Can supplemental nitrate in cured meats be used as a means of increasing residual and dietary nitrate and subsequent potential for physiological nitric oxide without affecting product properties?

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1. Introduction

Sodium nitrate and sodium nitrite have been used in meat products as curing agents and preservatives for centuries. However, nitrate is seldom used today because it must be converted to nitrite to be effective, which is a slow process achieved by microbial reductase. Nitrite is used in cured meats because it provides products with improved quality characteristics including color, shelf life, and flavor. The addition of sodium nitrite also contributes to meat product safety by reducing the potential for outgrowth of several microbial pathogens including Clostridium botulinum and Listeria monocytogenes in cured meat products (Cammack et al., 1999; Sebranek & Bacus, 2007). Sodium nitrite provides these benefits to cured meat by undergoing reactions within the meat system to form nitric oxide. Nitric oxide production from nitrite is the necessary step to achieve cured characteristics (Sebranek, 2009).

The use of nitrite for meat curing became a major concern in the 1980s when it was discovered that nitrite has potential to form carcinogenic nitrosamines when combined with secondary amines in a suitable environment (Butler, 2015). However, changes in meat curing formulations and procedures have significantly improved the control and greatly reduced nitrite concentrations in finished cured meats (Cassens, 1997; Bedale, Sindelar, & Milkowski, 2016). Thus, human exposure to nitrite from cured meat is very limited. Further, it is important to note that nitrate is inert in cooked meat products because nitrate-reducing bacteria have been eliminated (Honikel, 2008). Nitrate, by itself, is considered a benign, inactive ingredient (Butler, 2015).

On the other hand, in the 1980s, it was discovered that nitric oxide, derived from nitrite and nitrate, is very important to many human physiological functions, and dietary nitrate as a source of nitric oxide has been shown in several human clinical studies to have significant health benefits (Bedale et al., 2016). Butler (2015), for example, concluded that “The presence of nitrite in food is free of danger and a diet high in nitrate is beneficial to the health”.

While cured meats contribute a very small portion of human dietary intake of nitrate and nitrite, the human body derives nitrate and nitrite through two methods: endogenously through the nitric oxide synthase (NOS) pathway and exogenously through dietary consumption (Bryan, 2009). In the diet, nitrate and nitrite can be found in vegetables, water and some meats (Archer, 2002). The ingestion of nitrate from food leads to the conversion of nitrate to nitrite through bacteria in the mouth and subsequently to nitric oxide. Consequently, once a product containing nitrate or nitrite is ingested, the body's nitric oxide levels have been shown to increase as a result, provided the ingested amount is sufficient (Lundberg & Weitzberg, 2010). The discovery of nitric oxide, along with follow-up research, has made it clear that nitric oxide is one of the primary active substances in meat products.

The use of nitric oxide in meat products has been studied extensively over the past few decades. However, the effects of nitric oxide on meat quality characteristics, such as color, shelf life, and microbial growth, have been less studied. This study aimed to determine the effects of formulated sodium nitrate plus supplemental nitrate (SN) from celery juice powder on residual nitrate, residual nitrite, rancidity, microbial growth, color, sensory properties, and proximate composition of frankfurters, cotto salami and boneless ham during storage (1 °C) were studied. The products were assigned one of two treatments, which were each replicated twice: control (156 ppm sodium nitrite) or SN (156 ppm sodium nitrite and 1718 ppm sodium nitrate in combination with 2% VegStable 502). Sensory parameters and proximate composition were measured once for each replication. All other analytical measurements were conducted at regular intervals for 97–98 days. The SN showed no increase in residual nitrite compared to the control. No changes (P > 0.05) were observed in residual nitrate during storage for any of the products. The results showed that addition of SN did not significantly alter most physical, chemical or microbial properties of cured meat products during refrigerated storage, but some product dependent sensory effects were observed.

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of the most important signaling molecules in the human body for regulation of physiological functions such as blood flow to the tissues and organs (Bryan, 2009).

The ability to form nitric oxide and the important physiological role of this molecule, has established a new context for nitrate and nitrite. Incorporating sufficient nitrate concentration with nitrite in the meat system has potential to provide consumers with a meat product that could have a physiological impact similar to leafy green vegetables, derived from the increase in dietary nitrate that is provided by vegetable products. Manufacturing a product that contains nitrate in combination with nitrite will allow a typical curing reaction by nitrite to provide the necessary cured meat characteristics. At the same time, because nitrate is inert in cooked meat (Honikel, 2008; Sebranek, 2009), it is not typically depleted in a cooked meat system during storage time and distribution and, thus, can act as a source of dietary nitrate. This dietary source of nitrate could ultimately lead to increased nitric oxide production in vivo and contribute to reduced risk of cardiovascular diseases, such as heart attack and stroke (Bryan, 2006). Recent published literature has showcased the benefits of dietary nitrate and nitrite as well as the potential effects on human health (Butler, 2015; Bedale et al., 2016). However, it is clear that current negative perceptions of nitrate and nitrite in cured meat regarding health and safety need to be overcome before the use of supplemental nitrate (SN) in food can be accepted.

