Meat and Muscle Biology™

Nitrite-Embedded Film Develops Cured Color in Nitrite-Free Bologna Sausage Packaged Following Thermal Processing[a]

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Abstract: Curing of cooked nitrite-free bologna sausage utilizing nitrite-embedded film (NEF) was investigated. The objectives were to determine the effects of NEF following thermal processing of nitrite-free bologna with and without an added reductant for characteristics important to cured meat, including instrumental color (surface and internal color during lighted and dark storage), surface and sub-surface cured pigment concentration and sub-surface color changes, residual nitrite, and lipid oxidation. Six bologna treatments were produced, with combinations of formulation and package type as follows: a conventionally cured (with sodium nitrite) control formulation packaged in (1) conventional ("CON-CF") and (2) nitrite-embedded ("CON-NEF") film; a nitrite-free formulation packaged in (3) conventional ("UC-CF") and (4) nitrite-embedded ("UC-NEF") film; and a second nitrite-free formulation with an added reductant—cherry powder, a natural source of ascorbic acid—packaged in (5) conventional ("UCC-CF") and (6) nitrite-embedded ("UCC-NEF") film. In-package surface and internal instrumental a^* values, residual nitrite, cured pigment concentration, and cured pigment color development from the product surface to the interior were significantly greater ($P < 0.05$) for UC-NEF and UCC-NEF compared to their conventional film counterparts, UC-CF or UCC-CF, respectively. Lipid oxidation (2-thiobarbituric acid-reactive substances [TBARS] values) was significantly greater ($P < 0.05$) for UC and UCC formulations but was not different ($P > 0.05$) over storage time. Purge (%) was significantly lower ($P < 0.05$) for CF-packaged formulations and increased ($P < 0.05$) over storage time. The added reductant in the UCC formulation increased the rate of change and degree of difference for color attributes, residual nitrite, pigment concentration, and development of cured color between respective packaging films (NEF or CF). However, the same differences were observed for the nitrite-free formulation (UC) without any added reductant; consequently, the reductant was not required to generate cured meat attributes when packaged in NEF.

Key words: nitrite, nitrite-embedded film, cured meat, nitrite-free, bologna, color
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Introduction

Meat preservation is a well-understood process, which in ancient times took place by salting and drying meat to provide a more consistent and dependable protein source. Curing meat to form processed meat products resulted from the observation that certain salts, typically from coastal regions, exhibited better preservation properties. In the early 20th century, these salts were determined to contain saltpeter, also known as potassium nitrate (Pearson and Gillett, 1996;

Honikel, 2008). Much of the cured meat research in the early 20th century focused on understanding the impact of nitrate, nitrite, and the essential derivative nitric oxide on cured meat properties (Hoagland, 1908, as cited in Binkerd and Kolari, 1975).

Meat color is determined by the state and abundance of the heme-containing protein myoglobin. The oxidation state of the heme iron is the major factor determining the observed color. The oxidation state of iron can be either ferrous (Fe^{2+}) or ferric (Fe^{3+}), with the most prevalent forms of myoglobin being oxymyoglobin (Fe^{2+}), deoxymyoglobin (Fe^{2+}), and metmyoglobin (Fe^{3+}). The bright red color of ferrous iron bound with oxygen (oxymyoglobin) is considered the most desirable fresh meat color by consumers and is, thus, the desired form for retail meat products (Suman et al., 2014).

To produce cured meat products, sodium nitrite (or related nitrite-rich ingredient) is typically added to meat and forms nitric oxide myoglobin, which upon heat-based denaturation forms nitrosylhemochrome pigment. However, the number of uncured and alternatively cured meat products, produced with alternative, natural sources of nitrite, continues to increase in retail markets, due to negative consumer perceptions of meat cured with conventional chemical sources of nitrate (NO_3) and nitrite (NO_2). Due to their lower nitrite concentrations, many of the alternative products may not achieve the expected levels of cured meat color, microbial control, and antioxidant properties typical of traditionally cured meat products. Nitrite-embedded film (NEF) packaging offers the potential to improve cured meat characteristics of uncured and alternatively cured meat products.

In vacuum-packaged fresh meat, the addition of nitrite to the packaging film (NEF) has been shown to improve the display color of *longissimus lumborum*, *psoas major*, and *semitendinosus* in beef (Claus and Du, 2013), dark cutting beef (Ramanathan et al., 2018; Denzer, 2020), fresh pork sausage (Yang et al., 2016), *longissimus lumborum* and ground *rhomboideus* in bison (Roberts et al., 2017), and bison steaks (Narváez-Bravo et al., 2017). The resulting color of fresh meat packaged in NEF is a bright red color, no different than uncooked, vacuum-packaged corned beef, which exhibits nitric oxide myoglobin, a bright red pigment while held under vacuum. NEF contains a low amount of nitrite (113 mg NO_2/m^2) for the meat contact surface, with residual nitrite concentration on the surface of NEF-packaged beef steaks reported to be less than 2 ppm (Claus and Du, 2013), which is less than the amount of nitrite necessary to result in cured color after fresh meat is cooked. NEF has Generally Recognized as Safe status in the U.S. (GRAS Notice 228, FDA, 2007) and is permitted for the packaging of fresh beef and pork products (FDA, 2007, 2010). Moreover, the sodium nitrite embedded in NEF is considered a processing aid because the nitrite does not become a significant component of the product (USDA, 2018). In processed meat products, despite the low amount of nitrite imparted by NEF to the packaged product, NEF has been demonstrated to effectively improve the surface and internal and cured color of both alternatively cured (Cropp, 2018; Cropp et al., 2020) and nitrite-free cooked bologna during simulated retail display (Cropp et al., 2023).

Therefore, the objective of the present study was to determine the ability of NEF to produce cured meat properties after cooking, following the associated heat-based protein denaturation. We hypothesized that adding nitrite via NEF to cooked meat post-thermal processing will result in the development of cured meat characteristics without the typical "curing time" needed for nitrite in contact with uncooked meat prior to thermal processing. Cooked nitrite-free bologna with and without an added reductant was evaluated for instrumental color (surface and internal), lipid oxidation, purge and residual nitrite, as well as pigment type, pigment concentration, and progressive development of cured pigment color resulting from the migration of nitrite from the surface to the interior of the cooked product.

Materials and Methods

Formulations and raw materials

Three formulations of large-diameter beef bologna, shown in Table 1, were manufactured in the Iowa State University (ISU) Meat Laboratory (Ames, IA, USA). The control formulation (CON) was a conventionally cured control containing sodium nitrite and the reducing agent sodium erythorbate. The other two formulations (UC and UCC) were both truly uncured (i.e., free of any form of nitrite), with the difference being that UCC contained ascorbic acid, a natural reducing agent, from cherry powder (VegStable 515, Florida Food Products, Inc., Eustis, FL, USA).

Frozen 80% lean beef trimmings were obtained from the Iowa State University Meats Laboratory, and frozen 50% lean beef trimmings (from boneless beef navels) were obtained from a commercial beef processor. The meat materials were stored at −20°C until needed. Spices, salt (Cargill flake salt), and

Table 1. Bologna formulations (as percent of total meat weight)

Ingredient	CON[1]	UC[2]	UCC[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	1.77[4]	2.00	2.00
Dextrose	1.00	1.00	1.00
Mustard	1.00	1.00	1.00
Sodium phosphates[5]	0.50	0.50	0.50
Spices and herbs[6]	0.81	0.81	0.81
Modern Cure (6.25% $NaNO_2$)	0.25	-	-
Sodium erythorbate	0.054	-	-
Cherry powder[7]	-	-	0.50

[1]CON: conventionally cured control.

[2]UC: uncured, nitrite-free.

[3]UCC: uncured, nitrite-free with cherry powder.

[4]Salt concentration was adjusted to account for salt contribution from Modern Cure.

[5]Blend of sodium tripolyphosphate and sodium hexametaphosphate.

[6]Blend of coriander (45%), black pepper (37%), red pepper (9%), and garlic powder (9%).

[7]VegStable 515 (Florida Food Products, Inc., Eustis, FL, USA).

Modern Cure were provided by A.C. Legg, Inc. (Calera, AL, USA). Ingredients used for both replications of the study were from the same production lot.

Product preparation

Prior to use, beef raw materials were thawed at 4.4°C for 2 d, placed in refrigerated storage at 1°C for 24 to 48 h, and ground through a 12.7-mm plate (Biro Manufacturing Company, Marblehead, OH, USA). To ensure uniform distribution of the lean and fatty materials, portions of each were mixed separately for 2 min using a dual-shaft mixer (Leland Southwest, Fort Worth, TX, US). Each batch consisted of 7.48 kg of 50% lean trimmings and 15.20 kg of 80% lean trimmings, for a total of 22.68 kg of meat. The lean trimmings were added to a vacuum bowl chopper (Kramer & Grebe GmbH and Co., KG, Biendenkopf-Wallau, Germany), followed by salt, curing ingredient (if applicable; Table 1), half of the water/ice mixture, and remaining dry ingredients (Table 1). The mixtures were chopped to a batter temperature of 4.4°C, the fat trimmings and remaining water/ice were added, and chopping continued until the batter reached a temperature of 15°C. The meat batters were then moved to a vacuum stuffer (Handtmann VF 608 Plus, Lake Forest, IL, USA) and stuffed into 17.8 cm × 121.9 cm prestuck fibrous casings (L7S Fibrous P Clear, Kalle GmbH, Wiesbaden, Germany). Stuffed bologna logs were weighed individually, placed horizontally on smoke racks in a random arrangement, and thermally processed in a single-truck oven (Alkar Inc., Lodi, WI, USA) to an internal product temperature of 71°C, following a large-diameter bologna thermal processing schedule with stepwise temperature increases (approximately 6 h).

