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INVESTIGATION OF QUALITY ATTRIBUTES AND INHIBITION OF FOODBORNE PATHOGENS IN "NO-NITRATE OR NITRITE-ADDED" BACON

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by

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ABSTRACT

Processed meats manufactured using natural curing ingredients may exhibit color, flavor and shelf-life similar to traditional products. Naturally-cured meat products are labeled "no-nitrate or nitrite-added" and research has focused on hams and cooked sausages. There is limited research on the ability of these ingredients to inhibit foodborne pathogens. The objective of the research was to investigate quality attributes and inhibition of foodborne pathogens of "no-nitrate or nitrite-added" bacon. Two natural cure ingredients were investigated: one that required the reduction of nitrate (natural nitrate) during manufacturing, and the other, was a product that has been pre-reduced (natural nitrite). Three studies were conducted. Preliminary research found that the reduction of nitrate may be inhibited by salt. Nitrate reduction for 12 hours produced 48 ppm nitrite without salt vs 1 ppm nitrite with salt. Pre-converting without salt may save time during manufacturing. A second study investigated the quality attributes of "no-nitrate or nitrite-added" bacon. Bacon cured with natural nitrate with starter culture could have similar residual nitrite levels as sodium nitrite-cured bacon (44 ppm for naturally-cured vs 38 ppm for conventionally-cured at day 35). Natural nitrate or natural nitrite-cured bacon faded in color more quickly than bacon cured with sodium nitrite (a* value 3.14 vs 5.37) ($P < 0.05$) than all other treatments. The final study investigated the inhibition of foodborne pathogens: *Salmonella* Typhimurium (ST), *Escherichia coli* O157:H7 (EC), *Listeria monocytogenes* (LM), *and Clostridium perfringens* (CP) in brine and bacon. The brine study we found that brines with natural nitrate, natural nitrite, and sodium nitrite all decreased pathogen growth (ST, LM, CP) in solution to a similar degree (less than $\log_{10} 2$ CFU/mL) ($P > 0.05$) within 24 hours. The bacon phase showed no differences in pathogen reduction in a bacon system at the end of the 21-day shelf-life study for all cures. However, when treatments were pooled over storage time sodium nitrite was more effective at slowing CP growth than all other treatments ($\log_{10}$ 1.9 CFU/g for naturally-cured vs $\log_{10}$ 0.6

CFU/g for sodium nitrite-cured). We concluded that “no-nitrate or nitrite-added” bacon is less red and inhibits CP less than conventionally-cured bacon.

Keywords: bacon, natural, nitrate, nitrite, color, foodborne pathogens

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Chapter 1

Introduction

The people involved in the industry of cured/processed meats have made many advances from its conception. Meat curing is one of the world's oldest forms of food preservation and began around 4000 B.C. At the time, salt contaminated with saltpeter (salt most likely contaminated with potassium nitrate) was used to cure/preserve meat. Many things have changed when it comes to meat curing, but the same basic concepts still apply today.

Sodium nitrite was approved for use by the USDA in 1925 (Kerr et al., 1926). It was not until the late 1960s that nitrite came under attack because of its link to nitrosamine formation and a possible link to cancer (Newberne, 1979). Bacon is a special situation, due to concern for possible nitrosamine formation during frying. The regulations for pumped or massaged bacon specify a target of 120 ppm in-going nitrite, in combination with 550 ppm of erythorbate in-going, or an equivalent reducing agent. Dry-cured products are allowed to have 625 ppm sodium nitrite in-going. The establishment of minimum ingoing nitrite concentration is considered critical to subsequent product safety (Sebranek & Bacus, 2007). Since in-going nitrite and nitrate are quite variable in natural and organic cured meat products, the safety of the product can be a concern as in-going and residual nitrite are important at inhibiting pathogen growth. Thus, USDA requires such products to carry special labeling that highlights the fact that these are not typical cured meat products (9 CFR 319.2).

In recent years, naturally-cured meat products have been introduced into the market place and into the news media. Consumers are questioning whether naturally-cured meat products are also cured with nitrite (Applegate, 2011). Applegate Farms has petitioned the USDA to reconsider their position on the labeling of naturally-cured products, requesting that products

made with natural cure ingredients no longer be labeled as “uncured”. Applegate Farms concedes that their natural processed meat products contain nitrate and/or nitrite (even though they are naturally-occurring) (Applegate, 2011). Applegate Farms has petitioned the USDA-FSIS to add curing agents made from vegetable juices to the chart of approved substances in the preparation of meats (9CFR424.21(6)(c) and/or the Directive 7120.1 “Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products” as an alternative ingredient for the purpose of curing meats. Applegate would also like the term “uncured” removed from the label if vegetable juices or powders are added to the list. The term “uncured” is misleading as naturally-occurring nitrates and nitrites are found in these products and the products have conventionally-cured meat characteristics. The USDA is considering the request and that ”uncured” may be confusing and misleading to consumers (USDA, 2011). Currently, the USDA has delayed making a ruling until further research can be conducted, with respect to the inhibition of *Clostridium botulinum,* in naturally-cured products.

Current research concerning natural cure ingredients has focused on ham and cooked sausage. However, bacon is an interesting product with respect to naturally-cured products. USDA regulations do not permit the direct addition of nitrate in bacon because of the possibility of nitrosamine formation, due to residual nitrite. In the United States, bacon is cured with sodium nitrite. Yet, naturally-cured bacon may be manufactured with unregulated amounts of nitrate from vegetable juice powder or other natural flavorings. The amount of residual nitrite in naturally-cured bacon becomes a concern because of the potential of nitrosamine formation in fried bacon.

Currently in the United States, products in which part of their standard identity is from the addition of nitrate or nitrite are permitted to be made without nitrate or nitrite (9 CFR 319.2). However, the term “uncured” in the same size and style of lettering as the rest of the standard

name, must appear on the label. In addition, the term "no-nitrate or nitrite-added except those naturally occurring in …." must appear on the label. The product needs to be similar in size, flavor, consistency, and general appearance as the product made with nitrate or nitrite (9 CFR 319.2). The "uncured" product is labeled as "no-nitrate or nitrite-added" because it was not made with sodium or potassium nitrate or nitrite, even though natural curing ingredients contain nitrate and nitrite. As previously mentioned, it has been proposed to eliminate the "uncured" and "no-nitrate or nitrite-added" from the label of products that have been naturally-cured (Applegate, 2011). Throughout the dissertation, "no-nitrate or nitrite-added" and "naturally-cured" will be used interchangeably as there is continuing debate on the labeling of these products.

Research is warranted in regard to bacon as residual nitrite is a concern because of the possible formation of nitrosamines during cooking. Additionally, the USDA wants more research in regard to foodborne pathogen inhibition (Applegate, 2011). Therefore, the overall objective in the following investigation was to investigate the quality attributes and inhibition of foodborne pathogens in "no-nitrate or nitrite-added" bacon.

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Chapter 2

Review of Literature

History of meat curing

Curing is an ancient process, whereby meat is preserved by the addition of salt. In their textbook, "The Meat We Eat," Romans et al., (2001) traced the origins of salt curing of meat to the Sumerian culture, which emerged in the Tigris and Euphrates valleys, about 4000 B.C. It is commonly accepted that the salt used in early meat curing was contaminated with salt peter (potassium or sodium nitrate), which contributed to the sustained red color of cured meat. Sea salt, a form of salt easily available in early times, contains up to 1.78 ppm of nitrate (Cantoni et al., 1978). Therefore, the multifunctional contributions of curing ingredients to cured meats began to become clearer.

The term "cure" can be used as a noun or a verb. According to Sebranek (2010) and Pegg and Shahidi (2000), "to cure" means to add nitrite and/or nitrate with salt to a meat product to achieve preservation. Additionally, the term "cure" is often used to describe the chemical entities of nitrite and/or nitrate especially when utilized with salt for meat preservation. Moreover, the terms "curing" or "cure" are typically used when nitrite and/or nitrate is added to a product.

Meat curing has been associated traditionally with processed meats for the purpose of altering color, flavor, safety, and shelf-life characteristics (Sebranek & Fox, 1985). Such characteristics make these products unique, when compared to fresh meat products. It is not clear from published literature when meat processors became aware that nitrate was a critical ingredient for successful meat curing. However, experiments in the late 1800s demonstrated that nitrate in the cure was converted to nitrite by nitrate-reducing bacteria as nitrite was found in cured meat and in curing pickle when only nitrate was added initially (Polenske, 1891). Additionally, Lehman (1899) and Kisskalt (1899), both concluded that typical color of cured meats could be attributed to nitrite and not to nitrate. Nitrite, added directly or

derived from nitrate, is required for meat curing reactions. There is no known substitute for nitrite that imparts similar color, flavor and antimicrobial actions in cured meats (Sebranek & Bacus, 2007b).

The use of nitrate and nitrite for meat curing became controversial in the 1970's following a report that carcinogenic nitrosamines might be formed in cured meat products (Lijinsky and Epstein, (1970). The nitrite controversy led to much research in the past 50 years, most of which focused on the development of a meat curing system, including cure accelerators, new equipment, water-binding ingredients, and USDA regulations governing water retention and addition in processed meats.

Nitrate

Nitrate may be reduced to nitrite in raw meat when a microorganism is present (Polenske, 1891; Hustad et al., 1973). When nitrate is consumed as part of the diet, about 16% of it is converted to nitrite in the oral cavity (Van Maanen et al., 1996). Nitrite and the remainder of nitrate pass into the digestive tract where they may react with other digesta or be absorbed from the digestive tract, into the blood. Dietary nitrate is rapidly absorbed into the bloodstream. The dietary nitrate mixes with endogenous nitrate from the nitric oxide (NO) pathway (Gladwin et al., 2005). A large portion of nitrate is then taken up by salivary glands, secreted with saliva and reduced to nitrite by symbiotic bacteria in the oral cavity. Nitrite derived from saliva is further reduced to NO and other biologically active nitrogen oxides in stomach acid. Any remaining nitrite is absorbed rapidly and accumulates in tissue, where it serves to regulate cellular functions through the reduction to NO or by direct reactions with protein and lipids. Nitrite and NO are eventually oxidized to nitrate, which again enters the enterosalivary circulation or is excreted in urine.

Nitrate in food may also be subject to reduction to nitrite. Certain types of bacteria have the ability reduce nitrate to nitrite over a wide temperature range. However, the time it takes to convert the nitrate to nitrite varies (Cassaburi et al., 2005). Meat mixtures are typically contaminated with bacterial cultures, which can be encouraged to grow by providing an extended period of time after salt and curing

agents have been added (Leroy et al., 2006). The salt in the meat system will inhibit many spoilage bacteria, while allowing salt-tolerant cocci and coagulase-negative *Staphylococcus* stains to grow and reduce nitrate to nitrite (Leroy et al., 2006).

By the early 1920s, relatively little nitrate was being used in cured meats (Binkerd and Kolari, 1975). Today nitrate is used in specialty products, such as dry-cured hams and dry sausage (Pegg and Shahidi, 2000). The use of nitrate is necessary in products like these because of the long, slow curing processes that require a long-term source of nitrite that can be slowly released over the course of the process (Pegg and Shahidi, 2000).

The following is a diagram of the reduction of nitrate to nitrite adapted from Bacus (2006). Nitrate is reduced to nitrite by bacteria that are capable of producing nitrate reductase. In an acidic environment, nitrite is reduced to nitrous acid and through a series of chemical reactions; nitrate, nitric oxide, and water are formed.

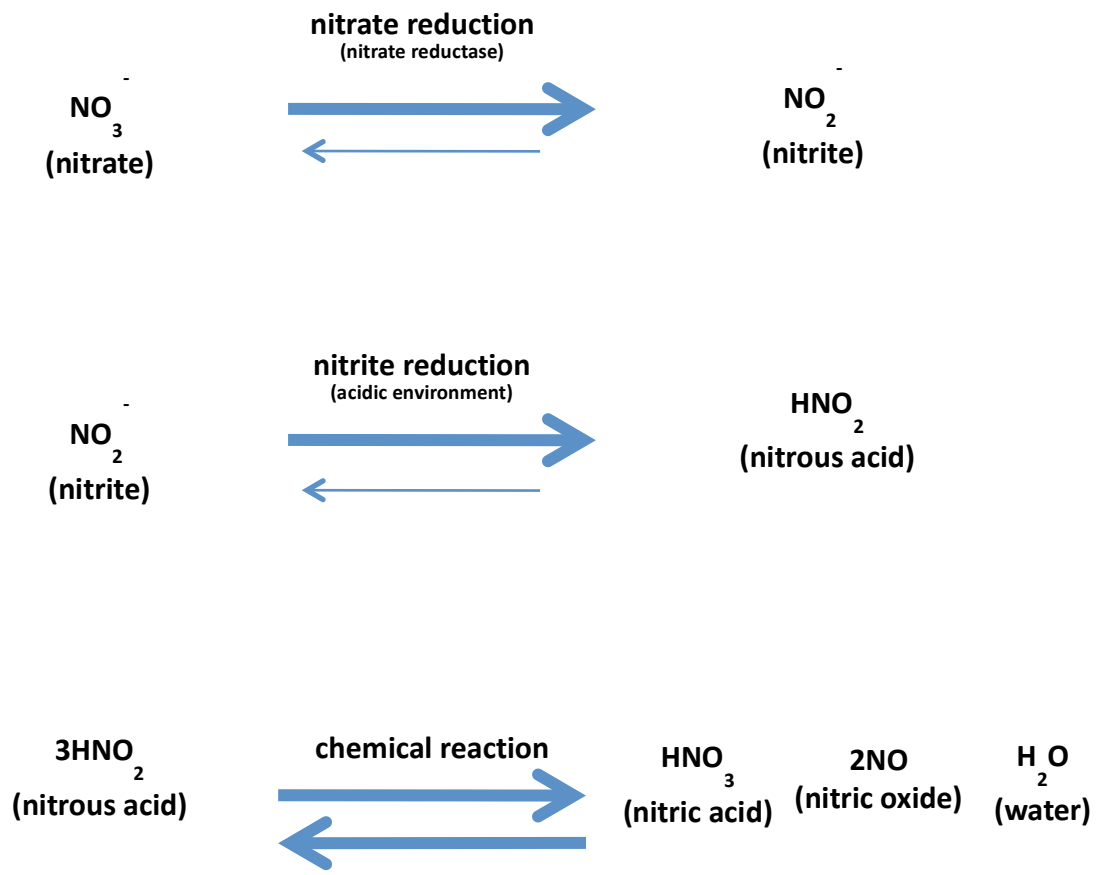

Figure 2-1 Reduction of nitrate to nitrite.

Regulations

The USDA permits the maximum use of nitrate at 1,718 ppm for comminuted products (ground products such as hot dogs) based on meat block (weight of meat only, also known as green weight) (USDA, 1995). Massaged (brine is incorporated into meat with tumbling) and pumped products (meat injected with brine) are limited to700 ppm in-going nitrate, based on meat block. Dry-cured meat products are allowed to have 2,187 ppm in-going nitrate. However, the combination of nitrate and nitrite cannot exceed 200 ppm of analytically-measured nitrite, calculated as sodium nitrite in the finished product.

Nitrite

The use of nitrite along, with or in place of nitrate, allows for much faster production of cured meats in modern commercial processing facilities (Cassaburi et al., 2005). Addition of nitrite in one form or another, results in the distinctive characteristics of cured meat (Cassens, 1990a), including the development of cured color and flavor, as well as antioxidant and antimicrobial properties (Shahidi and Pegg, 1992). Nitrite was approved for use in meat by the USDA in 1925 (Pearson and Tauber, 1984). Kerr et al. (1926) conducted the research that enabled the USDA to come to this landmark regulation. The authors investigated residual nitrate and nitrite levels, spoilage, and sensory qualities. It was determined that the use of nitrite alone resulted in less residual nitrite, less spoilage, and similar sensory qualities as hams and bacon cured with nitrate.

Nitrite is a highly reactive compound that can function as an oxidizing or reducing agent. Under favorable conditions, nitrite may be reduced to nitric oxide, a nitrosylating agent. Nitrite can be converted to a variety of related compounds in meat including nitrate, nitrous acid, and nitric oxide (Honikel, 2004). Møller and Skibsted (2002) observed that the formation of nitric oxide (NO) from nitrite is a necessary prerequisite for the meat curing reaction. It has been suggested that a portion of nitrite added to meat during the curing process is actually converted to nitrate (Cassens et al., 1979). The addition of nitrite to meat results in formation of nitrate and nitrogen gas, as well as a reaction with carbohydrates and lipids (Honikel, 2004; Pegg and Shahidi, 2000). However, these are minor pathways when compared to the formation of nitric oxide.

Regulations

USDA regulates nitrite based on the amount going into the product during manufacture and sets limits based on the weight of meat and meat by-product, not total formulation (weight of meat and non-meat ingredients) or finished product weight. The regulations allow up to 200 ppm of in-going nitrite for

pumped or massaged cured meats except bacon and up to 156 ppm of in-going nitrite for comminuted cured meats (USDA, 1995). For most cured meat products, USDA policies hold nitrite to no less than 120 ppm in-going. Bacon is a special situation because of the concern for possible nitrosamine formation during frying. The regulations for pumped or massaged bacon specify a target of 120 ppm in-going nitrite in combination with 550 ppm of erythorbate or equivalent reducing agent. Dry-cured products are allowed to have 625 ppm sodium nitrite in-going. The establishment of minimum ingoing nitrite concentration is considered critical to subsequent product safety (Sebranek & Bacus, 2007b). Since in-going nitrite and nitrate are quite variable in natural and organic cured meat products, the safety of the product can be a concern. Thus, USDA requires such products to carry special labeling that draws attention to the fact that these are not typical cured meat products (9 CFR 319.2) (USDA, 2006).

Residual nitrite

Following normal manufacturing processes, the amount of detectable residual nitrite in a finished cured meat product is usually about 10-20% of the initial added amount (Cassens, 1997a). Some residual nitrite is essential in maintaining typical cured meat properties during extended product storage, such as color and antimicrobial properties (Cassens, 1997b). Residual nitrite declines over the storage time of the cured product until it is often undetectable (Eakes and Blumer, 1975; Skjelkvale and Tjaberg, 1974). Nitrite is converted to nitric oxide and dissipates into the air during storage. When surveyed in 1997, residual nitrite in retail cured meat products was in the range of 1 to 16 ppm (Cassens, 1997b).

Antimicrobial

According to Tarr (1941, 1942), it appears that nitrite reactivity is key to microbial inhibition (strongly dependent on pH). In addition, in-going and residual nitrite are important for antimicrobial effects (Szczawinski et al., 1989). Effectiveness of nitrite as an antimicrobial agent is dependent on pH

(Tarr, 1941 and 1942), with a lower pH being more effective. Additionally, the nitrite reaction sequence generates nitric oxide (NO) and other reaction products, which makes nitrite a more effective antimicrobial. The effects of nitrite and its inhibitory properties most likely differ by bacterial species (Tompkin, 2005). Reaction sequences involving NO are probably an important part of the antimicrobial role of nitrite in cured meat (Sebranek & Bacus, 2007). Tompkin (2005) also suggests that nitrous acid and/or nitric oxide may be responsible for inhibitory effects of nitrite. According to Tompkin (2005), the residual nitrite present at the time of temperature abuse is critical to the antibotulinal effect. In addition, the depletion of residual nitrite during product storage will reach a point in which inhibitory effects are also depleted, which is all dependent on ingoing nitrite level (Tompkin, 2005).

Pathogens of concern

Clostridium perfringens is a Gram-positive anaerobic spore-forming rod (Jay, 2000). It has been associated with gastroenteritis since 1895. *C. perfringens* spores have a wide range of heat resistance. Outbreaks related to *C. perfringens* are often associated with meats prepared one day and eaten the next, when foods are heated, cooled, and reheated. If the cooked product is improperly cooled (not cooled quickly enough within a specified time frame), spores, which survive the heating process may germinate and grow to the point of toxin production. Therefore, Appendix B (Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products) must be followed during the manufacturing of cured meat products (USDA, 1995). Partially-cooked and fully-cooked products must be cooled from 54°C to 26°C in 1.5 hours and 26°C to 4°C within 5 hours. However, ready-to-eat meat and poultry products that contain a minimum of 100 ppm in-going sodium nitrite must be cooled from 54°C to 26°C in 5 hours and 26°C to 7°C in 10 hours.

Listeria monocytogenes is a Gram-positive, facultative anaerobic, non-spore forming rod. *L. monocytogenes* is of concern in ready-to-eat (RTE) meat products because it can survive and grow when products are held at refrigerated temperatures for long periods of time. When RTE meat products are

heated, *L. monocytogenes* is killed. However, *L. monocytogenes* is commonly found in the cool, moist environment present in a meat plant and may be reintroduced to the cooked product surface during handling and before packaging. It can thrive during refrigerated storage in the vacuum package where many competing microorganisms have been eliminated. *L. monocytogenes* causes gastrointestinal symptoms, such as nausea, vomiting, and diarrhea, may proceed to more serious forms of listeriosis; intrauterine infections often lead to spontaneous abortion. Listeriosis mortality rate is 20-30%; quite high for foodborne illnesses.

