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Naturally cured meats: Quality, safety, and chemistry

Gary Anthony Sullivan
Iowa State University

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Gary Anthony Sullivan

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Program of Study Committee:
Joseph Sebranek, Co-major Professor
Kenneth Prusa, Co-major Professor
Joseph Cordray
Aubrey Mendonca
Dennis Olson

Iowa State University

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CHAPTER 1. GENERAL INTRODUCTION

Consumers are becoming more aware of their food choices and are willing to pay a premium for foods that meet their specific desires. As a result, the natural and organic foods markets have grown rapidly over the past two decades. Often times, consumers perceive the natural and organic foods as healthier alternatives. Research findings from the 1940's to the 1970's created a public perception that consuming foods that contain nitrate and nitrite would cause deleterious health effects and at times even death. In response, some manufactures began eliminating sodium nitrite and nitrate from their products. These products would not possess typical cured meat color, flavor, aroma, or antimicrobial control that the addition of sodium nitrite provides. In the 1970's, the United States Department of Agriculture created a special labeling class for these products that contained no added sodium nitrate or nitrite and required "Uncured" to be placed on the label following the common name. Now, this labeling practice is commonly used in the natural and organic foods sectors since sodium nitrite or nitrate are recognized as preservatives and are not allowed. Meat processors have began utilizing natural nitrate sources such as celery juice, celery powder, and sea salts and nitrate reducing starter cultures to indirectly add nitrite to their products. Because sodium nitrite is not added directly, these products are required to be labeled as "Uncured" even though they have typical cured meat color and flavor and contain residual nitrite and nitrate.

Nitrites and their reduction by-products are required for nitrosation/nitrosylation reactions and are responsible for typical cured meat color, flavor, aroma, antioxidant activity and antimicrobial activity. The antimicrobial activity is most well known for inhibiting *Clostridium botulinum* but also inhibits other pathogens such as *Listeria monocytogenes* which is of great concern in ready-to-eat processed meats. Greater ingoing nitrite

concentrations are required to provide antimicrobial activity than the other cured meat characteristics. Natural and organic cured meats typically have lower ingoing nitrite and the nitrite is slowly formed by bacterial reduction. Additionally, many ingredients used to enhance the curing process or for microbial inhibition are not allowed in natural or organic products. Although these products have many typical cured meat characteristics, the combination of lower ingoing nitrite concentrations and limited use of antimicrobials such as organic acids could provide an environment more susceptible to pathogen growth. Many natural antimicrobials have been identified as potential alternatives to antimicrobials commonly used in meat processing. Additionally, the nitrosation/nitrosylation reactions could be altered due to the slow formation of nitrite and the limited used of cure accelerators. This could shift the typical distribution of nitrosation/nitrosylation reactions products in meat curing and impact product characteristics. The first overall objective of these studies was to determine factors that impact pathogen growth and effectiveness of natural antimicrobials in naturally cured meats. The second overall objective was to evaluate influences of curing system, natural of conventional, on nitrosation/nitrosylation reactions that occur during meat curing using a model system.

Dissertation Organization

This dissertation is organized into six chapters. Chapter 1 provides a general introduction and Chapter 2 provides a review of relevant literature. These chapters are formatted using the style for *Meat Science*. Chapter 3 is entitled **"**Comparison of commercially available naturally and conventionally cured frankfurters, ham, and bacon for physio-chemical properties that affect bacterial growth" and has been prepared as a manuscript for submission to *Meat Science*. Chapter 4 is entitled "Inhibition of *Listeria monocytogenes* using natural antimicrobials in no-nitrate-or-nitrite-added ham" and has been prepared as manuscript for submission to the *Journal of Food Protection*. Chapter 5 is entitled "Nitrosylation of myoglobin and nitrosation of cysteine by nitrite in a model system simulating meat curing" and has been prepared as a manuscript for submission to the *Journal of Agricultural and Food Chemistry*. The final chapter will provide a general summary of findings.

CHAPTER 2. REVIEW OF LITERATURE

History of Meat Preservation

Meat and food preservation was an essential development so that early hunter-gatherers could maximize the harvest and extend the food supply by delaying spoilage. Freezing, salting, and drying foods was used by early humans and the method of preservation was dependent on the surrounding environment (Wentworth, 1956). Sun drying initially was developed as an effective preservation practice in areas of low humidity; later meat was dried and smoked over or alongside fires (Aberle et al., 2001). Freezing, as method of preservation, was limited to geographical climate and season. On the Arabian Peninsula and in coastal regions, early civilizations discovered salting meats was an effective preservation technique (Binkerd & Kolari, 1975; Wentworth, 1956).

Earliest records referring to saltpeter (potassium nitrate) were found around 2200 BC and certain passages from the bible are thought to reference saltpeter (Barnum, 2003). Records date Chinese alchemists' familiarity with saltpeter to the 5th century but it could be as early as the 2nd century AD (Tien-Chin, Ping-Yo & Needham, 1959). Natural nitrate deposits are found in parts of the world and but also it can formed on walls of buildings covering nitrogen rich soils (Barnum, 2003). The advent of gun powder greatly increased the demand and aided in the development of potassium nitrate production in the Far East. In the 1500's, Europeans were producing saltpeter by layering of nitrogen rich soil with lime and water and nitrate concentrations could surpass 10,000 ppm (Barnum, 2003; Williams, 1975). In the 1840's, nitrosation of amines was first identified and research continued to discover other nitrosated/nitrosylated compounds (Williams, 2004).

Cured meats, as known today, are believed to have originated from preserving meat with nitrate contaminated salts. It is unknown when nitrates were first intentionally used but the earliest records of nitrate's reddening effect date to the 10th century, late in the Roman Empire (Binkerd & Kolari, 1975; Pearson & Gillett, 1999). During the late 1890's, researchers noted that nitrite, not nitrate, was responsible for cured meat characteristics (Lewis, Vose & Lowry, 1925). Identification of cured pigment was reported in 1901 (Haldane, 1901). Much research has been conducted to investigate the characteristic color, flavor/aroma, antioxidant activity, and antimicrobial activity associated with cured meats. Concerns over the safety of consuming nitrates and nitrite arose in the middle of the 20th century (Comly, 1945; Gray, 1976). These findings reinvigorated research of nitrite and nitrate and were further propelled by the discovery that nitric oxide is an endogenously produced bioactive molecule. Nitrite is currently considered an essential and safe ingredient necessary to provide high quality and safe cured meats (Sebranek & Bacus, 2007)

Major Nitrogen Oxide Compounds

In order to fully understand the nitrite reactions in cured meats, one must have an understanding of the underlying chemical mechanisms that occur. Nitrogen has three outer electrons and is often found as an inert, diatomic gas, N_2, that comprises over 78% of the atmosphere. However, molecules of nitrogen and oxygen form a complex group of biologically important and chemically reactive compounds. The oxidation and reduction of these compounds range from nitrate in the fully oxidized state to ammonia in the fully reduced state. Nitrate and ammonia are more stable and less reactive compounds than the intermediate nitrogen oxide redox compounds. These will be discussed in greater detail in the following sections.

Nitrosation is the addition of nitric oxide to a non-metal such as sulfur or carbon the result of which is a nitroso compound where as nitrosylation is the addition of nitric oxide (NO) to a metal such as iron or copper to form a nitrosyl compound (Pegg & Shahidi, 2000; Stamler, 1994). Many oxidation, reduction, and nitrosation/nitrosylation reactions of nitrogen oxide compounds are essential in biological systems and the production of cured meats. Uses and understanding of nitrogen oxide compounds have evolved from being considered beneficial, to being considered harmful, and now many researchers have demonstrated them to be beneficial again. Many new roles and functions of nitrogen oxide compounds have been found, yet many questions remain to be answered.

Nitrate

Nitrate, NO_3^-, is the fully oxidized nitrogen oxide compound. The pK_a of nitric acid, HNO_3, is -1.6, meaning when nitrate is dissolved in water, nearly all exists as nitrate anion (Honikel, 2008). Many bacteria possess nitrate reductase activity (Harrison, 1929; Tavares, Pereira, Moura & Moura, 2006). In 2000, Fukuto and others (2000) stated that nitrate "has little or no physiologically relevant chemistry." However since then, nitrate has been shown to be a reserve and precursor for nitric oxide and other biologically important nitrogen oxide compounds (Lundberg et al., 2009; Lundberg, Weitzberg & Gladwin, 2008). However, bacterial reduction is necessary for nitrate to have biological activity (Lundberg & Weitzberg, 2010). Nitrate can be readily formed in biological conditions through the oxidation reactions of lower nitrogen oxide compounds (Lundberg et al., 2008; Miranda et al., 2000; Tannenbaum, Fett, Young, Land & Bruce, 1978). As in biological systems, nitrate must be reduced to nitrite in order to be reactive and for meat to develop traditional cured

characteristics during processing (Terns, Milkowski, Claus & Sindelar, 2011a). Honikel even states that "nitrate is useless and superfluous" unless meat products are allowed to cure and ferment for extended periods (Honikel, 2004).

Nitrite

Nitrite, in comparison to nitrate, is a much more reactive compound. Like nitrate, the pK_a of nitrous acid, HNO_2, is relatively low, 3.3. Most of this compound would be found as the nitrite anion, NO_2^-, in biological or meat curing conditions but some of nitrous acid would be found. The nitrite ion must be reduced to act as a nitrosating/nitrosylating agent (Honikel, 2004). Acidification of nitrite provides one of the best methods to form nitric oxide. In the presence of mineral acids and other reducing compounds, nitrite can be non-enzymatically reduced to nitric oxide (Williams, 1988). However, the anhydrous form of nitrous acid, dinitrogen trioxide, N_2O_3, is thought to be the one of the main nitrosating compounds (Fukuto et al., 2000). Reduction of nitrite in meat curing systems is essential to provide nitrosation/nitrosylation reactions and is impacted by many factors including pH, temperature, endogenous compounds, and other added ingredients (Cassens, 1997). In vivo, the nitrite produced by bacteria in the oral cavity is readily reduced to nitric oxide in the acid conditions of the stomach and provides a supplement nitric oxide source (Lundberg et al., 2008). Further examples of reducing reactions will be discussed in later sections.

Early researchers noted the color change of hemoglobin with the addition of nitrite (Gamgee, 1868). This reaction is now known to form nitosylmetmyoglobin which renders hemoglobin unable to transport oxygen. Cyanosis caused by ingesting sodium nitrite or nitrite containing food has been reported throughout medical literature (Aquanno, Chan &

Dietzler, 1981; Bakshi, Fahey & Pierce, 1967; Barton, 1954; Bradberry, Gazzard & Vale, 1994; Harvey, Cave & Chanwai, 1976; Oppé, 1951; Simon, 1966; Walley & Flanagan, 1987; Wilson, 1976). Some of these cases were treatable and others fatal. Sodium nitrite intake of 33-250 mg per kg of body weight can result in death (Honikel, 2004). Due to this, sodium nitrite is tightly regulated during meat processing. It is most often added as a 6.25% sodium nitrite/sodium chloride blend and dyed pink to prevent accidental addition of excess sodium nitrite.

Nitric Oxide

Over 200 years ago, nitric oxide was first indentified and early researchers found that it readily reacted to from other nitrogen oxide compounds (Gow, 2006). Unlike most free radicals, nitric oxide does not self dimerizes (Fukuto et al., 2000) and likely contributes to the ability of nitric oxide to be such a potent nitrosylating/nitrosating agent as it is more likely to react with other compounds. Nitric oxide has a solubility of 2 nM in water but has higher solubility and reactivity in hydrophobic environments (Liu, Miller, Joshi, Thomas & Lancaster, 1998). Depending on the environment, nitric oxide can serve as an oxidizing, reducing or nitrosylating/nitrosating agent (Henry, Ducastel & Guissani, 1997; Wink et al., 2001). Furthermore, nitric oxide is able to terminate free radical reactions such as those found in lipid oxidation (Miranda et al., 2000). These unique properties allow nitric oxide to act as an important regulatory molecule in biological systems and to provide the typical cured meats properties.

Nitrogen Dioxide

Nitrogen dioxide, also a free radical molecule, is often found as a brown colored gas. But unlike nitric oxide, nitrogen dioxide readily dimerizes and forms dinitrogen tetraoxide, N_2O_4 (Williams, 2004). Peroxynitrite, a reaction product of nitric oxide and super oxides O_2^- which can act as a nitrating compound, decomposes to form nitrogen dioxide (Fukuto et al., 2000; Ignarro, 2000). In the presence of water, nitrogen dioxide hydrolyzes to form nitrite and nitrate. Nitrogen dioxide can act as an oxidizing agent but this process is slow and reducing compounds limit the reaction (Fukuto et al., 2000). It is likely that nitrogen dioxide has a minimal role in cured meats production.

Nitroxyl

Even though nitroxyl, HNO, was identified early in the 20th century, it has not been investigated to the extent of other nitrogen oxide compounds until recently (Fukuto, Switzer, Miranda & Wink, 2005). The pKa of nitroxyl is around 11.4 and would exist almost exclusively in the protonated form (Williams, 2004). It is not known if nitroxyl is formed endogenously although many chemical mechanisms have been proposed (Flores-Santana et al., 2009). The biological importance is not known but some have suggested pharmacological possibilities (Miranda, 2005). One difficultly of identifying nitroxyl in vivo is that it forms slowly and rapidly dimerizes or decomposes (Fukuto et al., 2005). Nitroxyl reacts with many of the same compounds as nitric oxide resulting in some similar and some different end products (Flores-Santana et al., 2009). These authors report that nitroxyl can form myoglobin products similar to nitric oxide but in the presence of sulfhydryl groups, nitroxyl forms sulfanilamides not S-nitrosothiols. The reaction of nitric oxide and other

nitrosating compounds likely play a much greater role than nitroxyl in cured meats processing.

Chemistry of Nitrogen Oxide Compounds and Nitrosation Reactions

As a composite group, nitrogen oxide compounds have the ability to react with many compounds and functional groups. Some reactions form permanent covalent bonds while others serve primarily as reactionary intermediates. In some cases, nitric oxide acts directly as a nitrosating agent while a different nitrosating reagent is required for others (Williams, 1988). Nitrogen oxide compounds have been shown to react with and nitrosylate/nitrosate many different elements including oxygen, nitrogen, carbon, sulfur, transition metals, and halides.

Oxygen

Reactions of nitrogen oxides with oxygen are probably the most well-researched nitrosation mechanism. Nitric oxide reacts with either dioxygen gas, O_2, or superoxide, O_2^-, at nearly 10^{10} M^{-1} s^{-1} and are only limited by the rates of diffusion (Ford & Lorkovic, 2002). In aqueous, oxygenated solutions with excess nitric oxide, oxygen and nitric oxide react and decompose to reform nitrite (Henry et al., 1997; Wink, Darbyshire, Nims, Saavedra & Ford, 1993). Similarly, nitric oxide and superoxide rapidly react to form peroxynitrite, $ONOO^-$. This can have mutagenic and carcinogenic properties but also can further react with nitric oxide to reform nitrite and nitrite (Ignarro, 2000; Wink & Mitchell, 1998). Metmyoglobin can also act as a catalyst to decompose peroxynitrite to form nitrate and deoxymyoglobin (80% of peroxynitrite decomposition) and nitrogen dioxide and hypervalent heme iron (20% of peroxynitrite decomposition) (Bourassa, Ives, Marqueling, Shimanovich & Groves, 2001).

Nitric oxide also reduces hypervalent heme iron to metmyoglobin and nitrite (Miranda et al., 2000). Although peroxynitrite can irreversible modify molecules in vivo, these authors suggest that reactive oxygen species likely cause more harm than peroxynitrite. During times of immune response, nitrate and nitrite are endogenously produced by the macrophage (Stuehr & Marletta, 1985) an event explained by peroxynitrite formation and decomposition (Miranda et al., 2000). Hydrogen peroxide, H_2O_2, oxidizes nitric oxide and forms nitrate via a peroxynitrous acid, HOONO, intermediate (Williams, 2004). The ability of nitric oxide to consume oxygen and react with other reactive oxygen species explains one aspect of nitric oxide's antioxidant capacity.

Nitrosation reactions also occur with oxygen containing functional groups. Alcohol nitrosation occurs by replacing the alcohol hydrogen with a nitroso- group to produce an alkyl nitrite, RONO (Williams, 2004). Halide ions serve as catalyst in the reaction. Through the reverse of this same mechanism, alkyl nitrites can serve as a nitrosating agent (Williams, 1988). Nitrosation of carboxylic acids has been demonstrated but little research has been conducted to determine biological significance of these reactions (Williams, 2004). Nitrosation of other carbonyl compounds results in the addition of NO on the adjacent carbon atom not oxygen (Williams, 1988). Nitrosation of alcohols and carboxylic acids likely have less importance in meat processing than other oxygen reactions.

Transition Metals

Of all the nitrosation reactions with transition metals, iron plays the most important role in mammalian systems (Ford & Lorkovic, 2002) but reactions with other metals have been identified (Hughes, 2008; Miranda et al., 2000). Reactions with heme iron proteins

have been the most widely investigated (Salerno, 1996) and early research described the color change of blood after the addition of nitrite (Gamgee, 1868). At the turn of the 20th century, nitrosylhemochromogen was identified as the pigment responsible for cured meat color (Haldane, 1901). Nitrosylation of heme iron is responsible for the production of cured meat color and regulation of many heme iron containing enzymes (Ford & Lorkovic, 2002; Miranda et al., 2000). Nitrosylation of ferrous heme iron occurs more rapidly and forms a more stable complex than with ferric iron (Ford & Lorkovic, 2002; Miranda, 2005). Uniquely, nitric oxide has the ability to oxidize, reduce, and nitrosylate heme-iron (Pantopoulos & Hentze, 2000; Wink & Mitchell, 1998).

Nitric oxide also reacts with non-heme iron and plays an important role in enzyme and iron regulation in biological systems (Butler & Megson, 2002; Pantopoulos and Hentze, 2000). Iron-sulfur complexes, Fe_4S_4, Fe_3S_4, and Fe_2S_2, play important roles in many metabolic enzymes and are often bound through organic and inorganic sulfur bonds (Salerno, 1996). In the 1850's, Roussin produced iron-sulfur-nitrosyl compounds (Williams, 2004) similar to those formed by nitrosylation of iron-sulfur compounds found in many metalloproteins (Butler, Glidewell, Hyde & Walton, 1985). Nitric oxide has also been shown to react with many other transition metals (Mo, Mn, Co, Ni, Cu, Ru) that are found in enzyme active sites (Henry et al., 1997; McCleverty, 2004). The redox chemistry of transition metals and nitric oxide provide regulatory functions in many biological processes and is thought to be one of the methods of bacterial inhibition by nitrite.

Sulfur

Sulfur compounds are readily nitrosated and play an important regulatory function in biological systems. Nitrosation of sulfhydryl groups, as in S-nitrosoglutathione, is one of the most studied S-nitrosation reactions but also nitrosation of thiocarbonyls, organic and inorganic sulfides, sulfinic acids, sulfites, and thiosulfates occur (Williams, 2004). Nitrosation of cysteine can inhibit or activate enzyme function in biological systems (Gow, 2006). When nitrosocysteine is formed in aqueous systems, nitrous anhydride, N_2O_3, not nitric oxide, serves as the nitrosating agent (Miersch & Mutus, 2005). However for reactions with other sulfur compounds, nitric oxide can serve as a nitrosating agent but these reactions are thought to play less of a role in biological systems (Williams, 2004).

Many S-nitrosothiols (SNO) are relatively unstable compounds, readily release nitric oxide, and can function as nitrosating/nitrosylating agents (Oae & Shanhama, 1983). In the presence of oxygen, iron, copper, or other transition metals, SNO stability is greatly reduced (Miersch & Mutus, 2005). Cysteine is found in the active site of many enzymes and can be inhibited by nitrosation (O'Leary & Solberg, 1976; Zhang, 2009). SNO compounds function as reducing agents and as nitric oxide donating compounds. It is these that properties allow for SNO to serve as bioregulatory molecules and offer many potential pharmaceutical applications.

Carbon

Nitrosation of carbon has been used historically and is continued to be used in laboratory and industrial processes to produce many compounds (Williams, 2004). Mechanisms of nitrosation have been identified for carbon at double bounds, adjacent to carbonyls, or on nearly any alkane (Williams, 1988). In reviewing the reactions and required

environmental conditions, few would have relevance to biological systems. Nitrosyl chloride and other halides possess carbon nitrosating capabilities (Williams, 2004) and could be formed in curing brines (Sebranek & Fox, 1985). Alkenes and aromatic rings readily undergo nitrosation in the presence of nitrosyl chloride at points of unsaturation and can further react to form ring closures, oximes, or dimers (Williams, 1988, 2004;). Nitrite reacts with lipid and nitrosation is known to occur on linolenic acid (Woolford and Cassens, 1977).

