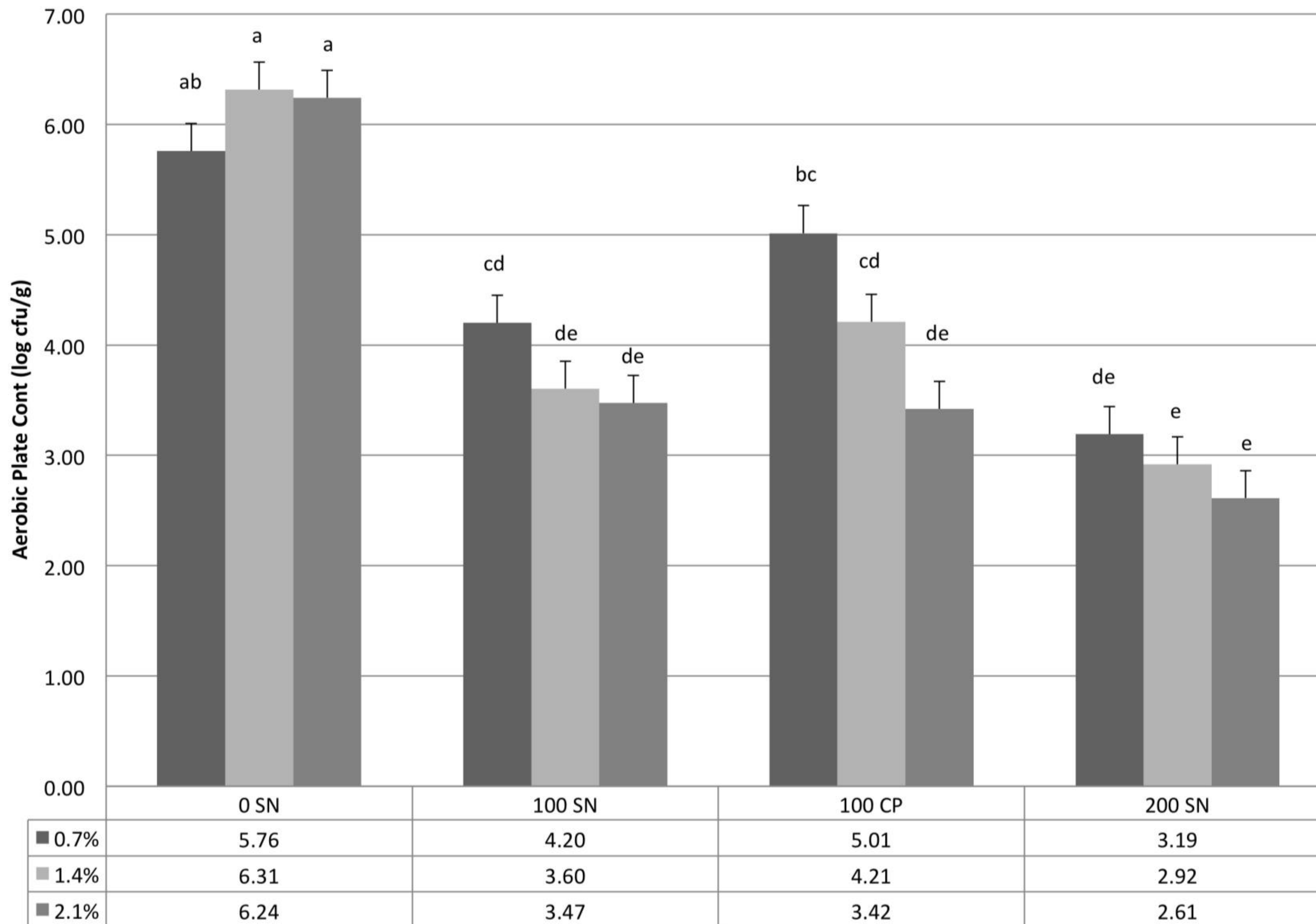


Figure 5.7: Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P <$ 0.01) on gumminess for 0.7, 1.4, and 2.1% salt and 0 SN, 100 SN, 100 CP, and 200 SN products. Bars with different superscripts ($^{a-b}$) are significantly ($P \leq 0.05$) different.



