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Effects of Conventional and Alternative Curing Methods on Processed Turkey Quality Traits

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EFFECTS OF CONVENTIONAL AND ALTERNATIVE CURING METHODS ON
PROCESSED TURKEY QUALITY TRAITS

by

Amy Lynn Redfield

A THESIS

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Effects of Conventional and Alternative Curing Methods on Processed Turkey Quality Traits

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This study compared physicochemical and sensory qualities of deli-style turkey breast produced pre-converted celery juice powder (CP; for alternative curing) or sodium nitrite (SN; for conventional curing). Formulas were designed to include 0, 50, 100, 150, and 200 ppm ingoing sodium nitrite or the equivalent from CP or SN, and 3 replicates of products were manufactured. Turkey and curing brines were tumbled, stuffed, and cooked to an internal temperature of 73.9°C. Products were stabilized and sliced into 12 mm slices (physicochemical trait analysis) and 2 mm slices (sensory trait analysis).

Physicochemical traits measured only on d 0 were cured meat pigment (CMP), total meat pigment (TMP), salt concentration, and water activity (factorial design: 2 nitrite sources x 5 nitrite concentrations) and traits measured on d 0, 7, 14, 21, 28, 35, and 42 were color, pH, and residual nitrite (repeated measures factorial design: 2 nitrite sources x 5 nitrite concentrations x 7 time points). Untrained sensory panelists analyzed cured meat color, color acceptability, cured meat flavor, turkey flavor, off-flavor, flavor acceptability, and overall product acceptability for the 50, 100, 150, and 200 SN and CP products.
Products made with 0 ppm nitrite had lower ($P \leq 0.05$) $a^*$ values and cured meat pigment concentrations than products containing nitrite. The interaction of nitrite concentration and source affected ($P \leq 0.05$) $b^*$ values, pH, and residual nitrite. Products made with SN and CP had similar ($P > 0.05$) residual nitrite concentrations for every ingoing nitrite concentration except 200 ppm (200 SN product had more ($P \leq 0.05$) residual nitrite). Residual nitrite was also affected ($P = 0.022$) by the nitrite concentration*day interaction: less ingoing nitrite and more storage time led to less residual nitrite in products. Between d 0 and 42, the decrease in pH was significant ($P \leq 0.05$) but minimal. Untrained sensory panels suggested an overall disliking for 150 or 200 ppm nitrite from CP. Overall, conventionally and alternatively curing were similarly effective for several cured meat traits, but ingoing nitrite from celery juice powder appeared to be limited to 100 ppm (0.46% addition) for acceptable deli-style turkey breast production.
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1. **Introduction**

Since ancient times, meat preservation has incorporated salt for its antimicrobial and flavor-enhancing effects (Binkerd & Kolari, 1975; Honikel, 2008). Over time, certain salts were noticed to impart a particular and desirable flavor and color to meat, perhaps due to inherent impurities in the salt sourced from seawater or mines (Binkerd & Kolari, 1975; Parthasarathy & Bryan, 2012). One impurity of note was “saltpeter” (potassium nitrate), and the understanding that this ingredient, perhaps due to nitrate’s reduction to nitrite, imparted desirable safety and quality traits encouraged its use in cured meat processing (Binkerd & Kolari, 1975; Honikel, 2008). At the turn of the 20th Century, nitric oxide was discovered to be responsible for the pink color of cured meats (Haldane, 1901). However, direct addition of nitric oxide to meat products was not feasible, and processors experienced extensive trial and error as they experimented with nitrate use in products (Cassens, 1990). In the 1920s, experiments that revealed advantages of nitrite over nitrate in meat processing led the Bureau of Animal Industry to allow direct addition of nitrite to meat products in 1925 (Kerr, 1926; Lewis, Vose, & Lowry, Jr., 1925; United States Department of Agriculture [USDA], 1925).

Along with the allowance of nitrite in meat products, restrictions on the use of nitrate and nitrite were established. In 1925, the USDA restricted the levels of ingoing nitrate, nitrite, or the combination of both to 0.25 oz. per 100 lbs. of meat to prevent a finished product from containing more than 200 ppm of nitrite (USDA, 1925). In 1931, the restrictions were modified: 0.25 oz. of sodium nitrite and 2.75 ounces of sodium nitrate could be used per 100 lbs. of meat (Cassens, 1990; Sindelar & Milkowski, 2012).
Eventually, use of sodium nitrate for most cured products fell out of favor when excessive residual nitrite problems from nitrite use were mitigated with reducing agents, and now sodium nitrate is mostly used in products such as dry-cured hams that undergo a long-term curing process (Sebranek & Bacus, 2007a; Sindelar & Milkowski, 2012).

Nitrite or nitrate can be added to processed meats in several ways, and the type of product made and the curing method used will determine how much nitrite or nitrate can be added. Nitrite or nitrate salts and other non-meat ingredients such as salt, phosphate, sugar, and reducing agents, are added to meat through immersing, massaging, pumping, direct addition, or a “dry rub” method (Lechowich et al., 1978; Sebranek & Bacus, 2007a). When sodium nitrite encounters the mildly acidic environment of a meat system, the sodium and nitrite ions dissociate and the nitrite becomes protonated, forming nitrous acid (Honikel, 2008). Two nitrous acid molecules can combine to form a water molecule and dinitrogen trioxide (Pegg & Shahidi, 1997). Dinitrogen trioxide can then dissociate into nitric oxide and nitrogen dioxide (Honikel, 2008). In this pathway, the presence of reducing agents can increase the rate of nitrite reduction to nitric oxide (Fox, 1966), and reducing agents can also allow for the direct reduction of nitrite to nitric oxide (Barbieri, Bergamaschi, Barbieri, & Franceschini, 2013). The nitric oxide can then bind to the iron atom held in myoglobin’s heme ring (Parthasarathy & Bryan, 2012). During thermal processing, the heme ring separates from the protein portion of myoglobin, which partially surrounds the heme ring, to form nitrosylhemochrome, the pigment that imparts a pink color to cured products (Pegg & Shahidi, 1997).
In addition to encouraging cured color development, nitrite is instrumental for developing other traits of cured meats. The flavor of cured meats, which is noticeably different from that of uncured meats, can be attained with as little as 40-50 ppm ingoing nitrite, and greater nitrite concentrations may not increase the intensity of the flavor (Sebranek & Bacus, 2007a). Nitrite also possesses antimicrobial properties. Nitrite has been observed to limit the ability of germinated *Clostridium* spores to divide and to grow, thereby reducing chances for toxin production by *Clostridium* species in anaerobic, shelf-stable cured meat products (Duncan & Foster, 1968a). The inclusion of nitrite may also increase the lag phase of growth for *Listeria monocytogenes*, effectively reducing exponential growth of this pathogen in refrigerated, ready-to-eat products (Duffy, Vanderlinde, & Grau, 1994). Nitrite and nitric oxide can also act as antioxidants in meat products. Igene, Yamauchi, Pearson, & Gray observed decreased oxidation rates when nitrite interacted with lipid membranes (1985), and MacDonald, Gray, & Gibbins suggested nitrite can deter the oxidation of non-heme iron released during cooking (1980a). Due to nitric oxide’s status as a free radical, it can terminate oxidation chains, and when nitric oxide is bound to a heme ring’s iron atom, that atom cannot initiate oxidation (Kanner, Harel, Shagalovich, & Berman, 1984). These antioxidant properties can contribute to the absence of a warmed-over flavor when fully cooked cured products are reheated, and improve consumer acceptability of meat products (Skibsted, 2011; Yun, Shahidi, Rubin, & Diosady, 1987).

Despite the many benefits of using nitrite to cure meat products, nitrite use has been criticized for various reasons. Just as with myoglobin, nitric oxide can combine
with the heme ring of hemoglobin and prevent the attachment and transport of oxygen to body tissues, thereby inducing cyanosis (Archer, 2002). The formation of N-nitroso compounds from ingested nitrite may also increase the risk of certain cancers (Santarelli, Pierre, & Corpet, 2008). Due to these and other health concerns, many consumers have increased their demand for meat products without chemical additives including nitrite and nitrate salts. Traditionally cured products can be made without a curing agent but must be labeled as “Uncured;” “Not Preserved—Keep Refrigerated Below 40°F At All Times” must also be on the label if product safety is not bolstered with an effective pH, water activity level, or thermal processing (Code of Federal Regulations [CFR], 2013a). If products are alternatively cured through means other than direct addition of nitrite or nitrate salts, but still labeled as “Uncured,” the presence of naturally sourced nitrates and nitrites must be disclosed on the products’ label to avoid false or misleading labeling (CFR, 2013b; CFR, 2013c).

A meat or poultry product may be labeled as “natural” if it is minimally processed and contains no artificial ingredients (USDA, 2005). Though nitrite was once argued to be an artificial colorant, its interaction with myoglobin leads to color fixation, so the inclusion of naturally sourced nitrate and nitrite is still allowed in “natural” products (Dryden & Birdsall, 1980). To develop cured characteristics in meat products without using conventional curing agents such as sodium/potassium nitrite and sodium/potassium nitrate, as listed in the Processing Inspectors’ Calculations Handbook (USDA, 1995), different methods and ingredients may be used. Celery juice and other derivatives of leafy vegetables such as Swiss chard, spinach, broccoli, and lettuce are potentially rich
sources of nitrate and may be used as an alternative source of nitrate and nitrite for meat curing (Santamaria, Elia, Serio, & Todaro, 1999). Celery has advantages over other vegetables, however, since its juices and powders do not contribute distracting flavors or colors to meat products (Sebranek & Bacus, 2007a). Natural nitrate sources can be added with other dry ingredients or incorporated into curing brine, and starter cultures with nitrate reducing capabilities can be used to convert nitrate to nitrite (Sebranek & Bacus, 2007b). However, extra time and care are needed to allow bacteria to reduce nitrate into nitrite, and this can extend the curing process. For example, “incubation periods” may extend smokehouse cooking times for small diameter cooked sausages, and uneven or inadequate brine delivery can leave injected products with uncured spots (Sebranek & Bacus, 2007a).

Alternative curing methods using a natural nitrate source and starter culture are not known for delivering equivalent concentrations of ingoing sodium nitrite as those used in conventional curing methods. For example, Sindelar, Cordray, Sebranek, Love, and Ahn (2007) observed that brines containing 46.6 ppm and 81.0 ppm nitrate from a celery juice powder before brine incubation contained only 19.5 and 36.1 ppm residual nitrite, respectively. Further comparisons among products made with incubated brines, non-incubated brines, and sodium nitrite (applied at an ingoing nitrite concentration of 200 ppm) revealed significant differences in products’ residual nitrite, residual nitrate, and color (Sindelar et al., 2007). Also, Canadian bacon made with sodium nitrite contained more residual nitrite over a course of twelve weeks than Canadian bacon made with a celery juice powder and starter culture (Baseler, 2009). Though decreasing the
amount of residual nitrite in cured products is one purpose of alternative curing, the author did concede that such low amounts of residual nitrite in the alternatively cured products may not provide sufficient antibotulinal and antioxidant activity (Baseler, 2009).

Another option for alternative curing involves a pre-converted celery juice powder (PC-CJP) containing a standardized amount of nitrite. This powder can be easily incorporated into formulas similar to those used for conventional curing, so time, labor, and equipment for developing nitrite from nitrate and bacteria are not needed (Krause, Sebranek, Rust, & Mendonca, 2011). The amount of ingoing nitrite can be more accurately calculated with PC-CJP than with a nitrate source due to varying extents of nitrate reduction to nitrite. Comparing the results of simulated curing with sodium nitrite or a nitrate source-starter culture treatment, Sullivan and Sebranek (2012) demonstrated that curing was affected more by nitrite concentration than the rate of nitrite formation, strengthening the potential of PC-CJP as a curing agent. Inclusion of PC-CJP may become the preferred method for alternative curing, as Terns et al. (2011) concluded that the incubation time and amount of nitrate-reducing bacteria for nitrate reduction are proportional to the level of useful nitrite developed during incubation, and converting adequate levels of nitrite could be too costly and inefficient.

The purpose of this study was to compare the physicochemical and sensory attributes of conventionally and alternatively cured deli-style turkey breast formulated to be cured with equivalent ingoing concentrations of sodium nitrite. For conventional curing, a 6.25 percent sodium nitrite, 93.75 percent sodium chloride curing agent and sodium erythorbate (a common reducing agent) were used, and for alternative curing, PC-
CJP (curing agent) and cherry powder (reducing agent) were used. After quantification of the nitrite concentration in the PC-CJP, equivalent ingoing sodium nitrite concentrations could be calculated for both curing methods.

2. Review of Literature

2.1 History of Meat Preservation and Curing

Mankind has valued meat as a rich source of nutrients for millennia, but mankind has also known the ease at which fresh meat can spoil. Preservation of meat for future meals became a necessary function, and this was accomplished in several ways, many of which included salt. In 1600 BC, Jewish people utilized salt from the Dead Sea, and in 900 BC Europeans excavated salt mines (Binkerd & Kolari, 1975). These and other peoples realized that salt dehydrated meat and fish, effectively deterring the growth of microorganisms in the food (Honikel, 2008). The salt that came from the sea or mines often contained impurities like potassium and sodium nitrates and nitrites, and though perhaps the effects of the impure salt, such as a distinct color and flavor, were noticed, they were long from being understood (Binkerd & Kolari, 1975; Parthasarathy & Bryan, 2012).

During the 19th Century, the component of impure salt known as “saltpeter” (potassium nitrate) was hypothesized to impart the characteristic longevity and pink color to cured meats (Honikel, 2008). Saltpeter became a necessary ingredient in recipes for hams, cured sausages, and other meat products, and the process of bacterial conversion of nitrate to nitrite was elucidated in the growing fields of Quality Assurance and Meat
Science (Binkerd & Kolari, 1975). In the late 1800s, Edward Smith noted that when salt alone was used for curing with a dry rub method, meat lost its color, but the inclusion of saltpeter produced a stable red color (Smith, 1873). At the turn of the 20th Century, the pink pigment of cured meat products was discovered to be formed with nitric oxide (Haldane, 1901). However, nitric oxide could not be directly added to meat products, and the addition of sodium nitrate remained the only way to obtain a cured product. When nitrate was intentionally added to meat products in the early 1900s, the meat industry saw a wide gamut of problems: too little nitrate produced dull brown products while too much nitrate resulted in green products (Cassens, 1990). In the early 1920s, researchers carried out experiments that led them to advocate the direct addition of sodium nitrite, since the reliance on nitrite formation from nitrate in a pickling solution had no obvious advantages over direct addition (Kerr, 1926). These findings persuaded the Bureau of Animal Industry to allow the direct addition of nitrite in 1925 (United States Department of Agriculture [USDA], 1925).

The need for regulations on preservatives became obvious when Tomhave (1925) described saltpeter’s positive impact on meat color fixation and preservation but warned it must be used in limited quantities. The USDA responded, and in 1925 it officially restricted the levels of ingoing nitrate, nitrite, or the combination of both to 0.25 oz. per 100 lbs. of meat to prevent a finished product from containing more than 200 ppm sodium nitrite (USDA, 1925). In 1926, use of nitrate was further discouraged when legislation limited the level of nitrate in pickling solutions to 1 percent (Cassens, 1990). Further knowledge of curing chemistry led to a momentous USDA ruling in 1931: a cure
mix applied to 100 lbs. of meat could contain, at the most, 0.25 oz. of sodium nitrite and 2.75 oz. of sodium nitrate (Cassens, 1990; Sindelar & Milkowski, 2012). Eventually, between 1970 and 1980, nitrate use grew rarer still as the benefits of sodium nitrite became well comprehended and cure accelerators, which effectively reduced problems associated with residual nitrite, became more widely used (Sindelar & Milkowski, 2012). Today, nitrate is mostly restricted to products that require a reservoir for nitrite formation throughout a very long curing process (Sebranek & Bacus, 2007a).

### 2.2 Chemistry of Nitrate, Nitrite, and Nitric Oxide

While researching meat color, Haldane (1901) proposed that the “nitre” found in salt used for meat products was reduced to nitrite. Understanding the transformation of nitrate into nitrite and nitrite into nitric oxide began with an early observation of nitrate (NO$_3^-$) being reduced to nitrite (NO$_2^-$) by bacteria equipped with nitrate reductases (Jones, 1933). Some species of nitrate-reducing bacteria studied included species of the genera *Staphylococcus*, *Micrococcus*, *Aerobacter*, *Lactobacillus*, and *Pseudomonas* (Harrison, 1929). These bacteria were suggested to be present on meat processing equipment, in water, and in the meat itself, making nitrate reduction a very likely occurrence (Kerr, 1926). In a rudimentary study by Lewis, Vose, and Lowry, decreases in sodium nitrate and increases in sodium nitrite over time in brines used for ham and beef tongue curing supported this idea of bacterial reduction of nitrate into nitrite (1925). In an acidic environment, nitrous acid (HNO$_2$) can be formed from nitrite and free
hydrogen ions, but with a pKa value of only 3.37, most nitrous acid is dissociated in a meat system with an approximate pH of 5.5 (Honikel, 2008).