After cooking, the bologna logs were chilled overnight (approximately 17 h) at 1 ± 2°C, and weighed for cook and chill yield calculations (yield % = [chilled weight/raw weight] x 100). After removal of casings, the product was sliced (Puma Treif, Treif Maschinenbau GmbH, Oberlahr, Germany) into slices 3 mm in thickness and packaged in conventional, high-barrier film (CF) or in NEF (Bemis Company, Inc., Oshkosh, WI, USA) for a total of 3 conventional film-packaged treatments (CON-CF, UC-CF, and UCC-CF) and 3 NEF-packaged treatments (CON-NEF, UC-NEF, UCC-NEF). Each combination of formulation (CON, UC, or UCC) and package type (CF or NEF) is referred to as a treatment. Both films were made of the same materials and had the same thickness and barrier properties (177.8 µm thickness; oxygen transmission rate < 5.0 cm^3/m^2/24 h at 23°C/0% relative humidity; water vapor transmission rate < 8.3 cm^3/m^2/24 h at 38°C/100% relative humidity), except that NEF was embedded with 113 mg/m^2 of sodium nitrite (information provided by supplier). NEF was the film approved in the USA for use with fresh beef and pork (FDA, 2010). Both films were provided in roll stock form by Bemis Company Inc. (Oshkosh, WI, USA) and were manufactured into 15.25-cm × 20.32-cm pouches before use. Four stacked slices of bologna were placed inside each pouch, except for samples to be used for evaluation of progressive cured color development, which were packaged as described below. Pouches were sealed under vacuum (Ultravac UV 2100 packaging machine, UltraSource LLC, Kansas City, MO, USA) and stored at 3 ± 2°C for the duration of the study (126 d), with product packaging day designated as day 0. Samples from all treatments were stored either in the dark, by placing them inside cardboard boxes, or under lights, by placing them in single layers on shelves equipped with fluorescent lights (32W, 120V, 4100K, 2,950 lm; Philips F32T8/TL941/ALTO, Koninkljke Philips N.V., Amsterdam, Netherlands) suspended 254 mm above the package surfaces. Illuminance at the package surface was 2,850 ± 500 lx, measured with a URCERI Light Meter MT-912 (URCERI, Shenzhen Huanhui E-commerce Co., Ltd, Shenzhen, China). Various locations throughout the storage area were selected for illuminance

measurements. Sample locations were rotated randomly every 4 wk to provide uniform light exposure. Samples for evaluation of progressive cured color development were stored in the dark only. In-package instrumental surface color measurements (lighted and dark storage) were conducted on refrigerated samples on each sampling day. Packaged samples to be used for internal color, 2-thiobarbituric acid-reactive substances (TBARS), residual nitrite, and pigment concentration measurements were frozen at −80°C on each respective sampling day of storage and thawed for 24 h at 2°C in darkness prior to analysis.

Packaging of samples for progressive cured color development

Due to analytical sampling constraints, bologna slices for cured color development were vacuum-packaged in bulk on day 0 and frozen at −40°C. After approximately 4 mo, the bologna slices were thawed and separated into stacks of 12 slices each. A circular piece of packaging film (CF or NEF), with a diameter equal to that of the bologna slices, was placed on the top slice, and the stacks were vacuum-packaged in CF only.

Analytical procedures: Proximate composition, pH, and salt concentration

Samples of the finished raw batters after chopping, and of cooked sliced bologna were randomly collected, frozen at −80°C, and used for proximate analysis at a later date. Raw meat batters and cooked products from each production batch were finely chopped using a food processor (KitchenAid, St. Joseph, MI, USA) for triplicate measurement of proximate composition (fat, moisture, and protein) as well as pH and salt concentration. For pH measurement, 10 g of sample were mixed with 90 mL of distilled water, mixed thoroughly, filtered through Whatman Grade 1 filter paper (GE Health Care Life Sciences, Pittsburgh, PA), and pH of the filtrate measured with a Mettler Toledo SevenMulti pH meter with a InLab solids pH probe (Mettler Toledo, Columbus, OH). Sodium chloride content was measured using the Quantab method (AOAC International, 2019). Fat and moisture content were measured according to the AOAC method 2008.06, where fat was measured by the CEM ORACLE System (CEM Corporation, Matthews, NC, USA), and moisture by the CEM SMART 6 System (CEM Corporation, Matthews, NC, USA). Protein content was measured according to the AOAC method 2011.04, with a CEM Sprint Rapid Protein Analyzer (CEM Corporation, Matthews, NC, USA).

Analytical procedures: Color

Color measurements were conducted following the AMSA Guidelines for Meat Color Measurement (King et al., 2023), with a HunterLab MiniScan EZ 4500L colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) using illuminant D65 (daylight at 6,500 K) at an observer angle of 10°, with an aperture of 2.54 cm. The Commission Internationale de l'Eclairage (CIE) $L^*a^*b^*$ color space and resulting color values were used to calculate chroma [$C^* = a^{*2} + b^{*2}$] and hue angle [HA = arctangent (b^*/a^*)(180/π)]. Color measurements were recorded on days 0, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and 126 of storage.

In-package surface color (with the packaging film intact) of samples in lighted and dark storage was measured on 22 randomly selected packages of each treatment (3 scans per package, taken in random locations). The same designated packages were repeatedly measured at each sampling day for the duration of the display period, for a total of 264 measured packages (22 packages × 6 treatments × 2 replications) on each sampling day. For this measurement, the colorimeter was calibrated with the black and white calibration tiles covered by a segment of the packaging film (CF or NEF) used to package the samples. Product measurements were then conducted by placing the nose cone of colorimeter directly onto the light-exposed surface of each package.

Internal color was measured after opening the package, removing the packaging film and the top 2 slices, then measuring the color of the slice surface at the interface of slices 2 and 3 of the 4-slice stack. Measurements were conducted on one randomly selected light-stored package per treatment (6), per sample day (12), per replication (2) for a total of 144 packages measured during the display period. A standard instrument calibration (no packaging film covering the calibration tiles) was performed, and 3 scans were taken in random locations of slice 3 of the 4-slice stack. For both in-package and internal color, 3 measurements were taken on each sample and were averaged.

Data for progressive color development were collected on days 0, 14, 28, 42, and 56, using the packaged stack of 12 slices. After opening the package, the 12 slices within each package were separated into 3 sections: top (slices 1–4), middle (slices 5–8), and bottom (slices 9–12), and 3 measurements were collected on each of the 12 slices.

Analytical procedures: Residual nitrite

Residual nitrite was measured on days 0, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and 126 of storage, and done

in accordance with AOAC Method 973.31 (AOAC International, 2019). Briefly, previously described samples were finely chopped using a food processor (KitchenAid, St. Joseph, MI, USA), and 5.0 ± 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 for 2 h, swirling them every 30 min. The flasks were then cooled to room temperature (approximately 23°C) and filled to volume with distilled water. Approximately 30 mL was filtered through Whatman #42 filter paper (GE Health Care Life Sciences, Pittsburgh, PA, USA) into a 50-mL volumetric flask, reagents added as described in the procedure, flasks filled to volume, and absorbance read at 540 nm with a spectrophotometer (Genesys 150, Thermo Fisher Scientific, Madison, WI, USA). When generating the standard curve, in addition to the dilutions of the 1 ppm nitrite working solution described by the AOAC International method, which correspond to nitrite concentrations on the standard curve of 0.2, 0.4, 0.6, and 0.8 ppm, respectively), greater dilutions of the working solution (0.01, 0.02, 0.05, and 0.10 ppm of nitrite, respectively) were also included to extend the linear standard curve to lower concentrations, in order to improve the consistency of measurements of product nitrite concentrations that were below 20 ppm.

Analytical procedures: Cured and total meat pigments

Cured pigment and total meat pigment concentrations were measured in duplicate by extracting cured pigments in 80% acetone and total pigments in acidified acetone, as described by Hornsey (1956), with the modifications described by Sindelar et al. (2007). Pigment concentration was measured for storage days 0, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and 126. Sample preparation for cured and total pigments was conducted in reduced lighting conditions at 22°C.

Cured pigment extractions utilized 40 mL acetone and 3 mL water added to 10 g of each sample, followed by homogenization using a Polytron mixer (PT 10/35, Kinematica GmbH, AG, Switzerland) for 1 min at speed 7 (approximately 12,500 rpm). The samples were immediately filtered through Whatman #42 filter paper (Whatman, GE Health Care Life Sciences, Pittsburgh, PA, USA), and absorbance was measured at 540 nm with a Genesys 150 spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA). Nitrosylhemochrome pigment was calculated as $A_{540} \times 290$ and reported in ppm. Cured pigment extracts were also scanned from 470 to 570 nm for confirmation of the respective cured meat pigments.