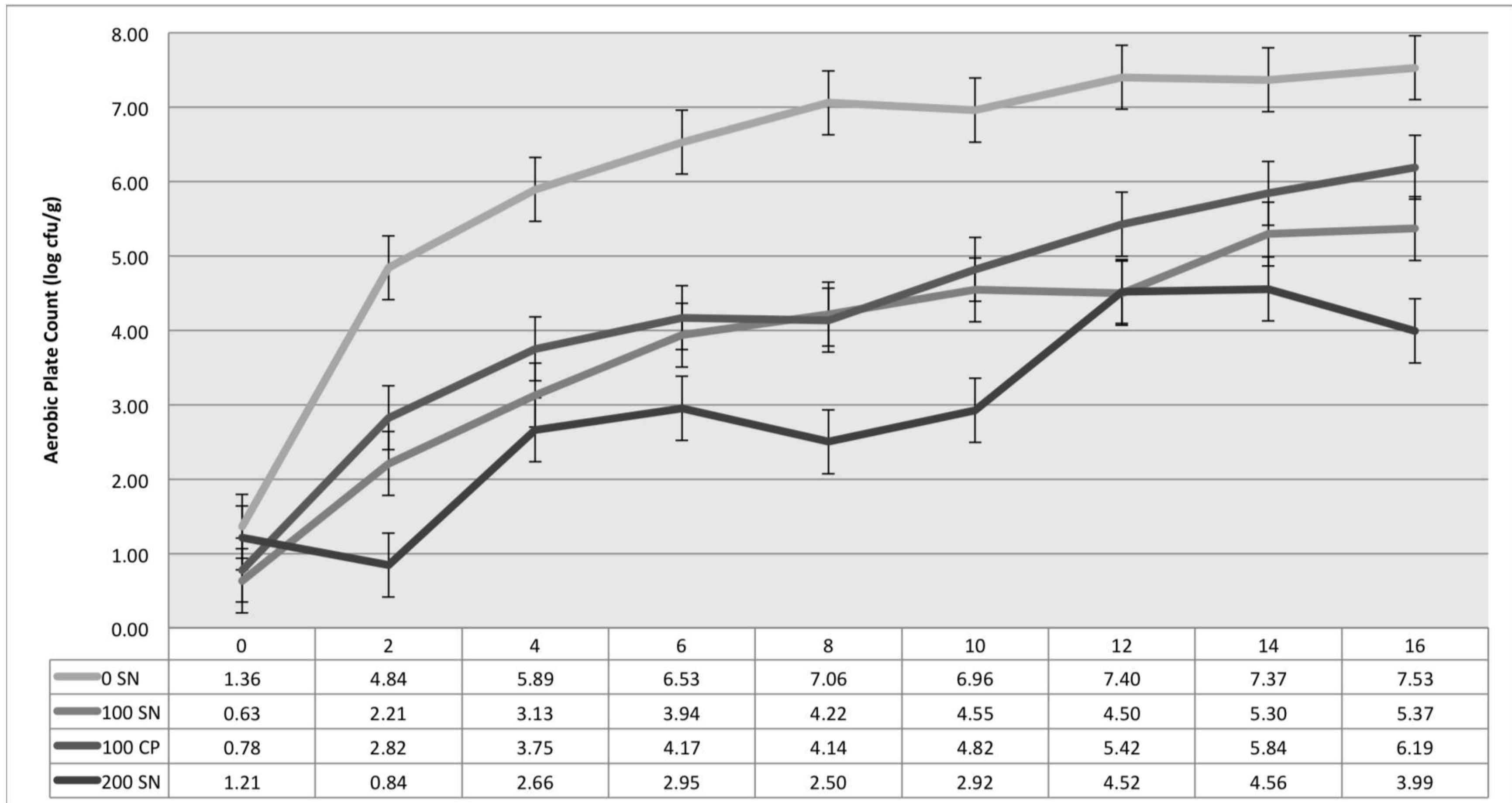
	0	2	4	6	8	10	12	14	16
0 SN	1.55	1.55	1.53	1.13	0.65	1.30	1.78	1.91	2.06
100 SN	41.71	57.63	39.53	28.05	31.01	22.97	15.30	13.26	10.79
100 CP	49.47	67.03	46.26	35.47	25.48	21.09	17.01	16.97	12.21
200 SN	94.88	101.20	84.17	66.60	64.76	51.50	46.05	38.12	33.10

Figure 5.8: Least square means for the effect of nitrite concentration by week of storage interaction ($P < 0.01$) on residual nitrite for 0.7, 1.4, and 2.1% salt and 0 SN, 100 SN, 100 CP, and 200 SN products.



	0 SN	100 SN	100 CP	200 SN
0.7%	5.76	4.20	5.01	3.19
1.4%	6.31	3.60	4.21	2.92
2.1%	6.24	3.47	3.42	2.61

Figure 5.9: Least square means for the effect of ingoing salt concentration by ingoing nitrite concentration (ppm) and source ($P <$ 0.01) on aerobic plate count for 0.7, 1.4, and 2.1% salt and 0 SN (sodium nitrite), 100 SN, 100 CP (100 ppm sodium nitrite equivalent of celery powder), and 200 SN products. Bars with different superscripts ([a-e]) are significantly ($P \leq 0.05$) different.



	0	2	4	6	8	10	12	14	16
0 SN	1.36	4.84	5.89	6.53	7.06	6.96	7.40	7.37	7.53
100 SN	0.63	2.21	3.13	3.94	4.22	4.55	4.50	5.30	5.37
100 CP	0.78	2.82	3.75	4.17	4.14	4.82	5.42	5.84	6.19
200 SN	1.21	0.84	2.66	2.95	2.50	2.92	4.52	4.56	3.99

Figure 5.10: Least square means for the effect of nitrite concentration by week of storage interaction ($P < 0.01$) on aerobic plate count for 0.7, 1.4, and 2.1% salt and 0 SN (sodium nitrite), 100 SN, 100 CP (100 ppm equivalent to sodium nitrite of celery powder), and 200 SN products.