During dissociation, two molecules of nitrous acid can form water and the acid’s anhydride, dinitrogen trioxide (N$_2$O$_3$), which Pegg and Shahidi (1997) determined to be the rate-determining step in the production of nitric oxide from nitrite. The anhydride then exists in equilibrium with nitric oxide (NO) and nitrogen dioxide (NO$_2$) (Honikel, 2008). The nitrite ion can also interact with reducing substances such as cysteine and ascorbate to produce NO, so knowing how native and added components in meat interact with nitrite is vital for maximizing nitrite’s positive influences in meat curing (Barbieri, Bergamaschi, Barbieri, & Franceschini, 2013). While too much residual nitrite (nitrite remaining in a cooked meat product) can increase the risk of nitrosamine formation (Sebranek & Bacus, 2007a), this leftover nitrite can serve as a reservoir for NO production and thus reduce discoloration and auto-oxidation in cooked products (Dryden & Birdsal, 1980). Nitric oxide can ultimately combine with myoglobin and influence the color of cured meat (Parthasarathy & Bryan, 2012) or can combine with other proteins such as albumin and myosin (Woolford, Cassens, Greaser, & Sebranek, 1976). A summary of nitrogen-containing compounds’ reactions in the curing process is shown in Figure 2.1.

### 2.3 Nitric Oxide and Myoglobin

In a live animal, the major heme protein is hemoglobin (found in blood), but after exsanguination and removal of most hemoglobin, myoglobin becomes the major heme
protein in meat (Sebranek & Fox, 1985). The porphyrin ring of myoglobin contains an iron atom bound to four nitrogen atoms surrounding it, is bound to a histidine residue, and can be coordinated with a variable ligand (Mancini & Hunt, 2005). Possible ligands include diatomic molecules such as oxygen (O$_2$), carbon monoxide (CO), or nitric oxide (NO). A water molecule bound to a distal histidine residue discourages ligand binding, and displacement of this water must be achieved for a ligand to bind to the iron atom (Quillin, Arduini, Olson, & Phillips, Jr., 1993). Whether the iron atom is in its ferrous (Fe$^{2+}$) or ferric (Fe$^{3+}$) state and to which ligand it is bound will determine the color the myoglobin projects. For most meat products, packaging and display conditions affect meat color, and curing agents further contribute to product coloration.

When nitrite is added to meat, myoglobin (Fe$^{2+}$) becomes oxidized to metmyoglobin (Fe$^{3+}$), thereby changing the meat’s color from red to brown, and myoglobin subsequently reduces nitrite to nitric oxide (Skibsted, 2011). Reducing agents such as erythorbate, NADH, or ascorbate may reduce metmyoglobin to myoglobin, thus increasing the porphyrin ring’s affinity for nitric oxide and allowing for the formation of nitrosylmyoglobin (Dryden & Birdsall, 1980; Skibsted, 2011). In addition, NO can bind to the oxidized protein and form the intermediate nitrosylmetmyoglobin, which may then be reduced by a reducing agent (Dryden & Birdsall, 1980). Myoglobin has an affinity for NO similar to that which it shows for water in its ligand-free, native state, so this interaction forms a stable complex (Olson & Phillips, 1997). Until the meat product is cooked, the nitrosylmyoglobin, in its hexacoordinate form, will stay intact as a ligand-bound protein, and during cooking it will denature and form nitrosylhemochrome with a
pentacoordinate form (Bonnett, Chandra, Charalambides, Sales, & Scourides, 1980; Fox, 1966).

2.4 Effects on Curing from Other Additives and Factors

The purpose of reducing agents is to speed up the conversion of nitrite to nitric oxide, thus accelerating the process of meat curing, encouraging more complete color formation, and maintaining cured color during storage of cooked product (Sebranek, Jackson-Davis, Myers, & Lavieri, 2012). Limits placed on commonly used reducing agents are summarized in Table 2.1. For the production of alternatively cured products labeled “natural” or “organic,” natural sources of these reducing agents can be added (e.g., cherry juice powder used for a source of ascorbic acid) to ensure adequate curing (Terns, Milkowski, Rankin, & Sindelar, 2011).

The reducing agent ascorbic acid can combine with nitrous acid to form an ascorbate-nitric oxide intermediate, which then dissociates into ascorbate and nitric oxide (Fox, Sebranek, & Phillips, 1994). This first step may be bypassed through the direct addition of ascorbate. Fox (1966) observed that when ascorbate was the only reducing agent in a meat system, the reduction of nitrosylmetmyoglobin to nitrosylmyoglobin was constant with respect to time, and the addition of cysteine did not improve the reduction rate. In a study by Reith and Szakaly, the inclusion of ascorbate reduced the presence of metmyoglobin, improved color stability and deterred nitrosylmyoglobin breakdown, and similar effects were seen with ascorbate’s isomer erythorbate (1967). When Bowen, Cerveny, and Beibel investigated ascorbate’s effect on nitrite’s deterrence of Clostridium
*botulinum*, they found that ascorbate did not weaken nitrite’s ability, thus making ascorbate an acceptable cure accelerator to pair with nitrite (1974). Inclusion of reducing agents may also reduce risks to consumer health, as Gray and Dugan observed greater inhibition of nitrosamine formation due to higher concentrations of ascorbic acid added to a meat product model (1975).

A component found in nearly all processed meats is sodium chloride, and the influence of sodium chloride on nitrite has been investigated. Reith and Szakaly (1967) observed that of two solutions containing nitrosylmyoglobin, the solution with sodium chloride had a less intense color than the solution without sodium chloride. When Nordin and others considered control of *Clostridium sporogenes* P.A. 3679, growth rate decreased when salt was increased and pH and nitrite values were unchanged (1975). Salt and nitrite likely worked synergistically in this case since salt by itself can deter the growth of *Clostridium* species only at levels that would make meat products unpalatable (Lechowich, Brown, Beibel, & Somers, 1978). Lee and Cassens (1980) studied the effect of sodium chloride levels on residual nitrite levels and found that a model containing 156 ppm of sodium nitrite and 3.5 percent sodium chloride had the least amount of residual nitrite compared to models with less sodium chloride. Sebranek and Fox suggested that formation of nitrosyl chloride may increase color formation rate and suppress bacterial growth, and that sodium chloride’s presence does not increase nitrosamine formation (Sebranek & Fox, 1985). In fact, sodium chloride has been suggested to inhibit the formation of nitrosamines (Bulushi, Poole, Deeth, & Dykes, 2009).
The pH of a meat product can greatly affect product quality and can impact reactions involving nitrite (Table 2.2). Reducing agents reduce nitrite to nitric oxide at lower pH levels, while interactions of nitric oxide with metmyoglobin or myoglobin appear to be independent of pH (Fox, 1966). Byler, Gosser, and Susi (1983) observed that the nitrosylation of cysteine’s sulphhydryl group was more efficient at lower pH values (3.62 compared to 4.24), though this reaction did occur at 5.50, the typical pH of a cured meat product. In meat models of different pH levels, Reith and Szakaly (1967) noticed that at a higher pH, less metmyoglobin was formed, nitrosylmyoglobin was more stable after exposure to light, and little nitrous acid was present. However, at a lower pH, nitrite reacted faster with myoglobin (Reith & Szakaly, 1967).

Realizing ingredients’ effects on pH is important when deciding which ones to include in a meat product. Though cherry powder, which contains ascorbic acid, can be used for antioxidant applications, it has little impact on pH unlike acidulants such as vinegar and lemon juice (Sebranek & Bacus, 2007a). However, understanding the effect of components on brine pH is more critical than knowing how directly added ingredients affect pH due to the buffering abilities of meat (Sebranek & Bacus, 2007a). The pH of brines for alternatively cured products can also be greatly affected by the incubation time during which nitrate is reduced to nitrite. Sindelar et al. (2007) saw lower pH values in brines with greater incubation times and attributed this to the growth of lactic acid bacteria within the brine. In addition to technical properties of meat products at certain pH levels, other consequences must be considered. The pH can greatly affect the flavor,
microorganism viability, and potential for nitrosamine formation (Lechowich et al., 1978).

2.5 Cured Color

A very distinctive characteristic of cured meats is the pink color which results from nitric oxide’s interaction with myoglobin. Raw meat may be either dark red, bright red, or brown due to the presence of deoxymyoglobin (no ligand; ferrous iron), oxymyoglobin (oxygen ligand; ferrous iron), or metmyoglobin (no ligand; ferric iron), respectively, and none of these pigments remains stable through heat treatment (Reith & Szakaly, 1967). Inclusion of nitrite, subsequent reduction to nitric oxide, and formation of the nitrosylmyoglobin that denatures into a protein portion and NO-porphyrin structure causes cured meats to develop a pink color (Honikel, 2008). The color imparted by this pigment is much more stable than that given by oxymyoglobin (Dryden & Birdsall, 1980), which partially contributes to a longer shelf life for cured meat than for fresh meat. While the pink color from nitrosylmyoglobin may be seen briefly in raw nitrite-treated products, it can easily fade, so cooking the meat to a temperature of 150°F or greater is a vital part of cured meat production to ensure a stable color (Fox, 1966; Hornsey, 1956). The color’s stability may be due to the pentacoordinate arrangement of nitric oxide and the porphyrin ring, which may be trapped in the denatured protein portion of the myoglobin (Bonnett et al., 1980).

While many cured meat products are treated with ingoing sodium nitrite levels of 120-200 ppm, satisfactory color development can still occur at levels as low as 40-50 ppm (Froehlich, Gullett, & Usborne, 1983). Coloration at such low levels allows for the
successful color production in alternatively cured meats when bacterial reduction of nitrate produces ingoing nitrite levels much lower than those used for conventionally cured products (Sebranek & Bacus, 2007b). Despite the reliability of coloration from nitrite, discoloration can occur due to deviations during production. Excessive nitrite addition, or overproduction of nitrite from nitrate via bacteria, may lead to a green pigment either on the surface or inside a cured product (Deibel & Evans, 1957). Also, exposure of cured products to light and oxygen can cause oxidation of the heme group and development of a brownish-gray color (Aberle, Forrest, Gerrard, & Mills, 2001).

2.6 Cured Flavor

As with color, the unique cured meat flavor can develop from ingoing nitrite levels as low as 40-50 ppm (MacDonald, Gray, Kakuda, & Lee, 1980b). In a study by Froehlich, Gullett, and Usborne, untrained panelists rated ham samples with 50 ppm and 150 ppm as equally desirable but more desirable than ham lacking nitrite (1983). The researchers also noted that a trained panel found hams made with greater levels of both salt and nitrite had more intense “cured meat flavor,” suggesting salt may enhance the effect of nitrite on flavor (Froehlich et al., 1983). The antioxidative role of nitrite may contribute to cured flavor, as cooked pork treated with sodium nitrite and other antioxidants (butylated hydroxyanisole and tert-butylhydroquinone) had more acceptable flavor than pork not treated with antioxidants (Yun et al., 1987).

Cured flavor may be attributable to hydrocarbons such as 2,2,4-trimethylhexane, 1,2,4-trimethylcyclohexane, and 1,3-dimethylbenzene, which were detected in cured, but
not uncured, beef and chicken (Ramarathnam, Rubin, & Diosady, 1991). Differences in volatile compound production were also observed for cured and uncured pork, though such compounds might more readily affect aroma than flavor (Ramarathnam, Rubin, & Diosady, 1993). In addition to heated products, flavor differences have been observed between cured and uncured products not thermally processed (Noel, Briand, & Dumont, 1990). However, as different cured products carry distinct flavors, a “cured flavor” is difficult to define, and the chemical definition of a cured flavor has yet to be determined (Noel et al., 1990; Sebranek & Bacus, 2007b).

### 2.7 Antimicrobial Properties

Perhaps the most vital role of nitrite in cured meats is to act as an antimicrobial. Though many possible antimicrobial replacements for nitrite have been tested, none has matched the effectiveness, affordability, safety, and practicality offered by nitrite (Pierson & Smoot, 1982). For example, salt, a traditional ingredient in meat preservation, may inhibit anaerobe spore outgrowth but only at very high levels that would make the product unpalatable (Duncan & Foster, 1968b). Extensive research into how nitrite acts as an antimicrobial has been performed, with several conclusions being reached. Duncan and Foster (1968a) observed nitrite to deter the outgrowth of cells from germinated anaerobe spores and division of newly emerged cells, with nitrite being more effective at pH 6.0 than pH 7.0. O’Leary and Solberg (1976) concluded that at an acidic pH, nitrous acid may interact with and modify a vital cellular component, perhaps through its sulphydryl groups, and decrease the functionality of this component, thereby leading to
the cell’s death. Castellani and Niven (1955) observed a complex being formed from pyruvate/fumarate and sulfhydryl substances and then being made inaccessible by nitrite, which compromised the health of *Staphylococcus aureus* strains.

One of the greatest abilities of nitrite is to suppress the development of *Clostridium* species and the deadly toxins these species can produce. In the anaerobic and neutral or slightly acidic environment of many shelf-stable meat products, *Clostridium botulinum* and *Clostridium perfringens* can form extremely resilient spores which can then germinate in the presence of adequate heat and nutrients (Cammack et al., 1999). However, the presence of nitrite in processed meats can deter growth of both *C. botulinum* and *C. perfringens* (Sebranek et al., 2012). Christiansen (1980) suggested that both adequate ingoing and residual nitrite levels are needed to control *Clostridium* growth, and sufficient depletion of nitrite in a meat product can allow spore outgrowth.

In a study by Bowen, Cerveny, and Beibel (1974), hot dogs made with 0, 15, 30, 50, 100, or 150 ppm sodium nitrite were inoculated with *C. botulinum* and observed for toxicity. Hot dogs made with 50 ppm or less of sodium nitrite developed toxicity after seven days, only one hot dog made with 100 ppm sodium nitrite showed toxicity after fifty-six days, and none of the 150 ppm sodium nitrite hot dogs ever displayed toxicity (Bowen et al., 1974). The interaction of ingoing and residual nitrite with an optimal mix of pH, sodium chloride, heat treatment, and initial bacterial load greatly determine the potential of a meat product to host spore germination and cell development (Archer, 2002).

A bacterium infamous for its presence in ready-to-eat (RTE) meat products is *Listeria monocytogenes*. This pathogen, responsible for listeriosis, can grow at
refrigerated temperatures and a pH range of 4.7-9.2, making growth upon many RTE meat products a major concern, so antimicrobial ingredients and methods must be engaged to prevent or reduce *L. monocytogenes* growth (Cammack et al., 1999). The combination of nitrite with other non-meat ingredients or production methods can effectively reduce the potential for *L. monocytogenes* growth. In a study by Myers et al. (2013), hams inoculated with *L. monocytogenes* were subjected to high hydrostatic pressure (HHP) and nitrite at different levels and combinations. At 0 or 400 MPa HHP, hams with 200 ppm sodium nitrite had less bacterial growth than hams made without sodium nitrite or with 50 or 100 ppm nitrite derived from natural sources, and at 600 MPa HHP, nitrite source did not affect bacterial growth, but the exclusion of nitrate or nitrite allowed for greater bacterial growth (Myers et al., 2013). In a different study, the presence of nitrite (70-140 ppm) in inoculated cooked meat samples (pH 5.90 to 6.20; \(a_w\) 0.960 to 0.993) increased the lag phase of *L. monocytogenes*, and the inclusion of sodium ascorbate increased the lag time even further (Duffy, Vanderlinde, & Grau, 1994). While other preservatives and antimicrobials, such as sodium or potassium lactate and diacetate, can be implemented to control *L. monocytogenes* growth, many of these substances are not allowed in “natural” or organic meat products, so sufficient levels of naturally sourced nitrite, as well as other acceptable ingredients or processes, should be used to retard *L. monocytogenes* growth in these products (Schrader, Cordray, Sebranek, Dickson, & Mendonca, 2010).
2.8 Antioxidative Properties

Another benefit of nitrite inclusion in cured meat products is the antioxidative action of nitrite and nitric oxide. Nitrite can interact with membrane lipids and reduce the extent to which they are oxidized (Igene, Yamauchi, Pearson, & Gray, 1985). Arendt, Skibsted, and Andersen observed in a model system that nitrite lowered rates of metmyoglobin denaturation and suppressed lipid peroxidation, perhaps by “blocking” myoglobin’s entrance to the heme cavity (1997). When lipid oxidation is inhibited, cured meats such as ham, bacon, and sausage are not characterized with the warmed-over flavor associated with re-heated, uncured meat, and sensory panels have suggested traditionally cured meat products outranking the same but uncured products (Skibsted, 2011; Yun, Shahidi, Rubin, & Diosady, 1987). Also, nitrite can deter the oxidation of non-heme iron released during cooking, thereby preventing warmed-over flavor (MacDonald et al., 1980a). Reducing agents, such as sodium ascorbate, found in cured meats can also act synergistically with sodium nitrite to deter oxidation (Yun et al., 1987). Since nitrite can act as a very effective antioxidant at levels permissible under law, its inclusion in cured meat products reduces the need for other antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (Sindelar & Milkowski, 2012). Perhaps this is due to the extreme effectiveness of nitrite, as it can outperform butylated hydroxyanisole and citric acid in terms of lipid oxidation deterrence (MacDonald et al., 1980b).