Although a different reaction, it should be noted that nitration, the addition of NO_2, can occur in the presences of strong nitrating compounds and reactive oxygen species. Nitrogen dioxide or peroxynitrite are capable of nitrating compounds but not lower nitrogen oxides such as nitric oxide. Enzyme inactivation by nitration is usually irreversible and targets different complexes as compared to nitrosation (Cassina & Radi, 1996). However, heme and other porphyrin metal complexes convert peroxynitrite and other nitrating compounds primarily to nitrite and nitrate (Bourassa et al., 2001; Radi, 2004).

Nitrogen

Nitrosation and formation of N-nitrosamines can occur in both primary and secondary amines but only those formed from secondary amines are stable (Honikel, 2004). Tertiary amines can be nitrosated but the reaction is much slower (Williams, 2004). Many, but not all, N-nitrosamines are carcinogenic (Wolff & Wasserman, 1972). Feeding dimethylnitrosamine to rats was found to cause liver tumors (Magee & Barnes, 1956) and it was identified as the compound in nitrite-preserved herring meal that caused liver disease in livestock and rodents (Ender et al., 1964; Koppang, 1964). In low pH (<5.5) or high temperature (>130 °C) environments, N-nitrosamines can be formed from the reaction of nitrite and proteins during

food production, preparation, and in vivo (Crosby, 1976; Fine, Ross, Rounbehler, Silvergleid & Song, 1977; Gray, 1976; Honikel, 2004; Hotchkiss, 1989; Sen, Iyengar, Donaldson & Panalaks, 1974; Walters, Dyke, Saxby & Walker, 1976; Wong, 1989). While frying bacon, many nitrosamines volatilize but higher concentrations may still remain than amounts found in other cured meat products (Wong, 1989). N-nitrosamine formation has been found to be blocked or reduced by ascorbic acid or ascorbate (Mirvish, Wallcave, Eagen & Shubik, 1972; Scotter & Castle, 2004) and reduced by salt (Theiler, Sato, Aspelund & Miller, 1981). Due to nitrosamine formation, concerns arose over the consumptions of nitrite and nitrate. The meat industry has modified production practices and reduced residual nitrite to limit the N-nitrosamine formation (Cassens, 1997; Sen & Baddoo, 1997; USDA, 2010c). Although nitrite and nitrate have now been shown to be beneficial to health, public concerns persist.

Cured Meats: Ingredients, Functionality, and Regulations

Nitrite and Nitrite

Sodium or potassium salts of nitrite and nitrate can be used in curing meats. It was not until the 1890's that nitrite, not nitrate, was identified as the curing agent (Lewis et al., 1925). In 1926, the use of sodium or potassium nitrite was allowed by the USDA at levels described by Kerr and others (1926). Current meat processing uses sodium nitrite almost exclusively but nitrate is still used in some dry cured and dry or semi-dry products that have extended curing, drying, or fermentation periods (Honikel, 2004; Pearson & Gillett, 1999). Nitrite is an essential ingredient for cured meats to provide cured color, cured flavor and aroma, antioxidant activity and antimicrobial activity. However, the nitrite anion alone does not function as a nitrosating/nitrosylating agent and nitric oxide must be reduced for nitrosation/nitrosating reactions to occur.

Regulations concerning ingoing nitrite and nitrate concentration are found in the Processing Inspector's Calculations Handbook (USDA, 1995). Limits of ingoing nitrite vary by product type and calculations are based on product green weight. Both sodium and potassium nitrite and nitrate are allowed to be added but the addition of potassium salts results in lower ingoing nitrite anion concentration than when sodium salts are used. To ensure product safety, USDA policy "requires a minimum 120 ppm of ingoing nitrite to all 'Keep Refrigerated' products" unless other preservation processes are verified and implemented to assure safety. The direct addition of nitrite to comminuted products is limited to 156 ppm. For products manufactured with brine added through emersion, massaging, or injection, 200 ppm of nitrite is allowed. The addition of 625 ppm of nitrite is allowed in the manufacture of dry cured products. Nitrate is limited to 700, 1718, and 2187 ppm for injected, comminuted and dry cured products, respectively, although use is usually limited to products that have extended fermentation and drying periods to serve as a nitrite reserve formed via bacterial reduction. Bacon has a separate regulation in order to limit N-nitrosamine formation during frying. Injected or brine cured bacon products are produced with the addition of 120 ppm of nitrite, 550 ppm sodium erythorbate or ascorbate, and the use of nitrate is prohibited (USDA, 2010c).

Salt

Along with nitrite, salt is an essential ingredient found in all cured meats and provides multiple functions. Salt is the most common ingredient in food processing. Sodium chloride is the most commonly used salt but potassium chloride can be added at up to 50% without negative sensory characteristics (Pearson & Gillett, 1999). Meat and meat products supply

20-21% of sodium intake in American and European diets (Desmond, Kenny & Ward, 2002). Due to relationship of sodium and hypertension, efforts have been taken to reduce sodium intake over the past half century but little change in intake has occurred (Bernstein & Willett, 2010).

As originally used in meat preservation, salt has the capability to act as a drying agent but at lower concentrations can increase moisture content. For whole muscle products in brines, moisture uptake reaches a maximum around 5% salt content and 15% or greater will result in moisture loss (Schmidt, Carciofi & Laurindo, 2008). The chloride anion is responsible for myofibrillar protein extraction during processing. Minimum salt concentrations of 1.4% or 1.75% for normal and low fat products, respectively, are required to achieve acceptable product bind and quality (Ruusunen & Puolanne, 2005). Without proper protein extraction, fat and moisture retention is diminished and product texture can be soft or crumbly. The addition of 1.7-1.8% salt is required for lean sectioned and formed products for proper binding (Ruusunen & Puolanne, 2005).

Salt is one of the five primary tastes found. Sodium in an aqueous solution, including saliva, interacts with the taste bud to produce a taste response (Spielman, 1990). Research has shown that the addition of salt increased the rate of salivation (Neyraud, Prinz & Dransfield, 2003) and could explain the diminished taste found in products with low salt formulations. In addition to the direct taste, salt can act as a flavor enhancer and is essential for product flavor and overall palatability (Doyle & Glass, 2010). While excess salt can have a harsh flavor, too little salt will also negatively impact consumer acceptability (Martin, 2001). Salt is considered as a self limiting ingredient as over use will result in an unpalatable product.

Historically, the salting of meat not only provided a desiccation action but effectively increased ionic strength and decreased water activity. These conditions play an important role in salt's antimicrobial activity in processed meats. Salt does lower the water activity of a product but this does not fully explain the preservative affect (Jay, 2000; Sperber & Peck, 1983). In order to inhibit pathogen growth by lowering water activity alone, 9 to 11% salt would need to be added (Aberle et al., 2001). Salt content in meat products is lower than these inhibitory levels and but some preservative effect is maintained. Chloride toxicity has been suggested to explain this extra control (Taormina, 2010). In combination with other ingredients, treatments, and storage, salt can serve as a hurdle in bacterial inhibition (Doyle & Glass, 2010). Still, not all bacteria are equally sensitive to salt. *Campylobacter* species have optimal growth conditions with 0.5% salt content while *Staphylococcus aureus* can grow with greater than 20% salt present (Doyle & Glass, 2010). Decreased salt in products will generally result in increased bacterial growth and reduced shelf life.

Nitrite and salt are essential in meat curing and provide synergistic effects for bacterial control and development of cured meat characteristics. Nitrosyl chloride, a strong nitrosating agent, is formed from the reaction of acids with nitrite. However, dinitrogen tetraoxide, N_2O_4, or nitric oxide can replace the acid during nitrosyl chloride formation (Beckham, Fessler & Kise, 1951). Sebranek and Fox (1991) found that chloride ions increased the rate of nitric oxide formation during curing. These reactions can be further accelerated with increased acidity. The addition of 0.5% or more salt has also been shown to decrease N-nitrosamine formation (Theiler et al., 1981).

Along with all the positive roles of salt in meat processing salt also acts as a prooxidant. The addition of salt will increase the rate of metmyoglobin formation (Chem,

Huffman, Egbert & Smith, 1992) and increase lipid oxidation in meat products (Andersen & Skibsted, 1991). While salt alone will promote oxidation, trace contamination with metal ions can further increase the rate of oxidation (Townsend & Olson, 1987). This could be of concern in the use of sea salts. It is important to use high purity salt to limit this effect.

Sweeteners

Many different ingredients can be used as sweeteners and the type used impacts flavor, color characteristics, and microbial growth. Sugar, brown sugar, dextrose, and corn syrup are commonly used (Martin, 2001) but honey, maple syrup, and molasses can be used to provide specific flavor characteristics (Pearson & Gillett, 1999). The primary role of sweeteners in processed meats is to counteract and balance the harsh flavor of salt (Townsend & Olson, 1987). Additionally, flavor and color is impacted the Maillard browning reaction of sugars during thermal processing (Pearson & Gillett, 1999). Different sugars have different levels sweetness and browning properties.

Sugars can lower water activity and are used during the production of jams and preserves. Antibacterial effects of sugars are similar to salt and increase ionic strength and decrease water activity (Jay, 2000). However the concentrations of sugars used in meat products are too low to provide much impact (Pearson & Gillett, 1999). Simple sugars provide an energy source for bacterial fermentation and lactic acid production. Sugars can increase moisture retention in meat products and corn syrup, only about 40% as sweet as sucrose, is commonly added for this purpose. Previously, corn syrup addition was limited (Pearson & Gillett, 1999). As with salt, sugars and sweeteners are considered self limiting and no regulations exist for levels of addition.

Sodium Phosphates

German meat processors began using phosphates to increase moisture retention in sausage products before research began investigating the swelling properties of sodium phosphates (Bendall, 1954). Phosphates vary in chain length and properties and are applied for different functions depending upon type of processed meat. The primary function of alkaline phosphates is to increase water binding and retention in processed meats. Bendall (1954) explained that meat with added phosphate would swell due to cleaving of the actomyosin bond. This, in addition to increased pH of alkaline phosphates, allows for greater moisture absorption and retention in brine cured products. In comminuted meats, increased protein extraction allows for greater moisture and fat binding and creates a more stable emulsion (Townsend & Olson, 1987). Alkaline phosphates can retard cured color development due to increased pH, while acid phosphates increase color formation but have less effect on water retention (Pearson & Gillett, 1999). The slightly acidic pH environment created with acid phosphates increases the rate of nitric oxide formation and explains the increased rate of color formation (Pegg & Shahidi, 2000).

Phosphates have the capability to form complexes with many metal ions found in meat products (Wazer & Campanella, 1950). Many of these metal ions including iron and copper are capable of promoting oxidation and phosphates serve as a secondary antioxidant by sequestering these molecules (Aberle et al., 2001). The chelation of metal ions essential for bacterial growth has been identified as a possible antimicrobial property of phosphates (Elliott, Straka & Garibaldi, 1964).

The addition of sodium phosphates is limited to 0.5% in the finished product (USDA, 1995). When high levels of phosphates are used, products can have a rubbery texture and

soapy off-flavor (Pearson & Gillett, 1999). Sodium phosphates are slow to dissolve and must be added to the brine first to ensure full solubility and functionality. Hard water will decrease the solubility. Phosphates can recrystalize in meat products if not properly dissolved resulting in whiskers or glass-like crystals (Townsend & Olson, 1987).

Spices

As technologies improved, processing and storage conditions reduced bacterial growth and meat processors were able to lessen the amount of salt added to products. This allowed greater diversity of flavors and subtle use of spices because salt did not overpower the other flavors (Aberle et al., 2001). Flavor is the primary purpose of spices and herbs, however some have been shown to have antimicrobial properties (Tajkarimi, Ibrahim & Cliver, 2010; Tiwari et al., 2009). Similarly, many spices and extracts contain phenolic compounds that have been shown to have antioxidant activity (Sasse, Colindres & Brewer, 2009). Rosemary extract is a commonly used antioxidant in the food industry. It is unlikely that without the use of extractives, that the levels of spices used in meat products can provide significant reduction of oxidation or microbial growth. As with salt and sugar, most spices are considered self-limiting from a regulatory standpoint.

Smoke

Smoked meats probably originated when nomadic tribes dried meat next to fires (Townsend & Olson, 1987). Smoke is composed of many compounds including phenols, alcohols, organic acids, carbonyls, hydrocarbons, and gasses and is contributes to product flavor, aroma, color, antimicrobial activity, antioxidant activity, and skin formation (Aberle et al., 2001; Pearson & Gillett, 1999). Natural smoke is produced by heating moist sawdust

at a smoldering temperature. In liquid smoke, the smoke compounds are collected and concentrated and can be applied to products internally or externally.

Color and flavor characteristics are the most important results of smoke applications. The mahogany color of smoke is produced by the Maillard reaction of smoke carbonyls, exterior proteins, and the cured meat pigment (Ellis, 2001; Pearson & Gillett, 1999). Temperature, moisture, and humidity all have large impacts on the color deposition. Phenolic compounds react with sulfhydryl groups and short chain carbonyls react with amino acids to provide much of the smoked flavor characteristics (Ellis, 2001; Pearson & Gillett, 1999). Flavor thresholds of many major smoke compounds are low and range from 90 ppb to 1.85 ppm (Wasserman, 1966). Alcohols from smoke are oxidized to organic acids and responsible for the protein skin formation on the exterior of products (Rust, 1987). Phenols and other aromatic compounds in smoke have antioxidant properties due to their ability to donate electrons (Soldera, Sebastianutto & Bortolomeazzi, 2008). Many of smoke compounds also have bacteriostatic or bacteriocidal properties (Aberle et al., 2001) and formaldehyde and phenolic compounds are thought to provide much of this effect (Ellis, 2001; Urbain & Campbell, 1987).

Antimicrobial Ingredients and Processes

Many compounds are added to cured meats for their antimicrobial activity. Organic acids are among the most common and effective and are often added as sodium or potassium salts (Theron & Lues, 2007). Although many organic acids have antimicrobial properties, the combination of lactate and diacetate is most commonly used in the meat industry. Organic acids effectively inhibiting many pathogens but *Listeria monocytogenes* control is of

greatest concern due to high mortality rates and the capability of this organism to induce miscarriages (FDA, 2003; Swaminathan, 2001). Thermal processing effectively decreases bacterial and pathogen load but contamination post-processing is of concern in ready-to-eat products since they are not reheated. Due to this, the USDA has a zero-tolerance for *L. monocytogenes* for these products (USDA, 2010a). As part of the regulation, processors have stringent *L. monocytogenes* environmental and product testing requirements unless they utilize an antimicrobial, a post thermal processing treatment or the combination of both. Organic acids are commonly used to meet the antimicrobial requirement. The use of organic acids is also effective in extending the shelf life by delaying the growth of spoilage organisms.

Other ingredients, processing treatments, and packaging have been investigated for bacterial inhibition. Many natural antimicrobials have been identified but are not as commonly used or effective as organic acids (Beuchat, 2007; Naidu, 2000) when applied in concentrations that do not negatively impact product quality. Processing and packaging technology can be applied to provide bacterial inhibition. In addition to limiting product quality deterioration, packaging in an anaerobic environment limits some bacterial growth (Jay, 2000). Some packaging manufactures have begun impregnating films with known antimicrobials to increase product shelf life (Vartiainen, Skytta, Enqvist & Ahvenainen, 2003). High pressure processing is increasing in use as an effective method to increase product safety and extend shelf life without the addition of other ingredients (Rendueles et al., 2011).

Nitrite Reducing Agents

Sodium ascorbate or erythorbate are commonly used in cured meat systems to increase the rate and extent of curing. Sodium ascorbate and erythorbate are isomers and provide the same function and activity in cured meats (Pearson & Gillett, 1999). Ascorbic or erythorbic acid can also be used (USDA, 1995) but are not as common as the sodium salts due to decreased product pH. Since the 1950's, ascorbic acid or ascorbate have been used to increase the rate and extent of cured color development (Watts & Lehmann, 1952) by increasing the rate of nitric oxide production and nitrosylmyoglobin formation (Fox & Ackerman, 1968; Fox, Sebranek & Phillips, 1994). Excess ascorbate serves as an antioxidant, decreases lipid oxidation, and increases stability of cured meat color (Pearson & Gillett, 1999). It has been suggested that ascorbate can increase the antimicrobial effectiveness of other organic acids (Golden, Buchanan & Whiting, 1995). N-nitrosamine formation has been shown to be decreased or inhibited by ascorbate or erythorbate (Mirvish et al., 1972; Theiler et al., 1981). Sodium ascorbate and erythorbate are limited to 550 ppm and are required at that concentration for bacon (USDA, 1995, 2010c).

Similar to the increased cured color found with the addition of acid phosphates, glucono delta lactone (GLD) lowers brine pH and increases the rate of the curing reaction (Fox, 1987). GLD is limited to 0.5% of green weight (United States Department of Agriculture (USDA), 1995).

Nitrosation and Nitrosylation Reactions in Cured Meats

Nitrite Reduction and Nitrosation/Nitrosylation Agents in Cured Meats

While nitrate was originally used in curing meats, it was recognized in the 1890's that nitrate must first be reduced to nitrite to act as a curing agent (Lewis et al., 1925). In the

1920's, several bacteria species with nitrate reductase capacity were isolated and identified (Harrison, 1929). However, in distilled water, nitrite is stable and has little nitrosating/nitrosylating capabilities (Williams, 1988). In order for nitrosation/nitrosylation to occur, nitrite must be reduced to nitric oxide or form intermediates with other compounds. The most effective method of increasing nitrosating/nitrosylating activity is through acidification. The nitrite ion and nitrous acid form in equilibrium and acidic conditions shift this toward nitrous acid (Fox, 1987). Through dehydrolysis and the intermediary compound dinitrogen trioxide, N_2O_3, nitric oxide, nitrite, and nitrous acid are found in equilibrium (Honikel, 2004). In a meat system, lower muscle pH resulted in decreases residual nitrite in finished products as a result of the acid induced shift in equilibrium (Lee, Cassens & Fennema, 1976).

Many compounds are able to reduce nitrite. Sodium erythorbate and sodium ascorbate are commonly added to increase the rate and extent curing reaction. Ascorbate or erythorbate function by quickly forming a nitrosated intermediate which decomposes to release nitric oxide (Fox & Thomson, 1963). Halide salts, like sodium chloride, can form nitrosyl halides and have been shown to increase the rate of nitric oxide formation in curing systems (Fox et al., 1994; Sebranek & Fox, 1991).

Muscle tissue contains endogenous compounds capable of nitrite reduction (Walters & Taylor, 1964). Myoglobin and hemoglobin are capable of reducing nitrite through the oxidation of the heme iron (Brooks, 1937; Doyle, Pickering, DeWeert, Hoekstra & Pater, 1981; Shiva et al., 2007). Cysteine and other sulfhydryl compounds can form S-nitrosothiols in aqueous solutions (Miersch & Mutus, 2005) and can serve as nitrosating agents or can release nitric oxide (Williams, 1999). It has been suggested that mitochondria may reduce

nitrite (Walters & Taylor, 1965). However, while some enzymes were inactivated, the addition of mitochondria did not function as reducing agents affecting nitrosylation of myoglobin (Fox & Ackerman, 1968).

Nitrite concentrations decline in meat mixtures during processing. Many factors influence the rate of nitrite reduction in meat. Greenwood (1940) proposed six factors that influence nitrite loss:

1. Time and temperature employed during processing
2. Amount of protein, fat, and carbohydrate
3. Concentration of salt
4. Concentration of nitrate and nitrite
5. Number of microorganisms
6. Acidity

Inclusion of reducing agents began after Greenwood proposed the above factors and also impacts nitrite reduction and residual nitrite concentration. Residual nitrite concentrations also decline over time during storage (Pérez-Rodríguez, Bosch-Bosch & Garciá-Mata, 1996, 1997). It is difficult to determine ingoing nitrite from residual nitrite but it has been estimated nitrite concentration decreases by 50-65% during production and thermal processing (Greenwood, 1940; Honikel, 2008). The decline in nitrite that occurs during meat curing is due to nitrate formation, nitrosation/nitrosylation reactions, and loss of dinitrogen and nitric oxide gases (Cassens, Greaser, Ito & Lee, 1979; Cassens, Ito, Lee & Buege, 1978; Cassens, Izumi, Lee, Greaser M & Lozano, 1981; Honikel, 2008). Reducing compounds, endogenous or added, and physiochemical properties such as pH all influence the curing reaction. Due to differing activity of nitrosation/nitrosylation compounds, it is possible that reducing agents could alter the reactions that occur during curing (Sebranek & Fox, 1985).