The United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) currently limits ingoing (added) nitrate (as sodium nitrate) in chopped meat and poultry to 1718 parts per million (ppm) (USDA, 1995), which is equivalent to 137 mg of nitrate ion in a 112 g portion of finished meat product. Because significantly greater concentrations of dietary nitrate (over 200 mg) have been studied in human clinical trials (Larsen, Ekbom, Sahlin, Lundberg, & Weitzberg, 2006; Webb et al., 2008; Bryan, 2009; Liu et al., 2013; Kapil, Khambata, Robertson, Caulfield, & Ahiwuaila, 2015; Velmurugan et al., 2016) with several positive health effects including blood pressure reduction, it is likely that SN would be necessary to achieve a comparable level of dietary nitrate from cured meat. Utilizing celery powder concentrate, as currently approved without restriction by USDA-FSIS (Sebranek, Jackson-Davis, Myers, & Lavieri, 2012), provides a means of adding SN. A practical limitation, however, could be the impact, at the necessary concentration, of the added celery concentrate on product quality characteristics.

This study was initiated to test the hypothesis that the addition of SN to cured meat, utilizing celery powder in addition to formulated sodium nitrate to achieve a nitrate concentration that could potentially impact nitric oxide concentrations in consumers, will introduce no significant changes in meat product quality or microbial characteristics. Therefore, the objectives of the present study were to evaluate the physical, chemical and microbial effects of SN in cured meat products, when manufactured to contain 220 mg or more nitrate per 112 g serving of cured meat.

2. Materials and methods

2.1. Experimental design

The experimental design consisted of two treatments of three different products, each of which were replicated twice. A control and a SN treated were manufactured using boneless ham, cotto salami and frankfurter products. All products were manufactured in the Iowa State Meat Laboratory under USDA inspection. A.C. Legg (A.C. Legg, Inc., Calera, AL, U.S.A.) provided spices, and celery powder (VegStable 502) was provided by Florida Food Products, Inc. (Florida Food Products, Inc., Eustis, FL, U.S.A.) to be used as a SN source. The SN treated products were formulated to achieve a target of 220 mg or more nitrate per 112 g serving (1964 ppm as nitrate ion) of cured meat. This concentration was achieved by including sodium nitrate at 1718 ppm as permitted by the USDA as well as including 2% celery powder containing 30,000 ppm nitrate according to the supplier. However, the combination must not result in >200 ppm of nitrate, calculated as sodium nitrite, in the finished product (USDA, 1995). For the SN products, potassium chloride was substituted for 8% of the sodium chloride used in the control treatment to compensate for the additional sodium content of the sodium nitrate and to keep the sodium content of the two treatments similar. Two percent celery powder as used in this study is a higher concentration than typically used in processed meats, but was chosen to achieve the desired amount of added nitrate.

2.1.1. Product manufacturing for ham treatments

Boneless ham (semitendinosus, semimembranosus, biceps femoris, and gracilis) was obtained from and processed in the Iowa State University Meat Laboratory. Control ham treatments were manufactured with salt (2.2%), sodium tripolyphosphate (0.28%), sugar (1.3%), modern cure (0.25%) (6.25% nitrite, 156 ppm sodium nitrite), and sodium erythorbate (0.05%, 547 ppm). The SN treatments included identical ingredients as the control with the addition of VegStable 502 (2%), sodium nitrate (0.17%, 1718 ppm), salt (2%) and potassium chloride (0.17%). The replications of the control and SN treatments were processed separately, but in the same manner. Boneless ham was ground through a Biro® grinder (Model 7.5 424852, The Biro® Manufacturing Co., Marblehead, OH, U.S.A.) fitted with a 0.95 cm plate. The ground ham was mixed with water/ice and nonmeat ingredients in a Higashimoto Kikai paddle mixer (Model 90.3.3, Nava, Japan) for 5 min. The mixture was ground a second time in the Biro® grinder with a 0.64 cm grinder plate to provide product uniformity. The product was loaded into a vacuum filler (RS 1040C, Risco U.S.A. Corp., South Eaton, MA, U.S.A.) and stuffed into 23 × 66 cm clear, fibrous casings (Kalle, Gurnee, IL, U.S.A.). The stuffed ham was hung on a smoke truck and placed into a Maurer (Maurer AG, Reichenau, Germany) oven with a natural smoke generator (Raucherzeuger Goliat 11, Reichenau, Germany), for conventional thermal processing. The two treatments in each replication were thermally processed together in the same oven for approximately 5 h using a standardized smokehouse processing schedule for hams until reaching an internal temperature of 71 °C. After cooking was completed, the products were cooled at 1 °C for 12 h overnight and subsequently stored at 1 °C. Ham was sliced (3.7 mm thickness) the following day using a Bizerba slicer (Model No. 10191442, Piscataway, NJ, U.S.A.) and vacuum packaged into half-pound packages using high barrier bags (Crovyac Sealed Air Corporation, 6 × 12, Duncan, SC, U.S.A.) with an oxygen transmission rate of 3–6 cm² at 23 °C (m², 24 h atm @ 23 °C, 0 RH) and a water vapor transmission rate of 0.5–0.6 g at 38 °C (100% RH, 645 cm², 24 h), with a Ultravac Model UV 2100 packaging machine (Koch, Kansas City, MO, U.S.A.). All ham treatments were stored in boxes at 1 °C.