Due to the free electron in the outermost shell of nitric oxide’s nitrogen atom, nitric oxide is a very reactive radical and can easily combine with other substances
The introduction of nitric oxide into a biological system can reduce lipid peroxidation and mitigate oxidation due to iron (Kelley, Wagner, Buettner, & Burns, 1999). As long as nitric oxide stays bound to the iron of the porphyrin ring, the iron atom cannot act as a catalyst and will not encourage lipid oxidation and off-flavor development (Kanner, Harel, Shagalovich, & Berman, 1984). Nitrosylmyoglobin can also limit fatty acid peroxidation initiated by metmyoglobin (Møller, Sosniecki, & Skibsted, 2002). In addition to coupling with heme, Kanner et al. (1984) noticed that NO can bond with a cysteine-iron complex, interact with radical compounds, and terminate the oxidation chain.

2.9 Nitrite and Nitric Oxide’s Positive Impacts on Health

While the formation of nitric oxide from nitrite allows for curing reactions to occur in meat products, it can also promote physiological well-being within the human body. The NO molecule can perform several functions such as promoting cardiovascular health, maintaining nervous system signaling, destroying pathogenic and cancerous cells, regulating mucosal blood flow, producing mucus, and prohibiting platelet activity (Milkowski, Garg, Coughlin, & Bryan, 2010; Lundberg, Weitzberg, & Gladwin, 2008; Lundberg & Govoni, 2004). The NO molecule was discovered to move quickly from endothelial cells to its targeted muscle cells, thereby making it an effective and powerful messenger (Wells, 2000). Nitric oxide appears to be made from L-arginine using nitric oxide synthase in aerobic conditions and can be made through the nitrate-nitrite-nitric oxide pathway when oxygen is limited (Lundberg et al., 2008). This reduction pathway
may serve as a “backup” system to ensure adequate levels of NO to be present in stressed conditions. For example, Webb et al. observed the NO made from nitrite by the xanthine oxidoreductase enzyme under ischemic conditions to protect human and rat myocardia from ischemia-reperfusion injury (2004). Under hypoxic conditions, nitrite may serve as an alternative electron acceptor for the mitochondrial electron transport chain, and the NO subsequently produced may up-regulate certain genes to combat hypoxic stress (Castello, David, McClure, Crook, & Poyton, 2006).

Also, the amount of nitrate and nitrite available for reduction within the human body can be influenced by food and water intake (Milkowski et al., 2010). Approximately 80 percent of ingested nitrate comes from vegetables, while drinking water provides about 10-15 percent of daily nitrate intake, though this value could be higher in countries with unregulated water supplies (Archer, 2002; Lundberg et al., 2008). After consumption of nitrate, bacteria reduce nitrate to nitrite and secrete the new compound in the saliva; this reduction can account for up to 93.0 percent of daily nitrite consumption for humans (Sindelar & Milkowski, 2012). Of the total amount of nitrite in saliva, about 7 percent is attributed to nitrite from the diet and not endogenously formed from nitrate (Archer, 2002). Nitrite can then act as a substrate in several different reactions that create NO (Lundberg & Govoni, 2004). For example, nitrite may interfere with the attraction of electrons to oxygen, thus reducing the presence of superoxide ions and increasing the presence of NO (Lundberg et al., 2008). Even if NO reverts to nitrite, the regeneration of NO from nitrite may be possible (Lundberg & Govoni, 2004).
Though regulations are in place to limit the ingoing levels of nitrate and nitrite in meat products, these regulations may be overly stringent if a goal exists to minimize dietary intake of nitrate and nitrite (Milkowski et al., 2010). This is because limits set for dietary nitrate intake are surpassed by normal consumption of fruits and vegetables (Hord, Tang, & Bryan, 2009). Whether consumption of meat products containing higher levels of nitrate or nitrite, as found in some other countries, is advantageous for increasing NO formation, or disadvantageous for possibly increasing risks for cyanosis or cancer, is debatable (Keeton, Osburn, Hardin, Bryan, & Longnecker, 2009).

2.10 Use of Nitrite in the Meat Industry

Though nitrite provides unique color, flavor, and safety qualities for cured meats, over-consumption of nitrite can have deleterious consequences. Not long after the legalization of the direct addition of sodium nitrite for meat curing, members of the American Public Health Association expressed concern over the presence of nitrite in meat even at levels below 200 ppm (Ravenel, et al., 1926). Just as nitric oxide formed from nitrite can interact with myoglobin, it can also interact with hemoglobin, and excessive binding between NO and hemoglobin’s heme ring in living tissues can greatly reduce oxygen transport and induce cyanosis (Archer, 2002). Also, ingested nitrite may, with help from heme, form N-nitroso compounds that have been suggested to increase the risk for colorectal cancer (Santarelli, Pierre, & Corpet, 2008). Nitrates present in vegetables can be endogenously reduced to nitrite, thus raising total nitrite consumption (Archer, 2002; Tannenbaum, Fett, Young, Land, & Gruce, 1978). However, whether this
heightened concentration of nitrite raises the chances of cancer or other conditions is questionable. Still, despite the possible risks associated with processed meat consumption, meat products can provide many beneficial nutrients, some of which may combat cancer development (Ferguson, 2010). Therefore, limits on ingoing nitrate and nitrite levels exist to keep consumers safe from overexposure to nitrite as well as to promote product quality.

Meat is cured when adequately exposed to the cure components usually consisting of salt, nitrite and/or nitrate, phosphate, sugar, and a reducing agent (Lechowich et al., 1978). The cure can be added in a dry form or a liquid form known as a “pickle” administered through immersing, massaging, or pumping the meat with the curing ingredients (Lechowich et al., 1978; Sebranek & Bacus, 2007a). Limits for nitrite and nitrate vary by curing method and product due to varied interaction between meat and cure components, but a minimum of 120 ppm ingoing sodium nitrite in all cured products labeled as “Keep Refrigerated” is required to ensure product safety (USDA, 1995). Regulated limits of ingoing nitrate and nitrite for meat products based on product type and curing method are presented in Table 2.3. These levels must be in relation to the initial weight of the meat block since relating them to the finished weight of a product may lead to an excess of nitrate or nitrite within the product (USDA, 1995).

As shown in Table 2.3, bacon is a product with special regulations regarding cure components. Ordinarily, an ingoing concentration of 120 ppm sodium nitrite is required, but ingoing sodium nitrite can be lowered to 100 ppm or 40-80 ppm when safety parameters are incorporated (USDA, 1995). Also, 550 ppm ingoing sodium ascorbate
must be included in bacon formulations to reduce the likelihood of nitrosamine formation (USDA, 1995). The high temperature at which bacon is fried can catalyze the formation of nitrosamines, and the ingoing level of nitrite has been positively correlated with the level of nitrosamine development (Sen, Iyengar, Donaldson, & Panalaks, 1974). Due to the unpredictable extent of nitrate reduction to nitrite, and therefore an uncertain true ingoing level of nitrite, nitrate is not an acceptable cure component for bacon (USDA, 1995).

2.11 Alternative Curing

Despite the benefits of nitrate and nitrite to ensure cured meat product quality and safety, as well as to serve as precursors for nitric oxide, consumers’ demands for products made without conventional curing agents is increasing. This demand began in the late 1960s when the discovery that nitrosamine formation either within cured products during cooking, or in vivo after consumption, triggered distrust of conventional curing since nitrosamines were shown to be carcinogenic (Cassens, 1990). Now, to meet demand for meat products without allegedly “unwholesome” nitrite, variations of traditionally cured meat products can be made without a conventional curing agent but with an alternative curing agent. If nitrite or nitrate is not directly added to a meat or poultry product, but is indirectly added to achieve characteristics of a cured product, the product must be labeled as “Uncured” in a style similar to the product name (Code of Federal Regulations [CFR], 2013a). If a product is claimed as “Uncured,” its label must also bear the statement “Not Preserved—Keep Refrigerated Below 40°F At All Times,” unless certain pH, water
activity, or thermal processing thresholds are met to provide additional safety measures for the product (CFR, 2013a). However, a disclosure on the label regarding the inclusion of naturally sourced nitrates or nitrites in alternatively cured meat and poultry products is required so the term “Uncured” is not false or misleading to consumers (CFR, 2013b; CFR, 2013c).

A meat or poultry product may be labeled as “natural” if no artificial ingredients are included and the product has not endured more than minimal processing (USDA, 2005). Though some have argued that nitrite is an artificial colorant, its interaction with myoglobin allows for color fixation rather than production of an unnatural color, so the inclusion of naturally sourced nitrate and nitrite is still allowed in “natural” products (Dryden & Birdsall, 1980). Recently, demand for alternatively cured meat and poultry products that classify as “natural” has grown, and this might be due to a misconception that conventionally cured products present more health hazards than alternatively cured products (Sebranek et al., 2012). As a result, alternatively cured “natural” meat and poultry products specifically have experienced rapid growth in the market due to consumer willingness to pay a higher price for seemingly “healthier” food (Nath, 2012).

To develop cured characteristics without conventional additives in meat products, different methods and ingredients may be used. Ingredients used in “natural” meat and poultry products must not identify as artificial colors, flavors, or sweeteners; synthetic preservatives; emulsifiers; hydrogenated oils; stabilizers; or other artificial additives (USDA, 2005). An unpublished survey of 56 alternatively cured products by Sindelar revealed listed ingredients not normally found in conventionally cured products: sea salt,
raw sugar, evaporated cane juice, natural flavorings, lactic acid starter culture, and celery juice (Sebranek & Bacus, 2007a). Celery juice and other derivatives of leafy vegetables such as Swiss chard, spinach, broccoli, and lettuce are rich sources of nitrate and may be used for alternative curing (Santamaria, Elia, Serio, & Todaro, 1999). Celery has advantages over other vegetables, however, since its juices and powders contribute less distracting flavors and colors to meat products (Sebranek & Bacus, 2007a). Nitrate alone cannot deliver the cured qualities and safeguards that nitrite imparts, however, and vegetable-sourced nitrate must be converted into nitrite to be effective (Sebranek et al, 2012).

To make alternatively cured products, production methods may be more complex and lengthy than those for conventional curing. Natural nitrate sources can be added with other dry ingredients or incorporated into the brine, and the starter cultures must be treated carefully to maintain their nitrate-reducing power (Sebranek & Bacus, 2007b). Extra time and care are needed to allow bacteria to reduce nitrate into nitrite. For example, “incubation periods” may extend smokehouse cooking times for small diameter cooked sausages, and uneven or inadequate brine delivery can leave injected products with uncured spots (Sebranek & Bacus, 2007a). Another option for alternative curing involves a pre-converted celery juice powder (PC-CJP) containing a standardized amount of nitrite. This PC-CJP can be easily incorporated into formulas similar to those used for conventional curing, so time, labor, and equipment for developing nitrite from nitrate and bacteria are not needed (Krause, Sebranek, Rust, & Mendonca, 2011). Also, the amount of ingoing nitrite can be more accurately calculated with PC-CJP than with a nitrate
source. In a study by Sindelar and others (2007), the going sodium nitrate levels provided by celery juice powder of 69 or 120 ppm would have hardly reached the minimum going nitrite level of 120 ppm required in products labeled “Keep Refrigerated” (USDA, 1995). In this study, the treatments with the lower level of celery juice powder had more desirable sensory traits than the treatments with the greater levels of celery juice powder, suggesting that product safety would need to be compromised for consumer acceptance of “natural” products (Sindelar et al., 2007). Comparing the results of simulated curing with sodium nitrite and a nitrate source-starter culture pair, Sullivan and Sebranek (2012) demonstrated that curing was affected more by nitrite concentration than the rate of nitrite formation. This would support the use of PC-CJP which delivers all possible nitrite immediately upon addition of powder to the other components. Inclusion of PC-CJP may become the preferred method for alternative curing, as Terns et al. (2011) concluded that the incubation time and bacteria level for nitrate reduction are proportional to the level of useful nitrite developed during incubation. Waiting for bacteria to convert nitrate to an acceptable concentration of nitrite would cost companies more resources than would be needed with products cured with pre-converted natural nitrite sources.

### 2.12 Issues with Alternative Curing

Though consumers are steadily embracing alternatively cured meat products, concerns about product quality and safety being inferior to those of conventional products still exist. If, due to “natural” product criteria, going nitrite levels are lower than those
for conventional products, and certain antimicrobials are excluded, pathogenic control within a product may be weakened (Sullivan et al., 2012). Exclusion of sodium nitrite or inadequate concentrations of ingoing nitrite may allow for pathogens, including *C. botulinum* and *C. perfringens* to grow within products (Jackson, Sullivan, Kulchaiyawat, Sebranek, & Dickson, 2011; Sebranek & Bacus, 2007a). Ironically, alternatively cured meats, desired by consumers wishing to avoid the risk of nitrosamine consumption, may be greater sources for nitrosamines: variable rates of nitrite formation when the nitrate source-starter culture method is used may lead to abnormally high levels of residual nitrite within the alternatively cured product (Sebranek & Bacus, 2007a). For example, Jackson et al. observed residual nitrite concentrations in alternatively cured frankfurters, ham, and bacon similar to and greater than concentrations found in conventionally cured frankfurters, ham, and bacon (2011). Residual nitrite may then combine with secondary amines at a low pH and high temperature to form nitrosamines (Honikel, 2008). When conventional ingredients, such as the reductants ascorbic acid and erythorbic acid, and the antioxidant alpha-tocopherol, are not included in “natural” products, the probability for nitrosamine formation could even be enhanced (Parthasarathy & Bryan, 2012). Overall, the lack of conventional ingredients can contribute to reduced shelf-life, which is not desirable for producers or consumers (Sebranek & Bacus, 2007b).

Since investigations in the 1960s and 1970s into the risks of nitrosamines from cured meat consumption, meat processors have worked on reducing the possibility of nitrosamine formation, and as a result, residual nitrite levels in meat are approximately one-fifth of what they were thirty-five years ago (Sebranek & Bacus, 2007a; Cassens,
1997). The chances for nitrosamine formation from conventionally cured meat products are low, as Sindelar and Milkowski point out, since nitrosamines can only form when the combination of secondary amines, adequate nitrite, appropriate pH, and high temperatures occurs, such as when bacon is fried (2012). Milkowski and others even conclude that the amount of nitrite consumed from conventionally cured meats accounts for only a small portion of nitrite intake and presents few adverse health effects to consumers (2010).

2.13 Current Research and Unanswered Questions on Alternative Curing

Despite the fact that the ingoing concentrations of nitrite for conventionally cured meat products have been declared as safe, consumers are currently pushing for the replacement or reduction of nitrite in these products (Weiss, Gibis, Schuh, & Salminen, 2010). One way to cure meat with less nitrite could be through the inclusion of lactate, as McClure, Sebranek, Kim, and Sullivan suggested that lactate, which is normally added to meat products for antimicrobial purposes, could increase the rate of metmyoglobin reduction (2011). Efforts to change production methods merely to lower residual nitrite levels may be somewhat futile however, as a recent survey found few differences in residual nitrite between commercial conventionally cured products and alternatively cured counterparts (Nuñez De González et al., 2012; Sullivan et al., 2012). Due to challenges associated with reworking formulations and processing methods, some value-added meat processors might refrain from experimenting with alternative curing. Difficulties experienced by Sindelar, Terns, Meyn, and Boles (2010) to produce a whole
muscle jerky with characteristics similar to conventionally cured jerky exemplify problems that still exist with current alternative curing technologies. Additional processing steps to increase alternatively cured product safety and quality may be needed, as Horsch et al. (2014) found that adjusting the pH of commercial celery juice powder kept pH’s of alternatively cured hams at lower, more acceptable levels. However, while the pH’s of conventionally and alternatively cured hams could be kept at similar levels, celery juice powder contributed to greater b* values for the alternatively cured hams, affecting a different aspect of product quality (Horsch et al., 2014). To enhance the safety of alternatively cured products, post-processing techniques may be employed. For example, when Myers et al. (2013) applied 600 MPa during high pressure processing to conventionally and alternatively cured hams, similar levels of \textit{L. monocytogenes} retardation were observed for both product types despite source or level of ingoing nitrite.