For total pigment extractions, 40 mL acetone, 2 mL water, and 1 mL 12.1 N HCl were added to 10 g of sample, followed by homogenization as described above. The samples were allowed to stand approximately 45 min in a dark cabinet, then filtered through Whatman #42 filter paper and absorbance measured at 640 nm. Total pigment was calculated as $A_{640} \times 680$ and reported in ppm.

Analytical procedures: Lipid oxidation

Lipid oxidation of the packaged slices was measured on days 0, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and 126 of storage by the 2-thiobarbituric acid-reactive substances (TBARS) test (Tarladgis et al., 1960) as modified for cured meat by Zipser and Watts (1962). Results were reported as mg of malondialdehyde per kg of sample.

Analytical procedures: Purge

Purge or drip loss of the packaged formulations was measured to determine if purge differed between treatments or during storage. The CF and NEF films utilized were formed into pouches manually and thus varied slightly in weight/size. To compensate for this, all pouches of both film types (CF and NEF) were individually weighed before packaging. The packages designated for purge measurement were weighed and opened, after which the bologna sample was removed, allowed to drip for 3–5 ss, and the weight of the bologna and pouch with purge were recorded.

Experimental design and statistical analysis

The study was replicated twice, with replications produced on separate, consecutive days. Manufacturing and thermal processing occurred on the same day for each replication. The experiment was designed as a split-split plot, with formulation (CON, UC, UCC) as whole plot factor, packaging type (CF, NEF) as subplot factor, and storage time (day) as sub-subplot factor. The data were analyzed as a mixed model using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), with formulation, packaging film, and storage time as fixed factors and replication as a random blocking factor. Pairwise comparisons were performed by the Tukey-Kramer pairwise adjustment. Statistical significance was established at $P < 0.05$; consequently, P values are generally not shown when effects are stated as significant. P values were considered as trending when

Table 2. *P* values[1] of fixed main effects and interactions

	Main effects			Interaction effects			
Dependent variable	Formulation (F)	Packaging film (P)	Storage time (T)	F×P	F×T	P×T	F×P×T
Raw fat, %	**0.035**	–	–	–	–	–	–
Raw moisture, %	0.778	–	–	–	–	–	–
Raw protein, %	0.342	–	–	–	–	–	–
Raw pH	0.875	–	–	–	–	–	–
Raw salt, %	0.504	–	–	–	–	–	–
Yield, %	**0.003**	–	–	–	–	–	–
Cooked fat, %	**<0.001**	0.243	–	0.997	–	–	–
Cooked moisture, %	**<0.001**	0.881	–	0.939	–	–	–
Cooked protein, %	0.084	0.489	–	0.859	–	–	–
Cooked pH	0.775	0.387	–	0.824	–	–	–
Cooked salt, %	0.984	0.937	–	0.988	–	–	–
Light storage *L**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.019**
Light storage *a**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Light storage *b**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Light storage C*[2]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Light storage HA[3]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Dark storage *L**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Dark storage *a**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Dark storage *b**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Dark storage C*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Dark storage HA	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Internal *L**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.048**	**<0.001**
Internal *a**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Internal *b**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Internal C*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.008**	**<0.001**
Internal HA	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Progressive development of *L**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.042**	**0.006**	0.504
Progressive development of *a**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Progressive development of *b**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Progressive development of C*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.013**	**<0.001**
Progressive development of HA	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Residual nitrite, ppm[4]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.234
Purge, %	0.254	**0.043**	0.483	0.339	0.645	0.203	1.000
Cured pigment, ppm	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Total pigment, ppm	**<0.001**	0.111	**0.013**	0.805	0.058	0.431	0.893
TBARS[5]	**<0.001**	**<0.001**	**<0.001**	0.118	0.943	0.987	1.000

[1]Statistical significance established at $P < 0.05$ (shown in bold).

[2]C*: chroma [$= a^{*2} + b^{*2}$].

[3]HA: hue angle [= arctangent $(b^*/a^*)(180/\pi)$].

[4]ppm: parts per million.

[5]TBARS: 2-thiobarbituric acid-reactive substances.

$0.05 \leq P \leq 0.10$. All *P* values for the effects of the fixed main factors and their interactions are shown in Table 2.

Results and Discussion

Proximate composition, pH, and salt content

Mean treatment effects for proximate composition (Table 3) of raw batters and cooked/packaged bologna showed no significant differences in raw product moisture, protein, pH, or salt content, respectively. Raw fat content was lower in the CON formula compared to other formulations. Cooked moisture was higher, and cooked fat and yield were lower, in the control formulation (CON-CF and CON-NEF). However, the differences in moisture and fat were less than 1% and were not considered to be of practical relevance to our study objectives.

Table 3. Least-squares means for treatment (formulation by package type) effects on proximate composition, pH, salt, and yield of bologna

	Raw					Cooked					
Treatments	Moisture (%)	Fat (%)	Protein (%)	pH	Salt (%)	Moisture (%)	Fat (%)	Protein (%)	pH	Salt (%)	Yield (%)
CON-CF	65.15	18.06^{b}	14.51	6.22	1.26	64.07^{a}	17.99^{b}	15.32	6.16	1.68	94.76^{b}
CON-NEF	65.15	18.06^{b}	14.51	6.22	1.26	64.14^{a}	17.89^{b}	15.30	6.17	1.66	94.76^{b}
UC-CF	65.11	18.48^{a}	14.78	6.24	1.27	63.53^{b}	18.76^{a}	15.20	6.15	1.66	96.27^{a}
UC-NEF	65.11	18.48^{a}	14.78	6.24	1.27	63.52^{b}	18.64^{a}	15.11	6.18	1.68	96.27^{a}
UCC-CF	64.86	18.59^{a}	14.66	6.23	1.18	63.30^{b}	18.62^{a}	15.31	6.17	1.66	96.13^{a}
UCC-NEF	64.86	18.59^{a}	14.66	6.23	1.18	63.30^{b}	18.46^{a}	15.33	6.18	1.66	96.13^{a}
SEM	0.31	0.31	0.12	0.03	0.10	0.42	0.25	0.15	0.01	0.05	0.75

CON: conventionally cured control; UCC: uncured, nitrite-free; UC: uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film; SEM: standard error of the mean.

^{a,b}Means in the same column with no letter in common are significantly different (Tukey HSD; $\alpha = 5\%$).

Surface and internal color

For a^* values (Figure 1), all two- and three-way interaction effects were significant, regardless of lighting conditions and sampling location (external vs. internal) (Figure 1), and indicated an improvement in redness in both uncured formulations (UC and UCC) when packaged in NEF. On day 0 of storage, both uncured formulations had the same a^* value regardless of packaging film, which was much lower than in the nitrite-containing CON treatments. During early storage, however, a^* values increased significantly in the NEF-packaged treatments but remained low and unchanged in those packaged in conventional film (UC-CF, UCC-CF). This increase occurred during the first 14 d under light storage (Figure 1A) and was considerably slower under dark storage, whereas it took at least 28 d of storage in UCC and 42 d in UC (Figure 1B). Internal a^* values followed a pattern similar to dark stored samples (Figure 1C). It was also evident that red color developed faster in UCC-NEF than in UC-NEF, as a result of the added cherry powder, a natural source of ascorbic acid. Cropp et al. (2020) showed that NEF improved a^* values in alternatively cured bologna during lighted display compared to a conventional-film-packaged counterpart and suggested that a commercial ingredient included in that study, and which contained fruit and spice extracts, likely contained ascorbic acid. ≈It is also evident, as noted above, that light exposure increased the rate of surface redness development, regardless of the presence of a reducing agent. To our knowledge, this has never before been reported in meat products. While a mechanism for this effect is, therefore, unknown, we propose that it is the result of photolysis of nitrite, which, in the ultraviolet region ($\lambda \approx 200$–400 nm, $\lambda_{max} \approx 360$ nm) of the electromagnetic spectrum is known to result in formation of nitric oxide (Mack and Bolton, 1999). If the light source used in this study emitted in the upper part of this region ($\lambda < 400$ nm), it could have played a role in catalysis of this reaction. This theory remains to be tested and confirmed by further research.