7. Appendices

7.1 Production Protocol for Deli-Style Ham (for each of three replications)

1. Freeze denuded ham inside muscles upon arrival.
2. Temper ham muscles at -1°C for 48 hours prior to manufacture.
3. Coarse grind muscles through ½" plate.
4. Fine grind ham through 3/16" plate.
5. Weigh ham into 12 batches (25 lbs each).
    - 0.7% salt, 0 ppm sodium nitrite
    - 1.4% salt, 0 ppm sodium nitrite
    - 2.1% salt, 0 ppm sodium nitrite
    - 0.7% salt, 100 ppm sodium nitrite
    - 1.4% salt, 100 ppm sodium nitrite
    - 2.1% salt, 100 ppm sodium nitrite
    - 0.7% salt, 200 ppm sodium nitrite
    - 1.4% salt, 200 ppm sodium nitrite
    - 2.1% salt, 200 ppm sodium nitrite
    - 0.7% salt, 100 ppm of sodium nitrite equivalent from Celery juice powder
    - 1.4% salt, 100 ppm of sodium nitrite equivalent from Celery juice powder
    - 2.1% salt, 100 ppm of sodium nitrite equivalent from Celery juice powder
6. Make brine for each batch, then mix with meat block for 3 minutes.
7. Stuff ham logs in 6Mx42" fibrous casings using a Vemag vacuum stuffer.
    a. Each stick should be tagged.
    b. Be sure to get initial weights before putting it in the smokehouse and final weights after thermal processing and chilling to calculate cook yields on this product.
8. Hang ham logs on smoke rack and cook using the "Turkey Roll" cycle on the smokehouse, and chill overnight in the cooler with fans.
    a. Use data loggers to record temperature and cooling curve of ham overnight in coolers to meet USDA Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products. http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/95-033F/95-033F_Appendix%20B.htm
9. Slice into 2mm and 13mm slices for analysis.
    a. Place 2 slices of one thickness side-by-side into a 3 mil vacuum package bag and seal. Store packages in a lug with a lid for dark refrigerated storage.

7.2 Ham Formulations

Product Name:	0.7% Salt, 0ppm NaNO2 Deli Ham TRT1			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.725125**	**2596.8709**	**22.90%**	**18.32%**
Non-Meat Ingredients	**0.425**	**192.7766**	**1.70%**	**1.36%**
Salt	0.175	79.3786	0.70%	0.56%
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:	0.099875	45.302501	0.40%	0.32%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11577.879**		

Product Name:	1.4% Salt, 0ppm NaNO2 Deli Ham TRT2			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.550125**	**2517.4923**	**22.20%**	**17.76%**
Non-Meat Ingredients	**0.6**	**272.1552**	**2.40%**	**1.92%**
Salt	0.35	158.7572	1.40%	1.12%
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:	0.099875	45.302501	0.40%	0.32%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11657.258**		

Product Name:	2.1% Salt, 0ppm NaNO2 Deli Ham TRT3			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.375125**	**2438.1137**	**21.50%**	**17.20%**
Non-Meat Ingredients	**0.775**	**351.5338**	**3.10%**	**2.48%**
Salt	0.525	238.1358	2.10%	1.68%
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:	0.099875	45.302501	0.40%	0.32%
Sodium Nitrite (6.25% curing salt)	0	0	0.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11736.636**		

Product Name:	0.7% Salt, 100ppm NaNO2 Deli Ham TRT4			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.7218472**	**2595.3841**	**22.89%**	**18.31%**
Non-Meat Ingredients	**0.3882778**	**176.11971**	**1.55%**	**1.24%**
Salt	0.1382778	62.721712	0.55%	0.44%
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:	0.139875	63.446181	0.56%	0.45%
Sodium Nitrite (6.25% curing salt)	0.04	18.14368	100.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11579.366**		

Product Name:	1.4% Salt, 100ppm NaNO2 Deli Ham TRT5			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.5465968**	**2515.8919**	**22.19%**	**17.75%**
Non-Meat Ingredients	**0.5635282**	**255.61187**	**2.25%**	**1.80%**
Salt	0.3135282	142.21387	1.25%	
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:	0.139875	63.446181	0.56%	0.45%
Sodium Nitrite (6.25% curing salt)	0.04	18.14368	100.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11658.858**		

Product Name:	2.1% Salt 100ppm NaNO2 Deli Ham TRT6			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.3713499**	**2436.4013**	**21.49%**	**17.19%**
Non-Meat Ingredients	**0.7387751**	**335.1025**	**2.96%**	**2.36%**
Salt	0.4887751	221.7045	1.96%	1.56%
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:	0.139875	63.446181	0.56%	0.45%
Sodium Nitrite (6.25% curing salt)	0.04	18.14368	100.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11738.349**		