Cured Meat Color

The formation of cured color is the most understood reaction in meat curing. Research of reaction of nitrite and heme pigments has been conducted since the 1860's (Gamgee, 1868) and nitric oxide hemochromogen was identified as the cured pigment at the turn of the 20th century (Haldane, 1901). Over the next several decades, the mechanisms of the redox reaction of myoglobin and nitrite or nitric oxide were described (Greenwood, Griffin & Lewis, 1939). A stepwise mechanism has been proposed for cured color development. Myoglobin reacts with nitrite to form metmyoglobin and nitric oxide which then react to form nitrosylmetmyoglobin (Giddings, 1977; Killday, Tempesta, Bailey & Metral, 1988). Scientists debate whether cured meat pigment is found as mononitrosylhemochromogen or dinitrosylhemochromogen (Cornforth, 1996; Pegg, Shahidi & Fox, 1997). Reducing agents cysteine, NADH, and ascorbate/erythorbate reduce the heme iron to form nitrosylmyoglobin (Fox & Ackerman, 1968). It has been suggested that NADH plays a limited role in metmyoglobin reductase activity because it is rapidly depleted postmortem (Madhavi & Carpenter, 1993). However, the addition of sodium lactate can activate lactate dehydrogenate and reduce NAD^+ to reform NADH (McClure, 2009). Following thermal processing, the globin protein is denatured forming nitrosylhemochromogen.

Increased salt content has been shown to increase the rate of nitrosylmyoglobin formation (Fox et al., 1994; Sebranek & Fox, 1991). The addition of S-nitrosocysteine to meat can produce typical cure color and other cured meat characteristics suggesting its role as an intermediary nitrosylating/nitrosating compound (Kanner & Juven, 1980). Satisfactory and stable cured color development can be achieved with the addition of 40 ppm of sodium

nitrite (USDA, 1995). Exposure to oxygen and light results in cured color fading (Andersen, Bertelsen, Boegh-Soerensen, Shek & Skibsted, 1988) although the presence of sufficient residual nitrite and reducing compounds slows this process.

Flavor and Aroma

The reactions responsible for cured meat flavor and aroma are not fully understood but it is thought to be primarily related to the limited formation of oxidation products. Cured meat products have fewer volatile compounds than their equivalent uncured cooked meats (Shahidi, Rubin, D'Souza, Teranishi & Buttery, 1986). Fewer hydrocarbons, ketone, alcohols, phenols, esters, furans pyrazines, aldehydes and other nitrogen containing compounds, and increased carboxylic acids, sulfur, and nitrite/nitrate containing compounds were found in cured versus uncured meat (Shahidi et al., 1986; Ramarathnam, Rubin & Diosady, 1993). Less than half of the total volatile compounds were found in cured meat products and much of the difference is thought to be due to the limited formation of lipid oxidation byproducts. Alcohols and phenols all undergo nitrosation reactions and also could impact volatile compounds. Increases in sulfur compounds are likely due to S-nitrosothiol formation and reduction to disulfide bonds during meat curing. The antioxidant role of nitrite, discussed in the next section, explains the reduction of oxidation products such as hexanal in cured meats (Ramarathnam, Rubin & Diosady, 1993). Although variations in sulfur compounds may be a result of nitrite, it seems more likely that cured flavor is a result of inhibiting formation of many volatiles. Further work needs to be conducted to more fully understand the reactions and volatiles responsible for cured meat flavor and aroma.

Antioxidant Activity

The increased oxidative stability of cured meats has been well established. Many reactions that take place in cured meats can extend product shelf life. Lipid oxidation can be initiated by many methods and once started, it exponentially increases by free radical reactions (Wong, 1989). Oxygen and other reactive oxygen species rapidly react with, and are sequestered by, nitric oxide (Ford & Lorkovic, 2002). Nitric oxide, as a free radical, can also terminate lipid autooxidation (Miranda et al., 2000; Pegg & Shahidi, 2000). Nitric oxide binds free iron and stabilizes heme iron (Bergamaschi, 2009) which can reduce lipid oxidation by limiting prooxidant activity of the iron. Unsaturated fatty acids are targets of lipid oxidation and the nitrosation of double bonds also could decrease lipid oxidation. The addition of 50 ppm of sodium nitrite has been shown to reduce lipid oxidation products by nearly 65% (Sato & Hegarty, 1971).

Antimicrobial Activity

Greater nitrite is required for antimicrobial activity than to provide other cured meat characteristics. Nitrite has the unique ability to inhibit outgrowth of *Clostridium botulinum* spores and historically has been the primary pathogen of investigation in studying nitrite's antimicrobial impact. More recently, *Listeria monocytogenes* has been of concern in ready-to-eat meats due to ability to grow in high salt and at refrigerated temperature environments (Swaminathan, 2001). In addition to nitrite, many nitric oxide-donating compounds have been studied and have shown antimicrobial activity similar to that found in cured meats (Cammack et al., 1999; Kanner & Juven, 1980). It has been proposed that nitrite targets bacteria at multiple sites by inhibiting metabolic enzymes, breaking the proton gradient, and limiting oxygen uptake (Yarbrough, Rake & Eagon, 1980).

Synthetic iron-sulfur complexes react with nitric oxide and form complexes similar to Roussin's black and red salts (Harrop et al., 2008). Ferredoxin and pyruvate:ferredoxin oxidoreductase are inactivated by nitrosylation of the iron sulfur complexes (Payne, Glidewell & Cammack, 1990; Rahman, 2007). Cytochrome-c is a heme centered protein that transports electrons from complex III to IV and is inhibited with the addition of nitrite (Walters & Taylor, 1964). Nitric oxide binding to iron regulates and limits iron availability which is necessary for enzyme functionality and bacterial metabolism and growth (Pantopoulos & Hentze, 2000; Tompkin, 2005). Due to high reactivity of iron and nitrite, iron sulfur complexes and heme iron centers of enzymes are often the targets of nitrite (Cui, Joannou, Hughes & Cammack, 1992).

Cysteine is found in many enzymatic processes and signaling pathways (Gaston, 1999) and is thought to be another target of nitrite for inhibitory effects. Nitrosation of membrane sulfhydryl groups likely breaks the signaling pathway and the ability of the cell to react to external environment (Morris, Walsh & Hansen, 1984). Complex I of the mitochondria is the entry point of the electron transport chain and is inhibited by s-nitrosation (Shiva, 2010). Inactivation of the electron transport chain, the more efficient ATP production pathway, is suggested by the accumulation of pyruvate in bacteria grown in the presence of nitrite (Woods, Wood & Gibbs, 1981). Others found decreases in bacterial glycolytic enzymes that attributed to sulfhydryl group nitrosation (O'Leary & Solberg, 1976). These complexes are found in, and explain the inhibition of, *Clostridia*, *Listeria*, some *Staphylococcus aureus*, *Escherichia*, and some *Pediococcus* species by nitrite (Rahman, 2007; Tompkin, 2005). *Salmonella* species and most lactic acid producing bacteria are

among the bacteria that do not contain these complexes and are not inhibited by nitrite (Jay, 2000).

Many ingredients used in processed meats have synergistic antimicrobial effects with nitrite. Increased ingoing nitrite has shown to increase the antimicrobial activity of organic acids (Qvist & Bernbom, 2000). Similarly, lower levels of sodium chloride are needed for inhibition of *Clostridium botulinum* toxin production when nitrite is added (Tompkin, 2005). Anaerobic and more acidic environments increase the antimicrobial effectiveness of nitrite (Rahman, 2007). Tompkin (2005) identified many factors that impact the antimicrobial activity of nitrite and effect product safety and shelf life:

1. pH of the product during abuse
2. Injection level
3. Residual nitrite at point of abuse and the rate of depletion during abuse
4. Amount of viable botulinal spores and vegetative cells at the time of abuse
5. Temperature of abuse
6. Concentration of ascorbate or isoascorbate
7. Concentration of "available" iron in the product
8. Type of meat and other formulation ingredients
9. The thermal process applied to the product
10. The growth of competitive flora
11. The concentration and type of phosphate may play a role

Nitrogen Oxide Compounds and Human Health

Historically nitrogen oxide compounds were considered healthful compounds. A 4th century Chinese manuscript contained a description of using potassium nitrate as a remedy for chest pains (Lundberg et al., 2008). In the west, nitrate was recognized as medicine during the 12th century (L'hirondel & L'hirondel, 2001). Uses of nitrates for medicinal purposes declined through the 20th century but were never completely abandoned. Medicinal

value of nitroglycerine was identified in the mid 19th century and is used to this day as a nitric oxide donor to ease angina (Marsh & Marsh, 2000).

During the middle of the 20th century, the reputation of nitrates and nitrites was damaged by several findings. In 1945, Comly (1945) identified well water contaminated with nitrate and bacterial as the cause of cyanosis in infants. Other similar incidents were reported and led to regulations on allowable nitrate in drinking water. However, it is often forgotten that without bacterial reduction, nitrate has little biological function and now it has been suggested that it is inappropriate to link nitrate concentration of drinking water and cyanosis (Fewtrell, 2004). Nitrogen oxides were further vilified over the next few decades. In 1952, nitric oxide and nitrogen dioxide were identified as a component of photochemical smog (Butler & Nicholson, 2003). N-nitrosamines were identified as carcinogenic compounds (Magee & Barnes, 1956) and later, these compounds were found to be the causative agent of hepatotoxic liver disease in animals fed nitrite cured herring meal (Ender et al., 1964; Koppang, 1964).

In 1978 the United States Department of Agriculture (USDA) and Food and Drug Administration (FDA) announced nitrite would be banned in food production following a study that found dietary nitrite increased lymphatic cancer in rats (Laub, 1980). This study was later discredited and continued used of nitrite was allowed. The National Academies of Sciences (1981; 1982) determined that nitrite and nitrate were not carcinogen compounds and no other alternatives existed for their replacement. Still, in order to limit N-nitrosamine formation during bacon frying, processing regulations were altered (USDA, 2010c). A trend of decreased residual nitrite has been found across many cured meat products since the 1970's (Sen & Baddoo, 1997).

The Delaney Clause of the 1958 Food Additives amendment to the 1938 Food, Drug, and Cosmetics Act disallowed the use of any carcinogenic compounds in food, drugs, or cosmetics. The prior sanctions provision allowed the continued use of ingredients in concentrations, in conditions, and in products for which they were approved for prior to 1958 (FDA, 2010). A public interest group sued the USDA citing that the continued use of nitrate or nitrite violated the Delaney Clause because nitrite regulations for bacon processing had been changed and the prior exemption no longer applied (Public Citizen v. Foreman, 1980). The District of Columbia Court of Appeals found that the USDA acted lawfully and allowed the continued use of nitrite in bacon.

While there has been no direct link to cured meats, many researchers have suggested, and some epidemiological studies have indicated a link with nitrite to several forms of cancer (Demeyer & De Smet, 2010; Ferrucci et al., 2010; Santarelli, Pierre & Corpet, 2008). Much of the epidemiologic data suggests that increases in cancer incidence are below levels generally used to draw epidemiological conclusions. The lack of direct evidence was further supported by a recent study that investigated male and female rats and mice over 14 weeks or 2 year period. Sodium nitrite in drinking water at 1500 ppm or less had no carcinogenic effects within any part of the study (National Toxicology Program, 2001). Additionally, the animals with sodium nitrite in their drinking water had decreased incidences of mononuclear cell leukemia. This is further evidence discrediting the claim that consuming of nitrate, nitrite, or cured meats is deleterious to health.

Still, concerns about nitrite and nitrate persist. Saliva and vegetables provide the greatest amount of ingested nitrate and nitrite daily, while cured meats provide 9.4 and 21.2% of daily nitrate and nitrite consumption, respectively (White, 1975, 1976). Due to

concerns about nitrates, the European Food Safety Authority established limits of nitrate in vegetables (EFSA, 2008). Current recommends suggest intake should be less than 0.07 and 5 mg/kg of body weight for nitrite and nitrate, respectively (FAO/WHO ECFA, 2002).

Over the past three decades, many biological functions of nitrogen oxide compounds have been identified. In the 1980's, it was discovered that nitric oxide was produced endogenously and was a bioregulatory molecule (Ignarro, 2000; Ignarro, Buga, Wood, Byrns & Chaudhuri, 1987). It was recognized as the 'Molecule of the Year' by *Science* in 1992 (Koshland, 1992) and the researchers who discovered endogenous nitric oxide were awarded the Nobel Prize in 1998 (Marsh & Marsh, 2000). Three isoforms of nitric oxide synthases are present in humans (Ignarro, 2000). Bioregulatory roles of nitric oxide are involved in vasodilation, erectile dysfunction, platelet aggregation, enzyme regulation, antioxidant activity, iron regulation, immune response, metabolic regulation, apoptosis, and likely many others (Albina & Reichner, 2000; Darley-Usmar, Patel, O'Donnell & Freeman, 2000; Ignarro, 2000; Koshland, 1992; Pantopoulos & Hentze, 2000; Shiva, 2010). Many nitric oxide donors are being investigated for therapeutic roles (Butler & Feelisch, 2008)

Even with the ever expanding biological role of nitric oxide, many consider consumption of nitrite and nitrate to have deleterious effects. In a review, Lundberg and others (2008) began by stating:

> "Nitrite (NO_2^-) and nitrate (NO_3^-) are known predominantly as undesired residues in the food chain with potentially carcinogenic effects, or as inert oxidative end products of endogenous nitric oxide metabolism."

The role of dietary nitrite and nitrate has been reviewed by many (Addiscott & Benjamin, 2004; Gilchrist, Winyard & Benjamin, 2010; Hord, Tang & Bryan, 2009; L'hirondel, Avery & Addiscott, 2006; McKnight, Duncan, Leifert & Golden, 1999; Milkowski, Garg, Coughlin

& Bryan, 2010; Nair, Irving & Lanza, 2011; Walker, 1996) and has been shown that nitrite and nitrate provide nitric oxide reserves. Nitrate is concentrated in the saliva and bacterial reduction occurs in the oral cavity (Tannenbaum, Sinskey, Weisman & Bishop, 1974). Upon entering the acidic gastric juices, nitric oxide is readily produced and absorbed. Circulating plasma nitrite is reduced by myoglobin in times of anoxia for a cytoprotective effect (Hendgen-Cotta et al., 2008; Shiva et al., 2007) and it has been proposed that dietary nitrates can improve mitochondrial efficiency (Nair et al., 2011). Dietary nitrite and nitrate have been shown to provide nitric oxide homeostasis in nitric oxide synthase deficient animals (Bryan, Calvert, Gundewar & Lefer, 2008; Carlstrom et al., 2010). Research has shown numerous benefits and biological functions of dietary nitrite and nitrate. Lundberg and others (2008) end their review by stating:

> "We must now revise our long-standing view that nitrate and nitrite are only harmful substances in our diet or inert metabolites of endogenous nitric oxide. Instead, accumulating evidence suggests that the nitrate–nitrite–nitric oxide pathway critically subserves physiological hypoxic nitric oxide signalling, providing an opportunity for novel nitric oxide-based therapeutics."

Naturally Cured Meats

Production and Regulations

The USDA's standards of identity define traditional product characteristics and regulate common names. To address the public concerns about nitrite in the 1970's, some processors began eliminating sodium nitrite and nitrate from traditionally cured products such as frankfurters. These products would be produced with similar ingredients and processes to cured products, but no nitrite would be added. They were simply not cured

(Forest, 1979). To address the category of products, the USDA (2010b; 2010d) allowed for products:

> " which nitrate or nitrite is permitted or required to be added may be prepared without nitrate or nitrite and labeled with such standard name when immediately preceded with the term 'Uncured' in the same size and style of lettering as the rest of the standard name" and "shall bear the statement 'No Nitrate or Nitrite Added.'"

USDA authority to approve 'No Nitrate or Nitrite' labels has been upheld in the U.S. supreme court (Ensminger, Ensminger, Konlande & Robson, 1995). Much work has been conducted to find a substitute for nitrite but no single ingredient can replace all functions of nitrite (Pegg & Shahidi, 2000).

The natural and organic food markets have undergone rapid growth over the past two decades. In 2005, organic food sales reached $13.8 billion (Enis, 2010) and the natural foods category is expected to reach $30 billion in sales by 2014 (Nunes, 2011). The growth varies across products but one brand of natural ham has experienced 16% increase in annual sales since its release (Nunes, 2011). The persistent consumer concern about nitrite and nitrate are among those that have lead to the large growth of these sectors. Consumers of these categories have indicated a perceived health benefit as a primary motivation for purchase (Hughner, McDonagh, Prothero, Shultz II & Stanton, 2007; Magnusson, Arvola, Hursti, Åberg & Sjödén, 2003; Organic Trade Association, 2009) although published research does not suggest differences in natural or organic and conventional foods (Dangour et al., 2010).

In the Organic Food Protection Act of 1990, the USDA established acceptable and prohibited production practices, food ingredients and labeling policy for organic foods. Natural food regulations have similar restrictions on ingredients as organic food products, but

do not address production practices. The USDA allows meat and poultry products to be labeled as natural by meeting the following definition:

> "(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. Minimal processing may include: (a) those traditional processes used to make food edible or to preserve it or to make it safe for human consumption, e.g., smoking, roasting, freezing, drying, and fermenting, or (b) those physical processes which do not fundamentally alter the raw product and/or which only separate a whole, intact food into component parts." (USDA, 2005)

Nitrites and nitrates are classified as chemical preservative under this definition. Also, most commonly used antimicrobials, sodium phosphates, ascorbate, and erythorbate are not allowed in natural and organic products. Lactate, from a natural source, can be added at up to 2% level but must be applied for on an individual basis for use in natural products (USDA, 2005).

As stated earlier, nitrite is an essential ingredient in cured meats and by the definition of natural food, no traditionally cured meat product could be labeled as such. However, several natural meat products are being marketed that have physiochemical characteristics of cured meats but are made without the addition of sodium nitrite or nitrate (Sindelar, Cordray, Olson, Sebranek & Love, 2007a). Unlike products originally designated by the 'Uncured' label definition, chemical analysis revealed that these products contained residual nitrite (Sindelar et al., 2007a). Yet, these products must be labeled as "Uncured" and "No Nitrate or Nitrite Added" since no sodium nitrite or nitrate was added directly. Processors add celery juice/powder or other ingredients high in nitrate and nitrate reducing starter culture, to produce nitrite to naturally cure the meats (Terns et al., 2011a). These products have been shown to have similar sensory characteristics as traditionally cured meats (Sindelar, Cordray,

Sebranek, Love & Ahn, 2007b, c). The naturally curing meat utilizes technologies similar to those used prior to the approval of sodium nitrite in1926. To further add to the controversy, some ingredient manufactures have begun pre-converting celery juice with a bacterial reduction of nitrate prior to drying. This provides a natural ingredient already containing nitrite and allows natural meat processors to increase throughput and consistency by eliminating the need for the variable bacterial nitrate reduction step.

Challenges Associated with Naturally Cured Meats

Many ingredients commonly used in processed meats are prohibited in natural and organic meat products and ingoing nitrite is typically lower in naturally cured meats than in conventionally cured products (Sebranek & Bacus, 2007). The USDA (1995) identifies 120 ppm of ingoing nitrite as a necessary concentration to provide pathogen control but these concentrations are difficult to achieve in natural cured meats. Since cured meat color, flavor, and antioxidant characteristics are developed at lower concentrations of ingoing nitrite than necessary for antimicrobial activity, pathogen growth could occur if consumers handle these products in a similar manner as conventionally cured meats. Furthermore, during bacterial reduction of nitrate, nitrite is slowly added to the system and it has been suggested that this could alter nitrosation/nitrosylation reaction that occur in cured meats (Sebranek & Bacus, 2007). These variations in addition to the prohibited use of many antimicrobials typically used in cured meats may further limit pathogen control in these products. While some differences may exist in product quality in the naturally cured meats, safety is of greater concern.

Some natural ingredients have been identified to replace the prohibited ingredients as a method to maintain product quality and safety. Some plant-based products have been shown to have antimicrobial effects in a cured meat system (Xi, Sullivan, Jackson, Zhou & Sebranek, 2011) but addition at minimum inhibitory concentrations may impact product quality. Cherry powder, high in ascorbic acid, has been shown to reduce residual nitrite and could serve as a natural alternative to sodium ascorbate (Terns, Milkowski, Rankin & Sindelar, 2011b). No replacement for sodium phosphate has been identified but this compound primarily impacts product quality rather than safety.

In addition to lower ingoing nitrite in the naturally cured meats, nitrite is slowly formed from bacterial reduction of nitrate. It has been proposed that the slow rate of nitrite addition could shift nitrosylation/nitrosation reactions (Sebranek & Bacus, 2007). When nitrite is added at one time like traditional cured meats, it may result in a more heterogeneous mixture of nitrosated/nitrosylated compounds where slower rate of addition may result in more homogenous nitrite reaction products. Limited use of reducing compounds could also alter which compounds are nitrosylated/nitrosated (Sebranek & Fox, 1985).

Meat is a complex system that makes measuring all reactions difficult. Early work used ^{15}N isotopes to determine the fate of nitrite in cured meats (Sebranek et al., 1973; Woolford & Cassens, 1977; Woolford, Cassens, Greaser & Sebranek, 1976). Creating a model system could provide a simplified method to determine nitrite reactions associated with different curing systems. During curing, myoglobin and cysteine are known to undergo nitrosation/nitrosylation (Pegg & Shahidi, 2000). These compounds can be tracked and could provide insight into about possible changes in nitrosated/nitrosylated compounds in curing systems.

