



Use of Nitrite-Embedded Packaging Film for Color Stability of Alternatively Cured, Fully Cooked Bologna

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Abstract: Nitrite-embedded packaging film was investigated for potential effects on the color stability of alternatively cured meats. The impact of nitrite-embedded film on color stability of large-diameter sandwich bologna was assessed over a 125-d lighted display period. Five treatments of large-diameter bologna were manufactured: (1) a conventionally cured control packaged with conventional film (“CON-CF”), (2) an alternatively cured formulation (cultured celery juice powder plus cherry powder) packaged with conventional film (“CJP-CF”), and (3) an alternatively cured formulation (cultured celery juice powder plus cherry powder) packaged with nitrite-embedded film (“CJP-NEF”). An additional alternatively cured formulation (4) Natpre T-10 EML Plus S was packaged with conventional film (“NT10-CF”) and (5) with nitrite-embedded film (“NT10-NEF”). In-package surface a^* values were significantly higher ($P < 0.05$) in nitrite-embedded film, particularly for the bologna with low ingoing nitrite concentration. Reduced color stability was observed for bologna in conventional film packages during lighted display, while the nitrite-embedded film showed improved color stability. Surface and internal residual nitrite concentrations were significantly lower ($P < 0.05$) in the Natpre T-10 EML Plus S treatments for the first 13 d of storage. Further, residual nitrite in the nitrite-embedded film products was not increased ($P > 0.05$) by use of the packaging film and did not differ compared to the conventional film. Thus, nitrite-embedded film packaging technology can improve the color stability of alternatively cured meats without increasing the measurable nitrite concentration in the product.

Keywords: nitrite, cured meat, active packaging, nitrite-embedded film, color stability, color

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Introduction

The widespread consumer demand for alternatively cured meat products continues to rise, due to negative consumer perceptions of conventional, chemical sources of nitrate (NO_3) and nitrite (NO_2) as chemical preservatives. Alternatively cured meat products typically rely on natural sources of nitrate and/or nitrite, which are generally vegetable-based substitutes and which provide an alternative source of nitrate and nitrite that is perceived more positively by consumers. However, replacement of nitrate/nitrite with natural sources can lead to vegetable-like flavors and aromas in the final product (Sindelar et al., 2007b; Djeri and Williams, 2014). Thus, to compensate for these

potential vegetable-like flavors and aromas, processors typically reduce the usage level of the vegetable-based curing ingredient, which results in less ingoing nitrite in alternatively cured meats than in conventionally cured products (Sindelar et al., 2007a). The reduced nitrite concentration in the final product has potential to reduce the color stability of alternatively cured meat products, which can result in a reduced shelf life in retail display. Food packaging provides a widely used means of improving product appearance, quality, and food safety, and recent novel developments in packaging films may also offer a means of improving the color stability of alternatively cured meat products.

An area of current research in meat packaging is active packaging, in which the package environment

promotes a favorable interaction with the meat product (McMillin, 2017). A novel active packaging application that utilizes nitrite embedded in the packaging material (nitrite-embedded film) has been shown to improve and extend the color stability and subsequent shelf life of vacuum-packaged fresh beef, bison, and pork products compared to conventional overwrap packaging (Claus and Du, 2013; Yang et al., 2016; Narváez-Bravo et al., 2017; Roberts et al., 2017; Ramanathan et al., 2018). In the application for fresh meat, the nitrite-embedded film provides an extremely low nitrite concentration, which—in contact with the meat product surface in a vacuum package—then forms nitric oxide upon contact with the meat. The nitric oxide subsequently combines with myoglobin to form nitric oxide myoglobin, which is bright red in color, similar to that seen in vacuum-packaged corned beef in retail displays. The combination of vacuum packaging with highly desirable color results in extended shelf life for fresh meat. However, the resulting nitrite concentration in products packaged in nitrite-embedded film is extremely low, and after the package is opened, it does not result in cured meat color when the product is cooked. The nitrite-embedded film packaging material used for fresh meat contains 113 mg of sodium nitrite per square meter of film and is considered generally recognized as safe (Generally Recognized As Safe, Number 228) for fresh beef (FDA, 2007) and fresh pork (FDA, 2010). It is also categorized as a processing aid when used for fresh beef and fresh pork where nitrite is not a common constituent (USDA, 2018). However, nitrite-embedded film has not, to the best of our knowledge, been studied for cured meat applications and has not been considered for packaging of cured meat products. Therefore, the objective of the present study was to determine the impact and efficacy of nitrite-embedded film on the color stability and shelf life of alternatively cured, all-beef bologna, a cured and fully cooked meat product. This is a novel application of nitrite-embedded film that may offer a means of reducing cured color fading that can result when nitrite concentrations in cured meats are reduced, such as is often the case with alternatively cured processed meats.

Materials and Methods

This research did not include animal or human subjects and therefore did not require review and approval by institutional animal care or human subjects committees.

Experimental design and product preparation

Three product formulations of all-beef, large-diameter sandwich bologna were manufactured in the Iowa State University Meat Laboratory, Ames, Iowa, to provide 3 different concentrations of ingoing nitrite. The 3 formulations were packaged in conventional and nitrite-embedded film films as follows. (1) The first formulation (“CON-CF”) was a conventionally cured control (nitrite from Modern Cure, with sodium erythorbate, both provided by A.C. Legg Inc., Calera, AL), which was vacuum packaged with conventional, high-barrier film that is commercially available (Sealed Air Corporation, Duncan, SC). The second (“CJP-CF”) and third (“CJP-NEF”) formulations were an alternatively cured bologna formulation utilizing nitrite from cultured celery juice powder (VegStable 506) and cherry powder (VegStable 515) supplied by Florida Food Products, Inc. (Eustis, FL), with (2) one-half of the batch (CJP-CF) vacuum packaged in conventional film and (3) the second half of the batch (CJP-NEF) vacuum packaged in nitrite-embedded film (Bemis Company Inc., a division of Amcor Flexibles North America, Oshkosh, WI) pouches. An additional alternatively cured formulation was produced using Natpre T-10 EML Plus S (Productos Sur, S.A. [Prosur], San Ginés, Murcia, Spain), with (4) one-half of the batch (“NT10-CF”) vacuum packaged in conventional film and (5) the second half of the batch (“NT10-NEF”) vacuum packaged in nitrite-embedded film pouches. The Natpre T-10 EML Plus S was chosen for this study because it was a commercially available alternative cure that provided a very low ingoing nitrite concentration for the bologna. The nitrite concentration for the Modern Cure (CON-CF) ingredient was 62,500 parts per million (ppm) sodium nitrite, which resulted in 156 ppm ingoing sodium nitrite for the bologna as formulated; the cultured celery juice powder (VegStable 506) ingredient (CJP-CF and CJP-NEF groups) contained 22,500 ppm sodium nitrite, which resulted in 99 ppm ingoing sodium nitrite; and the Natpre T-10 EML Plus S ingredient (NT10-CF and NT10-NEF groups) contained 1,700 ppm sodium nitrite, which resulted in 21 ppm ingoing sodium nitrite. Nitrite concentrations of Modern Cure and celery juice powder were provided by respective commercial ingredient suppliers, and the nitrite concentration of Natpre T-10 EML Plus S was determined by analysis using AOAC Method 993.30 (AOAC International, 2005a). The ingoing nitrite concentrations were determined by calculation

based on quantities of the respective ingredients used (USDA, 1995).

$$\text{Ingoing ppm} = ((\text{weight of cure}) \times \% \text{nitrite in cure mix}) \\ \times 1,000,000 / \text{green weight of meat block}$$

All nitrite concentrations were determined and expressed as sodium nitrite. Treatment formulations are listed in Table 1. The experiment was replicated twice.

The beef used for this study was harvested from market weight animals obtained from university farms, fabricated and frozen by the Iowa State University Meat Laboratory. The meat was stored frozen at -20°C until use, then thawed at 4.4°C for 2 d and moved into refrigerated storage at 1°C for 24 to 72 h. All treatments were processed separately using the same procedure. Replications were manufactured on separate, consecutive days, and the treatment processing sequence was randomized prior to production. Manufacturing and thermal processing of all treatments occurred on the same day for each replication. The slicing and packaging order of each treatment was conducted in the same order as production.

Beef raw materials were first ground through a 12.7-mm plate (Biro Manufacturing Company, Marblehead, OH). Each formulation consisted of 45.36 kg of meat including 14.97 kg of beef 50s and 30.39 kg of beef 80s. Lean (beef 80s) and fat (beef 50s) portions were mixed separately using a double action mixer (Leland Southwest, Fort Worth, TX) to ensure uniform

distribution of fat and lean after grinding and to create a uniform blend of each. The lean ground beef (beef 80s) was then added to a vacuum bowl chopper (Kramer & Grebe GmbH and Co., KG, Bienenkopf-Wallau, Germany) along with salt, curing ingredient, half of the water/ice mixture, spices (A.C. Legg Inc., Calera, AL), and cure accelerator (if needed). For the experimental treatments of CON-CF, CJP-CF, and CJP-NEF, the cure accelerator (sodium erythorbate or cherry powder) was added to the spice blend and mixed prior to use. A cure accelerator was not included in the Natpre T-10 EML Plus S formulation. The mixtures were chopped until the batter temperature reached 4.4°C . The fat trimmings (beef 50s) were then added, along with remaining water/ice mixture, and chopping continued until the batter temperature reached 13°C . The meat batters were then moved to a vacuum stuffer (Handtmann VF 608 Plus, Lake Forest, IL) and stuffed into $14.25 \text{ cm} \times 114.3 \text{ cm}$ pre-stuck fibrous casings (Kalle: 6.5×45 Fibrous N Clear, Kalle, Wiesbaden, Germany). Each bologna log was individually weighed, placed horizontally on a smoke rack, and moved into a smokehouse (Alkar, The Middleby Corporation, Elgin, IL) for thermal processing, which included application of smoke. All treatments were thermally processed together in the smokehouse for each replication. Thermal processing utilized a standard large-diameter bologna thermal processing schedule with stepwise temperature increases to reach internal product temperature of 71°C (approximately 6 h).