It is well understood that residual nitrite is important as a source of nitric oxide during storage of cured meat to maintain the nitrosylhemochrome pigment. An equilibrium balance between nitrosyl-associated heme pigment and dissociated nitric oxide is expected with light exposure (Böhner and Rieblinger, 2016). Light catalyzes the dissociation of nitric oxide from the heme, which can result in color fading, and with a source of residual nitrite, color re-establishment can also occur (Andersen and Rasmussen, 1992; Møller et al., 2000; Houser et al., 2005), especially under vacuum packaging conditions (Møller and Skibsted, 2002, 2006). Further exemplifying this in the present study, a^* of CON-CF and CON-NEF in lighted storage (Figure 1A) were not significantly different at any time point, nor were they different within treatment across any storage time except for at day 0, where both were significantly lower than in all subsequent days. This is most likely attributed to light and oxygen-induced oxidation of the cured pigment at day 0, which would have occurred during slicing and packaging. By day 7, both treatments showed significantly improved redness, indicating color re-establishment occurred. For products in lighted storage, the surface redness of UCC-NEF was not significantly different from either CON-CF from day 14 through day 126, or from CON-NEF after day 21. UC-NEF was similar to UCC-NEF for increasing a^* value, however, UCC-NEF never quite reached the a^* values found in CON-CF and was significantly lower at each storage

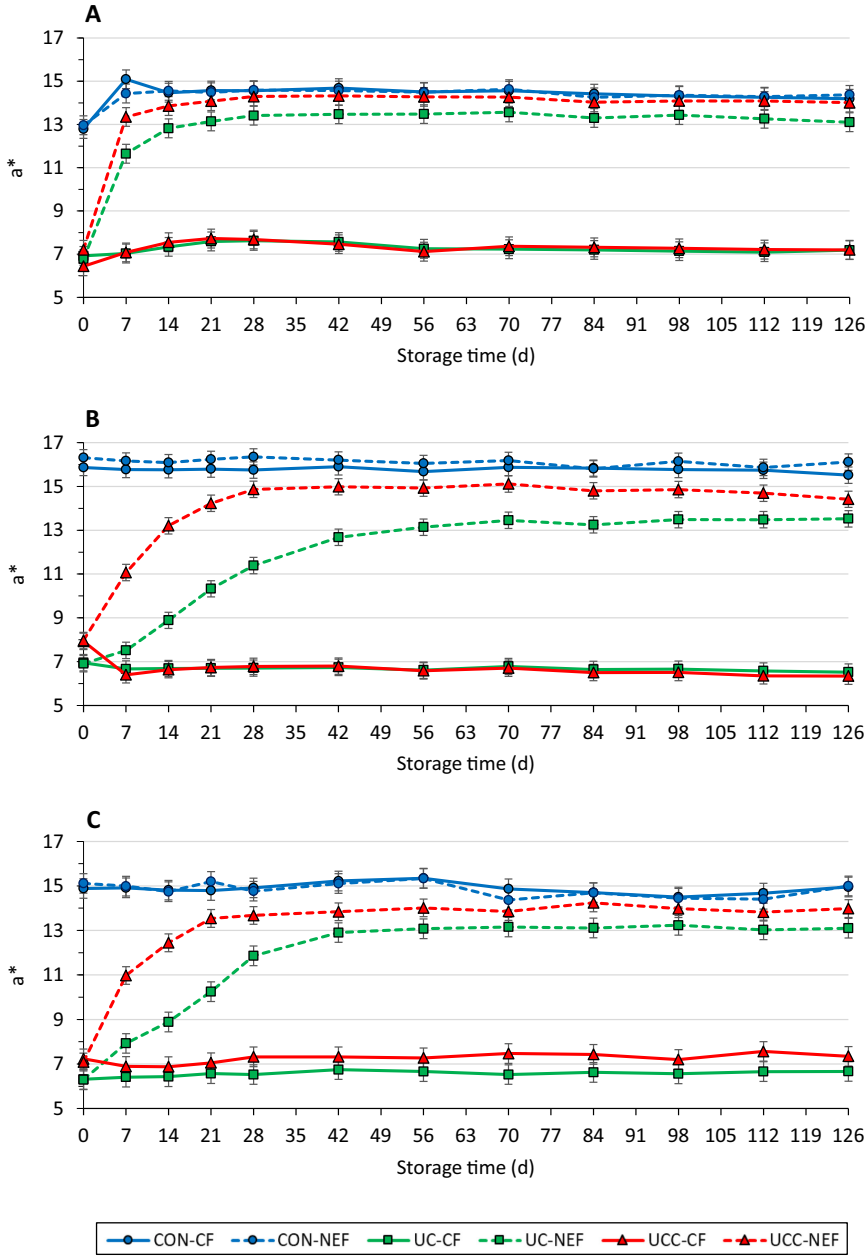

Figure 1. Least-squares means of *a** values of bologna treatments stored at 3 ± 2°C during 126 d. (A) External surface, lighted storage; (B) external surface, dark storage; (C) internal. CON: conventionally cured control; UC: uncured, nitrite-free; UCC: uncured, nitrite-free with cherry powder. CF: conventional film; NEF: nitrite-embedded film. Error bars represent ± standard error of the mean (SEM = 0.43 [A], 0.37 [B], and 0.40 [C]).

point. However, significant redness improvements were observed in both nitrite-free formulations packaged in NEF and both depicted *a** values that would typically be associated with cured color (*a** value >12) after 7 d of lighted storage or after 7 d and 28 d under dark storage for UCC-NEF and UC-NEF, respectively. Increased *a** values of the internal slices were also observed for nitrite-free formulations and were similar to those for dark storage surface measurements. The packages designated for internal color measurement were held in lighted storage until measurements were taken. No *a** value differences were observed for storage time within treatments for light, dark, and internal color measurements for CON-CF, CON-NEF, UC-CF, and UCC-CF, respectively. Notably, the *a** values were higher for CON formulation under dark storage. The color stability of cured meats stored in the dark is well documented (Andersen et al., 1988, 1990; Yen et al., 1988). These results support the findings of previous research (Cropp et al., 2020) and further support the hypothesis that NEF, when applied to cooked, vacuum-packaged, nitrite-free meat post-thermal

processing, will generate nitrosylhemochrome pigment. The subsurface development of cured color in the uncured, cooked and chilled bologna in this study resembles that of the "pink ring" typically observed below the surface of uncured barbequed meat cuts. However, Cornforth et. al. (1998) reported that the "pink ring" of barbequed meats is not likely the result of diffusion into the meat of nitric oxide gas formed during cooking, as is often thought, because nitric oxide has very limited solubility in water. Rather, it results from diffusion of nitrous acid formed from nitrogen dioxide gas, formed during cooking, and water on the meat surface. The nitrous acid, which is soluble in water, reforms nitric oxide during diffusion into the meat to form the "pink ring." Nitrite, on the other hand, is highly soluble in water and consequently, in the present study, diffuses readily from the packaging film to the product interior and provides nitric oxide for cured color development.

The *b** values for light and dark storage (Figure 2) were highest in UC-CF and UCC-CF, suggesting the most yellowness or discoloration in these treatments. Under light (Figure 2A) and dark (Figure 2B) storage, UC-NEF and UCC-NEF resulted in significantly lower

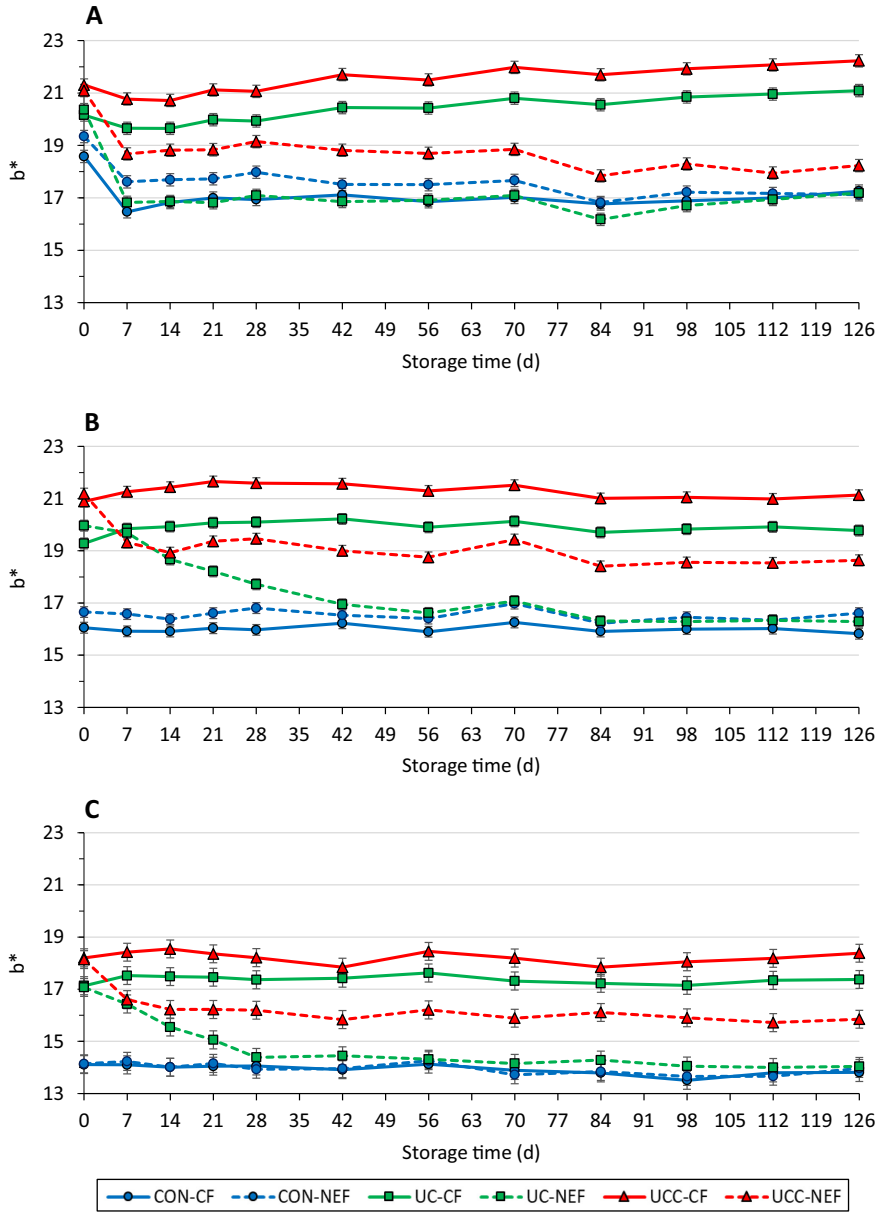

Figure 2. Least-squares means of *b** values of bologna treatments stored at 3 ± 2°C during 126 d. (A) External surface, lighted storage; (B) external surface, dark storage; (C) internal. (CON: conventionally cured control; UC: uncured, nitrite-free; UCC: uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film). Error bars represent ± standard error of the mean (SEM = 0.24 [A], 0.21 [B], and 0.34 [C]).

surface *b** values. In contrast, CON-CF resulted in lower *b** values than CON-NEF under both light (Figure 2A) and dark (Figure 2B) storage; however, for internal *b** values (Figure 2C), a significant difference between CON-CF and CON-NEF was not observed. Furthermore, the *b** value results over storage time (Figure 2A) show significant improvement (reduction in *b** values) from 0 d to 7 d under lighted storage, again suggesting that color re-establishment (nitrosyl-pigment equilibrium) occurred.