Product Name:	0.7% Salt, 200ppm NaNO2 Deli Ham TRT7			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.7185594**	**2593.8928**	**22.87%**	**18.30%**
Non-Meat Ingredients	**0.3515656**	**159.46734**	**1.41%**	**1.13%**
Salt	0.1015656	46.06934	0.41%	0.33%
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:	0.179875	81.589861	0.72%	0.58%
Sodium Nitrite (6.25% curing salt)	0.08	36.28736	200.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11580.857**		

Product Name:	1.4% Salt, 200ppm NaNO2 Deli Ham TRT8			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.5430581**	**2514.2868**	**22.17%**	**17.74%**
Non-Meat Ingredients	**0.5270669**	**239.07331**	**2.11%**	**1.69%**
Salt	0.2770669	125.67531	1.11%	0.89%
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:	0.179875	81.589861	0.72%	0.58%
Sodium Nitrite (6.25% curing salt)	0.08	36.28736	200.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11660.463**		

Product Name:	2.1% Salt, 200ppm NaNO2 Deli Ham TRT9			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.3675637**	**2434.6839**	**21.47%**	**17.18%**
Non-Meat Ingredients	**0.7025613**	**318.6762**	**2.81%**	**2.25%**
Salt	0.4525613	205.2782	1.81%	1.45%
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:	0.179875	81.589861	0.72%	0.58%
Sodium Nitrite (6.25% curing salt)	0.08	36.28736	200.00	PPM
Sodium Erythorbate	0.012375	5.613201	495.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11740.066**		

Product Name:	0.7% salt, 100ppm Cel506 Deli Ham TRT10			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.6229417**	**2550.5214**	**22.49%**	**17.99%**
Non-Meat Ingredients	**0.4133509**	**187.49268**	**1.65%**	**1.32%**
Salt	0.1633509	74.094682	0.65%	0.52%
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:	0.2137074	96.935955	0.85%	0.68%
Sodium Nitrite (2.17% 506 Celery Powder)	0.1152074	52.257143	100.00	PPM
Sodium Erythorbate (Cherry Powder 11%)	0.011	4.989512	440.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11624.229**		

Product Name:	1.4% Salt, 100ppm cel506 Deli Ham TRT11			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.4478681**	**2471.1094**	**21.79%**	**17.43%**
Non-Meat Ingredients	**0.5884246**	**266.90467**	**2.35%**	**1.88%**
Salt	0.3384246	153.50667	1.35%	1.08%
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:	0.2137074	96.935955	0.85%	0.68%
Sodium Nitrite (2.17% 506 cel powder)	0.1152074	52.257143	100.00	PPM
Sodium Erythorbate(11% cherry powder)	0.011	4.989512	440.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11703.641**		

Product Name:	2.1% Salt, 100ppm cel506 Deli Ham TRT12			
Meat Block:	25			
Percent Pump	25%			
	lbs	g	% of meat block	%total formulation
Meat Ingredients:	**25**	**11339.8**	**100.00%**	**80.00%**
Ham	25	11339.8	100.00%	80.00%
	0	0		0.00%
	0	0		0.00%
	0	0		0.00%
Water	**5.2675954**	**2389.3391**	**21.07%**	**16.86%**
Non-Meat Ingredients	**0.7629293**	**346.05863**	**3.05%**	**2.44%**
Salt	0.5129293	232.66063	2.05%	1.64%
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:	0.2194753	99.552255	0.88%	0.70%
Sodium Nitrite (2.17% 506 cel powder)	0.1209753	54.873443	100.00	PPM
Sodium Erythorbate(11% cherry powder)	0.011	4.989512	440.00	PPM
Sodium Phosphate	0.0875	39.6893	0.35%	0.28%
Brine Total	6.25	2834.95	25.00%	20.00%
Totals	**31.25**	**11785.411**		

7.3 Water Activity

Reference: AquaLab Quick Start Guide (2015). Decagon Devices, Inc. Retrieved from <
http://manuals.decagon.com/Quick%20Start%20Guides/13909_Series%204.pdf>.
Decagon Devices, Inc. Pullman, WA.

1. Materials needed:
    a. Food processor, cutting board, and knife
    b. AquaLab meter
    c. Water activity cups
2. Calibrate AquaLab meter using calibration vials (0.760 a_w: 6.0mol/kg NaCl in H_2O; 0.984 a_w: 0.5mol/kg KCl in H_2O).
3. Pack cup about halfway full with sample processed to fine particles.
4. Read cup in meter.
5. Samples were measured in duplicate.

7.4 Salt Concentration

Reference: Sebranek, J. G., Lonergan, S. M., King-Brink, M., Larson, E., & Beerman, D. H. (2001). *Meat Science and Processing* (pp. 275). Peerage Press, Zenda, MN.

