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The potential role of nitrite-embedded film technology in extending the color stability and shelf life of alternatively-cured meat products

Michael Stephen Cropp
Iowa State University

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The potential role of nitrite-embedded film technology in extending the color stability and shelf life of alternatively-cured meat products

by

Michael Stephen Cropp

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Meat Science

Program of Study Committee:

Joseph G. Sebranek, Major Professor

Rodrigo Tarté

James S. Dickson

The student author and the program of study committee are solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2018

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NOMENCLATURE

NEF	Nitrite-embedded Film
CF	Conventional Film
CJP	Cultured Celery Juice Powder
NT10	Natpre T-10 EML Plus S
CIE	Commission Internationale de l'Eclairage
CFU	Colony Forming Unit
NO ₃	Nitrate
NO ₂	Nitrite
NO	Nitric Oxide
PPM	Parts Per Million
GRAS	Generally Recognized As Safe
GNP	Gross National Product
MAP	Modified Atmosphere Packaging
VSP	Vacuum Skin Packaging
NH ₃	Ammonia Gas
N ₂	Nitrogen Gas
HNO ₂	Nitrous Acid
CO	Carbon Monoxide
CO ₂	Carbon Dioxide
O ₂	Oxygen
N ₂ O ₃	Dinitrogen Trioxide
H ₂ O	Water

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ABSTRACT

Meat preservation in the form of salting, curing, and packaging have been centric to meat consumption for centuries. Current research in processed meats is heavily focused on alternatively-cured meats. Alternatively-cured meats have a greater potential for reduced color stability and shelf life (compared to conventionally-cured counterparts), due to limiting vegetable-like flavors. Development of vegetable-like flavors and natural/organic labeling requirements, meat processors generally reduce the amount of ingredients used, which can lead to quality and shelf life concerns. This study was designed to determine the effects of nitrite-embedded film (NEF) technology on fully cooked all-beef bologna. Specific emphasis was given to NEF's ability to improve the cured color stability and shelf life of alternatively-cured meats. The research in the current study determined the impact of NEF on internal and external color, internal and external residual nitrate and nitrite, in addition to microbial growth over a 125-day simulated retail display period. An elevated temperature 21-day retail display was conducted on samples that were inoculated with a general unknown bacterial inoculum. Five treatments were manufactured (CON-CF, CJP-CF, CJP-NEF, NT10-CF, NT10-NEF); a conventionally-cured control (CON-CF) in vacuum packaged in conventional film, an alternatively-cured formulation (nitrite from cultured celery juice powder plus cherry powder) vacuum packaged into both conventional film (CJP-CF) and nitrite-embedded film (CJP-NEF). An additional alternatively-cured formulation was produced (Natpre T-10 EML Plus S) and vacuum packaged into both conventional film (NT10-CF) and nitrite-embedded film (NT10-NEF). NEF-packaged products demonstrated a significant increase ($P < 0.05$) for in-package external a^* (redness) values and greater

color stability. From day 41 through 125 of retail display, there were no significant differences ($P > 0.05$) in external and internal residual nitrite between treatments. NEF showed no significant difference ($P < 0.05$) for residual nitrate between any treatments. NEF-packaged product showed no difference ($P > 0.05$) in internal residual nitrite compared to vacuum packaged treatment counterparts. Reduced color stability was observed in CJP-CF, while CJP-NEF showed improved a^* values. NEF packaging demonstrated no difference ($P > 0.05$) in microbial growth under traditional conditions. NEF exhibited no difference ($P > 0.05$) between treatments for inoculated elevated temperature microbial growth.

CHAPTER 1. GENERAL INTRODUCTION

Cured meats fit into the broad category of processed meats, and traditionally contain ingredients such as sodium nitrate and/or its reduced, reactive derivative, nitrite. Nitrite is a common ingredient in cured meats for its multifactorial properties with regard to product quality, such as the unmistakable pink color, cured aroma, flavor and extended shelf life, in conjunction with its unique inhibition of certain potentially deadly microbial pathogens.

Consumer perceptions of nitrite have led to significant growth in the volume of natural and organic meat products available in the marketplace. Consumers are looking for cured meat product characteristics, while also not wanting them to be conventionally-cured via traditional (direct) application of nitrate and or nitrite. Nitrite is a multifactorial irreplaceable ingredient in cured meat and at this point in time, cured meat product characteristics in a nitrite-free (truly uncured) product is unrealistic.

As a result, the meat industry currently produces products that provide cured meat characteristics while still aligning with consumers “uncured” labeling desires. This category of products is commonly referred to as “alternatively-cured,” because it largely contains natural nitrate/nitrite substitutes which are primarily derived from vegetable sources. Due to the unrefined nature of the natural sources of nitrate/nitrite, there is greater potential for negative quality attributes, such as development of vegetable-like flavors, inconsistencies in curing, reduced color stability and decreased shelf life.

Consumer demand for alternatively-cured meat products doesn’t appear to be decreasing in the near future; therefore, it is important to investigate potential

interventions that can offer improved product quality and safety while aligning with demand.

Sodium nitrite and product processing methods play crucial roles in changing meat composition through modification of inherent intrinsic factors (water activity, growth inhibitors, oxygen/reduction potential, pH, and nutrient content). Another aspect of meat preservation beyond product composition are extrinsic factors, such as light, temperature, relative humidity, gas presence and microorganisms. The combination of understanding the intrinsic factors and controlling the extrinsic factors lead to an improved shelf life. Managing many of the extrinsic factors on meat products can be accomplished by controlling the packaging environment.

Current commonly used cured meat packaging conditions include vacuum packaging or modified atmosphere packaging. However, recently many newer technologies in the area of active packaging have emerged. Potential active packaging solutions have primarily focused on improving the quality and shelf life of fresh meat with little emphasis towards cured meat. The research aim of the current study was to evaluate the effects of nitrite-embedded film technology on the color stability and shelf life extension of alternatively-cured meat products. It was hypothesized that alternatively-cured meats packaged with NEF technology would offer greater color stability, not affect microbial growth, and provide a longer shelf life, without surpassing normal levels of residual nitrate and nitrite. Two different formulations of alternatively-cured meat products were produced and packaged in NEF to determine its potential role in alternatively cured meat products.

CHAPTER 2. REVIEW OF LITERATURE

History of Cured Meats

Gathering of meat through scavenging and hunting are known to be ancestral behaviors that early *homo sapiens* adapted for survival. The exact historical timepoint of the development of meat preservation is lost in antiquity; however, it is understood that gathering and preserving of meat for later use is a key distinction that differentiated *homo sapiens* from other hominids. Early meat preservation techniques included salting, drying, and freezing to achieve a more consistent food supply (Edward and Wentworth 1956). Salting of meat was able to lower the water activity, which decreased the spoilage rate to provide a safe, consistent protein source over a longer period of time.

Salting was known to be common for fish dating back to as early as 3500 B.C., and it is believed that by the 19th century saltpeter (potassium nitrate) was differentiated from normal salt because of the observation that certain salts led to reduced spoilage (Pearson and Gillett 1996; Honikel 2008).

It is understood that the role of nitrates in food preservation is an indirect result of saltpeter impurities in salt (Pearson and Gillett 1996; Pegg and Shahidi 2000). The actual discovery of the chemical reaction occurring in meat during curing were discovered in the early 1900's (Haldane 1901). After the chemical mode of action for curing was discovered there were later discoveries with regard to certain bacterial species converting nitrate (NO_3) to nitrite (NO_2), which can be reduced further to nitric oxide (NO), and react with the color-producing, water-soluble protein myoglobin (Binkerd and Kolari 1975; Bryan 2006; Sebranek and Bacus 2007). Bacterial species capable of reducing nitrate to nitrite have a molybdopterin-containing nitrate reductase (Heath-Pagliuso et al.

1984; Cammack et al. 1999; Lejay et al. 1999). Commercially produced bacterial cultures are commonly used to convert nitrate to nitrite for the production of many cured meat products.

Environmental N-Containing Sources

Nitrate is very common in the environment, with dinitrogen gas (N_2) being present in the atmosphere and converted to various nitrogen-containing products such as ammonia, nitrate, and nitrite through nitrogen fixation. Bacteria that contain the enzyme nitrogenase are capable of fixing atmospheric dinitrogen gas to more useable forms (Doty et al. 2016). In particular, legume plants form a symbiotic relationship with anaerobic nitrogen fixing bacteria of the *Rhizobium* species through unique oxygen binding nodules in the root system, which allows for conversion of dinitrogen gas to ammonia (Doty et al. 2016). Other nitrifying bacteria ubiquitous in soil can convert ammonia to nitrite and nitrate for plants to use and/or store in a process referred to as nitrification (Prosser 2007). It is common to find increased levels of nitrate in soil, groundwater, and in many plants, especially certain vegetables. Some vegetables known to have increased levels of nitrate include celery, lettuce, radishes, swiss chard, spinach, and beets (Santamaria et al. 1999; Fujihara et al. 2001; Sindelar 2006; Sebranek and Bacus 2007). Nitrogen atoms contain three valence electrons which is why nitrogen gas (N_2) is very stable. When nitrate (NO_3) is formed, nitrogen is considered fully oxidized and when ammonia (NH_3) forms, it is considered fully reduced. The intermediate nitrogen-containing compounds are more reactive and are very relevant for an in-depth understanding of cured meat products. The key intermediate nitrogen-containing compounds nitrite (NO_2^-), nitric oxide (NO), nitrous acid (HNO_2) and dinitrogen trioxide (N_2O_3) will be discussed in later sections.

Nitrite and its Role in Meat Processing

Sodium nitrite plays a fundamental role in cured meat products because of its irreplaceable role in offering distinctive food safety and quality attributes. Nitrite has been shown to impart a unique cured flavor and color, and to delay rancidity development during storage as well as inhibit outgrowth of *Clostridium botulinum* (Binkerd and Kolari 1975). *Clostridium botulinum* is an anaerobic, gram-positive, spore-forming bacterium that can be a deadly pathogen if not properly controlled. Ingestion of *C. botulinum* toxin can result in the deadly neurological disease known as botulism. Many cooked and/or cured meats are packaged with low oxygen content, which is the favorable environment for the outgrowth of *C. botulinum*. However, nitrite is capable of preventing outgrowth of *C. botulinum*. In addition to inhibition of *C. botulinum*, nitrite also provides protection under certain conditions against *Listeria monocytogenes*, a gram-positive, psychrophilic pathogen (Buchanan and Philips 1990; McClure et al. 1991; Duffy et al. 1994; Buchanan and Golden 1995; Myers et al. 2013). Generally, nitrite has some inhibitory capacity against gram-positive microorganisms; however, nitrite has not been proven to be effective against gram-negative enteric bacteria such as *Salmonella enterica* or *Escherichia coli* (Tompkin 2005; Toldrá 2017).

Meat curing is a well-understood mechanism that provides inimitable food safety and quality characteristics to cured meat products. In cooked, uncured (nitrate and/or nitrite-free) meat, salt is a pro-oxidant compound that can promote fatty acid oxidation (Toldrá 2017). However, in cured meat nitrite has a significant antioxidant role and prevents rancidity through stabilization of fatty acid molecules, even with increased salt concentrations, especially when products are vacuum-packaged (Binkerd and Kolari 1975; Parthasarathy and Bryan 2012). Sato and Hegarty found that nitrite prevents a

warmed-over flavor, which is commonly associated with cooked meats (nitrate-and/or nitrite-free) during storage (Sato and Hegarty 1971). The antioxidant properties of nitrite have been attributed to chelation of free heme iron to prevent prooxidant activity (Morrissey and Tichivangana 1985). Morrissey and Tichivangana showed that even a very low ingoing amount of nitrite (20-50 mg/kg NO₂) was able to significantly reduce lipid oxidation in cured meat products (Morrissey and Tichivangana 1985). The application of smoke to cured meats is common and natural phenolic compounds in the smoke contribute to delaying rancidity, especially those phenolics with an elevated boiling point (Pearson and Gillett 1996).

Meat Color

Meat color is a critical aspect for consumer appeal, since it is usually the first impression of the product to the consumer. For most consumers, the color of a meat product is thought to be highly correlated with its perceived quality (Fox 1987). Therefore, an understanding of the chemical and biological properties that impact meat color are fundamental for maintaining consumer appeal.

Fresh Meat Color

The color of fresh meat is largely determined by the composition and state of myoglobin. A key function of myoglobin in living muscle is storage and transport of oxygen (Pegg and Shahidi 2000; Poulson et al. 2012). Myoglobin and hemoglobin work together for the transport and storage of oxygen and thus have similar color-fixing characteristics (Govindarajan and Snyder 1973). Hemoglobin has similar oxygen-binding

properties as myoglobin, although it is a relatively insignificant component of meat color in well-bled carcasses.

The structure of intact myoglobin consists of a globin portion attached to a heme group, which is an iron-containing protoporphyrin ring structure. Heme iron forms a ring structure and can form six bonds, four with pyrrole nitrogen atoms (positions 1 through 4), one with the globin portion (proximal histidine-93), while the most notable position is the ligand binding 6th position (Mancini and Hunt 2005; Faustman and Suman 2017). The 6th coordination site has the unique capability to reversibly bind certain diatomic ligands. The 6th position binding site of heme iron along with the valence state of heme iron is responsible for fresh meat color. The most notable diatomic ligands that may bind to the 6th position of heme iron in myoglobin are oxygen, carbon monoxide, and nitric oxide (Mondal and Meuwly 2018). With regard to fresh meat color, oxygen is a key ligand of importance to determine the form of myoglobin, although the oxidation state of heme iron is also critical. Additionally, carbon monoxide and nitric oxide can play a role in fresh beef and pork color under specific environmental conditions (Krause et al. 2003; Claus and Du 2013; Suman et al. 2014; Yang et al. 2016; Roberts et al. 2017). The five main forms of myoglobin that are important in fresh meat are oxymyoglobin, deoxymyoglobin, metmyoglobin, carboxymyoglobin and nitrosylmyoglobin.

When no ligand is bound to the 6th position of myoglobin in ferrous (Fe^{2+}) iron the resulting form is deoxymyoglobin. The visual color of deoxymyoglobin is generally described as purplish-red and is commonly found in vacuum packaged or very freshly cut meat. When myoglobin containing ferrous (Fe^{2+}) iron becomes exposed to diatomic oxygen the resulting form is oxymyoglobin and the reaction is known as blooming (Mancini and Hunt 2005). Blooming depends on multiple extrinsic and intrinsic factors

such as temperature, pH, partial pressure of oxygen and oxygen competition, through other respiratory processes such as bacterial respiration (Mancini and Hunt 2005). The blooming phenomenon is visually perceived as a bright cherry-red color and is a hallmark for consumer acceptability of fresh meat (Suman et al. 2014).

Metmyoglobin forms as a result of iron oxidation (loss of an electron) and forms when ferrous iron (Fe^{2+}) loses an electron to become ferric iron (Fe^{3+}). Iron in a ferric state can be formed from deoxymyoglobin and oxymyoglobin (Mancini and Hunt 2005). Metmyoglobin does not bind ligands such as oxygen; instead it remains unbound or bound to a hydroxyl molecule or water. The resulting color of metmyoglobin is generally described as brown, tan, or yellow. The oxidation of deoxymyoglobin or oxymyoglobin to form metmyoglobin is dependent on the reducing ability of the meat system. Oxymyoglobin formation is an ongoing process where oxygen is constantly binding and dissociating from myoglobin. The bloomed, reduced state of myoglobin depends greatly on the metmyoglobin-reductase-reducing agent present in order for it to persist, and the eventual reducing agent depletion results in metmyoglobin formation. Other factors that impact metmyoglobin formation are temperature, pH, microbial activity and oxygen pressure (Mancini and Hunt 2005).

When deoxymyoglobin is exposed to carbon monoxide (CO) gas the open 6th coordination site will bind CO and produce a bright cherry-red color similar to that of oxymyoglobin (Suman et al. 2006). Furthermore, in living muscle when CO enters the body it binds tightly to myoglobin and hemoglobin. The resulting carboxymyoglobin or carboxyhemoglobin can result in asphyxiation. Carboxymyoglobin can improve the color stability of fresh meat. Krause and others (2003) showed that CO-packaged pork improved redness and suppressed lipid oxidation during extended storage (Krause et al.

2003). However, there are concerns that the CO-packaged product may appear fresh but contain a high bacterial load (Cornforth and Hunt 2008).

An additional form of myoglobin is nitrosylmyoglobin, which forms when nitric oxide is incorporated under anaerobic conditions (deoxymyoglobin) with iron being in the ferrous (Fe^{2+}) state (Fox and Ackerman 1968). The resulting color of nitrosylmyoglobin is a bright cherry-red color similar to that of oxymyoglobin. Anaerobic packaging systems are highly desirable by processors because they can prevent growth of aerobic spoilage bacteria and mitigate lipid oxidation, although appearance of deoxymyoglobin is less attractive to consumers (AMSA 2012). A study conducted by Aaslyng and others (2010) found that consumers preferred the eating quality of steaks packaged anaerobically as opposed to those packaged aerobically (Aaslyng et al. 2010). Nitrosylmyoglobin formed under anaerobic conditions has the potential to improve the color stability and shelf life of fresh meat. Nitrosylmyoglobin can be formed by applying nitrite directly to the product surface prior to vacuum packaging or by embedding the nitrite in the packaging material. Both nitrite applications methods have been shown to improve the color stability and shelf life of fresh beef and pork products (Claus and Du 2013; Song et al. 2015; Yang et al. 2016). Forming nitrosylmyoglobin offers a bloomed-like appearing product while under anaerobic conditions, which can mitigate spoilage concerns.