2.1.2. Product manufacturing for cotto salami treatments

Beef 90 trim (60% of meat block) and pork 50 trim (40% of meat block) were obtained from and processed in the Iowa State University Meat Laboratory. Control treatments were manufactured with caseinate (3.5%), salt (2.7%), garlic (0.09%), black pepper (0.25%), cracked black pepper (0.19%), cardamon (0.12%), modern cure (0.25%) (6.25% nitrite, 156 ppm sodium nitrite), and sodium erythorbate (0.05%, 547 ppm). The SN treatments included identical ingredients as the control with the addition of VegStable 502 (2%), sodium nitrate (0.17%, 1718 ppm), salt (2.5%) and potassium chloride (0.22%). The two replications of the cotto salami were processed separately, similar to the hams. Beef and pork were ground separately through a Biro® grinder (Model 7.5 424852, The Biro® Manufacturing Co., Marblehead, OH, U.S.A.) fitted with a 0.95 cm plate. The ground beef was mixed with the salt and half of the water/ice in a Higashimoto Kikai paddle mixer (Model 90.3.3, Nava, Japan) for 5 min. The pork and the rest of the water and nonmeat ingredients were added to the mixer and mixed for 5 min. The mixture was then ground a second time in the Biro® grinder with a 0.64 cm grinder plate, loaded into a vacuum filler (RS 1040C, Risco
2.2. Analytical procedures

2.2.1. Residual nitrite analysis

Samples were first ground, and then homogenized to achieve sampling uniformity. Residual nitrite analysis was conducted according to AOAC method 973.31 (AOAC, 2005e) for colorimetric measurement of nitrite concentration. Sample absorbance was read using a spectrophotometer at 540 nm (Model 4320940, DU 640, Beckman, Fullerton, CA, U.S.A.). Duplicate measurements for each package of sliced product were conducted for each sample on days 1, 6, 13, 27, 41, 55, 69, 83, and 97 after packaging.

2.2.2. Residual nitrate analysis

Hormel Laboratories (Hormel Foods, LLC, Austin, MN, U.S.A.) conducted residual nitrate testing (AOAC, 2005c). Samples were frozen at Iowa State University on days 1, 6, 13, 27, 41, 55, 69, 83, and 97 after packaging, and shipped overnight to Hormel for analysis. Frozen samples were combined and shipped monthly. Once received by Hormel the samples were held at −17 °C until 18 h before evaluation and then tempered at 3 °C before analyses. Testing was typically conducted within 1 to 2 weeks of sample arrival. Samples were prepared by weighing 1 g of homogenized meat into a 100 ml volumetric flask and adding 50 ml of hot, deionized water. Flasks were placed on a steam bath for 60 min, after which 10–20 ml of room-temperature deionized water was added. Flasks were then cooled in a cold water bath for 15 min and brought to volume with room-temperature deionized water. The fat layer was then discarded and the sample filtered first using Whatman GF/C paper followed by a Dionex OnGuard II RP 2.5 cm² cartridge (Thermo Fisher Scientific Inc., Sunnyvale, CA, U.S.A.). Data was collected using Chromleleon 7 (Thermo Scientific Chromleleon 7, Thermo Fisher Scientific Inc., Sunnyvale, CA, U.S.A.).

2.2.3. TBA analysis

The 2-thiobarbituric procedure of Tarladgis, Watts, Younathan, and Dugan (1960) modified by Zipser and Watts (1962) was used to measure oxidative rancidity. Absorbance of sample distillate was read on a spectrophotometer at 532 nm and multiplied by a factor of 7.8 to achieve mg malonaldehyde per kg of meat (Model 4320940, DU 640, Beckman, Fullerton, CA, U.S.A.). Duplicate measurements of each sample were conducted on days 1, 4, 7, 14, 28, 42, 56, 70, 84, and 98 days after packaging.