Staphylococcus aureus is a Gram-positive, non-spore forming cocci, facultative anaerobe that occurs in irregular clusters. *S. aureus* produces a heat stable enterotoxin. Therefore, reheating of food containing enterotoxin is generally not effective for deactivating the toxin. Refrigerating food properly can prevent the growth and toxin production of *S. aureus*. Bayne and Michener (1975) concluded that the use of nitrite in vacuum-packaged meat reduced the growth of *S. aureus*.

Clostridium botulinum was first discovered in 1896 in an outbreak associated with raw, salted pork. It is a Gram-positive, anaerobic, spore-forming rod. The spores can germinate, grow, and produce toxins in meat products. *C. botulinum* spores are extremely heat-resistant. *C. botulinum* grows only in the absence of oxygen. Vacuum packaged meat products may allow for growth of *C. botulinum* if the packaging film has exceptionally high barrier properties. If consumed, the toxin causes botulism, which is one of the most lethal of all foodborne diseases, with a 20% to 50% mortality rate.

Research

Research of the 1920s and 1930s focused mainly on the use nitrite to cure meat, prevent sour hams, and control bacterial growth in temperature-abused (10°C of higher), perishable, canned hams (Tompkin, 2005). In 1934, Jensen et al. concluded that a mixture of nitrate and nitrite was more inhibitory of bacterial growth in canned hams, when compared to that of nitrate- or nitrite- only cured hams. In addition, Tanner and Evans (1934) stated that one cannot place reliance on nitrite to prevent

clostridia in canned ham. Research in the 1940s was controversial as researchers determined that nitrate and nitrite could inhibit *C. botulinum*. Jensen and Hess (1941a, 1941b) thought nitrite was destroyed during heating and thereby not able to prevent the growth of anaerobes. Yet, Yesair and Cameron (1942) and Stumbo et al. (1945) determined that nitrite inhibited the outgrowth of *C. botulinum.*

In the 1960s, research began to focus more on nitrite's inhibitory properties. Schmidt and Segner (1964) concluded there was an increased delay in growth of *C. botulinum* as nitrite levels increased from 0 to 100 µg/g. In 1967, Perigo et al. suggested an inhibitory substance was formed from nitrite that disappeared during thermal processing. Now known as the Perigo Factor, researchers in the late 1960s and 1970s attempted to confirm or debunk this theory (Ashworth and Spencer, 1972; Moran et al., 1975, Lee et al., 1978). The debate also focused on whether ingoing/initial nitrite or residual nitrite was more important at inhibiting pathogens. Several researchers concluded that initial nitrite content was more important than residual nitrite (Bowen and Deibel, 1974; Christiansen et al., 1973). However, Christiansen et al. (1978) determined that outgrowth of *C. botulinum* was dependent upon residual nitrite levels and surviving botulinal cells.

The ability of nitrite to limit the outgrowth of botulinum spores is an important justification for the continued use of nitrite in cured meat products. Residual nitrite level at the time of temperature abuse is a primary determinant of nitrite's antibotulinal capability (Tompkin, 2005). Depletion of residual nitrite over storage time will eventually lead to a point at which the inhibitory effects will be depleted. In 1955, Scott determined that nitrate was a poor antimicrobial and Henry et al. (1954) determined that nitrite's inhibitory properties were influenced by pH. The lower pH was more effective at inhibiting *C. botulinum* than a higher pH.

Szczawinski et al. (1989) discussed the importance of residual nitrite in nitrite-cured, pasteurized, and irradiated pork meat. The authors showed that the extent of *Clostridium botulinum* spore inhibition is related to the degree of residual nitrite depletion. Spore outgrowth was completely inhibited only when a residual nitrite concentration of 100 ppm or higher was present at the time of spore inoculation.

Nitrite provides a degree of protection from botulism but the protection provided depends on the concentration of residual nitrite present, the duration of temperature abuse, and amount of contamination. Nitrite retards microbial spoilage of cured meats and inhibits anaerobic and aerobic spore-forming bacteria. Nitrite is very inhibitory to the growth of anaerobic bacteria (i.e. *botulinum*) (Pierson and Smoot, 1982) and helps control aerobic pathogenic microorganisms (i.e. *Listeria monocytogenes*) (Pichner et al., 2006). The antibotulinal effect of nitrite may be its most important function in vacuum-packaged, refrigerated meat products because it provides assurance of the safety of the products in the event that product temperature is not well controlled (Tompkin, 2005). Yet, nitrite is ineffective for control of Gram-negative enteric pathogens (i.e. *E. coli &* Salmonella) (Tompkin, 2005). However, in a study conducted by Pichner et al. (2006), *E. coli* survived longer and reached higher counts in salami without nitrite than in salami with added nitrite.

Antioxidant and flavor

Lipid oxidation is considered to be a major reason for quality deterioration in meat products (Vasavada and Cornforth, 2005; Yun et al., 1987). Oxidation often results in the development of rancidity and subsequently, warmed-over flavors, which can be minimized by the addition of nitrite (Vasavada and Cornforth, 2005; Yun et al., 1987). Nitrite also helps control oxidation and stabilizes the oxidative state of lipids in meat products (Shahidi and Hong, 1991). To achieve a significant effect on lipid oxidation, it takes as little as 50 ppm added nitrite (Morrissey and Techivangana, 1985).

Nitrite controls lipid oxidation within the meat system by several mechanisms. First, nitrite is thought to limit the catalytic function of iron in lipid oxidation reactions, since nitric oxide binds to iron, forming dinitrosyl hemochrome (Townsend and Olson, 1987; Shahidi et al., 1991). Second, when nitrite is added to a meat batter, it is partially oxidized to nitrate, thus sequestering oxygen in a form unavailable for lipid oxidation (Honikel, 2008). A third mechanism involves the reaction of nitric oxide with free radicals produced in the lipid oxidation cascade. This free radical serves as a reaction terminator in the

autoxidation pathway (O'Donnell et al., 1997). Another proposed mechanism for the antioxidant effects of nitrite includes a reaction with heme proteins and metal ions since the radical chelation activity by nitric oxide and formation of nitroso and nitrosyl compounds have antioxidant properties (Pegg and Shahidi, 2000).

Thiobarbituric acid (TBA) analysis is a commonly-used technique for monitoring the extent of lipid oxidation in food products. A TBA number of 0.5 to 1.0 mg of TBA reactive substance per Kg of food is considered to be the threshold for oxidized odor, whereas the threshold for oxidized flavor is 1.0 to 2.0 mg/kg (Tarladgis et al., 1960). Nitrite addition in cooked meat can reduce TBA values greatly for beef, chicken, pork, and fish. Nitrite added at 100 ppm reduced TBA values by 57-72% and at 200 ppm, reduced TBA values by 87-91% (Morrissey and Techivangana, 1985).

The distinctive flavor that is developed in nitrite-cured meat is the least understood aspect of nitrite chemistry (Pegg and Shahidi, 2000). Some of the flavor differences in cured meat may be due to the suppression of lipid oxidation by nitrite. With the addition of 50 ppm of nitrite, sufficient cured meat flavor and antioxidant protection can be produced in hams (MacDonald et al., 1980a; MacDonald et al., 1980b; MacDonald et al., 1980c).

In a study conducted by Gray et al. (1981), panelists were able to differentiate between samples manufactured with different levels of nitrite (10, 156, and 200 ppm). Noel et al. (1990) observed through sensory analysis that there was a difference in nitrite versus no nitrite-added dry sausage. The authors determined that nitrite played an important role in the development of specific flavor notes. Thuringer sausage (fermented sausage), with the addition of nitrite above 50 ppm, resulted in reduced off-flavor development and improved flavor quality (Dethmers and Rock, 1975). In addition, the authors concluded that no nitrite-added thuringer sausages were considered to be the most rancid and exhibited the poorest flavor quality.

Color

Much of the research in the late 19th century and early 20th century focused on color development. Haldane (1901) and Hoagland (1914) determined that cured meat color was formed from the reduction of nitrate to nitrite; in turn, fixing the color.

Nitric oxide will react with the iron of both myoglobin (Fe^{2+}) and metmyoglobin (Fe^{3+}) to form cured meat pigments and establish cured color (Pegg and Shahidi, 2000). The fixation of desirable color is a critical component affecting consumer retail purchases (Cornforth and Jayasingh, 2004). Research conducted in the 1970s showed that 25-50 ppm of in-going nitrite was sufficient to develop relatively stable cured color (National Academy of Sciences, 1982). However, as little as 2 to 14 ppm nitrite can induce pink coloration in cooked meats (Cornforth & Jayasingh, 2004). Yet, at these levels, the color is often sporadic and likely to fade in a short time.

The following is a diagram of the color reactions in nitrite cured meat products and is adopted from Cornforth and Jayasingh (2004). The development of cured color can start with several different states of myoglobin. When myoglobin is in the deoxy- (purplish-red in color), oxy- (red in color), or metmyoglobin (brown in color) states, and sodium nitrite is added to the system, nitric oxide (NO) is formed. Nitric oxide then combines with iron to the porphyrin ring to form nitric oxide metmyoglobin (brown in color). NO-metmyoglobin is reduced, forming NO-myoglobin and with the application of heat, nitrosohemochrome (cured pink color) is formed. Nitrosohemochrome, however, can fade over the shelf-life of the product, especially when the products are exposed to light and oxygen.

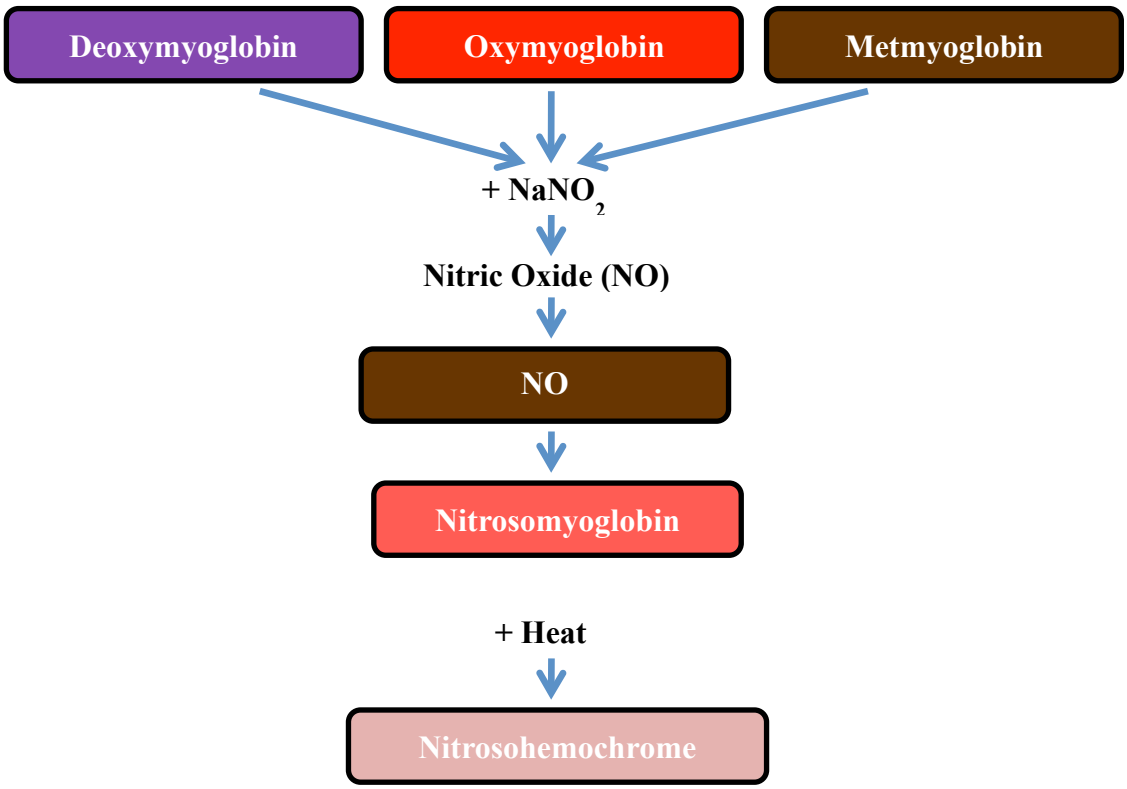

Figure 2-2. Formation of cured color.

Nitrate and nitrite in the human diet

Nitrates and nitrites are consumed in many foods within the diet. Leafy greens, root vegetables, and drinking water are sources of nitrate that humans consume on a daily basis (Cassens, 1997b). Nitrates and nitrites are part of the nitrogen cycle of plants and are by-products of green plant photosynthesis (Bednar and Kies, 1994). According to White (1975), an estimated 81.2% of nitrate intake and 1.6% of nitrite intake are derived from vegetable consumption. Conversely, the National Academy of Sciences (1981) reported that 85% of dietary nitrate came from vegetables, while 39% of dietary nitrite was from cured meat, 34% from baked goods and cereals, and 16% from vegetables. According to American Meat Institute (2003), Archer (2002), Cassens (1997a), and Milkowski (2006), less than 5% of dietary nitrite comes from cured meats, with the remainder coming from vegetables and saliva, primarily via nitrate reduction in the oral cavity. In fact, 90% of the nitrite entering the stomach

comes from saliva (Archer, 2002; Cassens, et al., 1979). The nitrite in saliva comes from previously ingested nitrate that is swallowed and absorbed into blood plasma. Nitrate in the blood is then re-circulated to the oral cavity where it reenters as saliva. With each passage through the oral cavity, a portion of the nitrate is reduced to nitrite by bacteria present in the oral cavity (Archer, 2002; Eisenbrand et al., 1980).

Health risk

In the 1960's and 1970's, it became apparent that nitric oxide could react with secondary amines to form carcinogenic n-nitrosamines in cured meat. In 1969, bacon was linked with nitrosamines. Research demonstrated that a significant factor in nitrosamine formation in bacon was residual nitrite concentration (Lijinski and Epstein, 1970). Therefore, nitrate was eliminated from most curing processes and replaced with nitrite to achieve better control over residual nitrite concentrations (Pegg and Shahidi, 2000). Also, in the 1970's, a study was published, suggesting that nitrite was a carcinogen (Newbern, 1979). Even though this paper was subsequently withdrawn, concerns about nitrite carcinogenicity continue. In 2006, the International Agency for Research on Cancer stated that ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Coughlin, 2006). However, increasing knowledge of the critical role of nitric oxide homoeostasis in human health is currently diffusing some of the popular fear of nitrite (Milkowski et al., 2010). Two summary reports generated from research and testing published by the National Academy of Sciences (1981, 1982) concluded that nitrite-cured meat did not pose a human health risk. Nevertheless, epidemiological studies continue to link consumption of cured meats with childhood leukemia and brain cancer (Peters et al., 1994; Preston-Martin and Lijinsky, 1994; Preston-Martin et al., 1996; Sarasua and Savitz, 1994).

Other traditional processed meat ingredients

While nitrate and nitrite are the main focus of this work, a number of other ingredients commonly used in meat curing are worthy of mention. Only a limited selection of ingredients may be used in organic or naturally-cured meats, but familiar ingredients will be discussed, even if they are not allowed in such products.

One of the most common ingredients in processed meat products is salt. Salt is used largely because it imparts a desirable flavor note in meat. It is also provides some preservation effect, extending the refrigerated shelf life of the product. As a low molecular weight ionic material, salt is effective at raising the ionic strength of a meat product and thus, its water binding ability. Salt also has some undesirable properties. While salt itself is probably not a pro-oxidant at the concentrations typically used in meat products (Castell et al., 1965), it typically contains metal contaminants, which promote lipid oxidation. Therefore, salt may contribute to lipid oxidation in cured meat products (Sindelar and Houser, 2010).

Other ingredients are ones that act as processing aids used to bind water. Phosphate is one of these ingredients. Phosphates have an important function as contributors to antioxidant activity in processed meat products (Molins, 1991; Sebranek, 2010). However, phosphates are not typically classified as antioxidants, despite their positive contribution to lipid stability (Detienne and Wicker, 1999). Phosphates are known for their ability to promote water binding and for improving cooking yield, texture, tenderness, and juiciness (Sebranek, 2010).

Cure accelerators are often used in cured meat products to speed up the curing reaction. Acidulants (i.e. fumaric acid, sodium acid pyrophosphate and glucono-delta-lactone) favor the curing reaction by lowering the pH to a more desirable range. Sodium ascorbate and erythorbate are sodium salts of the ascorbic and erythorbic acids, respectively. They are commonly used reducing agents, which favor the curing reactions by raising the redox potential of the commercial meat curing system (Sebranek, 2010).

Natural and organic food sectors

The sales of organic food and beverages in the U.S. have grown from $1 billion in 1990 to $29.22 billion in 2011 (OTA, 2012). Even with the current recession in the U.S., consumers still chose to purchase more expensive organic foods. From 2009 to 2010, organic food sales grew 7.7% (Organic Trade, 2011). Organic fruits and vegetables experienced the most growth of all organic segments, up 11.8% from 2009 to 2010. In 2011, the organic industry grew by 9.5% and reached $31.5 billion in sales (OTA, 2012).

Organic foods now account for over 4% of total food sales (OTA, 2012 and Winter and Davis, 2006). Consumers typically pay a premium price of 10-40% for organic foods over conventional foods (Winter and Davis, 2006). Under some circumstances, consumers are willing to pay 200% more for organic meat and poultry products, over conventional (Bacus, 2006). Within the natural and organic food sectors, the fastest growing segment in 2005 was meat, poultry, and seafood [growing by 55.4% (Mitchell, 2006)]. With the growth of organic foods, there has been rapid proliferation of new products and increased marketing by retailers (Petrak, 2005). It has been documented that consumer preferences for organic and natural foods are based on concerns about antibiotics, pesticides, hormones, genetic modifications in plants and animals, and chemical additives that consumers associate with conventionally-produced foods (Bourn and Prescott, 2002; Dreezens et al., 2005, Siderer et al., 2005; Saher et al., 2006; Winter & Davis, 2006; Devich et al., 2007).

Regulations

According to the USDA Food Standards and Labeling Book (August 2005), natural meat products and their ingredients cannot be more than minimally processed (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 those physical processes which do not fundamentally alter the raw

product and/or which only separate a whole, intact food into component parts, e.g., grinding meat, separating eggs into albumen and yolk, and pressing fruits to produce juices) (USDA, 2005). Natural products may not contain any artificial flavorings, coloring ingredients, or chemical preservatives, or any other artificial or synthetic ingredients (21 CFR 101.22) (USDA Food Standards and Labeling Book, 2005). However, there is no clear definition for "natural", as some natural ingredients, such as salt have dual functions as flavorings or "natural" preservatives (Sebranek & Bacus, 2007a). Beets, an arguably "natural" source of pigment, are disallowed as coloring agents in natural products, while paprika, also a natural source of pigment, is considered acceptable by USDA as a seasoning ingredient. As for meat products manufactured using "natural" sources of nitrate for curing, the 2006 US Code of Federal Regulations (9 CFR 319.2) requires processors to label the products as "uncured" and "no nitrates or nitrites added except those naturally occurring in ...(celery juice powder or other ingredient)".

"Uncured" products

With respect to meat products such as ham, bacon or cooked sausage, which are typically cured, there are two types of analogous "uncured" products within the meat industry. They are: 1) those manufactured with the intention of replacing nitrate and nitrite using a natural nitrate source to simulate typical curing (so-called naturally-cured products) and 2) those that do not include any significant source of nitrate or nitrite (uncured products). The naturally-cured products may exhibit properties typical of a regular-cured product. The uncured products lack the typical cured color and flavor of the corresponding cured meat product. (Sindelar et al., 2007c).

Ingredients used in naturally cured products

As explained earlier, there are no new categories of approved ingredients for naturally-cured meats. Thus, ingredients commonly used in these products must fit into existing categories. The unique

ingredients used in naturally-cured products may be chosen for functional purposes, such as development of cured meat color and flavor, or because they elicit a favorable consumer perception. Some ingredients commonly used in naturally-cured meat products are sea salt, evaporated cane juice, raw or turbinado sugar, lactic acid starter culture, and natural flavorings, such as celery juice, celery juice concentrate and vegetable juice powder.

Source of cure

A variety of natural plant or vegetable ingredients could be used as well for a nitrate source, but their distinctive flavors or colors limit their use. Vegetable or plant ingredients are chosen for their ability to supply nitrate, but nitrate concentrations vary widely among types of plants and plant parts (Lorenz, 1978). Dried vegetable juice powders may contain as much as 2.5% nitrate or more than 25,000 ppm (Sindelar, 2007c). Commercial celery juice powder has approximately 27,462 ppm nitrate (~2.75%) (Sindelar et al.,2007c). According to the National Academy of Sciences (1981), vegetables contain 1,500 to 2,800 ppm nitrate. Sebranek (2006) reported the nitrate contents of several vegetable juices: carrot with 117 ppm nitrate, celery with 2,114 ppm nitrate, beet with 2,273 ppm nitrate, and spinach with 3,227 ppm nitrate. Celery juice powder appears to be highly compatible with processed meat products because it has very little vegetable pigment and a mild flavor profile (Sebranek and Bacus, 2007). With these attributes, celery does not detract from the finished product flavor or appearance.