The natural and organic meats category is rapidly growing and these products are readily found in the marketplace. Some work has been conducted on product quality but little has been done investigating safety of these products. The objectives of the following studies are to:

1) Determine differences in physio-chemical characteristics of naturally and conventionally cured commercial products that impact pathogen growth.
2) Evaluate the effect of natural antimicrobials on *Listeria monocytogenes* growth and product quality in naturally cured ham.
3) Determine effect of natural or conventional curing system on nitrite reactions in a myoglobin and cysteine model system.

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CHAPTER 3. COMPARIONS OF COMMERCIALLY AVAILABLE NATURALLY AND CONVENTIONALLY CURED FRANKFURTERS, HAM, AND BACON FOR PHYSIO-CHEMICAL CHARACTERISTS THAT AFFECT BACTERIAL GROWTH

A paper to be submitted to Meat Science

Gary A. Sullivan, Armitra L. Jackson, Kohl D. Schrader, Yuan Xi, Joseph G. Sebranek, James S. Dickson

Abstract

Natural and organic food regulations limit the use of sodium nitrite/nitrate and other antimicrobials. Consequently, processors began to use natural nitrate/nitrite sources to manufacture products with cured meat characteristics but without sodium nitrite. The objective of this study was to compare physio-chemical characteristics that affect *C. perfringens* and *L. monocytogenes* growth in commercially available naturally and traditionally cured frankfurters, hams, and bacon. Generally, naturally cured frankfurters were leaner and contained less salt. Ham and bacon had fewer differences than frankfurters. Correlations of specific product characteristics to pathogen growth varied between products and pathogens though, water activity, salt, and product composition were common intrinsic factors correlated to pathogen growth across products. Other frequently correlated traits were related to curing reactions. Residual nitrite and nitrate were only significantly correlated to *C. perfringens* growth in ham. Many naturally cured products provide an environment susceptible to pathogen outbreak.

Keywords: naturally cured, frankfurters, ham, bacon, *Clostridium perfringens*, *Listeria monocytogenes*.

Introduction

Since 1990, the organic foods sector has had 20% annual growth and reached $13.8 billion in US sales in 2005 (Winter & Davis, 2006). Although consumer's finances have tightened during the recent recession, the organic sector still grew by 12% in 2009 and was boosted by increases in consumer purchasing of food to prepare at home (Enis, 2010). Organic food consumers often identify healthfulness, safety and perceived quality as major motivations to purchase organic foods while price, usually at a 20-30% premium, is the main deterrent (Enis, 2010; Hughner, McDonagh, Prothero, Shultz II & Stanton, 2007). The United States Department of Agriculture (USDA) defines acceptable and prohibited practices and ingredients in organic food production; first established in the Organic Foods Protection Act of 1990. Natural food products are defined by the United States Department of Agriculture (USDA, 2005) as those that are minimally processed and do not contain artificial flavor or flavoring, coloring ingredient, or chemical preservatives and follow many of the same regulations as organic products, but without the consideration for production practices.

In processed meats, one obstacle to producing natural or organic products is replacing sodium nitrite which is classified as a chemical preservative. Concerns about the safety of nitrite and nitrate began many years ago with reports of cyanosis caused by nitrate and bacterial-contaminated well water (Comly, 1945) and later with the possible formation of carcinogenic N-nitrosamine compounds in cured meats (Crosby, 1976). Since then, nitric oxide, nitrite and nitrate have been shown to have many important biological functions (Lundberg, Weitzberg & Gladwin, 2008). However, persistent consumers' concerns about the safety of nitrate, nitrite, and other chemical food additives have created a growing niche for "No Nitrate or Nitrite Added" "Uncured" processed meats. Sebranek and Bacus (2007)

described the production of these naturally cured products that use ingredients naturally high in nitrate and a starter culture with nitrate reductase activity to subsequently produce nitrite and cured meat products without directly adding sodium nitrite. Due to USDA regulations, these products must be labeled as 'Uncured' and must include "No Nitrate or Nitrite Added except those naturally occurring in … (list of natural sources of nitrate/nitrite)" on the label because no sodium nitrite is added (USDA, 2010c, d). In an effort to simplify production of naturally cured products, celery juice/powder suppliers began fermenting celery juice during production to provide celery juice containing nitrite without the need to ferment the meat product during production. These naturally cured products have been shown to have typical cured product characteristics (Sindelar, Cordray, Olson, Sebranek & Love, 2007), but often have much lower ingoing nitrite levels (Sebranek & Bacus, 2007). Lower ingoing nitrite and limited use of antimicrobials could result in increased pathogen and spoilage bacteria growth. The objective of this study, in conjunction with paired pathogen challenge studies, was to compare commercially available no-nitrate-or-nitrite-added naturally cured and traditionally cured processed meats for physio-chemical characteristics and determine which factors impact pathogen growth.

Materials and Methods

Experimental Design and Statistical Analysis

This study was conducted with paired pathogen challenge studies to evaluate commercially available naturally cured and traditionally cured frankfurters, hams and, bacon. The *C. perfringens* challenge group evaluated pathogen growth on frankfurters (n=12, 2 control brands), ham (n=11, 4 control brands) and bacon (n=10, 1 control brand) for 10 days. Three replicates were conducted. The *L. monocytogenes* challenge group evaluated growth

on frankfurters (n=10, 2 control brands) and ham (n=8, 3 control brands) for 35 days. Two replicates were conducted. Further information on methods used in bacterial challenge studies can be found in Jackson, Sullivan, Kulchaiyawat, Sebranek and Dickson (2011) for *C. perfringens* and Schrader (2010) for *L. monocytogenes*. Codes assigned to brands in this study match those used by Jackson and others (2011).

Data from the challenge studies were combined with the physio-chemical data reported here and evaluated for brand and where applicable day and day by brand interactions using PROC GLIMMIX procedure (SAS version 9.2, SAS Institute, Cary, NC). When significant brand, day or brand by day effects were found ($P < 0.05$), means separation was conducted using LSMEANS function with Tukey's honestly significant difference adjustment. Correlation of product physio-chemical characteristics on day 0 to mean pathogen growth over all sampling days was conducted using PROC CORR function of SAS.

Product Procurement, Preparation, and Analysis

Commercially available naturally cured brands and traditionally cured controls were identified and purchased at retail establishments and transported in coolers or purchased directly from distributors and shipped to the Iowa State University Meat Laboratory. Within a replication, all packages within a brand had the same use by/sell by dates. Packages were randomly assigned to pathogen challenge or analytical analysis. Packages were opened, separated into smaller quantities, and vacuum packaged. Packages were kept in dark storage in a walk-in cooler (4° C) until appropriate day of analysis. Product analysis first occurred on day 0, day of paired study sample inoculation, and then on future days corresponding with pathogen sampling. On days of analysis, samples were first analyzed for color, and then

homogenized using a food processor (Model KFP715, Kitchenaid, St. Joseph, MI) and stored in a covered insulated cooler on ice while conducting analyses.

Color Analysis

Color analysis, CIE L*, a*, and b*, was conducted using a Hunter LabScan XE (HunterLab, Reston, VA) using illuminant A, 10° observer. Color was measured using a 1.27 cm viewing port at three randomly selected locations for ham slices and for internal frankfurter color. Frankfurters were sliced longitudinally prior to measurement for internal color. Bacon color was evaluated using a 0.64 cm viewing port and two reading were conducted on each of the primary and secondary lean. Packages were opened immediately prior to reading and samples were covered in Saran plastic wrap (SC Johnson & Sons, Racine, WI) for evaluation. The colorimeter was calibrated using a standardized white tile, X = 80.45, Y = 85.37 and Z = 90.79 covered with the same plastic wrap. All samples were evaluated on day 0.

Water Activity

Water activity was measured on homogenized samples using a pa_wkit water activity meter (Decagon, Pullman, WA). Samples were placed in disposable sample cups, covered and allowed to equilibrate to room temperature (5-10 min) prior to reading. Two readings from each sample were taken. Calibration was performed using 0.25 and 0.76 sodium chloride water activity standards. Water activity was evaluated on days 0, 4 and 10 for the *C. perfringens* study and days 0, 7, 14, 21, 28, and 35 for the *L. monocytogenes* study.

pH

Product pH was measured by placing a pH probe directly into homogenized samples (FC200 ph probe, Hanna Instruments, Woonsocket, RI; Accumet 925 pH/ion meter, Fisher Scientific, Waltham, MA) after equilibration to room temperature. Calibration was conducted using phosphate buffers of pH 4.0 and 7.0. Duplicate reading were taken for each product and was evaluated on days 0, 4 and 10 for *C. perfringens* studies and days 0, 7, 14, 21, 28, and 35 for *L. monocytogenes* studies.

Residual Nitrite

Residual nitrite determination was conducted as described by AOAC method 973.31 (1990c). Samples were evaluated in duplicate on days 0, 4 and 10 for *C. perfringens* samples and days 0, 7, 14, 21, 28, and 35 for *L. monocytogenes* samples.

Residual Nitrate

Residual nitrate was determined using High Pressure Liquid Chromatography (Witter & Balish, 1979; Witter, Gatley & Balish, 1982) with modifications as described by Ahn and Mauer (1987). Samples were prepared in duplicate and evaluated on days 0, 4 and 10 for *C. perfringens* samples and days 0, 14, and 35 for *L. monocytogenes* samples.

Salt

Salt concentration was determined using Quantab high range chloride titrator strips (Hach Co., Loveland, CO.) as described by Sebranek, Lonergan, King-Brink, Larson and Beermann (2001). Chloride content was measured and used to calculate sodium chloride (salt) content. Samples were evaluated in duplicate on day 0 for all studies.

Total, Cured and Percent Cured Pigment

Homogenized samples were evaluated for nitrosylhemochromogen (cured pigment), and total pigment using acetone extraction modified from Hornsey (1956) as described by Sindelar et al. (2007). For bacon samples, lean portion of the slices was separated and used for analysis. Samples were prepared in duplicate and measured on day 0 for all studies. Percent cured meat pigment was calculated using the ratio of cured pigment: total pigment.

Proximate Analysis

Proximate analysis was conducted for moisture, fat, and protein using AOAC methods 950.46, 960.63, and 992.15, respectively (1990a; 1990b; 1993). Samples were prepared in duplicate and measured on day 0 for all studies.

Results and Discussion

Comparing commercial products is challenging due to variability in raw materials, formulation, processing, and time following production, but also is representation of retail products as experienced by consumers. More statistically significant differences were found between brands of frankfurters than ham and more in ham than in bacon, probably reflecting more variability in the products with less statistical significance detectable with greater variance. In general, frankfurters, as an emulsified product, resulted in greater homogeneity within brand than whole muscle products such as ham and bacon. Ham products fall into four different labeling categories: ham, ham with natural juices, ham water added, and ham and water product X percent of weight is added ingredients (United States Department of Agriculture (USDA), 2010a) and this may explain some of the variation observed among

products. Bacon had the greatest variability in measured traits partially due to inherent differences within and between bellies.

Frankfurter Physio-chemical Properties

Four brands of frankfurters were organic products, six were natural products, and two brands were traditionally cured controls. All brands (Table 1), except B and C, were all beef franks. Brand B contained beef and mechanically separated chicken and brand C was an all-turkey frankfurter. Of the 10 natural or organic brands, all were labeled uncured but only one, brand B, made no attempt to replace nitrate or nitrite with an alternative source to provide cured meat characteristics. Six brands' ingredient statements included lactic acid starter culture for nitrate reduction. It is likely that the remaining three brands, without lactic acid starter culture, used a 'pre-converted' celery juice/powder as a nitrite source. Three of the uncured brands contained sodium lactate. Producers using sodium or potassium lactate, from a natural source, and up to 2% level for flavoring, must petition the USDA for approval on a case-by-case basis for use in natural products (USDA, 2005). One brand included cherry powder, a source of natural ascorbic acid, which has been shown to decrease residual nitrite (Terns, Milkowski, Rankin & Sindelar, 2011). Both traditionally cured controls contained sodium nitrite, sodium diacetate, sodium or potassium lactate, sodium phosphates, and ascorbic acid (although sodium ascorbate or erythorbate are more commonly used).

Frankfurter physio-chemical properties are found in Tables 1 and 2. Little difference was observed for water activity as all brands measured 0.94-0.95, though statistical analysis showed both controls to be lower than brand B ($P < 0.05$). Frankfurter brand F had the greatest amount of residual nitrite, 60.1 ppm, while all remaining brands had residual nitrite

concentrations statistically similar ($P > 0.05$) to both controls. Brand G had the greatest amount of residual nitrate and brands B, C, and D were statistically lower ($P < 0.05$) than either control. A day by brand interaction was found for pH where brands D and H had significantly lower pH ($P < 0.05$) on days 28 and 35 and brand G on day 35 than on day 0 (data not shown). No other brand had a statistically significant decline in pH over time. Two uncured brands, C and D, contained less salt than either control ($P < 0.05$). Six of the brands contained less than 2.0% salt. No naturally cured brands were different from the controls for L*, though brands B, C and E were less red ($P < 0.05$) than either control. Of these, brand B did not contain any source of nitrite and brand C was a turkey frankfurter which could contribute to lower a* values due to lower pigment concentration. Brand E was less yellow (b*, $P < 0.05$) than either control. Brands A and E had a greater ($P < 0.05$) total pigment (myoglobin) concentration than either control. As with L*, large variations in total pigment were found among the controls. Brand A had the greatest amount of cured pigment and was among the highest in total pigment content. Brand B had the lowest cured pigment, which was no surprise because this product did not attempt to replace sodium nitrite. Analysis for percent cured pigment showed that brands B and C had significantly lower nitrosylation of myoglobin to form nitrosylhemochromogen. These brands were also among the lowest for residual nitrite and a*. Large differences were found in proximate composition. Nine brands of the naturally cured products had greater moisture, six had less fat, and five had greater moisture content than either control ($P < 0.05$).

Ham Physio-chemical Properties

Of the ham brands tested, two brands were organic, four were labeled natural, one was labeled uncured but was not labeled natural or organic, and four were traditionally cured controls. Five brands met the labeling classification of ham, two uncured and two control brands were classified as ham with natural juices. Of the remaining two controls, one was labeled as ham-water added and one as ham and water product: 35% of weight is added ingredients. Of the seven uncured ham brands tested, five contained starter culture and a natural nitrate source while two brands likely used pre-converted, nitrite-containing ingredients to provide cured meat characteristics. Two of the uncured brands contained sodium or potassium lactate, and the naturally cured brand not labeled natural or organic contained sodium phosphates. The control brands all contained sodium nitrite, sodium erythorbate or ascorbate, sodium or potassium lactate and diacetate, and sodium phosphate.

Physio-chemical traits for ham are found in Tables 3 and 4. As with frankfurters, little variation in water activity was found, ranging from 0.94-0.96. A significant brand difference ($P < 0.05$) was found but after Tukey's adjustment no means separation of brands occurred. Residual nitrite ranged from 4.2-12.0 ppm, and all brands were similar ($P > 0.05$) to at least one of the controls. Brand E had less residual nitrate than any control. A brand x day interaction was also found for ham. Brand B had significantly ($P <0.05$) lower pH on day 35 than on day 0 (data not shown). Brand C had less salt than any control ($P <0.05$) and was one of two brands with less than 2.0% salt. All brands had L* and a* values similar ($P <0.05$) to at least one of the control brands. No significant brand effect was found for b*. Brand F had greater total pigment content than any control and also had the lowest L* value. All brands were similar to at least one of the controls for cured pigment content and no

significance was found for percent cured pigment. All brands were similar to at least one of the controls for moisture and fat content. Brand H was the only brand significantly different ($P < 0.05$) than any control for protein.

Bacon Physio-chemical Properties

Of the bacon brands evaluated, two were organic, seven were labeled natural, and one was a traditionally cured control. Eight of the uncured brands contained starter culture and a natural nitrate source; one had no starter culture and likely used a pre-converted nitrite source. One uncured brand contained sodium lactate. The traditionally cured control contained sodium nitrite and sodium erythorbate.

Physio-chemical traits for bacon can be found in Tables 5 and 6. Two brands, I and E, had lower water activity than the control brand. Brand E had greatest amount of residual nitrite ($P <0.05$). Brand J had greater residual nitrate than the control while five brands had less ($P <0.05$). Two brands, C and H, had lower pH than the control. The low pH of brand H may have been caused by growth of lactic acid spoilage bacteria because the product had the highest water activity, lowest residual nitrate and nitrite, and only 1% salt. Furthermore, when the packages were opened in all three replications, milky purge and off-odors were present in brand H. The control brand was similar in L* values to all brands. Interestingly, the control was among the least red, which suggests less developed cured color. Brands I and J were more red than the control ($P <0.05$). The control had a lower b* value ($P <0.05$) than brands B, E, and J. No brand significant brand effects were found for total pigment, cured pigment, or percent cured pigment. Also, no brand differences were found for moisture, fat

or protein content. Overall, bacon had the greatest standard errors for physio-chemical traits, which may be expected due to natural variability in quality and composition of pork bellies.

Sindelar et al. (2007) analyzed four commercially available, naturally cured brands of frankfurters, hams, and bacon for various quality attributes. Their findings were in a similar range as found in this study for most traits. The present study had similar cured pigment, but higher total pigment concentrations than found in previous work resulting in differences in percent cured pigment. Also, the current findings report greater upper limits for residual nitrite and nitrate concentrations suggesting that some manufacturers may have modified their processes or ingredients to increase ingoing nitrate and conversion to nitrite.

Correlation of Measured Physio-chemical Properties to C. perfringens Growth

Pearson's correlation coefficients of physio-chemical traits on day 0 to mean *C. perfringens* growth over 10 days can be found in Table 7. For frankfurters, significant correlations ($P < 0$ .05) with the pathogen were found for nine of the traits measured which can be separated into two groups, known intrinsic factors and traits relating to the curing reaction. In general, greater *C. perfringens* growth has been observed in naturally cured frankfurters than in traditionally cured control frankfurters (Jackson et al., 2011). These experiments found pH was the most highly correlated to *C. perfringens* growth (r=0.735). While no product was below minimum growth pH for *C. perfringens*, reducing pH below the optimal range can result in longer lag times and decreased growth rate (McClane, 2001). Product composition was strongly correlated to pathogen growth. Increased moisture and protein and decreased fat were correlated to increased *C. perfringens* growth. These three traits are interrelated as amount of fat and added moisture is regulated in emulsified sausages

(USDA, 2010b) and more lean tissue would be added to decrease fat content in product formulation. Moisture content and other ingredients impact availability of water. Decreased salt and increased water activity were associated with greater *C. perfringens* growth. The remaining significantly correlated traits are related to the extent of the curing reaction that occurred. One major characteristic of cured meats is the stable pink color formed by nitric oxide binding to myoglobin (Honikel, 2008). Products that were more red, with increased cured pigment, and greater percent cured pigment had less *C. perfringens* growth. This suggests that the curing reaction occurred to a greater extent and could be related to higher ingoing nitrite concentration. However, no correlation was found for residual nitrite or nitrate. This is not surprising because nitrate and nitrite are typically depleted by the cure reactions in formulation and thermal processing of these products, and continue to be depleted during subsequent storage (Cassens, 1997). It has been suggested that residual nitrite level, as long as it is not completely depleted, is not as important in controlling bacterial growth as other factors (Tompkin, 2005; Tompkin, Christiansen & Shaparis, 1979). Further more, residual nitrite can be impacted by many product factors including ingoing nitrite, other added ingredients, meat source, pH, thermal processing temperature, and time of storage (Cassens, 1997). Commercial products were not evaluated at a common day post production and this would influence measured residual nitrite which declines over time (Pérez-Rodríguez, Bosch-Bosch & Garciá-Mata, 1996, 1997).

Fewer significant results were found for ham and bacon than frankfurters. Unlike the results in frankfurters, lower residual nitrate ($P < 0.05$) and nitrite ($P < 0.10$) were significantly correlated to increased *C. perfringens* growth in ham. Residual nitrate had the greatest correlation (r=-0.622) to *C. perfringens* growth in ham. While nitrate is directly

added to the naturally cured meats, sodium nitrate is no longer commonly used in traditionally cured products, but is produced during the curing reaction. Nitric oxide oxidation can reform nitrate and may consume up to 40% of the ingoing nitrite (Honikel, 2008). Ham and other brine-injected products (excluding bacon) are allowed higher ingoing nitrite concentrations than those made with the direct addition of sodium nitrite to the product mixture such as frankfurters (USDA, 1995). The higher ingoing concentration and subsequent nitrate formation may account for this correlation. It may be an indication of the extent of nitric oxide reaction in traditionally cured controls as opposed to incomplete bacterial conversion of nitrate in naturally cured products. As expected, lower water activity (P <0.05) and higher salt content (P <0.10) were related to less *C. perfringens* growth. Increased protein (P <0.05) and total pigment (P <0.10) indicated greater pathogen growth and can likely be explained by differences in ham classification. The traditionally cured controls were classified as ham with natural juices, ham water added, and ham and water product where as the naturally cured products were all ham or ham with natural juices. The naturally cured hams, which exhibited greater *C. perfringens* growth, have higher protein and of total pigment concentration due to less dilution by added ingredients. As with frankfurters, percent cured pigment (P <0.10) was an indicator of pathogen growth and is also an indicator of extent of curing reaction in the product. Greater yellow color (increased b*) was also significantly correlated (P <0.10) to *C. perfringens* growth in ham.