### 2.14 Summary

Nitrite is a multi-functional, highly regulated ingredient in cured meat products, though some public health concerns have been raised over nitrite consumption. Alternative curing with natural nitrate sources and starter cultures is one method processors employ, though this method has not proved effective at delivering similar levels of nitrite used for conventional curing. With the development of pre-converted celery juice powders, calculating the ingoing level of nitrite for alternatively cured products can be much simpler than when nitrate from natural sources needs to be converted prior to or during product manufacture. Still, natural sources of nitrate/nitrite
contribute other components than curing compounds. For example, Djeri (2010) concluded a pre-converted celery juice powder contained minerals and carbohydrates that could affect products in ways that conventional curing salts do not. Comparing conventionally and alternatively cured products with equivalent amounts of ingoing sodium nitrite will be vital for determining the acceptability of these products for both safety and quality reasons.
Figure 2.1: Reactions involving nitrogen compounds in meat curing. Sodium nitrate (NaNO₃) can be added directly to a meat curing system through a rub or brine (1), though this technique is rarely used today. Nitrate (NO₃⁻) can be reduced (2) to nitrite (NO₂⁻) which can then be added to a meat curing system (3). Sodium nitrite (NaNO₂) may be added to a meat curing system (4) wherein it dissociates into sodium (Na⁺) and NO₂⁻ ions. NO₃⁻ added to a meat curing system is reduced (5) to NO₂⁻. NO₂⁻ combines with a hydrogen ion (H⁺) in the relatively acidic environment (6) to produce nitrous acid (HNO₂), and two HNO₂ molecules can combine (7) to form the acid’s anhydride compound (N₂O₃). The N₂O₃ molecule exists in equilibrium (8) with nitric oxide (NO) and nitrogen dioxide (NO₂). Due to its low pKa value, HNO₂ quickly dissociates into H⁺ and NO₂⁻ (9). Then, NO₂⁻ can be reduced by a reducing agent (HRd) to form NO (10). The NO molecules formed through reactions (8) and (10) can eventually combine with the iron atom of the porphyrin ring of myoglobin (Mb) and influence the color of the cured meat product (11).
Table 2.1: Ingoing limits of select cure accelerants. To speed the transformation of nitrite ion into nitric oxide, cure accelerants are included in formulations under certain limitations (USDA, 1995).

<table>
<thead>
<tr>
<th>Cure accelerator</th>
<th>Ingoing limit</th>
<th>Comment</th>
</tr>
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<tbody>
<tr>
<td>Ascorbic acid</td>
<td>469 ppm</td>
<td></td>
</tr>
<tr>
<td>Erythorbic acid</td>
<td>469 ppm</td>
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<tr>
<td>Sodium ascorbate</td>
<td>547 ppm</td>
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<tr>
<td>Sodium erythorbate</td>
<td>547 ppm</td>
<td></td>
</tr>
<tr>
<td>Citric acid or sodium</td>
<td>Can replace up to ½ of ascorbic or erythorbic acid, or sodium ascorbate or sodium erythorbate</td>
<td>Only allowed in cured, comminuted meat and poultry products</td>
</tr>
<tr>
<td>Glucono δ-lactone</td>
<td>5000 ppm (1% or 10,000 ppm in Genoa salami)</td>
<td>1% or 10,000 ppm in Genoa salami</td>
</tr>
<tr>
<td>Sodium acid pyrophosphate</td>
<td>5000 ppm (alone or in combination with another accelerant)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2: Effects of pH on nitrite reactions. In several studies, the rates and extents of meat curing reactions involving nitrite have been influenced by pH.

<table>
<thead>
<tr>
<th>High pH</th>
<th>Low pH</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slower reduction of NO$_2^-$ to NO</td>
<td>Faster reduction of NO$_2^-$ to NO</td>
<td>Fox, 1966</td>
</tr>
<tr>
<td>Less CysSNO made from NO$_2^-$ and CysSH</td>
<td>More CysSNO made from NO$_2^-$ and CysSH</td>
<td>Byler et al., 1983</td>
</tr>
<tr>
<td>Less MetMb formed</td>
<td>More MetMb formed</td>
<td>Reith &amp; Szakaly, 1967</td>
</tr>
<tr>
<td>NO-Mb more stable after light exposure</td>
<td>NO-Mb less stable after light exposure</td>
<td></td>
</tr>
<tr>
<td>Less HNO$_2$ present</td>
<td>More HNO$_2$ present</td>
<td></td>
</tr>
<tr>
<td>Slower NO$_2^-$ reaction with Mb</td>
<td>Faster NO$_2^-$ reaction with Mb</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3: Ingoing nitrate and sodium or potassium nitrite levels allowed in processed meat products. Special regulations exist for bacon to reduce the likelihood of nitrosamine formation (Sebranek & Bacus, 2007a; USDA, 1995).

<table>
<thead>
<tr>
<th>Meat Product</th>
<th>Curing Method</th>
<th>Ingoing Nitrate (ppm)</th>
<th>Ingoing Nitrite (ppm)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any, excluding bacon</td>
<td>Immersion/massage/pump</td>
<td>700</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Any, excluding bacon</td>
<td>Dry cured</td>
<td>2187</td>
<td>625</td>
<td></td>
</tr>
<tr>
<td>Any, excluding bacon</td>
<td>Direct addition</td>
<td>1718</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td>Bacon, skin off</td>
<td>Massage/pump</td>
<td>Not permitted</td>
<td>120 (NaNO₂)</td>
<td>At least 120 ppm ingoing sodium nitrite needed when other safety measures are not applied.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40-80 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>148 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>123 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>49-99 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immersion</td>
<td>Not permitted</td>
<td>120 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>148 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td>Bacon, skin off</td>
<td>Dry cured</td>
<td>Not permitted</td>
<td>200 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>246 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td>Bacon, skin on</td>
<td>Massage/pump¹</td>
<td>Not permitted</td>
<td>108 (NaNO₂)</td>
<td>At least 120 ppm ingoing sodium nitrite needed when other safety measures are not applied.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36-72 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>133.2 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>110.7 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>44.1-89.1 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Immersion</td>
<td>Not permitted</td>
<td>108 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>133.2 (KNO₂)</td>
<td></td>
</tr>
<tr>
<td>Bacon, skin on</td>
<td>Dry cured</td>
<td>Not permitted</td>
<td>180 (NaNO₂)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>221.4 (KNO₂)</td>
<td></td>
</tr>
</tbody>
</table>

¹ For pumped and massaged bacon, ingoing nitrite and ascorbate/erythorbate concentrations can be within a range of plus or minus 20 percent of the given maximum or minimum concentration.

² Permissible if additional parameters are in place to control quality.

³ Permissible if a sugar and lactic acid starter culture are included in the formula.
3. Materials and Methods

3.1 Celery Juice Powder Nitrite Content Determination

Prior to product manufacture, the concentration of nitrite in VegStable™ 506 (CP; Florida Food Products, Inc., Eustis, FL), a pre-converted celery juice powder and natural nitrite source, was determined. As per the method in Appendix 2a, 0.002%, 0.003%, 0.004%, and 0.005% (w/v) dilutions were made with CP and double-distilled deionized (DDD) water. Nitrite content was determined using an adapted version of the AOAC 973.31 method (AOAC, 1990; see Appendix 1). Briefly, 4 ml of sample solution was reacted with 0.22 ml sulfanilamide and 0.22 ml N-(-1-naphthyl) ethylenediamine dihydrochloride (NED) solutions. Absorbance values of the developed color at a wavelength of 540 nm were compared with absorbance values for standard solutions containing 0.00, 0.20, 0.40, 0.60, and 0.80 ppm nitrite. To compare CP and standard solutions, 200 µl of each reacted CP and standard solution was loaded into a well of a 90-well plate (Nunc™ MicroWell™ Plates with Nunclon™ Delta Surface; ThermoScientific, Waltham, MA) and the absorbance of each solution at 540 nm was measured with a plate reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Winooski, VT). A linear formula developed from the standard solutions was used to calculate the nitrite concentrations for the sample dilutions. From the average concentrations of dilutions, the initial nitrite concentration of CP was determined to be equivalent to 21,617.74 ppm of sodium nitrite. This was comparable to the producer’s
reported value of 22,500 ppm, so the determined value of 21,617.74 ppm was accepted and used to calculate equivalent ingoing sodium nitrite concentrations for alternatively cured products.

To calculate the amount of CP needed for each ingoing sodium nitrite concentration equivalent for an 11.34 kg meat block, the series of equations in Appendix 2b were used. The amount of conventional curing agent (6.25% sodium nitrite, 93.75% sodium chloride) needed for each ingoing sodium nitrite concentration was calculated using the equations found in the Processing Inspectors’ Calculations Handbook (USDA, 1995). These equations are displayed in Appendix 3. The determination of nitrite in the CP and use of equations (7.1) through (7.9) allowed for products to be conventionally and alternatively cured with equivalent ingoing sodium nitrite concentrations.

3.2 Treatments and Product Formulations

To evaluate the effect of ingoing sodium nitrite concentration and source on product quality, ten treatments were designed. Ingoing equivalent sodium nitrite concentrations of 0, 50, 100, 150, and 200 ppm based on meat block weight were sourced from a conventional curing agent (6.25% sodium nitrite, 93.75% sodium chloride; SN) and a pre-converted celery juice powder (VegStable™ 506; CP). 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’s weight: sodium chloride (1.80% w/w), sugar (1.00% w/w), an agglomerated sodium phosphate blend (Brifisol® 85 Instant, Simi Valley, CA; 0.35%
3.3 Turkey Product Manufacture

Frozen boneless, skinless turkey breasts were received from a distributor and held in frozen storage at -20°C until approximately 5 d before product manufacture. The meat was then moved to refrigerated storage and tempered at -1°C. On the day of manufacture, the tempered turkey breasts were ground with a kidney plate (Model 4732, Hobart; Troy, OH) and weighed into ten 11.34 kg batches. Cold water volumes specified for each treatment were mixed with sodium phosphate, salt, sugar, curing agent, and reducing agent (in that order) using an immersion blender (WSB 120VAC; Waring, Torrington, CT) to make curing brines. In a vacuum tumbler (DVTS R2-50; Daniels Food Equipment, Parkers Prairie, MN), 11.34 kg of turkey and a specific treatment brine were combined. The vacuum was pulled to 67.73 kPa, and the meat and brine were tumbled for 90 min. Four tumblers were available for use, and two were designated to tumble SN treatments, and the other two to tumble CP treatments. Production progressed in the order of increasing ingoing nitrite concentration (from 0 to 200 ppm).

After 90 min, the meat mixture was removed from the tumbler, placed in a lug, covered with a plastic protectant sheet, and stored in refrigeration until stuffed into casings. Using a hydraulic piston stuffer (Talsa H31P, Talsabell S.A., Valencia, Spain) the meat mixtures were stuffed into 6M x 106 cm pre-stuck, fibrous casings (Kalle, Wiesbaden, Germany) and clipped. Four to five logs of equal length were made for each treatment. The logs were laid on grid-style screens on a smokehouse truck, and after all
treatments were stuffed, the truck was moved into a commercial-grade smokehouse (Alkar-Rapid Pak; Lodi, WI). The logs were thermally processed to a final internal temperature of 73.9°C to meet Appendix A mandates (USDA, 1999). Product was stabilized overnight in a 0°C cooler to meet FSIS Appendix B mandates (USDA, 1999) for cooked, cured poultry products (100 SN, 150 SN, and 200 SN) and cooked, uncured poultry products (0 SN, 50 SN, 0 CP, 50 CP, 100 CP, 150 CP, and 200 CP). Three replications were manufactured to test sensory traits, and three replications were later manufactured to test physicochemical traits. Formulations for both sets of replications were identical.

On the day after manufacture, the fibrous casings were removed and 12 mm-thick slices were taken from two logs within one treatment (SE 12D manual slicer; Bizerba, Piscataway, NJ). Two slices (one from each log) were placed side by side in a 3 mil, 20.32 cm x 38.10 cm vacuum pouch (Ultravac Solutions, LLC, Kansas City, MO), vacuum sealed (Model #C500, Sepp Haggenmuller GmbH and Co. KG, Wolfertschwenden, Germany), and stored at 0°C until physicochemical trait analysis.

Samples used for sensory analysis—thirty 2 mm-thick slices—were taken from each of two turkey logs for all treatments except 0 SN and 0 CP. Slices were vacuum-packaged in 3 mil vacuum pouches and kept in dark storage at 0°C until sensory analysis.

### 3.4 Physicochemical Trait Analysis

Immediately after products were sliced and packaged, d 0 analyses for objective color (L*, a*, b*), cured meat pigment (CMP), total meat pigment (TMP), water activity
(a<sub>w</sub>), salt, residual nitrite, and pH were performed. Color, residual nitrite, and pH were further tested on d 7, 14, 21, 28, 35, and 42. After color was measured, the packages were opened and the slices were removed. On d 0, the slices were ground in a dark room to protect the integrity of meat pigments. Slices were ground for 20 s in a food processor (Handy Chopper; Black & Decker, Shelton, CT) to attain a fine particulate composition. On d 7, 14, 21, 28, 35, and 42, samples were ground in a lit room.

### 3.4.1 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 illuminant. The calibration plate was read through a 3 mil vacuum pouch since the slices were still within the packaging during the color measurement. The color of three locations characterized by a consistent and true color (i.e., no blood splashes or other colorations not caused by curing) on each of two slices was measured, and the resulting measurements were averaged to calculate the color values for each treatment. Color was measured on d 0, 7, 13, 21, 28, 35, and 42.

### 3.4.2 Cured Meat Pigment (CMP)

Cured meat pigment (CMP) was measured using acetone extraction (Hornsey, 1956) with modifications described by Sindelar et al. (2007). In a 125 ml Erlenmeyer flask, 10 g of sample, 40 ml acetone, and 3 ml DDD water were combined and homogenized (PT 10-35, Kinematica, Bohemia, NY) at medium speed for one minute.
The solution was then filtered through Q2 filter paper (Fisher Scientific, Pittsburgh, PA) into a glass beaker wrapped in foil. While the sample was being filtered, the flask was swirled and uncorked, and more contents of the flask were poured into the filter. This was repeated until all flask contents had been poured into the filter. Before and after being poured, the Erlenmeyer flask was corked with a rubber stopper to deter acetone from evaporating from the solution.

The resulting filtrate was poured through another Q2 filter into a foil-wrapped glass beaker to ensure a clear filtrate. The beaker was covered in Parafilm (American National Can, Chicago, IL) until the filtrate was measured to protect against acetone evaporation. A blank solution of 80% DDD water and 20% acetone, and filtrates were measured at 540 nm using a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) with a sipper flow cell. Filtrate absorbance values were multiplied by 290 to determine the concentration of nitrosylhemochrome in ppm. Measurements for CMP were made in duplicate and only on d 0.

3.4.3 Total Meat Pigment (TMP)

Total meat pigment (TMP) was measured using acetone extraction (Hornsey, 1956) with modifications described by Sindelar et al. (2007). In a flask, 10 g of sample, 40 ml of acetone, 2 ml DDD water, and 1 ml concentrated HCl were combined and homogenized (PT 10-35, Kinematica, Bohemia, NY) at medium speed for one minute. The flask was then corked with a rubber stopper and set aside for one hour to allow conversion of meat pigments to one form. After one hour, the solution was poured into a
cone of Q2 filter paper (Fisher Scientific, Pittsburgh, PA) and into another foil-wrapped, 125 ml Erlenmeyer flask, and the original flask was recorked. While the sample filtered, the original flask was swirled and uncorked, and more solution was poured into the filter until all contents of the flask were poured. Before and after being poured, the original flask remained corked to protect the solution from acetone evaporation.

A cork was placed in the new flask to protect the filtrate from acetone evaporation. A blank solution of 80% acetone, 18% DDD water, and 2% concentrated HCl, and filtrates were measured at 640 nm and 512 nm using a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) with a sipper flow cell. Filtrate absorbance values at 640 nm ($A_{640}$) were multiplied by 680 to calculate TMP in ppm. Then, the 512 nm absorbance value ($A_{512}$) was divided by its corresponding $A_{640}$ value to determine the extent of pigment conversion. The desired $A_{512}/A_{640}$ value was less than or equal to 1.90. Measurements were made in duplicate and only on d 0.