1. Materials needed:
    a. Food processor, cutting board, and knife
    b. Plastic beakers
    c. Electric hot water kettles
    d. Glass stir rods
    e. Whatman #1 filter paper
    f. Plastic funnels
    g. Quantab® strips (high chloride range Chloride titration strips; Hach Company, Loveland, CO)
2. Boil distilled water in electric kettle.
3. Homogenize samples into fine particles using food processor.
4. Weigh 10 g of sample into plastic beaker (2 beakers per treatment).
5. Add 90 ml of boiling water to beaker.
6. Stir for 30 s; wait 60 s; stir 30 s using a glass stir rod.
7. Fold circle of filter pater into a cone shape, set in beaker and allow liquid to permeate paper.
8. Place Quantab® strip in solution in cone.
9. Leave strip in place until yellow strip at top turns blue.
10. Locate white peak along scale and convert to percentage salt.
11. Multiply percentage of salt from the Quantab® unit conversion table by 10 to adjust for dilution.
12. Samples were measured in duplicate.

7.5 Proximate Analysis: Protein, Moisture, Ash, and Fat

7.5.1 Protein: LECO FP-528 Sample Preparation and Analysis

Reference: [AOAC] Association of Official Analytical Chemists. 1990. Crude protein in meat. Official Method 960.39. Official Methodss of Analysis (15th ed.), Arlington, VA, 0.937.

Preparation using foil method:

1. Place sample cup holder with tin foil cup on the balance and tare.
2. Weigh out into foil 0.10g EDTA (if for a standard) or 0.25 g (powdered meat sample), record weight.
3. Remove foil from the sample cup holder and twist to seal.
4. Set the analysis method parameters and system control parameters on FP-528.
5. Turn the gas supplies, including the carrier gas ON.
6. Select the proper analysis mode: Nitrogen or Protein.
7. If unit has not run in a while, run enough blanks (blank on air) to stabilize the machine (10-15) before loading in standards and samples.
8. Once the machine is stable, load 5 standards followed by samples into the autosampler.
9. The autosampler will continue to run, dropping samples into the analyzer as needed.
10. Make sure to enter sample ID, sample weight, and nitrogen factor into computer program for calculations to be accurate
11. Samples were measured in duplicate.

Combustion Furnace Temperature: 850°C.
Reduction Heater: 750°C.
Gas Conversion Timeout: 15 sec.
Carrier Gas: Helium.
Atmospheric Gas: Oxygen.
Nitrogen Conversion Factor: 6.25.

7.5.2 Moisture and Ash

Reference: [AOAC] Association of Official Analytical Chemists. 1990. Ash of meat. Official Method 920.153. Official Methods of Analysis (15th ed.), Arlington, VA, p.932.

1. Turn on the TGA 701 Gravimetric analyzer, computer and printer. Select the ANALYZE screen on the TGA 701.
2. Open up the TGA program on the computer.
   a) This should contain the parameters to run moisture and ash.
3. Type in sample ID in the sample column, then either A or B, or 1 or 2, in the second column to designate number of replicates.
4. Select FILE, then SAVE AS and type in the run name (e.g. ham1).
5. Select ANALYZE. The program will now prompt you to load the crucibles. Use only clean, oven-dried crucibles that have been cooled down in the dessicator.
6. Load empty crucibles in the oven. There is always a reference crucible in the first position. The maximum number of sample crucibles in each oven is 19. We usually use 18 (9 samples in duplicate for a full run).
7. After loading, the analyzer screen will prompt you to press any key. The analyzer will then count and tare the crucibles.
8. Load 1 g of sample using the loading spoon (if using liquid nitrogen-powdered sample, use a spoon cooled in liquid nitrogen, then return samples to -80°C freezer).
9. After all samples are loaded and weighed, the analysis will begin.
10. When the analysis is complete, export data to a flash drive. The oven must be at 25°C before you can use it to analyze another set.
11. Remove crucibles after they have cooled down, wash in soapy water, and allow crucibles to dry in a drying oven for at least 90 minutes.
12. Samples were run in duplicate.

Parameters for moisture and ash:

Name	Covers	RampRate	RampTime	StartTemp	EndTemp
Moisture	Off	6 d/m	17 min	25 °C	130 °C
Ash	Off	20 d/m	30 min	130 °C	160 °C

Name	Atmosphere	Hold Time	Const. Wt.	Const. Wt. Time	Flow Rate
Moisture	N	0 min	0.05%	9 min	High
Ash	O	0 min	0.05%	9 min	High

7.5.3 Fat Extraction: Soxhlet Method

Reference: [AOAC] Association of Official Analytical Chemists. 1990. Fat (Crude) or ether extract in meat. Official Method 960.39. Official Methods of Analysis (15th ed.), Arlington, VA, p.931.