2.2.4. Color analysis

Color measurements (L, a, b) were conducted on the surface of the ham, cotto salami and frankfurters using a HunterLab LabScan instrument (Model LS 1500, Hunter Associated Laboratories Inc., Reston, VA, U.S.A.) using illuminant D65 (daylight @ 6500 K) and 10° observer angle. The instrument was standardized by covering the white standard with Saran Wrap™. Ham and cotto salami slices were covered with Saran Wrap™ and surface measurements were taken on the surface of the sliced samples using a 1.00 cm port insert. Frankfurters included an additional color measurement to include both external surface and internal surface color. Frankfurters were cut in half length-wise and wrapped with Saran Wrap™. External and internal frankfurter measurements were then taken using a 0.25 cm port insert. A total of three random surface measurements were collected for each product sample and averaged. Samples, designated for color analyses (15 samples for each control and treatment), were stored at 4 °C under fluorescent lighting (Sylvania Octron 3500 k, 32 W) until day of analysis. Color measurements were conducted on days 1, 6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, and 97 after packaging.

2.2.5. Total plate counts

Total plate counts were measured by first blending 10 g of sample in combination with 90 ml peptone water (Hardy Diagnostics, Cat no. D290, Santa Maria, CA, U.S.A.) in a stomacher bag (Whirl Pak, Jackson, WI, U.S.A.) using a lab blender (EasyMix, AES Laboratories, France) for 60 s. One milliliter of each sample was plated onto 3 M petrifilm (3 M Health Care, St. Paul, MN, U.S.A.) containing peptone dilluent (Becton, Dickinson and Company, Sparks, MD, U.S.A.). The petrifilms were incubated for 72 h at 23 °C and then counted (USDA, 2013). Total plate counts were conducted on day 0, 7, 14, 30, 60, and 90 days after packaging. Day 0 samples were immediately frozen following packaging then thawed and plated on day 8, with the counts recorded as day 0 as shown in Fig. 4.
2.2.6. Lactic acid bacteria

Lactic acid bacteria were enumerated by first blending 10 g of sample in combination with 90 ml peptone water (Hardy Diagnostics, Cat. no. D290, Santa Maria, CA, U.S.A.) in a stomacher bag (Whirl Pak, Jackson, WI, U.S.A.) using a lab blender (EasyMix, AES Laboratories, France) for 60 s. One tenth ml of sample containing peptone diluent (Becton, Dickinson and Company, Sparks, MD, U.S.A.) was plated onto 100 mm × 15 mm petri plates (Fisherbrand, Fisher Scientific, Chicago, IL, U.S.A.) containing MRS (deMan, Rogosa and Sharpe) agar (Becton, Dickinson and Company, Sparks, MD, U.S.A.). MRS agar was modified by adjusting the pH to 5.5 with the incorporation of acetic acid and was prepared according to the Compendium of Methods for the Microbiological Examination of Foods (APHA, 1992). The petri plates were incubated for 72 h at 23 °C and then counted (USDA, 2013). If no growth was observed, plates were incubated for an additional 72 h at 23 °C and then counted. Lactic acid bacterial counts were conducted on day 0, 30, 60, and 90 days after packaging. Day 0 samples were immediately frozen following packaging, then thawed and plated on day 8. Potentially nitrate-sensitive microorganisms such as enterobacteria or gram-negative bacteria were not enumerated because the control treatments were already relatively high in nitrate that would have likely impacted these populations if they were affected.

2.2.7. Sensory analysis

Sensory evaluation of the products was conducted using a nine-member trained panel. The panel was comprised of students, staff and faculty at Iowa State University. Three separate training sessions were held before evaluation of the first replication of each product and a brief re-training was conducted before panelists evaluated the second replication. Two sessions for evaluation of each replication were conducted to obtain sensory data. A three-digit code was randomly assigned to each sample of the products evaluated in each session. Panelists evaluated samples using a 15-cm line scale. Data was collected using Compusense five Release 5.6 sensory evaluation software (Ontario, Canada). Panelists evaluated cured aroma, “other aroma,” texture, cured flavor, “other flavor,” saltiness and intensity of pink color for each of the three products. For cotto salami and frankfurters, panelists evaluated the same properties as for hams (cured aroma, “other aroma,” texture, cured flavor, “other flavor,” saltiness, and intensity of pink color), but also included intensity of light to dark color. Panelists were asked to determine “other aroma” and “other flavor” as attributes that would be unusual or different from that expected for a typical product of that type. Additionally, panelists were instructed to evaluate “other flavor” in general to include rancidity and any other unusual flavor.

The ham and cotto salami slices were cut into 2.5 cm square pieces for evaluation. The pieces were mixed in a large mixing bowl to ensure panelists would receive a random sampling of the small pieces from each product. Four to five pieces were placed in a cup and covered with a lid. Ham and cotto salami treatments were held at room temperature for 5 min prior to being served. Frankfurters were heated in boiling water prior to evaluation. Frankfurters were placed in water, and the water was brought to a boil, after which the pot was removed from the heat and the frankfurters remained in the water for 7 min. Frankfurters were then cut into 2.5 cm pieces, and four to five pieces, each from a different frankfurter link from a given treatment or control, were placed into a cup and covered with a lid. In addition to the samples used for tasting, one whole slice of ham and cotto salami and one frankfurter link, were used for appearance and color evaluations by the panel. The frankfurter was cut lengthwise, one side was left intact and the other side was cut vertically into two pieces so panelists could view both external and internal color.

