The effect of lactate on nitrite in a cured meat system

By

Brooke N. McClure

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Meat Science

Program of Study Committee:
Joseph G. Sebranek, Major Professor
Dennis Olson
Leo Timms

Iowa State University
Ames, Iowa
2009
# Table of Contents

**General Introduction**................................................................................................................. 1  
**Thesis organization** .................................................................................................................... 2  
**Chapter 1. Literature Review** ....................................................................................................... 3  
  - **History of Lactate** ..................................................................................................................... 3  
  - **Antimicrobial Effects of Lactate** ............................................................................................. 4  
  - **Advantages of Lactic Acid/Lactate** .......................................................................................... 6  
  - **Increased Tenderness** .............................................................................................................. 8  
  - **Increased Cook Yields** ............................................................................................................ 8  
  - **Flavor Enhancer** .................................................................................................................... 9  
  - **Color** ................................................................................................................................... 10  
  - **History of Nitrite** .................................................................................................................. 12  
  - **Regulations of Nitrate and Nitrite** ........................................................................................ 14  
  - **Cured Meat Color** ................................................................................................................ 16  
  - **Cured Meat Flavor** .............................................................................................................. 19  
  - **Nitrite as an Antioxidant** ....................................................................................................... 20  
  - **Nitrite as an Antimicrobial** .................................................................................................. 22  
  - **Cured Meat Color Chemistry** .............................................................................................. 24  
  - **References** ............................................................................................................................ 25  

**Chapter 2. The effect of lactate on nitrite in a cured meat system** .............................................. 30  
  - **Abstract** .................................................................................................................................. 30  
  - **Introduction** ............................................................................................................................ 30  
    - **Meat Curing** ......................................................................................................................... 30  
    - **Lactate effect on meat color** .............................................................................................. 31  
  - **Materials and Methods** ......................................................................................................... 33  
    - **Model System** .................................................................................................................... 33  
      - **Role of lactate in metmyoglobin reduction** .................................................................... 33  
      - **Measurement of residual nitrite** .................................................................................... 34  
    - **Meat System** ..................................................................................................................... 35  
      - **Meat preparation** ............................................................................................................. 35  
      - **Nitrite analysis** ................................................................................................................ 36  
      - **Color measurement** ........................................................................................................ 37  
      - **pH measurement** ............................................................................................................. 37  
  - **Statistical Analysis** .................................................................................................................. 38  
  - **Results and Discussion** ......................................................................................................... 38  
    - **Model System** .................................................................................................................... 38  
      - **Reduction of metmyoglobin by addition of lactate** ..................................................... 38  
      - **Model system residual nitrite analysis** ...................................................................... 39  
    - **Meat System** ..................................................................................................................... 40  
      - **Residual Nitrite** ............................................................................................................... 40  
      - **Meat color analysis** ......................................................................................................... 42  
        - a* ....................................................................................................................................... 42  
        - L* ....................................................................................................................................... 44
GENERAL INTRODUCTION

Maintaining attractive color of fresh and processed meats has been an important issue facing the meat industry for decades. Meat surface color is a visual aspect that consumers instantly see when they approach a meat counter and if found unacceptable, they will not purchase the product. Appearance of meat color significantly influences consumer purchasing decisions. Though the specific color of a meat cut depends on the species, consumers are often aware of the color differences in similar cuts from the same species. If the color does not meet the consumer’s standards, then it is likely to be left on the shelf and eventually thrown away. Although the appearance may not be pleasing, it is not necessarily an indication that the meat is unfit for consumption. Meat processors are looking for ways to increase the color stability of products in order to get product off the shelf with less waste.

Lactates have been shown to help stabilize color. The addition of lactate to a meat system may help improve color stability by replenishing nicotinamide adenine dinucleotide (NADH, the reduced form of NAD\(^+\)) when lactate is converted to pyruvate via lactate dehydrogenase and therefore increasing the metmyoglobin reducing activity (Kim et al. 2006). Metmyoglobin reduction is essential to meat color life and this process requires NADH (Mancini and Hunt 2005). Because oxidation of myoglobin is one means by which nitrite is reduced to nitric oxide in meat curing, reduction of oxidized myoglobin may facilitate greater production of nitric oxide from nitrite by making more reduced deoxymyoglobin available for oxidation by nitirite. It is the goal of this research to determine if lactate affects production of nitric oxide from nitrite in cured
meat. Nitrite gives meat the cured pink color that is known and accepted in today’s industry. Not only is nitrite used for flavor and color attributes but it is also a widely accepted method to prevent microbial growth and extend the shelf life of products.

Thesis Organization

This thesis is organized into three chapters. Following a general introduction of meat color, lactates and nitrates/nitrites, the first chapter is a general literature review of the relevant topics pertaining to this research project. The second chapter is a manuscript to be submitted to Food Chemistry titled “The Effects of Lactate on Nitrite in a Cured Meat System”. The third chapter is a general summary of this research.
CHAPTER 1. LITERATURE REVIEW

History of Lactate

The use of lactic acid and lactate has been part of the food industry for quite some time and has many different applications. Lactic acid was first used as a pharmaceutical specialty in the early 19\textsuperscript{th} century and in 1881, Charles Avery established the ‘Lactate Company’ near Boston (Bogaert and Naidu 2000). It was the goal of this company to replace the use of tartric acid in bread making. About 1900, lactic acid was first successfully marketed for more general purposes by a company in Germany called the Boeringher Company (Bogaert et al. 2000). Lactic acid and lactates were being used in the production of wine, pickles and breads as early as 1969 (Reid 1969) and probably before according to some literature. It is around this time that lactic acid and lactates were being studied by the meat industry, as there was not much information available for meat applications. Use of lactates in the meat industry likely began around the early 1980s and was initiated by Oscar Mayer Foods Corporation due to a food safety concern in uncured poultry products. As of 2000, the market for lactic acid and its derivatives was estimated at approximately 100,000 metric tons each year and was growing by 12-15\% yearly (Bogaert et al. 2000).

The salt form of lactic acid is sodium lactate ($\text{C}_3\text{H}_5\text{O}_3\text{Na}$) which is now commonly used in the food industry, including meat products. Sodium lactate is a generally recognized as safe (GRAS) ingredient. It is used as an emulsifier, a flavor enhancer, a flavoring agent, a humectant, and a pH control agent (CFR184.1768)(USDA-FSIS 1996). Sodium lactate has the ability to extend shelf-life of meat products, though the mechanism is not clear (Bacus 1988). There are three proposed mechanisms by which
the sodium lactate ion can have an antimicrobial affect; the first is changing water activity \( (a_w) \), the second is the sodium lactate passing through the cell membrane and lowering intracellular pH, and the third is affecting cellular metabolism by inhibition of ATP generation (Bacus 1988; Maas, Glass and Doyle 1989). Although the exact mechanism is not known, there is strong scientific evidence to support the use of sodium lactate as an antimicrobial agent in meat products. There are no guidelines for this ingredient as long as it is used in food at levels not to exceed current good manufacturing practices (USDA-FSIS 1996). Lactate has also been shown to help improve color stability of fresh meat and has been reported to function as an antioxidant. Lactic acid is one of the most widely distributed acids and preservatives in nature (Shelef 1994).

**Antimicrobial Effects of Lactate**

Though lactate was used in other food products for many years before it was used in meat products, it has proven to be a very beneficial ingredient in the meat industry. Lactic acid has been used more extensively in the past for sensory qualities and is now being used more and more today for the antimicrobial properties (Doores 2005). There is continuing and growing evidence that sodium lactate has antimicrobial properties (Shelef 1994). Though other organic acids can be used as antimicrobials, lactate is successful as an antimicrobial because it is known to enhance meat flavor due to the salty taste that it provides, while retaining color, and contributing to increased water holding capacity (Doores 2005). It has been shown that sodium lactate, as well as potassium and calcium lactate, are all similar in their effectiveness for controlling the growth of aerobes and anaerobes in meat (Shelef 1994). As reported in Papadopoulos,
Miller, Acuff, Lucia, Vanderzant and Cross (1991a), H. Angersbach discovered in 1971, that adding sodium lactate at 0.3% to a nutrient medium would significantly inhibit the growth of three *Bacillus* strains that were isolated from meat products, *Bacillus cereus*, *B. subtilis*, and *B. circulans*. During the 1980s, the Oscar Mayer Foods Corporation investigated use of sodium lactate in meat products as an antimicrobial. During this time period, cook-in-bag, uncured turkey products were becoming very popular. The environment in which these meat products were placed was very favorable for the growth of *Clostridium botulinum*. According to Maas et al. (1989) uncured product contained ample nutrients, low levels of salt, a near neutral pH and an anaerobic environment which could support the growth and toxin production of *Clostridium botulinum*. The ability to control *C. botulinum* is a challenge and, in cured meat products, has been mostly done by the use of sodium nitrite. Little work had been done regarding the use of sodium lactate in meats, up to the time of the work published by Maas et al. (1989). They reported a study done by Krol in 1972 which showed that the growth of lactobacilli decreased in ham products formulated with sodium lactate. Krol proposed that the bactericidal effect observed was due to sodium lactate. The results reported by Maas et al. (1989) indicated that as the level of sodium lactate increased, the toxin production of *C. botulinum* was delayed. These researchers also believed that their work was the first report on the antibotulinal effect of sodium lactate (Maas et al. 1989). Not only is *C. botulinum* controlled by sodium lactate, but this ingredient has also been shown to affect *Listeria monocytogenes* (Choi and Chin 2003).