After cooking, the bologna logs were chilled overnight (approximately 19 h) at $1 \pm 2^{\circ}\text{C}$. Subsequently, bologna logs were weighed for cooked and chilled yield measurements, casings were removed, and the product was sliced (Bizerba, Piscataway, NJ) into 6.35-mm-thick slices and packaged. Four slices were stacked and placed into either conventional vacuum packages (CON-CF, CJP-CF, NT10-CF) (2-mil thick with an oxygen transmission rate of $1.5\text{--}3.5 \text{ cm}^3/0.06 \text{ m}^2/24 \text{ h}/1 \text{ atm}$) at 5°C and 0% relative humidity [RH]; water vapor transmission rate of $0.3\text{--}0.6 \text{ g}/0.06 \text{ m}^2/24 \text{ h}/1 \text{ atm}$ at 38°C and 100% RH; Cryovac, Sealed Air Corporation, Duncan, SC) or nitrite-embedded film vacuum packages (CJP-NEF, NT10-NEF) (7-mil thick with an oxygen transmission rate of $<0.3 \text{ cm}^3/0.06 \text{ m}^2/24 \text{ h}/1 \text{ atm}$ at 23°C and 0% RH; water vapor transmission rate of $<0.5 \text{ g}/0.06 \text{ m}^2/24 \text{ h}/1 \text{ atm}$ at 38°C and 100% RH; FreshCase, Bemis Company Inc., a division of Amcor Flexibles North America, Oshkosh, WI). The nitrite-embedded film was the same as that approved for use with fresh meat and included 113 mg of sodium nitrite per square meter

Table 1. Formulations for bologna treatments (as percent of meat block)

Ingredient	CON-CF ¹	CJP ²	NT10 ³
Beef 80 trim	67.00	67.00	67.00
Beef 50 trim	33.00	33.00	33.00
Water/ice	20.00	20.00	20.00
Salt	2.000	2.00	2.00
Spices	3.31	3.31	3.31
Modern Cure (6.25% NO ₂)	0.25		
Natpre T-10 EML Plus S			1.25
VegStable 506		0.44	
Sodium erythorbate	0.05		
VegStable 515		0.50	

¹CON-CF = control (1) conventionally cured and vacuum packaged in conventional film.

²CJP = formulation for treatments (2) CJP-CF and (3) CJP-NEF = alternatively cured with cultured CJP and packaged in conventional or nitrite-embedded film.

³NT10 = formulation for treatments (4) NT10-CF and (5) NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and packaged in conventional or nitrite-embedded film.

CJF = celery juice powder.

of film. Both package types (conventional film, nitrite-embedded film) were vacuum sealed (Ultravac UV 2100 vacuum chamber packaging machine, UltraSource LLC, Kansas City, MO) and immediately stored at $1 \pm 2^\circ\text{C}$ under continuously lighted (24 h/d) simulated retail display conditions using white fluorescent lights (32W, 120V; Sylvania, Danvers, MA) for the duration of the study. Packages were placed in single layers on shelves, with each shelf having fluorescent lights suspended immediately above the packages. The light source was placed approximately 254 mm from the package surface, and illuminance was $2,200 \pm 500$ lx. Illuminance was measured using an URCERI Light Meter MT-912 (URCERI, Shenzhen Huanhui E-commerce company, Ltd, Shenzhen, China). Multiple locations throughout the storage area were selected for illuminance measurements. Sample locations were routinely rotated (every 3 wk) to provide uniform light exposure. Product packaging day was considered day 0 of the experiment.

Analytical procedures: Color

Surface color and internal color of the stacks of slices were measured in 2 ways using Commission Internationale de l'Eclairage (CIE) $L^*a^*b^*$ color space (L^* = lightness; a^* = redness; b^* = yellowness). For in-package color, measurements were done with the packaging film in place on the products. Unpackaged color was measured on the products after opening the packages and removing the packaging film. In both cases, color measurements were done on days 1, 6, 13, 27, 41, 55, 69, 83, 97, 111, and 125 following packaging. All color measurements were taken at a 10° observer angle, using illuminant D65 (daylight at 6,500 K), and a 2.4-cm aperture size, with a HunterLab MiniScan EZ 4500L colorimeter (Hunter Associates Laboratory Inc., Reston, VA). For unpackaged color measurements, a standard instrument calibration (with no film covering the black or white tiles) was used, and measurements were conducted by placing the HunterLab MiniScan EZ instrument nose cone directly onto the product surface. For the in-package surface measurements, a modified colorimeter standardization procedure was utilized, in which the white calibration tile was covered with the respective packaging material (conventional film or nitrite-embedded film). Product measurements were then taken by placing the nose cone of the HunterLab MiniScan EZ instrument directly onto the package. This process was used to more accurately measure the visual color of the packaged product as observed by consumers.

For unpackaged products, both surface and internal color were measured. One package per treatment (5), per sample day (11), per replication (2) was randomly selected for both surface and internal color measurement for a total of 110 packages measured during the display period. Measurements were done by opening the package, removing the slices, and subsequently measuring the surface of the top slice (slice directly exposed to light source) for unpackaged surface color as well as measuring the color at the interface of slices 2 and 3 of the 4-slice stack for the internal color measurement.

For in-package surface color (with the packaging film intact), 22 packages of each treatment and each replication were selected for repeated measurement of the same designated packages on each sample date for the duration of the display period. This generated a total of 220 measurements (22 packages \times 5 treatments \times 2 replications) of in-package color on each sampling day of the 125-d display period. The mean of the 3 measurements on each of the 220 packages for both in-package and unpackaged products was calculated and used to represent the color values of each package at each time point of the display period.

Analytical procedures: Residual nitrite

Residual nitrite analysis was done in accordance with AOAC Method 973.31 (AOAC International, 2005b). Sampling was conducted in duplicate on days 1, 6, 13, 27, 41, 55, 69, 83, 97, 111, and 125 post packaging. Samples were prepared by separating the 2 exterior slices (slices 1 and 4 in the stack) from the 2 interior slices (slices 2 and 3 in the stack) of each package. The exterior and interior slices were finely chopped separately using a food processor (KitchenAid, St. Joseph, MI), and 5.0 g (± 0.01 g) was weighed into a beaker. Each 5.0 g sample was then added to a 500 mL volumetric flask with approximately 300 mL hot (approximately 50°C – 80°C) distilled water and placed into a 100°C water bath. Flasks remained in the water bath for 2 h and were swirled every 30 min. After heating, the flasks were cooled to room temperature (approximately 23°C), then filled to volume (500 mL) with distilled water. Approximately 30 mL was filtered through Whatman #1 filter paper (Whatman Grade 1, GE Health Care Life Sciences, Pittsburgh, PA) into a 50 mL volumetric flask. Next, reagents were added as described in the AOAC International procedure, flasks were filled to volume, and absorbance at 540 nm was recorded using a Beckman DU 640 spectrophotometer (Model 4320940; Beckman, Fullerton, CA).

Analytical procedures: Residual nitrate

Residual nitrate analysis was conducted by Hormel Laboratories (Division of Hormel Foods, LLC, Austin, MN) using AOAC Method 993.30 (AOAC International, 2005a). Sampling was conducted on days 1, 6, 13, 27, 41, 55, 69, 83, 97, 111, and 125 post packaging. On each sampling day, collected samples were immediately frozen at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and shipped to Hormel Laboratories in insulated shipping containers with ice packs. The samples were held frozen until analysis and thawed overnight prior to sample preparation. Thawed samples were prepared by first separating the 2 exterior slices from the two interior slices. The exterior and interior slices were homogenized separately using a commercial grade food processor. One gram of each sample was weighed into a 100 mL Kohlrausch volumetric flask, and 50 mL of distilled hot water (approximately 50°C – 80°C) was added. Flasks were placed on a 100°C steam bath for 1 h and chilled in ice water (approximately 0°C) for 15 min. After chilling to room temperature (approximately 23°C), the flasks were filled to volume and shaken. Approximately 30 mL of sample was filtered through Whatman GF/C filter paper (Whatman Grade 1, GE Health Care Life Sciences, Pittsburgh, PA), and the filtrate was again filtered under vacuum through a Dionex OnGuard II RP 2.5 cc cartridge (Thermo Fisher Scientific, Inc., Waltham, MA) equipped with a 10-mL syringe pre-treated with 10 mL of methanol and 15 mL of distilled water. The first 6 mL of sample filtrate was discarded to ensure proper flushing of methanol and distilled water. A multisample Restek Resprep 12-Port Vacuum Manifold (Restek Corporation, Bellefonte, PA) was used under vacuum to ensure proper sample flow rate. Sample flow rate through the cartridge was approximately 1 mL per minute to provide a 1:100 dilution factor. The samples were then added to a Dionex High Pressure Ion Chromatography system (Thermo Fisher Scientific, Inc., Waltham, MA). Residual nitrate peaks from the resulting chromatograms were plotted, and the area under each peak used to calculate concentration in ppm.