### 3.4.4 Water Activity

An AquaLab 4TE water activity ($a_w$) meter (Decagon Devices, Inc., Pullman, WA) was calibrated using standards with $a_w$ values of 0.984 and 0.760 (Decagon Devices, Inc., Pullman, WA). For each treatment, ground meat was packed into disposable sample containers (Decagon Devices, Inc., Pullman, WA) so the bottom of the containers were covered but sample material did not fill the containers more than half full. Approximately 5 g of ground meat were needed. Samples were then read using the
water activity meter. Single measurements were taken for each treatment and only on d 0.

3.4.5 Sodium Chloride

The procedure followed the instructions written by Sebranek, Lonergan, King-Brink, Larson, and Beermann (2001). To a 150 ml plastic beaker, 10 g of ground meat was added. Next, 90 ml of DDD water boiled in an electric kettle (KT-1800 cordless electric kettle; Brentwood Appliances, Vernon, CA) was added to the beaker. The solution was stirred with a glass rod for 30 seconds, left undisturbed for 60 seconds, and stirred again for 30 seconds. A circular Whatman No. 1 filter paper (GE Healthcare UK Ltd., Buckinghamshire, UK) was folded into a cone and placed in the beaker. Once the meat and water solution had permeated the filter, a Quantab high chloride range titration strip (Hach Company, Loveland, CO) was placed so the end was submerged in the filtrate. When the yellow indicator bar at the top of the Quantab strip turned blue, the measured chloride concentration was converted to a sodium chloride concentration as per instructions on the bottle containing the Quantab strips. Measurements were made in duplicate and only on d 0.

3.4.6 pH

For each treatment, 10 g of ground meat was added to a 150 ml plastic beaker, and 90 ml DDD water was added to the beaker. The solution was homogenized (PT 10-35 GT, Kinematica, Inc., Bohemia, NY) at 23,000 RPM for 30 seconds, and then a
magnetic stir bar was placed in the beaker. A stir plate (Thermolyne® Cimarec®-top stirring hotplate; Barnstead Thermolyne, Dubuque, IA) was used along with the stir bar to allow for continuous motion of the solution while the pH was read with a pH meter (Orion 410Aplus; ThermoFisher Scientific, Waltham, MA) which had been calibrated with standards of pH 4.01, 7.00, and 10.01 (Orion 910104, 910107, 910110, respectively, ThermoScientific, Waltham, MA). Measurements for pH were made in duplicate on d 0, 7, 14, 21, 28, 35, and 42.

3.4.7 Residual Nitrite

Residual nitrite was measured using a method adapted from the AOAC 973.31 method (AOAC, 1990); production of the reagents, nitrite standard solutions, and standard curve for the assay is described in Appendix 1. For the treatment samples, 5 g of ground meat was measured into a 150 ml plastic beaker. Then, 50 ml of DDD water boiled in an electric kettle (KT-1800 cordless electric kettle; Brentwood Appliances, Vernon, CA) was added to the beaker. The solution was stirred with a glass rod and poured into a 500 ml volumetric flask. An additional 300 ml of boiling DDD water was used to transfer the entirety of the meat in the plastic beaker to the flask, and then the flask was corked with a rubber stopper. Duplicate flask solutions were made for each treatment. Once flask solutions had been prepared for every treatment, the flasks were placed in 82°C water baths for 2 hours. Every 30 min, the flasks were uncorked, swirled, and recorked to avoid pressure buildup within the flasks. After 2 h, the flasks were
removed from the baths and stored at 2°C for approximately 2.5 h to cool the solutions to room temperature.

Once the solutions were cooled and removed from cold storage, room temperature DDD water was added to the flasks to bring the solutions to volume. The flasks were then inverted to attain a homogeneous solution, and then approximately 40 ml of solution was poured through a Whatman No. 1 filter paper cone (GE Healthcare UK Ltd., Buckinghamshire, UK) into 150 ml plastic beakers. Next, 4 ml of filtrate was added to a test tube followed by 0.22 ml of sulfanilamide solution and a 5 min waiting period. Then, 0.22 ml NED solution was added to the test tube, and a 15 min waiting period followed to allow for the development of an azo dye. A blank solution of 4.5 ml DDD water, 0.25 ml sulfanilamide, and 0.25 ml NED was also made, and this was measured at 540 nm using a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) with a sipper flow cell. Assay solutions were then measured at 540 nm with the spectrophotometer being flushed with DDD water between the sets of SN and CP treatments. The linear formula developed from the standard curve was used to determine residual nitrite concentration from absorbance (A_{540}) values. Measurements were made in quadruplicate (two test tubes per flask; two flasks per treatment) on d 0, 7, 14, 21, 28, 35, and 42.

3.5 Sensory Trait Analysis

Sensory panels were conducted on three days with one replication evaluated per day. Only cured products (50, 100, 150, and 200 SN, and 50, 100, 150, and 200 CP)
were analyzed. Panels were conducted within 35 to 39 days after product manufacture. Slices were served on white paper plates marked with a three-digit blinding code representing the treatment. Each panelist received four treatments representing each source of nitrite for two nitrite concentrations (Figure 5.3) in a randomized order. The six possible combinations of samples were served in groups to ensure similar numbers of evaluation for each treatment.

Six untrained consumer sensory panels were conducted at the University of Nebraska-Lincoln Food Science and Technology Department (mornings of panel days) and Animal Science Department (afternoons of panel days) sensory laboratories and complied with guidelines set out by the University of Nebraska-Lincoln Institutional Review Board. University faculty, students, and staff at least 19 years of age could voluntarily participate after signing a consent form. Panelists were in a room separated from the sample preparation area and received samples through a sliding door or hood. Panelists were served samples one at a time and were encouraged to cleanse their palates with distilled water and unsalted crackers between samples.

Unstructured line scales (150.75 mm long with vertical anchors at each end) were used for panelists to evaluate 1) cured meat color (“absent (white)” to “intense (pink)”), 2) color acceptability (“unacceptable” to “acceptable,” with instructions to ignore dark spots attributed to blood splashing), 3) cured meat (“ham-like”) flavor \(^1\) (“absent” to “intense”), 4) turkey flavor (“absent” to “intense”), 5) off-flavor (“absent” to “intense”).

\(^1\) Cured meat flavor may result from multiple effects from curing components, and is difficult to describe precisely (Noel, Briand, & Dumont, 1990). Since most ham consumed is conventionally cured and has a very different flavor from uncured pork, the description of “ham-like” was added to “cured meat flavor” to give context for the panelists unfamiliar with the concept of cured meat.
6) flavor acceptability ("unacceptable" to "acceptable"), and 7) overall product acceptability ("unacceptable" to "acceptable"). Panelists wrote the 3-digit code for each treatment at the top of the questionnaire. After visually evaluating and tasting the product, panelists placed a mark on the 150.75 mm line according to their perceptions for each sensory trait.

To measure the distance from the start of the evaluation line to the mark placed by the panelist, a Westward® 6 in/150 mm electronic caliper (Grainger International Inc., Lake Forest, IL) was used, and distances were recorded in millimeters. Each questionnaire was numbered to represent each panelist. A total of 196 panelists submitted acceptable evaluations of the products, though not every panelist gave a score for every parameter on the questionnaire.

3.6 Statistical Analysis

Physicochemical trait data were analyzed according to a factorial design (2 nitrite sources x 5 nitrite concentrations) for traits only measured on d 0 (total meat pigment, cured meat pigment, salt, and water activity), and according to a repeated measures multifactorial design (2 nitrite sources x 5 nitrite concentrations x 7 test days) for traits measured on d 0, 7, 14, 21, 28, 35, and 42 (pH, objective color, and residual nitrite). Residual nitrite data was also analyzed with only the 50, 100, 150, and 200 ppm products considered (2 nitrite sources x 4 nitrite concentrations x 7 test days). The data for total and cured meat pigment, salt, and water activity were analyzed using analysis of variance (ANOVA) through PROC GLIMMIX on SAS 9.2 (SAS Institute, Inc., Cary, NC).
Significant differences between means from main effects or interactions \((P \leq 0.05)\) were separated through LSMEANS and DIF LINES functions. Data for pH, objective color, and residual nitrite were analyzed using ANOVA through PROC MIXED on SAS 9.2 (SAS Institute, Inc., Cary, NC). The LSMEANS function was used to separate significantly different means \((P \leq 0.05)\) from main effects or interactions.

Sensory trait data were analyzed using an incomplete block design: 196 blocks (total panelists from 6 panels) x 8 treatments x 4 treatments per block. Panelist was considered a random blocking factor. Through ANOVA and the PROC GLIMMIX procedure on SAS 9.2 (SAS Institute, Inc., Cary, NC) significance of effects was determined. Nitrite source and concentration were the main effects, and source*concentration was the possible interaction effect. When significantly different means \((P \leq 0.05)\) from main effects or interactions appeared, LSMEANS and DIF LINES functions were used to separate the means.

4. Literature Cited


5. Effects of conventional and alternative curing methods on processed turkey quality traits

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5.1 Abstract

Deli-style turkey breast cured with either a pre-converted celery juice powder (CP) or sodium nitrite (SN). Products were manufactured with ingoing sodium nitrite concentrations of 0, 50, 100, 150, and 200 ppm from SN or equivalent concentrations from CP, and 3 replicates of products were manufactured. Cured meat pigment (CMP), total meat pigment (TMP), salt, and water activity were measured on d 0, and color, pH, and residual nitrite were measured on d 0, 7, 14, 21, 28, 35, and 42. Untrained panelists evaluated sensory traits of 50, 100, 150, and 200 ppm products. Products made with 0 ppm nitrite had lower \((P \leq 0.05)\) a* values and CMP concentrations. The interaction of nitrite concentration and source affected \((P \leq 0.05)\) b* values, pH, and residual nitrite. Less ingoing nitrite and more time in storage resulted in less \((P \leq 0.05)\) residual nitrite in products. Decreases in pH over time were significant \((P \leq 0.05)\) but minor. Sensory results suggested an overall disliking for products made with 150 or 200 ppm nitrite from CP. Overall, conventionally and alternatively cured products were similar for several traits, but since there was less acceptance of products made with 150 and 200 ppm nitrite from CP, inclusion of ingoing nitrite from celery juice powder was apparently limited to 100 ppm nitrite (0.46% addition) for acceptable deli-style turkey breast.

5.2 Introduction

Since ancient times, meat preservation has incorporated salt for its antimicrobial and flavor-enhancing effects (Binkerd & Kolari, 1975; Honikel, 2008). Over time, certain salts were noticed to impart a particular flavor and color to meat, perhaps due to
inherent impurities in the salt sourced from seawater or mines (Binkerd & Kolari, 1975; Parthasarathy & Bryan, 2012). One impurity of note was “saltpeter” (potassium nitrate), and the understanding that this ingredient, perhaps due to nitrate’s reduction to nitrite, imparted desirable safety and quality traits encouraged its use in cured meat processing (Binkerd & Kolari, 1975; Honikel, 2008). At the turn of the 20th Century, nitric oxide was discovered to be responsible for the pink color of cured meats (Haldane, 1901). However, direct addition of nitric oxide to meat products was not feasible, and processors experienced extensive trial and error as they experimented with nitrate use in products (Cassens, 1990). In the 1920s, experiments that revealed advantages with the direct addition of nitrite led the Bureau of Animal Industry to allow the direct addition of nitrite to meat products in 1925 (Kerr, 1926; Lewis, Vose, & Lowry, Jr., 1925; United States Department of Agriculture [USDA], 1925).

With the allowance of nitrite in meat products, restrictions on the use of nitrate and nitrite were established. In 1925, the USDA restricted concentrations of ingoing nitrate, nitrite, or the combination of both to 156 ppm (0.25 oz. per 100 lbs. meat) to prevent a finished product from containing more than 200 ppm of nitrite (USDA, 1925). These restrictions were further refined in 1931, to 0.25 oz. of sodium nitrite and 2.75 oz. of sodium nitrate could be used per 100 lbs. of meat (Cassens, 1990; Sindelar & Milkowski, 2012). Eventually, use of sodium nitrate fell out of favor for most cured products and is mostly used today for products such as dry-cured hams that undergo a long-term curing process (Sebranek & Bacus, 2007; Sindelar & Milkowski, 2012).
Nitrite or nitrate can be added to processed meats in several ways, and the type of product made and the curing method used will determine how much nitrite or nitrate can be added. Through a series of reactions, nitric oxide is produced from the curing agents, and nitric oxide combines with myoglobin to produce a pink color characteristic of cured meats (Haldane, 1901). In addition to encouraging cured color development, nitrite contributes to cured meat flavor through as little as 40-50 ppm ingoing nitrite (Sebranek & Bacus, 2007). Nitrite also inhibits the outgrowth of germinated Clostridium botulinum spores (Duncan & Foster, 1968) and may retard growth of Listeria monocytogenes (Duffy, Vanderlinde, & Grau, 1994). Nitrite can also deter oxidation in products, thereby maintaining product quality (Igene, Yamauchi, Pearson, & Gray, 1985; MacDonald, Gray, & Gibbins, 1980).

Despite the many benefits nitrite provides for cured meat products, nitrite use has been criticized for various reasons. Just as with myoglobin, nitric oxide can combine with the heme ring of hemoglobin in living tissues, prevent the attachment and transport of oxygen throughout the body, and thereby induce cyanosis (Archer, 2002). The formation of N-nitroso compounds from ingested nitrite may also increase the risk of certain cancers (Santarelli, Pierre, & Corpet, 2008). Due to these and other health concerns, many consumers have increased their demand for meat products without chemical additives including nitrite and nitrate salts. In response, the USDA allowed for traditionally cured products to be made without conventional curing agents. These products must be labeled as “Uncured;” “Not Preserved—Keep Refrigerated Below 40°F At All Times” must also be on the label if product safety is not strengthened by an
effective pH, water activity, or thermal processing (Code of Federal Regulations [CFR], 2013a).

While complying with labeling policies, processors began to cure products without conventional curing agents but with ingredients that serve as alternative sources of nitrate or nitrite. Celery juice and other derivatives of leafy vegetables such as Swiss chard, spinach, broccoli, and lettuce are rich sources of nitrate and may serve as alternative sources of nitrate for meat curing (Santamaria, Elia, Serio, & Todaro, 1999). Celery has advantages over other vegetables, however, since its juices and powders contribute less distracting flavors and colors to meat products (Sebranek & Bacus, 2007). Nitrate from natural sources must first be reduced to nitrite by bacteria with nitrate reductase enzymes, or no cured meat characteristics will develop. Reduction of nitrate can occur outside (prior to processing) or within the meat product (during processing).

Another option for alternative curing involves a pre-converted celery juice powder (PC-CJP) containing a standardized amount of nitrite. Bacterial reduction of naturally sourced nitrate to nitrite occurs during the production of PC-CJP, removing the reduction step for the meat processor. This powder can be easily incorporated into formulas similar to those used for conventional curing, so time, labor, and equipment for developing nitrite from nitrate and bacteria are not needed (Krause, Sebranek, Rust, & Mendonca, 2011). Also, the amount of ingoing nitrite can be more accurately calculated with PC-CJP than with a natural nitrate source. If a product is alternatively cured through means other than direct addition of nitrite or nitrate salts, but still labeled as “Uncured,”
the presence of naturally sourced nitrates and nitrites must be disclosed on the product’s label to avoid false or misleading labeling (CFR, 2013b; CFR, 2013c).

For many studies that have compared experimental, alternatively and traditionally cured products, the amounts of ingoing nitrite are often dissimilar due to the curing method. Often, the ingoing nitrite sourced from sodium nitrite is added at regulatory limits whereas ingoing nitrite from natural sources is added at much lower concentrations to reflect commercial processing techniques. The purpose of this study was to compare the physicochemical and sensory attributes of conventionally and alternatively cured deli-style turkey breast formulated with equivalent ingoing concentrations of sodium nitrite.