*Listeria monocytogenes* is a common, well known foodborne pathogen that causes listeriosis, a foodborne disease that has a high death rate (George, Richardson
Listeria is a gram-positive, non-spore-forming rod. It can be found anywhere in the environment and this is one of the reasons it is such a concern for the food industry. It has a wide pH range from about 4.1 to 9.6 in which it can grow (Jay 2000). It also has wide temperature range, 1°C - 45°C, in which it can grow. Because of Listeria’s ability to grow virtually anywhere and survive for a long period of time, the meat industry has been looking for other improved methods in which to control this pathogen, and one of the candidates is sodium lactate. According to Shelef (1994) L. monocytogenes was inhibited by 4% sodium lactate with a meat moisture content of 55%. In a study done by Choi et al. (2003), the addition of 3.3% sodium lactate showed potential to replace or supplement other preservatives for anti-listerial effects because it was able to delay the lag phase for L. monocytogenes growth by approximately 2 weeks.

Sodium lactate was found to be effective against the growth of aerobic microorganisms, psychotropic bacteria, lactic acid bacteria, and enterobacteriaceae, while maintaining chemical quality and extending shelf life of ground beef during refrigerated storage (Sallam and Samejima 2004). The fact that sodium lactate is capable of decreasing or slowing the growth of microorganisms not only increases the safety of the product but also increases the shelf life.

**Advantages of Lactic Acid/Lactate**

Lactate and lactic acid are finding their way into many different applications in the food industry. Both the fresh meat and processed meat sectors have found numerous benefits for using this organic compound. Today carcasses of beef, pork, and poultry are being rinsed with lactic acid. The Food Safety and Inspection Service (FSIS) has
approved using food grade FDA GRAS organic acid in aqueous solutions of 1.5% to 2.5% for carcass washing applications (USDA-FSIS 1996). These organic acids include acetic, citric and lactic acids applied to a skinned carcass as a mist, fog, or small droplet rinse (USDA-FSIS 1996). The reasoning behind using organic acids is that the acid has the ability to reduce the pH to levels which do not allow bacteria to initiate growth (Doores 2005). Pipek, Houska, Hoke, Jelenikova, Kyhos and Sikulova (2006) found that by using steam and lactic acid to decontaminate pork carcasses, the surface microbial count was reduced immediately and microbial growth was slowed throughout storage. The use of lactic acid along with a steam treatment also decreased and slowed microbial growth over time in a study done on chicken skins (Lecompte, Kondjoyan, Sarter, Portanguen and Collignan 2008). The use of lactic acid on beef carcasses with a hot water treatment also showed a decrease in microbial growth (Ozdemir, Yildirim, Kuplulu, Koluman, Goncuoglu and Inat 2006).

Lactate, the salt of lactic acid, is used in the production of processed meats and has been found to improve characteristics such as tenderness, shelf life, and color stability. It has been shown that raw muscle with a higher pH is darker in color (Ockerman and Cahill 1977). Yet, according to Papadopoulos, Miller, Ringer and Cross (1991c) sodium lactate darkened cooked meat color by a different mechanism than an altered pH. Another theory is that myoglobin color may be affected by the lactate ion concentration and once a certain cellular ionic concentration is achieved, myoglobin will be denaturated and past that point, the color will not change, regardless of any additional lactate (Papadopoulos et al. 1991c).
Increased Tenderness

Tenderness in beef is one of the characteristics that highly affects consumers’ eating satisfaction. According to Boleman et al. (1997) consumers are willing to pay extra for beef that is known to be tender, compared to less tender beef. Sodium lactate has also been shown to have a positive influence on meat tenderness. Vote et al. (2000) determined that sodium lactate enhancement of the longissimus dorsi muscle significantly improved tenderness and juiciness when compared to the control. Shear force measurements done on product injected with 0%, 1%, 2%, 3%, and 4% lactate showed a decrease in shear force with increased lactate from 0% to 3%, but a decrease from 3% to 4% lactate (Papadopoulos et al. 1991c). These authors also found that shear force measurements were consistent with tenderness scores from sensory panel evaluations. Significant differences in tenderness were found between untreated steaks and steaks treated with sodium lactate as determined by a sensory panel (McGee, Henry, Brooks, Ray and Morgan 2003). McGee et al. (2003) concluded that sodium lactate could be used on traditionally less tender cuts of beef and those cuts might be acceptable to consumers. The mechanism that causes sodium lactate to have an effect on increased tenderness and decreased shear force is not known (Papadopoulos et al. 1991c).

Increased Cook Yields

Sodium lactate has been shown to improve cook yields of meat products (Papadopoulos et al. 1991c). The addition of 3% sodium lactate, for example, increased cook yields by 12% (p<0.01) in beef top rounds (Papadopoulos et al. 1991a). Bloukas, Paneras and Fournitzis (1997) showed that 3% sodium lactate in low-fat
frankfurters had a higher (p<0.05) processing yield than frankfurters without sodium lactate. The processing yield increase with increasing sodium lactate could be due to a combination of increased levels of sodium ions and the humectant properties of sodium lactate (Bloukas et al. 1997).

**Flavor Enhancer**

Sodium lactate is known as a flavor enhancer. Seman (2001) discussed characteristics of flavor that sodium lactate helps to improve. Sodium lactate, when added to fresh meat, will delay the development of sour and off-flavors and seems to be a very prominent flavor enhancer with little negative effects (Seman 2001). One explanation for sodium lactate delaying the development of off-flavor is that it acts as a radical scavenger. It will bind to free radicals in meat which will then prevent lipid oxidation. Lipid oxidation is closely correlated to myoglobin oxidation. As lipid oxidation decreases, myoglobin oxidation decreases as well.

Processed or precooked meats have also displayed benefits from the addition of sodium lactate. Precooked products such as roasts had enhanced flavor notes, with a stronger beefy flavor, and decreased warmed-over flavor (Papadopoulos et al. 1991a). In the same way, off-notes such as warmed-over flavor, cardboard, fishy, and painty flavors were minimized when sodium lactate was added (Papadopoulos et al. 1991a). Addition of sodium lactate resulted in enhancement of overall flavor and beef flavor intensity, which ultimately increased the liking of overall flavor and beef flavor (Papadopoulos et al. 1991a). It has been suggested that by adding sodium lactate to a meat product, the amount of salt (NaCl) could be decreased and still maintain the desired level of salt flavor. O'Connor, Brewer, McKeith, Novakofski and Carr (1993)
found that, in combinations of NaCl and sodium lactate, the salt intensity of ground pork was higher with 3% sodium lactate, regardless of the sodium chloride level. O’Connor (1993) found that the salt intensity of ground pork samples containing 1.5% sodium lactate and 1% NaCl were similar to samples containing 0% sodium lactate and 2% NaCl. The sensory evaluation of O’Connor’s study showed that the addition of sodium lactate had no effect on off-flavor (O’Connor et al. 1993). This study ultimately suggested that the addition of sodium lactate to processed meat products has the potential to decrease the amount of sodium chloride in a product while still maintaining the level of salt flavor.

Color

Sodium lactate has repeatedly been shown to have an effect on both raw and cooked meat color. According to results by Maca, Miller, Bigner, Lucia and Acuff (1999), sodium lactate helped to stabilize the color of roast beef. Product with sodium lactate was darker and redder than the control (Maca et al. 1999). Results from Papadopoulos et al. (1991c) demonstrated that precooked beef roasts with the addition of sodium lactate appeared to be redder and darker than the control that did not have lactate. A study by Eckert, Maca, Miller and Acuff (1997) reported that as the storage time of uncooked beef increased, a* values decreased, meaning the samples were becoming less red. Since the red hue was found to decrease and become more of a reddish-purple (Eckert et al. 1997), it can be assumed that the meat was going from a state of oxymyoglobin to deoxymyoglobin. A study done by Kim et al. (2006) investigated the relationship between metmyoglobin, lactate to pyruvate conversion and the production of reduced nicotinamide adenine dinucleotide (NADH) via lactate
dehydrogenase (LDH). These authors discovered that MMb was reduced in a lactate-LDH-NAD system and if any one of the system components were left out, minimal reduction occurred (Kim et al. 2006). It was suggested that lactate promoted fresh meat color stability due to increased lactate dehydrogenase activity, by which lactate is converted to pyruvate while NADH is also regenerated. This regenerated NADH is thought to then be available to reduce metmyoglobin to deoxymyoglobin which can then form oxymyoglobin. This ultimately means that fresh meat will maintain a red color for a longer period of time. The following diagram by Kim et al. (2006) depicts the proposed process in which the lactate-LDH system generates NADH for metmyoglobin-reducing activity.

![Diagram of lactate-lactic dehydrogenase (LDH) system for generating NADH for metmyoglobin-reducing activity (MRA).](image)


Kim, Keeton, Smith, Maxim, Yang and Savell (2009) suggested that addition of lactate to fresh meat protected the meat pigment from myoglobin oxidation under oxidizing conditions, allowing formation of more red oxymyoglobin and longer retention of the oxymyoglobin pigment. Data from an early study by Cheah (1976) showed that bacon contained a functional lactate dehydrogenase capable of reducing NAD+ to NADH,
which could be re-oxidized by metmyoglobin which was concurrently reduced. However, the exact mechanism by which sodium lactate affects cooked muscle color is unknown (Papadopoulos, Miller, Acuff, Vanderzant and Cross 1991b; Maca et al. 1999).

**History of Nitrite**

Meat curing is a process that has been utilized for meat preservation for thousands of years. Originally, it was not considered meat curing, but simply was a process which helped to preserve meat. It was not until this art of preserving meat evolved into a better understood procedure that it became known as meat curing (Pegg and Shahidi 2000). No one knows exactly when or how the method of curing meat was first developed or discovered, as it was likely being used before record keeping was established. The preservation of meat probably began with the use of salt alone which occurred centuries before the deliberate use of nitrate (Binkerd and Kolari 1975). It was discovered that meat could be preserved when packed in salt because it prevented microbial growth due to salt’s drying action on meat (Pegg et al. 2000). Some of the earliest Summerians of Mesopotamia, around 3,000 BC, ate salted meat and fish as a regular part of their diet (Binkerd et al. 1975). Research shows that meat preservation was first practiced in the deserts of Asia and that the salts from these areas were contaminated with nitrate. However, records did not show the reddening affects created by nitrates until late in the Roman Era (Binkerd et al. 1975). Saltpeter, as it later became known, is salt contaminated with potassium nitrate which became recognized as a means of improving the color of salted meat.