Cooking of fresh meat under aerobic conditions results in oxidation of iron ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$) to form metmyoglobin and thermal denaturation results in the formation of denatured metmyoglobin, which is referred to as ferrihemichrome (Fe^{3+}) and is visually described as brown, tan or grey (Tarladgis 1962). If ferrous myoglobin is denatured under anaerobic conditions, the resulting pigment is referred to as ferrohemochrome (Fe^{2+}),

which upon exposure to oxygen will oxidize to ferrihemochrome (Ghorpade and Cornforth 1993). When denaturation of carboxymyoglobin occurs (via heat-based denaturation) the resulting color can be described as pinkish-red due to presence of CO-hemochrome (Tappel 1957; John et al. 2004). Denaturing nitrosylmyoglobin (NOMb) results in a cooked pinkish-red pigment (Song et al. 2015).

Fresh Meat Color Conclusion

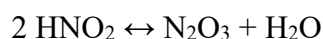
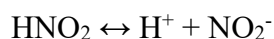
The iron state of myoglobin and presence or absence of certain diatomic ligands play crucial roles in meat color. Meat color can range from purplish-red in meat devoid of oxygen to bright cherry-red in the presence of oxygen, nitric oxide or carbon monoxide, with a brown color in oxidized fresh meat products.

Cured Meat Color

Nitrite (formed by reduction of nitrate) provides the unmistakable visual pink color, which is a hallmark of cured meat products. The curing processes and associated chemical reactions are complex. Originally, curing was achieved by the addition of nitrate (NO_3^-), although nitrate is relatively inert and requires bacterial reduction to form reactive nitrite (NO_2^-); Because of this, curing with via addition of nitrite is more common. Nitrite is primarily used for commercial production of many cured meats such as bologna, frankfurters, knockwurst, pastrami, bacon and many ham products, although dry-cured meat products (primarily hams and sausages) often contain significant amounts of nitrate to provide a source of nitrite over their extended curing period.

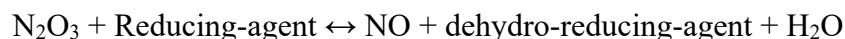
When nitrite is added to a meat system under aerobic conditions, it will oxidize the heme iron and generate nitric oxide. Nitric oxide is also generated from other nitrite derivatives that react with myoglobin. Nitrite ions are inherently negatively charged,

which allow nitrite molecules to donate an electron. Therefore, in an aqueous system the NO_2^- can react with positively charged hydrogen ions and form nitrous acid (HNO_2), which is a weak acid. Dinitrogen trioxide (N_2O_3) formation from nitrous acid is required to form nitric oxide (NO), which can bind to the 6th position of heme iron (Mondal and Meuwly 2018). Nitrous acid can form dinitrogen trioxide and water (H_2O). The subsequent dinitrogen trioxide in the presence of reductants will generate nitric oxide. Below are the understood reactions, adapted (with slight modifications) from Pegg and Shahidi (2000) and Sebranek (2009):



Nitric oxide + Myoglobin \rightarrow Nitric oxide myoglobin + Heat-based denaturation \rightarrow Nitrosylhemochrome

The ability to form nitric oxide from dinitrogen trioxide is dependent on the reducing ability present in the meat system and/or the addition of reducing ingredients. A common reducing ingredient in commercially produced conventionally-cured meats is erythorbate or its more expensive isomer ascorbate (Vitamin C). As pH is increased the reactivity of reducing agents increases respectively. The dinitrogen trioxide produced from nitrous acid while in the presence of a reducing agent results in formation of nitric oxide which is outlined below and adapted from Møller and Skibsted (2002):



Nitrite is considered a potent oxidizing agent targeting the heme iron complex. When nitrite is added to myoglobin under aerobic conditions (oxymyoglobin) the meat will quickly turn brown due to the oxidation of ferrous (Fe^{2+}) iron to ferric (Fe^{3+}) iron.

The resulting nitric oxide metmyoglobin undergoes auto-reduction by either endogenous (NADH) or exogenous reducing agents (ascorbate or erythorbate) to form nitric oxide myoglobin, and in the presence of heat, the nitrosylhemochrome pigment is formed (Hornsey 1956; Møller and Skibsted 2002).

Another important consideration is the formation of nitrate. The nitrous acid in the presence of a positively charged hydrogen ion can be a source of nitrate. The most common route for nitrite to take is to be converted to N_2O_3 and subsequently reduced to NO and H_2O , although there is some production of NO_3 and H^+ from HNO_3 (Sebranek 2009). Regarding the fate of nitrite in cured meat products, it is known that the production of nitric oxide is more favorable than that of nitrate, yet both will occur. Thus, finding nitrate in products which only contained nitrite in the formulation is not uncommon.

Cured Meat Color Conclusion

When nitrite is added to a meat system containing oxymyoglobin the resulting oxidation creates metmyoglobin and binding with nitric oxide yields nitrosylmetmyoglobin (Fe^{3+}), which is visually brown in color and is a key indicator of nitrite addition (Killday et al. 1988). Nitrosylmetmyoglobin is subsequently reduced by either endogenous or exogenous reducing agents to nitrosylmyoglobin (Fe^{2+}), which is red in color. Nitrosylmyoglobin in the presence of heat induced protein denaturation results in the formation of nitrosylhemochrome, which is pink in color (Hornsey 1956). The addition of elevated levels of nitrite to a raw meat system can result in formation of nitrimetmyoglobin, which is green in color (Fox 1987). However, following USDA mandated nitrate and nitrite regulations will prevent formation of nitrimetmyoglobin.

Meat color is very complex and slight changes in intrinsic and/or extrinsic properties can result in changes in fresh and cured meat color.

United States Regulations of Conventional Nitrate and Nitrite

In the 1970s, it was determined under certain conditions, nitrate and nitrite in cured meat products can form carcinogenic nitroso-compounds such as *N*-nitrosamines. The potential formation of *N*-nitrosamines is especially an issue in bacon (Shahidi et al. 1988). Bacon is commonly exposed to high-heat cooking (frying) and the interaction of nitrite and secondary amines can form carcinogenic compounds (Lijinsky 1999). Formation of *N*-nitrosamines has caused consumers to question the use of nitrite in meat products. The discovery that potentially harmful carcinogenic compounds can form in cured meat products led to tighter regulations regarding the use of nitrate and nitrite, especially in bacon products where there is a greater risk of *N*-nitrosamine formation due to cooking method. Formation of *N*-nitrosamines only occurs under specific conditions when nitrite and secondary amines are present, at close to neutral pH and cooking temperatures in excess of 130°C (Sindelar and Milkowski 2012). Current USDA-FSIS regulations regarding the use of nitrate and nitrite and preparation guidelines ensure consumer safety by mitigating the risk of carcinogenic compound formation in cured meat products (Pensabene et al. 1974; USDA 1995, 2013). Table 1 shows the current nitrate and nitrite usage levels mandated by USDA-FSIS.

Table 1. Maximum amount of ingoing nitrite and nitrate (in ppm) for cured meat products (except bacon products, which follow tighter regulations).

Curing Agent	Curing Method			
	Immersion Cured (ppm)	Massaged or Pumped (ppm)	Comminuted (ppm)	Dry Cured (ppm)
Sodium Nitrite	200	200	156	625
Potassium Nitrite	200	200	156	625
Sodium Nitrate	700	700	1718	2187
Potassium Nitrate	700	700	1718	2187

This reprint is from USDA-FSIS Processing Calculations Inspector's Handbook (FSIS Directive 7620.3)

In addition, USDA-FSIS has set a minimum requirement for products which contain nitrite. According to USDA-FSIS, all cooked products, labeled “keep refrigerated” must contain no less than 120 ppm of ingoing nitrite, unless the processor can validate an alternative safe process (USDA 1995). Thus, conventionally-cured meat products are required to contain 120 ppm of ingoing nitrite; however, alternatively-cured meats are not required to contain a standardized amount of ingoing nitrite. This lack of ingoing nitrite regulations can potentially lead to food safety and quality concerns in alternatively-cured meat products.

Alternatively-Cured Meat Products

Since the 1970s, consumer fear and perception of nitrate and nitrite revolving around nitrosamine formation have led to an increase in the quantity and variety of alternatively-cured meat products available in the marketplace. Alternatively-cured meats contain nitrate and/or nitrite, although they originate from natural ingredients. Vegetables are the most common source of nitrate/nitrite for alternatively-cured meat products. Some natural sources of nitrate and its commercial meat curing derivative nitrite include celery, lettuce, radishes, swiss chard, spinach, and beets (Santamaria et al. 1999; Fujihara et al. 2001; Sindelar 2006; Sebranek and Bacus 2007). Alternatively-cured meats follow the same curing principles as conventionally-cured meats. The natural sources of

nitrate/nitrite usually are less refined compared to conventional sources. Less refined sources of nitrate/nitrite subsequently require a greater amount of the natural replacement ingredient to achieve an equivalent ingoing amount comparable to conventional sources. Unfortunately, the natural sources of nitrite/nitrate can result in vegetable-like flavors in the finished product, especially when used at amounts that provide an equivalent amount of nitrite comparable to conventional sodium nitrite (Sindelar et al. 2007b; Djeri and Williams 2014). Sindelar and others (2007a) found that commercially produced alternatively-cured meat products had significantly less residual nitrite compared to conventionally-cured products. The impact of potential formation of vegetable-like flavors with natural nitrate/nitrite replacement ingredients, along with lower residual nitrite values found in commercially available alternatively-cured meat products, strongly suggest that processors have decreased the relative ingoing level of nitrate/nitrite to account for vegetable-like flavors. The reduced amount of nitrite in alternatively-cured meats can potentially lead to decreased color stability and shelf life.

“Uncured” Cooked Meat Products

Many cured meat products are required to contain nitrite to be labeled appropriately. For example, according to the Code of Federal Regulations, Title 9, §319.180, bologna must be cured with nitrite. If processors wanted to use other (natural/vegetable) sources of nitrite than the product would have to be labeled as “uncured bologna” and would fall into the alternatively-cured meat product category. Virtually all alternatively-cured meat products claim on the product label to be “uncured,” which can be confusing and is often misunderstood by general consumers. The general consumer might believe that the word “uncured” means there is chemically no nitrite or nitrate in the product formulation (nitrate/nitrite free), while the opposite is

more than likely the case. Thus, “uncured” meat products can be divided into two main categories.

The first category encompasses “uncured” products that aim to replace nitrite and/or nitrate. The replacement of nitrate/nitrite in these “uncured” products is usually achieved with a plant-based vegetable alternative that naturally contains nitrate/nitrite. These products would be expected to offer product characteristics such as aroma, flavor, color, and antibacterial properties similar to conventionally-cured meat products. Under current regulations in the United States, these products that contain known sources of nitrate/nitrite are required to contain a label disclaimer regarding the presence of nitrate and or nitrite.

A spin off from the first category of “uncured” includes those products that intend to replicate the typical characteristics (color, flavor, aroma and shelf life) of conventionally-cured meats while also not containing significant amounts of residual nitrate or nitrite. Therefore, these products do not require a label disclaimer when labeled as “uncured.” However, they still may contain nitrite but in much lesser levels. An example can be found in the Prosur[®] Product Guide, where it markets this type of cure replacement ingredients as “no-to-low residual nitrates/nitrites” (Wenda America, 2018). This category of “uncured” products is relatively novel and targets consumers that want cured meat characteristics without residual nitrate or residual nitrite in the finished product. The products may not contain significant amounts of residual nitrite; however, some ingoing nitrite is likely the case. Therefore, it still falls in the alternatively-cured category.

The second category of “uncured” products comprises those products that do not aim to replace nitrate or nitrite. Therefore, these products are not expected to exhibit the

typical aroma, flavor, color or antimicrobial protection that are associated with conventionally-cured meat products. These products do not need to state any label disclaimer regarding a nitrate or nitrite source. Inherently, there would be little to no detectable amounts of nitrate or nitrite in these products. Therefore, they are actually uncured and the most truthful representation of the respective category.

The two categories of “uncured” discussed above create great consumer confusion regarding the topic of “uncured” meat products. The traditional and alternative products that consumers are looking for exist in the marketplace, but the current labeling system can be confusing for consumers to navigate.

Regulations for Alternatively-Cured Meats

The term “alternatively-cured” is generally synonymous with “naturally cured” or “no nitrate or nitrite added.” Alternatively-cured meat products that have a natural label claim must follow the guidelines outlined by the USDA-FSIS Food Standards and Labeling Policy Book (USDA 2005). The USDA-FSIS regulations regarding labeling of products that claim to be “natural” read (p. 116-117):

“The term “natural” may be used on labeling for meat products and poultry products, provided the applicant for such labeling demonstrates that: (1) the product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and (2) the product and its ingredients are not more than minimally processed...All products claiming to be natural or a natural food should be accompanied by a brief statement which explains what is meant by the term natural, i.e., that the product is a natural food because it contains no artificial ingredients and is only minimally processed. This statement should appear directly beneath or beside all-natural claims or, if elsewhere on the principal display panel; an asterisk should be used to tie the explanation to the claim.”

According to the Code of Federal Regulations, Title 9, §317.17, alternatively-cured meat products that may or may not contain natural sources of nitrate or nitrite (for example

celery juice powder) instead of conventional nitrite, and are required to be named by a common, usual or descriptive name must be labeled as “uncured” and state “Not Preserved—Keep Refrigerated Below 40°F at All Times” unless they have met more stringent processing methods (CFR 1979). This section of the CFR (Title 9, §317.17) encompasses labeling for all categories of “uncured” meat products (i.e. those with intent of replicating cured product characteristics and those without intent of replicating cured product characteristics).

Packaging

In developed countries, packaging represents approximately two percent of the Gross National Product (GNP) and food packaging accounts for roughly fifty percent of the packaging GNP sector (Robertson 2013). Most food products sold to consumers come in some kind of package, with plastics being the dominant type of food packaging material. The plastics industry is believed to have originated in London, England around the early 1860s, where plastic was originally called “Parkesine,” which was created by altering the mixture of chemicals used to make gun cotton explosives (Robertson 2013). Packaging is an extensively used technique that can improve the product’s appearance, as well as extend safety and quality attributes.

Meat Packaging

The primary goal of meat packaging is to provide protection against extrinsic factors such as light, humidity, gases and microorganisms. The specific packaging requirements depend on the product’s intrinsic properties which include pH, water activity, nutrient content, growth inhibitors, and the oxygen/reduction potential. Virtually all meats are perishable under particular environmental conditions, thus creating motives

for packaging technology advancements. Unlike other consumer good producers, food and especially meat product manufacturers have an incentive to ensure product safety and quality (Osborn and Jenkins 1992). Packaging is a fundamental aspect of food production that has the potential to play a large role in product integrity. Packaging material selection is critical for maintaining and extending meat product quality. In particular, color stability of both fresh and processed meats is a critical quality indicator for consumers. The color stability also known as the product appearance throughout its shelf life, and is a key indicator of product quality for most consumers (Nannerup et al. 2004). Thus, packaging selection ultimately seeks to provide consumers with a safe, attractive, and wholesome product.

Modified atmosphere packaging (MAP) is a well-known meat packaging technology for both fresh and cured meat products. There are two primary categories of MAP, the first is active modification which is synonymous with the term “gas flushing” and the second is passive modification which in meat is largely seen as a negative outcome of respiration in the package (Robertson 2013). Passive modification, or “equilibrium-modified-atmosphere packaging,” is most commonly used for packaging fresh fruits and vegetables where respiration is likely to occur (Church 1994). High-oxygen MAP for fresh meat contains mixtures of oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) gas, with oxygen being the predominant gas at approximately 70-80% (Gill 1996; Sørheim et al. 1999; Djenane and Roncalés 2018). The oxygen gas allows for fresh meat to be in a consumer-favorable “bloomed” oxymyoglobin state, the carbon dioxide gas provides some antibacterial effect on microorganisms, and the nitrogen gas is a filler gas (Church 1994). It is worth noting that the carbon dioxide in MAP has not been shown to have a bacteriostatic effect against lactic acid producing bacteria, which are common

spoilage organisms of concern in conventional vacuum packages (Bjorkroth et al. 2000; Koort et al. 2005). Carbon monoxide (CO) MAP has also been shown effective toward developing the bright cherry-red carboxymyoglobin appearance on fresh beef and pork with approximately 0.4-0.5% CO, 60-70% CO₂ and 30-40% N₂ (Brewer et al. 1994; Sørheim et al. 1999; Jayasingh et al. 2001; Krause et al. 2003; Mancini and Hunt 2005).

In cured meats high oxygen MAP and carbon monoxide MAP are not beneficial, since it is important to limit the oxygen content, and the color is already fixed by nitric oxide. Therefore, the predominant MAP gas mixture for maintaining the color stability (nitrosylhemochrome pigment) of cured meat products contains 20-30% CO₂ and 60-70% N₂ (Møller et al. 2003; Nannerup et al. 2004; Rubio et al. 2008). One benefit for selecting MAP for both fresh and cured meat products is to inhibit certain common types of gram negative spoilage bacteria via implementing CO₂ (McMillin 2017).

Cured Meat Packaging

Cured meat packaging generally consists of two main types; conventional vacuum packaging or MAP. Recent packaging technology advances have led to development of intelligent and active packaging.