1. Weigh 2 g of each sample into filter paper, fold, and paperclip. Record weight of filter paper and paper clip, and weight of folded packet with sample.
2. Place folded filter paper packet with sample into Soxhlet tubes, arranging them so that no samples are above the level of the top bend in the narrower tubing on the outside of the Soxhlet. (The Soxhlet will only fill with the solvent up to this point before cycling back down into the boiling flask.) In general, the large soxhlets will hold about 20 two-gram samples and the small soxhlets from 4-6.
3. Fill the large (500 ml) boiling flasks with approximately 400 ml of solvent.
4. Fit the Soxhlet onto the boiling flask. The ceramic fiber sheet could be covering the bare metal surfaces of the burners completely.
5. Turn the heating element control dials between three and four. Each burner has its own dial. Ether has a very low boiling point and violent boiling is dangerous. Double check fittings, boiling stones, etc.
6. Fat extraction will take from 24 to 72 hours depending on the sample (Beef: 48 hours, Bacon: 72 hours). Check extractions twice daily while they are running.
7. When done, turn off the burners and let solvent cool completely before removing samples.
8. After it has cooled down, slowly uncouple the flask and Soxhlet tube from the condenser. Cover the top of the Soxhlet with one palm so as to reduce ether vapors while transporting it to the fume hood. Allow samples to air dry in the fume hood for two hours to get rid of the remaining ether in the samples. Pour ether back slowly into an approved container for reuse or discarding.
9. Place samples in the drying oven (105°C) for about 4 hours or overnight before weighing back.
10. Calculation: {[(Original weight including filter paper and paper clip – Fat extracted sample weight)/Sample weight]*100}-%Moisture=%Fat.
11. Samples were run in triplicate.

7.6 Texture Profile Analysis

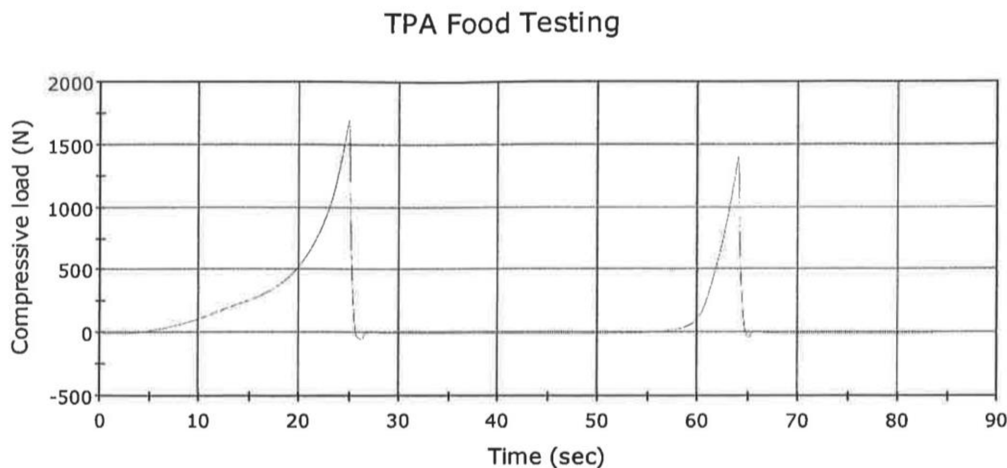
Reference: Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*(7), 62-66,72.

Materials needed:

a. 4.0x4.0cm square, cutting board, and knife
b. Instron Universal Testing Machine model 1123
c. 2,500 kg load cell
d. 140 mm plate

2. Cut ham samples to 4.0x4.0cm square that is 13mm thick.
3. Place sample square into Instron.
4. Run sample with a head speed of 30mm/min to 75% of its thickness, twice.
5. Obtain values from computer for hardness, springiness, cohesiveness, gumminess, and chewiness.
6. Samples were measured in duplicate.

Hardness: the maximum force during the first cycle of compression.
Springiness: the distance that the product is extended during decompression before separating from the probe.
Cohesiveness: the ratio of the positive force area during the second cycle of compression to that of the first cycle, calculated as (Area B/Area A).
Gumminess: calculated as hardness * cohesiveness.
Chewiness: calculated as gumminess * springiness.



7.7 pH

Reference: 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.

1. Materials needed
    a. Food processor, cutting board, and knife
    b. Plastic beakers
    c. Graduated cylinder
    d. Double Distilled Deionized (DDD) water
    e. Polytron
    f. pH meter and calibration liquids
2. Grind sample into fine particles
3. Weigh 10 g into plastic beaker (2 beakers per treatment)
4. Add 90 ml DDD water to beakers, and mix with Polytron on low speed for 1 minute.
5. Calibrate pH meter with calibration standards (pH values of 4.01, 7.00, and 10.01).
6. Read pH with pH meter while mixture is stirred with the stir bars.
7. Samples were measured in duplicate.

7.8 Color

Reference: Hunt, M. & King, A. (2012). Section X: Laboratory Procedures for Studying Myoglobin and Meat Color. *AMSA Meat Color Measurement Guidelines*, P.59.