Nitrate is converted to nitrite through the action of bacterial cultures. It is nitrite which gives meat the distinctive cured pink color and flavor. As cited by Pegg &
Shahidi, it was studies done in the late 19th century by Polenske in 1891, Kisskalt in 1899, and Lehmann in 1899 that demonstrated the importance of nitrite in meat curing. Polenske was the scientist responsible for providing the first technological advances in curing that led to the idea that nitrate is converted to nitrite by bacterial action. Some bacteria are natural nitrate reducers and are present in the environment. These bacteria were also present thousands of years ago, and were the reason meat was preserved and a cured pink color developed with the use of saltpeter and later, nitrate. The difference then was people were not aware of what was causing the pink color and how it developed. It was not long after the discovery by Polenske that Kisskalt and Lehmann showed that it was nitrite, not nitrate that gave meat the cured color (Pegg et al. 2000). Studies were done in the earlier part of the 20th century that allowed for the authorization of using nitrate and nitrite in meat. As reported by Cassens, Ito, Lee and Buege (1978), two scientists, Lewis in 1925 and Kerr in 1926, contributed to the USDA Bureau of Animal Industry authorization for nitrate and nitrite with the results they reported. Some of the rules that were part of the original authorization are still in use today. Though some of these rules still set the guidelines for today’s nitrite regulations, many of the regulations have changed as far as nitrate and nitrite concentrations are concerned. In the 1960s and 1970s, questions began to rise in regards to the usage of nitrites in cured meats products. Concerns that developed at that time involved the formation of carcinogenic N-nitrosamines which can be produced in some cured meat products if residual nitrite concentrations are unusually high.
Regulations of Nitrate and Nitrite

Since the early 19th century, the scientific knowledge on the use of nitrates and nitrites in the meat industry has expanded a great deal. Since that time, more information has allowed for changes in the amounts of nitrate and nitrite that a processor is able to use in their product. Though changes have been slow, progress has been made on the amounts needed for the curing process. Either sodium or potassium nitrate/nitrite can be used as a curing agent in meat products. The limits are the same for both salts and are based on weights. This means that since potassium is a heavier element by weight than sodium, there is less nitrite in a product when potassium salt is used as the carrier. There is an exception to the general limits of curing agents in cured meats and that is with bacon. The FSIS Directive 7620.3 requires a minimum of 120 parts per million (ppm) for all cured meat products that are not shelf stable (USDA 1995). However, certain processes may be validated as safe and therefore, exceptions to this amount can be made. Forty parts per million is recommended for preservation processes of shelf stable products, even though there is no minimum ingoing nitrite level set. Currently, the maximum levels of nitrate and nitrite added to products vary depending on what method of curing is used. The green weight of the meat block is what determines the nitrite concentration used in the formulation. The table below shows the maximum allowable limits of various curing agents that can go into meat products (USDA 1995). More stringent regulations are required for nitrite in bacon because of the concern of nitrosamine formation. Nitrosamines are carcinogenic N-nitroso compounds that are especially a concern in bacon. Nitrosamines are formed when a nitroso group (-N=O) is added to a nitrogen atom in
certain organic compounds (Price and Schweigert 1987). These compounds have been found to be very potent carcinogens to experimental animals and therefore, thought to have similar carcinogenic effects on humans. Bacon that is crisp and well done, as a result of high cooking temperature, has been found to have higher concentrations of nitrosamines than bacon cooked at lower temperatures (Price et al. 1987). Nitrate is no longer allowed in bacon, regardless of production type (pumped and/or massaged, dry cured, or immersion cured), because of the nitrosamine formation concern (USDA 1995). Because of this cancer concern, the specific curing method of bacon production that is being utilized will determine the ingoing amount of nitrite.

The above mentioned methods of production for bacon determine the limits of nitrite and all limits are based on the green weight of the belly with the rind off. For pumped and/or massaged bacon (rind off), the amount of 120 ppm ingoing sodium nitrite (148 ppm potassium nitrite) is required. Immersion cured bacon (rind off) limits sodium nitrite to 120 ppm maximum or the equivalent of potassium nitrite (148 ppm). Dry cured bacon (rind off) has a maximum limit of 200 ppm sodium nitrite and 146 ppm potassium nitrite. If the rind (skin) is left on the belly then additional adjustment must be made. The weight of the pork belly is about 10% skin and retains basically no cure solution or agent. This factor requires that the maximum amount of ingoing nitrite must be reduced by 10% in order to account for the weight difference. All of the above regulations can be found in the USDA-FSIS Processing Inspectors Calculation Handbook (USDA 1995).
<table>
<thead>
<tr>
<th>Curing Agent</th>
<th>Immersion Cured</th>
<th>Massaged or Pumped</th>
<th>Comminuted</th>
<th>Dry Cured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Nitrite</td>
<td>200</td>
<td>200</td>
<td>156</td>
<td>625</td>
</tr>
<tr>
<td>Potassium Nitrite</td>
<td>200</td>
<td>200</td>
<td>156</td>
<td>625</td>
</tr>
<tr>
<td>Sodium Nitrate</td>
<td>700</td>
<td>700</td>
<td>1718</td>
<td>2187</td>
</tr>
<tr>
<td>Potassium Nitrate</td>
<td>700</td>
<td>700</td>
<td>1718</td>
<td>2187</td>
</tr>
</tbody>
</table>

Table 1. Maximum limits (in ppm) of curing agents for meat and poultry products (not including bacon). From USDA FSIS Processing Calculations Inspector’s Handbook (FSIS Directive 7620.3).

Cured Meat Color

The pink color of cured meats is just as important to consumers as the bright cherry red color of fresh raw meat. It is this pink color that allows consumers to identify that the meat product is cured. Most consumers favor such a color when it comes to cured products such as frankfurters, ham and bologna. According to a study done in 1977, when the amount of nitrite added to frankfurters was decreased, consumer panel appeal was also decreased (Sebranek, Schroder, Rust and Topel 1977). However, it is the muscle pigment that gives cured meat its pink color, not the nitrite. Nitrite stabilizes myoglobin through a reversible chemical bond. Nitrite fixes the color of pigment but does not change the color (Dryden and Birdsall 1980). Consequently, it is important to understand myoglobin in a raw meat system before it can be understood in cured meat.

Myoglobin is the main pigment in muscle and postmortem muscle tissue. In living tissue, myoglobin is where oxygen is stored to be used for day to day biochemical
processes of the muscle. Myoglobin is a relatively complex structure and yet, it is a small protein. This complex protein structure has both a protein portion and a non-protein portion (Romans, Costello, Carlson, Greaser and Jones 2001). The non-protein portion is known as the heme group, which is comprised of two parts, an iron atom and a porphyrin ring. Myoglobin is a globular heme protein made up of a single polypeptide chain which consists of about 153 amino acids, depending on the species. The iron atom in the heme group has six binding sites. The heme group consists of four pyrrole groups centered on an iron atom. These four sites are used to stabilize the protein portion also known as the globin (Romans et al. 2001). The 5th and 6th binding sites are two additional bonds that are associated with the iron atom. The 5th binding site is used to link the protein portion to the heme group. The 6th binding site is free to interact with different chemical elements (Romans et al. 2001). This allows for different color states of the meat. The heme iron can exist at either the ferrous (Fe$^{2+}$) or ferric (Fe$^{3+}$) state depending on oxidation/reduction conditions which affects the chemical element that is attached to the 6th binding site. If nitrite, nitric oxide or other reductants are present, several pathways can lead to the formation of nitrosylhemochrome, the cured meat pigment (Fox 1966).

Cured meat color is one that is very distinctive to consumers and therefore it is important to maintain this pink color when meat is packaged and put in the supermarket. Properly cured meat should maintain the bright pink color throughout and not have any gray spots or other similar color defects. There are several factors that ultimately affect cured meat color and its ability to maintain this color. Some examples of these factors are pH, myoglobin content, amount and dispersion of nitrite, packaging, exposure to
light and oxygen, and the microbiological condition (Romans et al. 2001). It is necessary to understand the chemical reactions that take place in a cured meat system in order to know how each of these factors can affect cured meat color. In order to form the color in cured meat, nitrite (NO$_2^-$) must be converted to nitric oxide (NO). Nitrite itself does not act as a nitrosylating agent and must go through several reactions in which different intermediates are formed. When meat and nitrite (NO$_2^-$) are combined, the ultimate goal is for nitrite to be reduced to nitric oxide (NO). There are two forms of nitrite found in meat, the anion, NO$_2^-$ and the neutral nitrous acid, HNO$_2$ (Sebranek and Fox 1985). The pH of meat is around 5.6, which is higher than the pKa of nitrite (3.36) making the concentration of HNO$_2$ very low in cured meat. It is thought that the main reactive species is the anhydride of HNO$_2$, dinitrogen trioxide (N$_2$O$_3$). It is this dinitrogen trioxide that reacts with reductants naturally found in muscle tissue as well as added reductants to form nitric oxide (Pegg et al. 2000).

The formation of nitric oxide from nitrite can be affected by other additives and conditions including salt, reducing agents, pH, temperature, and time. Salt will accelerate the curing reaction due to the formation of nitrosyl chloride (NOCl), which is a more effective nitrosating agent than dinitrogen trioxide (Moller and Skibsted 2002). The reactants forming the nitrosating species (N$_2$O$_3$, NOCl) have to be in the protonated (acid) form, thus the lower the pH the faster the reaction occurs (Fox 1987). A low pH results in increased conservation of nitrous acid into nitric oxide. It has been shown by Ahn and Maurer (1989) that a pH increase, due to added phosphates, resulted in decreased nitric oxide production and higher levels of residual nitrite concentration in poultry products. Reducing agents, erythorbate and ascorbate, will accelerate reduction
of nitrite to nitric oxide. According to Lee and Cassens (1976), it takes at least two hours to convert 90% of nitrite to nitric oxide and bind with myoglobin thus forming additional nitrosomyoglobin.