Analytical procedures: Proximate composition

Duplicate samples of raw batters and cooked products from each production batch were finely chopped using a food processor (KitchenAid, St. Joseph, MI) for measurement of fat, moisture, and protein content. Fat content was analyzed by the

CEM ORACLE System (AOAC International, 2013; CEM Corporation Matthews, NC), moisture was evaluated with the CEM SMART 6 System (AOAC International, 2013; CEM Corporation), and protein content was measured with the CEM Sprint Rapid Protein Analyzer (AOAC International, 2011; CEM Corporation).

Analytical procedures: pH

Samples of raw batters and cooked products from each treatment were finely chopped using a food processor, as done for the proximate composition measurements. Ten grams of each sample were weighed into a beaker, and 90 mL of ambient-temperature distilled water were added. The meat and water were mixed thoroughly by hand with a glass stirring rod for 1 min, after which a filter paper disc (Whatman Grade 1, GE Health Care Life Sciences, Pittsburgh, PA) was folded and submerged into the sample. The pH of the filtered solution was measured with a Mettler Toledo SevenMulti pH meter (Mettler Toledo, Columbus, OH). Duplicate measurements of raw batters were done on the day of production, whereas the cooked products were measured in duplicate on day 14 following thermal processing and chilling of the products. The pH of some of the nonmeat ingredients included in the formulations was measured by first dispersing 5 g of each ingredient in 90 mL of distilled water, then measuring the pH as described previously.

Analytical procedures: Microbiology

Microbial populations of aerobic bacteria and of lactic acid bacteria on the products were monitored utilizing a shelf life procedure in which all treatments and replications were stored at $1^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The analyses were conducted on days 0, 7, 14, 30, 60, 90, and 120. Eleven grams of sample was aseptically removed from each package, and 99 mL of 0.1% peptone water (Hardy Diagnostics, Category Number D299, Santa Maria, CA) was added, creating a 1:10 dilution. The samples were then placed into a WhirlPak filter stomaching bag (Nasco, Ft. Atkinson, WI), homogenized in a stomacher with the peptone water for 1 min, and serially diluted, with the first 10-fold dilution coming from the stomaching bag and the remaining dilutions (to extinction) consisting of 1 mL into 9 mL of 0.1% peptone water. Subsequently, total aerobic bacterial populations were enumerated in duplicate on Aerobic Count Petrifilm (3M Health Care, St. Paul, MN) following the manufacturer's

instructions. Petrifilms were incubated aerobically at 21°C for 72 h to assess total aerobes, and populations were determined following the manufacturer's instructions. For lactic acid bacteria populations, enumeration was conducted anaerobically on the same days as the total aerobic populations and followed the procedure previously described, but with 0.1 mL of homogenized sample surface plated onto DeMan, Rogosa and Sharpe (MRS) agar (Becton, Dickinson and Company, Sparks, MD) in duplicate. Plates were incubated anaerobically at 31°C ± 2°C for 72 h before counting.

Due to the low bacterial growth observed during conventional storage at 1°C, an additional accelerated shelf life analysis was conducted to test the impact of the packaging environment on potential growth of spoilage bacteria. To achieve this, a spoilage inoculum of lactic acid bacteria was isolated from samples of commercial bologna. To create the inoculum, packages of 5 different commercial bologna products were purchased from retail stores. Two slices (approximately 60 g) were removed from each package, added to a bag with 1 L of tryptic soy broth (Becton, Dickinson and Company), and allowed to incubate at 20°C for 72 h. After incubation, 1 mL of broth was removed, added to 9 mL of MRS broth (Becton, Dickinson and Company), and incubated at 30°C for 48 h; 1 mL of the inoculated broth was then transferred to an additional 9 mL of MRS broth and again incubated at 30°C for 48 h. This bacterial transfer process was repeated 4 times. Finally, 1 mL of culture was added to 40 mL of MRS broth and incubated at 30°C for 48 h. Serial dilutions (to extinction) were made from the final culture and plated onto MRS agar to enumerate the population of the lactic acid bacteria inoculum. The culture was held at 1°C during this time. The culture was then diluted to achieve an approximate inoculation population of log₁₀3 colony-forming units (CFU)/g in the packages. The packages were inoculated with 1 mL of the spoilage inoculum by aseptic injection through a self-closing foam adhesive septum placed on each package. The inoculated packages were held at 10°C for the duration of the inoculation study. Inoculation occurred on day 158 post processing of the previously described products. For the inoculation study (denoted as I), day 158 post processing was designated as day 0(I). Analyses were conducted on days 0(I), 3(I), 6(I), 9(I), 12(I), 18(I), and 21(I) of storage at 10°C ± 2°C.

All microbiological data (conventional and inoculated shelf life) were collected and reported logarithmically as CFU/g.

Statistical Analysis

The study was replicated twice, with replications produced on separate but consecutive days. The experiment was designed as a randomized complete block design with treatment (CON-CF, NT10-CF, CJP-CF, NT10-NEF, CJP-NEF) and replication as whole-plot factors and day as subplot factor, with factors arranged in a (2 × 2) + 1 arrangement. The data were analyzed using a mixed model with SAS version 9.4 (SAS Institute Inc., Cary, NC) in which treatment and day were fixed factors and replication was a random factor and the interaction of treatment × day was investigated. In addition, a Tukey-Kramer pairwise adjustment was included for repeated measures. Statistical significance was determined at $P < 0.05$, and consequently, P values are generally not repeated when significance is described in the Results and Discussion, although Table 2 displays the actual P values for the main effects of treatment, day, and interaction of treatment × day for each dependent variable. Bacterial populations were transformed to log CFU/g, and direct comparisons were made between treatments during storage.

Results

Raw composition, processing attributes, and final composition

Mean treatment effects on proximate composition of raw and cooked bologna in addition to cooked yields (Table 2) showed no significant difference ($P = 0.133$; Table 3) for protein content of the raw products between any treatments. Fat content of raw CJP-CF and CJP-NEF treatments were not different, but both treatments had higher fat content than CON-CF, NT10-CF, and NT10-NEF treatments. Moisture content was higher for raw CON-CF than for all other treatments (CJP-CF, CJP-NEF, NT10-CF, and NT10-NEF), but there were no significant differences ($P > 0.20$) between the other treatments.

For cooked products, fat, moisture, and protein content in samples of the treatment groups followed trends similar to those of the raw product composition. Because there was relatively little numerical difference between treatments for raw or cooked composition, the composition was not expected to affect the outcome of this study. Cooked and chilled yield results were all within approximately 1% among the treatments, though the Natpre T-10 EML Plus S treatments resulted

Table 2. Means for raw and cooked proximate composition and yields of bologna treatments

	Raw			Cooked			
	% Moisture	% Fat	% Protein	% Moisture	% Fat	% Protein	% Yield
CON-CF	60.9 ^a	21.8 ^b	13.6	57.6 ^a	24.7 ^b	16.8 ^a	92.3 ^a
CJP-CF	59.9 ^b	23.0 ^a	13.4	56.6 ^b	25.8 ^{ab}	16.1 ^b	91.6 ^a
CJP-NEF	59.9 ^b	23.0 ^a	13.4	57.0 ^{ab}	25.0 ^{ab}	16.1 ^b	91.6 ^a
NT10-CF	60.3 ^b	21.5 ^b	13.7	56.6 ^b	25.8 ^a	15.9 ^b	91.6 ^b
NT10-NEF	60.3 ^b	21.5 ^b	13.7	56.5 ^b	25.1 ^{ab}	16.4 ^{ab}	91.6 ^b
SEM	0.01	0.01	0.00	0.01	0.00	0.00	0.00

^{a-b}Means in the same column with different letters are significantly different ($P < 0.05$).

CON-CF = control, conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

SEM = standard error of mean.

Table 3. *P* values of fixed main effects and interaction¹

Dependent Variable	Treatment	Day	Treatment × Day
Raw fat, %	0.012	—	—
Raw moisture, %	0.008	—	—
Raw protein, %	0.133	—	—
Cooked fat, %	0.162	—	—
Cooked moisture, %	0.083	—	—
Cooked protein, %	0.027	—	—
Yield, %	0.002	—	—
Raw pH	0.115	—	—
Cooked pH	<0.001	—	—
In-package surface color L*	<0.001	<0.001	0.004
In-package surface color a*	<0.001	<0.001	<0.001
In-package surface color b*	<0.001	<0.001	<0.001
Unpackaged surface color L*	0.002	0.542	0.908
Unpackaged surface color a*	<0.001	<0.001	0.014
Unpackaged surface color b*	<0.001	<0.001	0.760
Internal color L*	<0.001	0.172	0.957
Internal color a*	<0.001	<0.001	<0.001
Internal color b*	<0.001	<0.001	<0.001
Surface residual NO ₂ ppm	<0.001	<0.001	<0.001
Internal residual NO ₂ ppm	<0.001	<0.001	0.002
Surface residual NO ₃ ppm	<0.001	<0.001	0.794
Internal residual NO ₃ ppm	<0.001	<0.001	0.955
LAB ² 120-d growth	0.673	0.234	0.535
LAB ² inoculated 21-d growth	<0.001	<0.001	0.409
TAB 120-d growth	0.021	0.004	0.059
TAB inoculated 21-d growth	0.002	<0.001	0.176

¹Alpha level of 0.05 used for statistical significance. $P < 0.05$ shown in bold.