Source of nitrate reductase

When nitrate is used for meat curing it must be reduced to nitrite before curing reactions can proceed. The required nitrate reductase enzyme is a bacterial product derived from various nitrate reducing cultures. Lactic acid starter cultures used for fermented sausage such as *Lactobacillus plantarum* and *Pediococcus acidilactici* do not reduce nitrate (Olesen et a., 2004; Casaburi et al., 2005). However,

cultures of coagulase-negative cocci such as *Kocuria varians*, *Staphylococcus xylosus*, *Staphylococcus carnosus* and others, reduce nitrate to nitrite (Olesen et al., 2004; Casaburi et al., 2005). Typical incubation temperatures for commercial nitrate-reducing cultures are 38-42°C to minimize the time necessary for adequate nitrite formation (Casaburi, et al., 2005). Nitrate reduction can be achieved at temperatures as low as 15-20°C, but 30°C is a more effective temperature (Casaburi, et al., 2005).

Source of cure accelerator

The curing reaction is favored by reducing conditions and reduced pH. Thus, "natural" cure accelerators include acidifiers such as vinegar, lemon juice solids, and reducing agents such as cherry powder (Sebranek & Bacus, 2007). Cherry powder is high in ascorbic acid (Vitamin C), which functions as a strong nitrite reductant, but does not have the large impact on product pH as other ingredients. Cure accelerators, which reduce pH are generally not favored in processed meats due to reduced moisture retention. This observation is a special concern since phosphates and many other traditional water binders cannot be used for natural or organic products.

Other ingredients

Sea salt is another common ingredient in natural meat products. Sea salt is obtained by evaporation of sea water. It is usually unrefined without addition of free-flow additives and retains the natural trace minerals that are characteristic of the source (Heinerman & Anderson, 2001; Kuhnlein, 1980). Sea salt is a GRAS (generally recognized as safe) substance. Salt incorporated in food must comply with the Food Chemicals Codex tolerances for purity (Codex, 2006). Solar-evaporated sea salt must be at least 97.5% sodium chloride with specific limits on calcium/magnesium, arsenic, and heavy metals content (Codex, 2006). Sea salt has been sighted as a likely source of nitrate; however, limited analytical information suggested that the nitrate content of sea salt is relatively low (Sebranek and Bacus,

2007b). A study reported that Mediterranean seas salt contained 1.1 ppm of nitrate and 1.2 ppm of nitrite (Herrador et al., 2005). Cantoni et al. (1978) conducted an experiment that analyzed ten samples of three grades of sea salt for their nitrate and nitrite contents. The authors found that the sea salt samples contained 0.3 – 1.7 ppm nitrate and 0 – 0.45 ppm nitrite. These quantities would be insignificant for curing functions.

Another common ingredient used in naturally cured products is raw sugar/turbinado sugar. Turbinado sugar is obtained from the evaporation of sugar cane juice followed by centrifugation to remove molasses. However, there is no evidence that there are significant nitrate or nitrite concentrations in raw sugar (Sebranek & Bacus, 2007).

Current research in naturally cured meat products

Quality attributes

Celery powder, a naturally occurring nitrate source, combined with a starter culture, is one of the most commonly used sources of nitrite in natural and organic meat products, as it provides the most cured meat characteristics, when compared to other natural alternatives (Sindelar *et al*., 2007a; Sindelar *et al*., 2007b; Sindelar *et al*., 2007c). However, incubation time and vegetable juice concentration are important variables when using this alternative.

Sindelar et al. (2007b) conducted a study looking at the effects of incubation time and vegetable juice content in naturally-cured sausage. As incubation time increased, residual nitrate decreased ($p < 0.05$). In addition, as residual nitrate decreased in each treatment, nitrite increased. This finding was due to the conversion of nitrate to nitrite. Treatments with 0.2% vegetable juice powder differed from treatments with 0.4% vegetable juice powder for residual nitrate. Yet, the sodium nitrite-cured sausages differed from all treatments. However, residual nitrite in sausage diminished over the course of storage. There also was no significant difference for treatments or incubation time for lipid oxidation. As

incubation times increased, and at each vegetable juice concentration (0.2% and 0.4%), redness values increased ($p < 0.05$) at days 0 to 14. Sindelar et al. (2007a) determined that there was no difference in color for naturally- and conventionally-cured hams.

Naturally-cured products have shorter shelf life than nitrite-cured products because less nitrite is present and they do not contain other typical preservatives such as, lactates, curing accelerators, and antioxidants (Bacus, 2006). Research in recent years has focused on the use of "clean label" antimicrobials and natural antimicrobials in naturally-cured meats. Jackson et al. (2011) concluded that natural nitrate with antimicrobial and natural nitrite with antimicrobial can have similar residual nitrite to conventionally-cured products. Sullivan (2011) reported increased redness for natural nitrate and natural nitrite-cured hams when measured by a Hunter Colorimeter, but the differences would probably not be detectable by consumers. The natural antimicrobials used by Jackson et al. (2011) and Sullivan (2011) were a blend of cultured sugar and vinegar and a blend of cherry, lemon, and vinegar powder.

Sensory research has shown that the use of vegetable juice powder in naturally-cured meats can impart a vegetable taste. Sindelar et al. (2007a) concluded that hams cured with 0.4% vegetable juice powder had more vegetable flavor than hams cured with sodium nitrite. However, Sindelar et al. (2007b) concluded that cooked sausages cured with either 0.2% or 0.4% vegetable juice powder had no detectable vegetable flavor and aroma or objectionable flavor or aroma when compared to cooked sausages cured with sodium nitrite. The use of vegetable juice powder in certain cured meats may lead to a decrease in consumer acceptance, since consumers would not expect the vegetable flavor in a meat product.

Food safety

Over the past several years, more research has focused on the effectiveness of natural curing ingredients to inhibit foodborne pathogen-growth. However, research mainly has focused on *L. monocytogenes* and *C. perfringens.* Sullivan (2011) investigated the use of natural nitrate and natural nitrite along with natural antimicrobials (a blend of cultured sugar and vinegar and a blend of cherry,

lemon, and vinegar powder). It was concluded that natural nitrate and natural nitrite, along with the use of natural antimicrobials, exhibited similar growth of *L. monocytogenes* as sodium nitrite-cured ham. However, the use of natural nitrite with no antimicrobial had similar growth to uncured (no cure) ham. Jackson et al. (2011) determined that *C. perfringens* grew faster in uncured and natural nitrite cured hams and sausages with no natural antimicrobial (a blend of cultured sugar and vinegar and a blend of cherry, lemon, and vinegar powder) than conventionally-cured hams and sausages with natural nitrate with natural antimicrobials conventionally-cured. The above two research studies suggest that the use of natural nitrate and nitrite with natural antimicrobials can have similar growth of the two pathogens investigated. However, other pathogens and the whole curing process (injection to packaging) have not been investigated, leading to the potential for future research.

Challenges facing natural curing

As indicated earlier, Applegate Farms has petitioned the USDA to reconsider their position on the labeling of naturally-cured products (Applegate, 2011). Applegate Farms has asked that the product no longer be labeled "uncured" as the naturally-cured products have similar quality characteristics to sodium nitrite-cured products. Applegate Farms realizes natural products do contain nitrites (even though they are naturally occurring). However, the USDA has not yet published a ruling. The USDA is waiting for more research concerning the ability of naturally cure ingredients to inhibit pathogen growth, particularly *C. botulinum* (Applegate, 2011).

Naturally cured bacon

Bacon is an interesting product with respect to naturally-cured products. USDA regulations do not permit the use of nitrate in the manufacturing of bacon and limits the amount of nitrite to 120 ppm (versus 156 or 200 ppm for other cured products). Yet, naturally-cured bacon may be manufactured with

unregulated amounts of nitrate from vegetable juice powder or other natural flavorings. Therefore, the amount of residual nitrite that is found in naturally-cured bacon becomes a concern because of the potential of nitrosamine formation during cooking/frying. In recent years, meat scientists have focused their research of naturally-cured meat products on hams and cooked sausages, but not bacon. As such, research needs to be conducted in regard to bacon because of the special regulations regarding bacon. In particular, residual nitrite needs to be investigated because of the possible formation of nitrosamines during frying. Also, the USDA wants more research concerning the antimicrobial properties of natural cures currently being used within the industry. Therefore, the overall objective of the research discussed in this dissertation is to investigate the quality attributes and inhibition of foodborne pathogens in "no-nitrate or nitrite-added" bacon.

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Chapter 3

Investigation of nitrate and nitrite content of "no-nitrate or nitrite-added" bacon brine formulations

Abstract

In-going nitrite concentration is a primary determinant of ultimate cured meat properties and cured meat safety. Natural ingredients, such as celery juice powder, provide nitrite via microbial reduction of nitrate prior to or during manufacture of "no-nitrate or nitrite-added" meat products. However, such ingredients have variable nitrate contents. Therefore, the objective of this study was to investigate the nitrate and nitrite contents of "no-nitrate or nitrite-added" bacon brine formulations. Brines were all manufactured with 5% sugar. All no salt (NS) treatments were formulated with no-added salt and all salt (S) treatments were formulated with 20% salt. The curing ingredients investigated include vegetable juice powder with starter culture (V) (natural nitrate; treatments NSV2 (2%), NSV4 (4%), SV2 (2%), SV4 (4%)), pre-converted celery juice powder (C) (natural nitrite; treatments NSC, SC), and pre-converted celery juice powder with natural cure accelerator (CC) (natural nitrite with accelerator; treatments NSCC, SCC). All commercial ingredients were utilized at concentrations recommended by the supplier. Conventional-cure brine (NI) (treatments NSNI, SNI) contained 1200 ppm sodium nitrite and 5500 ppm sodium erythorbate. The No-Cure brine (NC) (treatments NSNC, SNC) contained sugar (5%) and water and salt (0 or 20%) respectively. Results demonstrated that treatments NSV2, NSV4, SV2, and SV4 had lower ($P < 0.05$) pH's than all other treatments (NSC, NSCC, NSNI, NSNC, SC, SCC, SNI, SNC). As expected, conventional treatments (NSNI and SNI) had greater ($P < 0.05$) nitrite concentrations than all other treatments. Treatments NSV2 and NSV4 had increasing nitrite concentrations over the first 6 hours, then those concentrations decreased thereafter. Additionally, nitrate concentrations were higher in treatments NSV2 and NSV4 (no

salt, natural nitrate with starter culture) than all other treatments. These data suggest that salt may inhibit the reduction of nitrate when pre-incubating brines containing natural nitrate. Pre-incubating the brine for 4-6 hours, without salt, prior to injection, may help manufacturers eliminate the need for an incubation step during the cooking/smoking process and shorten the time needed for processing "no-nitrate or nitrite-added bacon.

Keywords: bacon, natural, nitrate, nitrite

Introduction

Ingoing nitrite concentration is a primary determinant of ultimate cured meat properties and cured meat safety (Cassens, 1997). Natural ingredients such as celery juice powder provide nitrite via microbial reduction of nitrate prior to or during manufacture of "no-nitrate or nitrite-added" meat products. However, such ingredients have variable nitrate contents. That variation, along with environmental variables such as temperature, pH, ionic strength etc. during nitrate reduction, could lead to highly variable concentrations of nitrite in curing brines or products.

Conventionally-cured meat products use a precise quantity of sodium nitrite and usually a reducing agent such as sodium erythorbate, in order to quickly produce consistent cured meat products (Sebranek and Bacus, 2007). Nitrites in cured meat products help develop characteristic color, flavor, safety, shelf stability, antioxidant, and antimicrobial properties. Manufacturers intending to capitalize on consumer desire for products with "natural" in the name often rely on ingredients such as celery juice powder, to supply nitrate and a system, such as a microbial culture, i.e. *Staphylococcus carnosus*, to reduce it to nitrite. The reduction of nitrate to nitrite by *S. carnosus* is due to a membrane bound type of nitrate reductase that is involved in respiratory energy conservation (Neubauer and Götz, 1996; Pantel et al., 1998). *S. carnosus* expresses its nitrate reductase capability best in anaerobic conditions and expresses maximal activity during

the exponential growth phase. The formation of nitric oxide is the result of the further reduction of nitrite by nitrite reductase. Gøtterup et al. (2007) investigated several different types of *Staphylococcus* and concluded that *S. carnosus* had the ability to help form the most nitrosylmyoglobin, which is important in cured color development. When products are cured with nitrate, bacteria that are capable of producing nitrate reductase reduce to nitrite (Bacus, 2006). In an acidic environment, nitrite is reduced to nitrous acid and through a series of chemical reactions; nitrate, nitric oxide, and water are formed. Regulating production of nitrite in this system is difficult, leading to substandard cured color, flavor, or safety.

Salt concentration is one important factor influencing microbial nitrate reduction in brines when reduction takes place during a pre-incubation process and before injection (Krause et al. 2011). Researchers concluded that pre-incubating ham brines at 38°C without salt allowed for the most rapid reduction of nitrate. Therefore, salt could be added easily following the pre-incubation phase and prior to injection of hams. However, ingredient suppliers who sell celery juice powder or other natural nitrate-source ingredients typically recommend an incubation temperature of 43°C. In work reported by Krause et al. (2011), the highest temperature investigated was 38°C, even though manufacturers of starter cultures used in natural products recommend a temperature of 43°C during the incubation step of manufacturing a meat product containing vegetable juice powder and a starter culture. Therefore, the objective of this research was to investigate reduction of nitrate to nitrite during pre-incubation of bacon brines with and without added salt at 43°C.

Materials and Methods

Manufacture of brine

Twelve bacon brine formulation treatments (six without salt (NS) and six with salt (S)) were produced and incubated in order to measure pH, nitrate concentration, nitrite concentration, and aerobic plate counts of brines using various nitrate or nitrite sources. Bacon brine formulations are summarized in Table 3.1. Vegetable juice powder with starter culture (CS-299 Bactoferm, Chr Hansen, Inc., Milwaukee, WI) (V) (natural nitrate; Vegetable Juice Powder, Symrise, Teterboro, NJ) used at two levels (2% (V2) and 4% (V4)); pre-converted celery juice powder (C) (natural nitrite; Celery Baste, Newly Weds, Chicago, IL); and pre-converted celery juice powder with natural cure accelerator (CC) (natural nitrite with accelerator; Celery Baste and Cherry Baste Aid, Newly Weds, Chicago, IL) were used in treatments brines. All commercial ingredients were utilized at concentrations recommended by supplier. Conventional cure (NI) brine contained 1200 ppm sodium nitrite and 5500 ppm sodium erythorbate. The no-cure (NC) brine contained only salt, sugar, and water. All brines were formulated with 5% sugar and either no added salt (NS) or 20% salt (S).

Brines were formulated in 1liter batches using distilled water (ca. 27°C) and were mixed until all ingredients were dissolved. All brines were held at 43°C for 72 hours (Precision Scientific Incubator Model 805, General Electric, New York, NY), with samples taken at hours 0, 2, 4, 6, 12, 24, 48, and 72. At each sampling hour, 40 mL of brine was obtained and used for pH, aerobic plate count (APC), nitrate concentration, and nitrite concentration analyses.

Nitrate and nitrite analyses, pH, APC

Brine samples (10 mL each) for nitrate analysis were frozen (-15°C) in screw cap plastic tubes for up to 30 days until analysis was completed. Nitrate concentration was measured using the AOAC method number 935.48 (AOAC, 2010). The 10 mL sample was thawed and mixed with distilled water. The sample was heated and filtered. Various concentrations of bromocresol green, sulfuric acid, potassium permanganate, and phosphotungstic acid solution were used to convert nitrites to nitrates. Silver-ammonium hydroxide solution was used to precipitate out excess phosphitungstic acid and salt. M-xylenol was added to bind with the nitrate and turn yellow in color. The solution was then distilled; precipitant was collected and diluted and absorbance was read with a spectrophotometer. Five mL of brine sample was used for nitrite determination. Nitrite concentration was conducted using the AOAC method number 973.31 (AOAC, 2010). The five mL sample was mixed with distilled water and heated. The distilled water and sample was diluted and filtered. NED and sulfanilamide reagents were used develop a pink color if nitrites were present. The absorbance was read with a spectrophotometer. Nitrate and nitrite analyses were run in duplicate at the same time. Measurement of pH was recorded at each sampling time using a glass electrode with a silver/silver chloride reference (Testo 206; Testo AG, Germany). For APC, samples were serially diluted in sterile BPW and 1 mL was plated on duplicate APC petrifilm (3M, St. Paul, MN). Petrifilm was incubated at 37°C for 24-48 h, enumerated, and counts were reported as log colony forming units per mL (CFU/mL).

Statistical analysis

The experiment was analyzed as a repeated measure design since the measurements were taken on the same brine over time with three replications. Brine was the experimental unit with

main effects of formulation treatment and day of storage. The PROC MIXED procedure of Statistical Analysis System (SAS; version 9.3, SAS Institute Inc., Cary, NC) was used for statistical analysis. Main effects of brine formulation and incubation time were tested along with the formulation by time interaction effect. Means separation was conducted using LSMEANS function of SAS and Fisher's least significant difference (LSD) were performed for nitrite concentration, nitrate concentration, pH, and APC. Statistical significance was set at $P < 0.05$.

Results and discussion

The pH (Table 3-2) of a product can affect its stability and shelf-life. Treatments NSV2, NSV4, SV2, and SV4 had lower ($P < 0.05$) pH values than all other treatments (NSC, NSCC, NSNI, NSNC, SC, SCC, SNI, SNC). This observation is probably due to the processing of the vegetable juice powder. Since the pH did not change much over time, it is unlikely to be due to the addition of the starter culture. The starter culture was in the death phase over time, therefore not affecting the pH at the end of the time evaluated. Conversely, the treatments NSC, NSCC, SC, and SCC (natural nitrite) had higher ($P < 0.05$) pH values for the first 24 hours. This observation may be attributed to the process that is used to convert nitrate to nitrite or other items added (i.e. anti-caking agents) prior to being sold to meat manufactures. The celery baste contains celery juice powder, sea salt, and turbinado sugar, while cherry powder contains cherry powder, turbinado sugar, and anti-caking agent. As a point of reference, the pH of celery is 5.7 to 6.0 (Engineering Tool Box, 2012).

Treatments NSNI and SNI had greater ($P < 0.05$) nitrite concentrations than all other treatments (Table 3-3). This observation is to be expected since sodium nitrite was added directly into the brine as Modern Cure™. Nitrite concentration increased ($P < 0.02$) within the first 6 hours, then decreased numerically over time for both NSNI and SNI treatments. One would

expect nitrite to decrease over time due to the conversion of nitrite to nitric oxide (NO) or nitrate. However, this reduction could be attributed to the type of system used since it was a brine and not a meat system with fat, protein, and other elements for the nitrite to interact with. Additionally, this observation could be due to the reduction of nitrate, thus accumulating nitrite. Nitrite then can be further reduced to nitric oxide or can be converted back to nitrate. There was no significant change for nitrite ($P = 0.71$) for NSNI as it did not change from 0 hours to 72 hours (474 ppm vs 463 ppm, respectively), but there was a significant change ($P < 0.0001$) in nitrite for SNI from 0 hours to 72 hours (586 ppm to 467 ppm, respectively). No Salt, natural nitrate treatments (NSV2 and NSV4) had numerically greater nitrite concentrations than their salt counterparts, even though the values were not statistically different ($P > 0.05$). Yet, NSV2 did increase ($P = 0.06$) in nitrite concentration from 0 to 6 hours. As expected, nitrite concentrations did increase numerically then decrease over time for treatments NSV2 and NSV4 since nitrate is reduced to nitrite and nitrite is further reduced to nitric oxide or converts back to nitrate. After 72 hours, treatments NSV4, NSC, NSCC, NSNC, SV2, SV4, SC, SCC, and SNC had lower ($P < 0.05$) nitrite concentrations than treatments NSV2, NSNI, and SNI. *S. carnosus* is considered salt-tolerant, but Krause et al. (2011) concluded that the greatest amount of nitrite was produced from natural nitrate when salt was absent from the formulation. Eddy and Kitchell (1961) conducted an experiment that evaluated nitrate and nitrite metabolism in a bacon-curing brine and their relation to the bacterial population. The authors found that when nitrate was added to the brine, the destruction of nitrite after accumulation was greater than the destruction of nitrite when added alone. In other words, when nitrate is added and reduced to nitrite, the nitrite is reduced faster than when sodium nitrite is added as the cure ingredient. Patterson (1963) found that many strains of *Micrococcus* reduced nitrate to nitrite, but the number of bacteria strains capable of reducing nitrate decreased with increasing salt concentrations.