Vacuum packaging removes oxygen and other gases after which the package is sealed. Vacuum packaging creates an anaerobic environment which limits the growth of many spoilage bacterial species, which results in a longer shelf life. For example, the aerobic bacterial species *Pseudomonas ssp.* is a common spoilage microorganism in meat, although it can be prevented through vacuum packaging (Kerry and Tyuftin 2017). A more novel variety of vacuum packaging is vacuum skin packaging (VSP), where the product is placed in high gas barrier trays and heat shrinking film is placed over the tray (Belcher 2006). An advantage of VSP is that there are less air pockets and wrinkles

which can contain purge and allow for increased bacterial growth (Kerry and Tyuftin 2017). Both cooked and cured meat products are packaged in VSP, although it is less common than conventional vacuum packaging.

In cured meat, it is important to eliminate the oxygen gas present to prevent light-induced color fading. Ideal cured meat MAP gas mixtures are ~0.5% oxygen (as low as possible), 35% carbon dioxide, and nitrogen gas as needed (Church 1994). Additionally, in cured meat utilizing MAP, Møller and others (2003) showed that it is important to keep the headspace oxygen low along with minimizing the package headspace volume (Møller et al. 2003). Cured meat MAP is somewhat utilized, although the packages are bulkier and can be more costly than conventional vacuum packages (McMillin 2008).

Cured Meat Packaging Problems and Solutions

The cured meat pigment (nitrosylhemochromogen) after cooking is considered heat stable but is extremely susceptible to color fading resulting from specific extrinsic factors. The extrinsic factors primarily responsible for color fading via photooxidation of nitrosylmyoglobin are light and oxygen (Møller and Skibsted 2002). Munk and others (2010) showed that oxidation of nitrosylheme to ferric heme occurs as a two-step process involving both oxygen and light presence (Munk et al. 2010). Color fading begins with light energy catalyzing the separation or excitation of nitric oxide from heme iron. In the presence of oxygen and light, the open ligand binding site on heme iron will allow oxygen to bind, which results in formation of denatured metmyoglobin or ferrihemichrome, which is brown, tan, yellow, or colorless. Cured color stability is an important area of research, especially with products that contain reduced amounts of ingoing nitrite such as many alternatively-cured meat products. The decreased color

stability can be mitigated by utilizing certain packaging techniques described in the following sections.

Novel Meat Packaging Concepts

Recent packaging advancements have been mostly in the realm of intelligent and active packaging. Intelligent packaging primarily encompasses product composition and/or monitoring the package environment for food quality and safety detection during storage. Some examples of intelligent packaging concepts are indicators of freshness, package integrity, temperature abuse, and biological reactions (Realini and Marcos 2014). Active packaging is an additional novel realm of research with the potential to solve, prevent, and/or decrease multiple food safety and quality related problems. Active packaging allows the product, package, and packaging environment to interact favorably (McMillin 2017). Active packaging generally results in some kind of response that would not occur with conventional packaging. Active packaging is a broad category that encompasses carbon dioxide emitter pads, oxygen scavenger packets, moisture absorbers, microwave susceptors, along with antimicrobial, antioxidant and nitrite-embedded films, which all can potentially play a role towards extending the safety and quality of food products (Realini and Marcos 2014). In summary, intelligent packaging is focused on detection and communication, while active packaging is focused on preventing deterioration or imposing a positive change on the product.

A novel active packaging technology that has the potential to increase consumer appeal and shelf life of alternatively-cured meats is the use of nitrite-embedded film (NEF). Currently, there is no published research on NEF with regard to its effects on cured meat products. NEF technology has been shown to be effective for improving the shelf life and color stability of fresh beef and pork products (Claus and Du 2013; Yang et

al. 2016; Roberts et al. 2017; Ramanathan et al. 2018). Specifically, NEF has been shown effective for improving the color of dark cutting beef (Ramanathan et al. 2018), *longissimus lumborum*, *psoas major*, and *semitendinosus* muscles in aged beef (Claus and Du 2013), *longissimus lumborum* and ground *rhomboides* in bison meat (Roberts et al. 2017) along with fresh pork sausage (Yang et al. 2016). NEF film is generally recognized as safe (GRAS No. 228) with a low nitrite loading of 113 mg/m² NO₂ embedded in the film, and is considered a processing aid for fresh meat (USDA 2018). According to the Code of Federal Regulations, a processing aid may be used within allowable limits with no labeling required (CFR 2017). Currently NEF is only approved to maintain the color stability of fresh meat; however, a similar application with regard to cured meat products is a viable possibility. Considering that NEF is allowable on fresh meat where nitrite is not a commonly associated component, it is within reason to suggest a use for NEF on conventionally-cured and/or alternatively-cured meat products, where nitrite is a frequent component. If NEF provides improved color stability and shelf life to cured meat products, then it is possible that it may be an advantageous route for processors to improve their products' integrity and consumer appeal. There is also the potential for processors to utilize NEF to generate cured color in nitrite-free products.

Summary

Nitrite is an irreplaceable ingredient in cooked, cured meat products. To date there is no perfect alternative to nitrite that can replicate the cured color, antimicrobial, flavor, and antioxidant properties. There is no published literature that has studied the effect of NEF technology on cured or alternatively-cured meat products. Utilizing nitrite-embedded film may provide a unique solution to meat processors to extend the color stability and shelf life of conventionally-cured, alternatively-cured and even potentially

nitrite-free cooked meat products. The objective of this study was to determine the efficacy of NEF technology in extending the color stability and shelf life of alternatively-cured, all-beef bologna, a cured, cooked meat product. We hypothesize that NEF-packaged product will offer greater color stability, and reduced growth of lactic acid bacteria, without increased residual nitrite or nitrate levels.

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CHAPTER 3. THE POTENTIAL ROLE OF NITRITE-EMBEDDED FILM TECHNOLOGY IN EXTENDING THE COLOR STABILITY AND SHELF LIFE OF ALTERNATIVELY-CURED, COOKED MEAT PRODUCTS

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Michael S. Cropp¹, James S. Dickson¹, Rodrigo Tarté^{1,2} Joseph G. Sebranek^{1,2*}

¹Department of Animal Science, ²Department of Food Science and Human Nutrition

Iowa State University, Ames, IA 50011, United States

Abstract

The effects of nitrite-embedded film (NEF) technology on fully cooked all-beef bologna were investigated for its potential application for improving the color stability and shelf life of alternatively-cured meats. The study was designed to determine the impact of NEF on external and internal color properties, external and internal residual nitrate, external and internal residual nitrite, and microbial growth over a 125-day simulated, retail display period. Five treatments were manufactured; a conventionally-cured control (CON-CF) in vacuum packaged with conventional film, an alternatively-cured formulation (nitrite from cultured celery juice powder plus cherry powder) vacuum packaged into both conventional film (CJP-CF) and nitrite-embedded film (CJP-NEF). An additional alternatively-cured formulation was produced (Natpre T-10 EML Plus S) and vacuum packaged into both conventional film (NT10-CF) and nitrite-embedded film (NT10-NEF). In-package external a^* (redness) values showed a significant increase ($P < 0.05$) for NEF packaged products. External and internal residual nitrite was not a significantly different ($P > 0.05$) between treatments from day 41 through 125 of retail display. NEF packaged product showed no difference ($P > 0.05$) in internal residual nitrite compared to vacuum packaged conventional film counterparts. NEF packaged

product showed no difference ($P < 0.05$) in external or internal residual nitrate compared to vacuum packaged conventional film counterparts. Reduced color stability was observed in CJP-CF, while CJP-NEF showed improved a^* values. NEF had no effect ($P > 0.05$) on microbial growth. NEF exhibited no difference ($P > 0.05$) between treatments for inoculated elevated temperature microbial growth. The results found in the present study showed that NEF technology has the potential to improve the color stability of alternatively-cured meats and suggest a novel means of generating cured color after thermal protein denaturation.

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

Introduction

Food packaging has been used to improve product appearance, increase quality and reduce food safety risks. The widespread consumer demand for alternatively-cured meat products continues to rise, due to negative consumer perception revolving around nitrates and nitrites. Alternatively-cured meat products typically rely on natural sources of nitrate and/or nitrite, which are generally vegetable-based substitutes; 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 account for potential vegetable-like flavors and aromas; processors tend reduce to the relative ingoing amount of nitrite in alternatively-cured meats (Sindelar et al. 2007a). The reduced nitrite composition in the final product, in conjunction with expectation of extended shelf life have led to a greater potential for reduced color stability in alternatively-cured meat products.

An area of current research in meat packaging is active packaging, where the package environment promotes a favorable interaction with the product (McMillin 2017). An active packaging application of nitrite embedded into the package surface (nitrite-embedded film) before packaging fresh beef and pork has been shown to improve and extend the color stability and shelf life (Claus and Du 2013; Yang et al. 2016; Ramanathan et al. 2018). Nitrite-embedded film (NEF) packaging material is generally recognized as safe (GRAS No. 228) and is considered a processing aid in fresh meat where nitrite is not a common constituent (USDA 2018) but has not been studied in cured meat applications. Therefore, the objective of the present study was to determine the impact of NEF in extending the color stability and shelf life of alternatively-cured, all-beef bologna, a cured cooked meat product.

Materials and Methods

Experimental Design

Three product formulations of all-beef bologna were manufactured in the Iowa State University Meat Laboratory, Ames, IA. The three formulations consisted of a conventionally-cured control (sodium nitrite from Modern Cure with sodium erythorbate – CON-CF) which was vacuum packaged with conventional film (Sealed Air Corporation, Duncan, SC, U.S.A), an alternatively-cured bologna formulation utilizing nitrite from cultured celery juice powder (VegStable 506) and cherry powder (VegStable 515) supplied by Florida Foods (Florida Foods Products, Inc., Eustis, FL, U.S.A) with one half of the batch vacuum packaged in conventional film (CJP-CF) and the second half vacuum packaged in nitrite-embedded film (Bemis Company Inc., Oshkosh, WI, U.S.A) pouches (CJP-NEF). An additional alternatively-cured formulation was produced using Natpre T-10 EML Plus S (Productos Sur, S.A. (Prosur), San Ginés, Murcia, Spain)

supplied by Wenda America (Wenda America, Inc., Naperville, IL, U.S.A) with one half of the batch vacuum packaged in conventional film (NT10-CF) and the second half of the batch was vacuum packaged in nitrite-embedded film pouches (NT10-NEF). The ingoing nitrite for the curing ingredients for Modern Cure (CON-CF) was 62,500 ppm NO₂, cultured celery juice powder (VegStable 506) ingredient (CJP-CF, CJP-NEF) contained 22,500 ppm NO₂, and Natpre T-10 EML Plus S (Natpre T-10 EML Plus S) ingredient (NT10-CF, NT10-NEF) contained 1,700 ppm NO₂. Treatment formulations are listed in Table 1. Products from each of the two alternatively-cured formulations were vacuum packaged in both conventional and nitrite-embedded film. The control formulation was vacuum packaged in conventional film. The experiment was replicated two times.

Product Manufacture

Table 1. All-beef bologna formulations.

Ingredient	CON-CF % of Meat Block	CJP % of Meat Block	NT10 % of Meat Block
Beef 80s	67.000	67.000	67.000
Beef 50s	33.000	33.000	33.000
Water/Ice	20.000	20.000	20.000
Salt	2.000	2.000	2.000
Spices	3.313	3.313	3.313
Modern Cure (6.25% NO ₂)	0.250		
Natpre T-10 EML Plus S			1.253
VegStable 506		0.444	
Sodium Erythorbate	0.054		
VegStable 515		0.500	

Beef was obtained from the Iowa State Meat Laboratory, thawed at 4.4°C for 2 days and then moved into refrigerated storage at 1°C for 2-3 days. All treatments were processed separately using the same procedure. Treatment manufacturing and thermal processing occurred on the same day for each replication. Replications were manufactured on consecutive days. The treatment processing sequence was randomized prior to production. Treatment manufacturing order for replication one was CJP-CF/CJP-

NEF, NT10-CF/NT10-NEF and CON-CF, and CON-CF, CJP-CF/CJP-NEF, and NT10-CF/NT10-NEF for replication two, respectively. Slicing and packaging order was inverse of manufacturing order.

Beef was ground through a 12.7mm plate (Biro Manufacturing Company, Marblehead, OH, U.S.A). Each formulation consisted of a 100 pounds of meat. Lean (beef 80 trim) and fat (beef 50 trim) portions were mixed separately after grinding using a double action mixer (Leland Southwest, Fort Worth, TX, U.S.A) to create a uniform mixture. The lean ground beef was added to a vacuum bowl chopper (Kramer & Grebe GmbH and Co., KG, Biedenkopf-Wallau, Germany) along with salt, curing ingredient (or replacer), half of the water/ice mixture, spices (A.C. Legg Inc., Calera, AL, U.S.A) and cure accelerator (if needed). For the applicable treatments (CON-CF, CJP-CF, and CJP-NEF) the cure accelerator (sodium erythorbate or cherry powder) was manually pre-mixed into the spice blend. The lean portion of beef was chopped until the batter temperature reached 4.4°C. Beef 50's 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 into a vacuum stuffer (Handtmann VF 608 Plus, Lake Forest, IL, U.S.A) and stuffed into 6.5 x 45" pre-stuck fibrous casings (Kalle, Wiesbaden, Germany). Each bologna log was individually weighed, laid flat on a smoke rack and moved into a smokehouse (Alkar Smokehouse, DEC International, Inc., Lodi, WI, U.S.A). Bologna was cooked according to the schedule shown in Table 2.

Table 2. Thermal processing for bologna treatments.

Step Type	Step Time (min)	Dry Bulb Temperature (°F)	Wet Bulb Temperature (°F)	Relative Humidity (%)	Main Blower Fan	Exhaust Fan	Exhaust Damper
Cook	1:00	100	89	65	8	Off	Auto
Cook	0:45	130	104	42	8	Off	Closed
Cook	0:45	150	115	34	8	On	Closed
Smoke	1:00	176	150	52	6	Off	Auto
Cook	0:01	176	158	64	10	Off	Auto
Cook	0:01	185	176	81	10	Off	Closed
Cold Shower	0:20	50	0	0	0	On	Auto

After cooking, the bologna logs were chilled overnight at $1 \pm 2^{\circ}\text{C}$. Subsequently, bologna logs were weighed, casings removed and sliced (Bizerba, Piscataway, NJ, U.S.A). The bologna logs were sliced to 6.35mm thick slices and 4 slices were placed into either conventional vacuum packages (CON-CF, CJP-CF, NT10-CF) (oxygen transmission rate of 1.5-3.5cc/m²/24hr/1 atm at 5°C; 0% relative humidity, and water vapor transmission rate of 0.3-0.6g/100in²/24hr/1 atm at 100°F/38°C; 100% relative humidity, Cryovac, Sealed Air Corporation, Duncan, SC, U.S.A) or nitrite-embedded film vacuum packages (CJP-NEF, NT10-NEF) (oxygen transmission rate of <0.3cc/0.06m²/24hr/1 atm at 23°C; 0% relative humidity, and water vapor transmission rate of <0.5g/0.06m²/24hr/1 atm at 38°C; 100% relative humidity; FreshCase, Curwood, Division of Bemis Company Inc., Oshkosh, WI, U.S.A). Both package types (conventional film – CF and nitrite-embedded film – NEF) were vacuum sealed (Ultravac UV 2100 vacuum chamber packaging machine, UltraSource LLC, Kansas City, MO, U.S.A) and subsequently stored at $1 \pm 2^{\circ}\text{C}$ under simulated, continuous retail display conditions using white fluorescent lights (32W, 120V, Sylvania, Danvers, MA, U.S.A) for the duration of the study. The light source distance was approximately 254mm from

the package surface. The packaged samples were held under 2200 ± 500 lux during retail display and samples were routinely rotated to guarantee uniform light exposure. Lux was measured using an URCERI Light Meter MT-912 (URCERI, Shenzhen Huanhui E-commerce company, Ltd, Shenzhen, China). Multiple locations were selected for lux light measurements throughout the storage area. Product packaging day was considered Day 0.

Color Analysis

In-package, external and internal color was measured post packaging on days 1, 6, 13, 27, 41, 55, 69, 83, 97, 111, and 125. The color measurements were conducted using the Commission Internationale de l'Eclairage (CIE) L^* (lightness), a^* (redness), and b^* (yellowness) system. Measurements were taken at a 10° observer angle using illuminant D65 (daylight at 6500K) with a 2.4cm aperture size HunterLab MiniScan EZ 4500L colorimeter (Hunter Associates Laboratory Inc., Reston, VA, U.S.A). Three color measurements were conducted for in-package external color, as well as external surface and internal color. The in-package external color consisted of 22 fixed packages per replication, per treatment, per sample day; thus, 220 packages were measured on each sample day of 125-day retail display period (22 packages x 5 treatments x 2 replications). The same 220 packaged were measured each time for in-package color throughout retail display period. In-package external color was measured using a modified standardization process where the white calibration tile was covered with the respective packaging material (conventional film or nitrite-embedded film). The product measurements were taken by placing the nose cone of the HunterLab MiniScan EZ instrument directly onto the package. This process was used to more accurately measure visual (consumer) color of the packaged product. For external surface and internal color of the slices, 1 package

was randomly selected and used for both external surface and internal color at each timepoint.

External surface and internal color were measured by opening the package, removing the top slice (slice directly exposed to light source) for external surface color, and internal slices were used for internal color. A standard instrument calibration (no film covering tiles) was used and measurements were conducted by placing the HunterLab MiniScan EZ instrument nose cone directly onto the product surface.