5.3 Materials and Methods

5.3.1 Celery juice powder nitrite content determination

Prior to manufacture, the concentration of nitrite in VegStable™ 506 (CP; Florida Food Products, Inc., Eustis, FL), a PC-CJP, was determined according to the method in Appendix 2a. First, sample 0.002%, 0.003%, 0.004%, and 0.005% (w/v) dilutions were made with CP and double-distilled deionized (DDD) water. Nitrite content was then determined using an adapted version of the AOAC 973.31 method (AOAC, 1990). Briefly, 4 ml of sample solution was reacted with 0.22 ml sulfanilamide and 0.22 ml N-(-1-naphthyl) ethylenediamine dihydrochloride (NED) solutions. Absorbance values of the developed color at a wavelength of 540 nm were compared with absorbance values for standard solutions containing 0.00, 0.20, 0.40, 0.60, and 0.80 ppm nitrite. To do this, 200 µl of each reacted sample and standard solution was loaded into a well of a 90-well plate
(Nunc™ MicroWell™ Plates with Nunclon™ Delta Surface; ThermoScientific, Waltham, MA) and the absorbance of each solution at 540 nm was measured with a plate reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Winooski, VT). The nitrite concentration of CP was determined to be equivalent to 21,617.74 ppm of sodium nitrite and was comparable to the producer’s reported value of 22,500 ppm. The calculated value was used to formulate products with 0, 50, 100, 150, and 200 ppm ingoing sodium nitrite equivalents from CP.

5.3.2 Treatments and product formulations

To evaluate the effect of ingoing sodium nitrite concentration and source on product quality, ten treatments were designed. Ingoing sodium nitrite concentration equivalents of 0, 50, 100, 150, and 200 ppm based on meat block weight came from both a conventional curing agent (SN; 6.25% sodium nitrite, 93.75% sodium chloride) and CP. 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’s weight: sodium chloride (1.80% w/w), sugar (1.00% w/w), an agglomerated sodium phosphate blend (Brifisol® 85 Instant, Simi Valley, CA, 0.35% w/w), and water, curing agents, and reducing agents of varying amounts listed in Table 5.1.

5.3.3 Product manufacture

Product manufacture was replicated three times to test physicochemical traits. An additional three replications of product were manufactured to test sensory traits. Product formulation and manufacturing method were identical for both sets of three replications.
Frozen boneless, skinless turkey breasts were received from a distributor and held in frozen storage at -20°C until 5 d before product manufacture. The meat was then moved to refrigerated storage and tempered at -1°C. On the day of manufacture, the tempered turkey breasts were ground with a kidney plate (Model 4732, Hobart; Troy, OH) and weighed into ten 11.34 kg batches. Curing brines were made according to formulations in Table 5.1. In a vacuum tumbler (DVTS R2-50; Daniels Food Equipment, Parkers Prairie, MN), 11.34 kg of meat and a specific treatment brine were combined. The vacuum was pulled to 67.73 kPa, and the meat and brine were tumbled for 90 min.

After 90 min, the meat mixture was removed from the tumbler, placed in a lug, covered with a plastic protectant sheet, and stored at -1°C until stuffed. Using a hydraulic piston stuffer (Talsa H31P, Talsabell S.A., Valencia, Spain), the meat mixtures were stuffed into 6M x 106 cm pre-stuck, fibrous casings (Kalle, Wiesbaden, Germany) and clipped. Four to five logs of equal length were made for each treatment. The logs were thermally processed in a smokehouse (Alkar-Rapid Pak; Lodi, WI) using the program shown in Table 5.2 to a final internal temperature of 73.9°C in accordance with Appendix A (USDA, 1999). Product was stabilized overnight in a 0°C cooler to meet FSIS Appendix B mandates (USDA, 1999) for cooked, cured poultry products (100 SN, 150 SN, and 200 SN) and cooked, uncured poultry products (0 SN, 50 SN, 0 CP, 50 CP, 100 CP, 150 CP, and 200 CP).

On the day after manufacture, the fibrous casings were removed and 12 mm-thick slices were taken from two logs within one treatment (SE 12D manual slicer; Bizerba, Piscataway, NJ). Two slices (one from each log) were placed side by side in a 3 mil,
20.32 cm x 38.10 cm vacuum pouch (Ultravac Solutions, LLC, Kansas City, MO), vacuum sealed (Model #C500, Sepp Haggenmuller GmbH and Co. KG, Wolfertschwenden, Germany) and stored at 0°C until physicochemical trait analysis. Samples used for sensory analysis—thirty 2 mm-thick slices—were taken from each of two turkey logs for all treatments except 0 SN and 0 CP. Slices were vacuum-packaged in 3 mil vacuum pouches and kept in dark storage at 0°C until sensory analysis.

5.3.4 Physicochemical trait analysis

5.3.4.1 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. The calibration plate was read through a vacuum pouch identical to the type used for packaging slices since the samples were still within the pouch during the color measurement. Three locations on each of two slices per treatment were evaluated and the average reading of six locations was recorded. Color was measured on d 0, 7, 14, 21, 28, 35, and 42. After color was measured, sample slices were finely chopped in a food processor (Handy Chopper; Black & Decker, Shelton, CT).

5.3.4.2 Cured Meat Pigment (CMP)

Cured meat pigment (CMP) was measured using acetone extraction (Hornsey, 1956) with modifications described by Sindelar, Cordray, Olson, Sebranek, and Love (2007). Throughout the procedure, glassware was wrapped in aluminum foil to limit
exposure to light. In a flask, 10 g of sample, 40 ml acetone and 3 ml DDD water were combined and homogenized (PT 10-35, Kinematica, Bohemia, NY) at medium speed for one minute and filtered twice through Q2 filter paper (Fisher Scientific, Pittsburgh, PA).

A blank solution of 80% DDD water and 20% acetone, and sample filtrates were measured at 540 nm using a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) equipped with a sipper flow cell. Absorbance values were multiplied by 290 to determine the concentration of nitrosylhemochrome in ppm. Measurements for CMP were made in duplicate and only on d 0.

### 5.3.4.3 Total Meat Pigment (TMP)

Total meat pigment (TMP) was measured using acetone extraction (Hornsey, 1956) with modifications described by Sindelar et al (2007). Throughout the procedure, glassware was wrapped in aluminum foil to avoid exposure to light. In a flask, 10 g of sample, 40 ml of acetone, 2 ml DDD water, and 1 ml concentrated HCl were combined and homogenized (PT 10-35, Kinematica, Bohemia, NY) at medium speed for one minute. The flask was then corked with a rubber stopper and set aside for one hour to allow conversion of meat pigments to one form. After one hour, the solution was filtered through Q2 filter paper (Fisher Scientific, Pittsburgh, PA).

A blank solution of 80% acetone, 18% DDD water, and 2% concentrated HCl, and the sample filtrates were measured at 640 nm and 512 nm using a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) equipped with a sipper flow cell. The absorbance value at 640 nm ($A_{640}$) was multiplied by 680 to calculate
TMP in ppm. Then, the 512 nm absorbance value ($A_{512}$) was divided by its corresponding $A_{640}$ value to determine the extent of pigment conversion. The desired $A_{512}/A_{640}$ value was less than or equal to 1.90. Measurements were made in duplicate and only on d 0.

5.3.4.4 Water Activity

Water activity ($a_w$) was measured according to the manufacturer’s protocol for the AquaLab 4TE water activity meter (Decagon Devices, Inc., Pullman, WA). Only one measurement was taken for each treatment and only on d 0.

5.3.4.5 Sodium Chloride

Sodium chloride concentration was measured according to the protocol from Sebranek, Lonergan, King-Brink, Larson, and Beermann (2001) using Quantab high chloride range titration strips (Hach Company, Loveland, CO). Measurements were made in duplicate for each treatment and only on d 0.

5.3.4.6 pH

For each treatment, 10 g of ground meat was added to a 150 ml plastic beaker, and 90 ml DDD water was added to the beaker. The solution was homogenized (PT 10-35 GT, Kinematica, Inc., Bohemia, NY) at 23,000 RPM for 30 seconds. A stir plate (Thermolyne® Cimarec®-top stirring hotplate; Barnstead Thermolyne, Dubuque, IA) was used along with a stir bar to allow for continuous motion of the solution while the pH
was read with a pH meter (Orion 410Aplus; ThermoFisher Scientific, Waltham, MA) which had been calibrated with standards of pH 4.01, 7.00, and 10.01 (Orion 910104, 910107, 910110, respectively; ThermoScientific, Waltham, MA). Measurements for pH were made in duplicate on d 0, 7, 14, 21, 28, 35, and 42.

5.3.4.7 Residual Nitrite

Residual nitrite was measured according to a modified version of AOAC 973.31 (AOAC, 1990). Flasks containing 5 g of ground meat and approximately 350 ml of hot DDD water were heated in 82°C water baths for 2 h and were uncorked, swirled, and recorked every 30 min. The flasks were then removed from the baths and stored at 2°C for approximately 2.5 h to cool the solutions.

After being cooled and removed from cold storage, room temperature DDD water was added to bring the flask solutions to volume, and the solutions were filtered through Whatman No. 1 filters (GE Healthcare UK Ltd., Buckinghamshire, UK). In a test tube, 4 ml of filtrate was added followed by 0.22 ml of sulfanilamide solution. After a 5 min waiting period, 0.22 ml NED solution was added to the test tube, and a 15 min waiting period followed to allow for the development of an azo dye. A blank solution of 4.5 ml DDD water, 0.25 ml sulfanilamide, and 0.25 ml NED was also made, and this was measured at 540 nm with a spectrophotometer (DU 800 Spectrophotometer; Beckman Coulter, Fullerton, CA) equipped with a sipper flow cell. Assay solutions were then measured at 540 nm with the spectrophotometer being flushed with DDD water between the sets of SN and CP treatments. The linear formula developed from the standard curve
made with standard sodium nitrite solutions was used to determine residual nitrite concentration from absorbance (A<sub>540</sub>) values. Measurements were made in quadruplicate (two test tubes per flask; two flasks per treatment) on d 0, 7, 14, 21, 28, 35, and 42.

**5.3.5 Sensory Trait Analysis**

Sensory panels were conducted on three days with one replication evaluated per day. Panels were conducted within 35 to 39 days after manufacture. Slices were served on white paper plates marked with a three-digit blinding code representing the treatment. Each panelist received four treatments representing each source of nitrite for two nitrite concentrations (Figure 5.3) in a randomized order. The six possible combinations of samples were grouped to ensure similar numbers of evaluations for each treatment.

Six untrained consumer sensory panels were conducted at the University of Nebraska-Lincoln Food Science and Technology Department (mornings of panel days) and Animal Science Department (afternoons of panel days) sensory laboratories and complied with guidelines set out by the University of Nebraska-Lincoln Institutional Review Board. University faculty, students, and staff at least 19 years of age could voluntarily participate after signing a consent form. Panelists were in a room separated from the sample preparation area and received samples through a sliding door or hood. Panelists were served samples one at a time and were encouraged to cleanse their palates with distilled water and unsalted saltine crackers between samples.

Unstructured line scales (150.75 mm-long line with vertical anchors at each end) were provided for panelists to evaluate 1) cured meat color (“absent (white)” to “intense
(pink”), 2) color acceptability ("unacceptable” to “acceptable” with instructions to ignore dark spots attributed to blood splashing), 3) cured meat (“ham-like”) flavor\(^2\) ("absent” to “intense”), 4) turkey flavor (“absent” to “intense”), 5) off-flavor (“absent” to “intense”), 6) flavor acceptability ("unacceptable” to “acceptable”), and 7) overall product acceptability (“unacceptable” to “acceptable”). Panelists wrote the 3-digit code for each treatment at the top of the questionnaire. After visually evaluating and tasting the product, panelists placed a mark on the 150.75 mm line according to their perceptions for each sensory trait.

5.3.6 Statistical Analysis

Physicochemical trait data were analyzed according to a factorial design (2 nitrite sources x 5 nitrite concentrations) for traits only measured on d 0 (total meat pigment, cured meat pigment, salt, and water activity), and according to a repeated measures multifactorial design (2 nitrite sources x 5 nitrite concentrations x 7 test days) for traits measured on d 0, 7, 14, 21, 28, 35, and 42 (pH, objective color, and residual nitrite). Residual nitrite data was also analyzed with only the 50, 100, 150, and 200 ppm products considered (2 nitrite sources x 4 nitrite concentrations x 7 test days). The data for total and cured meat pigments, salt, and water activity were analyzed using analysis of variance (ANOVA) through PROC GLIMMIX on SAS 9.2 (SAS Institute, Inc., Cary, NC). Significant differences (\(P \leq 0.05\)) between means from main effects or interactions

\(^2\) Cured meat flavor may result from multiple effects from curing components, and is difficult to describe precisely (Noel, Briand, & Dumont, 1990). Since most ham consumed is conventionally cured and has a very different flavor from uncured pork, the description of “ham-like” was added to “cured meat flavor” to give context for the panelists unfamiliar with the concept of cured meat.
were separated through LSMEANS and DIF LINES functions. Data for pH, objective color, and residual nitrite were analyzed using ANOVA through PROC MIXED on SAS 9.2 (SAS Institute, Inc., Cary, NC). The LSMEANS function was used to separate significantly different \((P \leq 0.05)\) means from main effects or interactions.

To measure the distance from the start of the evaluation line to the mark placed by the panelist, a Westward® 6 in/150 mm electronic caliper (Grainger International Inc., Lake Forest, IL) was used, and distances were recorded in millimeters. Each questionnaire was numbered to represent each panelist. A total of 196 panelists submitted acceptable evaluations of the products, though not every panelist gave a score for every parameter on the questionnaire. An incomplete block design, with 196 blocks (total number of panelists from 6 panels) x 8 treatments x 4 treatments per block, was assumed. Data were evaluated through PROC GLIMMIX analysis using SAS 9.2 (SAS Institute, Inc., Cary, NC). Significantly different means \((P \leq 0.05)\) from main effects or interactions were separated through LSMEANS and DIF LINES functions.

5.4 Results

5.4.1 Objective color

Both nitrite source \((P < 0.0001)\) and ingoing concentration \((P = 0.001)\) created differences in lightness \((L^*)\) among products. Products made with SN were lighter than products made with CP, and greater ingoing nitrite concentrations led to lower \(L^*\) values (Table 5.3). The ingoing concentration of nitrite also affected redness \((a^*)\) in products \((P < 0.0001)\). Products made with 0 ppm nitrite had lower \(a^*\) values than products made
with any amount of nitrite (50, 100, 150, or 200 ppm; Table 5.3. The main effect of time had no \( P > 0.05 \) impact on color (Table 5.4). Yellowness \((b^*)\) values differed among products due to the nitrite concentration*source interaction \( P < 0.0001 \). Inclusion of SN led to the lowest \( b^* \) values, and inclusion of CP led to higher \( b^* \) values (Table 5.5). Products made with 0 ppm nitrite had higher \( b^* \) values than the products made with all other nitrite concentrations (Table 5.5).

### 5.4.2 Cured Meat Pigment

Cured meat pigment (CMP) varied among products according to nitrite concentration \( P < 0.0001 \) but not by source of nitrite \( P = 0.164 \) nor the concentration*source interaction \( P = 0.261 \). The quantity of CMP was the same for products containing nitrite (50, 100, 150, and 200 ppm) and was greater \( P \leq 0.05 \) than the quantity found in products made with 0 ppm nitrite. Values for CMP in each product are displayed in Table 5.3.

### 5.4.3 Total Meat Pigment

Total meat pigment (TMP) did not vary among products due to nitrite source or concentration \( P = 0.414, 0.492, \) respectively nor due to the concentration*source interaction \( P = 0.427 \). Values for TMP ranged from 16.86 to 20.50 ppm and are shown in Table 5.3. A “TMP ratio” (the pigment absorbance at 512 nm divided by the pigment absorbance at 640 nm) with a value of less than 1.90 indicated the complete conversion
of pigments to one type. TMP ratio values of 1.90 or less were not achieved with CP products possibly due to CP components affecting the absorbance at 512 nm.

5.4.4 Salt and Water Activity \( (a_w) \)

No differences resulted among products due to nitrite concentration, nitrite source, or concentration*source interaction for \( a_w \) \( (P = 0.943, 0.608, 0.967, \text{ respectively}) \) or for salt \( (P = 0.164, 0.741, 0.168, \text{ respectively}) \). Values for \( a_w \) ranged from 0.983 to 0.984, and values for salt concentration ranged from 1.30% to 1.46%. All values are displayed in Table 5.3.

5.4.5 pH

Neither nitrite source nor concentration caused differences \( (P > 0.05) \) among products for pH (Table 5.3). However, the pH of the products was affected both by time \( (P < 0.0001) \) and the interaction of nitrite concentration*source \( (P < 0.0001) \). On d 0, 7, 21, and 28, pH was higher than on day 42, while pH values on d 14 and 35 were similar to the values at all other time points (Table 5.4). The greatest pH value was observed for 200 CP and was greater than all other treatments except that of 150 CP (Table 5.5). The lowest pH value was observed for the 0 SN and 0 CP products (Table 5.5).