2.2.8 Salt content

Sodium chloride content was measured using the Quantab method (AOAC, 2005f) and was analyzed in duplicate for each treatment on day 6 after packaging.

2.2.9 Proximate analysis

Crude protein (AOAC, 2005a), moisture (AOAC, 2005d), and crude fat (AOAC, 2005b) were analyzed in duplicate for each treatment on day 6 after packaging.

2.3. Statistical analysis

The experiment was replicated twice with separate production days for each of the replications and all of the products were subsequently analyzed over a five-month period. Data were statistically analyzed using PROC MIXED by the Statistical Analysis System (SAS, v9.4). A P-value of 0.05 was used to establish least squares means. Total plate counts were analyzed using WINKS SDA Software (Texasoft, Cedar Hill, TX). Log transformation was used to report exponential growth rates of bacteria. A P-value of 0.05 was again used to establish least squares means. The fixed effects of the experimental design included product, trial, day and treatment. The interaction between treatment and day was analyzed. Results were compared for differences between treatments over the storage time.

3. Results and discussion

3.1. Residual nitrite

Residual nitrite measurements for frankfurters, cotto salami and ham are presented in Fig. 1. Residual nitrite in the frankfurters was significantly different (P < 0.05) between the control treatment and the SN treatment, with the control containing a greater concentration of overall nitrite content during storage time. Although the results were statistically different between the two treatments, the least squares means show the difference to be just 3.95 ppm overall between the two treatments. More importantly, the SN frankfurter treatment had a lower least squares means value than the control treatment, indicating slightly less residual nitrite than the control, and confirms that nitrate is not converted to nitrite in a cooked product. This was expected because nitrate is inert in cooked meat (Honikel, 2008), and therefore should not result in any additional residual nitrite levels compared with a similar product cured only with nitrite. The results showed no significant difference (P > 0.05) between the control treatment and the SN treatment when analyzing cotto salami and ham independently. Further, the residual nitrite concentrations observed for all the products are very typical and similar to those reported by others (Sindelar, Cordray, Sebranek, Love, & Ahn, 2007; Redfield & Sullivan, 2015). Thus, it is important to note that nitrate in combination with nitrite did not produce higher residual nitrite values than a similar product only containing only formulated nitrite. The results from both treatments within all of the three products showed a significant decline (P < 0.05) in residual nitrite during the storage time. This result was expected because as time in storage increases for cured meat products, residual nitrite is typically depleted. The relationship amongst the depletion of residual nitrite and increased storage time is an observation reported by numerous investigators (Hustad et al., 1973; Sindelar et al., 2007; Redfield & Sullivan, 2015). An interaction assessment conducted between storage time and treatment showed no significant difference (P > 0.05) between the two treatments as storage time increased for frankfurters, cotto salami or ham. This indicates that the residual nitrite in both treatments was depleted within each product at a similar rate.

3.2. Residual nitrate

Residual nitrate measurements, conducted by Hormel Laboratories, for frankfurters, cotto salami and ham are shown in Fig. 2 and were consistent and unchanged over time, again confirming the inert nature of nitrate in a cooked meat mixture. Results also clearly show a significant difference (P < 0.0001) between the control treatment and the SN treatment for all three products. The difference between treatments was expected due to the addition of nitrate in the SN treatment and clearly
shows that nitrate is not converted to nitrite in a cooked meat product. A low concentration of nitrate was observed in the control treatments to which only nitrite was added. Pérez-Rodríguez, Bosch-Bosch, and García-Mata (1996), who reported similar findings while investigating sodium nitrite and potassium nitrate in frankfurters, found that approximately 50% of added nitrite could be detected as residual nitrite and 10–15% was detected as nitrate. Sindelar et al. (2007) suggested that secondary oxidation involving nitrous acid during curing could be the explanation for the conversion of some nitrite to nitrate. Honikel (2008) also agreed that oxidation of nitrite to nitrate is why nitrate can be observed in products to which only nitrite was added. No significant difference \((P > 0.05)\) was observed for an interaction between treatment and storage time for either treatment within the three products. These results show that the amount of nitrate added in the supplemented treatment remained constant as storage time of the products increased. This again confirms the hypothesis that residual nitrate will remain constant even with the relatively high nitrate concentrations used in this study. Several researchers have reported constant residual nitrate levels when compared to conventional (not supplemented) ingoing levels of nitrate (Wierbicki & Heiligman, 1973; Shults, Cohen, Howker, & Wierbicki, 1977; Sindelar et al., 2007; Honikel, 2008). No significant differences \((P > 0.05)\) were observed independently with storage time, therefore confirming that no changes in residual nitrate levels occurred in these products during storage.