Table 3-4 depicts the nitrate concentration in the bacon brine formulations investigated in this study. As expected, nitrate concentrations were higher in treatments NSV2 and NSV4 (no salt, natural nitrate with starter culture) and decreased over time, except for NSV2 at 72 hours. However, this observation is due to an observation that was unusually high for one replication. Nitrate concentration decreased because of the expected reduction of nitrate to nitrite. The nitrate level in NSNI decreased from 6 h and 12 h possibly because the nitrate was converted to nitrite, then nitric oxide. The nitrate concentration of NSNI increased ($P = 0.14$) from 0 to 2 hours and could be attributed to nitrite being converted to nitrate. Treatments SV2, SV4, SC, SCC, SNI, and SNC measured lower in nitrate. This observation could be due to insufficient of reagent for precipitation of salt in the nitrate analysis. Patterson (1963) found that fewer strains of *Staphylococcus* and *Micrococcus* grew at higher salt concentrations (≥ 20%) than at lower salt concentrations (≤ 16%) in curing brines and bacon. Eddy and Kitchell (1961) also demonstrated that salt concentration and temperature affected the metabolism of nitrate and nitrite in the bacon brine. The authors found that increasing the salt concentration and decreasing the temperature lowered the metabolic rates for the reduction of nitrate. The salt concentration may inhibit the growth of nitrate reductase bacteria, which will inhibit the reduction of nitrate to nitrite. Without sufficient nitrite, it may lead to pale cured color and a decrease in antimicrobial and antioxidant properties, compared with sodium nitrite-cured meat.

Treatments NSV2, NSV4, SV2, and SV4 (treatments with the added starter culture) had higher ($P < 0.05$) APC counts (table 3-5) than all other treatments at 0 and 2 hours. Counts decreased over time. The decrease is probably attributable to bacteria dying and to the lack of key nutrients in the brine. Counts for these treatments remained higher than other treatments throughout the first 24 hours. This observation is due to the addition of the starter culture to reduce nitrate to nitrite. Eddy and Kitchell (1961) found that increasing population density of bacteria in bacon brines did not increase the conversion of nitrate to nitrite. Those authors cited

the need to be an adaptation period, which is in agreement with the findings in this study since nitrite did not accumulate immediately in treatments NSV2 and NSV4, until after some incubation time. However, after this incubation period, there was not much change in population counts; counts did not decrease to zero. Anderson and Hinrichsen (1995) found that microbial populations change during storage and manufacturing which may be a result of the nitrate reductase activity or bacteria going into the death phase. This agrees with our findings as the starter culture entered the death phase as counts decreased over time. Shaw and Harding (1978) found that microbial populations were not different between bacon cured with various levels of nitrate, but there were differences between nitrate- and nitrite-cured bacon. This finding is in agreement with the current study as there is a difference between brines manufactured with nitrate compared with brines manufactured with nitrite.

Conclusions

The preliminary brine study showed that even though *S. carnosus* is considered a salt-tolerant microorganism, the presence of 20% salt in the brine inhibited the reduction of nitrate to nitrite. Therefore, if manufacturers wish to pre-incubate brines containing natural nitrate with starter cultures, it should be done without salt to allow for the most reduction of nitrate. Pre-incubating the brine for approximately 6 hours may allow a processor to inject the brine into meat and reduce or eliminate the incubation step during processing. Forty ppm nitrite is sufficient for cured color development (National Academy of Sciences, 1982) and 50 ppm is sufficient for antioxidant/flavor properties (Morrissey and Techivangana, 1985). However, the nitrite concentration for antimicrobial properties is still unknown. The data suggest that pre-incubating brines containing natural nitrate and salt inhibits the reduction of nitrate, leading to minimal nitrite accumulation. Therefore, by pre-incubating the brine without salt prior to injection,

reduction of nitrate can occur and allow for the accumulation of nitrite. This step may help manufacturers reduce or eliminate the need for an incubation step during the cooking/smoking process. As a result, products may be manufactured more quickly since larger volumes of brine can be made beforehand.

Table 3-1 Brine formulations of "no-nitrate or nitrite-added" bacon.

	% Formulation								
TRT[a]	**H_2O**	**Salt**	**Sugar**	**Modern Cure**	**Sodium Erythorbate**	**Natural NO_2**	**Natural NO_3**	**Starter Culture**	**Natural Cure Accelerator**
NSV2	92.87	-	5	-	-	-	2	0.13	-
NSV4	90.87	-	5	-	-	-	4	0.13	-
NSC	90.26	-	3.75	-	-	5.99	-	-	-
NSCC	88.39	-	3.42	-	-	5.99	-	-	2.2
NSNI	93.05	-	5	1.9	0.05	-	-	-	-
NSNC	95	-	5	-	-	-	-	-	-
SV2	72.87	20	5	-	-	-	2	0.13	-
SV4	70.87	20	5	-	-	-	4	0.13	-
SC	74.00	16.26	3.75	-	-	5.99	-	-	-
SCC	73.01	15.38	3.42	-	-	5.99	-	-	2.2
SNI	74.75	18.3	5	1.9	0.05	-	-	-	-
SNC	75	20	5	-	-	-	-	-	-

[a] Treatment combinations: NSV2 = no salt, 2% natural nitrate source (vegetable juice powder), NSV4 = no salt, 4% natural nitrate source (vegetable juice powder), NSC = no salt, natural nitrite (prereduced celery juice powder), NSCC = no salt, natural nitrite with cure accelerator (cherry juice powder), NSNI = no salt, conventional cure (1200 ppm sodium nitrite with 5500 ppm sodium erythorbate), NSNC = no salt, no cure, SV2 = salt, 2% natural nitrate source, SV4 = salt, 4% natural nitrate source, SC = salt, natural nitrite, SCC = salt, natural nitrite with cure accelerator, SNI = salt, conventional cure, SNC = salt, no cure.

Table 3-2 pH of "no-nitrate or nitrite-added" bacon brine formulations during incubation at 43°C.

	Time[b]							
TRT[a]	**0**	**2**	**4**	**6**	**12**	**24**	**48**	**72**
NSV2	4.25^{e}	4.4^{f}	4.48^{f}	4.5^{f}	4.41^{f}	4.38^{f}	4.38^{f}	4.4^{f}
NSV4	4.18^{e}	4.31^{f}	4.34^{f}	4.39^{f}	4.34^{f}	4.32^{f}	4.36^{f}	4.31^{f}
NSC	9.0^{h}	9.05^{n}	8.89^{l}	8.78^{n}	8.56^{m}	8.29^{j}	5.82^{h}	5.31^{h}
NSCC	8.7^{h}	8.02^{l}	7.65^{j}	7.44^{l}	7.23^{k}	6.63^{i}	5.42^{g}	4.8^{g}
NSNI	7.56^{g}	7.2^{j}	6.96^{i}	6.77^{j}	6.52^{i}	6.38^{hi}	6.08^{ij}	5.86^{i}
NSNC	5.43^{f}	5.56^{g}	5.53^{g}	5.54^{g}	5.61^{g}	5.86^{g}	6.03^{i}	6.05^{j}
SV2	3.85^{e}	3.88^{e}	3.9^{e}	3.9^{e}	3.89^{e}	3.89^{e}	3.85^{e}	3.85^{e}
SV4	3.82^{e}	3.81^{e}	3.83^{e}	3.83^{e}	3.82^{e}	3.81^{e}	3.8^{e}	3.78^{e}
SC	8.55^{h}	8.6^{m}	8.46^{k}	8.38^{m}	8.23^{l}	7.95^{j}	7.7^{m}	7.56^{m}
SCC	8.48^{h}	7.74^{k}	7.4^{i}	7.12^{k}	6.96^{j}	6.71^{i}	6.61^{l}	6.72^{l}
SNI	7.24^{g}	6.81^{i}	6.61^{h}	6.52^{i}	6.42^{i}	6.34^{hi}	6.21^{jk}	6.21^{j}
SNC	5.46^{f}	5.62^{h}	5.66^{g}	5.75^{h}	5.89^{h}	6.14^{gh}	6.25^{k}	6.31^{k}
SEM[c]=	0.17	0.09	0.08	0.07	0.09	0.14	0.05	0.06

[a] Treatment combinations: NSV2 = no salt, 2% natural nitrate source (vegetable juice powder), NSV4 = no salt, 4% natural nitrate source (vegetable juice powder), NSC = no salt, natural nitrite (prereduced celery juice powder), NSCC = no salt, natural nitrite with cure accelerator (cherry juice powder), NSNI = no salt, conventional cure (1200 ppm sodium nitrite with 5500 ppm sodium erythorbate), NSNC = no salt, no cure, SV2 = salt, 2% natural nitrate source, SV4 = salt, 4% natural nitrate source, SC = salt, natural nitrite, SCC = salt, natural nitrite with cure accelerator, SNI = salt, conventional cure, SNC = salt, no cure.

[b] Brine incubation at 43°C.

[c] SEM = standard error of the mean.

[e – n] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

Table 3-3 Nitrite concentration (ppm) of "no-nitrate or nitrite-added" bacon brine formulations during incubation at 43°C.

	Time[b]							
TRT[a]	**0**	**2**	**4**	**6**	**12**	**24**	**48**	**72**
NSV2	1[e]	20[e]	41[e]	54[e]	48[e]	29[e]	32[e]	20[f]
NSV4	1[e]	9[e]	16[e]	12[e]	9[e]	10[e]	6[e]	1[e]
NSC	8[e]	9[e]	19[e]	7[e]	7[e]	19[e]	0[e]	0[e]
NSCC	9[e]	10[e]	32[e]	8[e]	6[e]	7[e]	0[e]	0[e]
NSNI	474[f]	621[f]	641[g]	629[f]	546[f]	577[f]	440[f]	463[g]
NSNC	0[e]	0[e]	1[e]	0[e]	1[e]	0[e]	0[e]	0[e]
SV2	2[e]	2[e]	3[e]	1[e]	1[e]	0[e]	0[e]	0[e]
SV4	2[e]	1[e]	2[e]	2[e]	9[e]	0[e]	0[e]	0[e]
SC	9[e]	7[e]	9[e]	11[e]	9[e]	9[e]	6[e]	7[e]
SCC	7[e]	7[e]	9[e]	9[e]	5[e]	7[e]	8[e]	8[e]
SNI	586[g]	568[f]	517[f]	653[f]	591[f]	812[g]	467[f]	467[g]
SNC	1[e]	0[e]	1[e]	0[e]	2[e]	0[e]	0[e]	0[e]
SEM[c]=	27.81	44.2	37.13	36.53	49.12	45.26	13.3	4.12

[a] Treatment combinations: NSV2 = no salt, 2% natural nitrate source (vegetable juice powder), NSV4 = no salt, 4% natural nitrate source (vegetable juice powder), NSC = no salt, natural nitrite (prereduced celery juice powder), NSCC = no salt, natural nitrite with cure accelerator (cherry juice powder), NSNI = no salt, conventional cure (1200 ppm sodium nitrite with 5500 ppm sodium erythorbate), NSNC = no salt, no cure, SV2 = salt, 2% natural nitrate source, SV4 = salt, 4% natural nitrate source, SC = salt, natural nitrite, SCC = salt, natural nitrite with cure accelerator, SNI = salt, conventional cure, SNC = salt, no cure.

[b] Brine incubation at 43°C.

[c] SEM = standard error of the mean

[e – g] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

Table 3-4 Nitrate concentration (ppm) of "no-nitrate or nitrite-added" bacon brine formulations during incubation at 43°C.

	Time[b]							
TRT[a]	**0**	**2**	**4**	**6**	**12**	**24**	**48**	**72**
NSV2	435^{g}	199^{e}	259^{efg}	295^{gh}	312^{g}	320^{f}	186^{k}	455^{h}
NSV4	1019^{h}	781^{f}	506^{g}	161^{efg}	283^{g}	77^{e}	110^{ij}	222^{g}
NSC	71^{e}	108^{e}	150^{e}	197^{fgh}	141^{f}	12^{e}	27^{efg}	193^{g}
NSCC	127^{ef}	56^{e}	102^{e}	153^{efg}	117^{f}	43^{e}	91^{hij}	52^{ef}
NSNI	299^{fg}	515^{f}	436^{fg}	380^{h}	0^{e}	0^{e}	0^{e}	0^{e}
NSNC	126^{ef}	103^{e}	105^{e}	176^{efg}	95^{f}	47^{e}	112^{ij}	208^{g}
SV2	65^{e}	36^{e}	46^{e}	42^{ef}	58^{ef}	25^{e}	77^{ghi}	98^{efg}
SV4	37^{e}	58^{e}	111^{e}	34^{ef}	76^{ef}	26^{e}	45^{efgh}	84^{efg}
SC	107^{e}	30^{e}	177^{ef}	67^{ef}	61^{ef}	25^{e}	58^{efghi}	38^{ef}
SCC	60^{e}	109^{e}	168^{ef}	61^{ef}	8^{ef}	39^{e}	143^{jk}	59^{ef}
SNI	0^{e}	0^{e}	0^{e}	0^{e}	2^{e}	0^{e}	0^{e}	0^{e}
SNC	136^{ef}	84^{e}	69^{e}	56^{ef}	75^{ef}	18^{e}	66^{fghi}	130^{fg}
SEM[c]=	67.26	102.76	98.47	67.51	32.35	27.33	21.64	44.38

[a] Treatment combinations: NSV2 = no salt, 2% natural nitrate source (vegetable juice powder), NSV4 = no salt, 4% natural nitrate source (vegetable juice powder), NSC = no salt, natural nitrite (prereduced celery juice powder), NSCC = no salt, natural nitrite with cure accelerator (cherry juice powder), NSNI = no salt, conventional cure (1200 ppm sodium nitrite with 5500 ppm sodium erythorbate), NSNC = no salt, no cure, SV2 = salt, 2% natural nitrate source, SV4 = salt, 4% natural nitrate source, SC = salt, natural nitrite, SCC = salt, natural nitrite with cure accelerator, SNI = salt, conventional cure, SNC = salt, no cure.

[b] Brine incubation at 43°C.

[c] SEM = standard error of the means for no-nitrate or nitrite-added and nitrite-added bacon brine formulations.

[e – j] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

Table 3-5 Aerobic Plate Counts (log_{10} Colony Forming Units/mL) of "no-nitrate or nitrite-added" bacon brine formulations during incubation at 43°C.

	Time[b]							
TRT[a]	**0**	**2**	**4**	**6**	**12**	**24**	**48**	**72**
NSV2	9.0^{e}	8.9^{e}	8.9^{e}	8.85^{e}	6.1^{e}	2.2^{g}	4.2^{f}	$<1^{f}$
NSV4	9.0^{e}	9.1^{e}	4.5^{fgh}	8.7^{e}	3.0^{fg}	$<1^{g}$	$<1^{g}$	$<1^{f}$
NSC	2.0^{gh}	3.7^{f}	$<1^{i}$	1.9^{gh}	$<1^{h}$	2.8^{f}	7.0^{ef}	$<1^{f}$
NSCC	4.5^{fg}	4.2^{f}	2.0^{ghi}	$<1^{h}$	$<1^{h}$	7.0^{e}	7.7^{e}	5.6^{e}
NSNI	4.6^{fg}	4.0^{f}	4.9^{fg}	4.1^{fg}	$<1^{h}$	$<1^{g}$	$<1^{g}$	$<1^{f}$
NSNC	4.25^{fg}	4.1^{f}	5.4^{f}	4.9^{f}	5.6^{ef}	5.6^{ef}	5.2^{ef}	$<1^{f}$
SV2	9.2^{e}	8.3^{e}	7.3^{ef}	5.5^{f}	2.5^{gh}	$<1^{g}$	4.2^{f}	$<1^{f}$
SV4	9.1^{e}	8.4^{e}	6.7^{ef}	4.9^{f}	1.9^{gh}	$<1^{g}$	$<1^{g}$	$<1^{f}$
SC	2.7^{fgh}	1.9^{fg}	$<1^{i}$	0^{h}	1.9^{gh}	2.0^{g}	$<1^{g}$	$<1^{f}$
SCC	5.0^{f}	1.9^{fg}	2.1^{ghi}	1.9^{gh}	$<1^{h}$	2.0^{g}	$<1^{g}$	$<1^{f}$
SNI	4.4^{fg}	$<1^{g}$	1.9^{hi}	$<1^{h}$	$<1^{h}$	$<1^{g}$	$<1^{g}$	$<1^{f}$
SNC	$<1^{h}$	$<1^{g}$	$<1^{i}$	$<1^{h}$	$<1^{h}$	$<1^{g}$	$<1^{g}$	$<1^{f}$
SEM[c]=	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[a] Treatment combinations: NSV2 = no salt, 2% natural nitrate source (vegetable juice powder), NSV4 = no salt, 4% natural nitrate source (vegetable juice powder), NSC = no salt, natural nitrite (prereduced celery juice powder), NSCC = no salt, natural nitrite with cure accelerator (cherry juice powder), NSNI = no salt, conventional cure (1200 ppm sodium nitrite with 5500 ppm sodium erythorbate), NSNC = no salt, no cure, SV2 = salt, 2% natural nitrate source, SV4 = salt, 4% natural nitrate source, SC = salt, natural nitrite, SCC = salt, natural nitrite with cure accelerator, SNI = salt, conventional cure, SNC = salt, no cure.

[b] Brine incubation at 43°C.

[c] SEM = standard error of the means for no-nitrate or nitrite-added and nitrite-added bacon brine formulations.

[e – i] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

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Chapter 4

Investigation of color and residual nitrate and nitrite of "no-nitrate or nitrite-added" bacon

Abstract

Consumers are concerned with the consumption of processed meats because of the nitrite content. Processed meats manufactured using natural curing ingredients may exhibit color, flavor, and shelf-life similar to conventional products. However, research is limited in regard to naturally-cured bacon. Therefore, the objective of this research was to investigate the quality attributes (color and residual nitrate and nitrite) of no-nitrate or nitrite-added (naturally-cured) bacon. Brine-injected bellies were used to evaluate the effects of natural curing ingredients on bacon color and residual nitrite. Four experimental and two control brine formulations were evaluated. Treatments were as follows: vegetable juice powder with starter culture (V) (natural nitrate; treatments V2 (0.2%), V4 (0.4%), pre-converted celery juice powder (treatment C) (natural nitrite), pre-converted celery juice powder with natural cure accelerator (treatment CC) (natural nitrite with natural cure accelerator), conventionally-cured (treatment NI) was composed of sodium nitrite (120 ppm) and sodium erythorbate (550 ppm), and no cure (treatment NC). In all instances, salt and sugar were added to 20% and 5% of brine formulation, respectively. Whole bellies were injected to a target pump of 10%, smoked, cooked, chilled, sliced, and vacuum packaged. Sampling occurred on day 0 (manufacturing day; injected raw belly), day 1 (packaging day), and days 3, 7, 14, 21, 28, 35, and 42. Two packages from each treatment were placed in coffin display cases (4°C) under continuous illumination to simulate retail display and were used for instrumental color measurement (L*, a*, b*). Remaining packages were refrigerated without illumination at 4°C for nitrite and nitrate analysis. Conventional cure,

treatment NI had significantly greater residual nitrite content (49 ppm – day 1) than all other treatments (0 to 8 ppm – day 1) at days 0, 1, 3, 7, 14, 21, 28, and 42. On day 35 of storage treatments, V4 and NI were not different from each other (44 ppm and 38 ppm, respectively), but had greater ($P < 0.05$) residual nitrite content than all other treatments (V2, C, CC, NC). Residual nitrate values were greater ($P < 0.05$) for treatments V2 and V4 than all other treatments at day 0 because vegetable juice powders can contain high levels of nitrate. There were no differences in redness of vacuum-packaged, sliced bacon on day 1. Treatment NI was more red ($P < 0.05$) at days 3, 7, 14, 21, 28, 35, and 42 than treatments C and NC. These findings demonstrate that even though natural nitrate-cured bacon can have similar residual nitrite as conventionally-cured bacon, natural nitrate bacon is less desirable in color which may lead to lower consumer acceptance.

Keywords: bacon, color, natural, nitrate, nitrite

Introduction

Natural and organic food segments are the fastest growing in the food industry (OTA, 2006). As more people become health conscious, they look for alternatives to traditional foods that are perceived to increase health risks. In 2005, meat, poultry, and seafood products were among the fastest growing components of organic foods (Sebranek and Bacus, 2007a), with sales for organic meat products increasing by 55.4% (OTA, 2006). To meet the increasing demand for natural and organic meat products, meat processors are looking for ways to make natural and organic versions of consumers' favorite conventionally-cured meats.

Conventionally-cured meat products use added nitrite, which is not permitted in natural, organic, or uncured meat products (Sebranek and Bacus, 2007b). To meet the consumer demand for natural and organic meat, there are two types of uncured meat products available currently in

the marketplace. First are products made with no intention of replacing nitrate or nitrite. And, second are products made with the intention of replacing nitrate or nitrite from a natural source. The current research deals primarily with the second option and will be referred to here as naturally-cured or "no-nitrate or nitrite-added". The USDA does not distinguish between the two classes of uncured products via labeling. Current USDA regulations require labeling products made with vegetable juice powders or other natural nitrate or nitrite sources as "uncured" and "no-nitrate or nitrite-added". In this chapter, "no-nitrate or nitrite-added" and naturally-cured will be used interchangeably.