²LAB = Lactic Acid Bacteria.

³TAB = Total Aerobic Bacteria.

ppm = parts per million.

in a small but significant reduction of the cooked yield for those products.

Table 4 shows that there was no difference ($P = 0.115$) between treatments for pH of the raw products. However, cooked pH was significantly lower ($P < 0.001$) in NT10-CF and NT10-NEF treatments than in the other treatments. The pH measurements of the nonmeat ingredients showed that the Natpre T-10 EML Plus S ingredient was considerably more acidic (pH 4.76) than Modern Cure (pH 7.84), sodium erythorbate (pH 7.50), celery juice powder (pH 8.67), or cherry powder (pH 6.03) and probably resulted in

Table 4. Means for raw and cooked pH for bologna treatments at day 14 post processing

	Raw pH	Cooked pH
CON-CF	6.16	6.19 ^a
CJP-CF	6.19	6.17 ^a
CJP-NEF	6.19	6.17 ^a
NT10-CF	6.15	6.09 ^b
NT10-NEF	6.15	6.07 ^b
SEM	0.03	0.11

^{a-b}Means in the same column with different letters are significantly different ($P < 0.05$).

CON-CF = control, conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

SEM = standard error of the mean.

the lower pH of the cooked products. The reduced product pH may have contributed to the lower cooking yields observed (Table 2) for those treatments.

In-package surface color

Table 5 shows the main effects for treatments during 125-d display, which were significant ($P < 0.001$); however, main effects are only briefly described later since the interaction was also significant ($P < 0.001$). Nitrite-embedded film resulted in significantly greater in-package surface a^* values (redness) in both the celery juice powder and Natpre T-10 EML Plus S formulations. Further, the CJP-NEF treatment was also significantly redder than the control despite a lower ingoing nitrite concentration (99 ppm vs. 156 ppm). However, the nitrite-embedded film treatment had the greatest impact on redness in the case of the two Natpre T-10 EML Plus S products in which ingoing nitrite was extremely low (21 ppm). The decreased redness resulting from the low nitrite concentration in the conventional film package of the NT10-CF ($a^* = 7.34$) in combination with the observed increased redness in the NT10-NEF treatment ($a^* = 12.02$) highlights the benefit of nitrite-embedded film. There was no difference for in-package surface a^* values for the conventional film treatments (CON-CF and CJP-CF) with ingoing nitrite concentrations of 156 and 99 ppm, respectively. The relative treatments effects for in-package surface a^* values were in the

following order: CJP-NEF > CJP-CF and CON-CF > NT10-NEF > NT10-CF (Table 5).

Figure 1 shows the in-package surface a^* values for treatment \times day effects. The impact of the nitrite-embedded film package on the very low ingoing nitrite formulation (NT10-NEF) compared to the conventional film package (NT10-CF) is clear, with a^* values for the NT10-NEF treatment approaching the other treatments that included much greater ingoing nitrite in the formulations. While not specifically denoted in Figure 1, the increase in NT10-NEF a^* value between 1 d and 27 d was significant ($P = 0.010$), suggesting an increase in cured pigment content during the first 27 d while in contact with the film for NT10-NEF. CJP-CF most notably showed significantly ($P < 0.001$) reduced a^* values between 1 d and 83–125 d of storage, while its nitrite-embedded film counterpart did not show any significant reduction from 1-d a^* value until after day 111. There was a significant decline (from day 1) toward the end of lighted display period (as would be expected) for CJP-NEF (after day 111), CON-CF (after day 83), and CJP-CF (after day 55). Treatment means for in-package surface L^* were significantly different as a result of the curing ingredients (NT10-CF and NT10-NEF > CON-CF > CJP-CF and CJP-NEF; Table 5) but not as a result of the packaging film. In-package surface L^* values for treatment \times day effects did not differ over time for any treatment (data not shown).

Table 5. Means for in-package surface color values for bologna treatment effects during 125-d lighted display

	L^*	a^*	b^*
CON-CF	65.09 ^b	13.76 ^b	16.21 ^c
CJP-CF	64.24 ^c	13.56 ^b	17.11 ^b
CJP-NEF	63.99 ^c	14.44 ^a	16.09 ^c
NT10-CF	65.73 ^a	7.34 ^d	20.70 ^a
NT10-NEF	65.71 ^a	12.02 ^c	16.82 ^b
SEM	1.70	0.84	0.09

^{a-d}Means in the same column with different letters are significantly different ($P < 0.05$).

CON-CF = control, conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

SEM = standard error of the mean.

Unpackaged surface and internal color

The results for unpackaged surface and internal CIE L^* , a^* , and b^* measurements for treatment effects are shown in Table 6 and are, in general, similar to the results observed for in-package surface color measurements. There were no differences between CJP-NEF, CJP-CF, and CON-CF or between CJP-CF and NT10-NEF for unpackaged surface a^* value. The Natpre T-10 EML Plus S treatments with very low ingoing nitrite again demonstrated the impact of nitrite-embedded film packaging for increasing product redness, but the nitrite-embedded film in this case did not have a significant effect on the formulation with celery juice powder, as it did for the in-package a^* values.

Unpackaged surface a^* values for treatment \times day effects are displayed in Table 7. There were no significant differences for CJP-NEF, CON-CF, or NT10-CF over the lighted display period (125 d). Most notable, again, is the effect of the nitrite-embedded film on the low nitrite product (Natpre T-10 EML Plus S).

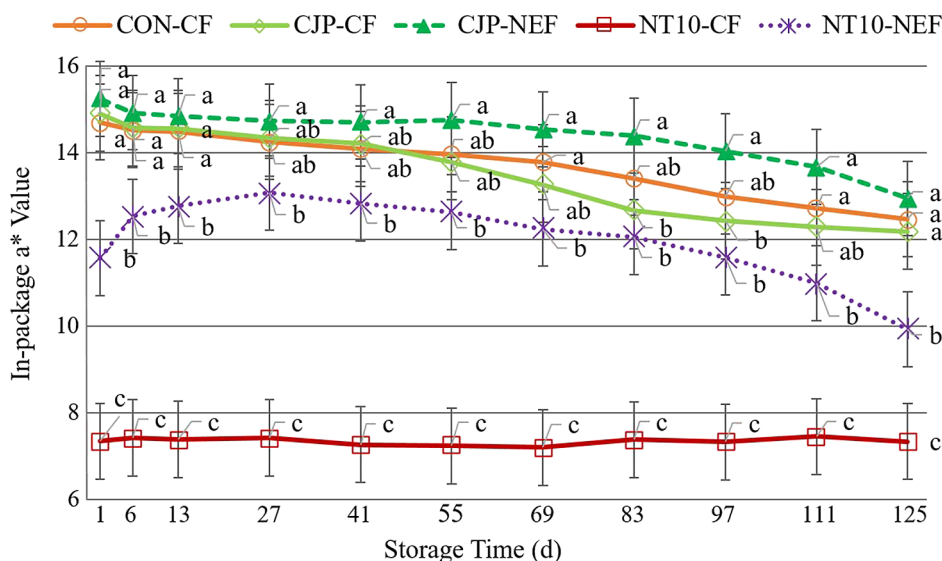


Figure 1. In-package surface a* value for bologna treatment × day effects during lighted display. CON-CF = control, conventionally cured and vacuum packaged in conventional film; CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film; CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film; NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film; NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film. Error bars represent ± standard error of the mean (SEM; 0.87). ^{a-c}Means from the same day with different letters are significantly different (*P* < 0.05).

Table 6. Means for unpackaged surface and internal color values for bologna during 125-d lighted display

	Unpackaged Surface			Internal		
	L*	a*	b*	L*	a*	b*
CON-CF	63.34 ^{ab}	13.74 ^a	15.79 ^b	64.45 ^{bc}	13.77 ^a	14.78 ^d
CJP-CF	63.12 ^{ab}	13.05 ^{ab}	16.67 ^b	63.79 ^c	13.82 ^a	15.45 ^c
CJP-NEF	62.64 ^b	14.06 ^a	16.14 ^b	63.52 ^c	13.91 ^a	15.34 ^c
NT10-CF	64.49 ^a	7.09 ^c	19.43 ^a	66.49 ^a	7.19 ^c	17.99 ^a
NT10-NEF	64.53 ^a	11.43 ^b	16.77 ^b	65.62 ^{ab}	10.41 ^b	16.17 ^b
SEM	1.68	0.83	0.26	1.54	0.60	0.13

^{a-d}Means in the same column with different letters are significantly different (*P* < 0.05).

CON-CF = control, conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

SEM = standard error of the mean.

The nitrite-embedded film package resulted in greater a* value than the conventional film for the Natpre T-10 EML Plus S formulation at all time points after day 1. Figure 2 shows the visual difference between NT10-CF and NT10-NEF unpackaged surface redness between

day 1 and 41. The visual appearance of both external and internal slice surfaces from nitrite-embedded film packages appear to be slightly redder at day 1 but are clearly redder at day 41, suggesting that further formation of nitrosylhemochrome pigment from nitrite and cooked meat pigments occurred during storage in the nitrite-embedded film package.