### 3.3. TBA analysis

The TBA (2-thiobarbituric acid) measurements are presented in Fig. 3. Frankfurters and cotto salami displayed a significant difference \((P < 0.05)\) between the two treatments. The control frankfurter treatment showed an overall TBA value of 0.38 compared to the SN treatment with a TBA value of 0.49, while the cotto salami control treatment showed an overall TBA value of 0.47 compared to the cotto salami SN treatment with a TBA value of 0.37. All of these TBA values are very low, well below the generally recognized threshold of 1.0, and because the control is lower for frankfurters, but higher for cotto salami this suggests that there is no practical effect of the added nitrate. An explanation for the statistical differences may be that different meat sources were acquired for each product replication and the meat ingredients may have had different initial TBA values. Frankfurter and cotto salami products both have relatively high fat contents, thus some variation between TBA values for raw materials for the two treatments is likely. Further, no significant difference \((P > 0.05)\) was observed for control and the SN treatments in ham where fat content was considerably less. Despite minor differences, TBA values for all products over storage time remained relatively low being below the generally recognized detection level, which was expected due to nitrite’s ability to function as an antioxidant (Sebranek, 2009) and the use of high barrier vacuum packaging. No significant differences \((P > 0.05)\) were observed for the effect of storage time of the treatments within the three products. The interaction between treatment and days of storage also showed no significant differences \((P > 0.05)\) for the treatments within the three products. These results indicated that as storage time increased, there was no change in TBA values for either the control or the SN treatments.

### 3.4. Color analysis

The results, shown in Table 1, indicate that the frankfurters (both internal and external) \((P > 0.05)\) and ham, while nearly significant \((P = 0.051)\) did not differ in L-values. However, the SN treatment of cotto salami had a significantly lower L-value \((P < 0.05)\) than the control cotto salami treatment. Redfield and Sullivan (2015) observed that turkey products cured with sodium nitrite had a lighter appearance than those cured with celery juice powder as a source of nitrite. Celery juice powder has a yellow-green pigment and this
natural coloring may contribute to a darker meat color when included in a meat mixture. None of the products showed an effect (P > 0.05) of storage time or for the interaction between storage time and treatment. Therefore, no changes in lightness were observed during the storage period.

The a-value results (Table 1) show that the supplemental nitrate treatment and the control were not different for the external color of the frankfurters (P > 0.05) or the cotto salami (P = 0.07). However, the a-value for hams and the internal frankfurter measurements were significantly different (P < 0.05) between the two treatments. The ham and the internal frankfurter measurements showed the control treatment with a higher a-value than the SN treatment. This means that the internal surface of the control treatment for hams and the frankfurters had a redder appearance. All three products showed no significant difference (P > 0.05) for the effect of storage time or for the interaction between storage time and treatment. Therefore, no changes in redness occurred during storage time.

Product b-value color results (Table 1) displays that the SN treatment and the control were again not different for the external frankfurter color (P > 0.05) or for the cotto salami (P = 0.07). And again, as with the a-values, the b-values for ham and for the internal frankfurter measurements were different (P < 0.05). The ham and the internal frankfurter measurements resulted in the SN treatment having a higher b-value than the control treatment, meaning a more yellow appearance. A greater b-value in products with the added nitrate was not unexpected because of the yellow-green color of the celery juice powder that was added to increase nitrate content. All three products showed no significant effect (P > 0.05) of storage time or for the interaction between storage and treatment. Hue is also displayed in Table 1 to demonstrate color differences between treatments.

Overall, there was no significant effect (P > 0.05) of storage time or for the interaction between storage and treatment for any of the color measurements in this study. Redfield and Sullivan (2015) also concluded that time did not have an impact (P > 0.05) on any CIE L*, a* and b* color measurements when evaluating turkey products cured with sodium nitrite or with celery juice powder as a source of nitrite, which supports this study’s findings in that no changes in color development occurred during storage time.

### 3.5. Total plate count

Total aerobic plate count analyses were conducted for frankfurters, cotto salami and ham and are shown in Fig. 4. Plate counts were conducted to determine if any microbial differences might develop between the control and the supplemental nitrate treatments for the frankfurters, cotto salami and ham, independently. Microbial data was transformed into logs to interpret the exponential growth rates of bacteria. A value of 5 CFU/g was used as the detection limit when plotting the results. The results showed no statistical difference (P > 0.05) for the counts between the control and the SN treatments in regard to all three products. For storage time effects, frankfurters were the only product that resulted in a statistical difference (P < 0.05) during storage with a small decrease in counts over time. Although the effect of storage time was significantly different, frankfurters showed no interaction (P > 0.05) between storage time and treatment. Likewise there was no interaction between storage time and treatment for cotto salami and ham (P > 0.05). No significant microbial growth was expected in either treatment due to sodium nitrite’s ability to perform as a bacteriostatic and bacteriocidal agent (Sebranek & Bacus, 2007) especially in cooked, vacuum-packed products stored at 1 °C.