Residual Nitrite Analysis – Colorimetric Method

Residual nitrite testing was in accordance with AOAC method 973.31 (AOAC 2005c). Sampling was conducted post packaging on days 1, 6, 13, 27, 41, 55, 69, 83, 97, 111, and 125. A duplicate of each sample was analyzed for every sampling day. Samples were prepared by separating the exterior slices from the interior slices. The two exterior and two interior slices were finely chopped separately using a food processor (KitchenAid, St. Joseph, Michigan, USA) and 5.0 g (± 0.01 g) of sample from each weighed into a beaker and stirred with hot (approximately 50-80°C) distilled water. Each sample was added to a 500mL volumetric flask with approximately 300mL hot (approximately 50-80°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), filled to volume (500mL) with distilled water and stirred. Approximately 30mL was filtered through a Whatman #1 filter (Whatman Grade 1, GE Health Care Life Sciences, Pittsburgh, PA, U.S.A) into a 50mL volumetric flask. Next, 2.5mL of sulfanilamide were added to each sample, followed by 2.5mL of N-(1-Naphthyl) ethylenediamine dihydrochloride (NED) reagent

after 5 min. Samples were filled to volume (50mL) with distilled water, mixed, allowed to rest for 15 min and absorbance at 540nm read with a spectrophotometer (Beckman, Fullerton, CA, U.S.A).

Residual Nitrate and Nitrite Analysis – Ion Chromatographic Method

Residual nitrate including nitrite testing was in accordance with AOAC method 993.30 (AOAC 2005a). Testing was conducted by Hormel Laboratories (Division of Hormel Foods, LLC, Austin, MN, U.S.A). The primary focus for analysis was centered around capturing residual nitrate; however, nitrite was measured as well. Sampling was conducted post packaging on days 1, 6, 13, 27, 41, 55, 69, 83, 97, 111, and 125. The samples were immediately frozen at $-20 \pm 5^{\circ}\text{C}$ and sent to Hormel Laboratories in five grouped shipments. The samples were held frozen until testing. The sample were thawed overnight prior to testing. Thawed samples were prepared by separating the exterior slices from the interior slices. The exterior and interior slices were homogenized separately using a commercial grade food processor. One g of each sample was weighed into a 100mL Kohlrausch volumetric flask and 50mL of distilled hot water were added. Samples were placed on a steam bath for 1 h and chilled in cold water for 15 min. After chilling, the 100mL Kohlrausch volumetric flasks were filled to volume and shaken. Approximately 30mL of sample were filtered through a Whatman GF/C filter (Whatman Grade 1, GE Health Care Life Sciences, Pittsburgh, PA, U.S.A) and filtrate was filtered under vacuum through a Dionex OnGuard II RP 2.5cc cartridge (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A) equipped with a 10mL syringe pretreated with 10mL of methanol and 15mL of distilled water. The first 6mL of sample filtrate was discarded to ensure proper flushing of methanol and distilled water. A multi sample

Restek Resprep 12-Port Vacuum Manifold (Restek Corporation, Bellefonte, PA, U.S.A) was used under vacuum to ensure proper sample flow rate. Sample flow rate through the cartridge was approximately 1mL per minute to provide a 1:100 dilution factor. The samples were run through a Dionex High Pressure Ion Chromatography system (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A). Residual nitrate and nitrite peaks were plotted and the area under each peak was used to calculate concentration in parts per million.

Proximate Composition Analysis

Raw treatment batters and cooked products were analyzed for proximate composition (% fat, % moisture, % protein, and pH). All samples were finely chopped separately using a food processor. Fat content was analyzed by the CEM ORACLE System (AOAC 2005b; CEM Corporation Matthews, NC, U.S.A), moisture composition was evaluated with the SMART 6 System (AOAC 2005b; CEM Corporation Matthews, NC, U.S.A), and protein content was measured with the CEM Sprint Rapid Protein Analyzer (AOAC 2005d; CEM Corporation Matthews, NC, U.S.A), where the protein composition is measured based on amino acid residues bound to Crocein Orange dye utilizing absorbance (Leebler et al. 2008; Moser and Herman 2011). Fat, moisture, and protein composition were analyzed in duplicate for raw batters and cooked products from each treatment for both replications.

Measurement of pH Analysis

The raw batters and finished product pH were measured for all replications. Samples were finely chopped separately using a food processor. 10g of each sample were

weighed into a beaker and 90mL of room temperature distilled water was added. The meat and water were mixed thoroughly by hand with a glass stir rod for 1 min and a filter paper (Whatman Grade 1, GE Health Care Life Sciences, Pittsburgh, PA, U.S.A) was submerged into the sample and pH was measured with a Mettler Toledo SevenMulti pH meter (Mettler Toledo, Columbus, O.H., U.S.A). Duplicate measurements were conducted for all samples.

Microbiological Analysis

Microbial spoilage was conducted utilizing a traditional shelf life sampling procedure where all treatments and replications were subsequently stored at $1^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Microbial analysis was conducted on days 0, 7, 14, 30, 60, 90, and 120.

An additional microbial analysis was conducted using an undefined lactic acid bacteria spoilage inoculum. Inoculation occurred on day 158 of product shelf life. For the inoculation study (I), day 158 was determined day 0(I). Bacterial analysis was conducted on days 0(I), 3(I), 6(I), 9(I), 12(I), 18(I), and 21(I) of storage at 10°C . All microbial data (traditional and inoculated shelf life) was collected and reported as log (Log) per colony forming unit (CFU) per gram (g).

Total Plate Counts

Total plate count analysis was conducted on days 0, 7, 14, 30, 60, 90, and 120 for the traditional shelf life and on days 0(I), 3(I), 6(I), 9(I), 12(I), 18(I), and 21(I) for the inoculation experiment. Sample preparation consisted of aseptically extracting 11g of sample from the package and adding 99mL of 0.1% peptone water (Hardy Diagnostics, Cat no. D299, Santa Maria, CA, U.S.A) to a stomacher bag (Whirl Pak, Jackson, WI, U.S.A) and stomached (EasyMix, AES Laboratories, France) for 1 min. Next, 1 mL was

pipetted on 3M aerobic plate count petrifilm (3M Health Care, St. Paul, MN, U.S.A) containing peptone diluent (Becton, Dickinson and Company, Sparks, MD, U.S.A) and petrifilms were incubated at $21 \pm 2^{\circ}\text{C}$ for 72 h before colonies were counted.

Lactic Acid Bacteria

Lactic acid bacteria analysis was conducted on days 0, 7, 14, 30, 60, 90, and 120 for the traditional shelf life and on days 0(I), 3(I), 6(I), 9(I), 12(I), 18(I), and 21(I) for the inoculation experiment. Sample preparation consisted of aseptically extracting 11 g of sample from the package and adding 99mL of 0.1% peptone water (Hardy Diagnostics, Santa Maria, CA, U.S.A) in a stomacher bag (Whirl Pak, Jackson, WI, U.S.A) and stomached (EasyMix, AES Laboratories, France) for 1 minute. Next, 0.1mL was pipetted on 100mm x 15mm petri plates (Fisherbrand, Fisher Scientific, Inc., Waltham, MA, U.S.A) containing MRS (DeMan, Rogosa and Sharpe) agar (Becton, Dickinson and Company, Sparks, MD, U.S.A) and petri plates were incubated at $31 \pm 2^{\circ}\text{C}$ for 72 h and then counted.

Bacterial Inoculum

Five different commercial bologna packages were purchased to create the spoilage inoculum. Two slices (approx. 60g) were removed from each package and added into a large stomaching bag. 1 L of tryptic soy broth (Becton, Dickinson and Company, Sparks, MD, U.S.A.) was added and allowed to incubate at 20°C for 72 h. After incubation, 1mL was removed and added to 9mL of MRS (DeMan, Rogosa and Sharpe) broth (Becton, Dickinson and Company, Sparks, MD, U.S.A.) and incubated at 30°C for 48 h. 1 mL of inoculated broth was transferred to an additional 9mL of MRS broth and

incubated at 30°C for 48 h. Bacterial transfer process was repeated four times. Next, 1mL of culture was added to 40mL of MRS broth and allowed to incubate at 30°C for 48 h. Serial dilutions (to extinction) were made from the culture and plated on MRS agar to determine the population of the lactic acid bacteria inoculum. The culture was held at 1°C while population was determined. After population was determined, the culture was diluted to 10^{-3} log and meat packages were inoculated with 1mL of the undefined lactic acid bacteria spoilage inoculum. Inoculation occurred aseptically using a 1mL syringe and self-closing septum on package. The packages were held at 10°C for the duration of the study. Inoculation occurred on day 158 of product shelf life. For the inoculation study (I), day 158 was determined day 0(I). Bacterial analysis was conducted using MRS agar (Becton, Dickinson and Company, Sparks, MD, U.S.A.) and 3M aerobic plate count petrifilm (3M Health Care, St. Paul, MN, U.S.A) on days 0(I), 3(I), 6(I), 9(I), 12(I), 18(I), and 21(I). Plating, incubation and counting protocol for the inoculation experiment followed the same procedure as for the traditional shelf life discussed earlier.

Statistical Analysis

The study consisted of five treatments with two replications. Replications were produced on separate but consecutive days. The data was analyzed using a mixed procedure with SAS version 9.4 (SAS Institute Inc., Cary, NC, U.S.A). The experimental design consisted of a 2x2+1 factorial design with the fixed effects being treatment (CON-CF, NT10-CF, CJP-CF, NT10-NEF, CJP-NEF) and replication was a random effect. For determining differences between treatments over time the fixed effects were treatment, day, and treatment x day interaction with a Tukey-Kramer pairwise adjustment. A P-value of less than 0.05 was used to determine significance.

Results

Raw Composition, Processing Attributes and Final Composition

Mean treatment effects in proximate composition for raw and cooked bologna in addition to cook yield treatment effects are displayed in Table 3. There was not a significant difference ($P > 0.05$) for raw protein content between any treatment (CON-CF – conventionally-cured formulation in conventional film, CJP-CF –alternatively cured with cultured celery juice powder in a conventional film, CJP-NEF –alternatively cured with cultured celery juice powder and packaged in nitrite-embedded film, NT10-CF – alternatively cured with Natpre T-10 EML Plus S in a conventional film, NT10-NEF – alternatively cured with Natpre T-10 EML Plus S and packaged in nitrite-embedded film). Mean treatment effects in raw fat composition for CJP-CF and CJP-NEF were not significantly different ($P < 0.05$) but both treatments were significantly higher ($P < 0.05$) than CON-CF, NT10-CF, and NT10-NEF. Mean treatment effects in raw moisture content was significantly greater ($P < 0.05$) for CON-CF compared to all treatments (CJP-CF, CJP-NEF, NT10-CF, and NT10-NEF). There was not a significant difference ($P > 0.05$) in mean raw moisture content between CJP-CF, CJP-NEF, NT10-CF, NT10-NEF.

Mean treatment effects in cooked protein content was significantly greater ($P < 0.05$) for CON-CF compared to CJP-CF, CJP-NEF, and NT10-CF; while NT10-NEF mean cooked protein was not significantly different ($P > 0.05$) from any treatment. Mean cooked fat content for treatment effects was significantly greater ($P < 0.05$) for NT10-CF compared to CON-CF, but not significantly different ($P > 0.05$) from any other treatment. Mean cooked moisture content was significantly greater ($P < 0.05$) for CON-CF compared to CJP-CF, NT10-CF, and NT10-NEF. Mean cooked moisture was not

significantly different ($P > 0.05$) between CJP-CF, CJP-NEF, NT10-CF, or NT10-NEF. Mean percent cook yield for CON-CF, CJP-CF, CJP-NEF was significantly greater ($P < 0.05$) than NT10-CF and NT10-NEF.

There was no significant difference ($P > 0.05$) between treatments for raw pH and cooked pH (Table 4). However, cooked pH was significantly less ($P < 0.05$) for NT10-CF and NT10-NEF than for other treatments.

Instrumental Color

In-Package External Color

The means for in-package external color treatment effects are shown in Table 5. The mean in-package external color results for treatment x day effects are shown in a Tables 6 and 7 in addition to Figure 2. Results presented in Table 5 show that nitrite-embedded film (NEF) offered significantly greater ($P < 0.05$) mean in-package external a^* value for treatment effects of CJP-NEF (compared to CJP-CF) and NT10-NEF (compared to NT10-CF). There was no significant difference ($P > 0.05$) for mean in-package external a^* value of treatment effects for CON-CF or CJP-CF. NT10-CF had significantly lower ($P < 0.05$) mean in-package external a^* value than all other treatments. Mean in-package external a^* value was significantly lower ($P < 0.05$) in NT10-NEF compared to CJP-NEF, CJP-CF, and CON-CF.

Treatment means for in-package L^* were significantly different ($P < 0.05$) based on curing ingredient type (NT10-CF & NT10-NEF $>$ CON-CF $>$ CJP-CF & CJP-NEF). Treatment mean in-package external b^* value was not significantly different ($P > 0.05$) for CJP-CF or NT10-NEF. Treatment mean in-package external b^* value was not significantly different ($P > 0.05$) for CON-CF or CJP-NEF. However, mean in-package

external b^* value for NT10-CF was significantly higher ($P < 0.05$) than for all other treatments.

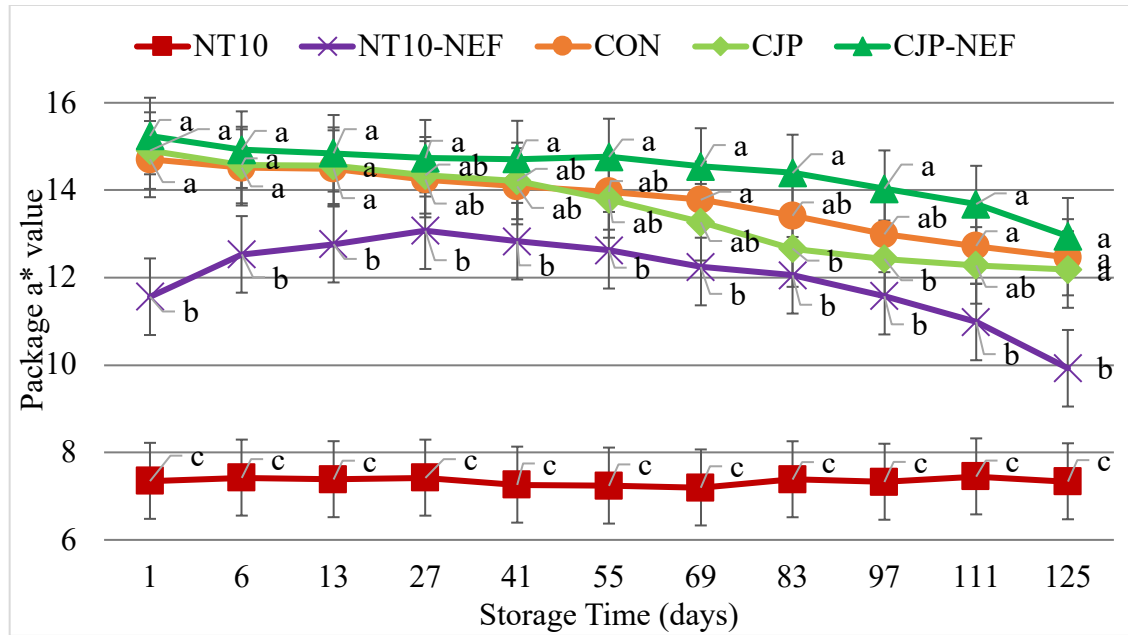


Figure 1. In-package external a^* value for bologna treatment x day effects under retail display. (SEM = 0.86) ^{a-c} Means from the same day with different letters are significantly different ($P < 0.05$)

Figure 1 displays mean in-package external a^* value for treatment x day effects.

The graph displayed in Figure 2 for mean in-package external a^* value for treatment x day effects show that there was not a significant change ($P < 0.05$) over retail display period (125 days) for CJP-NEF, CON-CF, NT10-NEF, or NT10-CF. CJP-NEF had a significantly higher ($P < 0.05$) mean in-package external a^* value than NT10-NEF and NT10-CF at every time point during retail display. There was no significant change ($P > 0.05$) over retail display for mean in-package external a^* value for CJP-NEF, CON-CF, NT10-NEF, or NT10-CF. In contrast, CJP-CF had a significantly lower ($P < 0.05$) mean in-package external a^* value at days 83 and 97. Day 27 through day 111 showed no significant difference ($P > 0.05$) in mean in-package external a^* value between CJP-CF

and NT10-NEF. CON-CF was not significantly different ($P < 0.05$) than NT10-NEF at days 27-55, 83 and 97, respectively.

The results presented in Table 6 depict mean in-package external L^* value for treatment x day effects under retail display. There was no significant difference ($P > 0.05$) over time for any treatment. However, certain treatments were significantly different ($P < 0.05$) at specific storage time points. NT10-NEF was not significantly different ($P > 0.05$) from CON-CF at any storage time point during retail display. NT10-NEF was not significantly different ($P > 0.05$) from NT10-CF at any storage time point. However, NT10-CF had significantly higher ($P < 0.05$) mean in-package external L^* value compared to CON-CF on day 1 through 13. CJP-CF and CJP-NEF were not significantly different ($P > 0.05$) at any storage time point during retail display. NT10-CF and NT10-NEF were significantly different ($P < 0.05$) from CJP-CF and CJP-NEF at every storage time point. CJP-CF and CJP-NEF were not significantly different ($P > 0.05$) from CON-CF at day 1 through 69. Additionally, CJP-NEF mean in-package external L^* value was significantly lower ($P < 0.05$) than CON-CF from day 83 through 125. CJP-CF was not significantly different ($P > 0.05$) from CON-CF or CJP-NEF at any storage time point during retail display.