In-package surface *L** values under both light and dark storage (Figure 3A, 3B) differed only slightly over time with a mean range of less than 3 units between treatments, with the primary effect being that UC-CF tended to be lighter than all other treatments throughout the entire 126-d storage period. A similar trend was observed for internal *L** values (Figure 3C), with *L** values being lower in UC and UCC when packaged in NEF, and not different in CON regardless of film type, evidently owing to the fact that the samples

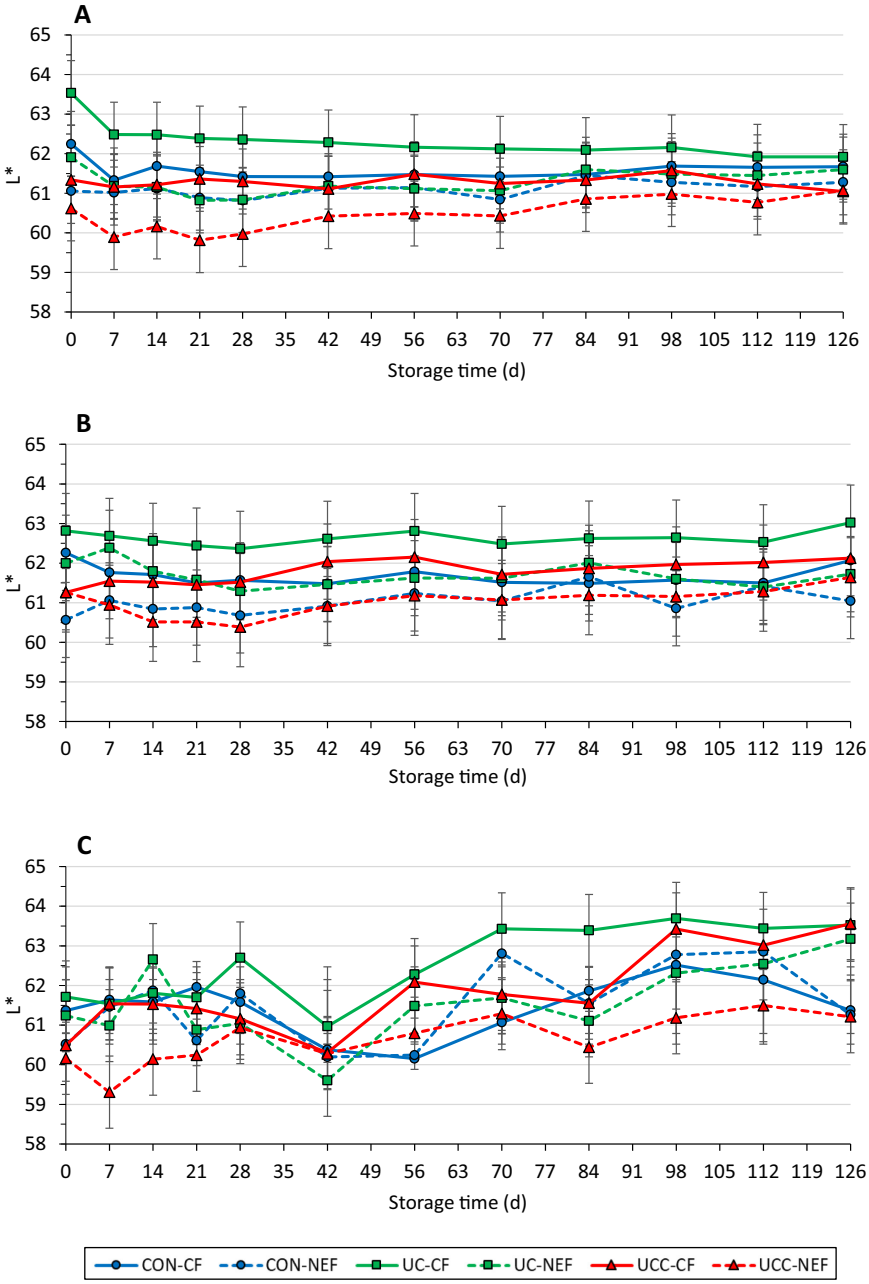

Figure 3. Least-squares means of *L** values of bologna treatments stored at 3 ± 2°C during 126 d. (A) External surface, lighted storage; (B) external surface, dark storage; (C) internal. (CON: conventionally cured control; UC: uncured, nitrite-free; UCC: uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film). Error bars represent ± standard error of the mean (SEM = 0.82 [A], 0.95 [B], and 0.91 [C]).

contained nitrite in their formulations. Hue angle (HA), which is an indication of true color observed (such as red, green, blue, yellow, etc.) and chroma (C*), a measure of color intensity (or saturation index), were also determined, with all two- and three-way interactions being significant for both (except for internal C*, in which none of the interaction effects were significant) (Table 2). Under light storage, surface HA values (Figure 4A) were highest in UC-CF and UCC-CF and lowest in both CON treatments. HA values of UC-NEF and UCC-NEF were initially as high as UC-CF and UCC-CD, but had decreased markedly by day 7 of storage, after which they did not change and remained just slightly higher than in CON-CF and CON-NEF. A similar pattern was observed under dark storage (Figure 4B), except that HA values in UC-NEF and UCC-NEF, while decreasing, did not stabilize completely until day 56 of storage. Beyond that time, they remained markedly higher than in both CON treatments, owing mostly to the fact that HA values for the

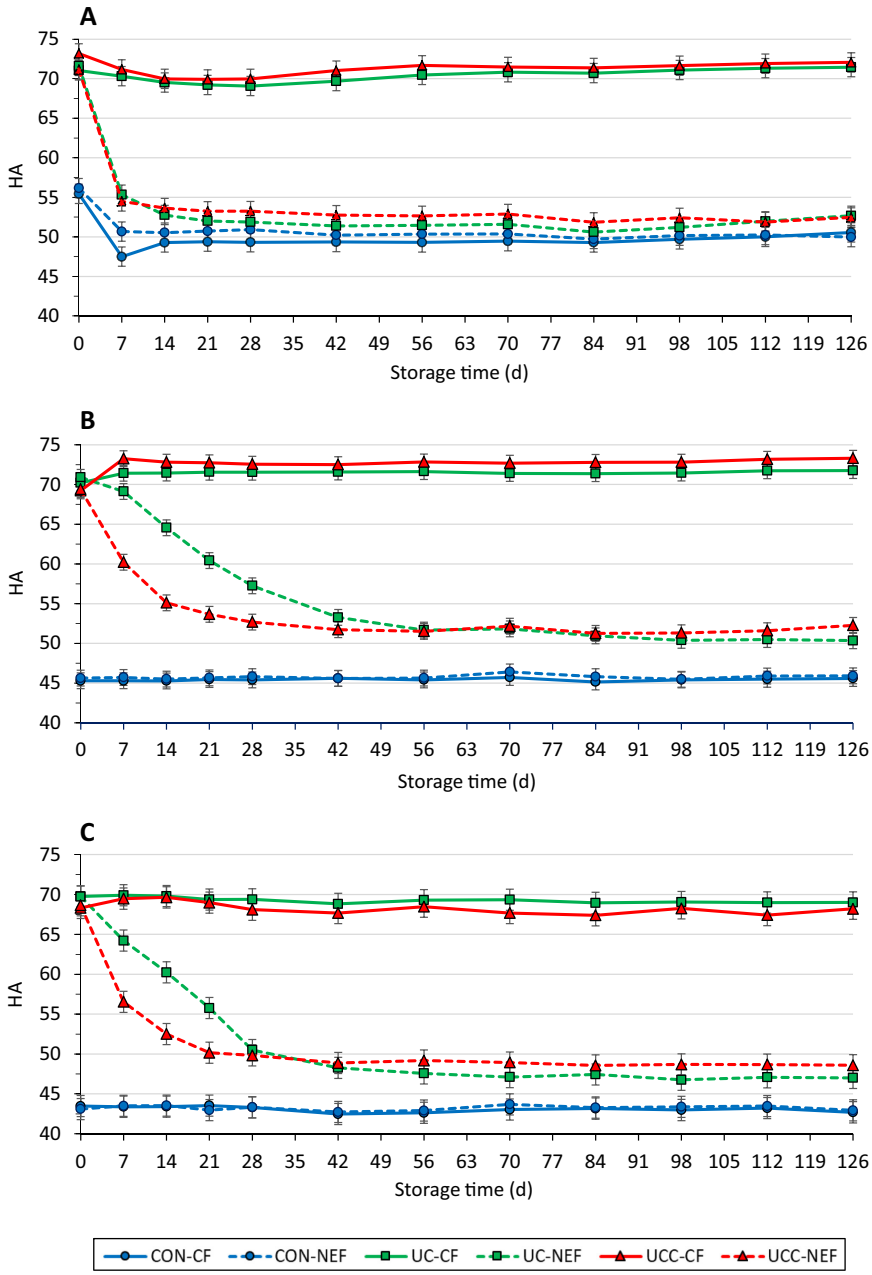

Figure 4. Least-squares means of hue angle (HA) values of bologna treatments stored at 3 ± 2°C during 126 d. (A) External surface, lighted storage; (B) external surface, dark storage; (C) internal. (CON: conventionally cured control; UC: uncured, nitrite-free; UCC: uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film). Error bars represent ± standard error of the mean (SEM = 1.22 [A], 1.00 [B], and 1.33 [C]).