For bacon, increased total pigment (P <0.05) was an indication of decreased pathogen growth which is opposite of that found in ham. As with frankfurters and ham, increased salt content and decreased water activity were significantly correlated (P <0.10) to *C. perfringens* growth for bacon.

Across all products, decreased salt and increased water activity were correlated to greater *C. perfringens* growth. For both ham and frankfurters, greater percent cured pigment and less protein delayed growth. While protein content was positively correlated to *C. perfringens* in both ham and frankfurters, different reasons likely explain the results. The naturally cured frankfurters were leaner and the naturally cured hams had less added water and ingredients as indicated by the label resulting in higher protein concentration in both products. The controls, in each case, had lower protein content and decreased growth. Percent cured pigment was negatively correlated to *C. perfringens* growth and provides an indication of curing reaction.

While these traits provide valuable insight, they cannot fully account for all variation in pathogen growth. Ingoing nitrite may be one of the most important traits. It has been estimated that only 50 to 70% of ingoing nitrite can be recovered immediately following formulation and mixing and between 20 and 80% is lost during thermal processing (Cassens, Ito, Lee & Buege, 1978). Honikel (2008) estimates a loss of 65% of ingoing nitrite from formulation through thermal processing regardless of ingoing nitrite levels. It has been suggested that 100-180 ppm of nitrite is required for the inhibition of *C. perfringens* (O'Leary & Solberg, 1976). Many ingredients reduce nitrite and impact the rate of curing (Cassens, 1997). Increased salt content has been to shown to decrease residual nitrite content (Fox, Sebranek & Phillips, 1994; Sebranek & Fox, 1985) and lower minimum inhibitory concentration of nitrite for *C. sporogenes* (Ashworth, Hargreaves & Jarvis, 1973). While ingoing nitrite is generally lower in naturally cured products (Sebranek & Bacus, 2007), the exact levels cannot be determined.

Correlation of Measured Physio-chemical Properties to L. monocytogenes Growth

Pearson's correlation coefficients of physio-chemical traits on day 0 to *L. monocytogenes* growth over 35 days can be found in Table 7. Schrader found that more *L. monocytogenes* grew on many of the naturally cured brands of frankfurters and ham than on traditionally cured controls (Schrader, 2010). Fewer significant correlations were found between physio-chemical traits and *L. monocytogenes* growth than were found with *C. perfringens*. Increased water activity and moisture and protein content were again correlated (P <0.10) to greater *L. monocytogenes* as for *C. perfringens*, but no significant correlations were found for salt, residual nitrite, residual nitrate, or traits relating to extent of curing reaction in frankfurters. As discussed in relationship to *C. perfringens* growth, leaner frankfurters replace fat with lean tissue, which resulted in greater moisture and protein content. In ham, higher protein and lower salt content were indicators of increased *L. monocytogenes* (P <0.10) growth. *L. monocytogenes* growth was depressed with increased ingoing nitrite up to 150 ppm (Xi, Sullivan, Jackson, Zhou & Sebranek, 2011) and greater ingoing nitrite improves the *L. monocytogenes* inhibitory effects of lactate (Qvist & Bernbom, 2000). *L. monocytogenes* is capable of growth over a wider range of conditions than *C. perfringens* (McClane, 2001; Swaminathan, 2001) and this could be the result of fewer significant correlations of physio-chemical traits to bacterial growth. Also, two replicates were conducted for *L. monocytogenes* challenge studies while three were used for *C. perfringens* resulting in reduced statistical power to find significant correlations with *L. monocytogenes* which could partially explain the reduced number of significant correlations.

Conclusions

Many of the naturally cured processed meat products have been observed to support greater pathogen growth than the traditionally cured controls. Naturally cured frankfurters examined in this study were generally leaner and contained less salt than conventionally cured controls. Fewer differences were found when comparing controls and naturally cured products for ham and bacon. Frankfurters had the greatest number and largest correlations (r values) of traits with *C. perfringens* growth in these studies and could be grouped into traits that are known intrinsic inhibitors and traits that indicate extent of curing reaction. Salt content and water activity were related to *C. perfringens* growth in all products. For frankfurters and ham, protein content and percent cured pigment were also related to growth of *C. perfringens*. Water activity, moisture and protein were related to *L. monocytogenes* growth in frankfurters where it was related to salt and protein content in ham. For both pathogens, water activity, protein and moisture content were correlated to pathogen growth while protein and salt content were for ham products. However, these traits do not explain all the differences in pathogen growth. Regardless of source, added nitrite is depleted during curing and thermal processing and is required to provide cured meat characteristics. Higher ingoing nitrite concentrations are required to provide microbial inhibition than cured color, flavor and antioxidant activity.

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Tables

Table 1. Means and range of values for water activity, residual nitrite and nitrate, pH and salt in commercially available no-nitrate-or-nitrite added frankfurters and conventionally cured controls.

Frankfurters Brand Code	Water activity	Residual Nitrite ppm	Residual Nitrate ppm[y]	pH[z]	Salt %
P-value	<0.0001	<0.0001	<0.0001	--	<0.0001
A	0.952^{ab}±0.004	16.1^{b}±2.6	34.5^{c}±3.5	6.17±0.06	1.7^{de}±0.1
B	0.950^{ab}±0.003	2.0^{c}±1.7	4.6^{e}±2.7	6.18±0.04	1.8^{de}±0.1
C	0.951^{ab}±0.004	2.0^{c}±2.6	18.0^{de}±3.5	6.20±0.06	1.5^{e}±0.1
D	0.951^{ab}±0.003	8.7^{bc}±1.7	19.6^{d}±2.7	5.87±0.04	1.7^{e}±0.1
E	0.950^{ab}±0.003	4.7^{c}±1.7	41.4^{bc}±2.7	5.98±0.04	1.7^{de}±0.1
F	0.945^{ab}±0.003	60.1^{a}±1.7	51.0^{ab}±2.7	6.10±0.04	2.4^{ab}±0.1
G	0.949^{ab}±0.003	13.9^{b}±1.7	57.8^{a}±2.7	5.87±0.04	2.2^{bc}±0.1
H	0.950^{ab}±0.003	4.8^{c}±1.7	31.6^{cd}±2.7	5.64±0.04	1.7^{de}±0.1
I	0.953^{a}±0.003	4.1^{c}±1.7	35.0^{c}±2.7	5.92±0.04	2.0^{cd}±0.1
J (Control)	0.937^{b}±0.003	7.6^{bc}±1.7	41.6^{bc}±2.7	5.94±0.04	2.1^{bcd}±0.1
K	0.943^{ab}±0.003	10.0^{bc}±1.7	32.4^{c}±2.7	5.91±0.04	2.2^{bc}±0.1
L (Control)	0.938^{b}±0.002	6.9^{bc}±1.4	31.3^{c}±2.1	5.88±0.03	2.5^{a}±0.1
Ranges for Naturally Cured:	0.943-0.953	2.0-60.1	4.6-57.8	5.64-6.20	1.5-2.4
Controls:	0.937-0.938	6.9-7.6	31.3-41.6	5.88-5.94	2.1-2.5

[a-e] Means without common superscript are significantly different ($P < 0.05$). Standard errors are displayed following ± for each brand.

[y] Due to difference in sample numbers and power, Brand L was significantly different ($P < 0.05$) than brands C and D while brand H, numerically higher than brand L, was statistically similar to brands C and D.

[z] Significant ($P < 0.05$) brand by day interaction was found for pH.

Table 2. Means and range of values for CIE color, total, cured and percent cured pigment, and proximate composition for commercially available no-nitrate-or-nitrite added frankfurters and conventionally cured controls.

Frankfurter Brand Code	L*	a*	b*	Total Pigment ppm	Cured Pigment ppm	% Cured Pigment	Moisture %	Fat %	Protein %
P-value	<.0001	<.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A	56.7^{bc}±1.3	24.0^{ab}±1.1	22.4^{ab}±1.1	357.3^{a}±17.7	160.2^{a}±10.0	44.9^{ab}±3.8	59.51^{cde}±1.02	22.74^{abc}±1.25	14.78^{bcde}±0.51
B	58.9^{bc}±1.2	13.1^{f}±1.0	15.6^{cd}±0.9	236.3^{defg}±13.7	4.1^{f}±7.8	1.7^{d}±3.0	68.17^{a}±0.79	11.35^{f}±0.97	16.70^{ab}±0.40
C	68.8^{a}±1.3	12.3^{f}±1.1	15.6^{cd}±1.1	182.0^{g}±17.7	38.6^{ef}±10.0	20.6^{c}±3.8	64.94^{a}±1.02	12.52^{ef}±1.25	18.41^{a}±0.51
D	57.3^{bc}±1.0	22.7^{ab}±0.9	23.2^{a}±0.9	334.4^{ab}±13.7	108.1^{bcd}±7.8	32.5^{abc}±3.0	64.41^{ba}±0.79	17.93^{cde}±0.97	14.50^{cde}±0.40
E	56.2^{c}±1.2	14.9^{ef}±1.0	12.7^{d}±0.9	358.8^{a}±13.7	111.4^{cd}±7.8	31.3^{bc}±3.0	60.84^{bcde}±0.79	17.00^{de}±0.97	17.40^{a}±0.40
F	59.2^{bc}±1.0	17.8^{cde}±0.9	16.1^{cd}±0.9	312.1^{abc}±13.7	108.3^{bcd}±7.8	34.6^{abc}±3.0	63.73^{bc}±0.79	16.76^{de}±0.97	15.45^{bc}±0.40
G	62.0^{b}±1.0	15.8^{def}±0.0	21.4^{ab}±0.9	224.0^{efg}±13.7	71.3^{de}±7.8	31.8^{bc}±3.0	61.88^{bcd}±0.79	19.92^{bcd}±0.97	15.08^{bcd}±0.40
H	61.6^{b}±1.0	19.8^{bcd}±0.9	15.3^{cd}±0.9	278.2^{bcde}±13.7	106.9^{bcd}±7.8	39.0^{ab}±3.0	59.62^{cde}±0.79	23.71^{ab}±0.97	13.67^{cde}±0.40
I	60.5^{bc}±1.0	18.2^{cde}±0.9	14.9^{cd}±0.9	296.2^{abcd}±13.7	118.2^{abc}±7.8	40.4^{ab}±3.0	57.37^{ef}±0.79	25.55^{a}±0.97	13.32^{de}±0.40
J (Control)	67.5^{a}±1.0	19.5^{bcd}±0.9	18.9^{bc}±0.9	204.3^{fg}±13.7	81.3^{cde}±7.8	39.7^{ab}±3.0	52.11^{g}±0.79	27.30^{a}±0.97	11.35^{f}±0.40
K	59.9^{bc}±1.0	20.5^{abc}±0.9	15.7^{cd}±0.9	262.2^{cdef}±13.7	121.0^{ab}±7.8	46.2^{a}±3.0	58.80^{de}±0.79	23.37^{ab}±0.97	13.19^{def}±0.40
L (Control)	58.0^{bc}±0.8	24.3^{a}±0.7	24.2^{a}±0.7	282.9^{bcde}±10.8	113.3^{bc}±6.1	40.8^{ab}±2.4	55.16^{fg}±0.62	27.08^{a}±0.77	12.89^{ef}±0.31
Ranges for Naturally Cured:	56.2-68.8	12.3-24.0	12.7-23.2	182.0-358.8	4.1-160.2	1.7-46.2	57.37-68.17	11.35-25.55	13.19-18.41
Controls:	58.0-67.5	19.5-24.3	18.9-24.2	204.3-282.9	81.3-113.3	39.7-40.8	52.11-55.16	27.08-27.30	11.35-12.89

[a-g] Means without common superscript are significantly different ($P < 0.05$). Standard errors are displayed following ± for each brand.

Table 3. Means and range of values for water activity, residual nitrite and nitrate, pH and salt in commercially available no-nitrate-or-nitrite added ham and conventionally cured controls.

Ham Brand Code	Water activity[y]	Residual Nitrite ppm	Residual Nitrate ppm	pH[z]	Salt %
P-value	0.0141	<.0001	<.0001	--	<.0001
A	0.954±.003	9.0^{abc}±0.8	12.2^{de}±1.2	5.88±0.04	2.4^{ab}±0.2
B	0.951±.003	8.6^{abc}±0.8	19.9^{ab}±1.2	5.78±0.04	2.7^{ab}±0.2
C	0.957±.003	10.7^{ab}±0.8	12.5^{de}±1.2	6.12±0.04	1.6^{c}±0.2
D	0.958±.005	6.5^{bcd}±1.3	14.4^{cd}±1.2	5.85±0.06	1.7^{bc}±0.2
E	0.954±.003	4.2^{d}±0.8	7.3^{e}±1.2	5.89±0.04	2.5^{ab}±0.2
F	0.950±.003	5.9^{cd}±0.8	15.7^{cd}±1.2	5.89±0.04	3.0^{a}±0.2
G (Control)	0.953±.003	9.9^{ab}±0.8	18.3^{bc}±1.2	6.03±0.04	2.5^{ab}±0.2
H	0.950±.005	9.9^{ab}c±1.3	15.2^{cd}±1.2	5.89±0.06	2.7^{ab}±0.2
I (Control)	0.948±.005	5.0^{cd}±1.3	15.6^{cd}±1.2	5.91±0.06	2.3^{abc}±0.2
J (Control)	0.944±.003	8.7^{abc}±0.8	23.2^{ab}±1.2	6.06±0.04	2.9^{a}±0.2
K (Control)	0.942±.003	12.0^{a}±0.8	24.8^{a}±1.2	6.12±0.04	2.9^{a}±0.2
Ranges for Naturally Cured:	0.950-0.958	4.2-10.7	7.3-19.9	5.78-6.12	1.6-3.0
Controls:	0.942-0.953	5.0-12.0	15.6-24.8	5.91-6.12	2.3-2.9

[a-e] Means without common superscript are significantly different ($P < 0.05$). Standard errors are displayed following ± for each brand.

[y] Significant brand effects were found for water activity but following Tukey's honestly significant difference adjustment, no means separation was found.

[z] Significant ($P < 0.05$) brand by day interaction was found for pH.

Table 4. Means and range of values for CIE color, total, cured and percent cured pigment, and proximate composition for commercially available no-nitrate-or-nitrite added ham and conventionally cured controls.

Ham Brand Code	L*	a*	b*	Total Pigment ppm	Cured Pigment ppm	% Cured Pigment	Moisture %	Fat %	Protein %
P-value	<.0001	0.0180	0.0614	<.0001	0.0011	0.0582	<0.0001	<0.0001	<0.0001
A	61.3^{abcd}±1.2	17.1^{ab}±0.7	10.0±0.5	146.0^{abc}±10.7	43.3^{ab}±5.2	32.1±3.2	73.88^{ab}±0.56	1.76^{c}±0.49	19.60^{abc}±0.60
B	64.1^{ab}±1.2	15.6^{ab}±0.7	10.7±0.5	124.8^{bcd}±10.7	36.0^{b}±5.2	26.5±3.2	71.98^{b}±0.56	2.82^{bc}±0.49	19.24^{abc}±0.60
C	65.3^{ab}±1.2	15.6^{ab}±0.7	9.9±0.5	128.0^{bcd}±10.7	34.8^{b}±5.2	28.3±3.2	73.95^{ab}±0.56	2.43^{bc}±0.49	20.10^{abc}±0.60
D	66.6^{a}±1.5	14.2^{ab}±0.9	11.3±0.7	176.4^{ab}±13.8	56.4^{ab}±6.8	32.1±4.1	71.00^{b}±0.73	4.85^{ab}±0.63	21.81^{ab}±0.78
E	64.7^{ab}±1.2	15.5^{ab}±0.7	9.4±0.5	111.2^{cd}±10.7	28.3^{b}±5.2	26.5±3.2	68.37^{c}±0.56	1.77^{c}±0.49	19.37^{abc}±0.60
F	56.7^{d}±1.2	17.9^{a}±0.7	10.9±0.5	193.6^{a}±10.7	64.6^{a}±5.2	37.3±3.2	72.45^{b}±0.56	2.44^{bc}±0.49	19.61^{abc}±0.60
G (Control)	65.8^{a}±1.2	13.9^{b}±0.7	9.1±0.5	104.2^{cd}±10.7	33.5^{b}±5.2	31.8±3.2	73.98^{ab}±0.56	2.82^{bc}±0.49	19.15^{bc}±0.60
H	57.7^{d}±1.5	16.8^{ab}±0.9	11.7±0.7	127.8^{bcd}±13.8	43.9^{ab}±6.8	34.4±4.1	68.37^{c}±0.73	4.09^{abc}±0.63	22.52^{a}±0.78
I (Control)	59.2^{bcd}±1.5	17.2^{ab}±0.9	9.6±0.7	104.9^{cd}±13.8	45.7^{ab}±6.8	43.8±4.1	68.81^{c}±0.73	6.12^{a}±0.63	18.63^{bcd}±0.78
J (Control)	64.3^{ab}±1.2	16.0^{ab}±0.7	9.7±0.5	93.0^{d}±10.7	33.2^{b}±5.2	36.2±3.2	72.93^{b}±0.56	2.82^{bc}±0.49	18.20^{cd}±0.60
K (Control)	62.5^{abc}±1.2	16.0^{ab}±0.7	9.6±0.5	135.6^{bcd}±10.7	46.0^{ab}±5.2	38.1±3.2	73.18^{ab}±0.56	3.28^{bc}±0.49	15.29^{d}±0.60
Ranges for Naturally Cured:	57.7-66.6	14.2-17.9	9.4-11.7	111.2-176.4	28.3-64.6	26.5-37.3	68.37-73.95	1.76-4.85	19.15-22.52
Controls:	59.2-65.8	13.9-17.2	9.1-9.7	93.0-135.6	33.5-46.0	31.8-43.8	68.81-73.98	2.82-6.12	15.29-19.15

$^{a-d}$ Means without common superscript are significantly different ($P < 0.05$). Standard errors are displayed following ± for each brand.

Table 5. Means and range of values for water activity, residual nitrite and nitrate, pH, and salt in commercially available no-nitrate-or-nitrite added bacon and conventionally cured controls.

Bacon Brand Code	Aw	Residual Nitrite ppm	Residual Nitrate ppm	pH	Salt %
P-value	<0.0001	<.0001	<.0001	<0.0001	<0.0001
A	0.954^{abcd}±0.007	11.7^{b}±1.9	13.2^{e}±3.0	5.90^{ab}±0.05	2.1^{bc}±0.2
B	0.972^{ab}±0.007	4.5^{b}±1.9	12.9^{e}±3.0	5.85^{ab}±0.05	1.6^{cd}±0.2
C	0.969^{abc}±0.007	9.7^{b}±1.9	27.5^{bcd}±3.0	5.56^{cd}±0.05	2.4^{abc}±0.2
D	0.962^{abcd}±0.007	5.2^{b}±1.9	19.6^{cde}±3.0	5.86^{ab}±0.05	2.0^{bc}±0.2
E	0.941^{cd}±0.007	27.2^{a}±1.9	38.0^{ab}±3.0	5.99^{a}±0.05	3.3^{a}±0.2
F	0.950^{abcd}±0.007	5.4^{b}±1.9	15.0^{de}±3.0	5.81^{ab}±0.05	2.3^{bc}±0.2
G (Control)	0.972^{ab}±0.007	11.3^{b}±1.9	33.2^{bc}±3.0	5.99^{ab}±0.05	2.5^{abc}±0.2
H	0.978^{a}±0.007	3.6^{b}±1.9	10.2^{e}±3.0	5.38^{d}±0.05	1.0^{d}±0.2
I	0.942^{bcd}±0.007	5.4^{b}±1.9	12.7^{e}±3.0	5.75^{bc}±0.05	2.6^{ab}±0.2
J	0.937^{d}±0.007	7.6^{b}±1.9	50.8^{a}±3.0	5.83^{ab}±0.05	2.8^{ab}±0.2
Ranges for Naturally Cured:	0.937-0.978	3.6-27.2	10.2-50.8	5.38-5.90	1.0-3.3
Control:	0.972	11.3	33.2	5.99	2.5

$^{a-e}$ Means without common superscript are significantly different ($P < 0.05$). Standard errors are displayed following ± for each brand.

Table 6. Means and range of values for CIE color, total, cured and percent cured pigment, and proximate composition for commercially available no-nitrate-or-nitrite added bacon and conventionally cured controls.