The internal a* values for treatment effects are also presented in Table 6 and show similar results as for unpackaged surface a* values. The nitrite-embedded film again resulted in significantly greater internal a* value than the conventional film for the Natpre T-10 EML Plus S (very low nitrite formulation) but lower internal a* values than the CJP-NEF, CON-CF, and CJP-CF treatments, all of which included greater ingoing nitrite concentrations.

Internal a* values for treatment × day effects also did not differ among CJP-NEF, CJP-CF, CON-CF, or NT10-CF treatments during the display period (125 d) (Table 8), but internal a* values for NT10-NEF increased from day 1 to day 27, with no further increase following day 27. It is notable that the nitrite-embedded film package resulted in increased a* values over time in the cooked, finished product with very low nitrite ingoing concentration, suggesting significant formation of cured pigment from the nitrite embedded in the film, after packaging was completed.

There were no differences between NT10-CF, NT10-NEF, CON-CF, or CJP-CF for unpackaged

Table 7. Surface a^* values for unpackaged bologna treatment \times day effects during 125-d lighted display

	Day 1	Day 6	Day 13	Day 27	Day 41	Day 55	Day 69	Day 83	Day 97	Day 111	Day 125
CON-CF	14.50 ^a	14.35 ^a	14.46 ^a	14.32 ^a	14.11 ^a	14.25 ^a	13.67 ^a	13.15 ^a	12.96 ^a	12.71 ^a	12.68 ^{ab}
CJP-CF	14.33 ^{av}	14.29 ^{av}	14.25 ^{av}	14.11 ^{av}	13.61 ^{av}	13.59 ^{avw}	13.15 ^{avw}	12.52 ^{avwx}	11.84 ^{avwx}	11.18 ^{avwx}	10.68 ^{ax}
CJP-NEF	14.68 ^a	14.56 ^a	14.23 ^a	14.23 ^a	14.33 ^a	14.20 ^a	13.85 ^a	13.85 ^a	14.08 ^a	13.32 ^a	13.35 ^{ab}
NT10-CF	6.59 ^b	7.31 ^b	7.00 ^b	7.06 ^b	7.17 ^b	7.02 ^b	7.22 ^b	7.34 ^b	7.21 ^b	7.07 ^b	7.05 ^b
NT10-NEF	9.94 ^{bv}	11.75 ^{aw}	12.43 ^{aw}	12.99 ^{aw}	12.50 ^{aw}	12.08 ^{avw}	10.96 ^{avw}	11.20 ^{avw}	11.10 ^{avw}	10.82 ^{avw}	9.99 ^{avw}

^{a-b}Means in the same column with different letters are significantly different ($P < 0.05$).

^{v-x}Means in the same row with different letters are significantly different ($P < 0.05$).

CON-CF = control, conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

SEM = standard error of the mean (0.91).

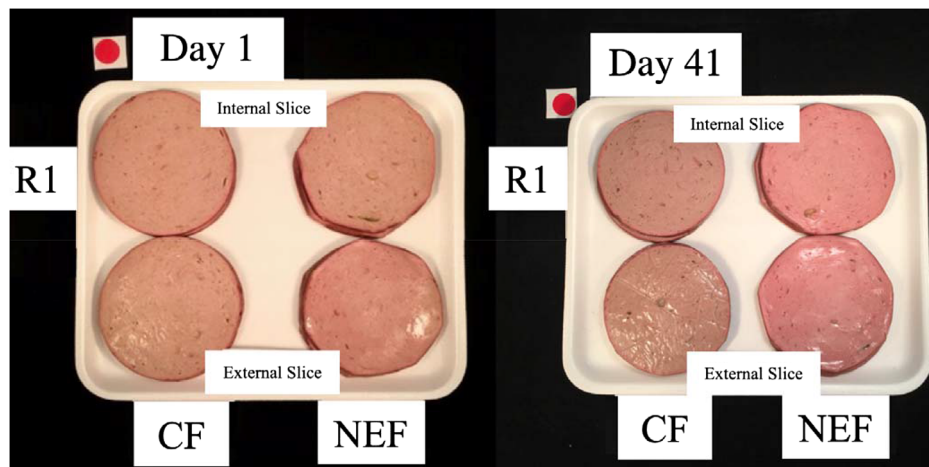


Figure 2 Visual appearance of the unpackaged surface of NT10-CF and NT10-NEF treatments opened at day 1 or day 41 of lighted display storage. NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film; NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

Table 8. Internal a^* values for bologna treatment \times day effects during 125-d lighted display

	Day 1	Day 6	Day 13	Day 27	Day 41	Day 55	Day 69	Day 83	Day 97	Day 111	Day 125
CON-CF	13.84 ^a	14.04 ^a	13.67 ^a	13.79 ^a	13.79 ^a	13.79 ^a	13.75 ^a	13.56 ^a	13.96 ^a	13.70 ^{ab}	13.59 ^{ab}
CJP-CF	13.91 ^a	13.80 ^a	13.81 ^a	14.00 ^a	13.74 ^a	13.69 ^a	13.82 ^a	13.73 ^a	13.73 ^a	13.90 ^a	13.87 ^a
CJP-NEF	13.83 ^a	14.24 ^a	13.85 ^a	13.90 ^a	13.76 ^a	13.76 ^a	14.01 ^a	13.80 ^a	14.10 ^a	13.82 ^a	13.94 ^a
NT10-CF	6.42 ^b	7.12 ^b	6.96 ^c	7.44 ^c	7.34 ^c	7.53 ^c	7.38 ^c	7.23 ^a	7.11 ^c	7.56 ^c	7.01 ^c
NT10-NEF	6.86 ^{by}	7.70 ^{by}	9.43 ^{bw}	10.37 ^{bw}	11.04 ^{bv}	11.36 ^{bv}	11.24 ^{bv}	11.48 ^{bv}	11.47 ^{bv}	11.86 ^{bv}	11.75 ^{bv}

^{a-c}Means in the same column with different letters are significantly different ($P < 0.05$).

^{v-y}Means in the same row with different letters are significantly different ($P < 0.05$).

CON-CF = control, conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

SEM = standard error of the mean (0.65).

external surface L^* value treatment effects (Table 6). However, CJP-NEF was darker than either NT10-CF or NT10-NEF. Internal L^* values followed a similar trend with NT10-CF significantly lighter than CON-CF, CJP-CF, and CJP-NEF but not different than NT10-NEF.

Unpackaged surface b^* value (Table 6) for NT10-CF was higher than for all other treatments; however, there was no difference between the other treatments. Additionally, NT10-CF was also greater than the other treatments for internal b^* values. However, for the internal b^* values, although NT10-CF was again highest, there were differences between the other treatments. In this case, NT10-NEF was second highest, while CJP-CF and CJP-NEF followed, with CON-CF having the lowest b^* value.

Unpackaged surface and internal L^* values for treatment \times day effects during lighted display showed no difference ($P > 0.90$) between any other treatments during the display period (125 d) (data not shown). Internal b^* values for treatment \times day effects also did not differ in CJP-NEF, CJP-CF, CON-CF, or NT10-CF during the display period (125 d) but were lower for NT10-NEF for days 1 through 125 (data not shown).

Surface and internal residual nitrite

Slices that were in direct contact with the packaging material (slices 1 and 4 of each package) and internal slices that were in the center of each package (slices 2 and 3) were analyzed separately for residual nitrite. Slices 1 and 4 were combined to represent the residual nitrite in the surface slices, while slices 2 and 3 were combined to represent the internal residual nitrite concentration.

Surface residual nitrite values for treatment \times day effects are displayed in Figure 3A. The impact of formulated ingoing nitrite is clear with the control (CON-CF) and celery juice powder formulations resulting in the greatest concentration of residual nitrite on day 1. The CJP-NEF was slightly higher than the CJP-CF initially, but this was significant only on day 6. This does, however, again suggest that the nitrite-embedded film package transfers a small amount of nitrite to the product to potentially improve and extend cured color stability during product storage. NT10-CF and NT10-NEF, on the other hand, were consistently very low in residual nitrite and did not differ at any day of the display period. It is notable that the nitrite-embedded film package did not increase the measurable residual nitrite in the product with very low ingoing nitrite concentration. CJP-CF was not different from CON-CF or

CJP-NEF except at day 6. None of the treatments were different at day 27 and after.

Internal residual nitrite concentrations as a result of treatments are shown in Figure 3B, and the results are very similar to those for surface residual nitrite. Table 9 shows the means for treatment effects and confirms the similarity of the surface and internal nitrite concentrations. Consequently, while the nitrite-embedded film packages impacted the redness (a^* value) of the product produced with very low ingoing nitrite concentration, the film did not contribute to the residual nitrite concentration in the product.

Surface and internal residual nitrate

Surface and internal residual nitrate for each treatment are shown in Table 10. The internal nitrate concentration for celery juice powder formulations was greater than all the other products, probably due to increased nitrate concentrations commonly found in cultured celery juice powder. Surface nitrate concentrations followed a similar pattern though differences are not as clearly defined. There were no treatment or day effects for either surface or internal residual nitrate during the 125-d display period (data not shown).

Microbiological results

The total aerobic bacterial populations (log CFU/g) in conventional shelf life conditions (1°C) showed essentially no growth in any of the treatments during the 120 d of storage (data not shown). However, there was a treatment effect at day 30, with NT10-NEF being significantly higher for total aerobic counts than CON-CF but not significantly different from NT10-CF, CJP-CF, or CJP-NEF. Given that this does not reoccur after day 30, this was most likely due to sampling error or some other procedural effect rather than a treatment effect. Lactic acid bacteria populations (log CFU/g) for conventional shelf life conditions also showed no measurable counts for any of the treatments (data not shown).