latter were lower under dark storage than under light storage. Internal HA values (Figure 4C) followed a pattern similar to that of surface HA values of dark-stored samples. C* values (data not shown) of samples under light and dark storage were similar (all close to 20; $P > 0.05$), while internal C* values were greater (indicating greater brightness) in UCC-NEF, CON-CF, and CON-NEF than in UCC-CF, which, in turn, were higher than in UC-NEF and UC-CF.

Previous observations (Cropp et al., 2020) suggested that a progressive development of cured color from the surface to the interior of cooked, uncured bologna occurred with use of NEF packaging. Results for *a** values (Figure 5) show that CON-CF and CON-NEF were not different, but both had significantly greater *a** values than all other treatments at all storage periods. UC-NEF during storage resulted in significant *a** increases during storage time for both the top and middle sections of the 12-slice stacks used to assess color development, with less redness at day 0 and day 14 than at day 42 and day 56, respectively. While more redness was observed in top and middle sections of UC-NEF samples (24 mm total redness penetration), the bottom section did not change over time. While redness increases were significant in the UC-NEF packages, larger redness increases in *a** values were observed in top and middle sections of the UCC-NEF samples. Both the top and middle sections reached an approximate numerical mean *a** value of 10.08 by day 56. Comparatively, UC-NEF top and middle sections had an approximate numerical mean *a** value of 8.20 by day 56. Further, on day 0, for the UCC-CF and UCC-NEF samples, the top, middle and bottom section numerical *a** measurements were all between 5.50 and 7.14. By day 56, for UCC-CF, the top, middle and bottom sections still had mean *a** values of close to 7.57, and the sections were not different; however, for UCC-NEF by day 56, the top, middle, and bottom section *a** values of the 12-slice stack were 10.27, 9.86, and 7.75, respectively.

Both UC-NEF and UCC-NEF resulted in significant *a** increases in top and middle sections over time, and, again, showed the effect of the added reductant in the UCC formulation. It is possible that freezing prior to packaging samples used for assessing the progressive color development might have impacted the ability or rate of nitrite reduction to nitric oxide to generate nitrosyl pigments. Also, these stacks were 36 mm thick, where each section (top, middle, bottom) was 12 mm thick, with each section containing 4 slices, which were the same thickness and slice count (4 slices) as the standard packages utilized in this study. Nevertheless, the importance of this observation is that it shows the ability for the cured color/pigment to develop in the interior of the sliced and stacked product when only NEF was present on the top surface slice, and indicates that color development from NEF was not solely a surface effect. These results demonstrate

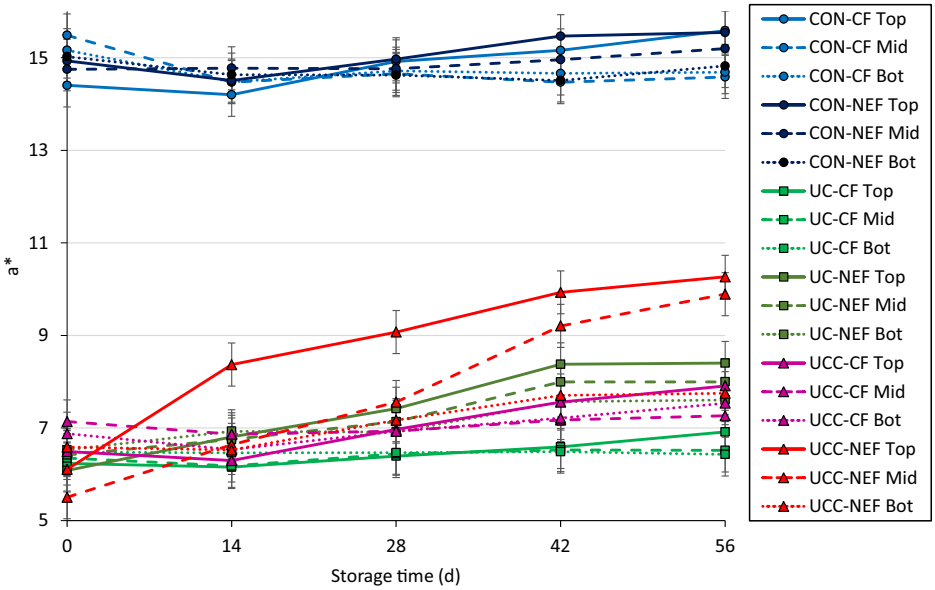

Figure 5. Least-squares means for development of *a** values in bologna stored at 3 ± 2°C during 56 d. Changes shown from film surface through 12 slices divided into 3 sections: Top: top (slices 1–4); Mid: middle (slices 5–8); Bot: bottom (slices 9–12). (CON: conventionally cured control; UC: uncured, nitrite-free; UCC: uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film). Error bars represent ± standard error of the mean (SEM = 0.46).

the ability of NEF to generate nitrosyl pigments after thermal processing, with or without an added reductant (cherry powder, a natural source of ascorbic acid, in the present study).

Residual nitrite

Residual nitrite values were significantly higher in NEF than in CF for all formulations (Figure 6). Our results showed the typical reductions in residual nitrite over time that are expected of meat products cured with nitrite. The decline of residual nitrite concentration during storage of cured meat is well understood and documented (Hustad et al., 1973; Dethmers et al., 1975; Jantawat et al., 1993; Ahn et al., 2002; Sindelar et al., 2007; Krause et al., 2011; Xi et al., 2012; Myers et al., 2013; Redfield & Sullivan, 2015; Usinger et al., 2016; Cropp et al., 2020). As discussed earlier, residual nitrite during storage is important for nitrosylhemochrome pigment stabilization, and for the equilibrium balance between nitrosyl-associated heme pigment and dissociated nitric oxide that is expected with light exposure and color re-establishment. It is understood that during storage some residual nitrite is also converted to nitrate (Sindelar et al., 2007; Honikel, 2008), which is another explanation for the decreased residual nitrite observed during storage. It does appear that NEF is able to serve as a source of residual nitrite in the product during storage, which improves color stability during retail display. Reductions in color stability were not observed in the present study; however, color stability during lighted retail display is a frequent and well-understood challenge for cured meat products.

Cured and total meat pigment

Measurement of cured meat pigment concentrations (Table 4) shows that CON formulations (CON-CF, CON-NEF) were not different and did not change over storage time. Similarly, the nitrite-free, CF-packaged treatments, UC-CF and UCC-CF, were not different, and no pigment changes over storage were

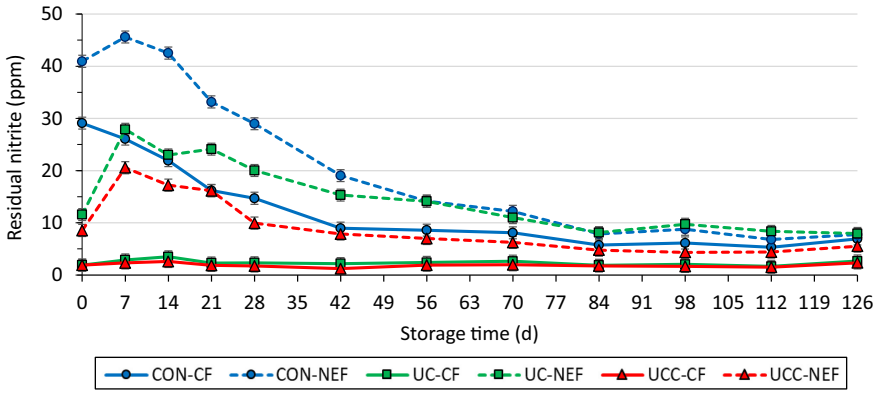

Figure 6. Least-squares means of treatment effects on residual nitrite concentration of bologna stored at 3 ± 2°C. (CON: conventionally cured control; UC: uncured, nitrite-free; UCC: uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film). Error bars represent ± standard error of the mean (SEM = 1.15).