1. Materials needed:
   a. Minolta Colorimeter (Konica Minolta Chroma Meter CR-400, Ramsey, NJ)
2. Set colorimeter to the following settings:
   a. Printer→On
   b. Color Space→Off
   c. Protect→On
   d. Auto Average→6
   e. Illuminant→D65
   f. Back Light→Off
   g. Buzzer→On
3. Calibrate colorimeter to the white tile for D65:
   a. Y=93.13
   b. x=0.3164
   c. y=0.3330
4. Read L*, a*, and b* values on both slices per treatment, 3 measurements per slice, for an average of 6 measurements.
5. Calculate a/b ratio where a/b ratio = a*/b*.
6. Calculate Hue angle as HA = [arctangent(b*/a*)]. Larger values are indicative of a less red, more cooked color.
7. Calculate Saturation Index, or chroma, as C = [(a*2+b*2)^1/2]. Larger values are indicative of more saturation of the hue of the sample. This is useful for indicating intensity of the hue of the product.
8. Calculate ΔE as $\Delta E^*_{ab}=\sqrt{[(L_2-L_1)^2+(a_2-a_1)^2+(b_2-b_1)^2]}$. This is useful for showing color differences over time with one value. While various periods of time can be compared depending on your selection of timepoints, in this study, all weeks 2-16 were compared to week 0, to measure the change of color over time.

7.9 Nitrite Determination

Reference: [AOAC] Association of Official Analytical Chemists. 1990. Nitrites in cured meat. Official Method 973.31. Official Methods of Analysis (15th ed.), Arlington, VA, p.938.

Reagents, Standard Curve, and Residual Nitrite

1. The reacting solutions sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride (NED) were prepared.
    a. 0.50 g sulfanilamide was dissolved in 150 ml 15% (v/v) glacial acetic acid and stored in a brown glass bottle.
    b. 0.20 g NED was dissolved in 150 ml 15% (v/v) glacial acetic acid and stored in a brown glass bottle.
2. Nitrite standard solutions were prepared to make a standard curve.
    a. For the stock solution (1000ppm), 0.50 g sodium nitrite was dissolved in approximately 100 ml double-distilled deionized (DDD) water, poured into a 500 ml volumetric flask, and brought to volume with DDD water.
    b. For the intermediate solution (100 ppm), 50 ml of stock solution was added to 450 ml DDD water in a 500 ml volumetric flask.
    c. For the working solution (1 ppm), 5 ml of intermediate solution was added to 495 ml DDD water in a 500 ml volumetric flask.
3. Standard curve solutions were made by adding 0, 10, 20, 30, and 40 ml of working solution to 50 ml volumetric flasks.
    a. To each flask, 2.5 ml of sulfanilamide solution was added, and allowed to react for 5 mins.
    b. 2.5 ml NED solution was added to each flask and 15 min was allowed for color development.
    c. To each flask, DDD water was added to bring the solution to volume.
    d. The sodium nitrite concentrations for these solutions were 0, 0.20, 0.40, 0.60, and 0.80 ppm, respectively.
4. The 0 ppm solution was read as a blank at 540 nm, and the absorbance (A_{540}) of each standard solution was evaluated at 540 nm.
5. Simple linear regression was used to develop a linear formula (y=mx+b) to relate nitrite concentration (x) to A_{540} (y).
6. Residual nitrite concentrations (in duplicate) were determined in the following manner:
    a. 5 g of ground meat sample was placed in a 150 ml plastic beaker.
    b. 50 ml of hot DDD water was added to the beaker, and the mixture was stirred with a glass rod.
    c. The beaker's contents were transferred into a 500 ml volumetric flask, and an additional 300 ml hot water was added to the beaker and then poured into the flask to ensure entire transfer of the 5 g meat sample.
    d. Flasks were corked and placed in an 82°C water bath for 2 h where flasks were uncorked, swirled, and recorked, every 30 minutes.

e. After 2 h, the flasks were stored at room temperature for 2 h to cool to room temperature.
f. After 2 h, the flasks were removed from cold storage and room temperature DDD water was used to bring the solution to a 500 ml volume.
g. Approximately 40 ml of flask solution was filtered through a Whatman No. 1 filter paper cone (GE Healthcare UK Ltd., Buckinghamshire, UK) into a 150 ml plastic beaker.
h. In a test tube, 4 ml of filtrate was added to 0.22 ml of sulfanilamide solution and vortexed.
i. After 5 min, 0.22 ml NED solution was added to the tube, vortexed, and 15 min passed to allow color development.
j. A blank solution of 4.5 ml DDD water, 0.25 ml sulfanilamide solution, and 0.25 ml NED solution was prepared.
k. The blank was measured at 540 nm, and absorbance values at 540 nm (A_{540}) for sample solutions were recorded. The standard curve produced earlier was used to solve the unknown nitrite concentration for each A_{540} value using the equation $x = (y - b)/m$.

7.10 Celery Juice Powder Nitrite Determination

Nitrite equivalent to sodium nitrite concentration was determined using modification of the procedure in Appendix 7.9.

Dilutions of celery juice powder (CP) for nitrite determination were produced in the following manner:

1. 1.0, 1.5, 2.0, or 2.5 g CP was added to 500 ml double-distilled deionized (DDD) water to make 0.2, 0.3, 0.4, or 0.5% (w/v) CP dilutions, respectively.
2. 5 ml of 0.2, 0.3, 0.4, or 0.5% dilutions was combined with 495 ml DDD water to make 0.002, 0.003, 0.004, or 0.005% (v/v) dilutions, respectively.
3. A blank of 4.5 ml DDD water, 0.25 ml sulfanilamide, and 0.25 ml NED was produced.
4. Four sets of 200 µl of 0.2, 0.4, 0.6, and 0.8 ppm sodium nitrite standard solutions (As described in Appendix 7.9) were pipetted into individual tubes.
5. Absorbance values at 540 nm were measured for all solutions using a spectrophotometer.
6. Through simple linear regression, a linear formula was created from the standard sodium nitrite solutions.
7. Absorbance values of the CP dilutions and the standard curve were used to determine the unknown nitrite concentration of the CP.