Nitrites in cured meat products help develop characteristic properties, which include: color, flavor, safety, shelf stability, antioxidant effects, and antimicrobial effects. To date, no single alternative to nitrite has been discovered that can produce the same characteristics as conventionally-cured meat products (Sindelar et al., 2007a). Ingredients often used in natural and organic products include: sea salt, turbinado sugar, natural spices or flavorings, sodium lactate (from corn), vinegar, lemon juice, vegetable juices, and cherry powder. Some of these ingredients contain natural-occurring nitrates that can be converted to nitrite with the use of a starter culture.

There are several options available for manufacture of natural- and organic-processed meat products. Most products are manufactured using vegetable juice powder along with a starter culture capable of reducing nitrate to nitrite. A newer ingredient is a pre-reduced, vegetable juice powder in which the nitrate is already reduced to nitrite. These natural ingredients supply the nitrite for meat curing reactions that give the characteristic color and flavor that consumers prefer.

Over the past decade, research has shown that natural nitrates (in celery or vegetable juice powders) with a starter culture can be used to manufacture products that have quality attributes similar to those made with sodium nitrite (conventionally-cured) cured meat products. The initial color of naturally-cured hams and sausages can be similar to that of conventionally-

cured products, but color intensity and shelf life may be reduced in naturally cured items (Sindelar et al. 2007a, 2007b, 2007c; Sullivan et al., 2011; Krause et al., 2011).

To our knowledge, no one has investigated the use of natural cure ingredients in bacon. Bacon is of concern because of the possible formation of nitrosamines during cooking if residual nitrite is too high. Furthermore, processors and consumers are interested in the use of natural nitrate or nitrite sources that can be utilized during the curing process to manufacture products similar to conventionally cured products. Therefore, the objective of this study was to investigate the quality attributes of "no-nitrate or nitrite-added" bacon including color, residual nitrate, and residual nitrite.

Materials and Methods

Bacon was manufactured by injecting one of six brine treatments (four experimental and two control treatments; table 4.1), which were produced to quantify residual nitrate and nitrite and instrumental color in bacon . Brines were formulated to obtain bacon composition targets of 2% salt and 0.5% sugar. In addition, vegetable juice powder (natural nitrate; Vegetable Juice Powder, Symrise, Teterboro, NJ) used at two levels (0.2% (V2) and 0.4% (V4); final bacon composition) with starter culture (CS-299 Bactoferm, Chr Hansen, Inc., Milwaukee, WI), pre-converted celery juice powder (natural nitrite (C); Celery Baste, Newly Weds, Chicago, IL), and pre-converted celery juice powder with natural cure accelerator (natural nitrite with accelerator (CC); Celery Baste and Cherry Baste Aid, Newly Weds, Chicago, IL) were used as natural curing agents. All commercial ingredients were utilized at concentrations recommended by the supplier. Conventional curing brine was formulated to give bacon with in-going concentrations of 120 ppm

sodium nitrite and 550 ppm sodium erythorbate. The No-Cure brine contained only salt, sugar, and water.

Manufacturing of bacon

Pork bellies were obtained from a local processor in Pennsylvania and bacon was produced at the Pennsylvania State University Meats Laboratory using formulations found in Table 4.1. Brine formulations (Table 1) were mixed until all ingredients were dissolved. Bellies were pumped to 10% of starting weight using a hand-operated multiple stitch injection system (Koch Supplies, North Kansas City, MO). Treatments with natural nitrate and starter culture (treatments V2 and V4) were placed in a single-truck smokehouse (Jet Smoke Maxi 3001, Kerres GmbH, Backnang, Germany) for incubation at 43°C for 75 minutes to allow for reduction of nitrate to nitrite by the starter culture. All other treatments (C, CC, NI, NC) were placed in the smokehouse after the 75 minutes and a temperature probe inserted into treatment NI (conventionally-cured). All products were heated to an internal temperature of 53°C. The bacon was placed in a 4°C cooler overnight to cool and stabilize. Green weights (weight of meat block), pumped weights, and cooked weights were taken and percent pump, smokehouse yield, and process yield were calculated (Table 4.2). After chilling, the bellies were sliced into 3 mm thick slices using a manual slicer (Model GSP, Bizerba USA, Piscataway Township, NJ). Slices, 5 per package, were vacuum-sealed in 3 mil polyethylene pouches (Prime Source, Kansas City, MO).

Color and chemical analyses

Two packages from each belly were placed into coffin display cases (4°C) under continuous illumination (GE Ecolux F40SP35, 3500K, 3870 lux) to simulate retail display

conditions for 42 days. A 5-cm portion was removed from the cranial end of the belly following pumping, but before smoking/cooking for initial (day 0) color, nitrate, and nitrite measurements. Color was measured on storage days 0, 1, 3, 7, 14, 21, 28, 35, and 42. Product color was measured at five, pre-determined locations on each package using CIE L*, a*, and b*, Illuminate D65, 10° standard observer and a 1.27 cm port (MiniScan EZ, HunterLab, Reston, VA). The five pre-determined locations were selected with focus on maximizing the lean in the area and marked on day 1 to ensure that the same location was measured on each storage day.

All other packaged samples were held in dark storage at 4°C in a walk-in cooler to mimic typical bacon storage since it often arrives in boxes and is put out for display as needed. Samples were removed from refrigerated storage on day 1 (day of packaging), 3, 7, 14, 21, 28, 35, and 42 and were frozen (-15°C) for up to 60 days before being analyzed for residual nitrate and nitrite. Two packages on each sampling day were selected and each package was homogenized separately, using a food processor (Cuisinart Mini Food Processor, East Windsor NJ). From the homogenized bacon, 10 g was used to determine nitrate concentration. Nitrate concentration was measured using the AOAC method number 935.48 (AOAC, 2010). Procedures can be found in the Appendix. However, the color standard was diluted to 100 mL instead of 500 mL because of the high concentration of nitrate in some samples. Five grams of homogenized bacon was used to determine nitrite concentration using the AOAC method number 973.31 (AOAC, 2010). Procedures can be found in the Appendix.

Statistical analysis

The experiment was analyzed as a repeated measure design since the measurements were taken on the same belly, over time, with three replications. Belly was the experimental unit with main effects of brine formulation treatment and day of storage. The PROC MIXED procedure of

Statistical Analysis System (SAS; version 9.3, SAS Institute Inc., Cary, NC) was used for statistical analysis. Color, residual nitrate, and residual nitrite were analyzed for brine formulation treatment effects by day. Means separation was conducted using LSMEANS function of SAS and Fisher's least significant difference (LSD) were performed for main effects or interactions. Statistical significance was set at $P < 0.05$.

Results and Discussion

Research conducted in the 1970s showed that 25-50 ppm of in-going nitrite was sufficient to develop relatively stable cured color (National Academy of Sciences, 1982). However, as little as 2 to 14 ppm nitrite can induce pink coloration in cooked meats (Cornforth & Jayasingh, 2004). Yet, at these concentrations, the color is often sporadic and likely to fade in a short time. Some of the flavor differences in cured meat may be due to the suppression of lipid oxidation by nitrite. With the addition of 50 ppm of nitrite, sufficient cured meat flavor and antioxidant protection can be produced in hams (MacDonald et al., 1980a; MacDonald et al., 1980b; MacDonald et al., 1980c). In the current study, conventional-cure, treatment NI (Table 4.3), had significantly greater residual nitrite content than all other treatments at days 0, 1, 3, 7, 14, 21, 28, and 42. However, treatments V2, V4, C, CC, and NC were not different from each other for days 0, 1, 7, and 42. On day 35 of storage, treatments V4 and NI were not different from each other, but had greater ($P < 0.05$) residual nitrite content than all other treatments (V2, C, CC, NC). On days 21 and 35, treatments V2 and V4 had greater ($P < 0.05$) residual nitrite content than treatments C, CC, and NC. As expected, nitrite concentration changed numerically from day 0 to day 1 since nitrite on day 0 was in a raw product versus smoked/cooked sliced bacon on day 1. It is important to note that we formulated the conventionally-cured bacon to contain 120 ppm nitrite in the raw product and 114 ppm nitrite was determined analytically.

Following the thermal process, the residual nitrite dropped to 49 ppm. Research conducted by Sindelar et al. (2007a, 2007b) found that residual nitrite levels for natural nitrate with starter culture hams and cooked sausages decreased over time. However, in the present study, residual nitrite levels increased in treatments V2 and V4. This observation may be due to the reduction of nitrate to nitrite during storage. The minimal heat treatment of bacon (53°C) may allow survival of some nitrate-reducing bacteria. This observation is in agreement with Jackson et al. (2011) who found that natural nitrate with starter culture in hams had greater residual nitrite values than conventionally-cured hams because of the reduction of nitrate. Sullivan et al. (2011) concluded that hams cured with natural nitrate using a starter culture had the highest levels of residual nitrite, when compared to conventionally-cured hams. The authors also found that residual nitrite decreased during days (14 days) of storage. Nitrite concentration is known to decline with time since nitrite can be reduced to nitric oxide or be converted back to nitrate. However, Xi et al. (2011) concluded that conventionally-cured frankfurters had higher residual nitrite values than naturally-cured frankfurters. In the current study, treatments C and CC had low levels of residual nitrite throughout days (10 days) of storage. This finding could be due to the fact that the natural nitrite brine supplied only 7-11 ppm of nitrite (Table 3.3) and that treatment CC contained cherry powder, which provides reducing conditions that further deplete nitrite. Terns et al. (2011) and Xi et al. (2011) reported that the addition of cherry powder reduced residual nitrite since the cherry powder acts as a cure accelerator, much like sodium ascorbate or erythorbate. Ahn et al. (2002) noted that packaging (aerobic vs vacuum) also affected residual nitrite levels. The authors stated that vacuum-packaged sausage had lower residual nitrite than samples stored under aerobic conditions. The authors suggested the difference was caused by the vacuum environment being in the reduced state, allowing the conversion of nitrite to nitric oxide. Woolford and Cassens (1977) found that the level of residual nitrite in sodium nitrite-cured bacon decreased during processing and storage. In the current study, residual nitrite decreased from day 0 to day 42, in

treatment NI. Residual nitrite can lead to the possible production of nitrosamines during cooking/frying of bacon. A residual nitrite concentration of 26 ppm in sliced bacon would be a concern in a commercial production facility because of possible nitrosamine formation (Edward Mills, personal communication, 2012).

Residual nitrate values were greater ($P < 0.05$) for treatments V2 and V4 than all other treatments at day 0 because vegetable juice powders can contain high levels of nitrate (Table 4.4). In the current study, bacon cured with vegetable juice powder exhibited nitrate concentrations of 435-1019 ppm (Table 3.4). However, the manufacturer of this vegetable juice powder does not state what the nitrate concentration is on the label. Additionally, residual nitrate increased ($P < 0.05$) until day 14 of storage and then decreased for treatment NI, suggesting that nitrite was oxidized to nitrate. For all treatments, residual nitrate changed over time as nitrate can be reduced to nitrite and nitrite can convert back to nitrate or be reduced to nitric oxide. Woolford and Cassens (1977) concluded in their sodium nitrite study that residual nitrate increased over time, even though the bacon was not formulated with nitrate. Herring (1973) also stated that nitrate levels in whole bacon increased during storage. Jackson et al. (2011) found that residual nitrate was higher in conventionally-cured and natural nitrate with starter culture-cured hams than natural nitrite-cured hams. Sindelar et al. (2007c) found that conventionally-cured bacon found at retail stores had greater residual nitrate than its naturally-cured counterparts. This finding may be due to different days of manufacturing and the shelf-life of the product. However, in another study, Sindelar et al. (2007a) concluded that hams cured with 0.4% vegetable juice powder had greater residual nitrate than hams cured with 0.2% vegetable juice powder and conventionally-cured hams. This finding is a concern because the reduction of nitrate can result in higher residual nitrite values. In another study, Anderson and Hinrichsen (1995) conducted an experiment that evaluated two curing systems (tank and vacuum package) in which bacon was cured with potassium nitrate and sodium nitrite. These authors found that bacon cured in tanks

decreased in residual nitrate content, but increased in residual nitrite over the 21 days of storage. This finding may be attributed to the reduction of nitrate.

In the following discussion of color, results for day 0 values represent injected, raw bacon before thermal processing (cooking). These values provide a point of reference, but are not expected to represent typical bacon color.

There were no differences ($P > 0.05$) in lightness (L*) for day 0 and 1 among all treatments (Table 4.5). On day 7, 14, 21, and 35 L* values were greater ($P < 0.05$) for treatment C than treatment NI, while all other treatments (V2, V4, CC, NC) were not different. Treatment C was lighter ($P < 0.05$) than treatment NI on days 3, 7, 14, 21, 28, 35, and 42. Another study found that L* values were lower in frankfurters cured with natural nitrite than frankfurters cured with sodium nitrite or natural nitrate (Xi et al., 2011). Sullivan et al. (2012) concluded that conventionally cured hams had higher L* values than natural nitrate-cured hams. However, these studies were not conducted under simulated retail display. Sindelar et al. (2007a and 2007b) did not find any significant interactions for treatment by day. This finding may be due to the fact that the color/shelf-life portion was not conducted using simulated retail display. In the current study, L* values numerically increased with thermal processing but did not change appreciably during retail storage.

There were no differences on day 1 for a* (redness) values (Table 4.6). Treatments C, NI, and NC were less red ($P < 0.05$) than treatments V2 and V4 for initial (day 0) color. Treatment NI was redder ($P < 0.05$) at days 3, 7, 14, 21, 28, 35, and 42 than treatments C and NC. Krause et al. (2011) also found that ham slices cured with sodium nitrite were redder at the end of a 42-d storage time than those cured with natural nitrate and starter culture. However, Sullivan et al. (2011) found that there were no differences in redness for ham slices cured with natural nitrite, natural nitrate, and conventional-cure. Yet Xi et al. (2011) concluded that conventionally-cured frankfurters had higher a* values than natural nitrite or natural nitrate-cured frankfurters.

Sindelar et al. (2007b) found that sausages cured with 0.4% vegetable juice powder or sodium nitrite were redder than sausages cured with 0.2% vegetable juice powder. Redness did not change ($P > 0.2$) over time for V2, V4, C, CC, and NC from day 1 to day 42, however a* values did increase, then decrease during storage. It is known that the cured meat pigment fades when exposed to light (Cornforth and Jayasingh, 2004).

Initially b* (yellowness) (Table 4.7) values were greater ($P < 0.05$) in treatments V2 and V4 than treatments (C, NI, and NC). Days 14, 21, 28, 35, and 42 had no differences in yellowness for all treatments. The differences in yellowness of bacon on day 0 could be due to the pigmentation of the vegetable juice powder. Krause et al. (2011) also found that cooked hams cured with natural nitrate with starter culture were more yellow than cooked hams cured with sodium nitrite conventional cure because of the vegetable pigment found in the vegetable juice powder.

Paquette et al. (1980) investigated the color of bacon cured with sodium nitrite and potassium sorbate at various levels. Consumers evaluated uncooked color over a 49-day shelf-life for vacuum-packaged and non-vacuum-packaged bacon. Vacuum-packaged bacon cured with sodium nitrite (40, 80, 120 ppm) had significantly higher color desirability scores than bacon without sodium nitrite. There were no significant differences in desirability of color among the three treatments, which included sodium nitrite . Herring (1973) also concluded that bacon formulated without sodium nitrite received lower color scores than sodium nitrite-formulated bacon. Paquette et al. (1980) did note that non-vacuum-packaged bacon had lower color desirability scores than vacuum-packaged bacon. The authors suggested that the poor color stability of non-vacuum-packaged bacon was exaggerated by continuous fluorescent illumination.

Conclusion

Naturally-cured bacon achieved color characteristics, which were measurably different from those of sodium nitrite-cured bacon. Redness of sodium nitrite-cured bacon was always numerically greater than that of the no cure and naturally-cured products, though sometimes the differences were not significant. The redness faded more rapidly in the no-nitrate or nitrite-added bacon than in the sodium nitrite-cured bacon. This finding could lead to lower consumer acceptance or may be accepted by consumers who prefer the product for other reasons.

Concerns about possible nitrosamine formation during frying of natural nitrate cured bacon are not supported by the current findings. Even though the results were not always statistically significant, residual nitrite concentrations in natural nitrate-cured bacon were always numerically lower than in the sodium nitrite-cured bacon. With such low residual nitrite concentrations, naturally-cured bacon would pose no more risk than traditional bacon. Further research should be conducted concerning the shelf-life stability and safety of naturally-cured bacon.

Table 4-1 Brine formulations of "no-nitrate or nitrite-added" bacon.

	% of Formulation								
TRT[a]	**H_2O**	**Salt**	**Sugar**	**Modern Cure**	**Sodium Erythorbate**	**Natural NO_2**	**Natural NO_3**	**Starter Culture**	**Natural Cure Accelerator**
V2	72.87	20	5	-	-	-	2	0.13	-
V4	70.87	20	5	-	-	-	4	0.13	-
C	74.00	16.26	3.75	-	-	5.99	-	-	-
CC	73.01	15.38	3.42	-	-	5.99	-	-	2.2
NI	74.75	18.3	5	1.9	0.05	-	-	-	-
NC	75	20	5	-	-	-	-	-	-

[a] Treatment combinations: V2 = 0.2% of natural nitrate source, V4 = 0.4% of natural nitrate source, C = natural nitrite, CC = natural nitrite with natural cure accelerator, NI = Conventional Cure - 120 ppm sodium nitrite with 550 ppm sodium erythorbate, NC = no cure.

Table 4-2 Process Control Table of "no-nitrate or nitrite-added" bacon.

	Process		
TRT[a]	**% Pump**	**Smokehouse Yield**	**Process Yield**
V2	11.21	92.22	101.17
V4	10.12	93.30	100.22
C	11.51	93.61	102.67
CC	12.12	93.29	102.21
NI	12.02	92.25	101.21
NC	8.58	92.98	99.67

[a] Treatment combinations: V2 = 0.2% of natural nitrate source, V4 = 0.4% of natural nitrate source, C = natural nitrite, CC = natural nitrite with natural cure accelerator, NI = Conventional Cure - 120 ppm sodium nitrite with 550 ppm sodium erythorbate, NC = no cure.

Table 4-3 Residual nitrite (ppm) of "no-nitrate or nitrite-added" bacon during simulated retail display.

	Day[b]								
TRT[a]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**35**	**42**
V2	1^{e}	6^{e}	9^{f}	8^{e}	22^{e}	21^{f}	28^{f}	29^{f}	1^{e}
V4	1^{e}	5^{e}	2^{ef}	3^{e}	11^{f}	19^{f}	16^{ef}	44^{g}	3^{e}
C	9^{f}	8^{e}	4^{ef}	2^{e}	5^{fg}	4^{e}	5^{e}	4^{e}	2^{e}
CC	3^{ef}	3^{e}	1^{ef}	0^{e}	0^{g}	1^{e}	1^{e}	3^{e}	1^{e}
NI	114^{g}	49^{f}	29^{g}	39^{f}	32^{h}	40^{g}	56^{g}	38^{g}	38^{f}
NC	2^{e}	0^{e}	0^{e}	0^{e}	0^{g}	0^{e}	0^{e}	0^{e}	2^{e}
SEM[c]=	2.71	3.32	2.89	3.7	2.79	4.06	7.37	2.62	2.33

[a] Treatment combinations: V2 = 0.2% of natural nitrate source, V4 = 0.4% of natural nitrate source, C = natural nitrite, CC = natural nitrite with natural cure accelerator, NI = Conventional Cure - 120 ppm sodium nitrite with 550 ppm sodium erythorbate, NC = no cure.
[b] Vacuum-packaged samples held at 4°C.
[c] SEM = standard error of the means.
[e – h] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

Table 4-4 Residual nitrate (ppm) of "no-nitrate or nitrite-added" bacon during simulated retail display.

	Day[b]								
TRT[a]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**35**	**42**
V2	86^{g}	20^{ef}	5	64^{ef}	26	14^{e}	23	8	0^{e}
V4	122^{g}	25^{f}	63	95^{f}	55	59^{f}	24	9	14^{f}
C	27^{ef}	1^{e}	32	48^{ef}	29	27^{ef}	18	11	2^{e}
CC	25^{ef}	15^{ef}	42	38^{e}	34	28^{ef}	29	15	0^{e}
NI	0^{e}	0^{e}	30	43^{e}	64	34^{ef}	12	1	0^{e}
NC	38^{f}	12^{ef}	47	28^{e}	25	9^{e}	32	18	12^{ef}
SEM[c]=	11.0	7.4	20.4	16.5	19.5	11.6	14.2	6.2	4.3

[a] Treatment combinations: V2 = 0.2% of natural nitrate source, V4 = 0.4% of natural nitrate source, C = natural nitrite, CC = natural nitrite with natural cure accelerator, NI = Conventional Cure - 120 ppm sodium nitrite with 550 ppm sodium erythorbate, NC = no cure.
[b] Vacuum-packaged samples held at 4°C.
[c] SEM = standard error of the means.
[e – g] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

Table 4-5 CIE L* (lightness) of "no-nitrate or nitrite-added" bacon during simulated retail display.