External Surface and Internal Color

The mean results for external surface and internal CIE L^* , a^* , b^* measurements for treatment effects are shown in Table 8. Figure 2 shows the visual difference between NT10-CF and NT10-NEF external surface and internal redness between day 1 and 41. There was not a significant difference ($P > 0.05$) between CJP-NEF, CJP-CF or CON-CF and between CJP-CF and NT10-NEF for mean external surface a^* value. However, NT10-NEF was significantly different ($P < 0.05$) from CJP-NEF, CON-CF, and NT10-

CF. CJP-CF and NT10-NEF were not significantly different ($P > 0.05$) and NT10-NEF had significantly higher ($P < 0.05$) mean external surface a^* value compared to NT10-CF. There was not a significant difference ($P > 0.05$) in mean external a^* value between CJP-NEF and CJP-CF.

The mean internal a^* values for treatment effects are presented in Table 8 and followed similar results as mean external surface a^* value treatment effects. The main difference is that the mean internal a^* value results show that NT10-NEF was significantly different ($P < 0.05$) from all treatments (which contrasts mean external surface a^* value results, specifically compared to CJP-CF). NT10-NEF showed significantly greater ($P < 0.05$) internal a^* value than NT10-CF. NT10-NEF had significantly reduced ($P < 0.05$) mean internal a^* value compared to CJP-NEF, CON-CF, and CJP-CF.

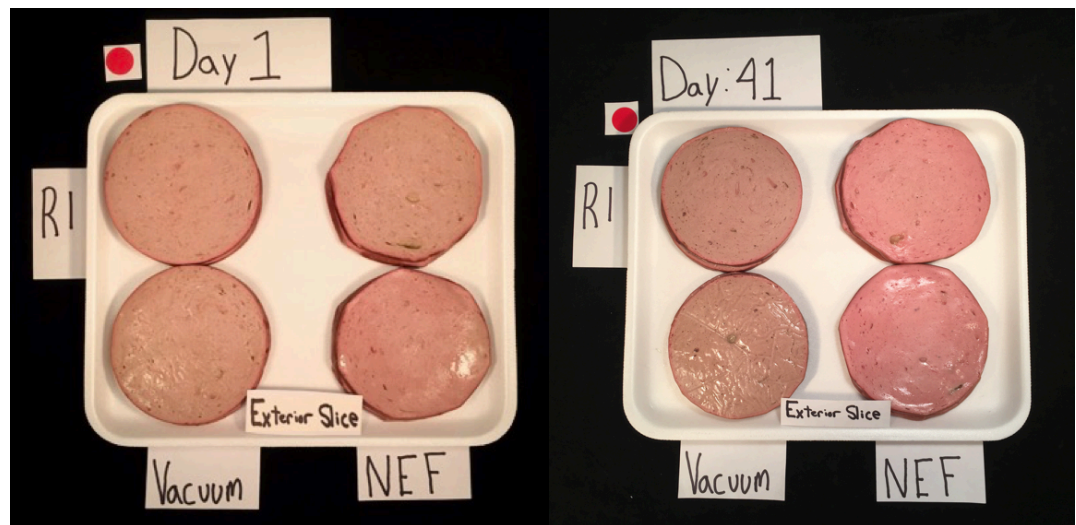


Figure 2. External surface and internal visual appearance of NT10-CF and NT10-NEF at day 1 versus day 41 of simulated, retail display storage.

External surface b^* value (Table 8) for NT10-CF was significantly higher ($P < 0.05$) for all other treatments; however, there was no significant difference ($P > 0.05$) between NT10-NEF, CJP-CF, CJP-NEF or CON-CF. Additionally, NT10-CF was

significantly greater ($P < 0.05$) than all treatments for mean internal b^* value for treatment effects. NT10-NEF mean internal b^* value was significantly different ($P < 0.05$) from all treatments. Likewise, CON-CF was significantly different ($P < 0.05$) from all treatments for mean internal b^* value treatment effects. There was no significant difference ($P > 0.05$) between CJP-NEF and CJP-CF for mean internal b^* value treatment effects.

There was not a significant difference ($P > 0.05$) between NT10-CF, NT10-NEF, CON-CF, or CJP-CF for mean external surface L^* value treatment effects. However, CJP-NEF had a significantly lower ($P < 0.05$) mean external surface L^* value than NT10-CF and NT10-NEF. In contrast, mean treatment results for internal L^* value, NT10-CF was significantly higher ($P < 0.05$) than CON-CF, CJP-CF, CJP-NEF but not significantly different ($P > 0.05$) than NT10-NEF. Furthermore, NT10-NEF was not significantly different ($P > 0.05$) from CON-CF but was significantly higher ($P < 0.05$) than CJP-CF and CJP-NEF.

Mean external surface a^* value for treatment x day effects are displayed in Table 9. The results show that there were no significant differences ($P > 0.05$) for CJP-NEF, CON-CF or NT10-CF over retail display period (125 days). There was a significant decrease ($P < 0.05$) for CJP-CF over retail display period. Specifically, CJP-CF at days 1 through 97 had significantly higher ($P < 0.05$) compared to days 111 and 125. NT10-NEF had a significantly lower ($P < 0.05$) mean external surface a^* value at day 1 than at days 6 through 41. Days 1 and 55 through 125 for NT10-NEF mean external surface a^* value were not significantly different ($P > 0.05$). There was no significant difference ($P > 0.05$) between NT10-CF or NT10-NEF at day 1, however, NT10-CF was significantly lower ($P < 0.05$) at days 6 through 125.

Mean external surface b^* values for treatment x day effects over retail display are shown in Table 10. The results show that there was no significant difference ($P > 0.05$) between any treatments over retail display period (125 days). However, NT10-CF was significantly higher ($P < 0.05$) at each day than all other treatments. Mean external surface L^* values for treatment x day effects shown in Table 11 show that there was no significant difference ($P > 0.05$) between treatments over the 125-day retail display period.

Results for mean internal a^* values for treatment x day effects are shown in Table 12. There was no significant day effect ($P > 0.05$) in CJP-NEF, CJP-CF, CON-CF or NT10-CF over the retail display period (125 days). Internal a^* values for NT10-NEF were significantly higher ($P < 0.05$) over days 1 through 27, with no significant difference ($P > 0.05$) between days 27 through 125 (day 1 & 6 < 13 < 27 through 125).

Mean internal b^* values for treatment x day effects are shown in Table 13. Results show no significant difference ($P > 0.05$) in CJP-NEF, CJP-CF, CON-CF or NT10-CF over retail display period (125 days). Values were significantly lower ($P < 0.05$) for NT10-NEF over days 1 through 125. Mean internal L^* values for treatment x day effects are shown in Table 14. There were no significant differences ($P > 0.05$) between treatment over the retail display period (125 days). Day 69 showed treatment interactions for certain treatments; but there was no significant difference ($P > 0.05$) between NT10-CF, NT10-NEF, CON-CF, or CJP-CF. However, CJP-NEF was significantly higher ($P < 0.05$) than NT10-CF, but CJP-NEF was not significantly different ($P > 0.05$) from CJP-CF, CON-CF, or NT10-NEF.

External and Internal Residual Nitrite

Results for mean external and internal residual nitrite (colorimetric method AOAC 973.31 and ion chromatographic method AOAC 993.30) are presented in Table 15. Regardless of analytic method, external surface slices were in direct contact with the packaging material (slices 1 and 4) and internal slices were in the center of the package (slices 2 and 3). Ion chromatographic method was conducted primarily for determining residual nitrate, however, residual nitrite was measured as well.

External and Internal Residual Nitrite – Colorimetric Method

The colorimetric analysis was conducted at Iowa State University in accordance with AOAC approved methods for nitrite measurements (AOAC 2005c). Mean external and internal residual nitrite (NO_2) values for treatment effects are shown in Table 15 and mean external and internal residual NO_2 values for treatment x day effects over the retail display period are shown in Figures 3–6. Mean external residual NO_2 for treatment effects (Table 15) show that there was no significant difference ($P > 0.05$) between CON-CF, CJP-NEF or CJP-CF. There was no significant difference ($P > 0.05$) between NT10-NEF or NT10-CF; however, NT10-NEF and NT10-CF showed significantly lower ($P < 0.05$) mean external residual NO_2 values than CON-CF, CJP-NEF and CJP-CF, respectively.

Mean external residual NO_2 values for treatment x day effects are displayed in Figure 3 and results show that CON-CF and CJP-NEF were not significantly different ($P > 0.05$) at any day of the retail display period (125 days). NT10-CF and NT10-NEF were not significantly different ($P > 0.05$) at any day of the retail display period (125 days). CJP-CF was not significantly different ($P > 0.05$) from CON-CF or CJP-NEF at any day

of retail display, except day 6, where CJP-CF had significantly lower ($P < 0.05$) mean residual NO₂ than CJP-NEF and CON-CF. Day 27 through 125 showed no significant difference ($P > 0.05$) between any treatment for mean external residual NO₂.

Mean internal residual NO₂ values for treatment effects are shown in Table 15 and the results show the same significance pattern as presented for external residual NO₂. Mean internal residual NO₂ values for treatment x day effects are displayed in Figure 4. Results follow a similar pattern to external residual NO₂ results. One notable exception is that CJP-CF was not significantly different ($P > 0.05$) in mean internal residual NO₂ from CON-CF or CJP-NEF at any retail display point. An additional difference in internal results from external is that CJP-CF was not significantly different from NT10-CF or NT10-NEF from day 13 through 125 (compared to day 27 presented in mean external surface NO₂ results). Day 27 through 125 showed no significant difference ($P > 0.05$) between any treatment for mean internal residual NO₂.

External and Internal Residual Nitrite – Ion Chromatographic Method

Mean external residual NO₂ treatment effects is shown in Table 15 and the results show that CJP-NEF, CON-CF, and CJP-CF are not significantly different ($P > 0.05$). Mean external residual NO₂ for NT10-CF were significantly lower ($P < 0.05$) for all other treatments. NT10-NEF was significantly lower ($P < 0.05$) than CJP-NEF, CON-CF, and CJP-CF, however, NT10-NEF was significantly greater ($P < 0.05$) than NT10-CF.

The results for mean external residual NO₂ for treatment x day effects are shown in Figure 5. The overall results follow a similar pattern to colorimetric results with some notable differences. Mean external residual NO₂ values show that on day 1 CJP-CF was significantly lower ($P < 0.05$) than CJP-NEF and CON-CF. CJP-CF was not significantly different ($P > 0.05$) from CJP-NEF, CON-CF, or NT10-NEF at day 6 and 13 but was

significantly higher ($P < 0.05$) than NT10-CF at day 1 through 13. At day 13, CON-CF was significantly greater ($P < 0.05$) than NT10-NEF and NT10-CF. Day 27 showed that CON-CF and CJP-NEF were significantly higher ($P < 0.05$) than NT10-CF but not significantly different ($P > 0.05$) from CJP-CF and NT10-NEF. Mean external residual NO_2 for day 41 through day 125 were not significantly different ($P > 0.05$) in any treatment.

Mean internal residual NO_2 treatment effects are displayed in Table 15 and the results show similar trends to external residual NO_2 with only one major difference. Mean internal residual NO_2 treatment effects show that NT10-CF and NT10-NEF were not significantly different ($P > 0.05$) in mean internal residual NO_2 treatment effects; however, NT10-CF and NT10-NEF were both significantly lower ($P < 0.05$) than CJP-NEF, CJP-CF and CON-CF.

Results for mean internal residual NO_2 (ppm) treatment x day effects are shown in Figure 6. The overall results follow a similar pattern to the colorimetric internal results as well as external ion chromatographic results, with a few notable differences. At day 1, CJP-CF was not significantly different ($P > 0.05$) from CJP-NEF or CON-CF but was significantly greater ($P < 0.05$) than NT10-NEF and NT10-CF. Similar to colorimetric mean internal residual NO_2 results, there was no significant difference ($P > 0.05$) between any treatment for day 27 through 125 of the retail display period.

External and Internal Residual Nitrate

Results for mean external and internal residual NO_3 for treatment effects are shown in Table 16. Mean treatment effects for CJP-CF were significantly higher ($P < 0.05$) than for CON-CF, NT10-NEF, and NT10-CF, but not significantly different ($P > 0.05$) from CJP-NEF. NT10-NEF was not significantly different ($P > 0.05$) in mean

external residual NO_3 from NT10-CF or CON-CF. NT10-CF was significantly lower ($P < 0.05$) in mean external residual NO_3 than CJP-NEF, CJP-CF, and CON-CF. Internal residual NO_3 for treatment effects show that CJP-CF and CJP-NEF are not significantly different ($P > 0.05$) in mean internal NO_3 but both were significantly higher ($P < 0.05$) than CON-CF, NT10-NEF and NT10-CF. Additionally, NT10-CF, NT10-NEF and CON-CF are not significantly different ($P > 0.05$).

Treatment x day effects for external residual NO_3 are listed in Table 17 and results show no significant difference ($P > 0.05$) between any treatment at any day during the retail display period (125 days). The only significant effect is CON-CF showed a significantly greater ($P < 0.05$) mean residual NO_3 for day 6 compared to day 1, however day 1 and 6 were not significantly different from any other day during retail display period.

Treatment x day effects for internal residual NO_3 are listed in Table 18 and results show no significant difference ($P > 0.05$) between any treatment at any day during retail display period (125 days). The only significant effect is that CJP-NEF was significantly higher ($P < 0.05$) in mean residual NO_3 at day 27 and significantly lower ($P < 0.05$) at day 55.

Aerobic Plate Count – Traditional Shelf Life Experiment

All microbial results are reported logarithmically (log) in colony forming units (CFU) per g. Aerobic plate count values (log CFU/g) for mean treatment effects under normal shelf life conditions are reported in Table 19. Mean treatment effects showed no growth difference ($P > 0.05$) for CON-CF, CJP-CF, CJP-NEF or NT10-CF. However, NT10-NEF showed significantly higher ($P < 0.05$) mean aerobic plate counts than CON-CF but was not significantly different from NT10-CF, CJP-NEF, or CJP-CF.

Mean aerobic plate count values (\log_{10} CFU/g) for treatment x day effects under normal shelf life conditions is shown in Table 20. The results show that there was no significant day effect ($P > 0.05$) for CON-CF, CJP-CF, CJP-NEF or NT10-CF; however, there was a treatment effect at day 30, with NT10-NEF being significantly higher ($P < 0.05$) than CON-CF but not significantly different ($P > 0.05$) from NT10-CF, CJP-CF, or CJP-NEF. There was a day effect for NT10-NEF where day 30 was significantly greater ($P < 0.05$) in mean aerobic plate count than day 60. No additional differences in aerobic plate count were observed.

Lactic Acid Bacteria Count – Traditional Shelf Life Experiment

Mean lactic acid bacteria plate count values (\log CFU/g) for treatment effects under normal shelf life conditions are reported in Table 21. The data shows that there was no significant treatment effect ($P > 0.05$) for any treatment.

Mean lactic acid bacteria plate count values (\log CFU/g) for treatment x day effects under normal shelf life conditions are shown in Table 22. Results showed no significant difference ($P < 0.05$) between any treatment or day effects.

Aerobic Plate Count – Inoculated Shelf Life Experiment

Table 23 showcases mean aerobic plate count values (\log CFU/g) for inoculated treatment effects. The results show that CJP-CF and CON-CF had significantly higher ($P < 0.05$) mean aerobic plate count values than NT10-CF and NT10-NEF. CJP-NEF was not significantly different ($P > 0.05$) from any treatment.

Results presented in Table 24 show mean aerobic plate count values (\log CFU/g) for treatment x day effects. There was no significant difference between any treatment at

any specific day; however, all treatments did have significantly higher ($P < 0.05$) mean aerobic plate count values over retail display period (21 days).

Lactic Acid Bacteria Count – Inoculated Shelf Life Experiment

Results presented in Table 25 show mean lactic acid bacteria plate count values (log CFU/g) for treatment effects. There was no significant difference ($P < 0.05$) between CON-CF, CJP-CF, or CJP-NEF. NT10-CF and NT10-NEF were not significantly different ($P > 0.05$) in mean lactic acid-producing bacteria plate count values, however both were significantly less ($P < 0.05$) than CON-CF, CJP-CF and CJP-NEF.

Mean lactic acid bacteria plate count values (log CFU/g) for treatment x day effects are shown in Table 26. There was not a significant difference between any treatment at any day, but all treatments did have significantly greater ($P < 0.05$) mean lactic acid-producing bacteria plate count values over retail display period (21 days).

Discussion

Cooked protein content was greater in CON-CF compared to CJP-CF, CJP-NEF, and NT10-CF; while NT10-NEF was not different from any treatment. When considering the impact, cooked protein content variation across all treatments was 0.89%. There were only minor differences in cooked fat content with the only significant difference being that CON-CF was lower than NT10-CF (1.09% difference in means). The percent cook yield for CON-CF, CJP-CF, CJP-NEF was significantly higher than NT10-CF and NT10-NEF, yet the variation was 0.69% and cooked moisture variation was 1.04% across all treatments; the two measurements showed similar trends (greater % moisture resulted in greater % yield). Additionally, raw pH was not statistically different for any treatment and there was no difference between raw pH and cooked pH for any treatment. However,

cooked pH for NT10-CF and NT10-NEF was lower compared to other treatments. Natpre T-10 EML Plus S was the alternative cure replacement constituent in NT10-CF and NT10-NEF treatments and the ingredients contained in Natpre T-10 EML Plus S were salt, fruit and spice extracts which could account for the reduced pH value.