### 3.6. Lactic acid bacteria

Lactic acid bacteria were also enumerated for frankfurters, cotto salami and ham (data not shown). Lactic acid bacteria were counted to determine if differences in growth of these common spoilage organisms in vacuum-packaged, cooked, cured meat products were apparent between the control and SN treatments for the frankfurters, cotto salami and ham. The results for days 0, 30, 60, and 90 showed no statistical differences (P > 0.05) between the two treatments for lactic acid bacteria counts, for the effect of storage time, or for the interaction between storage time and treatment for all three products.

#### Table 1

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<th>Frankfurters internal control</th>
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<td>0.15</td>
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<td>0.17</td>
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<td>0.11</td>
<td>0.11</td>
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<td>Hue</td>
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<td>39.88</td>
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<td>42.69</td>
<td>46.28</td>
<td>46.66</td>
<td>46.97</td>
<td>40.63</td>
</tr>
</tbody>
</table>

*Standard error of means.

<sup>1</sup> Means within a given row with different letters are significantly different at P < 0.05.
3.7. Salt content and proximate analysis

Salt, protein, fat and moisture content were measured to determine if any proximate compositional differences occurred between the control and SN treatment for frankfurters, cotto salami and ham (data not shown). No statistically significant differences \( (P > 0.05) \) in regards to salt, protein, fat or moisture were found between the control and SN treatments for frankfurters, cotto salami and ham. These results indicate that the addition of sodium nitrate and VegStable 502 did not change the proximate composition of the products when compared to the control treatment. The control and SN treatment were identical in formulation except for the additional sodium nitrate, celery juice powder and the substitution of a portion of the sodium chloride with potassium chloride. Potassium chloride was substituted for 8% of sodium chloride in the supplemental nitrate products to keep sodium content of the two treatments similar because the additional sodium content from the addition of sodium nitrate would be likely to impact the sensory evaluations of these products.

3.8. Sensory analysis

The sensory results for frankfurters, cotto salami and hams are shown in Table 2. The cured aroma, “other aroma”, texture, cured flavor, “other flavor”, saltiness and intensity of internal light to dark color for frankfurters were not significantly different \( (P > 0.05) \) between the control and the SN treatment. However, panelists determined that the intensity of frankfurter internal pink color was significantly greater \( (P < 0.05) \) for the control treatment than the SN treatment, which agrees with the measured instrumental a-values for the internal redness of the frankfurters.

Cured aroma evaluations for cotto salami determined that the control treatment had a significantly greater \( (P < 0.05) \) cured aroma than the SN treatment. Panelists also determined there was a significant difference \( (P < 0.05) \) in the “other aroma” score between the control and SN treatments with the SN treatment having a greater “other aroma” than the control cotto salami. When evaluating texture, cured flavor and saltiness no statistical differences \( (P > 0.05) \) were found between the control and the SN treatment. However, panelists found a significant difference \( (P < 0.05) \) in “other flavor” between the two treatments. The SN treatment possesses a greater “other flavor” than the control treatment. It is possible that the “other aroma” panelists observed could have affected their expectation of the SN treatment and therefore, concluded it to have a greater “other flavor” as well. The intensity of pink color was not different \( (P = 0.07) \) between the control and SN treatments for cotto salami, but the SN treatment was significantly darker \( (P < 0.05) \) in color comparison to the control treatment.

The ham control and SN treatments showed no statistical difference \( (P > 0.05) \) for the attributes of saltiness, texture and “other flavor”, but there was a significantly increased \( (P < 0.05) \) cured aroma and cured flavor, as well as a more intense pink color in the control hams. Results for “other aroma” were not significant \( (P = 0.07) \), but the trend was suggestive. The differences in cured aroma and flavor that are most likely due to the inclusion of celery powder were not unexpected due to the mild flavor profile of ham. The ham treatments were manufactured with no spices or flavoring agents other than the curing ingredients, whereas the frankfurters and cotto salami each included typical spice blends for those products. Thus, the ability to detect unexpected flavors is typically greater for hams than cotto salami or frankfurters.