	Day[b]								
TRT[a]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**35**	**42**
V2	58.05	71.08	69.37^{efg}	68.92^{ef}	70.05^{ef}	71.23^{ef}	69.72^{ef}	74.32^{ef}	71.76^{ef}
V4	56.3	71.10	67.39^{ef}	67.07^{ef}	71.39^{ef}	70.26^{ef}	71.29^{f}	72.92^{ef}	73.58^{ef}
C	58.67	74.14	72.38^{g}	70.42^{f}	74.64^{f}	74.61^{f}	73.63^{f}	78.49^{f}	76.31^{f}
CC	54.95	69.72	70.67^{fg}	66.06^{er}	71.87^{ef}	71.39^{ef}	70.65^{f}	72.70^{ef}	72.84^{ef}
NI	59.18	71.30	64.45^{e}	65.64^{e}	66.19^{e}	66.24^{e}	65.42^{e}	69.52^{e}	68.10^{e}
NC	55.47	68.67	69.05^{efg}	68.99^{ef}	68.99^{ef}	70.86^{ef}	71.80^{f}	73.53^{ef}	74.17^{f}
SEM[c]=	1.76	2.08	1.76	1.59	2.03	2.0	1.69	2.23	2.14

[a] Treatment combinations: V2 = 0.2% of natural nitrate source, V4 = 0.4% of natural nitrate source, C = natural nitrite, CC = natural nitrite with natural cure accelerator, NI = Conventional Cure - 120 ppm sodium nitrite with 550 ppm sodium erythorbate, NC = no cure.
[b] Vacuum-packaged samples held at 4°C.
[c] SEM = standard error of the means.
[e – g] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

Table 4-6 CIE a* (redness) of "no-nitrate or nitrite-added" bacon during simulated retail display.

	Day[b]								
TRT[a]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**35**	**42**
V2	4.69^{gh}	4.40	5.43^{fg}	5.38^{fg}	4.45^{ef}	4.05^{ef}	4.79^{e}	3.14^{ef}	3.23^{e}
V4	5.52^{h}	3.90	5.65^{g}	5.47^{g}	4.05^{ef}	5.22^{fg}	4.85^{ef}	3.50^{ef}	3.14^{e}
C	2.23^{e}	2.69	3.67^{f}	3.43^{ef}	2.31^{e}	2.40^{e}	3.21^{e}	1.93^{e}	3.29^{e}
CC	3.83^{fg}	4.22	4.28^{fg}	5.67^{g}	3.32^{e}	3.88^{ef}	4.26^{e}	4.29^{fg}	3.66^{ef}
NI	2.18^{e}	4.20	7.61^{h}	6.98^{g}	6.52^{f}	6.58^{g}	6.86^{f}	5.47^{g}	5.37^{f}
NC	3.02^{gef}	3.37	1.68^{e}	3.22^{e}	3.78^{e}	4.09^{ef}	4.17^{e}	3.38^{ef}	2.97^{e}
SEM[c]=	0.49	0.80	0.65	0.70	0.88	0.81	0.73	0.65	0.71

[a] Treatment combinations: V2 = 0.2% of natural nitrate source, V4 = 0.4% of natural nitrate source, C = natural nitrite, CC = natural nitrite with natural cure accelerator, NI = Conventional Cure - 120 ppm sodium nitrite with 550 ppm sodium erythorbate, NC = no cure.
[b] Vacuum-packaged samples held at 4°C.
[c] SEM = standard error of the means.
[e – h] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

Table 4-7 CIE b* (yellowness) of "no-nitrate or nitrite-added" bacon during simulated retail display.

	Day[b]								
TRT[a]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**35**	**42**
V2	13.86^{hi}	10.12^{f}	10.26^{e}	10.92^{ef}	11.92	10.96	11.72	10.82	11.10
V4	14.79^{i}	10.48^{fg}	12.04^{f}	11.92^{f}	11.57	11.66	12.20	12.15	11.49
C	11.64^{fg}	10.03^{ef}	10.86^{ef}	11.75^{f}	11.52	10.85	11.96	10.83	11.08
CC	12.25^{gh}	10.97^{fg}	11.08^{ef}	11.65^{f}	11.74	11.09	11.36	10.97	11.28
NI	9.68^{e}	8.02^{e}	10.67^{ef}	10.04^{e}	11.96	11.22	12.03	11.64	11.80
NC	10.22^{ef}	11.82^{g}	12.15^{f}	11.74^{f}	12.42	11.68	11.67	11.38	13.71
SEM[c]=	0.57	0.49	0.57	0.38	0.84	0.56	0.58	0.55	1.06

[a] Treatment combinations: V2 = 0.2% of natural nitrate source, V4 = 0.4% of natural nitrate source, C = natural nitrite, CC = natural nitrite with natural cure accelerator, NI = Conventional Cure - 120 ppm sodium nitrite with 550 ppm sodium erythorbate, NC = no cure.
[b] Vvacuum-packaged samples held at 4°C.
[c] SEM = standard error of the means.
[e – i] Least Squares Means within the same column without a common superscript are different ($P < 0.05$).

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Chapter 5

Inhibition of foodborne pathogens in "no-nitrate or nitrite-added" bacon brine and bacon

Abstract

Processed meats manufactured using natural curing ingredients may exhibit color, flavor and shelf-life attributes similar to traditional products. However, few reports describe the effects of natural curing ingredients on survival and growth of foodborne pathogens in meat products. Therefore, the objective of this study was to evaluate the inactivation of vegetative *Clostridium perfringens* (CP), *Listeria monocytogenes* (LM), *Escherichia coli* O157:H7 (EC) and *Salmonella* Typhimurium (ST) inoculated in bacon brine formulations using natural nitrate with starter culture (vegetable juice powder), natural nitrite with a natural cure accelerator (celery juice powder with cherry juice powder), and traditional cure (sodium nitrite). Bellies were inoculated with a cocktail of the four foodborne pathogens, injected with respective brine treatments, and cooked to an internal temperature of 53°C. Bellies were sliced and vacuum-packaged on day 1. Samples were taken at 0, 1, 3, 7, 14, and 21 days after inoculation and evaluated for remaining bacterial populations using direct plating and/or enrichment procedures. Inoculated bellies treated with natural nitrate, natural nitrite, traditional cure, and no cure exhibited growth (7 $\log_{10}$ CFU/g) of LM, EC, and ST. Conventional cure was more effective at inhibiting CP than all other treatments, regardless of day of storage. Conventional cure was the only treatment that resulted in no colonies growing by direct plating method.

Key Words: bacon, foodborne pathogens, nitrite

Introduction

As consumers become more health conscious, they often try natural or organic food alternatives. Organic food sales grew 7.7% from 2009 to 2010 (Organic Trade Association, 2011). Meat, poultry, and seafood products account for 2% of the total organic food market (Organic Trade Association, 2010). In 2005, meat, poultry, and seafood products were the fastest growing components of organic foods markets (Organic Trade Association, 2006).

Conventionally-cured meat products use sodium nitrite and other ingredients (i.e. sodium phosphates, sodium erythorbate) that are not permitted in natural, organic, or uncured meat products (Sebranek and Bacus, 2007). Nitrites in cured meat products help develop characteristic color, flavor, safety, shelf stability, antioxidant and antimicrobial properties. Natural curing ingredients, such as vegetable juice powder, often contain nitrates that can be converted to nitrite with the use of a starter culture. Current research indicates that products cured with vegetable juice powder can develop similar color and flavor as sodium nitrite-cured products (Sindelar et al. 2007a; Sindelar et al., 2007b, Sindelar et al., 2007c). In work with hot dogs and hams, Jackson et al. (2011) observed various responses for conventionally-cured versus naturally cured products. In commercial products challenged with pathogens, conventionally-cured products had more inhibition. But manufactured test products, using a variety of natural curing systems showed inconsistent results when challenged with pathogens. Inhibition was sometimes more effective in naturally-cured (no-nitrate or nitrite-added) products compared to that of conventionally-cured items. Additional work is needed to develop a more complete understanding of the effects of natural cures for inhibiting microbial growth.

According to Sebranek & Bacus (2007b), nitrite reactivity is important for microbial inhibition. There are several factors that affect microbial inhibition of nitrite, including pH (Jensen et al., 1941; Jensen et al., 1934) and the nitrite reaction sequence that generates nitric

oxide and other reaction products (Tompkin, 2005). The effects of nitrite and its inhibitory properties differ with regard to bacterial species. According to Tompkin (2005), the residual nitrite present at the time of temperature abuse is critical for an antibotulinal effect. In addition, the depletion of residual nitrite during product storage will reach a point when inhibitory effects also are depleted. Therefore, nitrite is important to the shelf-life and stability of processed meat products. This is a special concern in natural products that use a natural nitrate source since the nitrite level is unknown at the time of manufacturing.

Nitrite retards microbial spoilage of cured meats, including Gram-positive, anaerobic spore-forming bacteria (i.e. *Clostridium botulinum*) (Pierson et al., 1982) and aerobic bacteria (i.e. *Listeria monocytogenes*) (Tompkin, 2005; National Academy of Sciences, 1982; Pierson et al., 1982; Pichner et al., 2006). Nitrite is considered ineffective for control of Gram-negative enteric pathogens (i.e. *Escherichia coli* and *Salmonella*) (Tompkin, 2005). However, in a study conducted by Pichner et al. (2006), *E. coli* survived longer and reached higher counts in salami without nitrite than in that with added nitrite. *Staphylococcus* spp. and *Salmonella* spp. growth were suppressed slightly in frankfurters with nitrite, as compared to frankfurters that did not contain the compound (Bayne et al., 1975). It has been suggested that nitrite, converted to nitric oxide, disrupts the iron-sulfur cluster of the protein (Meyer, 1981). This action inhibits nitrogenase activity. Given the small number of published studies and the lack of publications dealing with natural curing of bacon, the purpose of the current study was to evaluate the effectiveness of natural curing systems for inhibiting growth of pathogens in brine solutions and subsequently, in a bacon system. Brine systems were evaluated as brines are often recirculated during the manufacturing of bacon and other cured meat products that are pumped with a brine solution. In addition, LM can survive in brines

Materials and Methods

Brine study

Culture preparation

L. monocytogenes (LM; ATCC Scott A; American Type Culture Collection, Manassas, VA), *C. perfringens*, (CP; ATCC 10543), and *S.* Typhimurium (ST; ATCC 14028) were obtained from the Muscle Foods Microbiology Lab of the Food Science Department at The Pennsylvania State University. LM and ST were cultured separately in Tryptic Soy Broth (TSB, Becton Dickinson and Co., Sparks, MD) for 24 hours at 37°C. CP was cultured in Reinforced Clostridial Medium (RCM, Becton Dickinson and Co., Sparks, MD) for 36 hours at 37°C. Individual cultures were centrifuged (2000g, 15 min) and resuspended in 10 mL of buffered peptone water (BPW, Becton Dickinson and Co., Sparks, MD). The concentration of LM and ST was approximately 10^8 colony forming units per mL (CFU/mL), while CP was approximately 10^7 CFU/mL. Ten mL of each washed culture were combined to make a cocktail and 4 mL of the cocktail were used to inoculate each brine formulation (44 mL) after solutions were made, as described below.

Inoculation of brine solution

The compounds and final brine solutions used in this experiment were composed as follows and listed in Table 5.1. Treatment V4 (natural nitrate) Vegetable Juice Powder, (Symrise, Teterboro, NJ) with starter culture (Bactoferm CS-299, Chr. Hansen, Inc., Milwaukee, WI) and Treatment CC (natural nitrite) Celery Baste and Cherry Baste Aid, (Newly Weds Foods Inc., Chicago, IL) were utilized at concentrations recommended by the manufacturer and mixed with

sterile distilled water, as indicated in Table 5.1. Treatment NI was composed of sodium nitrite and sodium erythorbate in distilled water (conventionally-cured control) used at concentrations (1200 ppm nitrite and 5500 ppm erythorbate) approved for bacon by the United States Department of Agriculture. Treatment NC was composed of salt, sugar, and distilled water. In all instances, salt and sugar were added to 20% and 5% of the formulation, respectively. Sterile distilled water was added and the brine was mixed for at least two minutes to ensure all ingredients were dissolved in solution. Treatment WA consisted of distilled water. Using this procedure, the final concentration of the pathogens was approximately 7 $\log_{10}$ CFU/mL in the brines. Inoculated brines were incubated at 37ºC for 0, 4, 8, 12, and 24 h and sampled as described below.

Microbial sampling of solutions

Sampling of the brine solutions was conducted following the respective incubation times (0, 4, 8, 12, and 24 h) by removing 5 mL of the inoculated brine, serially diluting in BPW, and spread-plating on the appropriate selective agars, in duplicate, as follows: LM was isolated on Oxford medium base supplemented with Moxalactam (Remel, Lenexa, KS); CP was isolated on Perfringens Agar Base with egg yolk and Perfringens Selective Supplement (Oxoid Ltd, Baskingstoke, UK); and ST was isolated on Xylose Lysine Deoxycholate agar (Becton Dickinson and Co., Sparks, MD). All inoculated agar plates were incubated at 37°C for 24-48 h, counted manually, and reported as $\log_{10}$ CFU per mL. Plates spread plated for CP were incubated in anaerobic jars with GasPak^{TM} EZ (Becton Dickson, Sparks, MD) and dry anaerobic indicator strip (Becton Dickson, Sparks, MD).

Bacon study

Culture preparation

L. monocytogenes (LM; ATCC Scott A; American Type Culture Collection, Manassas, VA, ATCC 19115), *C. perfringens*, (CP; ATCC 10543 and ATCC 3626), *S.* Typhimurium (ST; ATCC 14028 and ATCC 13311), and *E. coli* O157:H7 (EC; ATCC 43895 and ATCC 43889) were obtained from the Muscle Foods Microbiology Lab of the Food Science Department at The Pennsylvania State University. LM, ST, and EC were cultured separately in TSB (Becton Dickinson and Co., Sparks, MD) for 24 hours at 37°C. CP was cultured anaerobically in Reinforced Clostridia Medium (Becton Dickinson and Co., Sparks, MD) for 24 hours at 37°C. The cultures of LM, ST, and EC were stored at -80°C in TSB and glycerol as stock cultures until needed. Cultures of CP were stored in Cooked Meat Medium (Becton Dickinson and Co., Sparks, MD) at -4°C until needed. In preparation for experiments, stock cultures were thawed briefly and 0.1 mL of each culture were transferred to 9.9 mL of fresh TSB or RCM (depending on pathogen) and incubated at 37°C for 24 h. The cultures were later streaked for isolation on selective agars for each respective pathogen and incubated at 37°C for 36 h. LM, CP, and ST were isolated on selective agars as described previously. EC was isolated on MacConkey Sorbital Agar (Becton Dickson and Co., Sparks, MD) with cefixime tellurite selective supplement (Oxoid Ltd, Baskingstone, UK).

Inoculation of bacon

A single colony of each culture was taken from the selective agar plate and transferred to a separate 500 mL bottle of sterile TSB or RCM (depending on pathogen) and allowed to grow for 24 h at 37°C to obtain a cell concentration of ~8 $\log_{10}$ CFU/mL. This process was done in

quadruplicate for LM, ST, EC, and CP. The pathogen cultures were mixed in equal volumes to prepare a non-diluted cocktail for each replication. Pathogen cultures were not washed due to large volumes used in this experiment (Pan and Schaffner, 2010).

Bacon manufacturing

The brine formulations (Table 5.1) were utilized in this experiment for manufacture of injected bacon (except treatment WA). Bellies were obtained from a local processor and frozen (ca. -18^{o}C) prior to use. Bellies were manufactured into bacon at The Pennsylvania State University (PSU) Food Science Food Safety Pilot Plant. Two bellies per replication were quartered and two quarters were used per treatment. The quarters were placed into a sterile bin for 30 minutes and immersed in the pathogen cocktail for inoculation. Every 10 minutes, the quarters were inverted to ensure uniform inoculation of the surface. After the 30-minute inoculation, bellies were allowed to dry for approximately 15 minutes under a laminar flow bio hood. Following drying, bellies were injected to a 10% target pump. Treatment V4 was placed in the smokehouse (Vortron Model TR2-850, Vortron Smokehouses LLC, Iron Ridge WI) at 43°C dry bulb and approximately 70% relative humidity for 75 minutes for incubation of the starter culture (*S. carnosus*) (Sebranek & Bacus, 2007a). Sixty minutes after incubation began for treatment V4, treatments CC, NI, and NC were placed into the smokehouse to allow them to come up to temperature. A temperature probe was inserted into a treatment NI belly. After 15 minutes, the temperature of the smokehouse was set at 63°C and approximately 70% relative humidity for 60 minutes. The smokehouse was then set to 57°C and approximately 55% relative humidity and the product was cooked to an internal temperature of 53°C. Bellies were refrigerated (4°C) overnight. The following day, the bellies were sliced into 2 mm slices (Globe Model 150, Globe Equipment Co. Bridgeport CT). Bacon slices, five per package, randomly

chosen from each belly, were vacuum-packaged in 3 mil polyethylene pouches (UltraSource, Kansas City MO). Vacuum-packaged samples were then placed in a 10°C incubator to simulate temperature abuse of the product.

Bacon Sampling

Day 0 samples (approximately 25 g for each belly) were taken following inoculation/injection, but before heating. Day 1 sampling was taken after cooking, overnight cooling at 4°C, and slicing. Subsequent sampling was conducted on days 3, 7, 14, and 21. On each sampling day, a vacuum package was removed from the incubator, slices were diced aseptically, and a 25 g sample was weighed aseptically into a filtered stomacher bag (BA6141/STR Filter Bag, Seward, Port St. Lucie, FL). Samples were diluted 10 times the weight of the bacon (~25 g) with BPW (~225 g) and stomached for 2 minutes at 230 rpm (Stomacher 400 Circulator, Seward, Port St. Lucie, FL). The pH was then taken with SP20 sympHony pH meter (Thermo Scientific Orion, Beverly, MA). Samples were serially diluted in sterile BPW and 0.1 mL was spread-plated in duplicate on the appropriate selective agars. In addition, 0.1 mL was spread-plated in duplicate on Baird Parker Agar Base (Remel, Lenexa, KS) with egg yolk tellurite enrichment (Remel, Lenexa, KS) for enumeration of *S. carnosus* (starter culture for treatment A). Additionally, when direct plating failed to detect pathogens, enrichments were done for each pathogen, as follows. One mL of the stomached sample was pre-enriched for LM in UVM Modified Listeria Enrichment Broth (Becton Dickson, Sparks, MD), incubated for 24 hr at 37°C and transferred to a secondary enrichment of Fraser Broth (Becton Dickson, Sparks, MD) for 24-48 hr at 37°C. Fluid Thioglycollate Medium (Remel, Lenexa, KS) was used for detection of CP following a transfer of 1 mL and incubation for 24-48 hr at 37°C. One mL of stomached sample was transferred to GN Broth, Hijna (Becton Dickson, Sparks, MD) and incubated at 37°C for 24-

48 hrs for isolation of EC. For detection of ST, 1 mL of stomached sample was pre-enriched in Lactose Broth for 24-48 hrs at 37°C, 1 mL then transferred to a secondary enrichment of Tetrathionate Broth (EMD Chemicals Inc., Darmstadt, Germany) with Iodine (Sigma Aldrich, St. Louis, MO) and Selenite Cystine Broth (Becton Dickson, Sparks, MD), and incubated at 37°C for 24-48 hrs. If there were no detectable colonies on plates during enumeration, enriched samples were streak-plated on their respective selective agars for pathogen isolation. Plates used to enumerate or detect CP were incubated anaerobically. All enumerable agar plates were incubated at 37°C for 24-48 h, duplicate plates were counted manually, averaged, and transformed to $\log_{10}$ CFU per mL. For aerobic plate counts and coliform/generic *E.coli* counts, samples were serially diluted in sterile BPW and 1 mL was plated on APC Petrifilm™ and Coliform/*E. Coli* Petrifilm™ (3M, St. Paul, MN). Duplicate plates were incubated for 24-28 hours at 37°C. Colonies were counted using a 3M™ Petrifilm™ Plate Reader (3M, St. Paul, MN), averaged, and transformed to $\log_{10}$ CFU per mL.

Statistical analysis

Brine study

The experiment used a repeated measure design as the measurements were taken on the same brine over time with three replications. The tube with the brine solution was the experimental unit with main effects of treatment and day of storage. The PROC MIXED procedure of Statistical Analysis System (SAS; version 9.3, SAS Institute Inc., Cary, NC) was used for statistical analysis. Pathogen counts were analyzed for treatment effects by day. Means separation was conducted using LSMEANS function of SAS and Fisher's least significant difference (LSD) was performed for main effects or interactions. Statistical significance was set at $P < 0.05$.

Bacon study

The experiment used a repeated measure design as the measurements were taken on the same belly over time with three replications. Belly was the experimental unit with main effects of treatment and day of storage. The PROC GLM procedure of Statistical Analysis System (SAS; version 9.3, SAS Institute Inc., Cary, NC) was used for statistical analysis. Pathogen counts and pH were analyzed for treatment effects by day. Means separation was conducted using LSMEANS function of SAS and Fisher's least significant difference (LSD) was performed for main effects or interactions. Statistical significance was set at $P < 0.05$.