In a meat system, reduced myoglobin (Fe\(^{2+}\)) is oxidized by nitrite to form metmyoglobin (Fe\(^{3+}\)) and the nitrite is concurrently reduced to nitric oxide (NO). When nitric oxide (NO) is formed in this reaction, the myoglobin oxidation state is changed, due to the oxidation of the heme iron atom. In order to convert as much nitrite to nitric oxide as possible and speed up the curing reaction, it would be helpful to continually convert metmyoglobin to the reduced state, thus allowing for more nitrite to react with reduced myoglobin and form nitric oxide.

The nitric oxide-myoglobin pigment is relatively unstable. In order to stabilize this bond, the product must be cooked. Heat from cooking will denature the globin and cause the bond between nitric oxide and the heme group to become stabilized. When this occurs, nitrosylhemochrome is formed, resulting in the cured pink color. The nitrosylhemochrome pigment is sensitive to oxygen, light and other factors. This means that in order to maintain the cured pink color, product must be packaged and stored properly in order to retain the best quality and consumer appeal.

**Cured Meat Flavor**

Flavor is a very important sensory attribute that determines overall acceptance and whether or not a customer will buy a product again. Overall flavor can be derived from several different characteristics, not only by taste, but also by odor, texture, and temperature (Brooks, Haines, Mioran, and Pace 1940; Lawrie and Ledward 2006). However, volatile compounds decide the aroma characteristics and also play a role in
establishing the cooked meat flavor (Mottram 1994). Meat curing practices today not only involve sodium nitrite and salt, but also sugar, reducing agents, phosphates and sometimes seasoning to give the characteristic properties to the final product (Ramarathnam and Rubin 1994). Yet, it is the nitrite and its effects that are the most important ingredient when it comes to curing meat.

Nitrite is responsible for the development of the distinct flavor of cured meat. Brooks et al. (1940) first discussed the relationship between nitrite and flavor in cured bacon and ham. Though there was no taste panel information presented for this study, Brooks et al. is still credited as the first person to describe cured meat flavor (Wasserman and Talley 1972). The role of nitrite in cured meat flavor is quite complex and not fully understood. Cured meat flavor is likely due to chemical changes that occur when nitrite is added to meat. Nitrite’s role in cured meat flavor development probably includes antioxidant activity. This activity slows the breakdown of unsaturated fatty acids, as well as secondary oxidation product formation that contributes to off-flavors (Pegg et al. 2000). Scientists have tried to determine some of the volatile and non-volatile compounds which could be responsible for some of the cured flavor. However, determining these compounds has been a struggle and is still an ongoing process. Consumer appeal and acceptability is decreased as the level of nitrite in cured meat is decreased (Sebranek et al. 1977). Salt is another ingredient that is likely involved in cured meat flavor. It has been shown that salt increases cured meat flavor (Froehlich, Gullett and Usborne 1983).

Another factor that has been found to affect cured meat flavor, is the rate of lipid oxidation that occurs after the cooking of meat that has had nitrite added. Nitrite is
known to retard lipid oxidation, and prevent off-flavor in meat (Gray, Macdonald, Pearson and Morton 1981; Romans et al. 2001). A review by Gray et al. (1981) showed that nitrite slowed lipid oxidation, but no specific compound was found to be responsible for the cured meat flavor. According to Gray et al. (1981) there is no known substitute for nitrite that can effectively produce the characteristic cured meat flavor.

**Nitrite as an Antioxidant**

One of the main factors limiting meat quality and consumer acceptability of meat and meat products is lipid oxidation (Morrissey, Sheehy, Galvin, Kerry and Buckley 1998). Lipid oxidation has been a long standing issue when it comes to meat quality and can lead to problems such as discoloration, drip loss, off-odor and off-flavor development (Morrissey et al. 1998). Nitrite and nitrate act as powerful antioxidants in cured meat which helps to prevent lipid oxidation, slow quality deterioration and improve the shelf life of meat and meat products.

Lipid oxidation of an unsaturated fatty acid occurs in three steps; initiation, propagation and termination. Initiation is caused by a hydrogen atom (H) being eliminated from an unsaturated fatty acid (RH) then can bond with oxygen (O$_2$) or other catalysts. The second step, propagation, results when a lipid free radical (R$^*$) reacts with oxygen and forms a peroxo radical (ROO$^*$). It is in this propagation phase that a chain reaction begins and further oxidation of remaining unsaturated fatty acids occurs when more radicals are produced (Morrissey et al. 1998). The peroxo radical formed will remove a hydrogen atom from a different unsaturated fatty acid molecule to produce a hydrogen peroxide (ROOH) and a new lipid radical that subsequently reacts with oxygen (Pegg et al. 2000). This chain reaction continues until there is no longer oxygen
or unoxidized lipids present (Pegg et al. 2000) or until radicals react with each other. When this happens, propagation is completed, and is known as the termination step.

The effect that nitrite has on rancidity is likely due to the same reaction that is responsible for color development in cured meats (Price et al. 1987). Nitrite prevents lipid oxidation by binding to the heme group and stabilizing it during the cooking process (Vasavada and Cornforth 2005). When the nitrite group is bound to the porphyrin ring, it becomes stabilized and the release of Fe$^{2+}$ is prevented (Pegg et al. 2000). Free iron (Fe$^{2+}$ and Fe$^{3+}$) will react with lipids to form free radicals. These free radicals react with oxygen and act as a catalyst in reactions causing lipid oxidation. A study done by Igene, King, Pearson and Gray (1979) among others has shown that the addition of nitrite significantly reduced lipid oxidation in cooked meat samples.

**Nitrite as an Antimicrobial**

Meat provides organisms with an ideal environment to grow. Meat is high in moisture, minerals and accessory growth factors, nitrogenous compounds and a fairly favorable pH of around 5.6, all of which are nearly ideal for the growth of microorganisms. It is for these reasons that meat must be packaged and stored properly in order to prevent meat from spoiling through microbial activity. Nitrite is one of the most effective antibotulinal compounds known to prevent the sometimes fatal food poisoning caused by botulism (Romans et al. 2001). Nitrite also provides bacteriostatic properties to help safeguard against other food-borne disease and meat spoilage. Therefore, because of the addition of nitrite the meat supply is safer for human consumption and the shelf life of meat is extended. The microbiological safety
that is provided through nitrite in meat is probably the most important function offered by the meat curing process (Romans et al. 2001).

Nitrite is known to have an inhibitory effect on many different microorganisms. While, each microorganism is affected differently by nitrite, one of the most important microorganisms that nitrite helps to prevent is *Clostridium botulinum*. This microorganism is a gram-positive, anaerobic spore-forming rod that was first discovered and isolated by Ermengem in 1896 (Jay 2000). It can grow and produce its deadly toxin in foods of low acid content such as meat products. One of the biggest concerns for *C. botulinum* spores is that the spores are extremely heat resistant. *C. botulinum* is anaerobic, which means that vacuum packaged meat provides an ideal growing condition. Botulism is the known disease that develops if this toxin is consumed and is the most deadly of all food-borne illnesses. Botulism was a severe problem in meat products before the use of nitrite as a curing agent (Archer 2002).

Nitrite’s antibotulinal properties are multifunctional. These properties entail several interactions of nitrite with various other factors, such as pH, salt, heat treatment, spore level, and the original or residual levels of nitrite within meat products (Archer 2002). Nitrite can exert its’ antibotulinal functions in two different parts of the *C. botulinum* life cycle. First, nitrite may play a role in retarding the growth of vegetative cells that survive from spores and, second, nitrite may prevent cell division in vegetative cells (Pierson and Smoot 1982). For these reasons, nitrite has and will likely continue to be used in cured meats unless another antimicrobial is found to be as effective.
Cured Meat Color Chemistry

As previously mentioned, nitrite gives meat its cured color. When nitrite is added to a meat system, the nitrite is converted to nitric oxide by a reducing agent. The nitric oxide will attach at the 6th ligand forming nitric oxide or nitrosomyoglobin which will eventually be converted to nitrosylhemochrome. One means of reducing nitrite to nitric oxide in meat is by oxidation of myoglobin to metmyoglobin. This oxidation-reduction coupling produces both nitric oxide and metmyoglobin. It has been suggested by Kim et al. (2006) that metmyoglobin can be converted back to deoxymyoglobin through metmyoglobin reducing activity (MRA), a reaction facilitated by lactate. It is the enzyme activity of LDH that helps convert lactate to pyruvate and produce more NADH. Hendgen-Cotta et al. (2008) suggested that deoxymyoglobin can convert nitrite to nitric oxide and the generation of more deoxymyoglobin is likely to result in more nitric oxide (NO) from nitrite and less residual nitrite. Therefore, the hypothesis for this project is that addition of lactate will generate NADH to convert metmyoglobin to deoxymyoglobin with the resulting greater concentration of reduced, “recycled” myoglobin available to again react with nitrite to produce more nitric oxide. The net result is hypothesized to be more production of nitric oxide which in turn speeds up the curing reaction.
References


USDA-FSIS (1996). Notice of policy change; achieving the zero tolerance performance standard for beef carcasses by knife trimming and vacuuming with hot water or steam; use of acceptable carcass interventions for reducing carcass contamination without prior agency approval. 61: 15024-15027.