Regarding proximate composition, treatments can be inconsistent due to potential differences in raw material variation, manufacturing irregularity, potential cook loss impacts, and ingredient functionality differences (Modern Cure with 6.25% sodium nitrite, cultured celery juice powder, and Natpre T-10 EML Plus S). Generally speaking, proximate composition (% fat, % moisture, % protein) is calculated on a percentage basis which can allow for significant differences. Thus, small (but significant) changes in proximate composition may not be truly impactful regarding the product functionality in this study. The additional salt content contained in Modern Cure and Natpre T-10 EML Plus S could account for the increased protein content due to greater myofibrillar protein extraction and even result in the improved cooked moisture in CON-CF.

Products packaged in nitrite-embedded film (NEF) had greater in-package external a^* values (Table 5) based on treatment effects. Both CJP-NEF and NT10-CF-NEF showed greater redness (in-package external a^* value) than their respective vacuum-packed counterparts (CJP-CF and NT10-CF). This demonstrates the potential role for NEF to extend and improve the retail display (cured) color. Additionally, a greater a^* value suggests that the nitrite from the film is interacting with heat-denatured (cooked) myoglobin to generate improved cured color (redness). In fresh meat, the innate reducing capacity of the meat product has been shown to reduce nitrite (NO_2) to nitric oxide (NO) to generate nitrosylmyoglobin (Song et al. 2015). A study conducted by Ramanathan and others (2018) found that fresh beef steaks dipped in rosemary extract and then packaged

in NEF were more effective (and rapid) in developing improved redness in dark cutting beef compared to undipped steaks packaged in NEF. These results highlight the importance of reducing ability of the meat system in NEF packaged meat products.

The NT10-CF treatment had greater in-package external b^* values than all other treatments which highlights the extent of discoloration in the NT10-CF (conventional film) product compared to NT10-NEF (NEF packaged). Additionally, NT10-NEF was not different from CJP-CF in mean in-package external b^* values; suggesting that yellowness (or discoloration) was similar between NT10-NEF and CJP-CF. L^* in-package values were different for treatment effects based on ingredient type in the following decreasing order: NT10-CF and NT10-NEF > CON-CF > CJP-CF and CJP-NEF. NT10-CF and NT10-NEF were lighter most likely as a result of the low nitrite content in the Natpre T-10 EML Plus S ingredient in contrast to CJP-CF and CJP-NEF. A previous study conducted by Usinger and others (2016) found similar results to the present study and concluded that the natural yellow-green pigment of celery juice powder may result in darker colored (lower L^* value) meat products. Additionally, Myers and others (2013) found that L^* values in no nitrite (nitrite-free) ham were significantly higher ($P < 0.05$) than in the treatments containing nitrite from cultured celery juice powder. Treatment effects on CIE L^* , a^* , b^* values during retail display showed that the film type had a significant effect on in-package external color. NT10-NEF was less discolored than NT10-CF and CJP-NEF was redder than CJP-CF. The CIE L^* , a^* , b^* results found in the present study are consistent with previous research that showed decreased a^* , increased b^* and reduced L^* during respective retail display periods (Yen et al. 1988; Møller et al. 2003; Nannerup et al. 2004).

In-package external a^* values over time (Figure S1) show that CJP-CF and NT10-NEF performed most similar with regard to stability during the display period. Results comparing NT10-NEF to NT10-CF for in-package external a^* value highlight that NT10-NEF a^* value increased over retail display. The highest numeric a^* value for NT10-NEF occurred at day 27, which suggests that the maximum increase in cured color formation from the film was reached. Significant reductions in a^* value (indicator for cured pigment) for treatments during retail display period are supported by previous research (Yen et al. 1988; Sindelar et al. 2007; Terns et al. 2011). An additional study by Fernández-Ginés and others (2003) showed that a^* values decreased during storage and determined that a^* was an indication of cured pigment. Our results for in-package external CIE L^* , a^* , b^* values depict differences for treatment effects with product performance in the following relative decreasing order CJP-NEF, CON-CF, CJP-CF, NT10-NEF and NT10-CF. The findings in the present study show both generation and degradation of cured pigment based on relative changes in a^* value during the display period.

NEF's unmistakable impact is highlighted by the result that in general, CJP-CF and NT10-NEF were not different, and NT10-NEF had greater external surface a^* value than NT10-CF. The results for external surface color reveal that the NEF film had a positive impact on color development over the retail display period, which shows similar results found in in-package color.

With regard to internal color, NT10-NEF had greater internal a^* value compared to NT10-CF, which shows that the color development (increased redness) occurred. Therefore, the film did not only provide a surface effect but also provided improved internal color in the NT10-NEF treatment (compared to NT10-CF). This demonstrates

that the nitrite was able to not only provide a surface effect but actually generate improved redness with interior slices which was a package depth of 12.70 mm (½”) from the contact surface. The increased external surface b^* values (greater yellowness) shown in NT10-CF indicate greater product discoloration (compared to NT10-NEF). The varying results for external surface and internal L^* values suggest that the NT10-CF and NT10-NEF are similar to each other as well as CJP-NEF, CON-CF and CJP-CF are similar. Some differences existed between CJP-NEF and NT10-NEF in external surface L^* value.

The external surface a^* values (Table 9), showed that after 97 days CJP-CF had decreased redness (a^* value) for the remaining retail display period (days 97 through 125). This supports the hypothesis that CJP-CF offers reduced color stability compared to CON-CF and CJP-NEF over an extended retail display period (125 days). Previous research on celery juice powder as a source of nitrite showed similar results in that celery juice powder resulted in light-induced color fading over retail display (Sindelar et al. 2007; Krause et al. 2011). In contrast, a study by Redfield and Sullivan (2015) using celery juice powder instead of conventional nitrite found that time (retail display period) had no effect on CIE L^* , a^* , b^* values. The contradicting results with regard to color stability potentially can be explained by differences in storage conditions (lighting and/or temperature), proximate composition (pH and/or myoglobin content), and/or the duration of the study.

NT10-NEF had reduced mean external surface a^* value at day 1 compared to days 6 through 41. The mean external surface a^* value in NT10-NEF compared to NT10-CF over the first 41 days of retail display shows that cured color was developed with time

in NEF packaged product. The external surface a^* value reached its peak value at day 27 and began declining in numerical value for the remaining retail display period.

Internal a^* values were not different during retail display for any treatment except NT10-NEF which increased in redness for the duration of retail display (a^* of 6.86 on day 1 to 11.75 on day 125). Similarly, internal b^* decreased in NT10-NEF during retail display (b^* value of 17.87 on day 1 to 15.62 on day 125). NT10-CF showed the greatest discoloration (greatest b^* value) throughout retail display. Based on internal b^* results, the latter half of the retail display period illustrated that NT10-NEF discoloration (b^* value) was similar to all other treatments (except NT10-CF). Internal color results illustrate the improvements in internal cured color (nitric oxide penetration) over time in NEF packaged product.

Treatment effects for residual external NO_2 (using colorimetric method) showed that NT10-CF and NT10-NEF had considerably less residual NO_2 present in the cooked, packaged meat product and that the film did not have an impact on external residual NO_2 when comparing CJP-CF and CJP-NEF, and NT10-CF and NT10-NEF. These results do not align with previous research conducted on fresh meat using NEF (Claus and Du 2013). One potential explanation regarding conflicting results is that in the study conducted by Claus and Du (2013) the method used was different and it was adapted from Bryan and Grisham (2007). The colorimetric method (AOAC 2005c) used in the present study relies on spectrophotometry, as opposed to the ion chromatography techniques used in the study conducted by Claus and Du (2013). There are slight differences between results for spectrophotometry and chromatography methods. The treatment x day results for external residual NO_2 (colorimetric method) show that film type (conventional film or NEF) did not have a significant impact at any storage point

during retail display, with one exception where CJP-CF was lower than CJP-NEF at day 6. Internal residual NO₂ (colorimetric method) results for treatment by day effects showed that there was no difference between any treatment from day 27 through 125 and CJP-CF there was no difference from CJP-NEF at any day.

The ion chromatographic method for external and internal residual NO₂ followed the same general trend as the colorimetric results, although in the present study, numerical value using chromatographic procedure is lower than colorimetric method (AOAC 2005a). The only significant difference that was contradictory between the methods is that there was a greater amount of external residual NO₂ (ion chromatography method) in NT10-NEF compared to NT10-CF. Colorimetric external residual NO₂ found no difference between NT10-CF and NT10-NEF. Potential reasons for the contradicting results are differences in the procedure (low residual nitrite content in Natpre T-10 EML Plus S was more observable using ion chromatography compared to spectrographic procedure), potential freeze-thaw effects on the film and/or meat product during shipping for ion chromatography analysis.

Regardless of method, results found in the present study showed that residual NO₂ was reduced over time during retail display. Reduction in residual NO₂ during retail display as observed in the present study 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. 2007; 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 NO₂ levels observed in the present study show that NEF had little (if any) impact on residual NO₂ levels even for low nitrite-containing meat products such as those containing Natpre T-10 EML Plus S. Previous research has shown that lower

ingoing levels of nitrate/nitrite from celery juice powder are common in alternatively-cured meats (Sindelar et al. 2007; Myers et al. 2013). Reduced ingoing nitrite concentrations can potentially lead to reduced color stability over extended retail display as residual nitrite is depleted. Our findings show that alternatively-cured meat products from cultured celery juice (CJP-CF) offering reduced color stability (Table 9) as residual nitrite decreased over retail display (Figures S3–S6). NEF provided a positive impact in cured color, which improved the color stability (CJP-NEF) and was capable of generating redness (NT10-NEF) during storage.

Mean external residual nitrate (NO_3) results for treatment effects showed that NO_3 was detected in CON-CF, however, only NO_2 was included in the formulation. The proposed mechanism explaining this nitrate formation phenomena is during processing some of the NO_2 forms NO_3 (Sindelar et al. 2007; Honikel 2008). It has been shown that NO_3 is non-reactive in fully-cooked meat (Honikel 2008). This conclusion explains why residual NO_3 values for NT10-CF and CON-CF were not different while at the same time residual NO_2 was dramatically reduced in NT10-CF compared to CON-CF; thus, nitrate being unreactive was not affected. As expected, internal residual NO_3 showed similar results and the same trends as external NO_3 . CJP-CF and CJP-NEF showed greater residual NO_3 compared to other treatments and this finding is potentially resulting from cultured celery juice powder containing a greater amount of nitrate compared to modern cure powder (CON-CF) and Natpre T-10 EML Plus S (NT10-CF and NT10-NEF). In the present study, the increased 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). The minor treatment x day

interactions can potentially be explained by variation in NO₃ present, sample preparation variation, storage variation or other extrinsic factors.

Treatment effects for aerobic plate count values under normal storage conditions showed that there was no difference in growth between CON-CF, CJP-CF, CJP-NEF or NT10-CF, while growth in NT10-NEF was greater than in CON. Bacterial growth data was converted into logarithmic form for analysis of growth rates and all treatments were ≤ 1.51 log CFU/g for aerobic plate count values. There were some differences in aerobic plate count, however, the very low growth makes significance interpretation difficult. There were no treatment or treatment x day effects for lactic acid bacteria plate count values.

In the inoculated products, the treatment by day effects for aerobic plate count showed no effects but there were significant increases in bacterial growth during storage period (21 days), which was expected with inoculation. Similar to aerobic plate count, lactic acid-producing bacteria treatment effects reveal no change between CON-CF, CJP-CF or CJP-NEF. However, NT10-NEF and NT10-CF showed less bacterial growth compared to all other treatments. Reduced lactic-acid bacteria growth in NT10-CF and NT10-NEF potentially is a result of cooked pH treatment differences as both treatments containing Natpre T-10 EML Plus S resulted in reduced cooked pH potentially from the increased fruit and spice extracts contained in alternative curing ingredient.

Conclusion

The results from this study show that nitrite-embedded film (NEF) improves external and internal redness in alternatively-cured meat products, especially those that contain low levels of residual nitrite. In the present study, a* value was used as an

indicator for cooked, cured meat pigment, and based on remarkable improvements in a* value for treatments with low residual nitrite in NEF packaged products, it appears that cured pigments (nitrosylhemochrome) were developed in these cooked products following processing. Results demonstrate the potential to not only improve retail display color but also suggests a potential mechanism to generate cured color, post-thermal processing. The proposed mechanism utilizes the reducing capacity of the meat system to both reduce denatured metmyoglobin and the nitrite embedded in the film. The generated nitric oxide then binds to heme iron to generate nitrosylhemochrome pigment. Further research should be conducted to investigate the proposed mechanism in addition to the of impact NEF technology on the safety, quality, and sensory impact in nitrite-free cooked meat products.

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Tables and Figures

Table 3. Means of raw and cooked proximate composition for bologna treatments effects.

	Raw Composition			Cooked Composition			
	% Moisture	% Fat	% Protein	% Moisture	% Fat	% Protein	% Yield
CON-CF	60.85 ^a	21.75 ^b	13.56	57.57 ^a	24.72 ^b	16.80 ^a	92.25% ^a
CJP-CF	59.92 ^b	22.95 ^a	13.43	56.59 ^b	25.77 ^{ab}	16.10 ^b	91.63% ^a
CJP-NEF	59.92 ^b	22.95 ^a	13.43	57.00 ^{ab}	24.98 ^{ab}	16.06 ^b	91.63% ^a
NT10-CF	60.25 ^b	21.52 ^b	13.68	56.56 ^b	25.81 ^a	15.91 ^b	91.56% ^b
NT10-NEF	60.25 ^b	21.52 ^b	13.68	56.53 ^b	25.14 ^{ab}	16.37 ^{ab}	91.56% ^b
SEM	0.01	0.01	0.00	0.01	0.00	0.00	0.00

^{a-c} 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

Table 4. Means of raw and cooked pH for bologna treatment effects.

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

^{v-z} Means in the same row with different letter 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

Table 5. Means for in-package external color values for bologna treatment effects under retail 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-c} 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

Table 6. Mean in-package external L* value for bologna treatment x day effects under retail 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	64.76 ^{bc}	64.92 ^{bc}	64.67 ^{bc}	64.87 ^{ab}	64.88 ^{ab}	65.06 ^{ab}	65.31 ^{ab}	65.09 ^{ab}	65.21 ^{ab}	65.51 ^{ab}	65.68 ^{ab}
CJP-CF	63.78 ^c	63.86 ^c	63.80 ^c	63.84 ^b	63.92 ^b	64.11 ^b	64.27 ^b	64.62 ^{bc}	64.67 ^{bc}	64.88 ^{bc}	64.90 ^{bc}
CJP-NEF	63.98 ^c	64.06 ^c	64.09 ^c	64.03 ^b	63.84 ^b	63.91 ^b	64.20 ^b	63.78 ^c	63.98 ^c	63.92 ^c	64.07 ^c
NT10-CF	66.24 ^a	66.16 ^a	65.86 ^a	65.71 ^a	65.63 ^a	65.61 ^a	65.80 ^a	65.49 ^a	65.56 ^a	65.60 ^a	65.39 ^a
NT10-NEF	65.43 ^{ab}	65.44 ^{ab}	65.54 ^{ab}	65.49 ^a	65.46 ^a	65.69 ^a	66.01 ^a	65.79 ^a	65.87 ^a	66.09 ^a	66.04 ^a

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

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

SEM = 1.71

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

Table 7. Mean in-package external b* value for bologna treatment x day effects under retail 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	15.74 ^{bw}	15.59 ^{bw}	15.50 ^{bw}	15.52 ^{bw}	15.60 ^{bw}	16.05 ^{bcv} _w	16.50 ^{bcv} _w	16.74 ^{bcv}	16.94 ^{bcv}	17.07 ^{bcv}	17.11 ^{bcv}
CJP-CF	16.58 ^{bw}	16.27 ^{bw}	16.34 ^{bw}	16.19 ^{bw}	16.46 ^{bw}	17.04 ^{bw}	17.36 ^{bvw}	17.71 ^{bvw}	17.86 ^{bvw}	18.15 ^{bvw}	18.26 ^{bv}
CJP-NEF	16.12 ^b	15.89 ^b	15.89 ^b	15.78 ^b	15.75 ^b	15.91 ^c	15.81 ^c	16.08 ^c	16.31 ^c	16.56 ^c	16.88 ^c
NT10-CF	19.90 ^{ax}	19.97 ^{awx}	20.37 ^{avw} _x	20.69 ^{avwx}	20.89 ^{avw} _x	20.87 ^{avw} _x	21.16 ^{av}	21.20 ^{av}	21.12 ^{avw}	21.07 ^{avw} _x	20.51 ^{avw} _x
NT10-NEF	16.95 ^{bv} _y	16.09 ^{bxy}	15.63 ^{bx}	16.02 ^{bwxy}	16.48 ^{bvw} _{xy}	16.72 ^{bcv} _{wxy}	17.01 ^{bvw}	17.35 ^{bv}	17.52 ^{bv}	17.56 ^{bcv}	17.63 ^{bcv}

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

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

SEM = 0.22

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

Table 8. Means for external surface and internal color values for bologna treatment effects under retail display.