Results and Discussion

Brine study

Natural nitrate with starter culture (V4) decreased populations of LM more effectively ($P < 0.05$) than all other treatments (CC, NI, and WA) at 4 and 8 h (Figure 5.1). This observation may be attributed to an acidic pH (4.01) versus other treatments (CC, NI, NC; pH > 4.8; Table 5.2). There were no significant differences ($P > 0.05$) between treatments V4 (natural nitrate) and NI (conventionally-cured control); but CC (natural nitrite), V4 (natural nitrate), and WA (water) were different from all other treatments at 4 and 8 h. All treatments containing nitrate or nitrite (V4, CC, NI) were not statistically different ($P > 0.05$) from each other at 24 h, but were lower than uncured (NC) and water (WA) brines at 24 h. Treatment CC demonstrated a slower reduction of LM than treatments V4 and NI. This observation may be due to pH differences of the brines. This finding corresponds to findings by Sullivan (2011) who also found that natural nitrite was less effective than conventional nitrite at inhibiting LM.

Results for *C. perfringens* survival in bacon brine showed that at 0 h, treatments V4, CC, and NI were not statistically different from each other, but were lower than NC and WA (Figure 5.2). Treatment WA (water) exhibited higher populations ($P < 0.0001$) than all other treatments (V4, CC, NI, NC) at all-time points. Treatments V4, CC, and NI were not different ($P > 0.05$) from each other at all-time points. Treatments with uncured brine (NC) exhibited higher ($P < 0.05$) bacterial populations at 4, 8, and 12 h than treatment V4 (natural nitrate). Bacterial populations following treatments with natural nitrite (CC) and conventionally-cured (NI) solutions resulted in counts which were lower than uncured (NC) treatment after 4, 8, and 12 h. Natural nitrite (CC) and uncured (NC) samples exhibited similar ($P > 0.05$) counts after 4, 8, and 24 hours, but were significantly different ($P < 0.05$) at 12 h. Conventionally-cured control samples (NI) had lower ($P < 0.05$) populations at 8, 12, and 24 h than uncured (NC). Jackson et al. (2011) found that natural nitrite was less effective at inhibiting CP growth than natural nitrate with a starter culture in hams. This information differs from our findings, but could be attributed to the fact that we evaluated brines, and not a meat system.

Figure 5.3 illustrates the survival of ST in water (WA), which was significantly greater ($P < 0.05$) than all other treatments (V4, CC, NI, NC) at all-time points. Natural nitrate with starter culture (V4), natural nitrite (CC) and conventionally-cured control (NI) had significantly lower bacterial populations after 4, 8, and 12 h than uncured (NC) treatments. There were no differences ($P > 0.05$) in ST bacterial populations among treatments V4, CC, NI, and NC after 24 h of incubation. Hinton (1999) demonstrated that high salt (750 mM NaCl) concentrations can reduce the viability of ST. This observation may explain why there was not a difference at 24 h between treatments V4, CC, NI, and NC. Peters et al. (1991) found that higher salt concentrations limited the range of growth (pH and temperature) for *S.* Typhimurium. In 7% salt solutions, growth was limited to a narrow pH range of 5.6 to 6.7 and temperature range of 24°C

to 29°C. A 6.5% salt solution allowed growth at pHs as low as 4.2 with optimum temperatures of 21°C to 29°C.

Bacon study

Table 5.3 shows the survival and growth of LM at 10°C over 21 days of storage in bacon made with different brines. There were no significant differences, due to type of brine, within any storage day. This observation is inconsistent with the brine study, but is understandable since brine effects may be mitigated by belly tissues. Specifically, pH differences observed in the bacon brine are quickly buffered to near neutral (pH ~6.8) in the pumped belly (Table 5.2). The current findings are consistent with those of Sullivan (2011), who found that natural nitrite with antimicrobials (a blend of cultured sugar and vinegar and a blend of cherry, lemon, and vinegar powder) and natural nitrate with antimicrobials (a blend of cultured sugar and vinegar and a blend of cherry, lemon, and vinegar powder) in hams allowed microbial growth similar to that in conventionally-cured hams with an antimicrobial (lactate). The use of antimicrobials in all cases provided an additional hurdle in combination with nitrite. Nyachuba et al. (2007) investigated the impact of nitrite on LM growth. The authors concluded that as nitrite was depleted, LM grew more, especially as residual nitrite dropped below 20 ppm. Residual nitrite in the products in this study ranged from 0 ppm to 49 ppm. Xi et al. (2012) investigated the use of natural antimicrobials at inhibiting LM in naturally-cured frankfurters at 4°C. The authors found that no significant growth occurred in the first 28 days. However, after that time LM grew more rapidly in natural nitrate-cured frankfurters than conventionally-cured frankfurters.

The growth of CP was affected by the curing treatment more than any other pathogen (Table 5.4). By day 1, treatment NI afforded the most reduction of CP, as compared to day 0. This observation is possibly due to the higher level of in-going nitrite that is shown in Table 4.3.

The inoculation of the first replication was lower than the second replication, which may have caused lower counts throughout the subsequent days of storage. However, there was a treatment effect. When pooled across sampling day, treatment NI had lower ($P < 0.05$) populations (log_{10} 1.4 CFU/mL) than treatments V4, CC, and NC (log_{10} 2.3 CFU/mL, log_{10} 2.5 CFU/mL, log_{10} 2.7 CFU/mL respectively). Yet, treatments V4, CC, and NC were not different from each other. Jackson et al. (2011) concluded that natural nitrite with an antimicrobial (blend of cultured sugar and vinegar or a blend of cherry, lemon, and vinegar powder) and natural nitrate with starter culture and an antimicrobial exhibited similar growth to traditional cure with an antimicrobial. Sofos et al. (1980) investigated the effects of potassium sorbate and sodium nitrite on CP in commercially-prepared bacon. They found that 120 ppm nitrite was more effective at inhibiting CP than 40 ppm sodium nitrite and 0.26% potassium sorbate. During the 1970s, several researchers concluded that initial nitrite content was more important than residual nitrite (Bowen and Deibel, 1974; Christiansen et al., 1973). However, a few years later, Christiansen et al. (1978) determined that outgrowth of *C. botulinum* was dependent upon residual nitrite levels and surviving botulinal cells. Residual nitrite level at the time of temperature abuse is a primary determinant of nitrite's antibotulinal capability (Tompkin, 2005). Depletion of residual nitrite over storage time will eventually lead to a point at which the inhibitory effects will be depleted. In 1955, Scott determined that nitrate was a poor antimicrobial and Henry et al. (1954) determined that nitrite's inhibitory properties were influenced by pH.

Salmonella Typhimurium (Table 5.5) and *Escherichia coli* O157:H7 (Table 5.6) counts were similar for all treatments, within each storage day. This finding is not surprising given that nitrite is not known to affect Gram-negative bacteria (Pichner, et al., 2006; Tompkin, 2005). Turanta and Unluturk (1993) found that nitrite does not inhibit *Salmonella*. Buchanan and Bagi (1994) concluded that nitrite's effect on *E. coli* was dependent on the pH of the product, with a

lower pH allowing nitrite to be more inhibitory. Hinton (1999) demonstrated that high salt (750 mM NaCl) concentrations also can reduce the viability of ST.

The fate of *Staphylococcus carnosus* was evaluated in treatment V4 (natural nitrate with starter culture). Only treatment V4 was evaluated as it was the only treatment in which a starter culture was added to the formulations. It was concluded that counts declined during heating then increased back to starting counts ($p = 0.21$) during subsequent storage (Table 5.7). As expected, day 0 (raw belly) had numerically higher counts of *S. carnosus* than day 1 (cooked bacon). The reduction of nitrate to nitrite by *S. carnosus* is due to a membrane-bound type of nitrate reductase that is involved in respiratory energy conservation (Neubauer and Götz, 1996; Pantel et al., 1998). *S. carnosus* expresses its nitrate reductase capability better in anaerobic conditions and has its maximal level in the exponential growth phase. The formation of nitric oxide is the result of the further reduction of nitrite by nitrite reductase. Gøtterup et al. (2007) investigated several different types of *Staphylococcus* and concluded that *S. carnosus* formed the most nitrosylmyoglobin, which is important in cured color development. For products cured with nitrate, nitrate is reduced to nitrite by bacteria that are capable of producing nitrate reductase (Bacus, 2006). In an acidic environment, nitrite is reduced to nitrous acid, and through a series of chemical reactions nitrate, nitric oxide, and water are formed. Poorly regulated production of nitrite in this system may lead to substandard cured color, flavor or safety etc.

Evaluation of aerobic bacteria, coliforms, and generic *E. coli* showed that there were no differences among the treatments for each day of storage (Tables 5.8,5.9,5.10). This observation is understandable since LM, ST, and *E. coli* O157:H7, which are aerobic bacteria) were not affected by nitrate or nitrite concentrations on each day of storage. As stated previously, nitrite has minimal effect on Gram-negative bacteria. Anderson and Hinrichsen (1995) measured microbial populations in two brine curing systems and found that microbial populations changed over time, with lactic acid bacteria growing to similar levels as total plate counts. Shaw and

Harding (1978) stated that the highest numbers of lactic acid bacteria were present on bacon containing the lowest initial nitrite concentration. Shaw and Harding (1978) believed that nitrite may delay the souring of vacuum-packaged cured bacon. This is useful in the current study as nitrite may have prevented lactic acid bacteria from growing to high populations.

Conclusions

Natural nitrite with a natural cure accelerator (CC), natural nitrate with starter culture (V4), and sodium nitrite (NI) were all effective at reducing populations of *L. monocytogenes, C. perfringens,* and *S.* Typhimurium in brine during 24 h of exposure. However, only natural nitrate with starter culture (V4) inhibited pathogen growth as effectively as sodium nitrite (NI, conventionally- cured). Conversely, the bacon study demonstrated that nitrate or nitrite did not have an effect on Gram-negative bacteria. Nitrite itself is probably not effective at inhibiting pathogens, such as LM, ST, and EC, but is most likely due to a hurdle effect with other ingredients, such as antimicrobials and salt, that allow for bacterial reductions in products containing nitrite. However, sodium nitrite is effective at reducing CP growth, when compared to natural nitrite, natural nitrate, and no cure. Further research needs to be conducted in this area to further investigate natural curing ingredients and their ability to inhibit pathogens and lactic bacteria in a variety of other meat products.

Table 5-1 – Brine formulations of "no-nitrate or nitrite-added" bacon.

	% of Formulation								
TRT[a]	**Distilled H_2O**	**Salt**	**Sugar**	**Modern Cure**	**Sodium Erythorbate**	**Natural NO_3**	**Natural NO_2**	**Starter Culture**	**Natural Cure Accelerator**
V4	70.87	20	5	-	-	4	-	0.13	-
CC	73.01	15.38	3.42	-	-	-	5.99	-	2.2
NI	74.75	18.3	5	1.9	0.05	-	-	-	-
NC	75	20	5	-	-	-	-	-	-
WA	100	-	-	-	-	-	-	-	-

[a] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure, WA = water

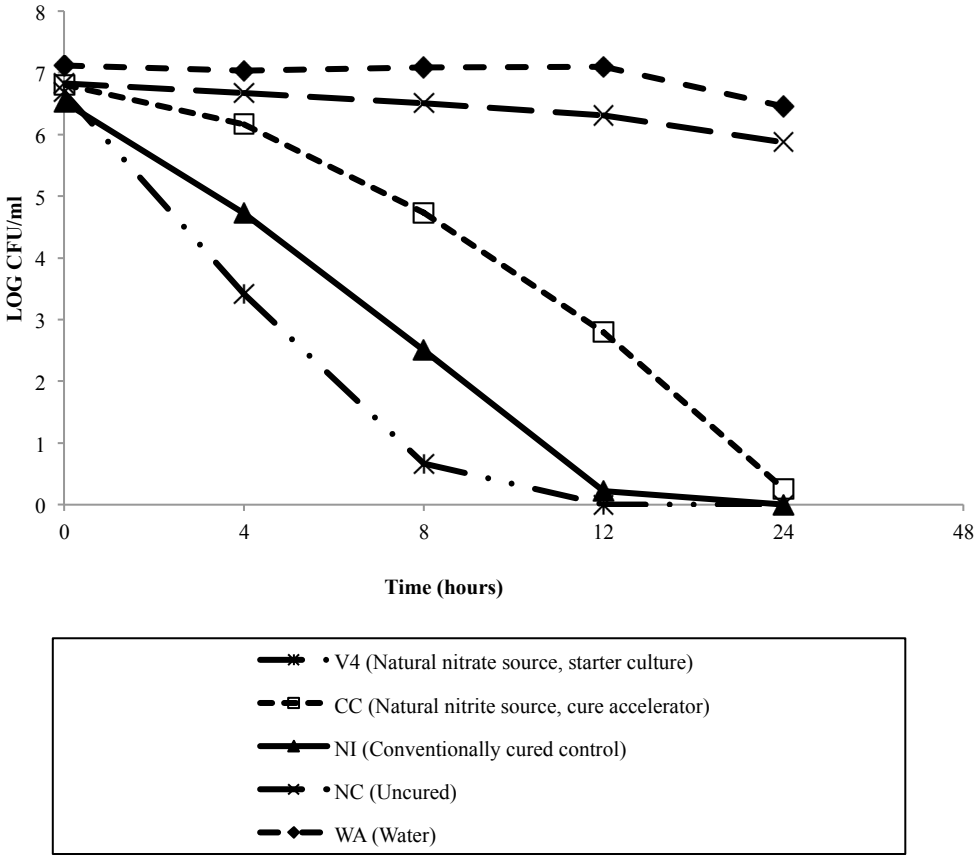

Figure 5-1 Effect of curing treatments[a] on survival of *L. monocytogenes* in bacon brines during storage at 37°C.

[a] Natural Nitrite = pre-converted celery juice powder and cherry juice powder, Natural Nitrate = vegetable juice powder with starter culture

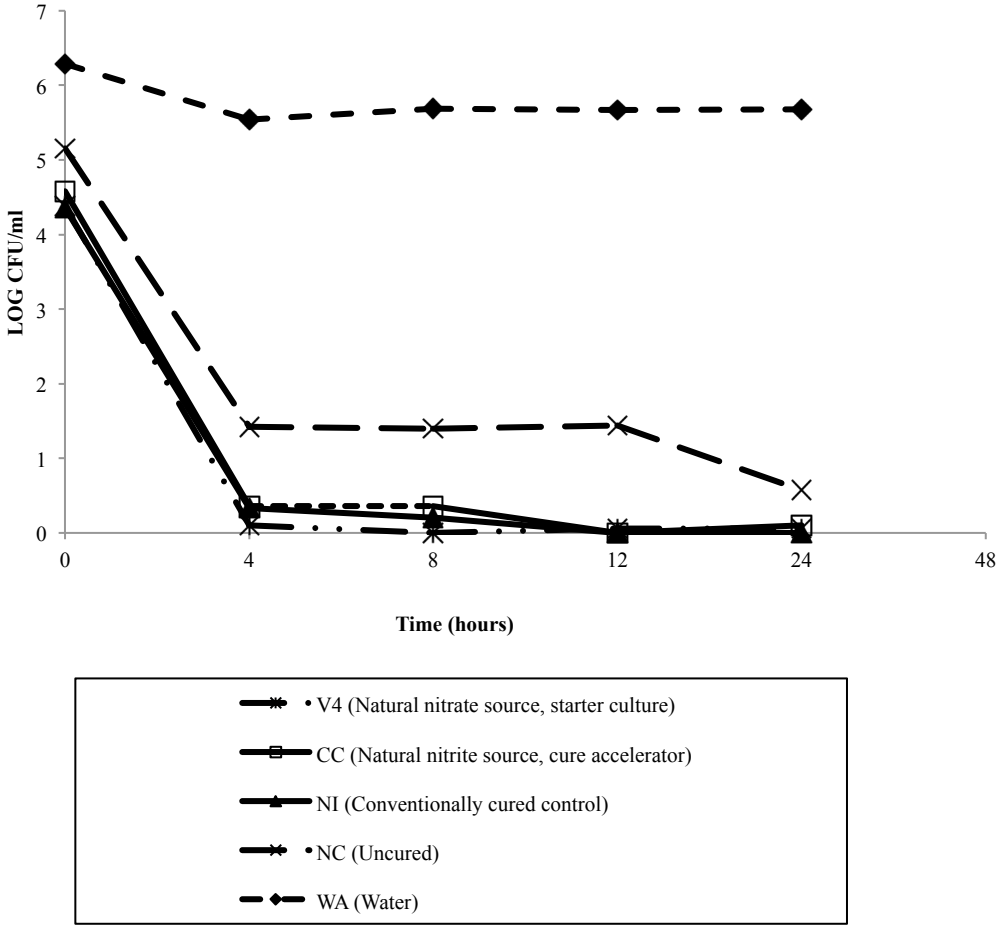

Figure 5-2 Effect of curing treatments[a] on survival of vegetative *C. perfringens* in bacon brines during storage at 37°C.

[a] Natural Nitrite = pre-converted celery juice powder and cherry juice powder, Natural Nitrate = vegetable juice powder with starter culture

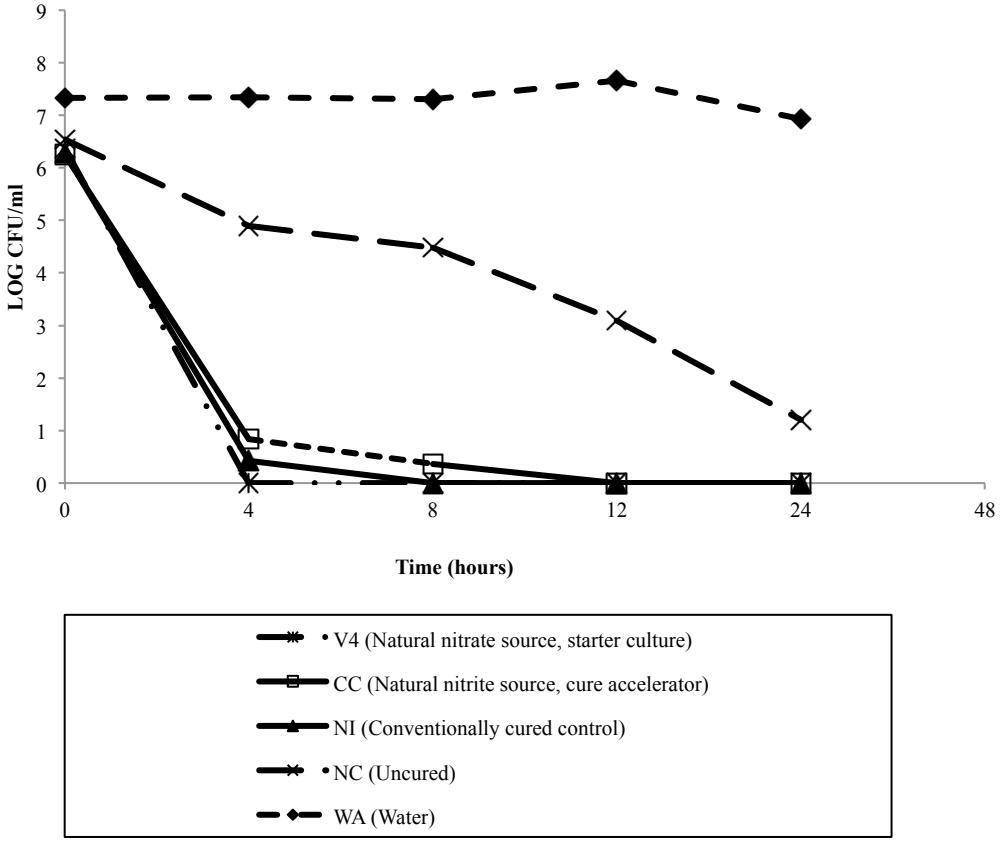

Figure 5-3 Effect of curing treatments[a] on survival of *S.* Typhimurium in bacon brines during storage at 37°C.

[a] Natural Nitrite = pre-converted celery juice powder and cherry juice powder, Natural Nitrate = vegetable juice powder with starter culture.

Table 5-2 pH of curing brines and bacon throughout 21-d storage of "no-nitrate or nitrite-added" bacon.

	Day[b]						
TRT[a]	Brine	0	1	3	7	14	21
V4	4.01[f]	7.04[c]	6.89[c]	6.85[c]	6.85[c]	6.79[c]	6.73[c]
CC	7.48[c]	7.03[cd]	6.88[c]	6.85[c]	6.86[c]	6.81[c]	6.75[c]
NI	5.58[d]	7.02[cd]	6.87[c]	6.85[c]	6.88[c]	6.85[c]	6.79[c]
NC	4.81[e]	6.92[d]	6.89[c]	6.83[c]	6.84[c]	6.80[c]	6.74[c]

[a] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[b] Vacuum-packaging and storage at 10°C.
[c-f] Means within the same column with different superscripts are different ($P < 0.05$).