CHAPTER 2. The effects of lactate on nitrite in a cured meat system

A paper to be submitted to Food Chemistry

B.N. McClure, J.G. Sebranek, Y.H. Kim, G.A Sullivan

Abstract

Two experiments were conducted to determine the effects of sodium lactate on nitrite during meat curing. In a model system, 8 reaction components were used to assess the effect of each on metmyoglobin reducing activity by excluding one component at a time. Excluding lactate, nicotinamide adenine dinucleotide (NAD), L-lactate dehydrogenase (LDH) or phenazine methosulfate (PMS) resulted in no reducing activity. A second experiment utilizing a meat mixture, lactate was added to ground beef at concentrations ranging from 2% to 6%, with or without nitrite, packaged in oxygen-permeable and impermeable materials, and stored for up to 96 hours. There was less residual nitrite when sodium lactate was added to the mixture. Results from both experiments supported the hypothesis that lactate generated NADH which reduced metmyoglobin to deoxymyoglobin, with the resulting greater concentration of reduced myoglobin reacting with nitrite to produce more nitric oxide, reducing nitrite concentration and accelerating the curing reaction.

1. Introduction

1.1 Meat curing

Meat curing is a process that has been utilized for meat preservation for thousands of years. However, it was not until the “art” of preserving meat evolved into a better understood procedure that it became known as meat curing (Pegg and Shahidi 2000). The preservation of meat probably began with the use of salt, centuries before the deliberate use of curing agents such as nitrate (Binkerd and Kolari 1975). Saltpeter, as it later became known, is salt contaminated with potassium nitrate which eventually became recognized as a means of improving the color of salted meat. Nitrate is
converted to nitrite through the action of nitrate-reducing bacteria and it is nitrite that
gives cured meat its distinctive color and flavor. In the curing process, nitrite is
converted to nitric oxide by added reductants such as erythorbate or ascorbate and
other compounds. The role of a reductant in cured meat is to convert nitrite to nitric
oxide which allows for a faster and more complete curing reaction. Nitrite not only gives
meat its cured color and flavor, but it also works as a strong antimicrobial and
antioxidant. The meat pH is also important to cured meats because at a lower pH,
nitrite is more reactive which speeds up the curing reaction. Nitrite is an important
antioxidant in cured meat because the nitric oxide produced from nitrite binds to the
heme group and stabilizes the iron during the cooking process (Vasavada and Cornforth
2005) consequently preventing lipid oxidation.

1.2 Lactate effect on meat color

Enhancement of fresh meat products is a common practice in the meat industry
today. The addition of sodium or potassium lactate with a small amount of added water
and salt (sodium chloride) can improve flavor, color, and tenderness, as well as
increasing antimicrobial and antioxidant properties (Papadopoulos, Miller, Acuff,
Vanderzant and Cross 1991a; Papadopoulos, Miller, Ringer and Cross 1991b; Maca,
Miller, Maca and Acuff 1997; Kim et al. 2006; Kim, Keeton, Smith, Maxim, Yang and
Savell 2009). Lactate is commonly used in fresh, cooked and raw meat products to
decrease off-flavor and off-odor developments and for inhibiting microbial growth
(Brewer, Rostogi, Argoudelis and Sprouls 1995). Lactate has also been studied by
several researchers whose results have consistently shown that addition of lactate is
very beneficial to the color of fresh meat. While lactate is also commonly used in cured
meats as an antimicrobial agent, there has been very little research on the effects of lactate on the color development of cured meat products.

Meat color is very important to the meat industry as it is one of the most significant factors for consumer appeal and purchasing decisions. The brown discoloration of fresh meat that is due to metmyoglobin formation on the surface of meat frequently results in consumer rejection. The consumer will begin to discriminate against products even when levels of metmyoglobin are relatively low (Renerre and Labas 1987). It is the brown, metmyoglobin color that consumers associate with old and spoiled meat. The oxidized form of metmyoglobin is converted back to deoxymyoglobin by means of metmyoglobin reducing activity (MRA) as long as the reducing system is active. After the pigment is converted to deoxymyoglobin, which is purple in color, it can then be oxygenated to form or reform oxymyoglobin. Oxymyoglobin in fresh meat is a bright cherry red color that consumers associate with fresh meat products. Ledward (1985) suggested that the most important intrinsic factor influencing the amount of metmyoglobin in meat at a given time is MRA. It is well known that the reduction of metmyoglobin occurs through both enzymatic and non-enzymatic reducing systems (Mancini and Hunt 2005). It is reduced nicotinamide adenine dinucleotide (NADH) that serves as a very important reducing substrate for both of the pathways. According to Kim et al. (2006), the addition of lactate to a fresh meat system increases the amount of NADH by conversion of lactate to pyruvate as a result of lactate dehydrogenase (LDH) activity. The increased NADH then serves to convert metmyoglobin to deoxymyoglobin. In fresh meat, this allows subsequent formation of oxymyoglobin for redder and longer lasting fresh meat color. It has long
been recognized in meat curing that addition of nitrite to fresh, raw meat results in formation of brown metmyoglobin color. Recent research has demonstrated that myoglobin oxidation provides nitrite reductase function to form nitric oxide in biological systems. Because one of the mechanisms for reduction of nitrite to nitric oxide during meat curing is the oxidation of deoxymyoglobin to metmyoglobin, we hypothesized that the increased MRA activity and production of more deoxymyoglobin will provide greater concentration of reduced, “recycled” myoglobin available to again react with nitrite to produce more nitric oxide. The net result is hypothesized to be depletion of residual nitrite, and assumed greater production of nitric oxide which in turn can be expected to speed up the meat curing reaction.

2. Materials and Methods

2.1 Model System

The model system is an experiment that is conducted to exemplify what would be occurring if the test was done in a meat system. The model system is a more controlled environment where all factors can be carefully monitored and changed easily according to results or desired outcome.

2.1.1 Role of lactate in metmyoglobin reduction

The role of lactate and LDH in metmyoglobin reduction was first assessed by adding various combinations (Table 1) of the following reaction components to disposable 1.5 milliliters (mL) polystyrene cuvettes: 0.3 mL of 0.5 mM equine metmyoglobin (MMb) in 30 mM phosphate buffer, 0.1 mL of 200 mM sodium lactate, 0.1 mL of 200 ppm sodium nitrite solution, 0.1 mL of 6.5 mM nicotinamide adenine dinucleotide (NAD+), 0.1 mL of 0.6 mM 2,6-dichloroindophenol (DCIP), 25 µL of 30 mM
phenazine methosulfate (PMS), and 1 µl of 40 unit L-lactate dehydrogenase (LDH) following the procedure from Kim et al. (2006) with modifications. These modifications included the addition of sodium nitrite and the deletion of citrate. The final reaction volume was 1.0 mL. The pH of the mixture was 7.5 and temperature was 23°C. PMS and DCIP are electron carriers that must be included in the model system in order for it to function, whereas meat naturally has electron carriers. Reagents were obtained from Sigma (St. Louis, MO). The cuvettes were then placed in a Beckman DU 640 spectrophotometer and the absorbance at 540 nm was recorded every 2 seconds for 30 minutes. Each combination of reaction components was measured in triplicate. The 8 combinations shown in Table 1 included all of the reaction components in the first treatment, followed by removal of one of the components, in turn, from each of the subsequent treatments. When one of the components was excluded, the specific volume of that component was replaced with distilled, de-ionized water (DDW) to bring the volume back to 1.0 mL. The activity was computed as nanomoles of metmyoglobin reduced in minutes for a linear phase of the analysis from Kim et al. (2006), using a difference in molar absorptivity of 12,000 mol-1 cm-1 at 540 nm. Activity is expressed as the average of triplicate samples. Different concentrations of nitrite (100, 200, or 300 ppm) and lactate (100, 150, or 200 mM) were also compared in order to determine the concentration effect of each of these components on the reaction.

2.1.2 Measurement of residual nitrite

Nitrite was determined by the AOAC method ([AOAC] Association of Official Analytical Chemists 1990) with modifications. One milliliter of the reagent solution from the model system was transferred to a 250 mL volumetric flask using a pipette. The
flask was then filled to volume with DDW. Approximately 30 mL of sample was then transferred to a 50 mL volumetric flask. Under a fume hood, 2.5 mL sulfanilamide reagent (0.5g sulfanilamide in 150 mL 15% acetic acid) was added. After 5 minutes, 2.5 mL NED reagent (0.2 g N-(1naphthyl) ethylenediamine dihydrochloride in 150 mL 15% acetic acid) was added and filled to volume with the original sample solution. Color was allowed to develop for 15 minutes. The solution was transferred to a spectrophotometer cuvette and absorbance was measured at 540 nm against a blank of 45 ml DDW, 2.5 ml sulfanilamide reagent, and 2.5 ml NED reagent. All nitrite assays were done in triplicate.

2.2 Meat System

2.2.1 Meat Preparation

Beef top rounds were used to obtain 80/20 ground beef. The top rounds were first ground (Biro MFG Co., Marblehead, Ohio, USA) using a 9.53 millimeter (mm) plate. On each day of sample preparation, four different batches of ground beef were made with one of three lactate concentrations. Sodium-(L)-lactate (60% food grade, Purac America, Lincolnshire, IL, USA) was added to the ground beef at 2%, 4%, or 6% by weight as lactate on a given day of sample preparation. Sodium nitrite and sodium erythorbate were included at 150 ppm and 550 ppm, respectively, for those batches in which these ingredients were included. For each lactate concentration, treatments included: control; lactate alone; nitrite and erythorbate; and lactate, nitrite and erythorbate (Table 2). Additionally, the effect of erythorbate on meat color and residual nitrite concentrations in the presence of lactate was assessed by comparing the treatments with erythorbate (Table 2) to similar treatments without erythorbate. These
treatments included a control; lactate alone; nitrite; and nitrite and lactate with lactate added in each case at 4% (Table 3). The ingredients for each of the treatments in all comparisons were added appropriately to the ground beef and mixed (K.K. Higashimoto Kikai, Model 20) for three minutes. The meat was then reground (Biro MFG Co., Marblehead, Ohio, USA) using a 3.18 mm plate. Four ounce beef patties were formed for each treatment using a hand patty press, 11.4 centimeters in diameter. The patties were then placed on styrofoam trays and packaged in either oxygen-permeable overwrap (Resinite Packaging Film, RMF 61-Hy, 1400 cc O2/100 in2/24 hr. at 23C; Borden Packaging Inc., North Andover, Mass., U.S.A) or 7X14 inch oxygen-impermeable vacuum packaging bags (Sealed Air, Cryovac, Curlon 861, 3 cc O2/645 cm2/24 hr. at 23C and 0% RH, Cryovac Division, W.R. Grace Co., Duncan, S.C., U.S.A.). Vacuum bags were evacuated and sealed using a double chamber machine (Multivac, model AG800). All packages were stored at 3ºC prior to product sampling. The entire process was repeated on a different day to complete a second replicate. Only one concentration of lactate was included on a single production day. Therefore, a total of eight days of sample preparation were required to complete the meat batch preparations with replication of each. Hunter color (L*, a*, b*) and residual nitrite concentrations were measured during storage to determine the effect of sodium lactate on nitrite curing reactions in ground beef.