5.4.6 Residual Nitrite

When data from all treatments were analyzed, residual nitrite in products was not influenced by main effects of nitrite concentration or source, or time (Table 5.3) but was
significantly affected by the interactions of nitrite concentration*source \( (P < 0.0001) \) and nitrite concentration*time \( (P = 0.022) \). Residual nitrite concentration was greatest in 200 SN products and lowest in 0 SN and 0 CP products (Table 5.4). The 150 SN and 200 CP products had similar residual nitrite concentrations, 100 SN, 100 CP, and 150 CP products had similar residual nitrite concentrations, and products made with 0 or 50 ppm nitrite from either source had similar amounts of residual nitrite (Table 5.4). Also, as ingoing nitrite concentration decreased and time in storage increased, residual nitrite decreased (Figure 5.1). When 0 ppm products were excluded in residual nitrite analysis, time \( (P < 0.0001) \) and the interaction of nitrite concentration*source \( (P < 0.0001) \) affected residual nitrite concentration in the products. When data from 0 ppm products were not included, 100, 150, and 200 SN products contained more residual nitrite than 50, 100, and 150 CP products, respectively (Figure 5.2).

### 5.4.7 Sensory Traits

Three of seven sensory traits were affected by nitrite concentration and/or source \( (P > 0.05; \text{Table 5.6}) \). Perception of cured meat color differed among treatments due to nitrite source \( (P = 0.013) \). Cured meat color was perceived as more intense for products made with SN than for products made with CP. Both source of nitrite and ingoing nitrite concentration affected product color acceptability \( (P = 0.048, 0.032, \text{respectively}) \). Color of products made with ingoing nitrite concentrations of 100 and 200 ppm was less acceptable than color of products made with an ingoing nitrite concentration of 150 ppm. The color of products made with 50 ppm was as acceptable as the color of products made
with all other concentrations of nitrite. Color of products made with SN was more acceptable than color of products made with CP. Similar to color acceptability, cured meat flavor was also affected by nitrite concentration and source ($P = 0.048$, $P < 0.0001$, respectively). Products made with 100 and 50 ppm ingoing nitrite had stronger cured meat flavor than products made with 150 ppm ingoing nitrite. Products made with 200 ppm ingoing nitrite had cured meat flavor similar to products made with 50, 100, and 150 ppm ingoing nitrite. Cured meat flavor was perceived as more intense for products made with CP than products made with SN. No differences due to nitrite source, ingoing nitrite concentration, or ingoing nitrite concentration*source interaction were observed for turkey flavor perception or flavor acceptability ($P > 0.05$).

Differences in off-flavor among products were affected by the ingoing nitrite concentration*source interaction ($P = 0.026$; Table 5.7). The 200 CP product had a more noticeable off-flavor ($P \leq 0.05$) than all other treatments. Also, off-flavor was stronger ($P \leq 0.05$) for the 150 CP products than for the 50 CP, 100 CP, and all SN products. The ingoing nitrite concentration*source interaction caused significant differences for overall product acceptability ($P = 0.008$). All products made with SN were equally acceptable whereas acceptability decreased as ingoing nitrite increased in CP products (Table 5.7). The 200 CP product was the least acceptable product, and the 50 CP product was more acceptable ($P \leq 0.05$) than 150 CP, 200 CP, 50 SN, and 150 SN products (Table 5.7).
5.5 Discussion

As expected, products manufactured with nitrite from sodium nitrite (SN) or celery juice powder (CP) had higher \((P \leq 0.05)\) concentrations of cured meat pigment (CMP) than products made with 0 ppm nitrite. As no significant differences in CMP among products containing nitrite were observed, these results support the theory that an ingoing nitrite concentration of 40-50 ppm is adequate for cured color development (Froehlich, Gullett, & Usborne, 1983). The CMP values for products made with nitrite (7.24 to 7.95 ppm) were much lower than those reported by Wesley, Marion, and Sebranek (1982) for frankfurters produced with sodium nitrite, but the fact that the frankfurters were composed of dark turkey meat—which contains more myoglobin than turkey breast meat—could explain this disparity for CMP. No significant \((P > 0.05)\) variations were observed among treatments regarding total meat pigment (TMP), which was expected since all treatments were produced with the same turkey meat block.

The observed water activity and salt values were to be expected from the production method and formula. Water activity was greater and salt content was lower than that of commercial bacon, ham, and frankfurters sampled by Sullivan et al. (2012), and as salt content has an inverse relationship to water activity, the “lower salt/higher water activity” relationship in this study’s products compared to the commercial bacon, ham, and frankfurters is logical. No differences in salt content among treatments also suggests the sodium chloride that partially constitutes both conventional and alternative curing agents has an insignificant impact on overall salt content in a product. Since these
water activity and salt values are within tolerable ranges for many foodborne microorganisms, precautions must be in place to retard possible microbial growth. Some fluctuations in pH were observed in weekly measurements, but on day 42, pH was lower ($P > 0.05$) than on day 0 (6.26 compared to 6.31). However, though the decrease in pH was statistically significant, it may have a minimal impact in overall product quality. Adjusting the pH of celery juice powder before incorporation into a meat system may lead to lower product pH values, as suggested by Horsch et al. (2014), who observed greater pH values for hams produced with celery concentrate unadjusted for pH than for hams produced with celery concentrate adjusted for pH. The pH values were higher than values for commercial bacon, ham, and frankfurters described by Sullivan et al. (2012), but these differences are supported by Kilic, Cassens, and Borchert (2001) who recorded greater pH values for poultry frankfurters than non-poultry frankfurters (6.30 compared to 6.00). Near the end of the testing period, some samples (usually those made with 0 or 50 ppm nitrite) had a noticeably “sour” smell and pH values lower than the values for other treatment samples, possibly indicating spoilage.

Products made with SN had greater L* values than products made with CP (77.92 compared to 74.68), which was probably due to differences in curing brine color. While the SN brines were colorless and clear, the brines made with reddish-brown cherry powder and (or, and without) yellowish-green CP were a dark brown color. The 0 ppm products had lower a* values than products made with nitrite (4.35 compared to a range of 6.32 to 6.67). This reflects the differences observed for CMP: CMP quantities for products lacking nitrite were also lower than those for products made with nitrite. This is
reasonable, since CMP projects a pink color which would increase a* values. Products made with SN had lower b* values (6.90 to 9.43) than products made with CP (9.78 to 10.96). For both nitrite sources, the products made with 0 ppm nitrite had greater ($P \leq 0.05$) b* values than the cured counterpart products. The only exception was the 0 CP product which had the same ($P > 0.05$) b* value as the 200 CP product. Again, the color of the curing brines likely affected product color, and the presence of CMP in products made with nitrite might have muted the yellowness of the cured products (except 200 CP). No effect from storage time on any color attribute might be due to storage of samples in covered opaque containers throughout the testing period.

Both depletion of nitrite in cured meat products over time and the positive relationship between quantities of ingoing nitrite and residual nitrite, as were observed in this study, are supported by previous studies. Krause et al. (2011) observed that residual nitrite concentrations in hams cured with sodium nitrite and vegetable juice powder decreased from 112.4 ppm to 26.1 ppm and from 40.8 ppm to 7.3 ppm, respectively, over 42 d. Terns, Milkowski, Rankin, and Sindelar (2011) observed cooked, cured sausages made with sodium nitrite had greater ($P \leq 0.05$) residual nitrite concentrations at each testing period over 84 d than sausages cured with four different combinations of natural nitrate source, starter culture, and cherry powder, though for all treatments, residual nitrite decreased over time ($P \leq 0.05$ between day 0 and 84). Sindelar et al. (2007) reported that hams conventionally cured with 200 ppm nitrite contained more residual nitrite over a period of 90 d than hams made with varied concentrations of natural nitrate source (0.20% and 0.35%) and pre-manufacture incubation times (0 or 20 min), but
residual nitrite still decreased over time for all treatments ($P \leq 0.05$ between d 0 and 90). Kilic et al. (2001) noticed similar declines in residual nitrite for frankfurters made with mechanically separated turkey and pork, 100 percent mechanically separated turkey, and whole muscle turkey (60 to 8 ppm, 56 to 3 ppm, and 54 to 4 ppm, respectively) over 49 d. In this study, since only products made with 200 ppm nitrite had differences in residual nitrite due to nitrite source ($P \leq 0.05$), both curing methods could be proposed to result in similar residual nitrite levels at ingoing nitrite concentrations less than 200 ppm.

However, ingoing nitrite concentrations restricted to less than 100 ppm could cause challenges in product quality (light fading, lipid oxidation), and safety and shelf life (lack of bacterial inhibition).

Sensory evaluation revealed cured meat color perception was affected by the nitrite source ($P = 0.01$), with SN contributing to a more intense (“pink”) color than CP. This might reflect the fact that products made with CP had greater ($P \leq 0.05$) $b^*$ values than products made with SN, and the yellow coloration might have muted the appearance of a cured color. Nitrite source also had an impact on color acceptability ($P = 0.048$), and the color of products made with SN was more acceptable than that of products made with CP. According to $L^*$ and $b^*$ values, products made with SN were less dark and less yellow than products made with CP, and, apparently, the panelists preferred lighter, less yellow products. These results are partially contrary to those of Terns et al. (2011), in which preferences among conventionally and alternatively cured sausages for internal color did not exist ($P > 0.05$). Evaluation of conventionally and alternatively cured hams by trained panelists also did not result in differences among treatments ($P > 0.05$).
for color intensity (Sindelar et al., 2007). However, the initially darker and redder qualities of beef and pork products used in these two studies might have masked subtle color differences that lighter turkey breast meat revealed in this study. Color acceptability was also affected by ingoing nitrite concentration; however, there was no proportional relationship between ingoing nitrite concentration and color acceptability.

Products made with CP had greater \( (P \leq 0.05) \) cured meat flavor than products made with SN. Also, products made with 50 and 100 ppm ingoing nitrite had the greatest \( (P \leq 0.05) \) cured meat flavor. During production, an aroma that could be described as “burnt” or “roasted” emanated from the celery and cherry powders. Due to the difficulty in describing or defining “cured meat flavor” (Noel, Briand, & Durmont, 1990), “cured meat flavor” was qualified as “cured meat (‘ham-like’) flavor” on the sensory questionnaire to guide panelists’ evaluation of the product. Compounds that contributed to the vegetable and cherry powders’ unique aroma, and the prompt to link ham-like qualities to the product, might have partially led panelists to rank alternatively cured products as having a more intense “cured meat flavor.” The impact of nitrite source on perceived cured meat flavor is easier to conjecture than ingoing nitrite concentration, as cured meat flavor perception was not proportionally related to nitrite concentration.

Froehlich et al. (1983) observed that hams with ingoing concentrations of 150 and 100 ppm nitrite to had similar \( (P > 0.05) \) but greater \( (P \leq 0.05) \) cured meat flavor than hams made with 50 ppm nitrite, supporting the idea of limited impact of nitrite concentration on cured flavor at greater ingoing concentrations. Neither turkey flavor perception nor flavor acceptability differed among treatments due to ingoing nitrite concentration, nitrite
source, or concentration*source interaction (P > 0.05). A lack of differences in flavor acceptability could imply that both curing methods and all ingoing nitrite concentrations could produce a suitable flavor for a deli-style turkey product.

Not surprisingly, the product made with 200 ppm of nitrite from CP had the greatest off-flavor of all products. A study by Sindelar et al. (2007) had similar results: trained panelists rated hams cured with 0.35% celery juice powder as having a greater (P ≤ 0.05) “vegetable flavor” than hams cured with sodium nitrite. Results from this study suggest that CP included up to 0.46% (100 ppm ingoing nitrite) could cure products without creating a noticeable, unacceptable off-flavor.

Values for overall product acceptability suggest that greater concentrations of CP (150 or 200 ppm nitrite) strongly reduced product acceptance but lower concentrations of CP (50 or 100 ppm nitrite) did not have a negative impact on product acceptability. Perhaps the compounds that contribute to celery juice powder and cherry powder aroma and flavor are advantageous at low levels but can detract from product acceptability when a certain quantity threshold is crossed. Overall, conventionally and alternatively cured products were similar for several traits, but inclusion of celery juice powder to achieve 150 or 200 ppm ingoing nitrite created unappealing sensory properties, thereby limiting celery juice powder to 100 ppm nitrite (0.46%) for acceptable deli-style turkey breast.
5.6 Literature Cited


6. Figures and Tables
Table 5.1: Treatment formulation. Percentages of non-meat ingredients (NMI) are relative to the meat block weight of 11.34 kg of turkey breast for each of ten treatments. The treatment abbreviations refer to ingoing nitrite concentration (0, 50, 100, 150, 200 ppm) and nitrite source (sodium nitrite, SN; celery juice powder, CP).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water (%)</th>
<th>Salt (%)</th>
<th>Sugar (%)</th>
<th>Sodium phosphate (%)</th>
<th>Sodium nitrite (6.25% sodium nitrite) (%)</th>
<th>Sodium erythorbate (%)</th>
<th>Total NMI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 SN</td>
<td>21.80</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.00</td>
<td>0.05</td>
<td>25.00</td>
</tr>
<tr>
<td>50 SN</td>
<td>21.72</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.08</td>
<td>0.05</td>
<td>25.00</td>
</tr>
<tr>
<td>100 SN</td>
<td>21.64</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.16</td>
<td>0.05</td>
<td>25.00</td>
</tr>
<tr>
<td>150 SN</td>
<td>21.56</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.24</td>
<td>0.05</td>
<td>25.00</td>
</tr>
<tr>
<td>200 SN</td>
<td>21.48</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.32</td>
<td>0.05</td>
<td>25.00</td>
</tr>
<tr>
<td>0 CP</td>
<td>21.42</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.00</td>
<td>0.43</td>
<td>25.00</td>
</tr>
<tr>
<td>50 CP</td>
<td>21.19</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.23</td>
<td>0.43</td>
<td>25.00</td>
</tr>
<tr>
<td>100 CP</td>
<td>20.96</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.46</td>
<td>0.43</td>
<td>25.00</td>
</tr>
<tr>
<td>150 CP</td>
<td>20.73</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.69</td>
<td>0.43</td>
<td>25.00</td>
</tr>
<tr>
<td>200 CP</td>
<td>20.50</td>
<td>1.80</td>
<td>1.00</td>
<td>0.35</td>
<td>0.92</td>
<td>0.43</td>
<td>25.00</td>
</tr>
</tbody>
</table>
Table 5.2: Process steps for turkey product heat treatment. These steps meet the requirements for USDA FSIS’s Appendix A for adequate reduction in microbial populations.

<table>
<thead>
<tr>
<th>Step</th>
<th>Dry Bulb set point (°C)</th>
<th>Wet Bulb set point (°C)</th>
<th>Time (min.)</th>
<th>Internal Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71.1</td>
<td>0</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>76.7</td>
<td>76.7</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>82.2</td>
<td>82.2</td>
<td>10a</td>
<td>73.9</td>
</tr>
<tr>
<td>4</td>
<td>15.6 (cold shower)</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

*Step 3 cooking continued for the longer of 10 minutes or time until internal temperature reached 73.9°C.*
Table 5.3: Least square means for main effects of nitrite source (sodium nitrite, SN, or celery juice powder, CP) and ingoing nitrite concentration (0, 50, 100, 150, or 200 ppm) for \( L^* \), \( a^* \), \( b^* \), cured meat pigment (CMP), total meat pigment (TMP), water activity (\( a_w \)), salt (%), pH, and residual nitrite (RN; measured in ppm). P-values denote a significant \((P \leq 0.05)\) or insignificant \((P > 0.05)\) effect from source or ingoing nitrite concentration on the physicochemical effects tested.

<table>
<thead>
<tr>
<th>Source</th>
<th>Ingoing nitrite concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Trait</td>
<td></td>
</tr>
<tr>
<td>( L^* ) ((P &lt; 0.0001))</td>
<td>77.92&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>( a^* ) ((P = 0.019))</td>
<td>6.15</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>( b^* ) ((P &lt; 0.0001))</td>
<td>7.56</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CMP ((P = 0.164))</td>
<td>5.96</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>TMP ((P = 0.414))</td>
<td>18.69</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>( a_w ) ((P = 0.607))</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt ((P = 0.741))</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>( pH ) ((P &lt; 0.0001))</td>
<td>6.28</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>( RN ) ((P &lt; 0.0001))</td>
<td>21.26</td>
</tr>
</tbody>
</table>

<sup>1</sup> Commission 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 (\( a^* > 0 \)) or greenness (\( a^* < 0 \)), and \( b^* \) indicates yellowness (\( b^* > 0 \)) or blueness (\( b^* < 0 \)).

<sup>2</sup> SEM = standard error of the means for conventionally and alternatively cured deli-style turkey breast.