Table 11 shows the overall lactic acid bacteria populations (log CFU/g) for the inoculated study (10°C for 21 d). The CON-CF, CJP-CF, and CJP-NEF treatments had significantly greater mean lactic acid bacterial populations than NT10-CF and NT10-NEF, though the differences were less than 0.37 log CFU/g. For treatment \times day effects, while growth occurred in all treatments for total aerobic and lactic acid bacteria populations (log CFU/g), there were no significant differences between any treatment at any specific day (data not shown). Lactic acid bacterial populations

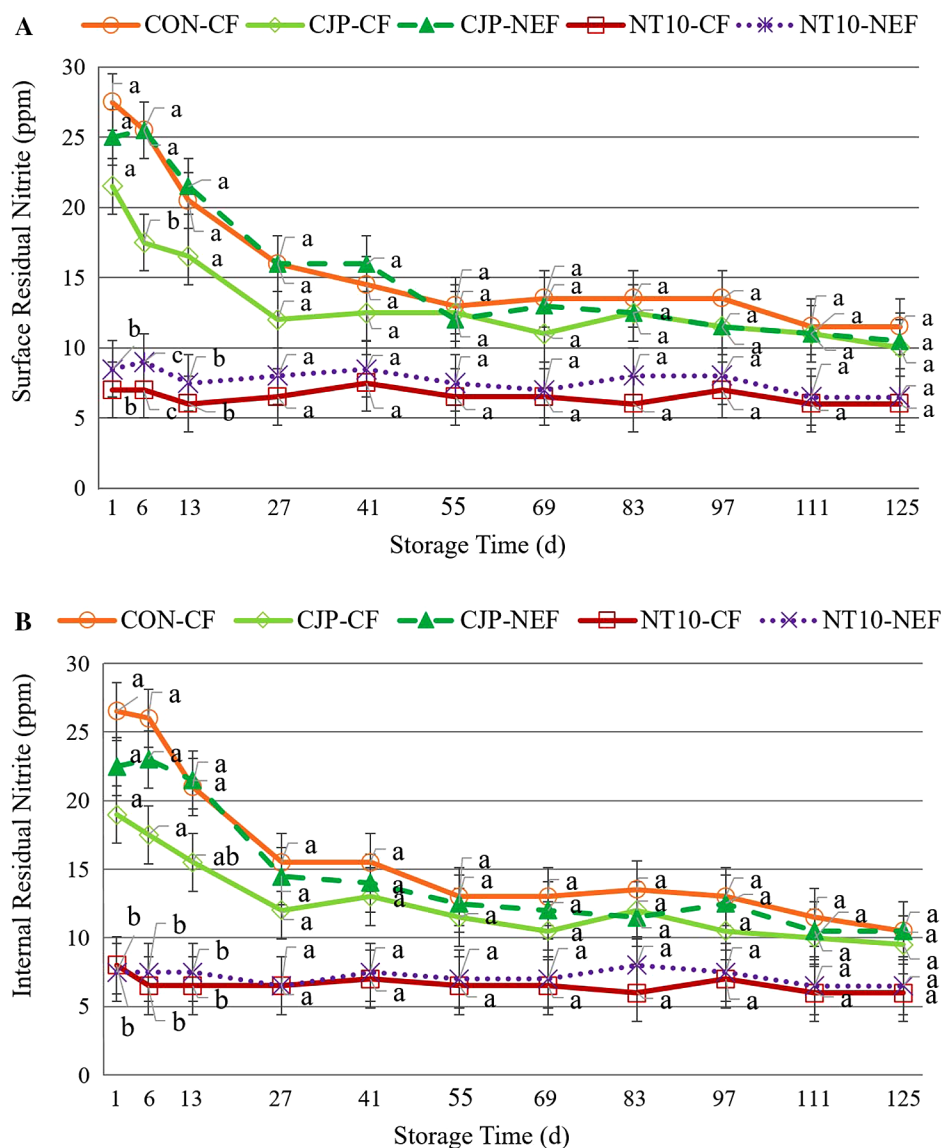


Figure 3 Residual nitrite (parts per million [ppm]) for bologna treatment × day effects under retail display. (A) Surface residual nitrite. (B) Internal residual nitrite. CON-CF = control, conventionally cured and vacuum packaged in conventional film; CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film; CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film; NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film; NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film. Error bars represent \pm standard error of the mean (SEM): (A SEM = 2.00), (B SEM = 2.10). ^{a-c}Means from the same day with different letters are significantly different ($P < 0.05$).

increased from log 3.70 CFU/g–3.95 CFU/g initially among the treatments to log 8.70 CFU/g–8.90 CFU/g, and total aerobic bacterial populations increased from log 2.80 CFU/g–3.25 CFU/g initially among treatments to log 7.90 CFU/g–8.20 CFU/g after 21 d at 10°C, respectively.

Discussion

Proximate composition of the bologna used in this study confirmed that formulation was consistent

among treatments with only minor differences observed for fat, moisture, and protein content. The small differences observed, though statistically significant, are not likely large enough or consistent enough to be truly impactful on the primary objectives of this study. The cooked yield of the Natpre T-10 EML Plus S formulations in both packaging treatments was lower than for the other formulations, likely due to the reduced pH of about 0.1-pH unit in that cooked product. However, the range in the cooked yield was just 0.69% from high to low. Natpre T-10 EML Plus S was the alternative cure replacement ingredient in

Table 9. Means for external surface and internal residual nitrite concentrations for bologna treatment effects under 125-d lighted display

	Surface Residual NO ₂ ppm	Internal Residual NO ₂ ppm
CON-CF	16.4 ^a	16.3 ^a
CJP-CF	13.5	12.8 ^a
CJP-NEF	15.9 ^a	15.0 ^a
NT10-CF	6.6 ^b	6.6 ^b
NT10-NEF	7.7 ^b	7.2 ^b
SEM	1.66	1.80

^{a-b}Means in the same column with different letters are significantly different ($P < 0.05$).

CON-CF = control, meat conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

SEM = standard error of the mean.

Table 10. External surface and internal residual nitrate concentrations for bologna treatment effects during 125-d lighted display

	Surface Residual NO ₃ ppm	Internal Residual NO ₃ ppm
CON-CF	40.2 ^{bc}	39.1 ^b
CJP-CF	46.6 ^a	46.3 ^a
CJP-NEF	45.5 ^{ab}	46.0 ^a
NT10-CF	33.6 ^d	33.4 ^b
NT10-NEF	38.6 ^{cd}	39.1 ^b
SEM	2.25	2.48

^{a-d}Means in the same column with different letters are significantly different ($P < 0.05$).

CON-CF = control, meat conventionally cured and vacuum packaged in conventional film.

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

ppm = parts per million; SEM = standard error of the mean.

the NT10-CF and NT10-NEF treatments; this ingredient contains fruit and spice extracts, and our analysis showed that it had a pH of 4.76, which could account for the lower product pH compared to the other

Table 11. Mean lactic acid bacteria populations (log CFU/g) for inoculated bologna treatment × day effects during 21-d display

	log CFU/g
CON-CF	6.53 ^a
CJP-CF	6.47 ^a
CJP-NEF	6.49 ^a
NT10-CF	6.10 ^b
NT10-NEF	6.10 ^b
SEM	0.09

^{a-b}Means in the same column with different letters are significantly different ($P < 0.05$).

CON-CF = control, conventionally cured and vacuum packaged in conventional film

CJP-CF = alternatively cured with cultured celery juice powder and vacuum packaged in conventional film.

CJP-NEF = alternatively cured with cultured celery juice powder and vacuum packaged in nitrite-embedded film.

NT10-CF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in conventional film.

NT10-NEF = alternatively cured with Natpre T-10 EML Plus S and vacuum packaged in nitrite-embedded film.

CFU = colony-forming units; SEM = standard error of the mean.

formulations. For comparison, Modern Cure and sodium erythorbate used as curing ingredients for the control product had pHs of 7.84 and 7.50, respectively. Additionally, there were no buffering ingredients (such as phosphates or carbonates) included in the formulations; therefore, an impact on pH from other ingredients such as curing ingredients and/or reductants would be more likely.