Bacon Brand Code	L*	a*	b*	Total Pigment ppm	Cured Pigment ppm	% Cured Pigment	Moisture %	Fat %	Protein %
P-value	0.0210	0.0002	0.0043	0.4031	0.4555	0.5300	0.0910	0.0737	0.1203
A	62.9^{a}±2.3	11.3^{bcd}±0.9	11.5^{ab}±0.8	139.3±21.22	38.9±7.9	28.5±7.8	44.17±3.9	38.16±4.8	12.83±1.3
B	58.7^{ab}±2.3	12.7^{abcd}±0.9	14.8^{a}±0.8	136.1±21.22	37.2±7.9	28.4±7.8	40.24±3.9	34.67±4.8	14.71±1.3
C	65.6^{a}±2.3	9.3^{d}±0.9	11.2^{ab}±0.8	109.4±21.22	45.3±7.9	45.6±7.8	44.49±3.9	37.69±4.8	12.83±1.3
D	61.0^{ab}±2.3	11.8^{bcd}±0.9	13.2^{ab}±0.8	148.8±21.22	60.5±7.9	39.6±7.8	32.76±3.9	53.72±4.8	9.86±1.3
E	58.6^{ab}±2.3	15.0^{abc}±0.9	15.0^{a}±0.8	191.3±21.22	47.9±7.9	26.3±7.8	46.72±3.9	33.09±4.8	14.30±1.3
F	57.8^{ab}±2.3	13.1^{abcd}±0.9	13.6^{ab}±0.8	143.7±21.22	41.4±7.9	29.4±7.8	39.83±3.9	44.14±4.8	12.35±1.3
G (Control)	60.0^{ab}±2.3	11.0^{cd}±0.9	10.1^{b}±0.8	132.8±21.22	45.4±7.9	39.3±7.8	50.86±3.9	30.72±4.8	14.97±1.3
H	62.9^{a}±2.3	14.0^{abc}±0.9	13.3^{ab}±0.8	144.7±21.22	56.1±7.9	39.8±7.8	51.07±3.9	31.61±4.8	15.35±1.3
I	56.7^{ab}±2.3	15.4^{ab}±0.9	13.7^{ab}±0.8	148.3±21.22	54.3±7.9	36.9±7.8	42.54±3.9	39.18±4.8	14.10±1.3
J	51.3^{b}±2.3	16.7^{a}±0.9	14.5^{a}±0.8	173.5±21.22	39.3±7.9	22.8±7.8	39.14±3.9	44.03±4.8	11.46±1.3
Ranges for Naturally Cured:	51.3-62.9	9.3-16.7	11.2-14.8	109.4-173.5	37.2-60.5	22.8-45.6	32.76-50.86	31.61-53.72	9.86-15.35
Controls:	60.0	11.0	10.1	132.8	45.4	39.3	50.86	30.72	14.97

$^{a-g}$Means without common superscript are significantly different ($P < 0.05$). Standard errors are displayed following ± for each brand.

Table 7. Pearson's correlation coefficients of physiochemical traits and pathogen growth.

	C. perfringens			***L. monocytogenes***	
Trait	Frankfurters	Ham	Bacon	Frankfurters	Ham
Water activity	0.462**	0.520**	0.347*	0.393*	0.341
pH	0.735**	-0.222	0.058	0.106	-0.136
Residual nitrite	0.053	- 0.321*	-0.043	-0.008	-0.013
Residual nitrate	-0.036	-0.622**	-0.268	0.252	-0.424
Salt	- 0.670**	-0.331*	- 0.309*	-0.172	-0.461*
L*	-0.076	0.066	0.101	-0.184	-0.219
a*	- 0.428**	0.061	-0.073	-0.355	0.236
b*	-0.121	0.323*	0.024	0.014	0.371
Total Pigment	0.135	0.398**	- 0.362**	0.318	0.338
Cured Pigment	- 0.383**	0.265	-0.273	-0.065	0.047
% Cured pigment	- 0.630**	- 0.348**	0.032	-0.209	-0.349
Moisture	0.737**	0.154	-0.05	0.410*	0.345
Fat	- 0.711**	-0.226	0.007	-0.298	-0.333
Protein	0.692**	0.566**	-0.022	0.397*	0.469*

** $P < 0.05$

* $P < 0.10$

CHAPTER 4. INHIBITION OF *LISTERIA MONOCYTOGENES* USING NATURAL ANTIMICROBIALS IN NO-NITRATE-OR-NITRITE-ADDED HAM

A paper to be submitted to the *Journal of Food Protection*.

Gary A. Sullivan, Armitra L. Jackson, Steven E. Niebuhr, Yuan Xi, Kohl D. Schrader, Joseph G. Sebranek, and James S. Dickson

Abstract

Consumers' demand for foods manufactured without the direct addition of sodium nitrite has resulted in a unique class of cured meat products. This study evaluated *Listeria monocytogenes* growth on ham manufactured with natural curing methods with added antimicrobials and assessed impacts on physio-chemical characteristics. Both of the natural antimicrobials evaluated inhibited growth similar to that of the traditionally cured control. Ham made with pre-fermented celery juice powder had lower residual nitrite concentrations and when no antimicrobial was added, *L. monocytogenes* growth was similar to that of the uncured control. Ham pH was influenced slightly by antimicrobials. Ham can be produced with natural curing methods and antimicrobials to inhibit *L. monocytogenes* growth with little changes to physio-chemical traits.

Index Terms—No-nitrate-or-nitrite-added, *Listeria monocytogenes*, natural antimicrobials, ham

Introduction

Nitrite and nitrate have been used in meat processing for thousands of years and are responsible for the color, flavor/aroma, antioxidant, and antimicrobial properties associated with cured meats. Chinese alchemists were investigating potassium nitrate (saltpeter) since at least the 5th century *(21)* and 4th century records described placing saltpeter under the tongue to ease chest pain *(6,10)*. However in 1945,

methemoglobinemia in infants (blue baby syndrome) was linked to nitrate concentrations and bacterial contamination of well water *(7)* and in 1964, carcinogenic n-nitrosamine compounds were isolated in herring meal produced with large amounts of sodium nitrite and shown to have deleterious effects on animal health *(8)*. These findings created a public concern about consuming cured meats that still persists even though the National Academy of Science has clearly stated that nitrate is ”neither carcinogenic nor mutagenic” and ”evidence does not indicate that nitrite acts directly as a carcinogen” *(11)*. Researches have also shown that nitrate, nitrite, and nitric oxide are important biological compounds that provide many healthful benefits *(10)*.

A recent survey by the Organic Trade Association *(12)* reported that 73% of US households at least occasionally purchase organic foods. These respondents cited health benefits as the major reason for organic food purchases and 47% indicated that they avoided artificial ingredients and preservatives in food to improve health. Currently regulations for natural foods prohibit the use of artificial flavoring, color, chemical preservatives, or synthetic ingredients *(23)*. Sodium nitrite and nitrate, classified as preservatives, are not allowed. However, processed meats are manufactured without the addition of sodium nitrite and with characteristics similar to traditionally cured products by utilizing natural nitrate/nitrite sources *(16)*. By United States Department of Agriculture (USDA) regulations, these products must be labeled as ”Uncured” and “No Nitrate or Nitrite Added”*(25, 26)*. Ingredients high in nitrate, such as vegetable powders and sea salts, are included along with nitrate reducing starter cultures to naturally reduce nitrate to nitrite. Furthermore, ingredient manufacturers have begun fermenting the celery juice with the nitrate reducing starter culture prior to drying, resulting a natural

nitrite source. When compared to traditionally cured products, ingoing nitrite levels are typically lower in these naturally cured meats *(15)*.

Because many antimicrobials are not allowed, and relatively low ingoing nitrite concentrations are typical, these products may be more susceptible to pathogenic bacterial growth than conventionally cured controls. The United States Department of Agriculture (USDA) has a zero tolerance for *Listeria monocytogenes* for ready-to-eat processed meats *(24)* because of the ability of this organism to grow during refrigerated storage. High mortality rates and prevalence of miscarriages found with Listeriosis cases *(18)* are also major concerns. Schrader *(14)* found that five of eight brands of commercial no-nitrate-or-nitrite frankfurters had greater *L. monocytogenes* growth than traditionally cured control frankfurters. Many natural antimicrobial alternatives have been identified but few have been assessed for effectiveness in naturally cured processed meats. The purpose of this study was to evaluate the effectiveness of natural curing systems and commercially available natural antimicrobials in inhibiting growth of *L. monocytogenes*.

Materials and Methods

Manufacture of Hams

Eight ham treatments (six experimental treatment combinations and two control treatments) were produced to evaluate the inhibition of *L. monocytogenes* growth by natural nitrate or nitrite sources and natural antimicrobials. Three independent replicates were produced. Celery juice powder (natural nitrate; VegStable 502, Florida Food Products, Eustis, FL) and pre-fermented celery juice powder (natural nitrite; VegStable 504, Florida Food Products, Eustis, FL) were used as natural curing agents. Two commercially available natural antimicrobials were evaluated: a blend of cherry, lemon and vinegar powder (Antimicrobial A; VegStable 507, Florida Food Products, Eustis, FL)

and a cultured sugar and vinegar blend (Antimicrobial B; Verdad 55, Purac, Lincolnshire, IL). While the latter is composed of natural ingredients, it is not clear at this point if this ingredient qualifies as natural in the eyes of the USDA. All commercial ingredients were utilized at concentrations recommended by the supplier.

Hams were produced at the Iowa State University (ISU) Meat Laboratory with pork inside ham muscles using formulations found in Table 1. The ham muscles were obtained from a local processor and frozen prior to use to ensure uniformity of raw material. The ham muscles were tempered to -2 °C, and then were coarse-ground through a 6.35 mm plate. Non-meat ingredients (Table 1) were added and mixed with ground ham for two minutes using a double action mixer (Leland Southwest, Fort Worth, TX, USA). Mixed samples were reground using a 3.18 mm plate and stuffed into a 35 mm fibrous casing with a rotary vane vacuum-filling machine (Risco vacuum stuffer, Model RS 4003-165). Treatments with natural nitrate (D, E, G) were placed in a single truck smokehouse (Thermal Processing Unit, Maruer-Atmos, Reichenau, Germany with Direct Digital Control, Alkar-RapidPak, Lodi, WI) for fermentation at 42 °C for 2 hours to convert nitrate to nitrite. Conventionally cured control (H) and treatments with natural nitrite (B, C, F) were placed in the smokehouse 90 minutes after beginning the fermentation to allow temperature to equilibrate prior to thermal processing. Treatment A (negative control) was processed in a separate smokehouse (Food Processing Oven with Direct Digital Control, Alkar-RapidPak, Lodi, WI) following the same thermal processing schedule excluding fermentation. All products were heated to an internal temperature of 73.9 °C. The hams were placed in a 0°C cooler overnight to stabilize. The next day, the hams were sliced to 1.5 mm thick slices using a fully automatic slicing machine (Bizerba, Model A-500, Piscataway, NJ., USA) and vacuum packaged (Ulma Packaging, MINI Series, Ball Ground, GA, USA). Hams were then transferred to the Food Safety Research

Laboratory or analytical laboratory at ISU to begin day 0 of the study. Three replications were produced.

Preparation of Inocula

L. monocytogenes strains H7969, H7764, H7769, H7762 and Scott A were obtained from the Food Safety Research Laboratory (FSRL) at Iowa State University (ISU). Each *L. monocytogenes* strain was cultured separately in trypticase soy broth supplemented with 0.6% yeast extract (TSBYE) for 24 hours at 35° C. A minimum of two consecutive 24 hour transfers of each strain to fresh TSBYE were preformed prior to each experiment. A 250 ml bottle of TSBYE was inoculated with 1 ml from each of the five *L. monocytogene*s strains and was incubated at 35° C for 24 hours to reach the stationary phase. The total concentration of the 5-strain mixture of *L. monocytogenes* was approximately 10^8 cells per ml. A 10 ml aliquot was removed from the inoculated broth and dispensed into a 90 ml 0.1% peptone bottle to achieve a 1:10 dilution. This diluted culture (5-strain mixture) was used to inoculate samples of ham.

Sample Inoculation

While in the FSRL, 25-gram samples of ham were placed in 5 X 16 in. vacuum bags (Cryovac Packaging, Duncan, SC., USA). A 0.1ml aliquot of the diluted (10^{-1}) culture was then aseptically transferred onto the ham in each bag for the various treatments. The cell concentration at inoculation was approximately 10^4 cells per gram. The bags were then vacuum sealed and stored at 4°C throughout the duration of the 35 day study. Sampling was conducted on day 0, 7, 14, 21, 28 and 35.

Microbiological Analysis

On the appropriate day, one package for each treatment was collected and opened

aseptically. Sampling was achieved by performing an initial 1:5 dilution using a diluter (Spiral System ASAP^{TM} Diluter, Cincinnati, OH). Each sample was homogenized in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, WI, USA) for 1 min in the laboratory blender (Stomacher 400, Seward Medical, London, UK). The product was further serially diluted, according to the sample date. An aliquot of 0.1 ml of the appropriate dilution was surface plated on modified Oxford medium base supplemented with modified Oxford antimicrobial supplement (Difco, Becton Dickinson, Sparks, MD). All inoculated agar plates were incubated at 35°C. After 24 – 48 hr, the plates were removed and colonies typical of *L. monocytogenes* were enumerated on duplicate plates. Numbers of bacterial colonies were converted to log colony forming units (CFU) per gram.

Analytical Analysis

Packaged samples were held in dark storage at 4 °C in a walk-in cooler. Samples were analyzed for residual nitrite, pH, CIE L*, a*, and b* on days 0, 8, 14, 21, 28 and 35. Samples from days 0, 8, 21, and 35 were frozen (-30°C) for up to 70 days before being analyzed for residual nitrate. Water activity and proximate composition were also analyzed on day 0. Samples during production were also collected after mixing and fermentation and were also evaluated for residual nitrite, nitrate and pH. Color was measured as described below and samples were then homogenized using a food processor (Model KFP715, Kitchenaid, St. Joseph, MI) to prepare for remaining analyses. Residual nitrite determination was conducted using the AOAC method number 973.31 *(4)*. Residual nitrate was measured using high performance liquid chromatography (HPLC) *(27, 28)* as described and modified by Ahn & Maurer *(1)*. The pH of ham samples was determined in a 9:1 water: sample slurry (Inlab Solids Pro probe; MultiSeven pH meter,

Metler Toledo Inc, Columbus, OH). Product color was measured at four random locations using CIE L*, a* and b*, Illuminate A, 10 ° standard observer and a 1.27 cm port (LabScan XE, HunterLab, Reston, VA). Water activity was determined using an Aqualab Series 3 water activity meter (Decagon, Pullman, WA). Moisture *(3)*, fat *(2)*, and protein *(5)* were determined by AOAC procedures 950.46, 960.39, and 992.15, respectively. Ash was calculated by difference. When not indicated, all analyses were conduced in duplicate.

Statistical Analysis

The PROC GLM (general linear models) procedure of Statistical Analysis System (SAS; version 9.2, SAS Institute Inc., Cary, NC) was used for statistical analysis. *L. monocytogenes* growth was analyzed for treatment by day effects. Analytical data was analyzed for treatment and where applicable day and treatment by day interactions were also analyzed. Where significant effects ($P<0.05$) were found, means separation was conducted using LSMEANS function of SAS and Fisher's least significant difference (LSD) adjustment for pathogen growth and Tukey's Honestly Significant Difference (HSD) adjustment for physio-chemical traits.

Results and Discussion

Listeria monocytogenes Growth

Treatments with natural antimicrobials (C, E, F, and G) and the natural nitrate alone with starter culture treatment (D) had *L. monocytogenes* growth similar to the traditionally cured control (H) over 35 days of storage at 4 °C (Figure 1). Treatments A (uncured control) and B (natural nitrite, no antimicrobial) showed similar growth and were significantly greater ($P<0.05$) than all treatments except C on day 28 and C and D on day 35. Although still statistically similar to the traditional cured product, treatments

C (natural nitrite, antimicrobial A) and D (natural nitrate, no antimicrobial) had an upward trend by day 35 (P = 0.0563 and 0.0826, respectively). All other treatments (E, F, G) had growth similar to the traditionally cured control (H). Schrader found that these antimicrobials reduced *L. monocytogenes* growth on naturally cured frankfurters with antimicrobial B exhibiting greater inhibitory effects and major differences in growth that were observed by day 28 of 120 days storage at 4° C *(14)*. However, the nitrite source had a greater influence on inhibition of *Clostridium perfringens* than antimicrobials in ham or frankfurters cured with natural nitrite or natural nitrate and starter culture treatments *(9)*. The antimicrobials used in this study contain natural compounds similar to those commonly used in processed meats. Vinegar, cultured sugar and lemon are natural alternatives to organic acid salts often used in the industry. Cherry powder contains high levels of ascorbic acid *(19)* which functions as a cure accelerator by reducing nitrite to nitric oxide and increases the rate and extent of the curing reaction. Organic acids have well-documented antimicrobial properties *(20)*. The difference in inhibitory effects between the organisms may be partially explained by differences in nitrite tolerance among bacterial species *(22)*.

Physio-chemical Traits

Physio-chemical traits can be found in Table 2. Ingoing nitrite concentration was one of the greatest differences among treatments. Preliminary research determined that about 50 ppm of ingoing nitrite can be achieved with the pre-fermented celery juice powder (0.4% addition). Treatments (D, E, G) with natural nitrate and starter culture had a reduction in nitrate concentration of 79.1 to 91.2 ppm from the amount added (94.7 to 99.0). This is equivalent to ingoing nitrite of 64.5 to 74.0 ppm. However this is likely an underestimate of ingoing nitrite due to nitric oxide oxidation reforming nitrate, which can

be re-reduced to nitrite. Lower residual nitrate concentrations in treatment D suggest that both antimicrobials may have slowed the bacterial reduction of nitrate. This appears to be much less than that found by Schrader in emulsified sausages *(14)*. The lower ingoing nitrite could explain the greater *L. monocytogenes* growth in pre-fermented celery juice than in the treatment with celery juice and starter culture samples when no antimicrobial was added. This is supported by Xi et al. *(29)* who found that increased ingoing nitrite resulted in decreased *L. monocytogenes* growth. Research has shown that ingoing nitrite concentrations may also impact the effectiveness of organic acids in *Listeria* control *(13)*.

No significant (P >0.05) treatment by day interactions were found for any traits measured. Residual nitrite concentrations were the highest in natural nitrate treatments, followed by the traditionally cured control. Pre-fermented celery juice treatments had the lowest residual nitrite. Terns et al. *(19)* and Xi et al. *(29)* both reported that the addition of cherry powder reduced residual nitrite but no difference in nitrite concentration was found in this study in treatments that included cherry powder. As expected, residual nitrite declined over time (data not shown). However, as long as residual nitrite is not depleted to non-inhibitory levels, impact on bacteria growth is likely to continue *(22)*.

Samples containing antimicrobial A had higher pH (P<0.05) than antimicrobial B when compared to the corresponding nitrate/nitrite source. Samples with antimicrobial A had the lowest L* which could be related to the higher ham pH. L* declined (P <0.05) with time (day 0 and 35, 67.2 to 65.8, respectively). Although means separation occurred among cured samples for a*, values ranged from 14.1-15.2. Therefore, it is unlikely this would impact visual appearance. Naturally cured samples with no antimicrobials had the lowest (P<0.05) b* values but again little numerical difference existed. No differences were found for water activity, fat, moisture, or protein but ash content differed (P < 0.05) among treatments based on amount of ingoing ingredients. Schrader found similar results

for these traits in emulsified sausage *(14)* and Sindelar et al. found no differences in color or pH in naturally and traditionally cured hams *(17).*

Consequently the use of natural curing ingredients combined with selected antimicrobials at least will result in hams that possess similar *L. monocytogenes* inhibitory properties when compared to traditionally cured controls during at least 35 day of storage. The method of natural curing impacted the amount of ingoing nitrite resulting in differences in pathogen growth. Increased pathogen growth in products with lower ingoing nitrite concentrations could be overcome with use of some natural antimicrobials. The natural antimicrobials and natural curing systems may have small impacts on physio-chemical traits of "naturally cured" ham but these are likely to be of little or no practical importance to these products.