<sup>a,b,x,y,z</sup> Means in the same row with different superscripts are significantly different \((P \leq 0.05)\).

<sup>‡</sup> Indicates a significant \((P \leq 0.05)\) nitrite concentration*source interaction for the trait.

<sup>‡</sup> Indicates a significant \((P \leq 0.05)\) nitrite concentration*day interaction for the trait.
Table 5.4: Least square means for main effects of time for L*, a*, b*, pH, and residual nitrite (RN; measured in ppm). P-values denote a significant ($P \leq 0.05$) or insignificant ($P > 0.05$) effect time on the physicochemical effects tested.

<table>
<thead>
<tr>
<th>Trait</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L^*$ ($P = 0.382$)</td>
<td>76.83</td>
<td>76.44</td>
<td>76.46</td>
<td>75.22</td>
<td>76.33</td>
<td>76.47</td>
<td>76.32</td>
</tr>
<tr>
<td>$a^*$ ($P = 0.054$)</td>
<td>6.20</td>
<td>6.28</td>
<td>6.07</td>
<td>6.05</td>
<td>6.06</td>
<td>6.07</td>
<td>6.06</td>
</tr>
<tr>
<td>$b^*$ ($P = 0.565$)</td>
<td>9.08</td>
<td>9.02</td>
<td>8.87</td>
<td>9.06</td>
<td>9.00</td>
<td>9.05</td>
<td>9.08</td>
</tr>
<tr>
<td>pH ($P &lt; 0.0001$)</td>
<td>6.31$^b$</td>
<td>6.31$^b$</td>
<td>6.28$^{ab}$</td>
<td>6.30$^b$</td>
<td>6.30$^b$</td>
<td>6.28$^{ab}$</td>
<td>6.26$^a$</td>
</tr>
<tr>
<td>$^2$RN ($P &lt; 0.0001$)</td>
<td>23.91$^c$</td>
<td>21.34$^{bc}$</td>
<td>18.10$^{ab}$</td>
<td>18.10$^{ab}$</td>
<td>16.16$^d$</td>
<td>14.68$^a$</td>
<td>14.31$^a$</td>
</tr>
</tbody>
</table>

1 Commision Internationale de l’Eclairage (CIE) L*, a*, b*, in which L* indicates lightness (0, “black,” to 100, “colorless”), a* indicates redness ($a^* > 0$) or greenness ($a^* < 0$), and b* indicates yellowness ($b^* > 0$) or blueness ($b^* < 0$).

2 Residual nitrite concentration measured in ppm.

3 When data from 0 ppm products are excluded, time affects the residual nitrite concentration in products. When data from 0 ppm products are included, there is a significant effect ($P \leq 0.05$) from the interaction of nitrite concentration*time on residual nitrite concentration.

3 SEM = standard error of the means for conventionally and alternatively cured deli-style turkey breast.

a-c Means in the same row with different superscripts are significantly different ($P \leq 0.05$).
Table 5.5: Least square means for interaction effect of nitrite concentration*source for b*, pH, and residual nitrite (RN).

<table>
<thead>
<tr>
<th>Trait</th>
<th>0 SN</th>
<th>50 SN</th>
<th>100 SN</th>
<th>150 SN</th>
<th>200 SN</th>
<th>0 CP</th>
<th>50 CP</th>
<th>100 CP</th>
<th>150 CP</th>
<th>200 CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>b*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b*</td>
<td>9.43^c</td>
<td>7.12^b</td>
<td>7.17^b</td>
<td>7.16^b</td>
<td>6.90^ab</td>
<td>10.96^g</td>
<td>9.78^d</td>
<td>10.24^c</td>
<td>10.51^f</td>
<td>10.94^g</td>
</tr>
<tr>
<td>pH</td>
<td>6.27^a</td>
<td>6.29^ab</td>
<td>6.27^ab</td>
<td>6.27^ab</td>
<td>6.29^ab</td>
<td>6.26^a</td>
<td>6.28^ab</td>
<td>6.31^b</td>
<td>6.32^bc</td>
<td>6.35^c</td>
</tr>
<tr>
<td>RN</td>
<td>0.01^a</td>
<td>7.77^b</td>
<td>18.46^c</td>
<td>30.67^d</td>
<td>49.4^c</td>
<td>0.02^a</td>
<td>6.22^b</td>
<td>13.88^c</td>
<td>21.88^cd</td>
<td>32.55^d</td>
</tr>
</tbody>
</table>

1 Treatments represent products made with sodium nitrite (SN) or celery juice powder (CP) with ingoing sodium nitrite concentration equivalents of 0, 50, 100, 150, or 200 ppm.
2 Commision Internationale de l’Eclairage (CIE) L*, a*, b*, in which b* indicates yellowness (b* > 0) or blueness (b* < 0).
3 Residual nitrite measured in ppm.
4 SEM = standard error of the means for conventionally and alternatively cured deli-style turkey breast.
5 Means in the same row with different superscripts are significantly (P ≤ 0.05) different.
Figure 5.1: Least square means for the effect of ingoing sodium nitrite (ppm) concentration*time ($P = 0.022$) on residual nitrite for 0, 50, 100, 150, and 200 CP and SN products. Time is measured in days following manufacture, and nitrite concentration is measured in ppm of sodium nitrite. Bars with different labels ($^{a-j}$) are significantly ($P \leq 0.05$) different.
Figure 5.2: Least square means for the effect of ingoing sodium nitrite concentration*source ($P < 0.0001$) on residual nitrite for 50, 100, 150, and 200 SN (sodium nitrite curing agent) and CP (celery juice powder curing agent) products (excludes 0 SN and 0 CP products). Bars with different labels (a–g) are significantly ($P \leq 0.05$) different.
Table 5.6 Least square means for main effects of nitrite source (sodium nitrite, SN, and celery juice powder, CP) and ingoing nitrite concentration (50, 100, 150, and 200 ppm) on sensory traits: cured meat color, color acceptability, cured meat flavor, turkey flavor, off-flavor, and overall product acceptability. These traits were measured by untrained panelists using line scales. P-values denote a significant ($P \leq 0.05$) or insignificant ($P > 0.05$) effect from source or ingoing nitrite concentration on the sensor traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source</th>
<th>Trait</th>
<th>Source</th>
<th>Ingoing Nitrite Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Cured Meat Color ($P = 0.014$)</td>
<td>SN</td>
<td>Cured Meat Flavor ($P = 0.048$)</td>
<td>CP</td>
<td>78.87$^b$</td>
</tr>
<tr>
<td>Cured Meat Color ($P = 0.048$)</td>
<td>SN</td>
<td>Cured Meat Flavor ($P = 0.048$)</td>
<td>CP</td>
<td>97.18$^b$</td>
</tr>
<tr>
<td>Cured Meat Flavor ($P &lt; 0.0001$)</td>
<td>SN</td>
<td>Cured Meat Flavor ($P = 0.048$)</td>
<td>CP</td>
<td>68.71$^a$</td>
</tr>
<tr>
<td>Turkey Flavor ($P = 0.519$)</td>
<td>SN</td>
<td>Turkey Flavor ($P = 0.387$)</td>
<td>CP</td>
<td>78.23</td>
</tr>
<tr>
<td>Off-flavor ($P = 0.051$)</td>
<td>SN</td>
<td>Off-flavor ($P = 0.046$)</td>
<td>CP</td>
<td>44.05</td>
</tr>
<tr>
<td>Flavor Acceptability ($P = 0.394$)</td>
<td>SN</td>
<td>Flavor Acceptability ($P = 0.157$)</td>
<td>CP</td>
<td>89.82</td>
</tr>
<tr>
<td>Overall Product Acceptability ($P = 0.670$)</td>
<td>SN</td>
<td>Overall Product Acceptability ($P = 0.090$)</td>
<td>CP</td>
<td>91.47</td>
</tr>
</tbody>
</table>

1 Cured meat color was measured from “absent (white)” (0.00) to “intense (pink)” (150.75).
2 Color acceptability was measured from “unacceptable” (0.00) to “acceptable” (150.75).
3 Cured meat flavor was measured from “absent” (0.00) to “intense” (150.75).
4 Turkey flavor was measured from “absent” (0.00) to “intense” (150.75).
5 Off-flavor was measured from “absent” (0.00) to “intense” (150.75).
6 Flavor acceptability was measured from “unacceptable” (0.00) to “acceptable” (150.75).
7 Overall product acceptability was measured from “unacceptable” (0.00) to “acceptable” (150.75).
8 SEM = standard error of the means for conventionally and alternatively cured turkey breast.
9 Indicates significant ($P \leq 0.05$) nitrite concentration*source interaction.
Table 5.7: Least square means for interaction effect of nitrite concentration*source on off-flavor and overall product acceptability ($P = 0.026, 0.008$, respectively) for conventionally and alternatively cured deli-style turkey breast as measured by untrained panelists.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment†</th>
<th>50 SN</th>
<th>100 SN</th>
<th>150 SN</th>
<th>200 SN</th>
<th>50 CP</th>
<th>100 CP</th>
<th>150 CP</th>
<th>200 CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off-flavor</td>
<td>44.20$^{ab}$</td>
<td>44.30$^{ab}$</td>
<td>42.40$^{ab}$</td>
<td>45.30$^{ab}$</td>
<td>43.60$^{ab}$</td>
<td>41.20$^{a}$</td>
<td>49.70$^{bc}$</td>
<td>56.90$^{c}$</td>
<td></td>
</tr>
<tr>
<td>SEM†</td>
<td>3.45</td>
<td>3.46</td>
<td>3.46</td>
<td>3.45</td>
<td>3.45</td>
<td>3.46</td>
<td>3.43</td>
<td>3.45</td>
<td></td>
</tr>
<tr>
<td>Overall Product Acceptability</td>
<td>90.50$^{ab}$</td>
<td>92.60$^{bc}$</td>
<td>90.20$^{ab}$</td>
<td>92.60$^{bc}$</td>
<td>99.80$^{c}$</td>
<td>94.50$^{bc}$</td>
<td>89.90$^{a}$</td>
<td>84.70$^{a}$</td>
<td></td>
</tr>
<tr>
<td>SEM†</td>
<td>3.09</td>
<td>3.10</td>
<td>3.08</td>
<td>3.09</td>
<td>3.09</td>
<td>3.10</td>
<td>3.07</td>
<td>3.09</td>
<td></td>
</tr>
</tbody>
</table>

† Treatments represent products made with sodium nitrite (SN) or celery juice powder (CP) at ingoing nitrite concentrations of 0, 50, 100, 150, or 200 ppm.

‡ Off-flavor was measured from “absent” (0.00) to “intense” (150.75).

§ SEM = standard error of the means for conventionally and alternatively cured deli-style turkey breast.

¶ Overall product acceptability was measured from “unacceptable” (0.00) to “acceptable” (150.75).
Figure 5.3: A representation of how treatments were paired according to ingoing nitrite concentration and served to panelists. The six possible combinations of pairs were arranged in groups prior to each sensory panel and served in such a way that a combination was not served again until the other five combinations were served to panelists.
7. Appendices
1. Nitrite Determination


**Reagents, Standard Curve, and Residual Nitrite**

1) The reacting compounds 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 (1000 ppm), 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; the sodium nitrite concentrations for these solutions were 0, 0.20, 0.40, 0.60, and 0.80 ppm, respectively.
   a. To each flask, 2.5 ml of sulfanilamide solution was added, and the solution was undisturbed for 5 min.
   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.

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 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 to 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 and were uncorked, swirled, recorked, and replaced every 30 minutes.
e. After 2 h, the flasks were stored in a 2°C room for 2.5 h to cool.
f. After 2.5 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 mixed with 0.22 ml sulfanilamide.
i. After 5 min, 0.22 ml NED was added to the tube, and 15 min passed to allow color development.
j. A blank solution of 4.5 ml DDD water, 0.25 ml sulfanilamide, and 0.25 ml NED was produced.
k. The blank was measured at 540 nm, and absorbance values at 540 nm ($A_{540}$) for sample solutions were recorded.
l. The standard curve produced earlier was used to solve the unknown nitrite concentration for each $A_{540}$ value.
2. **Celery Juice Powder Nitrite Determination**

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

- 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.
- 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.
- A blank of 4.5 ml H<sub>2</sub>O, 0.25 ml sulfanilamide, and 0.25 ml NED was produced.
- Four sets of 200 µl of 0.002, 0.003, 0.004, and 0.005% dilutions and the blank were pipetted into individual wells of a 90-well plate.
- Four sets of 200 µl of 0.2, 0.4, 0.6, and 0.8 ppm sodium nitrite standard solutions (as described in Appendix 1) were pipetted into individual wells of the same 90-well plate.
- Absorbance values at 540 nm were measured for all solutions using a spectrophotometric plate reader.
- Through simple linear regression, a linear formula was created from the standard sodium nitrite solutions.
- Absorbance values of the CP dilutions and the standard curve were used to determine the unknown nitrite content of the CP.
b. Equations used to determine the amount of VegStable™ 506 needed to deliver a desired concentration of nitrite based on a meat block of 11.34 kg.

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

\[ x \text{ ppm} = \frac{y \text{ mg nitrite}}{11.34 \text{ kg meat}} \quad (7.2) \]

\[ x \frac{\text{mg}}{\text{kg}} \times 11.34 \text{ kg} = y \text{ mg nitrite} \quad (7.3) \]

\[ \frac{y \text{ mg nitrite}}{z} = \frac{21,617.74 \text{ mg nitrite}}{1 \text{ kg CP}} \quad (7.4) \]

\[ (y \text{ mg nitrite}) \times (1 \text{ kg CP}) = z \times (21,617.74 \text{ mg nitrite}) \quad (7.5) \]

\[ \frac{(y) \times (1 \text{ kg CP})}{21,617.74} \times \frac{1,000 \text{ g}}{1 \text{ kg}} = z \quad (7.6) \]

- In equation (7.1), \( x \) represents the desired nitrite concentration (0, 50, 100, 150, or 200) in ppm.
- 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.
- Equation (7.4) establishes a ratio between \( y \) and an amount of CP (\( z \)) to the concentration of nitrite in 1 kg of CP.
- 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.
3. Sodium Nitrite Curing Agent Calculations


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.

\[
a_{\text{ppm}} = \frac{(\text{b lbs. cure mix}) \times (\% \text{ nitrite in curing agent}) \times 1,000,000}{\text{meat block (lbs.)}} \quad (7.7)
\]

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

\[
b \text{ g cure mix} = \left\{ \frac{(a \text{ mg}) \times 1.134 \text{ kg}}{(1 \text{ kg}) \times 62,500} \times \frac{1 \text{ lb}}{2.20 \text{ kg}} \times \frac{1 \text{ g}}{1,000 \text{ mg}} \right\} \quad (7.9)
\]

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.
4. Sensory Panel Questionnaire

Note: The line has been shortened from its original length (150.75 mm) for the sake of formatting.

Sample _________
Place a vertical mark on the line to indicate your response relative to the given range of reactions.
Examine the sample and respond to the first two questions before tasting the sample.

Cured Meat Color

| Absent (White) | Intense (Pink) |

Color Acceptability (disregarding dark spots)

| Unacceptable | Acceptable |

Cured Meat (“Ham-like”) Flavor

| Absent | Intense |

Turkey Flavor

| Absent | Intense |

Off-flavor

| Absent | Intense |

Flavor Acceptability

| Unacceptable | Acceptable |

Overall Product Acceptability

*Please cleanse palate with crackers and water before tasting next sample.*

Comments
8. Future Research Recommendations

This study suggested that meat could be cured with pre-converted celery juice powder (PC-CJP) rather than with sodium nitrite (SN) when the ingoing nitrite concentration from PC-CJP was limited to 100 ppm. This is important for processors who want to produce “natural” and organic products, or products with fewer chemical additives. Of course, processors must take into consideration how PC-CJP could affect the organoleptic, physicochemical, and safety characteristics of each product that could be alternatively cured. In this study, inherently light-colored turkey breast revealed the color differences imparted by SN and CP and cure accelerants sodium erythorbate and cherry powder, but other meats could possibly disguise these disparities. Also, the addition of spices could mask PC-CJP’s noticeable effects on flavor. Retail display trials could test whether the integrity of cured color might differ between alternatively and conventionally cured products. Challenge studies involving Clostridium species or L. monocytogenes could reveal whether the nitrite procured from PC-CJP can match the antimicrobial action of SN. The antioxidative effectiveness of PC-CJP could also be investigated, especially for products that are re-heated before consumption, to ensure warmed-over flavor is avoided in alternatively cured products. Thorough sensory panels will be essential for determining the palatability and marketability of alternatively cured products.