Table 4. Least-squares means for treatment effects on cured meat pigment (nitrosylhemochrome) concentrations (in ppm) of bologna stored under fluorescent lights at 3 ± 2°C

	Day											
Treatment	0	7	14	21	28	42	56	70	84	98	112	126
CON-CF	136^{a}	138^{a}	135^{a}	135^{a}	136^{a}	134^{a}	138^{a}	133^{a}	134^{a}	135^{a}	136^{a}	133^{a}
CON-NEF	146^{a}	137^{a}	136^{a}	137^{a}	135^{a}	132^{a}	136^{a}	136^{a}	133^{a}	133^{a}	134^{a}	132^{a}
UC-CF	6^{f}	5^{f}	4.4^{f}	6^{f}	4^{f}	6^{f}	6^{f}	6^{f}	6^{f}	7^{f}	7^{f}	7^{f}
UC-NEF	9^{f}	59^{e}	75de	95^{c}	100^{bc}	108^{bc}	108^{bc}	110^{bc}	109^{bc}	111^{bc}	114^{b}	113^{b}
UCC-CF	8^{f}	8^{f}	7^{f}	9^{f}	6^{f}	9^{f}	8^{f}	7^{f}	7^{f}	8^{f}	8^{f}	9^{f}
UCC-NEF	9^{f}	79^{d}	98^{bc}	102^{bc}	100^{bc}	100^{bc}	107^{bc}	105^{bc}	105^{bc}	108^{bc}	104^{bc}	104^{bc}

SEM: standard error of the mean (2.82).

CON: conventionally cured control; UCC: uncured, nitrite-free; uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film.

$^{a–f}$Means with different superscripts are significantly different ($P < 0.05$).

observed. On the other hand, cured pigment concentrations in UC-NEF and UCC-NEF increased rapidly (<7 d). UCC-NEF developed cured pigment faster and was significantly greater than UC-NEF at day 7 and day 14, but they were not different after day 14. These findings correspond well with the color results. When using colorimetric methods to determine concentrations of nitrosyl-pigments, absorbance at 540 nm is the typical wavelength for detection of nitrosylhemochrome (Brown and Tappel, 1957; Tappel, 1957). Some slight variability of nitrosyl-pigment absorbance peaks have been reported at 535 nm (Hornsey, 1956), 547 nm (Nam and Ahn, 2002) and 550 nm (Cornforth et al., 1986). Nevertheless, 540 nm is the most utilized wavelength. In this study, the nitrosyl-pigment absorbance spectra show maximum absorbance at wavelengths of 476, 535, and 563 nm, as determined by Hornsey (1956) (Figure 7). To confirm that the cured pigment reflectance values reported in the present study were nitrosylhemochrome pigment, scanning was conducted from 470 to 570 nm. Based on scans, the optical densities at day 0 (Figure 7A) reveal that only CON formulation treatments exhibit absorbance spectra that shows cured pigment, and all other treatments show essentially no absorbance (optical densities < 0.06). In contrast, by day 126 of storage (Figure 7B), the treatments with nitrite either in the formulation (CON) or from the film (NEF) show absorbance maxima that depict cured pigment presence, with maxima at wavelengths of 480, 540, and 562 nm, which is consistent with nitrosylhemochrome pigment.

These results show that nitrosyl-associated pigment was generated by NEF, which has been previously hypothesized (Cropp et al., 2020). Furthermore, they show that while the reductant helps speed up the process of color development, it is not required under the conditions of this study. Also, there is evidence that nitric oxide can bind to ferrous heme at a faster rate (close to 10^7 M^{-1} s^{-1}) when the distal histidine ligand is separated (Sharma et al., 1987), as it is thought to occur during thermal processing (Sun et al., 2009). Therefore, separation of the globin portion may explain the

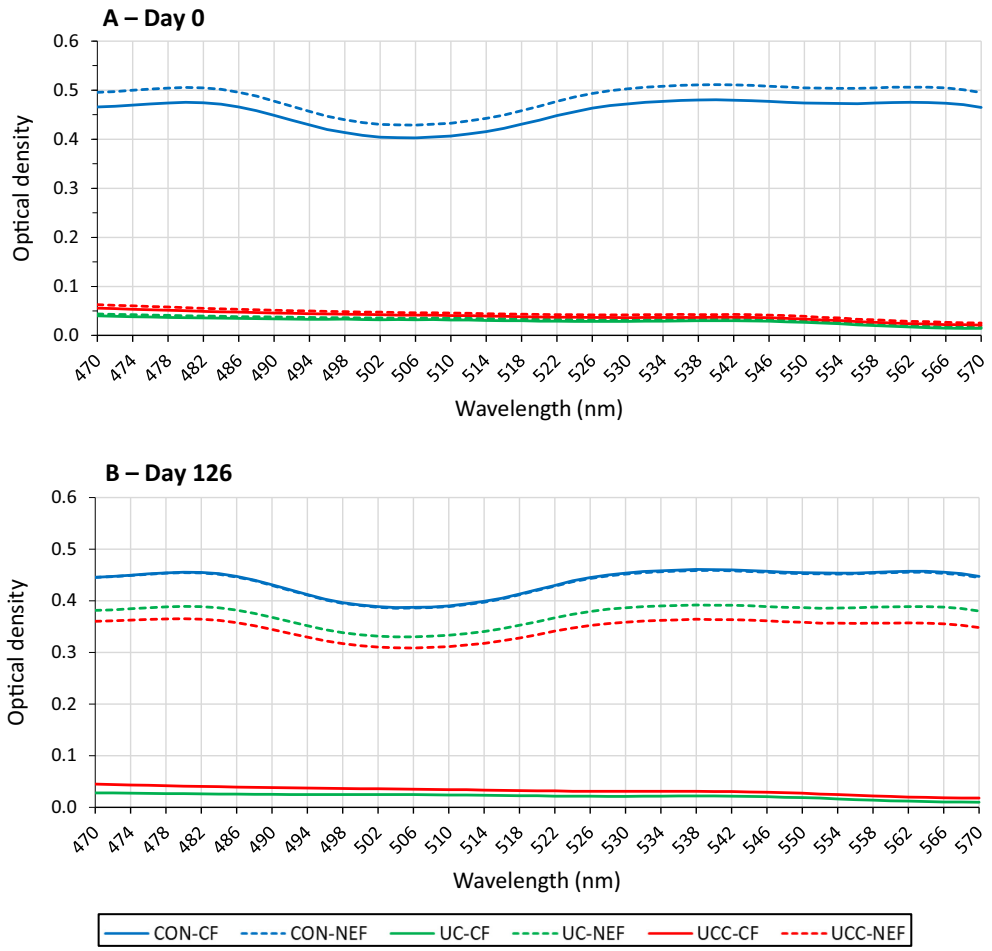

Figure 7. Effect of treatment on optical density of pigment extracts at various wavelengths. (A) Day 0; (B) day 126. (CON: conventionally cured control; UC: uncured, nitrite-free; UCC: uncured, nitrite-free with cherry powder; CF: conventional film; NEF: nitrite-embedded film).

seemingly faster generation of nitrosylhemochrome pigment from NEF with or without the reductant present.

Total pigment concentrations differed between formulations ($P < 0.05$), with CON > UCC > UC. While some formulation differences for total pigment were found, the mean pigment values (ppm) varied by less than 15 ppm across all treatments (data not shown). Over storage time, the ANOVA P value (0.013) was significant (Table 2). However, the post hoc multiple pairwise comparisons test using Tukey's procedure failed to identify a significant difference between pairs of means, so we conclude they were not different over storage time.

TBARS

No significant interaction effects for TBARS were observed. While the main effects of formulation, package type, and storage time were all significant ($P < 0.001$; Table 2), all TBARS values remained generally very low, ranging from 0.398 (CON/NEF/day 126) to 0.663 (UC/CF/day 14). Over the entire study, they were lower in CON (0.44) than in UC (0.54) and UCC (0.54), slightly lower in NEF (0.48) than in CF (0.53), and varied throughout the storage period from 0.47 on day 70 to 0.56 on day 14. Usually, increased TBARS values during lighted display are anticipated, especially in the absence of nitrite or an antioxidant. The results in the present study showed an antioxidant effect from nitrite in the formulation, as previously reported (Kanner et al., 1984; Shahidi and Hong, 1991; Sebranek, 2009) and suggest that NEF may play a role in reducing the rate and/or extent of lipid oxidation.

Purge

Purge results indicated no formulation effect ($P = 0.226$; Table 2). However, NEF-packaged bologna had significantly less purge (2.50% in NEF vs. 2.72% in CF, averaged across all storage time points). In general, purge increased over time throughout the storage period. There were no significant interactions observed.

Conclusions

Results of the present study demonstrate that nitrite-free cooked and chilled meat packaged in NEF can result in cured meat attributes, including cured pigment generation and cured color development, resulting from film contact with the product surface as well subsequent subsurface cured color development. To the best of our knowledge, we believe that this is the first report of evidence that cured meat color can be produced using an external nitrite source such as NEF in meat cooked and chilled prior to packaging, achieved by exposure to low concentrations of nitrite as the curing agent. The development of cured color, particularly the subsurface color, occurred despite the very limited amount of nitrite provided by the NEF. Additionally, in the case of alternatively cured meats, NEF may provide supplemental residual nitrite, important for maintaining nitrosyl-associated heme pigment equilibrium to extend color stability. Further research should be conducted with less saturated fat sources to determine the antioxidant capability of NEF, and investigate the food safety implications of nitrite-free cooked meat packaged in NEF.