Products packaged in nitrite-embedded film resulted in greater in-package external a^* values (greater redness), which demonstrated the potential role for nitrite-embedded film to affect cooked, cured meat color. This suggests that the nitrite from the film is providing nitric oxide for the heat-denatured (cooked) myoglobin to generate improved cured color (redness). In fresh meat, the innate reducing capacity of fresh meat has been shown to reduce nitrite to nitric oxide and generate nitric oxide myoglobin (Song et al., 2015), which forms cherry red color under vacuum. A study conducted by Ramanathan et al. (2018) reported that fresh beef steaks dipped in rosemary extract and then packaged in nitrite-embedded film developed improved red color more rapidly than undipped steaks packaged only in nitrite-embedded film. This also suggests that a reducing environment facilitates the development of nitric oxide myoglobin under vacuum. However, these effects have not, to the best of our knowledge, been demonstrated for nitrite-embedded film on fully cooked meat products in which the

pigment has been denatured prior to exposure to the nitrite embedded film. Honikel (2008) described the mechanism of nitric oxide formation from nitrite in a weak acid environment, such as exists in meat, as the result of nitrous acid formation and dissociation to form nitric oxide and nitrate. The nitric oxide is then available to combine with the heme group of meat pigments in fresh, uncooked meat. However, the reaction of nitric oxide with cooked, denatured meat pigment has not been considered to be important and has not been well studied. At the same time, the mechanism for cured color stability of cooked, cured meat in vacuum packages is commonly accepted to be due to an equilibrium between the nitrosylheme pigment and dissociated nitric oxide and heme that occurs with light exposure (Bohner and Rieblinger, 2016). In vacuum-packaged cured meat, a certain amount of residual nitrite has been considered to be important for providing a continual source of nitric oxide during storage to push the equilibrium toward the nitric oxide-associated nitrosyl pigment and thus avoid cured color fading. Møller et al. (2003) reported that nitrite content had a highly significant effect on cured color fading during exposure to light and oxygen, and Jongeward et al. (1988) found that rebinding of nitric oxide to heme iron after photodissociation occurred much faster than for other ligands such as carbon monoxide. Munk et al. (2010) also reported that rebinding of nitric oxide to myoglobin was very rapid following dissociation induced by light exposure. Thus, it appears—from the data in our study—that in a vacuum package, nitric oxide from nitrite that originates from nitrite-embedded film can also readily bind to cooked, denatured heme pigments to result in the nitrosylhemochrome pigment that is responsible for cured meat color.

In this study, in-package surface a^* values over time show that the NT10-NEF treatment performed similar to CJP-CF with regard to color stability during the display period despite considerably less ingoing nitrite (21 ppm vs. 99 ppm) in the product formulation. Further, comparing NT10-NEF to NT10-CF for in-package surface a^* value highlights the fact that NT10-NEF a^* value increased relative to NT10-CF during the display period. This again suggests that nitric oxide generated from nitrite in the film is resulting in additional formation of cured meat pigment in the product.

The increase in unpackaged surface a^* value in NT10-NEF compared to NT10-CF over the first 27 d of display (Table 7) also indicates that some additional generation of cured pigment occurred with time in nitrite-embedded film packaged product after cooking

and chilling was complete. Consequently, it appears that nitrite from the nitrite-embedded film may be forming additional nitrosyl pigment as well as reducing potential color fading. The greater in-package a^* value (Table 5) in the CJP-NEF treatment compared to the conventional film counterpart (CJP-CF) is additional support for the hypothesis that nitrite-embedded film would improve the color stability of alternatively cured products. Previous research on celery juice powder as a source of nitrite showed similar results in that celery juice powder resulted in greater light-induced color fading during retail display compared to controls (Sindelar et al., 2007b; Krause et al., 2011; Terns et al., 2011). In contrast, a study by Redfield and Sullivan (2015) using celery juice powder instead of conventional nitrite in deli-style turkey breast lunchmeat found that time (retail display period) had no effect on CIE L^* , a^* , and b^* values. These contradictory results with regard to color stability may be due to the relatively low myoglobin concentration of breast muscle compared to beef, in which the degree of change in color would be less because the myoglobin content is less.

Greater in-package surface b^* values in the NT10-CF relative to the other treatments suggests greater discoloration (yellowness) in the NT10-CF (conventional film) product compared to NT10-NEF. However, NT10-NEF was similar to CJP-CF for surface b^* values, which means that the nitrite-embedded film package provided for less discoloration in the low nitrite product, making the color stability of the low nitrite product similar to that of the products with celery juice powder that contained a greater amount of ingoing nitrite. Internal b^* values decreased in the NT10-NEF treatment during display (b^* value of 17.87 on day 1 to 15.62 on day 125), but NT10-CF showed the greatest discoloration (greatest b^* value) throughout the display period.

A surprising observation in this study was that internal color showed an improvement over time in nitrite-embedded-film-packaged product, presumably from penetration of nitrite/nitric oxide from the film into the product interior. It is important to note that all slices were 6.35-mm thick and the internal color was measured at the most interior slice surface (between slices 2 and 3), which was 12.7 mm from the contact point with the film. This is particularly noteworthy given the very low (21 ppm) ingoing nitrite in the Natpre T-10 EML Plus S treatments where this degree of internal color improvement was not expected.

The lighter color of the Natpre T-10 EML Plus S products relative to the celery juice powder treatments

was probably due to the celery juice powder. A previous study conducted by Usinger et al. (2016) found similar results and concluded that the natural yellow-green pigment of celery juice powder resulted in darker colored (lower L^* value) meat products. Additionally, Myers et al. (2013) found that L^* values in no-nitrite (nitrite-free) ham were significantly higher than in the treatments containing nitrite from cultured celery juice powder.

The CIE L^* , a^* , and b^* values observed in the present study over time are consistent with previous research that reported decreased a^* , increased b^* , and reduced L^* during respective display periods (Yen et al., 1988; Møller et al., 2003; Nannerup et al., 2004).

Residual nitrite in the surface slices of the bologna showed that Natpre T-10 EML Plus S treatments both had considerably less residual nitrite than the control or the celery juice powder products, as expected. More importantly, the nitrite-embedded film did not result in greater measurable nitrite in the product than in the product packaged in conventional film. Further, the film type (conventional film or nitrite-embedded film) did not have a significant impact over time during the display period, with one exception in that CJP-CF was lower than CJP-NEF at day 6. Thus, the nitrite-embedded film provided for improved cured color stability without affecting measurable residual nitrite concentrations.

As expected, the present study confirmed that residual nitrite in typical formulations decreased over time. Reduction in residual nitrite during storage has been well documented in previous research studies (Hustad et al., 1973; Dethmers et al., 1975; Jantawat et al., 1993; Ahn et al., 2002; Sindelar et al., 2007b; Krause et al., 2011; Xi et al., 2012; Myers et al., 2013; Djeri and Williams, 2014; Redfield and Sullivan, 2015; Usinger et al., 2016). Overall, the external and internal residual nitrite levels observed in the present study show that nitrite-embedded film had no impact on residual nitrite levels even for low nitrite-containing meat products such as those containing Natpre T-10 EML Plus S. Previous research has shown that reduced ingoing levels of nitrate/nitrite from celery juice powder compared to conventionally cured products are common in alternatively cured meats (Sindelar et al., 2007a; Myers et al., 2013). Reduced ingoing nitrite concentrations can potentially lead to reduced color stability over extended retail display because residual nitrite is depleted with time. Our findings confirm that alternatively cured meat products using cultured celery juice (CJP-CF) may experience reduced

color stability as residual nitrite is decreased during retail display. Nitrite-embedded film provided a positive impact in cured color, which improved the color stability (CJP-NEF), and was capable of generating greater redness during storage in the product with very low ingoing nitrite concentration (NT10-NEF).

Surface residual nitrate results for treatment effects showed that nitrate was detected in the control formulation (CON-CF), even though only nitrite was included in the formulation. This can be expected because, during the curing process, some of the nitrite forms nitrate by means of a mildly acidic environment (Sindelar et al., 2007b; Honikel, 2008). However, it has also been shown that nitrate is inert in fully cooked meat (Honikel, 2008). As expected, internal residual nitrate showed similar results and the same trends as external nitrate. CJP-CF and CJP-NEF resulted in greater internal residual nitrate compared to other treatments as might be expected because cultured celery juice powder typically contains a significant amount of nitrate with very little nitrite in either Modern Cure powder (CON-CF) or Natpre T-10 EML Plus S (NT10-CF and NT10-NEF). In the present study, the greater concentration of nitrate found in the CJP-CF treatments (CJP-CF and CJP-NEF) is most likely a result of unconverted nitrate in the cultured celery juice powder (or commonly referred to as pre-converted celery juice powder).

In the inoculated products, bacterial growth during elevated temperature storage (21 d) was achieved as expected. Anaerobic lactic acid bacterial populations were not different between control and celery juice powder treatments and there was no effect of nitrite-embedded film on bacterial growth. However, NT10-NEF and NT10-CF treatments showed lower bacterial populations compared to all other treatments. Both of these treatments contained Natpre T-10 EML Plus S, which includes fruit and spice extracts that may include microbial inhibitors such as organic acids, as well as other antimicrobial compounds.

Conclusions

The results from the present study demonstrate that nitrite-embedded film has potential to extend the color stability of alternatively cured meat products. Nitrite-embedded film improved both surface and internal redness in alternatively cured meat products, especially those that contained low concentrations of ingoing nitrite. In the present study, a^* value was used as an indicator for cooked, cured meat pigment, and—based

on the improvements in a* value for treatments with low ingoing nitrite in nitrite-embedded film packaged products—it appears that additional cured pigment (nitrosylhemochrome) was formed in these cooked products following thermal processing. This means that the film not only has the potential to improve retail display color but also provides a means of generating cured color, post thermal processing. The mildly acidic conditions surrounding cooked, cured products in an anaerobic package environment are conducive to nitric oxide formation from nitrite. The generated nitric oxide can then bind to heme iron to generate nitrosylhemochrome pigment as well as replace the nitric oxide that dissociates from the pigment during color fading. Further research should be conducted to clarify the proposed mechanism for nitrosylhemochrome formation following thermal processing and pigment denaturation, in addition to the impact of nitrite-embedded film technology on the safety, quality, and sensory impact in nitrite-free and alternatively cured, cooked meat products.

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Literature Cited

Ahn, H. J., J. H. Kim, C. Jo, C. H. Lee, and M. W. Byun. 2002. Reduction of carcinogenic N-nitrosamines and residual nitrite in model system sausage by irradiation. *J. Food Sci.* 67:1370–1373. <https://doi.org/10.1111/j.1365-2621.2002.tb10291.x>.