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Table 1. Ham formulations

TRT*	Ham kg	Water kg	Salt kg	Sugar kg	$NaNO_2$ ppm	Sodium Eryth[1] ppm	Natural NO_2[1] g	Natural NO_3[1] g	Starter Culture[1] g	LACT DIA[1] g	Antim A[1] g	Antim B[1] g
A	18.14	3.74	0.5	0.3	-	-	-	-	-	-	-	-
B	18.14	3.72	0.5	0.3	-	-	68.1	-	-	-	-	-
C	18.14	3.52	0.5	0.3	-	-	68.1	-	-	-	158.9	-
D	18.14	3.72	0.5	0.3	-	-	-	68.1	5.0	-	-	-
E	18.14	3.52	0.5	0.3	-	-	-	68.1	5.0	-	158.9	-
F	18.14	3.13	0.5	0.3	-	-	68.1	-	-	-	-	540.0
G	18.14	3.13	0.5	0.3	-	-	-	68.1	5.0	-	-	540.0
H	18.14	3.17	0.5	0.3	156	550	-	-	-	570.0	-	-

*Treatment description: A = Uncured control: B= Natural nitrite, no antimicrobial: C=Natural nitrite, Antimicrobial A: D=Natural nitrate, no antimicrobial: E=Natural nitrate antimicrobial A: F=Natural nitrite, antimicrobial B: G=Natural nitrate, antimicrobial B: H= Traditionally cured control

[1] Sodium Eryth = Sodium Erythorbate; Natural NO_2= Vegstable 504 (Florida Food Products, Inc.); Natural NO_3 = Vegestable 502 (Florida Food Products); Starter Culture = CS-Starter Culture299 Bactoferm (*Staphlococcus carnosus,*Chr. Hansen, Inc); LACT/DIA = Purasal Opti.Form PD.4 (Purac America); Antim A = Natural Antimicrobial A (vinegar, lemon and cherry powder blend; Vegstable 507) ; Antim B = Natural Antimicrobial B (Cultured sugar and vinegar blend; Verdad 55);

Table 2. Effect of curing treatment and antimicrobial on means of physiochemical properties of ham products during production and storage

	After Mixing			After Fermentation			Finished Product										
TRT*	Resid. nitrite ppm	Resid. nitrate ppm	pH	Resid. nitrite ppm	Resid. nitrate ppm	pH	Resid. nitrite ppm	Resid. nitrate ppm	pH	L*	a*	b*	water activity	Moisture %	Fat %	Protein %	Ash %
A	0.8^{c}	0.0^{c}	6.04	-	-	-	2.4^{e}	0.6^{e}	6.13^{de}	68.5^{a}	8.6^{d}	11.1^{a}	0.977	74.37	1.95	20.27	3.41^{d}
B	24.8^{b}	8.7^{c}	6.12	-	-	-	23.7^{d}	12.4^{bc}	6.21^{bc}	66.9^{bc}	14.1^{c}	9.5^{cd}	0.975	73.71	2.22	19.70	4.36^{bc}
C	24.4^{b}	13.9^{bc}	6.21	-	-	-	22.6^{d}	13.7^{bc}	6.32^{a}	64.4^{d}	14.7^{b}	11.0^{a}	0.974	73.57	2.08	19.62	4.73^{bc}
D	0.9^{c}	99.0^{a}	6.11	41.2	14.3	6.03	46.6^{a}	7.8^{d}	6.19^{bcd}	66.7^{bc}	14.4^{bc}	9.4^{d}	0.974	73.35	2.23	20.36	4.06^{cd}
E	0.7^{c}	94.7^{a}	6.07	40.4	15.1	6.11	42.8^{ab}	12.7^{bc}	6.25^{ab}	64.3^{d}	14.8^{ab}	10.8^{a}	0.962	72.53	2.22	20.20	5.06^{ab}
F	24.2^{b}	10.0^{c}	6.00	-	-	-	20.5^{d}	11.0^{cd}	6.16^{cde}	66.1^{c}	14.6^{b}	10.0^{b}	0.962	72.18	2.29	19.88	5.64^{a}
G	0.8^{c}	94.8^{a}	5.95	51.0	16.5	5.92	38.5^{b}	15.7^{b}	6.11^{e}	66.5^{c}	14.8^{ab}	10.2^{b}	0.969	71.94	2.33	19.95	5.78^{a}
H	68.1^{a}	30.8^{b}	5.91	-	-	-	31.2^{c}	21.9^{a}	6.09^{e}	67.7^{ab}	15.2^{a}	9.8^{bc}	0.967	71.90	2.34	19.99	5.77^{a}
SE	4.2	4.1	0.21	2.7	1.5	0.05	1.3	0.9	0.02	0.3	0.1	0.1	0.004	0.83	0.12	0.70	0.15

*Treatment description: A = Uncured control: B= Natural nitrite, no antimicrobial: C=Natural nitrite, antimicrobial A: D=Natural nitrate, no antimicrobial: E=Natural nitrate antimicrobial A: F=Natural nitrite, antimicrobial B: G=Natural nitrate, antimicrobial B: H=Traditionally cured control, sodium lactate and diacetate

$^{a\text{-}e}$Means with a common superscript within same column do not differ significantly (P > 0.05).

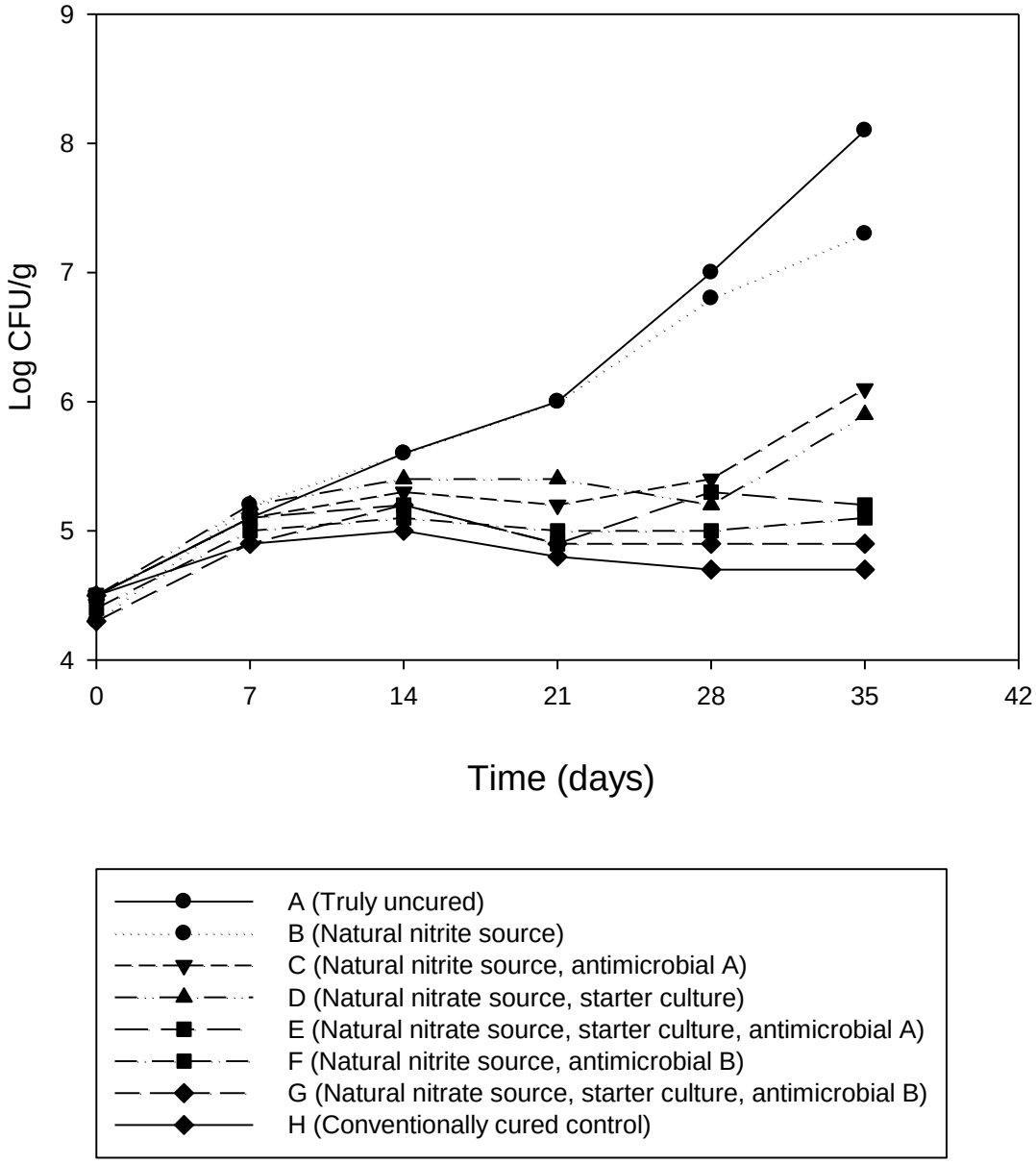

Figure 1. Effect of curing treatments and antimicrobial ingredients on growth of *L. monocytogenes* in ham during storage at 4°C.

CHAPTER 5. NITROSYLATION OF MYOGLOBIN AND NITROSATION OF CYSTEINE BY NITRITE IN A MODEL SYSTEM SIMULATING MEAT CURING

A paper to be submitted to the *Journal of Agricultural and Food Chemistry*.

Gary A. Sullivan and Joseph G. Sebranek

Abstract

Demand is growing for meat products cured without the addition of sodium nitrite. Instead of the direct addition of nitrite to meat in formulation, nitrite is supplied by bacterial reduction of nitrate which is added in the form of vegetable juice concentrate or powder. However the rate of nitrite formation in this process is relatively slow and total ingoing nitrite is typically less than when nitrite is added directly to the meat mixture in conventional curing processes. The objective of this study was to determine how the rate of addition and amount of nitrite added might impact nitrosylation/nitrosation reactions in a model system containing myoglobin and cysteine. Myoglobin was preferentially nitrosylated. No decreases in sulfhydryl groups were found until maximum nitrosylmyoglobin color was achieved. The cysteine plus myoglobin model retained a greater number of sulfhydryl groups than in the cysteine-only model. The rate of nitrite addition did not alter nitrosylation/nitrosation reactions. These data suggest that the amount of nitrite impacts the nitrosylation/nitrosation reactions that occur in a cured meat system but the rate of nitrite addition or formation is not important.

Keywords: Sodium nitrite, cysteine, myoglobin, nitrosylation, cured meat model

Introduction

Curing meat incorporates a complex set of chemical reactions some of which are not fully understood. Meat preservation by meat curing has been documented for over 5000 years and likely began by using salt contaminated with saltpeter (calcium or potassium nitrate) to preserve meat (*1*). In the 1890's, it was determined that nitrite, not nitrate, was necessary for cured meat production (*2*). Concerns about nitrate, nitrite, and n-nitrosamine formation surfaced following illnesses in animals fed fishmeal produced with sodium nitrite (*3, 4*) but the National Academy of Science has supported the safety and continued use of sodium nitrite and nitrate in food products (*5, 6*). Growing evidence now supports the importance of nitrite and nitrate in many biological functions (*7*). Still, a significant number of consumers have shunned the use of these and other common food ingredients as indicated by the rapid growth observed in the natural and organic food market (*8, 9*). Although research does not show health benefits in consuming organic versus conventionally produced foods (*10*), perception of improved healthfulness is one of the commonly cited reasons for purchasing these foods (*11, 12*).

Due to United States Department of Agriculture (USDA) regulations governing natural and organic foods, sodium nitrite and nitrate are among the many commonly used ingredients that are not allowed these classes of foods (*13-16*). However, by utilizing natural nitrate sources, primarily celery juice/powder, and a nitrate reducing starter culture, nitrite can be produced in natural and organic processed meats to produce characteristics of conventionally-cured products produced with direct addition of sodium nitrite (*17, 18*). While naturally cured products look and taste like traditionally cured meats, Jackson et al. (*19*) and Schrader (*20*) found increased growth of *Clostridium perfringens* and *Listeria*

monocytogenes, respectively, in naturally cured products. Many factors could impact pathogen growth but it is likely related at least in part to the curing process. Lower ingoing nitrite concentrations have been reported for naturally cured meats (*17*) but this observation could be affected by the rate that nitrite is formed or added to the curing mixture if concentration of nitrite impacts the curing reactions. For example, when using bacterial reduction of nitrate, nitrite is slowly being added to the system which could shift the various reactions in favor of those with greater substrate reactivity. The addition of all the nitrite at once, which occurs in conventional curing might, result in a different proportional distribution of nitrite amount the various reaction substrates in a meat mixture. This has implications for differential effects of nitrite for creating the typical cured meat properties of antimicrobial protection, color development, and flavor protection.

Meat is a complex system that makes measurement of chemical or biological reactions difficult. Early work used ^{15}N isotopes to determine the fate of nitrite in cured meats (*21-23*) and identified the partition of nitrite in a meat mixture but did not identify specific reactions and did not clarify the complexity. During curing, myoglobin and cysteine are known to undergo nitrosation/nitrosylation (*24*). Myoglobin-nitrite chemistry is among the most well understood of many cured meat reactions (*25, 26*). Cysteine has been shown to act as a nitrite reducing compound and nitrosating/nitrosylating agent in cured meats (*27, 28*). Creating a model system with these compounds could provide a simplified method to determine nitrite reactions as a result of the rate of addition of nitrite to the system. These compounds can be measured relatively easily and could provide insight into about alteration of nitrosated/nitrosylated compounds in natural and traditional meat curing systems. The objective of this study was to use a simplified model system of cysteine and myoglobin to

test the hypothesis that the amount or rate of addition of sodium nitrite will affect some of the reactions commonly occurring during meat curing.

Materials and Methods

Solution Preparation and Model System

A cysteine and myoglobin model was prepared to evaluate nitrosation/nitrosylation reactions. Final concentrations in the cysteine plus myoglobin model solution were cysteine (5.06 mM), myoglobin (0.029 mM), and nitrite (0, 0.72, 0.181, 0.362, 0.725, 1.087, 1.450, and 3.623 mM). Final concentrations in the cysteine-only model solutions were cysteine (5.06 mM), and nitrite (0, 0.72, 0.181, 0.362, 0.725, 1.087, 1.450, and 3.623 mM). Prior to the addition of nitrite in this study, the concentrations used in the cysteine plus myoglobin and cysteine-only model system were approximately half of those found in fresh ham. With these, the myoglobin and cysteine concentrations in the model solution would be about half of those typically found in fresh ham (*29, 30*). The nitrite concentrations were equivalent to ingoing sodium nitrite (based on a hypothetical meat block) of 0, 10, 25, 50, 100, 150, 200 and 500 ppm. United States regulations allow the addition up to 200 ppm of sodium nitrite to meat when nitrite is added in a brine solution (*31*).

Two 0.1 M phosphate (potassium phosphate, monohydrate) buffer solutions were prepared at pH 5.6 and 7.4. A 0.117mM stock myoglobin solution was prepared using 0.3 g myoglobin from equine skeletal muscle (Sigma Aldrich Co., St. Louis, MO) in 150 mL pH 5.6 phosphate buffer solution. A 20.25 mM stock cysteine solution was prepared with 0.7980 g L-cysteine hydrochloride hydrate (Acros Ogranic, Geel, Belgium) in 250 mL ph 5.6 phosphate buffer solution. Cysteine solutions were utilized immediately following

preparation to limit the reduction of sulfhydryl groups due to oxidation and disulfide bond formation.

Sodium nitrite stock solution was prepared by mixing 1 g sodium nitrite in 1 L distilled water (14.49 mM) and diluting with distilled water to 0.144, 0.362, 0.724, 1.449, 2.173, 2.899, and 7.246 mM solutions. Distilled water was used for 0 nitrite concentration. Ellman reagent was prepared with 0.1586 g 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) mixed with 20 mL phosphate buffer pH 7.4 (20 mM). Nitrite reagents, sulfanilamide and N-(1-naphtyhyl) ethylenediamine dihydrochloride (NED), were prepared as described by AOAC method 973.31 (*32*).

Stock cysteine solution was mixed 1:1 with stock myoglobin solution for the cysteine plus myoglobin model. Stock cysteine solution was also mixed 1:1 with pH 5.6 phosphate buffer for the cysteine-only model. Duplicate test tubes containing 5 mL of cysteine plus myoglobin or cysteine-only solutions were prepared for each nitrite concentration. Samples used to simulate traditional curing had all the sodium nitrite added at the beginning while, for the simulated natural cure, nitrite was slowly added over time to simulate bacterial reduction that occurs in natural curing. For tubes simulating traditional curing, 5 mL of diluted nitrite solution was added at the start of the experiment. To simulate natural curing, 1 mL of diluted nitrite solution was added to start. All nitrite concentrations were evaluated. All tubes were capped and placed in a 35 ° C water bath for 60 min to simulate a bacterial reduction step found in the natural curing. In the natural curing model, 1 ml of nitrite solution was added every ten minutes after the initial amount to reach 5 ml during the 60 min period. After 60 min, samples were placed in a 75 ° C water bath for 30 minutes to simulate cooking. Three

independent replicates were conducted and duplicate samples were prepared for each treatment combination in each replicate.

Sulfhydryl Concentration

Sulfhydryl concentrations in the model system mixtures were determined using a modified Ellman's Reaction (*33*). In test tubes, 2.97 ml of phosphate buffer pH 7.4 and 0.03 ml of the sample solution were combined with 0.015 mL DTNB. Samples were vortexed immediately following DTNB addition. After color development, samples were read using a spectrophotometer at 412 nm using a 1 cm cuvette with phosphate buffer as a blank. Absorbance of 0.015 DNTB in 3.0 ml phosphate buffer (7.4 pH) and 0.015 DTNB in 0.0725 mL myoglobin stock solution with 2.9925 ml phosphate buffer (pH 7.4) were recorded for measurement adjustment. A conversion factor of 1.415 M^{-1} cm^{-1} was used to determine sulfhydryl group concentration. Samples were read and recorded following the simulated bacterial reduction and cooking steps. Each sample was prepared in duplicate.

Cured Color

Cured color (nitric oxide myoglobin) was measured directly on the sample solution using absorbance at 535 nm in a 1 cm cuvette. Only samples in the cysteine plus myoglobin model were measured for cured color following the cooking step.

Residual Nitrite

Residual nitrite was measured using AOAC method 973.31(*28*) with modifications. For each sample, 3.6 mL water and 0.4 mL sample solution were placed in a test tube. Sulfanilamide reagent, 0.22 mL, was added to each test tube and mixed. After 5 min, 0.22

mL NED reagent was added, mixed and allowed to stand for 15 min. Samples were read at 540 nm in a 1 cm cuvette on a spectrophotometer. A solution of 4.5 ml water and 0.25 mL of each sulfanilamide and NED reagents was used for the blank. A standard curve to calculate residual nitrite was created as described in the original method. Residual nitrite was measured in the experimental samples following the simulated bacterial reduction and cooking steps.

Statistical Analysis

Data were analyzed using proc GLIMMIX procedure of SAS (v 9.2, SAS Corp, Cary, NC) in a factorial design including solution (cysteine-only or cysteine plus myoglobin), sodium nitrite concentration, and rate of sodium nitrite addition. When significant treatment effects (P <0.05) were identified, means separation was conducted using LSMEASN procedure. Results following the bacterial reduction and cooking simulations were analyzed separately.

Results

Nitrite reacts with myoglobin and cysteine during meat curing (*24*). Results of measurement of sulfhydryl groups remaining on cysteine in the presence of different concentrations and rates of addition of sodium nitrite are found in Figure 1. Reduction of sulfhydryl groups was used as an indication of nitrosocysteine formation. Following both simulated bacterial reduction and cooking, significant treatment effects were found for model, cysteine-only and cysteine plus myoglobin, and for ingoing nitrite concentration (P < 0.05). Sulfhydryl groups decreased as in going nitrite increased. Following the simulated bacterial reduction, 75.4% of the cysteine sulfhydryl groups were recovered when no nitrite

was added. No difference was found for the rate of addition of nitrite either following the bacterial reduction or cooking simulation steps ($P > 0.05$). Following the simulated reduction step, the cysteine plus myoglobin model had greater sulfhydryl groups remaining than the cysteine-only model. Equine myoglobin does not contain cysteine (*34*) and preliminary work showed myoglobin alone did not react with Ellman's reagent. Ingoing nitrite concentrations of 0, 10, and 25 ppm resulted in similar sulfhydryl concentration while all other concentrations were significantly different ($P < 0.05$) from each other. Following simulated cooking, similar results were found. The cysteine-only model had fewer sulfhydryl groups than the cysteine plus myoglobin model. Samples with 10 ppm ingoing nitrite resulted in the greatest number of sulfhydryl groups, followed by 0 and 25 which were similar. Within model and simulated processing step, all other ingoing nitrite concentrations were statistically different each other and declined with increased ingoing nitrite.

Cured color development was measured by absorption in the red visible region where cured meat pigment absorption maximum occurs (Figure 2). Ingoing nitrite ($P < 0.0001$) but not rate of addition ($P = 0.643$) had significant main effects. Regardless of rate of addition, 0 ppm ingoing nitrite had a significantly lower absorbance than all other nitrite concentrations ($P < 0.05$). Ingoing nitrite concentration of 200 and 500 ppm resulted in greater absorbance from nitrosylhemochromogen than 10 ppm ($P < 0.05$). All other ingoing nitrite concentrations (25 to 500 ppm) had similar absorbance. This plateauing effect of color formation is expected. General consensus suggests that 40-50 ppm of ingoing nitrite is required for stabile cured color but higher concentrations are needed for bacteria suppression (*31*).