	External 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-c} 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

Table 9. Mean external surface a* value for bologna treatment x day effects under retail 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 ^{awx}	10.68 ^{bx}
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 ^d
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-e} Means in the same column with different letters are significantly different ($P < 0.05$)

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

SEM = 0.91

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

Table 10. Mean external surface b* value for bologna treatment x day effects under retail 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	15.39 ^b	15.11 ^b	15.61 ^b	15.23 ^b	15.43 ^b	15.77 ^b	15.45 ^b	16.01 ^b	16.42 ^b	16.56 ^b	16.71 ^b
CJP-CF	15.96 ^b	15.66 ^b	15.70 ^b	15.83 ^b	16.17 ^b	16.34 ^b	16.43 ^b	17.29 ^b	17.68 ^b	18.23 ^b	18.14 ^b
CJP-NEF	16.17 ^b	15.53 ^b	15.70 ^b	15.76 ^b	15.89 ^b	15.52 ^b	16.03 ^b	16.39 ^b	16.97 ^b	16.63 ^b	17.03 ^b
NT10-CF	18.55 ^a	18.49 ^a	18.97 ^a	19.32 ^a	19.70 ^a	19.59 ^a	19.70 ^a	19.97 ^a	19.97 ^a	19.79 ^a	19.71 ^a
NT10-NEF	17.03 ^b	15.64 ^b	15.94 ^b	15.73 ^b	16.02 ^b	16.74 ^b	16.93 ^b	17.34 ^b	17.47 ^b	17.74 ^b	17.97 ^b

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

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

SEM = 0.43

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

Table 11. Mean external surface L* value for bologna treatment x day effects under retail 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	62.89	63.34	62.93	63.21	63.16	62.67	63.28	64.15	63.88	63.91	63.64
CJP-CF	62.17	62.75	62.58	62.78	63.24	63.15	63.03	63.33	64.09	63.43	63.78
CJP-NEF	62.06	62.33	62.69	62.54	62.88	63.03	62.51	62.74	62.53	63.30	62.51
NT10-CF	64.41	64.69	64.56	64.57	64.40	64.81	64.12	64.15	64.43	64.90	64.38
NT10-NEF	64.08	64.25	64.25	63.56	64.46	64.54	64.93	64.99	65.27	64.51	65.02

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

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

SEM = 1.75

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

Table 12. Mean internal a* value for bologna treatment x day effects under retail 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-e} Means in the same column with different letters are significantly different ($P < 0.05$)

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

SEM = 0.65

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

Table 13. Mean internal b* value for bologna treatment x day effects under retail 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	14.99 ^b	14.82 ^b	14.80 ^c	14.78 ^c	14.90 ^b	14.69 ^{bc}	14.65 ^b	14.70 ^c	14.90 ^b	14.76 ^c	14.63 ^b
CJP-CF	15.69 ^b	15.42 ^b	15.59 ^{bc}	15.53 ^c	15.49 ^b	15.39 ^{bc}	15.32 ^b	15.34 ^c	15.38 ^b	15.35 ^{bc}	15.46 ^b
CJP-NEF	15.37 ^b	15.39 ^b	15.46 ^c	15.43 ^c	15.41 ^b	15.32 ^c	15.38 ^b	15.24 ^c	15.54 ^b	15.14 ^{bc}	15.26 ^b
NT10-CF	18.00 ^a	18.06 ^a	17.89 ^a	17.86 ^a	18.19 ^a	18.17 ^a	18.20 ^a	17.79 ^a	18.00 ^a	17.77 ^a	17.97 ^a
NT10-NEF	17.87 ^{av}	17.07 ^{avw}	16.55 ^{bwx}	15.98 ^{bxy}	15.79 ^{bx}	15.84 ^{bx}	15.81 ^{bx}	15.77 ^{bx}	15.76 ^{bx}	15.81 ^{bx}	15.62 ^{bx}

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

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

SEM = 0.20

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

Table 14. Mean internal L* value for bologna treatment x day effects under retail 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	64.02	63.86	64.26	64.19	64.37	64.59	64.94 ^{ab}	64.88	64.33	64.64	64.91
CJP-CF	62.87	63.65	63.54	63.23	63.96	64.19	64.26 ^{ab}	64.03	64.29	63.72	63.94
CJP-NEF	63.12	62.32	63.35	63.42	64.17	63.95	63.52 ^b	63.83	63.61	63.78	63.62
NT10-CF	65.55	66.00	66.46	66.40	66.43	66.93	66.99 ^a	66.48	66.99	66.19	66.94
NT10-NEF	65.73	65.48	65.54	65.35	65.60	65.70	66.09 ^{ab}	65.45	65.95	65.30	65.70

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

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

SEM = 1.61

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

Table 15. Mean external surface and internal residual nitrite for bologna treatment effects under retail display.

	Colorimetric Method: AOAC 973.31		Ion Chromatographic Method: AOAC 993.30	
	External NO ₂ ppm	Internal NO ₂ ppm	External NO ₂ ppm	Internal NO ₂ ppm
CON-CF	16.41 ^a	16.27 ^a	11.64 ^a	11.18 ^a
CJP-CF	13.50 ^a	12.82 ^a	9.09 ^a	9.18 ^a
CJP-NEF	15.86 ^a	15.00 ^a	11.82 ^a	10.86 ^a
NT10-CF	6.55 ^b	6.59 ^b	0.23 ^c	0.09 ^b
NT10-NEF	7.73 ^b	7.18 ^b	3.68 ^b	2.91 ^b
SEM	1.66	1.80	1.22	1.39

^{a-c} 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

Table 16. Mean external surface and internal residual nitrate value (ion chromatographic method AOAC 993.30) for bologna treatment effects under retail display.

Ion Chromatographic Method: AOAC 993.30		
	External Residual NO ₃ ppm	Internal Residual NO ₃ ppm
CON-CF	40.18 ^{bc}	39.05 ^b
CJP-CF	46.59 ^a	46.32 ^a
CJP-NEF	45.45 ^{ab}	45.95 ^a
NT10-CF	33.64 ^d	33.41 ^b
NT10-NEF	38.55 ^{cd}	39.09 ^b
SEM	2.25	2.48

^{a-c} 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

Table 17. Mean external surface residual nitrate value (ion chromatographic method AOAC 993.30) for bologna treatment x day effects under retail 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	32.50 ^w	52.00 ^v	47.00 ^{vw}	41.50 ^{vw}	38.00 ^{vw}	35.50 ^{vw}	39.00 ^{vw}	42.50 ^{vw}	39.00 ^{vw}	36.50 ^{vw}	38.50 ^{vw}
CJP-CF	47.00	54.00	49.00	46.00	42.50	44.50	44.00	51.50	44.00	44.00	46.00
CJP-NEF	45.50	49.50	41.50	51.50	46.00	40.00	45.00	48.50	44.50	43.00	45.00
NT10-CF	30.50	33.50	32.50	36.00	35.00	30.50	33.00	36.50	36.00	35.00	31.50
NT10-NEF	31.00	46.50	42.00	38.00	38.00	34.50	42.00	40.50	36.50	37.00	38.00

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

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

SEM = 3.95

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

Table 18. Mean internal residual nitrate value (ion chromatographic method AOAC 993.30) for bologna treatment x day effects under retail 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	33.00	37.50	44.00	46.00	34.50	37.50	41.50	42.00	40.00	36.00	37.50
CJP-CF	48.00	52.00	46.00	50.00	43.00	45.00	44.50	52.00	44.50	41.50	43.00
CJP-NEF	39.50 ^{vw}	42.00 ^{vw}	48.50 ^{vw}	64.00 ^v	44.50 ^{vw}	39.00 ^w	45.50 ^{vw}	49.00 ^{vw}	44.00 ^{vw}	44.00 ^{vw}	45.50 ^{vw}
NT10-CF	31.00	34.50	30.00	39.50	30.50	30.00	35.00	37.00	33.00	36.50	30.50
NT10-NEF	32.50	39.00	45.00	44.00	36.50	36.50	41.50	42.00	37.50	38.50	37.00

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

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

SEM = 4.57

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

Table 19. Mean aerobic plate count values (log CFU/g) for bologna treatment effects under retail display.

	log CFU/g
CON-CF	1.02 ^b
CJP-CF	1.10 ^{ab}
CJP-NEF	1.10 ^{ab}
NT10-CF	1.10 ^{ab}
NT10-NEF	1.51 ^a

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

SEM = 0.11

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

Table 20. Mean aerobic plate count values (log CFU/g) for bologna treatment x day effects under retail display.

	Day 0	Day 7	Day 14	Day 30	Day 60	Day 90	Day 120
CON-CF	1.30	1.25	1.10	0.85 ^b	1.10	0.60	0.95
CJP-CF	1.55	1.35	1.00	1.25 ^{ab}	1.00	1.10	1.20
CJP-NEF	1.15	1.40	1.40	1.25 ^{ab}	0.60	1.15	0.70
NT10-CF	1.45	1.20	1.60	1.25 ^{ab}	0.50	0.70	0.95
NT10-NEF	1.40 ^{vw}	1.25 ^{vw}	1.35 ^{vw}	2.50 ^{av}	1.20 ^w	1.45 ^{vw}	1.40 ^{vw}

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

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

SEM = 0.23

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

Table 21. Mean lactic acid bacteria plate count values (log CFU/g) for bologna treatment effects under retail display.

	log CFU/g
CON-CF	0.05
CJP-CF	NG
CJP-NEF	0.05
NT10-CF	0.05
NT10-NEF	0.14

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

SEM = 0.09

NG = No Growth

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

Table 22. Mean lactic acid bacteria plate count values (log CFU/g) for bologna treatment x day effects under retail display.

	Day 0	Day 7	Day 14	Day 30	Day 60	Day 90	Day 120
CON-CF	NG	NG	NG	NG	0.35	NG	NG
CJP-CF	NG	NG	NG	NG	NG	NG	NG
CJP-NEF	NG	NG	NG	NG	0.35	NG	NG
NT10-CF	NG	NG	NG	NG	0.35	NG	NG
NT10-NEF	NG	NG	NG	1.01	0.35	NG	NG

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

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

SEM = 0.20

NG = No Growth

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

Table 23. Mean aerobic plate count values for inoculated bologna treatment effects under retail display.

	log CFU/g
CON-CF	7.43 ^a
CJP-CF	7.45 ^a
CJP-NEF	7.31 ^{ab}
NT10-CF	7.05 ^b
NT10-NEF	7.07 ^b

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

SEM = 0.09

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

Table 24. Mean aerobic plate count values (log CFU/g) for inoculated bologna treatment x day effects under retail display.

	Day 0(I)	Day 3(I)	Day 6(I)	Day 9(I)	Day 12(I)	Day 15(I)	Day 18(I)	Day 21(I)
CON-CF	3.80 ^y	5.20 ^x	7.20 ^w	8.10 ^{vw}	8.50 ^v	8.85 ^v	8.85 ^v	8.90 ^v
CJP-CF	3.85 ^y	5.25 ^x	7.40 ^w	8.05 ^{vw}	8.55 ^v	8.70 ^v	8.75 ^v	9.05 ^v
CJP-NEF	3.70 ^y	5.10 ^x	6.55 ^w	8.05 ^v	8.80 ^v	8.75 ^v	8.85 ^v	8.70 ^v
NT10-CF	3.80 ^z	4.95 ^y	6.50 ^{wx}	7.40 ^{vw}	7.70 ^{vw}	8.35 ^v	8.80 ^v	8.90 ^v
NT10-NEF	3.95 ^y	5.30 ^x	6.25 ^x	7.35 ^w	8.00 ^{vw}	8.45 ^{vw}	8.35 ^{vw}	8.75 ^v

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

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

SEM = 0.21

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

Table 25. Mean lactic acid bacteria plate count values (log CFU/g) for inoculated bologna treatment x day effects under retail 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

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

SEM = 0.09

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

Table 26. Mean lactic acid bacteria plate count values (log CFU/g) for inoculated bologna treatment x day effects under retail display.

	Day 0(I)	Day 3(I)	Day 6(I)	Day 9(I)	Day 12(I)	Day 15(I)	Day 18(I)	Day 21(I)
CON-CF	3.25 ^y	4.10 ^{xy}	6.00 ^w	7.25 ^{vw}	7.55 ^v	8.25 ^v	7.90 ^v	7.95 ^v
CJP-CF	2.90 ^y	4.15 ^{xy}	6.15 ^w	7.10 ^{vw}	7.55 ^v	7.80 ^v	7.90 ^v	8.20 ^v
CJP-NEF	2.80 ^y	4.15 ^{xy}	5.65 ^w	7.15 ^v	8.00 ^v	8.15 ^v	7.90 ^v	8.10 ^v
NT10-CF	2.85 ^z	4.05 ^{yz}	5.20 ^{xy}	6.50 ^{wx}	6.80 ^{vw}	7.60 ^{vw}	7.95 ^v	7.85 ^{vw}
NT10-NEF	2.95 ^z	4.25 ^{yz}	5.30 ^{wxy}	6.15 ^{wx}	7.15 ^{vw}	7.60 ^v	7.50 ^{vw}	7.90 ^v

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

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

SEM = 0.24

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

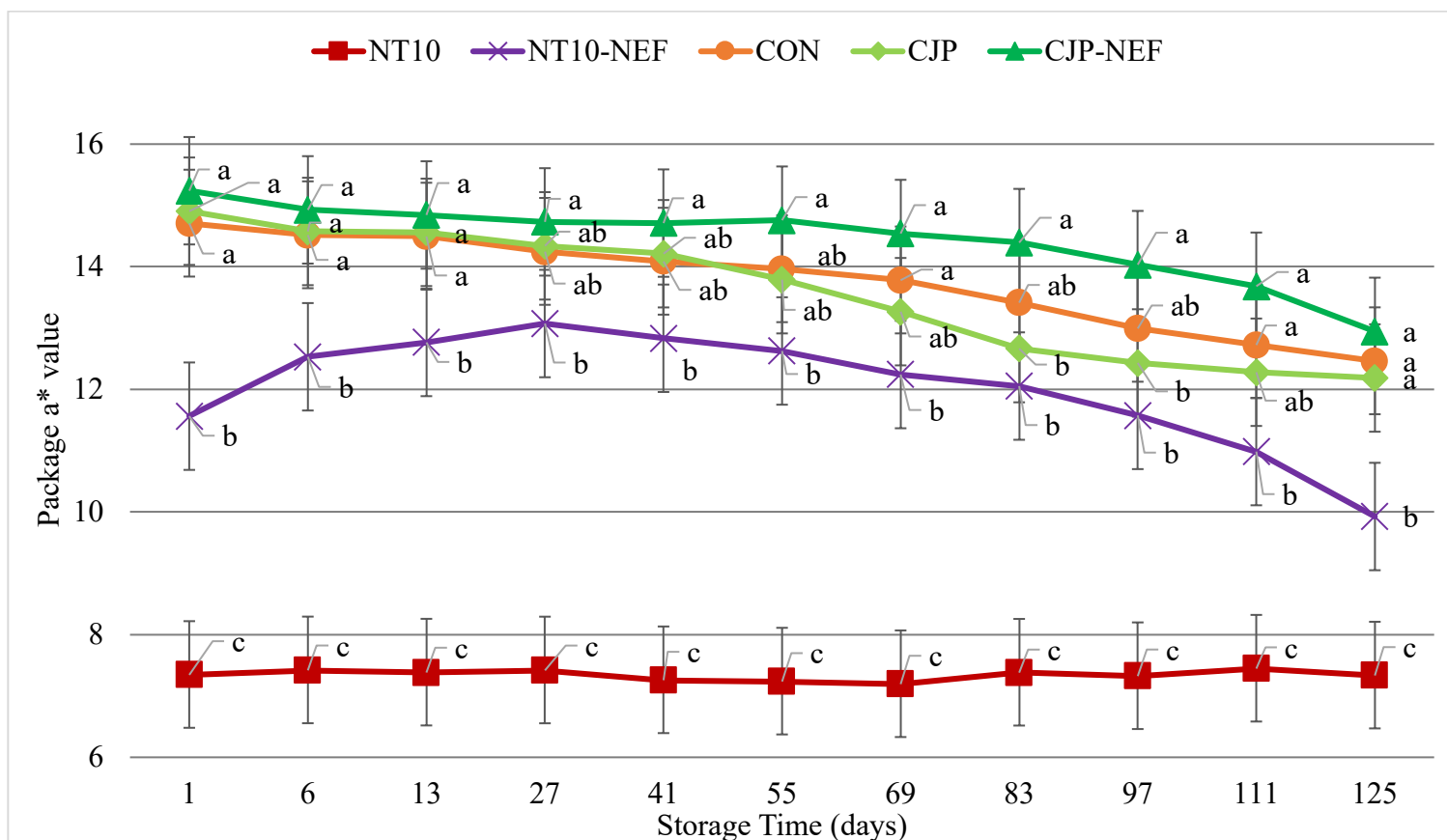


Figure 1. In-package external a* value for bologna treatment x day effects under retail display. (SEM = 0.86) ^{a-c} Means from the same day with different letters are significantly different ($P < 0.05$) (CON-CF = control, conventionally-cured and vacuum packaged in conventional film, CJP-CF = alternatively 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)