2.2.2 Nitrite Analysis

Residual nitrite was determined by the AOAC method (1990). Nitrite concentrations were measured at 1, 4, and 24 hours after the meat products were manufactured. All residual nitrite assays were done in duplicate.
2.2.3 Color measurement

Color measurements were performed by using a Hunterlab Labscan spectrocolorimeter (Hunter Associated Laboratories Inc., Va., U.S.A.). The spectrocolorimeter was standardized using plastic overwrap over the white standard tile. The beef patty samples were analyzed by using an Illuminant D75, 10° standard observer light source with a 2.54 cm port size. Commission International d’Eclairage (CIE) L*(lightness), a* (redness), and b* (yellowness) measurements were taken of the entire exposed area at three randomly selected locations on each patty and the resulting average was used in data analysis. Color was measured at 1 hour following preparation of the patties and then again at 4, 24, 48, 72 and 96 hours of storage. Samples were removed from packaging, covered with a plastic overwrap and color measurements were recorded immediately. A different beef patty was used for each time point throughout the color measurement process.

2.2.4 pH Measurement

The pH of the meat was measured for each treatment one hour after product was manufactured. The pH was measured with a pH/ion meter (Acumet 950: Fisher Scientific, Fair Lawn, N.J., USA) equipped with a combination electrode (Accumet Flat Surface Epoxy Body Ag/AgCl combination Electrode Model 13-620-289, Fisher Scientific, Fair Lawn N.J., USA) calibrated with phosphate buffers at pH 4.0 and pH 7.0. Measurements were taken in duplicate by placing the probe directly into the ground beef.
2.3 Statistical Analysis

Statistical analysis was not conducted on the model system; instead standard deviations were calculated for the activity and used for comparisons. For comparing the effects of lactate concentrations on residual nitrite, color and pH of the meat mixtures, data were analyzed using a 2X2X4 factorial design including two cure treatments, two packaging treatments and four concentrations of lactate for the meat system. Measures were repeated over time for color and residual nitrite and included in the model. Proc GLM of SAS (version 9.1, Cary, NC) was used for analysis of variance. Mean separation was conducted using the means function with the Tukey adjustment when level of significance indicated by ANOVA when \( p \leq 0.05 \).

3. Results and Discussion

3.1 Model System

3.1.1 Reduction of metmyoglobin by addition of lactate

In the model system, comparison of myoglobin reducing activity with and without the presence of nitrite showed similar patterns in that the initial metmyoglobin was reduced to oxymyoglobin then quickly oxidized in about 5 minutes, and followed by a slow reformation of pigment that is probably nitric oxide myoglobin (Figure 1). The treatment with ‘all’ components and without DCIP showed an increase in absorbance after about 300 seconds, while the treatment without nitrite did not. The results of the lactate treatment in this study are similar to the results found by Kim et al. (2006) for the effect of lactate on metmyoglobin and oxymyoglobin. The oxidation-reduction reaction appears to be the same in both cases. This suggests that the lactate in the system with nitrite is producing more nitric oxide myoglobin which is very similar in absorbance to
the oxymyoglobin observed in the Kim et al. (2006) study. Activity was calculated for all the eight combinations. The activity was computed (Kim et al. 2006) as nanomoles of metmyoglobin reduced in minutes for a linear phase of the analysis, using a difference in molar absorptivity of 12,000 mol-1 cm-1 at 540 nm (Table 1). Activity is expressed as the average of triplicate samples (Table 1). When either lactate, NAD, PMS or LDH was left out of the solutions with metmyoglobin, there was very little to no activity seen as each of these components is essential for the metmyoglobin reduction reaction to occur properly.

The effect of different concentrations of nitrite (100, 200, or 300 ppm) are shown in Figure 2. Figure 3 shows the different concentrations of lactate (100, 150, or 200 mM) and as the concentration increased, so did the metmyoglobin reducing activity.

### 3.1.2 Model System Residual Nitrite Analysis

The treatments with 'all' reaction components and without DCIP appeared to show a greater decrease in residual nitrite over 24 hours than did the other treatments. Residual nitrite was 56.7 parts per million (ppm), 54.9 ppm, and 49.5 ppm for 1, 5 and 24 hours, respectively (Table 4 and Figure 4). This decrease in nitrite concentrations suggests that when all the components were included in the system, a faster reduction of nitrite was occurring. This would occur if LDH activity regenerated NADH which then reduced metmyoglobin to deoxymyoglobin. In turn, subsequent deoxymyoglobin oxidation by nitrite would form nitric oxide which could then combine with the pigment. This is supported by Kim et al.(2006), who reported greater myoglobin reduction in the presence of lactate and by Hendgen-Cotta et al. (2008) who reported nitrite reductase activity by myoglobin. The exclusion of metmyoglobin, lactate, LDH, NAD or PMS
resulted in little to no change in residual nitrite. The samples without lactate initially had 59.8 ppm of nitrite at one hour and ended at 24 hours with 59.3 ppm, indicating that there was very little change in the amount of residual nitrite when lactate was not present. When LDH was excluded from the reaction, the amount of nitrite increased slightly from 56.4 at hour 1 to 58.2 at hour 24, suggesting that without LDH, the residual nitrite was not affected. Excluding NAD resulted in very little change as well. The amount of observed nitrite in this case was 58.5 ppm, 58.0 ppm, and 58.7 ppm at hour 1, 5, and 24 respectively. It is interesting to note that the lack of DCIP appears to result in a lower nitrite concentration at 24 hour when compared to the treatment with all the components, which suggests that DCIP is not important to the system. DCIP is an electron carrier, and because it was not essential to the reaction as indicated by the resulting residual nitrite concentration, could be left out of the model system experiment. However, the other electron carrier, PMS was important to the reaction because without it, there was little change in nitrite over the 24 hour time period.

3.2 Meat System

3.2.1 Residual Nitrite

Measurement of residual nitrite in this study was utilized as an indicator of how much nitrite had reacted with the meat system components.

In the ground meat mixture, residual nitrite was compared between treatments containing 0%, 2%, 4% and 6% sodium lactate. There was a significant difference between treatments with and without lactate, as well as over time. Meat samples (Table 5) without lactate showed a mean residual nitrite concentration of 87.9 ppm, while those
samples containing lactate had a mean value as low as 64.4 ppm, suggesting that lactate facilitated nitrite reactions and similar effects in cured meat products. This is supported by the results reported by Kim et al. (2006) who suggested that lactate increases lactic dehydrogenase activity, through the conversion of lactate to pyruvate and the regeneration of NADH. The regeneration of NADH in our study would have reduced metmyoglobin to deoxymyoglobin, providing a greater concentration of reduced myoglobin to subsequently react with nitrite in the system.

There was also a time effect of the lactate on residual nitrite, with the amount of residual nitrite decreasing over time as might be expected. The mean residual nitrite was 84.4 ppm at 1 hour after sample preparation which decreased to 72.5 ppm after a 24 hour time period across all treatments (Table 5). The decreasing amount of residual nitrite in the beef patties over time is typical of curing reactions but the faster depletion of nitrite in the presence of lactate suggests that there was continual regeneration of NADH for the metmyoglobin reducing activity. Generation of NADH converts metmyoglobin to deoxymyoglobin which then can reduce nitrite to nitric oxide. Therefore, over the 24 hour time period, it is likely that some of the decreased amount of residual nitrite was due to the reduction of nitrite to nitric oxide by deoxymyoglobin as recently reported (Hendgen-Cotta et al. 2008).

Residual nitrite was also measured for treatments without erythorbate (Table 6). Erythorbate is used in cured meat systems to reduce nitrite to nitric oxide more quickly and to allow for a more complete curing reaction. There were significant differences found for the lactate and time comparisons, independent of erythorbate. When measurements were analyzed comparing the treatments with 4% lactate, both with with
and without erythorbate, the difference for erythorbate approached significance (p-value .0583) for an effect on residual nitrite (Table 6). The mean residual nitrite for products without erythorbate was 90.3 ppm which decreased to 84.2 ppm with the addition of erythorbate (Table 6). Thus, both erythorbate and lactate reduced residual nitrite but there was no marked improvement when the two were used together. Because there was no significant difference, it appears that lactate and erythorbate acted as reductants independently of one another, which was expected, given the different reduction mechanisms of each. More investigation of these combinations is warranted.

The effects of the packaging treatments, with or without oxygen (overwrap or vacuum package) did not affect residual nitrite in these comparisons.

3.2.2 Meat Color Analysis

Color analysis was conducted to assess the effects of lactate in different packaging systems on cured and non-cured meat color. The $a^*$ color value provides a measure of the amount of redness in the meat, which is a prominent factor affecting consumer acceptability. $L^*$ is an indication of lightness of a meat product and $b^*$ indicates the intensity of yellow/blue color of a product. Comparisons between vacuum packaging and overwrapped packages were included in this study because the nitrite-deoxymyoglobin reaction in meat typically results in brown metmyoglobin in the presence of oxygen, but in anaerobic conditions, results in red nitric oxide myoglobin.