Hustad et al. (1973) researched sensory effects on frankfurters at different levels of added sodium nitrite as well as a combination of added sodium nitrite with sodium nitrate. Hustad et al. (1973) determined that the nitrite and nitrate used in combination at all concentrations tested did not have a significant effect \( (P > 0.05) \) on flavor quality when compared at any of the ingoing sodium nitrate levels tested. Thus, the addition of SN alone should not impact sensory properties of cured meat products. The findings of Hustad et al. (1973) correlate with what was observed in this study for the frankfurter treatments, where the only difference observed was for internal pink color. Frankfurter treatments in the current study were manufactured with a greater concentration of spices than the cotto salami or ham and received a greater amount of smoke deposition per unit weight given the greater

![Aerobic Plate Counts (log CFU/g) on frankfurters, cotto salami and hams during storage (Frankfurters S.E. = 0.23) (Cotto Salami S.E. = 0.54) (Hams S.E. = 0.80).](image)

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Frankfurters control</th>
<th>Frankfurters SN</th>
<th>Cotto salami control</th>
<th>Cotto salami SN</th>
<th>Hams control</th>
<th>Hams SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured aroma</td>
<td>9.69</td>
<td>8.93</td>
<td>8.89</td>
<td>7.79</td>
<td>8.65</td>
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<td>0.01</td>
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</tr>
<tr>
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<td>Texture</td>
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<td>Other flavor</td>
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<td>Saltiness</td>
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<td>0.17</td>
<td>0.76</td>
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<td>Pink</td>
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<td>7.01</td>
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<td>Light to dark</td>
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<td>6.65</td>
<td>9.87</td>
<td>–</td>
<td>–</td>
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<tr>
<td>S.E.a</td>
<td>1.88</td>
<td>1.88</td>
<td>0.48</td>
<td>0.48</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Standard error of means.

Means within a given row with different letters are significantly different at \( P < 0.05 \).

Not included for hams.
surface area of frankfurters. Consequently, this is probably why panelists did not observe any differences in aroma or flavor for the frankfurters, despite the addition of 2% celery juice powder. For the cotto salami in this study, differences in cured aroma and “other flavor” were observed, but cured flavor was not affected by nitrate supplementation. Both frankfurters and cotto salami included spices and smoke for a more intense flavor profile than for ham, thus making sensory changes due to the treatments, particularly the celery powder less noticeable. In the case of the hams, cured flavor as well as cured aroma was affected by the SN treatment and are most likely attributed to the characteristics of the celery juice powder. In order to manufacture meat products to contain 220 mg or more nitrate per 112 g serving of cured meat, the addition of 2% VegStable 502 in combination with 1718 ppm sodium nitrate was necessary and is likely to have greater impact on sensory characteristics of the products with a low-intensity flavor profile. Although celery juice powder is likely to affect color and sensory attributes, depending on the concentration used, celery juice powder is preferred for meat applications over other natural nitrate sources because of advantages it has over other vegetable products containing high nitrate concentrations. Celery is known to have mild flavors and a light pigment, thus its use is well accepted in meat processing (Sebranek & Bacus, 2007). Typical use, however, is 0.4%, considerably less than the 2% used in this study. These findings suggest that if celery juice powder were to be used to supplement nitrate concentrations in cured meat, the amount of celery juice powder added should be determined at some point of 2% or less of the meat block depending on the product in order to maintain sensory characteristics similar to a conventional product. With a proper spice formulation, smoke and similar flavor profile contributors, an acceptable level of SN using celery powder offers potential to provide a significant dietary source of nitrate.

The Hunter color results were supported by the evaluations collected from the trained panel. Panelists observed that the control treatments for ham and frankfurters displayed a greater intensity of pink color than the SN treatments. Hunter color results also showed that ham and internal color of frankfurters had a higher a-value, indicating that these products were redder in color. Color scores by the panelists and Hunter color results both showed the cotto salami SN treatment to be darker in color than the control, but also found no difference for lightness in the frankfurters. The color properties identified by panelists and Hunter color measurements that showed statistical differences between the control and SN treatments are most likely due to the addition of the celery powder concentrate.

4. Conclusions

This study has the potential to contribute to a new context for nitrate and nitrite. Nitrate added to cooked, cooked meat products remains unchanged following processing and would provide dietary nitrate to form nitric oxide after consumption. This has the potential to provide consumers with a physiological impact similar to leafy green vegetables as a result of increasing nitric oxide concentrations. Therefore, cured meat products could serve as a delivery vehicle for increasing dietary nitrate. Previous publications have shown positive health effects due to increased nitric oxide levels after the consumption of dietary nitrate from vegetable sources. While this holds promise, the next step needed to achieve this objective is a human feeding study, utilizing cured meat with added nitrate to demonstrate the expected impact of the dietary nitrate on human physiological parameters, such as nitrate, nitrite, and nitric oxide concentrations in blood plasma and associated effects on blood pressure and other physiological effects. Additionally, it is appropriate to continue safety evaluations of products with increased amounts of nitrate. While such cured meat products would have to overcome the challenges associated with the current perceptions of nitrite and nitrate in order to be commercially acceptable, the demonstration of potential effects of supplementary nitrate in cured meat for increasing physiological nitric oxide may be helpful for improving the current perceptions of cured meat.

Acknowledgements

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References