AOAC International. 2005a. Ions in water, modified for meat. Official method 993.30. In: Official methods of analysis. AOAC Int., Gaithersburg, MD.

AOAC International. 2005b. Nitrite in cured meat. Official method 973.31. In: Official methods of analysis. AOAC Int., Gaithersburg, MD.

AOAC International. 2011. Protein in raw and processed meats. Official method 2011.04. In: Official methods of analysis. AOAC Int., Gaithersburg, MD.

AOAC International. 2013. Moisture and fat in meats microwave and nuclear magnetic resonance analysis. Official method 2008.06. In: Official methods of analysis. AOAC Int., Gaithersburg, MD.

Bohner, N., and K. Rieblinger. 2016. Impact of different visible light spectra on oxygen absorption and surface discoloration of bologna sausage. *Meat Sci.* 121:207–209. <https://doi.org/10.1016/j.meatsci.2016.06.019>.

Claus, J. R., and C. Du. 2013. Nitrite-embedded packaging film effects on fresh and frozen beef color development and stability as influenced by meat age and muscle type. *Meat Sci.* 95:526–535. <https://doi.org/10.1016/j.meatsci.2013.05.029>.

Dethmers, A. E., H. Rock, I. Fazio, and R. W. Johnston. 1975. Effect of added sodium nitrite and sodium nitrate on sensory qualities and nitrosamine formation in thuringer sausage. *J. Food Sci.* 40:491–495. <https://doi.org/10.1111/j.1365-2621.1975.tb12512.x>.

Djeri, N. and S. K. Williams. 2014. Celery juice powder used as nitrite substitute in sliced vacuum-packaged turkey bologna stored at 4C for 10 weeks under retail display light. *J. Food Quality.* 37:361–370. <https://doi.org/10.1111/jfq.12102>.

FDA. 2007. GRN No. 228 [GRAS Notice]. U.S. Food and Drug Administration. <https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=228>. (Scessed 9 January 2019).

FDA. 2010. Agency Response Letter GRAS Notice No. GRN 000228. U.S. Food and Drug Administration. <https://wayback.archive-it.org/7993/20171031031644/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm221704.htm>. (Accessed 9 January 2019).

Honikel, K. O. 2008. The use and control of nitrate and nitrite for the processing of meat products. *Meat Sci.* 78:68–76. <https://doi.org/10.1016/j.meatsci.2007.05.030>.

Hustad, G. O., J. G. Cerveny, H. Trenk, R. H. Deibel, D. A. Kautter, T. Fazio, R. W. Johnston, and O. E. Kolari. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. *Appl. Microbiol.* 26:22–26. PMID:4580194.

Jantawat, P., J. Runglerdkriangkrai, C. Thunpithayakul, and R. Sanguandeekul. 1993. Effects of initial nitrite level, heating and storage on residual nitrite and spoilage of canned ham roll. *J. Food Quality.* 16:1–11. <https://doi.org/10.1111/j.1745-4557.1993.tb00095.x>.

Jongeward, K. A., D. Magde, D. J. Taube, J. C. Marsters, T. G. Traylor, and V. S. Sharma. 1988. Picosecond and nanosecond geminate recombination of myoglobin with CO, NO, and isocyanides. *J. Am. Chem. Soc.* 110:380–387. <https://doi.org/10.1021/ja00210a011>.

Krause, B. L., J. G. Sebranek, R. E. Rust, and A Mendonca. 2011. Incubation of curing brines for the production of ready-to-eat, uncured, no-nitrite-or-nitrate-added, ground, cooked and sliced ham. *Meat Sci.* 89(4):507–513. <https://doi.org/10.1016/j.meatsci.2011.05.018>.

McMillin, K. W. 2017. Advancements in meat packaging. *Meat Sci.* 132:153–162. <https://doi.org/10.1016/j.meatsci.2017.04.015>.

Møller, J. K. S., M. Jakobsen, C. J. Weber, T. Martinussen, L. H. Skibsted, and G. Bertelsen, 2003. Optimisation of colour

- stability of cured ham during packaging and retail display by a multifactorial design. *Meat Sci.* 63:169–175. [https://doi.org/10.1016/S0309-1740\(02\)00066-9](https://doi.org/10.1016/S0309-1740(02)00066-9).
- Munk, M. B., K. Huvaere, J. Van Bocxlaer, and L. H. Skibsted. 2010. Mechanism of light-induced oxidation of nitrosylmyoglobin. *Food Chem.* 121:472–479. <https://doi.org/10.1016/j.foodchem.2009.12.067>.
- Myers, K., J. Cannon, D. Montoya, J. Dickson, S. Lonergan, and J. Sebranek. 2013. Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. *Meat Sci.* 94:69–76. <https://doi.org/10.1016/j.meatsci.2012.12.019>.
- Nannerup, L. D., M. Jakobsen, F. Van Den Berg, J. S. Jensen, J. K. S. Møller, and G. Bertelsen. 2004. Optimizing colour quality of modified atmosphere packed sliced meat products by control of critical packaging parameters. *Meat Sci.* 68:577–585. <https://doi.org/10.1016/j.meatsci.2004.05.009>.
- Narváez-Bravo, C., A. Rodas-González, C. Ding, O. López-Campos, J. Galbraith, I. L. Larsen, J. Ye, D. Siegel, and J. L. Aulhus. 2017. Effects of novel nitrite packaging film on the bacterial growth of bison strip-loin steaks. *J. Food Process. Pres.* 41:1–7. <https://doi.org/10.1111/jfpp.13311>.
- Ramanathan, R., R. M. Mitacek, S. D. Billups, R. Jadeja, M. M. Pfeiffer, G. G. Mafi, and D. L. Vanoverbeke. 2018. Novel nitrite-embedded packaging improves surface redness of dark-cutting longissimus steaks. *Translational Animal Science.* 2:135–143. <https://doi.org/10.1093/tas/txy006>.
- Redfield, A. L., and G. A. Sullivan. 2015. Effects of conventional and alternative curing methods on processed turkey quality traits. *Poultry Sci.* 94:3005–3014. <https://doi.org/10.3382/ps/pev299>.
- Roberts, J. C., A. Rodas-González, J. Galbraith, M. E. R. Dugan, I. L. Larsen, J. L. Aalhus, and Ó. López-Campos, Ó. 2017. Nitrite embedded vacuum packaging improves retail color and oxidative stability of bison steaks and patties. *Meat and Muscle Biology.* 1:169–180. <https://doi.org/10.22175/mmb2017.03.0015>.
- Sindelar, J. J., J. C. Cordray, D. G. Olson, J. G. Sebranek, and J. A. Love. 2007a. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *J. Food Sci.* 72:551–559. <https://doi.org/10.1111/j.1750-3841.2007.00486.x>.
- Sindelar, J. J., J. C. Cordray, J. G. Sebranek, J. A. Love, and D. U. Ahn. 2007b. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *J. Food Sci.* 72:388–395. <https://doi.org/10.1111/j.1750-3841.2007.00404.x>.
- Song, X., D. Cornforth, D. Whittier, and X. Luo. 2015. Nitrite spray treatment to promote red color stability of vacuum packaged beef. *Meat Sci.* 99:8–17. <https://doi.org/10.1016/j.meatsci.2014.08.003>.
- Terns, M. J., A. L. Milkowski, S. A. Rankin, and J. J. Sindelar. 2011. Determining the impact of varying levels of cherry powder and starter culture on quality and sensory attributes of indirectly cured, emulsified cooked sausages. *Meat Sci.* 88:311–318. <https://doi.org/10.1016/j.meatsci.2011.01.009>.
- USDA. 1995. Processing inspector's calculation handbook. In: FSIS Dir. 7620.3. <https://www.fsis.usda.gov>. (Accessed 9 January 2019).
- USDA. 2018. Safe and suitable ingredients used in the production of meat, poultry, and egg products. In: FSIS Dir. 7120.1. <https://www.fsis.usda.gov>. (Accessed 22 January 2019).
- Usinger, E. L., E. M. Larson, S. E. Niebuhr, C. A. Fedler, K. J. Prusa, J. S. Dickson, R. Tarté, and J. G. Sebranek. 2016. 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? *Meat Sci.* 121:324–332. <https://doi.org/10.1016/j.meatsci.2016.06.022>.
- Xi, Y., G. A. Sullivan, A. I. Jackson, G. H. Zhou, and J. G. Sebranek. 2012. Effects of natural antimicrobials on inhibition of *Listeria monocytogenes* and on chemical, physical and sensory attributes of naturally-cured frankfurters. *Meat Sci.* 90:130–138. <https://doi.org/10.1016/j.meatsci.2011.06.013>.
- Yang, X., D. R. Woerner, K. R. McCullough, J. D. Hasty, I. Geornaras, G. C. Smith, J. N., and Sofos, K. E. 2016. An evaluation of the effectiveness of FreshCase technology to extend the storage life of whole-muscle pork and ground pork sausage. *J. Anim. Sci.* 94:4921–4929. <https://doi.org/10.2527/jas2016-0509>.
- Yen, J. R., R. B. Brown, R. L. Dick, and J. C. Acton. 1988. Oxygen transmission rate of packaging films and light exposure effects on the color stability of vacuum-packaged dry salami. *J. Food Sci.* 53:1043–1046. <https://doi.org/10.1111/j.1365-2621.1988.tb13525.x>.