3.2.2.1 $a^*$

The measurement of $a^*$ or redness showed an increase with time for the vacuum packaged products with nitrite (Figure 5 &6). Figure 5 shows the results of only the 4% sodium lactate, while the statistics are a combination of all 3 lactate percentages. This
indicates that the redness of the product increased over time out to 96 hours as is typical for cured meat in the absence of oxygen. The increase in redness of 4% sodium lactate appears to be considerably faster in the presence of erythorbate (Figure 5) than without erythorbate (Figure 6). In a early study done by Cheah (1976) involving cured bacon, it was reported that when faded bacon was vacuum packaged the surface color reverted to pink. Data in that study showed that bacon contained a functional lactate dehydrogenase capable of reducing NAD$^+$ to NADH, which then could be completely re-oxidized by metmyoglobin which was simultaneously reduced (Cheah 1976). In addition to lactate dehydrogenase, Cheah reported that NAD$^+$ and lactate were also present in the bacon, which are all necessary components for NADH generation from metmyoglobin reduction (Cheah 1976).

The vacuum packaged, uncured product without nitrite in our study was different as expected. Statistical analysis for the cure by hour effect showed at time 0, the mean a* value was 25.0 and decreased to 14.6 at 96 hours (data not shown). Because this product was not cured, the ground beef pigment was in the oxymyoglobin state initially and the product became less red within a few hours following removal of the oxygen by vacuum packaging.

Statistical analysis for the cure by hour effect of overwrapped beef patties with nitrite showed that for cured product, as time increased from 0 to 96 hours, there was a steady increase in mean a* value over time from 7.3 to 16.1 (data not shown).

Figure 5 shows the color change for the cured product, with nitrite and with and without 4% sodium lactate over time. The 4% sodium lactate, which is a common usage level in the meat industry, suggests a difference in the a* values between
packaging types. Uncured ground beef in overwrapped packages started at a higher a* value due to oxymyoglobin and decreased gradually over time as the color life was depleted (data not shown). On the other hand, the vacuum packaged meat without nitrite also started at a high level due to oxymyoglobin and then decreased rapidly in less than a 24 hour time period due to removal of oxygen and a predominance of reduced deoxymyoglobin (data not shown).

Figure 5 shows the change in color due to packaging differences for the cured meat samples with nitrite. Both overwrapped and vacuum packaged meats with nitrite and erythorbate started at a low a* value. The overwrapped products with nitrite maintained a low a* color with time due to metmyoglobin formation which is typical for raw cured meat in the presence of oxygen. The vacuum packaged products started with a low a* value of around 7 due to initial formation of metmyoglobin following addition of nitrite, but the a* increased rapidly in the first 24 hours due to the reduction of nitric oxide metmyoglobin to nitric oxide myoglobin which is cherry red and virtually identical to the color of oxymyoglobin. Sodium lactate added at 2% and 6% showed similar results to those shown for the 4% lactate treatments.

3.2.2.2 L*

A significant effect was observed in vacuum packaged samples for L* due to lactate (p<.0001) (Table 7) and time (p 0.0024), but no differences were found for nitrite effects. The lactate concentrations of 0% and 4% were different from 2% and 6%. It has been reported that as sodium lactate increased to 2%, the L* value of meat samples decreased. However, greater sodium lactate (over 2%) concentration did not further affect the L* value (Papadopoulos et al. 1991b). In the present study, the mean
L* value of 41.5 for 0% lactate decreased to 38.3 at 2% lactate followed by an increase to 41.4% at 4% lactate and then decreased to 37.3 at 6% lactate (Table 7). It was reported by Kim et al. (2006) that lactate enhanced steaks had a lower L* (darker red) than non-enhanced steaks. Papadopoulos et al. (1991b) reported that L* value fluctuated during storage time with no apparent pattern. Thus, previous research supports our observation that the addition of sodium lactate decreased the L* value, making the meat product darker in color but also that changing lactate concentrations may result in fluctuating L* values. Further, as time progressed from hour 0 to hour 96, the mean value decreased from 41.4 to 38.4, respectively, indicating that the product became darker as the time increased.

The L* value for overwrapped product showed similar results as the vacuum packaged samples. The fluctuating pattern with lactate concentrations was the same and as time increased the mean L* value decreased.

3.2.2.3 b*

In vacuum packaged samples, lactate showed a significant effect for b* in the meat system, but when the Tukey adjustment was made, no mean separation occurred. Mean b* value for samples without lactate was 16.3 and decreased gradually as percent lactate increased (Table 7). The cure by time interaction showed that at time 0, beef patties with nitrite had a mean b* value of 14.0 which increased to 16.1 over time to 96 hours. The opposite happened for the uncured product over time, with the mean b* value decreasing from 20.8 at time 0 to 14.7 at 96 hours.

The b* values for overwrapped patties were affected by the lactate and demonstrated a cure by time interaction. No lactate by time interaction occurred. This
was consistent with results of color evaluation by Papadopoulos et al. (1991b). The lactate effect was significant in the meat system, but when the Tukey adjustment was made, no mean separation was observed.

The results from color measurements showed that sodium lactate could be beneficial to color of both fresh and cured meat products. For fresh meat, Kim et al. (2009) suggested that addition of lactate protected the meat pigment from myoglobin oxidation under oxidizing conditions, allowing formation of more red oxymyoglobin and longer retention of the oxymyoglobin pigment.

3.2.3 \( L^*, a^*, b^* \) color measurements without erythorbate

Color was also evaluated on beef patties enhanced with 4% sodium lactate, but without erythorbate, because erythorbate is recognized as an effective reductant for metmyoglobin and for nitrite. The same type of packaging, vacuum packaging and overwrap, were used.

For vacuum packaged product with erythorbate, as the time increased mean \( a^* \) value increased from 7.6 initially to 8.7, 17.1, 16.8, 17.6 at 4, 24, 48 and 72 hours, respectively (data not shown). However, the patties without erythorbate in vacuum started at a much higher \( a^* \) value of 15.6 and then decreased gradually to 12.5 at 96 hours. The mean \( L^* \) value was significantly higher for vacuum packaged beef patties including erythorbate (42.4) relative to product without erythorbate (39.9). There were no significant differences in the \( b^* \) value for vacuum packaged product without erythorbate (data not shown).

Overwrapped patties without erythorbate and with 4% lactate were also measured for \( L^*, a^* \) and \( b^* \). The mean \( L^* \) value for patties with erythorbate was 42.7
which was lighter than product without erythorbate that had a mean of 39.9. There were no significant differences for \( a^* \) and \( b^* \) in these treatments.

The \( a^* \) values of the vacuum packaged and overwrapped product with 4% sodium lactate and with or without erythorbate had visual trends (Figure 5 and 6). For vacuum packaged patties with erythorbate, the \( a^* \) value increased rapidly and plateaued over time. Whereas, vacuum packaged samples without erythorbate slowly increased over time reaching nearly the same \( a^* \) value at 96 hours. All overwrapped sample maintained \( a^* \) values over time. Overwrapped samples with erythorbate had a slightly higher \( a^* \) value (statistical analysis was not run on 4% sodium lactate alone).

### 3.2.4 pH Determination

The pH was measured to determine whether lactate affected the pH of the meat mixtures and because pH is critical to nitrite reactions. Meat with a higher pH is also usually darker in color and the color is more stable (Ledward, Dickinson, Powell and Shorthose 1986). The pH of samples with lactate, at any level in this study, was higher than those that did not have sodium lactate (Table 8). Regardless of the sodium lactate level, there was a pH increase in the ground beef containing lactate when compared to ground beef without lactate (\( p<.0001 \)). The mean pH of beef without lactate was 5.64 and the pH of beef with lactate was 5.75, 5.76, and 5.83 for 2%, 4%, and 6% sodium lactate respectively (Table 8). This suggests that any effects of lactate on nitrite reactions were not due to a pH effect because it is well-recognized that higher pH alone typically slows nitrite reactions in cured meat products. Studies have found that as the sodium lactate increased so did the pH value (Papadopoulos et al. 1991b; Maca et al. 1997). Similarly, Kim et al. (2009) also found that steaks enhanced with lactate also
had higher pH values than non-enhanced steaks. According to Ledward et al. (1986), beef with a pH greater than 5.8 was more color stable than meat with a pH of 5.6, which is similar to the results found in the present study. The increase in pH with the addition of lactate could explain some of the color stabilizing properties of lactate.

When pH of beef without erythorbate was measured, no difference was found when compared to those treatments with erythorbate (data not shown). The pH of the samples with or without lactate was similar to those with erythorbate included. Because erythorbate did not have an impact on pH, all levels of lactate, with or without erythorbate were pooled for statistical analysis.

Conclusions (4.)

Lactate is commonly known to have an effect on a* values in fresh meat, however, we found that there was not a cure by lactate effect in product with nitrite and erythorbate, which suggests that any additional nitric oxide produced by reduction of nitrite may have become involved in other reaction in the meat mixture.