Equations used to determine the amount of VegStable TM 506 needed to deliver a desired concentration of nitrite based on a meat block of 11.34kg.

$$X \text{ ppm} = \frac{x \text{ mg nitrite}}{1 \text{ kg meat}} \quad (7.1)$$

$$X \text{ ppm} = \frac{y \text{ mg nitrite}}{11.34 \text{ kg meat}} \quad (7.2)$$

$$X \text{ mg/kg} * 11.34 \text{ kg} = y \text{ mg nitrite} \quad (7.3)$$

$$\frac{Y \text{ mg nitrite}}{Z} = \frac{21{,}696.7 \text{ mg nitrite}}{1 \text{ kg CP}} \quad (7.4)$$

$$(y \text{ mg nitrite}) * (1 \text{ kg CP}) = z * (21{,}696.7 \text{ mg nitrite}) \quad (7.5)$$

$$\frac{(y)*(1 \text{ kg CP})}{21{,}696.7} * \frac{1{,}000 \text{ g}}{1 \text{ kg}} = z \quad (7.6)$$

1. In equation (7.1), x represents the desired nitrite concentration (0, 50, 100, 150, or 200) in ppm.
2. Equation (7.2) defines y, the amount of nitrite necessary to achieve the desired nitrite concentration for 11.34 kg of meat, and is further defined in equation (7.3) when the ingoing concentration value is multiplied by the weight of the meat block.

3. Equation (7.4) establishes a ratio between y and an amount of CP (z) to the concentration of nitrite in 1 kg of CP.
4. Cross-multiplication leads to equation (7.5), and z, the amount of CP (in g) necessary for a particular concentration of nitrite for a meat block of 11.34 kg, is solved.

7.11 Sodium Nitrite Curing Agent Calculations

Reference: United States Department of Agriculture. (1995). Processing Inspectors' Calculations Handbook. FSIS Directive 7620.3. Retrieved from http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf.

1. Equations used to calculate the amount of curing agent (6.25% sodium nitrite, 93.75% sodium chloride) for a particular concentration of nitrite based on a meat block of 11.34 kg.

$$\text{a ppm} = \frac{(\text{b lbs. cure mix})*(\%\text{ nitrite in curing agent})*1{,}000{,}000}{\text{Meat block (lbs)}} \quad (7.7)$$

$$(\text{a ppm}) * 11.34\text{ kg} = (\text{b lbs. cure mix}) * 62{,}500 \quad (7.8)$$

$$\text{b g cure mix} = \frac{(\text{a mg})*11.34\text{ kg}}{(1\text{ kg}) * 62{,}500} * \frac{1\text{ lb.}}{2.20\text{ kg}} * \frac{1\text{ g}}{1{,}000\text{ mg}} \quad (7.9)$$

2. Equations (7.7), (7.8), and (7.9) allow b, the amount (g) of curing agent (6.25% sodium nitrite, 93.75% sodium chloride), needed for a, a particular ingoing concentration of nitrite, to be solved.

7.12 Deli Ham Microbial Plate Counts

Plate and peptone buffer preparation:

1. Add 47 g brain heart infusion agar to 1000 ml DDD water. Mix and microwave until boiling (be careful to avoid boiling over).
2. Add peptone buffer to 750 ml DDD water. Mix and microwave until particles are dissolved (be careful to avoid boiling over).
3. Autoclave the agar and peptone buffer.
4. Refrigerate peptone buffer until use.
5. Allow agar to cool at room temperature approximately 1 h, or until bottle can be handled.
6. Pour approximately 10 ml agar into a 10 cm petri dish until all agar is used (1000ml makes approximately 100 plates). Refrigerate plates once agar has set.

Sampling day:

1. Transfer meat sample (two 2mm thick slices per treatment) to a sterile WhirlPak bag in a sterile environment and weigh samples.
2. Add 50 ml peptone buffer to the WhirlPak bag, seal, and place in a paddle blender stomacher for 3 mins.
3. Add 2 ml sample solution to a test tube and perform serial dilutions as necessary (1:1, 1:10, 1:100, 1:1,000, and 1:10,000 were used).
4. Using a spiral plater, plate the sample solution of the appropriate dilution onto the plates (2 plates per sample for aerobic plate count, and 2 plates per sample for anaerobic plate count).
5. Cover and invert plates and store in the appropriate environment for 48 hours. Anaerobic plates will be stored in an anaerobic chamber with Oxygen absorbent packs. The Oxygen absorbers will need to be replaced at 24 hours after counting plates.
6. Count plates at 24 and 48 hours.
7. Convert counts to log CFU/g.