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Literature Cited

Ahn, H. J., J. H Kim, C. Jo, C. H. Lee, C. H., 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

Andersen, H., G. Bertelsen, L. Boegh-Soerensen, C. Shek, and L. Skibsted. 1988. Effect of light and packaging conditions on the colour stability of sliced ham. Meat Sci. 22:283–292. https://doi.org/10.1016/0309-1740(88)90067-8

Andersen, H. J., G. Bertelsen, A. Ohlen, and L. H. Skibsted. 1990. Modified packaging as protection against photodegradation of the colour of pasteurized, sliced ham. Meat Sci. 28:77–83. https://doi.org/10.1016/0309-1740(90)90021-W

Andersen, H., and M. Rasmussen. 1992. Interactive packaging as protection against photodegradation of the colour of pasteurized, sliced ham. Int. J. Food Sci. Tech. 27:1–8. https://doi.org/10.1111/j.1365-2621.1992.tb01172.x

AOAC International. (2019). Offical methods of analysis of AOAC International. AOAC International, Arlington, VA, USA.

Binkerd, E. F., and O. E. Kolari. 1975. The history and use of nitrate and nitrite in the curing of meat. Food Cosmet. Toxicol. 13:655–661. https://doi.org/10.1016/0015-6264(75)90157-1

Böhner, N., and K. Rieblinger, K. 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

Brown, W. D., and A. L. Tappel. 1957. Identification of the pink pigment of canned tuna. J. Food Sci. 22:214–221.

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

Cornforth, D. P., J. K. Rabovitser, S. Ahuja, J. C. Wagner, R. Hanson, B. Cummings, and Y. Chudnovsky. 1998. Carbon monoxide, nitric oxide, and nitrogen dioxide levels in gas ovens related to surface pinking in cooked beef and turkey. J. Agr. Food Chem. 46: 255–261. https://doi.org/10.1021/jf970474i

Cornforth, D. P., F. Vahabzadeh, C. E. Carpenter, and D. T. Bartholomew. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. J. Food Sci. 51:1132–1135. https://doi.org/10.1111/j.1365-2621.1986.tb13065.x

Cropp, M. S. 2018. The potential role of nitrite-embedded film technology in extending the color stability and shelf life of alternatively-cured meat products. M.S. thesis, Iowa State University, Ames, IA. (https://dr.lib.iastate.edu/handle/20.500.12876/30982)

Cropp, M. S., J. S. Dickson, R. Tarté, R., and J. G. Sebranek. 2020. Use of nitrite-embedded packaging film for color stability of alternatively-cured, fully cooked cologna. Meat Muscle Biol. 4:1–17. https://doi.org/10.22175/mmb.10379

Cropp M. S., R. Tarté, K. J. Prusa, J. S. Dickson, A. M. Shaw, T. A. Houser, R. I. Crowley, L. M. Reever, and J. G. Sebranek. 2023. Impact of nitrite-embedded packaging film on quality and sensory attributes of alternatively-cured and nitrite-free bologna. Meat Sci. 204:109289. https://doi.org/10.1016/j.meatsci.2023.109289

Denzer, M. 2020. Effects of enhancement and nitrite-embedded packaging on dark-cutting beef color. M.S. thesis, Oklahoma State University, Stillwater, OK. (https://hdl.handle.net/11244/328642)

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

FDA (U.S. Food and Drug Administration). 2007. GRN No. 228: Sodium nitrite embedded on one side of a food packaging film at a maximum level of 113 milligrams per square meter. U.S. Food and Drug Administration GRAS Notice Inventory. https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=228. (Accessed 17 October 2023).

FDA. 2010. Agency Response Letter GRAS Notice No. GRN 000228. U.S Food and Drug Administration GRAS Notice Inventory. https://wayback.archive-it.org/7993/20171031031644/https:/www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm221704.htm. (Accessed 17 October 2023).

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

Hornsey, H. C. 1956. The colour of cooked cured pork. I. Estimation of the nitric oxide-heam pigments. J. Sci. Food Agr. 8:547–552. https://doi.org/10.1002/jsfa.2740080910

Houser, T. A., J. G. Sebranek, A. U. Dong, P. M. Dixon, W. N. Maisonet, and J. C. Cordray. 2005. Irradiation-induced cured ham color fading and regeneration. J. Food Sci. 70:C281–285. https://doi.org/10.1111/j.1365-2621.2005.tb07174.x

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. Environ. Microb. 26:22–26. https://doi.org/10.1128/am.26.1.22-26.1973

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

Kanner, J., S. Harel, J. Shagalovich, and S. Berman. 1984. Antioxidative effect of nitrite in cured meat products: Nitric oxide-iron complexes of low molecular weight. J. Agr. Food Chem. 32:512–515. https://doi.org/10.1021/jf00123a023

King, D. A., M. C. Hunt, S. Barbut, J. R. Claus, D. P. Cornforth, P. Joseph, Y. H. Kim, G. Lindahl, R. A. Mancini, M. N. Nair, K. J. Merok, A. Milkowski, A. Mohan, F. Pohlman, R. Ramanathan, C. R. Raines, M. Seyfert, O. Sørheim, S. P. Suman, and M. Weber. 2023. American Meat Science Association guidelines for meat color measurement. Meat Muscle Biol. 6:12473, 1–81. https://doi.org/10.22175/mmb.12473

Krause, B. L., J. G Sebranek, R. E. Rust, and A. Mendonca, A. 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. 89:507–513. https://doi.org/10.1016/j.meatsci.2011.05.018

Mack, J. M., and J. R. Bolton. 1999. Photochemistry of nitrite and nitrate in aqueous solution: A review. J. Photoch. Photobio. A 128:1–13. https://doi.org/10.1016/S1010-6030(99)00155-0

Møller, J. K. S., J. S. Jensen, M. B. Olsen, L. H. Skibsted, and G. Bertelsen. 2000. Effect of residual oxygen on colour stability during chill storage of sliced, pasteurised ham packaged in modified atmosphere. Meat Sci. 54:399–405. https://doi.org/10.1016/S0309-1740(99)00116-3

Møller, J. K. S., and L. H Skibsted. 2002. Nitric oxide and myoglobins. Chem. Rev. 102:1167–1178. https://doi.org/10.1021/cr000078y

Møller, J. K. S., and L. H Skibsted. 2006. Myoglobins: The link between discoloration and lipid oxidation in muscle and meat. Quim. Nova 29:1270–1278. https://doi.org/10.1590/S0100-40422006000600024

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

Nam, K. C., and D. U. Ahn. 2002. Carbon monoxide-heme pigment is responsible for the pink color in irradiated raw turkey breast meat. Meat Sci. 60:25–33. https://doi.org/10.1016/S0309-1740(01)00101-2

Narváez-Bravo, C., A. Rodas-González, C. Ding, O. López-Campos, J. Galbraith, I. L. Larsen, J. Ye, D. Siegel, D., and J. L. Aalhus. 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

Pearson, A. M., and T. A. Gillett, editors. 1996. Processed meats. 3rd ed. Chapman & Hall, New York.

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. 2017. Nitrite embedded vacuum packaging improves retail color and oxidative stability of bison steaks and patties. Meat Muscle Biol. 1:169–180. https://doi.org/10.22175/mmb2017.03.0015

Sebranek, J. G. 2009. Basic curing ingredients. In: R. Tarté, editor, Ingredients in meat products: Properties, functionality and applications. Springer Science + Business Media, New York. p. 1–23. (https://link.springer.com/chapter/10.1007/978-0-387-71327-4_1)

Shahidi, F., and C. Hong. 1991. Evaluation of malonaldehyde as a marker of oxidative rancidity in meat products. J. Food Biochem. 15:97–105. https://doi.org/10.1111/j.1745-4514.1991.tb00147.x

Sharma, V. S., T. G Traylor, and R. Gardiner. 1987. Reaction of nitric oxide with heme proteins and model compounds of hemoglobin. Biochemistry-US 26:3837–3843. https://doi.org/10.1021/bi00387a015

Sindelar, J. J., J. C. Cordray, J. G. Sebranek, J. A. Love, and D. U. Ahn. 2007. 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

Suman, S. P., M. C. Hunt, M. N. Nair, and G. Rentfrow. 2014. Improving beef color stability: Practical strategies and underlying mechanisms. Meat Sci. 98:490–504. https://doi.org/10.1016/j.meatsci.2014.06.032

Sun, W. Q., G. H. Zhou, X. L. Xu, and Z. Q. Peng. 2009. Studies on the structure and oxidation properties of extracted cooked cured meat pigment by four spectra. Food Chem. 115:596–601. https://doi.org/10.1016/j.foodchem.2008.12.060

Tappel, A. L. 1957. Spectral studies of the pigments of cooked cured meats. J. Food Sci. 22:479–482. https://doi.org/10.1111/j.1365-2621.1957.tb17506.x

Tarladgis, B. G., B. M. Watts, M. T. Younathan, and L. Dugan. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44–48. https://doi.org/10.1007/BF02630824

USDA (U.S. Department of Agriculture). 2018. Safe and suitable ingredients used in the production of meat, poultry, and egg products – Revision 58. FSIS Directive 7120.1. https://www.fsis.usda.gov/policy/fsis-directives/7120.1. (Accessed 5 August 2023).

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. L. 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. Sofos, and 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

Zipser, M. W., and B. M. Watts. 1962. A modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats. Food Technol.-Chicago 16:102–104.