Residual nitrite concentration following simulated reduction and cooking are found in Figure 3 and Figure 4, respectively. Significant treatment effects were found for rate of

addition (P =0.020) and ingoing nitrite x model interaction (P = 0.002). When nitrite was added slowly to simulate a natural curing process, higher residual nitrite concentrations were found than in the traditionally cured simulation, 34.3 and 31.3 ppm, respectively across all concentrations and can be seen in Figure 3. A significant interaction of ingoing nitrite and model system was found following the simulated bacterial fermentation (P <0.05). Residual nitrite was similar between models with lower concentrations of ingoing nitrite but the cysteine-only model was significantly greater residual nitrite than the cysteine plus myoglobin model at 150, 200, and 500 ppm of ingoing nitrite. For results averaged across substrate and curing model treatments following the simulated fermentation step, the percentage of ingoing nitrite recovered as residual nitrite varied very little above 25 ppm ingoing nitrite with a range of 47.9 – 50.8 % but a greater portion of ingoing nitrite was recovered as residual nitrite (80.8% and 55.4%) for 10 and 25 ppm ingoing nitrite, respectively. Following the simulated cooking step, No significant treatment effects were found for the rate of addition for simulated cooking (P = 0.780). Similar to the reduction simulation, a significant ingoing nitrite and model interaction (P = 0.008) was found following simulated cooking. Residual nitrite in the cysteine-only model was greater than the cysteine plus myoglobin model only for 500 ppm of ingoing the nitrite and is likely due to the ratio of substrate to nitrite. For results averaged across substrate and curing system models following the simulated cooking step, the proportion of ingoing nitrite recovered ranged from 39.2 – 47.4% for the 100-500 ppm treatments. Residual nitrite changed less than 1 ppm for 10, 25, and 50 ingoing nitrite treatments between the simulated bacterial reduction and cooking steps.

The rate of addition of sodium nitrite was only significant for residual nitrite following the simulated reduction step. The rate of addition had no impact on sulfhydryl concentration at either time point, or on cured color formation and residual nitrite concentration following the cooking simulation step.

Discussion

S-nitrosothiol groups are formed through the reaction of a sulfhydryl group and a nitrosylating agent such as dinitrogen trioxide, N_2O_3, but not nitric oxide directly (*35*). Peterson et al. produced S-nitrosocysteine in mildly acidic conditions with equal molar concentrations of nitrite and cysteine and reported the formation of over 90% nitrosation of cysteine (*36*) in conditions similar to this experiment. Nitrosation of cysteine and other thiol groups have been shown to have many important biological functions as a cellular signaling molecules than can (*37*) release nitric oxide to regulate blood flow (*38*), modify metabolic rates and oxygen consumption (*39*) among many others. When aqueous solutions are exposed to oxygen, Rehder and Borges (*40*) found that disulfide bonds form non-enzymatically via a sulfenic acid, RSOH, intermediate. The presence of trace metals such as iron or copper increased the rate disulfide bond formation. The non-enzymatic disulfide bond formation observed by Rehder and Borges (*40*) may explain why only 75% of the ingoing cysteine sulfhydryl groups remained intact even when no nitrite was added. Interestingly in this experiment, with the addition of 10 ppm of sodium nitrite, greater remaining sulfhydryl concentrations were observed than with 0 ppm. Nitric oxide is able to stabilize heme and bind free iron (*41*) and rapidly consumes oxygen (*42*) which may limit the non-enzymatic disulfide bond formation. When greater than 10 ppm of nitrite was added, the

decrease in sulfhydryl groups was likely due to nitrosylation of cysteine. It is unlikely that nitrosocysteine remained in the mixture following the cooking simulation due to the thermal instability of nitrosocysteine and likely disulfide bond formation and nitric oxide release (*35*). In biological systems and cured meats, it is likely that S-nitrosothiols serve as reactionary intermediate and nitric oxide donor or reducing agent.

The effect of nitrite on heme pigments has been studied for over 140 years. In 1868, Gamgee reported the browning of blood that we know as methemoglobin formation when nitrite was added (*43*). At the turn of the 20^{th} century, Haldane characterized cured meat pigment as nitric oxide hemochromogen (*44*). Ingoing nitrite above 25 ppm did not provide increased cured pigment formation in this study which is the same ratio of nitrite to myoglobin as the current consensus that 40-50 ppm sodium nitrite provides acceptable cured color (*31*). No change in sulfhydryl concentration was observed until ingoing nitrite reached 25 ppm after cured pigment formation plateaued. Additionally, fewer sulfhydryl groups remained in the cysteine-only model than in the cysteine plus myoglobin model following nitrate reduction simulation. The binding rate constants for nitrosylation of sulfhydryl and heme groups, are 4.5×10^5 mol^{-1} Sec^{-1} and 2×10^7 mol^{-1} Sec^{-1}, respectively (*45, 46*). This suggests that myoglobin is nitrosylated more quickly than cysteine is nitrosated and is likely explains the formation of cured color prior to cysteine nitrosation. Previous research has shown that nitrosocysteine added to turkey provided cured color, antioxidant activity, and anticlostridial activity similar to those produced with sodium nitrite (*28*) suggesting that nitrosocysteine acts as a nitric oxide donor. Concentration of in going nitrite impacts pathogen growth. O'Leary and Solberg determined that between 100-180 ppm of ingoing nitrite inhibited *Clostridium perfringens* and decreased glycolytic enzyme activity by

sulfhydryl nitrosation (*47*). Xi et al. found that greater ingoing nitrite up to 150 ppm nitrite resulted in lower *Listeria monocytogenes* growth (*48*). The USDA recommends 120 ppm of ingoing nitrite to assure product safety in all 'Keep Refrigerated' cured meats unless other methods of microbial control are utilized (*31*). Greater ingoing nitrite concentrations are needed to provide antimicrobial control than for color development in cured meats. These data suggest that myoglobin is preferentially nitrosylated before cysteine is nitrosated and that the rate of addition of nitrite does not shift nitrosation/nitrosylation products formed during meat curing.

Differences were found for rate of addition of nitrite following simulated reduction but no differences were found following cooking. Cassens indentified many factors that impact residual nitrite (*49*) of which most were controlled in this model. While cysteine plus myoglobin have both been shown to have nitrite reducing capabilities (*27, 50*), the differences in residual nitrite within treatments and ingoing nitrite concentration was due to the total amount of nitrite reactive compounds (myoglobin and cysteine). In meat products, residual nitrite provides cured color stability and pathogen control during storage (*51, 52*).

Naturally cured products have similar cured meat characteristics (*18*) but these types of products have less pathogen controls (*19, 20*) than conventionally cured meats. The amount of ingoing nitrite affects the extent of nitrosation/nitrosylation reaction product formation. However, natural curing does not appear to result in a significant shift of nitrite reaction products and this would suggest that it is more important to increase the amount of ingoing nitrite than increase the rate of nitrite formation. This may not hold true for other nitrosation/nitrosylation reaction substrates found in meat or with the use of cure accelerators. Thus it appears that the slow release of nitrite in naturally cured products does

not affect or shift the amount of nitrite between reaction intermediate in this study but the addition of substrates and reactions should be investigated. This system provides a basis for additional research to evaluate reducing agents and substrates.

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Figures

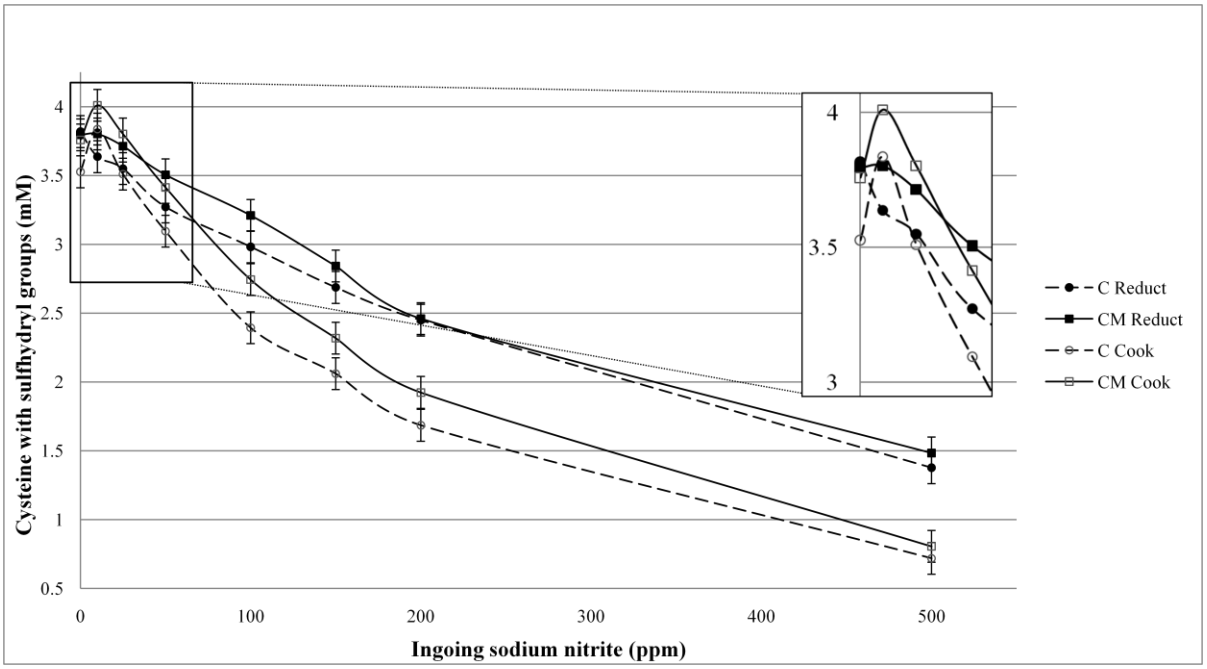

Figure 1. Concentration of cysteine with intact sulfhydryl groups. C = Cysteine-only model; CM = Cysteine plus myoglobin model; Reduct = Sample evaluated following simulated bacterial reduction; Cook = Sample evaluated following cooking simulation.

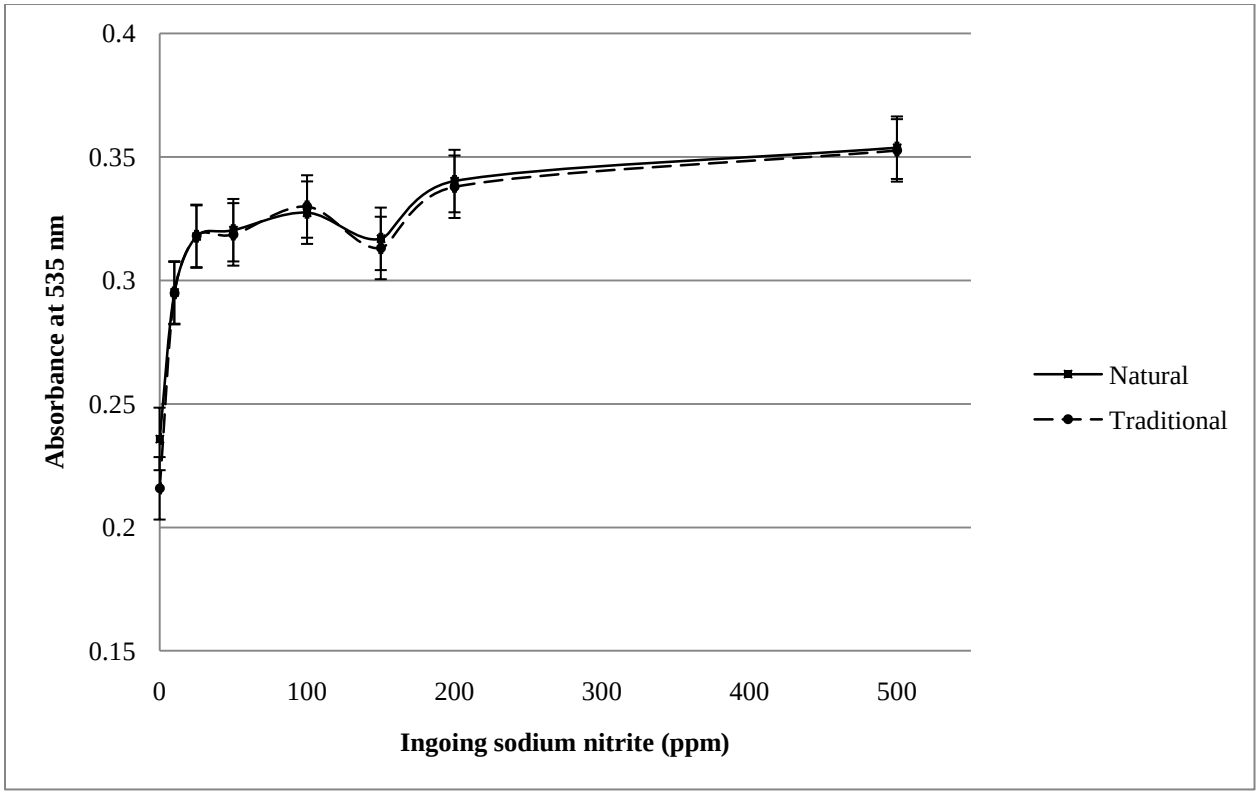

Figure 2. Absorbance of cysteine plus myoglobin model system as an indicator of nitrosylhemochromogen formation. Tradition = entire sodium nitrite solution added at beginning to simulate traditional curing; Natural = sodium nitrite solution added in 1 ml increments for simulated bacterial reduction of nitrate to simulate natural curing.

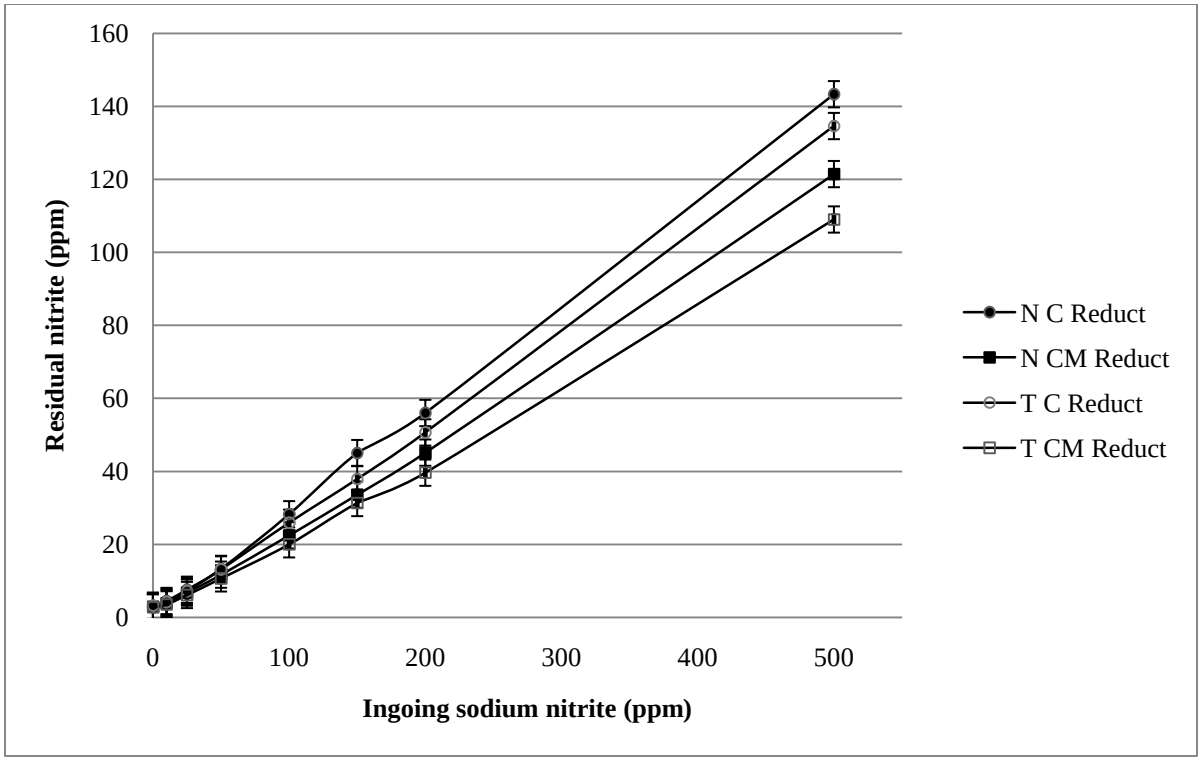

Figure 3. Residual nitrite content based on ingoing nitrite, rate of addition, and model system following simulated bacterial reduction of nitrate. C = Cysteine-only model; CM = Cysteine plus myoglobin model; N = sodium nitrite solution added in 1 ml increments for simulated bacterial reduction of nitrate to simulate natural curing; T = entire sodium nitrite solution added at beginning to simulate traditional curing.

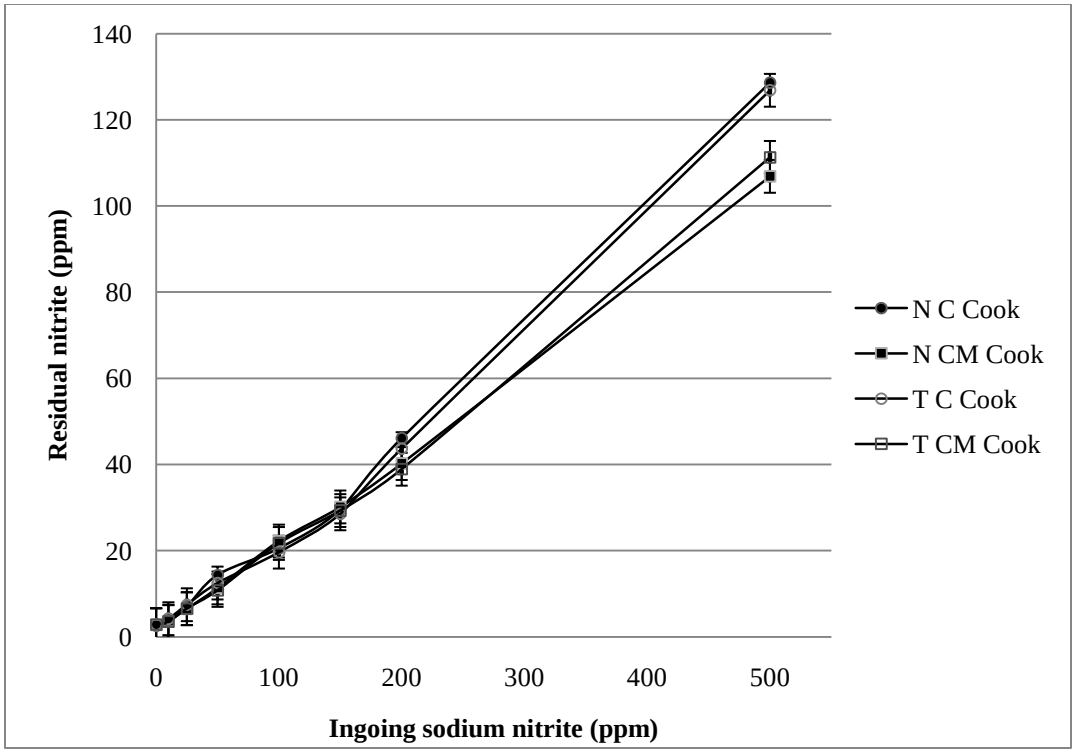

Figure 4. Residual nitrite content based on ingoing nitrite, rate of addition, and model system following simulated cooking. C = Cysteine-only model; CM = Cysteine plus myoglobin model; N = sodium nitrite solution added in 1 ml increments for simulated bacterial reduction of nitrate to simulate natural curing; T = entire sodium nitrite solution added at beginning to simulate traditional curing.

CHAPTER 6. GENERAL CONCLUSIONS

Consumers' concerns about nitrite and nitrate consumption led meat processors to begin manufacturing products without sodium nitrite or nitrate. The USDA created special labeling requirements for these products requiring 'Uncured' and the common name on the label. The original products within this class were different than traditional cured meats as processors eliminated but did not attempt to replace nitrite. Now, many products within this category utilize a natural nitrate/nitrite source and have traditional cured meat characteristics. Although these products appear cured, greater bacterial growth can occur and could result in foodborne illness outbreaks. Greater sodium nitrite concentrations are required for microbial suppression/inhibition than to provide stable cured meat characteristics. Changes in product composition, added ingredients, and traits related to the curing reaction all affect pathogen growth. Commercially available natural antimicrobials can suppress pathogen growth but the amount of sodium nitrite impacts the effectiveness of these antimicrobials. Although nitrite is slowly formed by bacterial reduction of naturally cured meats, it does not seem to affect nitrosation/nitrosylation reactions. As expected, the amount of sodium nitrite has an influence on the extent of nitrosation/nitrosylation reactions that would be found during curing. These data suggest that methods to increase ingoing nitrite concentrations could provide greater assurances of product safety than current naturally cured meats. Increased ingoing nitrite concentration in combination with natural antimicrobials may allow processors to produce naturally cured meats with quality and safety characteristics comparable to their conventionally cured counterparts.

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