Table 5-3 Effect of curing treatments on remaining population[a] of *L. monocytogenes* in bacon during storage at 10°C

	Day[c]					
TRT[b]	0	1	3	7	14	21
V4	6.5[e]	4.2[e]	4.9[e]	8.1[e]	7.8[e]	7.7[e]
CC	6.4[e]	4.7[e]	5.2[e]	8.3[e]	7.7[e]	7.9[e]
NI	6.5[e]	5.1[e]	5.0[e]	7.6[e]	7.9[e]	7.9[e]
NC	6.6[e]	4.8[e]	5.2[e]	6.6[e]	8.4[e]	9.1[e]
SEM[d] =	0.12	0.35	0.30	1.28	1.08	1.19

[a] $\log_{10}$ CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the least squares mean.
[e] Means within the same column with different superscripts are different ($P < 0.05$).

Table 5-4 Effect of curing treatments on remaining population[a] of *C. perfringens* in bacon during storage at 10°C.

	Day[c]					
TRT[b]	0	1	3	7	14	21
V4	5.2[e]	2.3[f]	2.2[ef]	1.7[e]	1.4[f]	1.3[e]
CC	5.2[e]	3.3[f]	2.5[ef]	1.5[e]	0.8[ef]	1.9[e]
NI	5.1[e]	0[eg]	1.7[e]	1.3[e]	0[eg]	0.6[e]
NC	5.1[e]	3.0[f]	2.6[f]	2.1[e]	1.6[f]	1.9[e]
SEM[d] =	1.48	0.72	0.65	0.49	0.36	0.47

[a] $\log_{10}$ CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the least square mean.
[e – f] Means within the same column with different superscripts are different ($P < 0.05$).
[g] Negative by the direct plating method, positive by the enrichment method.

Table 5-5 Effect of curing treatments on remaining population[a] of *S.* Typhimurium in bacon during storage at 10°C.

	Day[c]					
TRT[b]	0	1	3	7	14	21
V4	6.4[e]	3.7[e]	4.5[e]	4.9[e]	4.9[e]	5.1[e]
CC	6.6[e]	4.5[e]	5.3[e]	5.2[e]	5.3[e]	6.0[e]
NI	6.5[e]	4.0[e]	4.5[e]	4.3[e]	5.5[e]	5.6[e]
NC	6.6[e]	4.5[e]	4.8[e]	5.6[e]	6.2[e]	6.2[e]
SEM[d] =	0.09	0.35	0.51	0.80	0.58	1.07

[a] $\log_{10}$ CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the least square mean.
[e] Means within the same column with different superscripts are different ($P < 0.05$).

Table 5-6 Effect of curing treatments on remaining population[a] of *E. coli* O157:H7 in bacon during storage at 10°C.

	Day[c]					
TRT[b]	0	1	3	7	14	21
V4	6.1[e]	3.1[e]	3.9[e]	4.6[e]	4.5[e]	4.5[e]
CC	6.1[e]	3.7[e]	4.7[e]	4.8[e]	5.0[e]	5.1[e]
NI	6.1[e]	3.3[e]	4.3[e]	4.3[e]	4.5[e]	4.4[e]
NC	6.1[e]	3.4[e]	4.3[e]	4.8[e]	5.2[e]	5.3[e]
SEM[d] =	0.28	0.57	0.36	0.40	0.65	0.90

[a] $\log_{10}$ CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the least square mean.
[e] Means within the same column with different superscripts are different ($P < 0.05$).

Table 5-7 Effect of curing treatments on remaining population[a] of *Staphylococcus carnosus* in bacon during storage at 10°C.

	Day[c]						
TRT[b]	0	1	3	7	14	21	SEM[d]
V4	5.8[e]	4.9[e]	5.3[e]	5.6[e]	5.8[e]	5.7[e]	0.42

[a] CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the least square mean.
[e] Means within the same row with different superscripts are different ($P < 0.05$).

Table 5-8 Effect of curing treatments on remaining population[a] of aerobic bacteria in bacon during storage at 10°C.

	Day[c]					
TRT[b]	0	1	3	7	14	21
V4	7.5[e]	5.9[e]	6.1[e]	6.5[e]	6.7[e]	8.5[e]
CC	7.5[e]	5.5[e]	6.5[e]	5.2[e]	7.8[e]	8.1[e]
NI	7.7[e]	5.6[e]	5.6[e]	5.8[e]	6.7[e]	7.1[e]
NC	7.6[e]	5.5[e]	6.1[e]	6.8[e]	7.8[e]	8.9[e]
SEM[d] =	0.13	0.22	0.35	0.48	0.52	0.54

[a] $\log_{10}$ CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the least square mean.
[e] Means within the same column with different superscripts are different ($P < 0.05$).

Table 5-9 Effect of curing treatments on remaining population[a] of coliforms in bacon during storage at 10°C.

	Day[c]					
TRT[b]	0	1	3	7	14	21
V4	0.3[e]	3.8[e]	4.0[e]	4.9[e]	3.5[e]	2.9[e]
CC	0[e]	3.3[e]	3.0[e]	4.6[e]	5.2[e]	5.8[e]
NI	0.4[e]	2.8[e]	3.4[e]	4.7[e]	3.5[e]	4.7[e]
NC	0.3[e]	3.8[e]	3.7[e]	3.8[e]	6.0[e]	6.0[e]
SEM[d] =	0.29	0.36	1.06	0.69	0.03	1.02

[a] $\log_{10}$ CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the means.
[e] Means within the same column with different superscripts are different ($P < 0.05$).

Table 5-10 Effect of curing treatments on remaining population[a] of generic *E. coli* in bacon during storage at 10°C.

	Day[c]					
TRT[b]	0	1	3	7	14	21
V4	0[e]	0.3[e]	0.3[e]	0.4[e]	0[e]	0.8[e]
CC	0[e]	0.3[e]	0.5[e]	0[e]	1.4[e]	3.1[e]
NI	0[e]	0.7[e]	0.5[e]	0.5[e]	0[e]	2.1[e]
NC	0[e]	1.0[e]	1.0[e]	1.1[e]	1.1[e]	2.8[e]
SEM[d] =	0.28	0.57	0.36	0.4	0.65	0.9[e]

[a] $\log_{10}$ CFU/mL.
[b] Treatment combinations: V4 = natural nitrate source, CC = natural nitrite with cure accelerator, NI = conventional cure (120 ppm sodium nitrite with 550 ppm sodium erythorbate), NC = no cure.
[c] Vacuum-packaging and storage at 10°C.
[d] SEM = standard error of the least square mean.
[e] Means within the same column with different superscripts are different ($P < 0.05$).

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Chapter 6

Conclusions and Future Research

There is no doubt that consumers are driving the market for naturally-cured meat products. Meat processors are searching for ways to produce products that consumers want with the use of natural cure ingredients, but have similar characteristics to their conventionally-cured counterparts. The research presented in this dissertation investigated not only the quality attributes of no-nitrate or nitrite-added bacon, but also the ability of no-nitrate or nitrite-added bacon to inhibit foodborne pathogens. Bacon is the focus of this dissertation because of the lack of research on naturally-cured bacon and its residual nitrite.

The preliminary brine study showed that even though *S. carnosus* is considered a salt-tolerant microorganism, the presence of 20% salt in the brine inhibited the reduction of nitrate to nitrite. Therefore, nitrate reduction may be accelerated by pre-incubating the brine without salt, then adding the salt just before injection. This approach may help manufacturers eliminate the need for an incubation step during the cooking/smoking process, allowing products to be manufactured more quickly..

No-nitrate or nitrite-added bacon achieved color characteristics that approached those of sodium nitrite-cured bacon. However, the color faded more rapidly in the no-nitrate or nitrite-added bacon than in the sodium nitrite-cured bacon. This observation could lead to lower consumer acceptance or may be accepted by consumers who prefer the product for other reasons. Concerns about possible nitrosamine formation during frying of natural nitrate-cured bacon are not supported by the current study. Residual nitrite concentrations in natural nitrate-cured bacon were always numerically lower than in the nitrite-cured bacon, though sometimes the difference

was not significant. With such low residual nitrite concentrations, naturally-cured bacon would likely pose no more nitrosamine risk than for conventional bacon.

The brine portion study in Chapter 5 showed some promise that natural nitrate and natural nitrite brines might be able to slow or inhibit pathogens similar to sodium nitrite brines. However, this observation did not carry through with the bacon portion of the study. In the bacon study, nitrite-cured bacon was only effective at inhibiting *Clostridium perfringens*. The current study did not investigate interactions among ingredients so it is still possible that nitrite may be more effective when used in combination with other antimicrobials, such as a blend of cultured sugar and vinegar or a blend of cherry, lemon, and vinegar powder. Nevertheless, sodium nitrite is effective at reducing *Clostridium perfringens* growth when compared to natural nitrite, natural nitrate, or no cure.

There is still much research that needs to be conducted in reference to the use of natural cures in cured meat products. Further research needs to be conducted on the feasibility of pre-incubating brines versus incubating the injected or cured meat products in the smokehouse. The ability to pre-incubate brines prior to injection may allow for faster production, which will allow the meat processor to make more money.

Consumer and trained sensory panels need to be conducted to determine if the natural cures being used are imparting flavors that are unacceptable to consumers. Additionally, there still needs to be further research concerning the shelf life and safety of naturally-cured bacon and other meat products. Longer shelf life studies at refrigerated conditions need to be conducted to determine the color shelf life and microbial shelf life of the product. Sodium nitrite cured meat products are known to have a longer color and microbial shelf life than naturally cured meat products, but with the increasing number of natural antimicrobials and natural processing aids, naturally-cured products may be able to have a longer shelf life than they do currently. Various natural or clean label antimicrobials (such as a blend of cultured sugar and vinegar and a blend of

cherry, lemon, and vinegar powder) need to be investigated to see if they can help the color and microbial shelf life of naturally cured meats. A clean label antimicrobial is one that is made with natural ingredients or is one that consumers can easy understand and does not contain chemical names or the word "artificial". However, USDA and FDA do not define what "clean label" means; therefore, consumers have varying takes on the meaning. Additionally, natural ingredients that have antioxidant properties need to be investigated.

Furthermore, additional consumer studies need to be conducted determining if the flavor profiles of naturally-cured meat products are acceptable to consumers of various age, ethnic, and economic groups. Consumers panels relating to the products color also need to be conducted to make sure the color is acceptable to consumers.

Finally, more challenge studies need to be conducted to determine the ability of natural cure ingredients to inhibit foodborne pathogen growth. *Clostridium botulinum* is still a concern in the natural products because research is not conclusive with respect to the ability of natural cures to inhibit its growth. Natural and clean label antimicrobials need to be investigated further as a hurdle effect may be required for effective control for foodborne pathogens. To my knowledge, the research found in this dissertation concerning the inhibition of foodborne pathogens was the first to inoculate the meat product prior to injection; and as such, investigating the whole bacon manufacturing process. These types of challenge studies are important so the USDA-FSIS can make a ruling regarding the labeling of naturally-cured meat products.

Appendix

Nitrate Analysis

Procedures

1. 5-10 g finely comminuted & thoroughly mixed test portion with 80 mL warm distilled water in 140/150 mL beaker
2. Break lumps and heat on steam bath for 1 hour; stir occasionally
3. Transfer to 100 mL volumetric flask
4. Allow to cool
5. Dilute to volume with distilled water & mix
6. Filter or let settle
7. Pipette 40 mL filtrate or supernate into 50 mL volumetric flask
8. Add 3 drops of bromocresol green indicator
9. Add H_2SO_4 (1+10) dropwise until color changes to yellow
10. Oxidize nitrites to nitrates by adding 0.2M $KMnO_4$ (potassium permanganate) solution dropwise with shaking (shake while adding drops) until faint pink color remains for approximately 1 minute (time using timer)
11. Add 1 mL H_2SO_4 (1+10)
12. Add 1 mL phosphotungstic acid solution
13. Dilute to volume with distilled water & mix
14. Filter into 125 ml flask
15. Measure 20 mL of filtrate into 500 mL flask
16. Add enough Ag-NH_4OH solution to precipitate all chlorides & most excess phosphotungstic acid (~1-2 mL) (add 1 mL, then 0.5 mL at a time)
17. Add H_2SO_4 (3+1), approximately 3 times the volume of liquid (~60 mL)
18. Stopper flask
19. Mix
20. Cool to approximately 35°C (takes approximately 20-30 min)
21. Add 0.05 mL (1-2 drops) of the m-xylenol
22. Stopper
23. Shake
24. Hold at 30°-40°C for 30 minutes (water bath)
25. Add 150 mL of distilled water (wash off stopper)
26. Put in glass bulb to distill

27. Put 5 mL of NaOH (sodium hydroxide) in a graduated cylinder & place under distiller spot
28. Place glass on burner & hook up to distill
29. Plug burner in
30. Turn on water to the distill (cools off distillate)
31. Distill 40-50 mL liquid into the graduated cylinder that has the NaOH
    a. The liquid in the bulb should almost be clear
    b. The graduated cylinder will be at the 45 or above the kilmax on cylinder
32. Transfer distillate to 100 mL volumetric flask
33. Dilute to volume with distilled water
34. Determine nitrate N by comparing reading of color of suitable aliquot with standard curve prepared at ca 450 nm

Color standard

1. 10 mL nitrate standard solution into 500 mL flask
2. Add H_2SO_4 (3+1), approximately 3 times the volume of liquid (~30 mL)
3. Stopper flask
4. Mix
5. Cool to approximately 35°C (takes approximately 20 min)
6. Add 0.05 mL (1-2 drops) of the m-xylenol
7. Stopper
8. Shake
9. Hold at 30°-40°C for 30 minutes (water bath)
10. Add 150 mL of distilled water (wash off stopper)
11. Put in glass bulb to distill
12. Put 5 mL of NaOH (sodium hydroxide) in a graduated cylinder & place under distiller spot
13. Place glass on burner & hook up to distill
14. Plug burner in
15. Turn on water to the distill (cools off distillate)
16. Distill 40-50 mL liquid into the graduated cylinder that has the NaOH
    a. The liquid in the bulb should almost be clear
    b. The graduated cylinder will be at the 45 or above the kilmax on cylinder
17. Transfer distillate to 100 mL volumetric flask
18. Dilute to volume with distilled water
19. MAKING NITRATE STANDARD CURVE
    a. Put 10 mL into 50 mL volumetric flask

b. Put 20 mL into 50 mL volumetric flask
c. Put 30 mL into 50 mL volumetric flask
d. Put 40 mL into 50 mL volumetric flask
e. Dilute to volume with distilled water

Reading absorbance

1. Turn on spec (15 min warm-up)
2. Set wavelength to 450 nm
3. Zero the transmittance (left dial) – lid closed
4. Zero the absorbance with the nitrate blank

Nitrate reagents

- Silver-ammonium hydroxide solution
    1. Weigh out 5 g nitrate-free AG_2SO_4 (Silver-sulfate)
    2. Dissolve in 60 mL NH_4OH
    3. Heat to bp & concentrate to approximately 30 mL
    4. Cool & place in 100 mL volumetric flask
    5. Dilute to volume with distilled water
- Bromocresol green indicator
    1. Weigh out 0.1 g bromocresol green
    2. Dissolve in 1.5 mL 0.1 M NaOH
    3. Put in 100 mL volumetric flask
    4. Dilute to 100 mL with distilled water
- Nitrate Standard Solution
    1. 17.85 mL 0.1 M HNO_3
    2. Dilute to 1 L (1,000 mL)
- Low Concentrate Sulfuric Acid (1 + 10)
    1. 10 mL sulfuric acid (H_2SO_4)
    2. 100 mL distilled water
    3. Mix
- High Concentrate Sulfuric Acid (3 + 1)
    1. 300 mL sulfuric acid (H_2SO_4)
    2. 100 mL distilled water
    3. mix

Nitrite Analysis

Procedures

1. 5 g finely comminuted & thoroughly mixed test portion into 100 mL beaker
2. Add approximately 40 mL of 80°C distilled water
3. Mix thoroughly with glass rod & break up all lumps
4. Transfer to 500 mL volumetric flask
5. Rinse beaker & rod thoroughly with warm water
6. Add all washing to flask
7. Add enough warm water to bring volume to approximately 300 mL (will have to measure out 250 mL of warm water)
8. Put flask in steam bath (large water bath)
9. Let stand for 2 hours, shaking occasionally
10. Cool to room temperature (approximately 28°C)
11. Dilute to volume with distilled water & remix
12. Filter into 500 mL flask
13. If turbid – centrifuge
14. Place 40 mL of filtrate into 50 mL volumetric flask
15. Add 2.5 mL sulfanilamide reagent & mix
16. Wait 5 minutes
17. Add 2.5 mL NED reagent & mix
18. Dilute to volume with distilled water & mix
19. Let color develop for 15 min
20. Transfer portion of solution to photometer cell
21. Determine absorbance at 540 nm against blank

Nitrite standard

1. 10, 20, 30, and 40 mL nitrite working standard solution to 50 mL volumetric flasks
2. Add 2.5 mL sulfanilamide reagent & mix & wait 5 min

3. Add 2.5 mL NED reagent & mix & dilute to volume with distilled water & mix
4. Let color develop for 15 min
5. Transfer portion of solution to photometer cell and determine A at 540 nm against blank
6. Standard curve is straight line to 1 μg/mL $NaNO_2$ in final solution

Reagent blank

1. Add 45 mL distilled water in 50 mL volumetric flask
2. Add 2.5 mL sulfanilamide reagent & mix & wait 5 minutes
3. Add 2.5 mL NED reagent & mix & let color develop for 15 min
4. Use this as reagent blank

Reading absorbance

1. Turn on spec (15 min warm-up)
2. Set wavelength to 540 nm
3. Zero the transmittance (left dial) – lid closed
4. Zero the absorbance (right dial) with the nitrate blank

Nitrite reagents

- NED Reagent
    1. Weigh out 0.2 g N-(1-naphthyl) ethylenediamine·2HCl
    2. Dissolve in 150 mL 15% (v/v) Acetic Acid (CH_3COOH)
    3. Filter if necessary
    4. Store in glass-stoppered brown glass bottle
- Sulfanilamide Reagent
    1. Weigh out 0.5 g sulfanilamide
    2. Dissolve in 150 mL 15% (v/v) Acetic Acid (CH_3COOH)
    3. Filter if necessary
    4. Store in glass-stoppered brown glass bottle
- Nitrite Standard Solutions
    1. Stock Solution (1,000 ppm (C) $NaNO_2$)

        - Weigh out 1.0 g $NaNO_2$
        - Dissolve in distilled water
        - Dilute to 1 L (1,000 mL) with distilled water
    2. Intermediate Solution (100 µg/mL $NaNO_2$)
        - 100 mL stock solution
        - Dilute to 1 L (1,000 mL) with distilled water
    3. Working Solution (1 µg/mL)
        - 10 mL intermediate solution
        - Dilute to 1 L (1,000 mL) with distilled water
- TESTING FILTER PAPER
    1. Test 3-4 sheets at random
    2. Filter ca 40 mL distilled water through each sheet
    3. Add 4 mL sulfanilamide reagent & mix
    4. Let stand for 5 min
    5. Add 4 mL NED reagent & mix
    6. Let stand for 15 min
    7. If any sheets test positive, discard the entire box
        - Pink color

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EDUCATION

PhD, Animal Science, Penn State University, University Park, PA, 3.61 GPA, *Graduation Date* 12/12
- ***Animal Science – Emphasis: Meat Science***

M.S., Animal Science, Kansas State University, Manhattan, KS, 3.6 GPA, *Graduate* 12/08
- ***Animal Science – Emphasis: Meat Science***

B.S., Dual Degree, Kansas State University, Manhattan, KS, 3.7 GPA, *Graduate* 05/07
- ***Animal Science - Emphasis: Animal Products***
- ***Food Science – Emphasis: Food Business and Operations Management***
- ***Minor in Agricultural Economics***

A.A., General Agriculture, Merced College, Merced, CA, 3.8 GPA, *Honors Graduate* 12/03

RESEARCH EXPERIENCE

Masters Research: Department of Animal Science & Industry, Kansas State University, 2007- 08 (research advisors: Drs. Terry Houser & Melvin Hunt)
- Effects of distillers dried grains with solubles on pork loin quality and sow fat quality (Thesis research)
- Effects of aging on tenderness of *longissimus* muscle of cull cows under different management strategies (GEMS Project)

PhD Research: Department of Dairy & Animal Science, Pennsylvania State University, 2009 – present (research advisor: Dr. Edward Mills)
- Effects of transportation type on pig well-being and pork loin quality
- Impact of natural cures on microbial growth and cured meat quality attributes

ABSTRACTS
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TEACHING EXPERIENCE
- *Teaching Assistant*: Livestock Evaluation, Penn State, Sp & F of 2009-2011, Sp 2012
  - Organized lectures, labs, and planned trips to livestock producers ranches/farms
- *Instructor*: Advanced Meat Evaluation, Kansas State, Sp & F 2008
  - Organized and planned trips to packing plants, lectures and labs