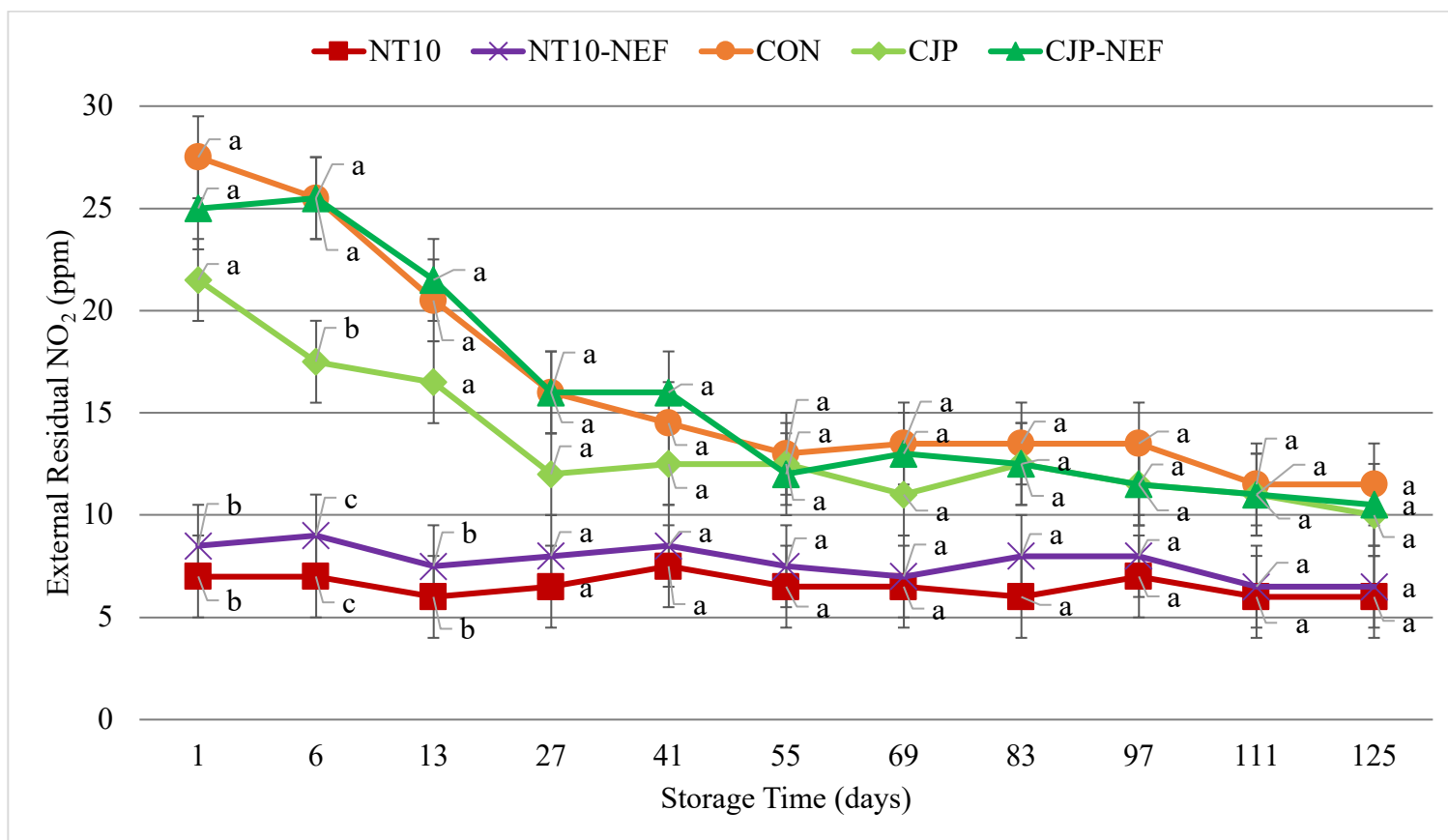


Figure 3. Mean external surface residual nitrite (AOAC 973.31) for bologna treatment x day effects under retail display. (SEM = 2.00) ^{a-c} Means from the same day with different letters are significantly different ($P < 0.05$) (CON-CF = control, conventionally-cured and vacuum packaged in conventional 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)

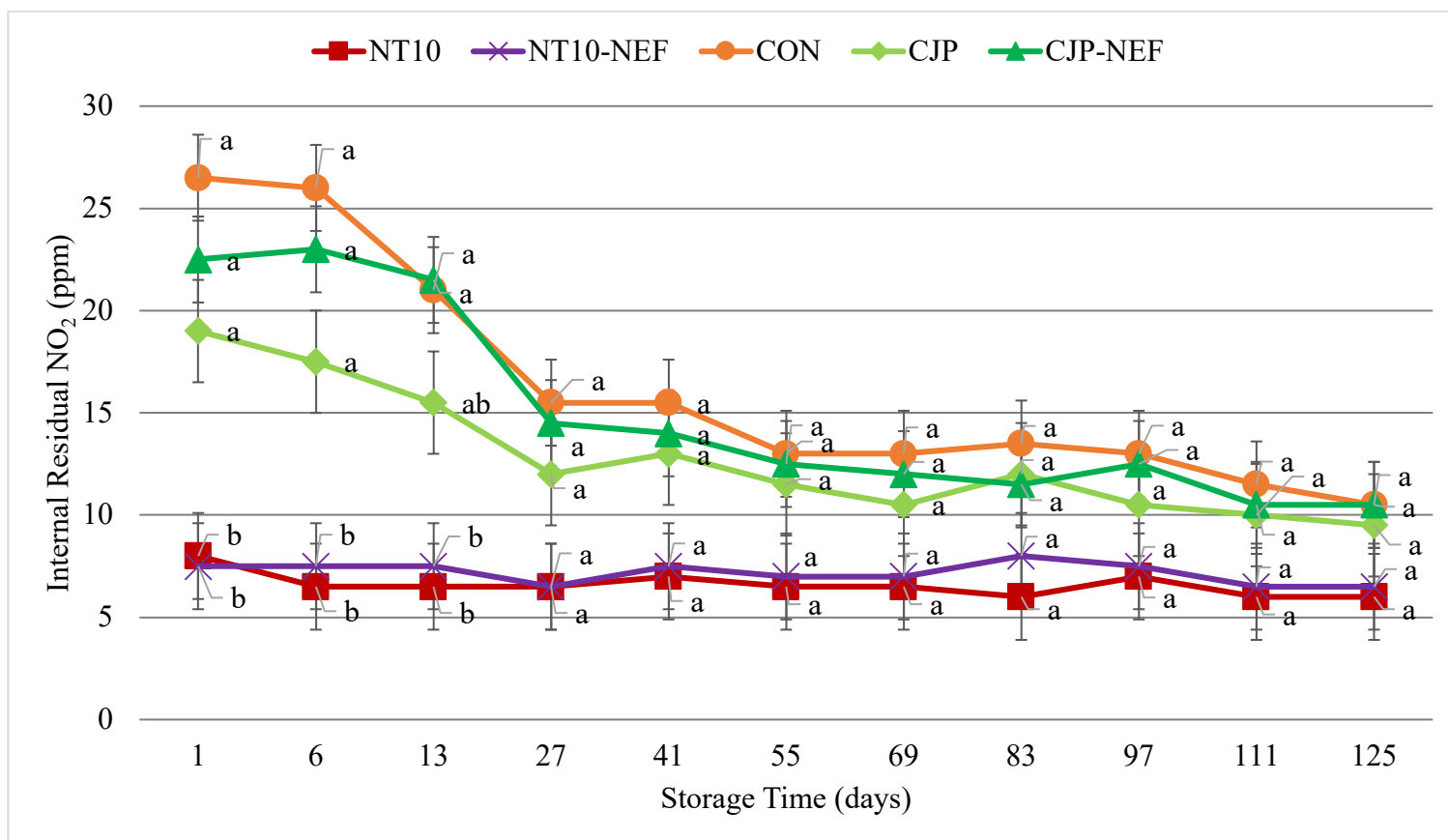


Figure 4. Mean internal residual nitrite (AOAC 973.31) for bologna treatment x day effects under retail display. (SEM = 2.10) Means from the same day with different letters are significantly different ($P < 0.05$) (CON-CF = control, conventionally-cured and vacuum packaged in conventional film, CJP-CF = 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)

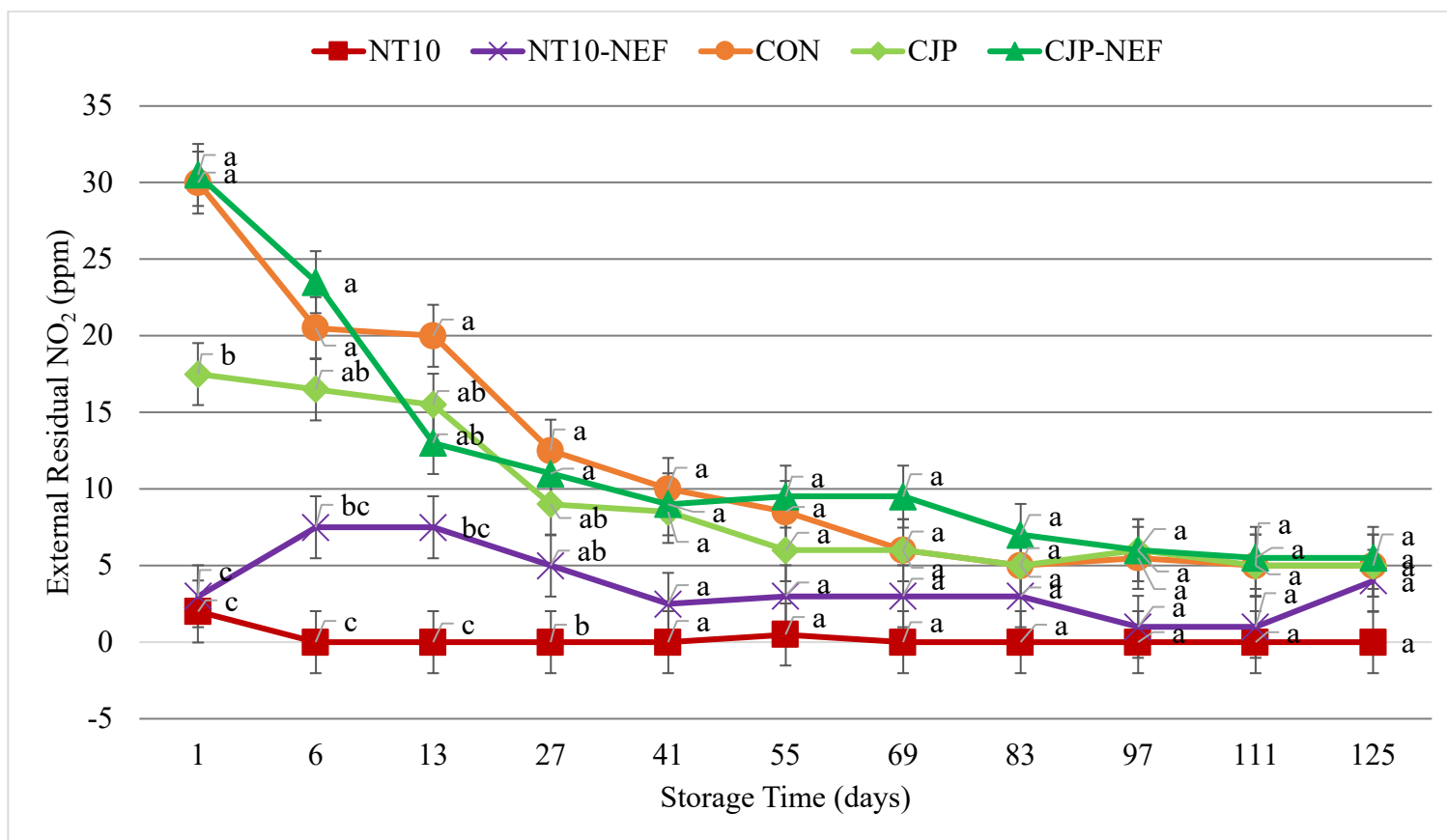


Figure 5. Mean external surface residual nitrite (AOAC 993.30) for bologna treatment x day effects under retail display. (SEM = 2.02) ^{a-c} Means from the same day with different letters are significantly different ($P < 0.05$) (CON-CF = control, conventionally-cured and vacuum packaged in conventional 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)

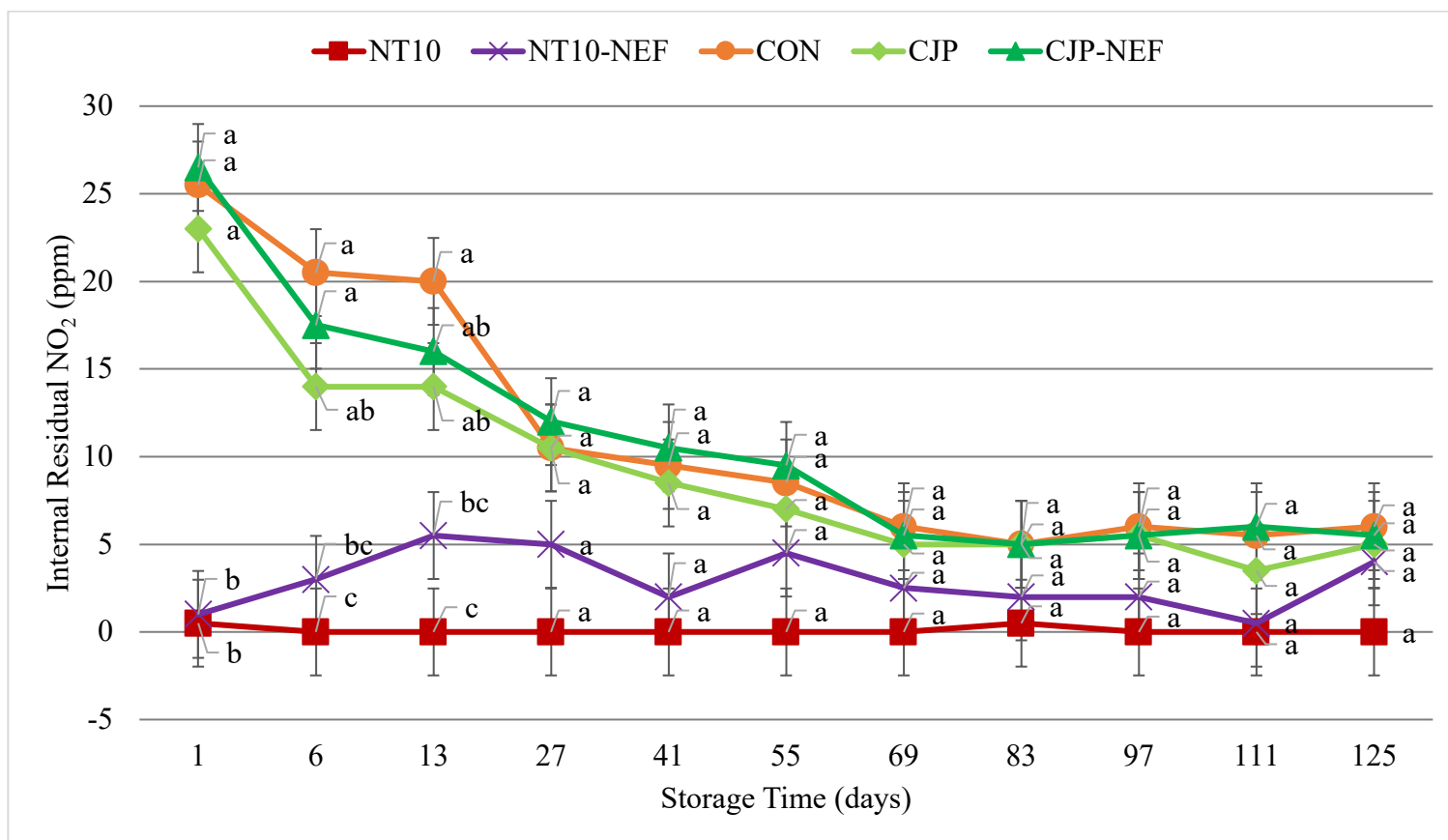


Figure 6. Mean internal residual nitrite (AOAC 993.30) for bologna treatment x day effects under retail display. (SEM = 2.48)

^{a-c} Means from the same day 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)

CHAPTER 4. GENERAL CONCLUSIONS

Conventional curing ingredients (sodium nitrite/nitrate) have stood the test of time and shown to be beneficial in providing irreplaceable food quality and safety attributes in meat products. However, modern day consumers have a negative perception regarding conventional curing ingredients and strive to find natural substitutes for nitrate and nitrite. There are some serious concerns with regard to product quality and shelf life due to negative sensory attributes and potential ingredient inconsistency with alternatively-cured meat products. Therefore, it is up to the meat industry to conduct research to evaluate potential solutions to extend the quality, shelf life and consumer appeal of alternatively-cured meats.

Active packaging is an area of current research that has the potential to solve many consumer driven product quality issues such as reduced color stability in alternatively-cured meat products. Nitrite-embedded film (NEF) has been shown to be an effective solution to improve the retail display color and extend the shelf life in fresh meat. However, no published research has looked into its effects on cooked, cured meat products.

The results of this study demonstrate that nitrite-embedded film can extend the color stability of alternatively-cured meat products. It is believed that the film is capable of generating cured color not only on the surface but throughout a package. Residual nitrite values were not affected by NEF using colorimetric method and only some effects with chromatographic methods (AOAC 2005a; AOAC 2005b). As expected, NEF had no impact on residual nitrate results. Furthermore, bacterial growth was not affected by NEF.

Further research should look into the effects of NEF on nitrite-free (truly uncured) cooked meat products, as well as quantify the nitrosylhemochrome pigment in NEF packaged product. Evaluation of food safety risks associated with adding nitrite post-thermal processing should be investigated along with the sensory and quality attributes associated with NEF packaged product.

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