The diagram below provides a suggested reaction sequence for what is believed to occur with addition of lactate to a meat curing mixture containing nitrite. When lactate is added to the system, NADH is increased due to conversion of lactate to pyruvate by LDH, which in turn results in metmyoglobin (MMb) reduction to deoxymyoglobin (DMb). Because deoxymyoglobin can convert nitrite to nitric oxide (Hendgen-Cotta et al. 2008) generation of more deoxymyoglobin is likely to result in more nitric oxide (NO) from nitrite and less residual nitrite remaining in the mixture.
The nitric oxide generated from reduction of nitrite by deoxymyoglobin then can be captured by the remaining deoxymyoglobin as iron-nitrosylated myoglobin (MbNO) (Hendgen-Cotta et al. 2008). We hypothesized that because lactate generates NADH to reduce metmyoglobin to deoxymyoglobin, the resulting greater concentration of reduced myoglobin will react with nitrite to produce more nitric oxide, which will then speed up the curing reaction. The depletion of nitrite in the presence of lactate observed in this study in both the model system and the meat mixtures supports the hypothesis of greater nitric oxide production. Consequently, addition of lactate to cured meats, a practice often used to improve bacterial control of cured meats, is likely to result in more complete reduction of nitrite to nitric oxide as part of meat curing reactions.
References


Table 1. Non-enzymatic metmyoglobin reducing activity in the model system, with and without each of the reaction components

<table>
<thead>
<tr>
<th>Solution components</th>
<th>Activity$^h$ (µmol/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMb$^a$ (0.5 mM)</td>
<td></td>
</tr>
<tr>
<td>Lactate$^b$ (200 mM)</td>
<td></td>
</tr>
<tr>
<td>Nitrite$^c$ (200 ppm)</td>
<td></td>
</tr>
<tr>
<td>NAD$^d$ (6.5 mM)</td>
<td></td>
</tr>
<tr>
<td>DCIP$^e$ (0.6 mM)</td>
<td></td>
</tr>
<tr>
<td>PMS$^f$ (30 mM)</td>
<td></td>
</tr>
<tr>
<td>LDH$^g$ (8.1 unit/µl)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>0.5±0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay was measured at pH 7.5 at a temperature of 23°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+) indicates the presence of the component</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-) indicates the absence of the component</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^a$equine metmyoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^b$sodium lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^c$sodium nitrite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^d$nicotinamide adenine dinucleotide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^e$2,6-dichloroindophenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^f$phenazine methosulfate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^g$L-lactate dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^h$activity was computed as nanomoles of metmyoglobin reduced in minutes for a linear phase of the analysis, using a difference in molar absorptivity of 12,000 mol$^{-1}$ cm$^{-1}$ at 540 nm. Activity is expressed as the average of triplicate samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Meat Formulations with different levels of lactate, with and without nitrite and lactate.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C  CL  L  NL</td>
<td>C  CL  L  NL</td>
<td>C  CL  L  NL</td>
<td></td>
</tr>
<tr>
<td>Ground beef</td>
<td>6 lb 6 lb 6 lb 6 lb</td>
<td>6 lb 6 lb 6 lb 6 lb</td>
<td>6 lb 6 lb 6 lb 6 lb</td>
<td></td>
</tr>
<tr>
<td>Sodium-(L)-Lactate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.8g 90.8g - -</td>
<td>181.7g 181.7g - -</td>
<td>272.4g 272.4g - -</td>
<td></td>
</tr>
<tr>
<td>Sodium Nitrite&lt;sup&gt;c&lt;/sup&gt;</td>
<td>- - .41g .41g</td>
<td>- - .41g .41g</td>
<td>- - .41g .41g</td>
<td></td>
</tr>
<tr>
<td>Sodium Erythorbate&lt;sup&gt;c&lt;/sup&gt;</td>
<td>- - 1.5g 1.5g</td>
<td>- - 1.5g 1.5g</td>
<td>- - 1.5g 1.5g</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatments:
C = Control
CL = Control and lactate
L = Lactate, nitrite, and erythorbate
NL = Nitrite and erythorbate

<sup>b</sup> Sodium nitrite added at 150 parts per million (ppm)
<sup>c</sup> Sodium erythorbate at 550 ppm

Table 3. Formulation for the meat mixtures without the addition of erythorbate.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Treatments&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C  CL  L  NL</td>
</tr>
<tr>
<td>Ground beef</td>
<td>6 lb 6 lb 6 lb 6 lb</td>
</tr>
<tr>
<td>Sodium-(L)-Lactate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>181.7g 181.7g - -</td>
</tr>
<tr>
<td>Sodium Nitrite&lt;sup&gt;c&lt;/sup&gt;</td>
<td>- - .41g .41g</td>
</tr>
<tr>
<td>Sodium Erythorbate</td>
<td>- - 1.5g 1.5g</td>
</tr>
</tbody>
</table>

<sup>a</sup> Treatments:
C = Control
CL = Control and lactate
L = Lactate and nitrite
NL = Nitrite

<sup>b</sup> 4% sodium lactate
<sup>c</sup> Sodium nitrite added at 150 parts per million (ppm)
Figure 1. Absorbance values measured at 540nm for non-enzymatic metmyoglobin reducing activity of the model system in various combinations at a pH of 7.5 with a temperature of 23°C for 30 minutes.
Figure 2. Effect of different concentrations of sodium nitrite in the model system on non-enzymatic metmyoglobin reducing activity. Assays were measured in triplicate at a pH of 7.5 with a temperature of 23°C.
Figure 3. Effect of different concentrations (100, 150 and 200 mM) of sodium lactate in the model system on non-enzymatic metmyoglobin reducing activity. Assays were measured in triplicate at a pH of 7.5 with a temperature of 23°C.
Table 4. Residual nitrite (ppm) for various combinations of the reaction components in the model system.

<table>
<thead>
<tr>
<th>Hour</th>
<th>Treatment</th>
<th>Residual nitrite (ppm±std)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All(^b)</td>
<td>56.7±0.01</td>
</tr>
<tr>
<td>5</td>
<td>All</td>
<td>54.9±0.01</td>
</tr>
<tr>
<td>24</td>
<td>All</td>
<td>49.5±0.01</td>
</tr>
<tr>
<td>1</td>
<td>No DCIP</td>
<td>54.1±0.01</td>
</tr>
<tr>
<td>5</td>
<td>No DCIP</td>
<td>52.5±0.01</td>
</tr>
<tr>
<td>24</td>
<td>No DCIP</td>
<td>44.8±0.00</td>
</tr>
<tr>
<td>1</td>
<td>No lactate</td>
<td>59.8±0.01</td>
</tr>
<tr>
<td>5</td>
<td>No lactate</td>
<td>60.0±0.01</td>
</tr>
<tr>
<td>24</td>
<td>No lactate</td>
<td>59.3±0.01</td>
</tr>
<tr>
<td>1</td>
<td>No LDH</td>
<td>56.4±0.02</td>
</tr>
<tr>
<td>5</td>
<td>No LDH</td>
<td>56.8±0.01</td>
</tr>
<tr>
<td>24</td>
<td>No LDH</td>
<td>58.2±0.01</td>
</tr>
<tr>
<td>1</td>
<td>No MMb</td>
<td>59.1±0.01</td>
</tr>
<tr>
<td>5</td>
<td>No MMb</td>
<td>58.6±0.01</td>
</tr>
<tr>
<td>24</td>
<td>No MMb</td>
<td>54.5±0.02</td>
</tr>
<tr>
<td>1</td>
<td>No NAD</td>
<td>58.5±0.01</td>
</tr>
<tr>
<td>5</td>
<td>No NAD</td>
<td>58.0±0.01</td>
</tr>
<tr>
<td>24</td>
<td>No NAD</td>
<td>58.7±0.01</td>
</tr>
<tr>
<td>1</td>
<td>No Nitrite</td>
<td>1.5±0.00</td>
</tr>
<tr>
<td>5</td>
<td>No Nitrite</td>
<td>1.1±0.00</td>
</tr>
<tr>
<td>24</td>
<td>No Nitrite</td>
<td>1.6±0.00</td>
</tr>
<tr>
<td>1</td>
<td>No PMS</td>
<td>57.5±0.01</td>
</tr>
<tr>
<td>5</td>
<td>No PMS</td>
<td>56.9±0.00</td>
</tr>
<tr>
<td>24</td>
<td>No PMS</td>
<td>56.9±0.02</td>
</tr>
</tbody>
</table>

\(^a\) Residual nitrite levels were measured in duplicate from the test cuvettes using various combinations of chemical components.

\(^b\) All components in the system.
Figure 4. Effects of removal of different reaction components from the model system on residual nitrite levels measured after 1, 5, and 24 hours.
Table 5. Mean residual nitrite concentrations (150 ppm added) for lactate and time effects in a meat system including erythorbate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lactate (%)</th>
<th>Mean residual nitrite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>87.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>72.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>73.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>64.4</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hour</th>
<th>Mean residual nitrite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>84.40</td>
</tr>
<tr>
<td>5</td>
<td>79.86</td>
</tr>
<tr>
<td>24</td>
<td>72.51</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0259</td>
</tr>
</tbody>
</table>

*a Hour indicates the time in which the residual nitrite test began after meat preparation

Table 6. Mean residual nitrite concentrations (150 ppm added) for the lactate, time, and erythorbate effects in a meat system without erythorbate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lactate (%)</th>
<th>Mean residual nitrite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>73.9</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hour&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean residual nitrite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94.1</td>
</tr>
<tr>
<td>5</td>
<td>87.8</td>
</tr>
<tr>
<td>24</td>
<td>79.8</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0029</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Erythorbate</th>
<th>Mean residual nitrite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.2</td>
</tr>
<tr>
<td>NE&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90.3</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0583</td>
</tr>
</tbody>
</table>

<sup>a</sup>Hour indicates the time in which the residual nitrite test began after meat preparation
<sup>b</sup>Erythorbate
<sup>c</sup>No Erythorbate
Figure 5. Hunter $a^*$ values measured over 72 hours for the meat mixtures with 4% sodium lactate, sodium nitrite and sodium erythorbate and packaged in oxygen permeable overwrap or oxygen impermeable vacuum bags.

LOW = Lactate, nitrite, erythorbate-overwrap
LVP = Lactate, nitrite, erythorbate-vacuum package
NLOW = Nitrite, erythorbate-overwrap
NLVP = Nitrite, erythorbate-vacuum package
Figure 6. Hunter $a^*$ values measured over 96 hours for meat mixtures with 4% sodium lactate, sodium nitrite and without erythorbate packaged in oxygen permeable overwrap or oxygen impermeable vacuum bags.

- LOW = Lactate, nitrite-overwrap
- LVP = Lactate, nitrite-vacuum package
- NLOW = Nitrite-overwrap
- NLVP = Nitrite-vacuum package
Table 7. The effect of varying concentrations of sodium lactate and two packaging types on Hunter $L^*$, $a^*$, $b^*$ color values for the meat mixtures.

<table>
<thead>
<tr>
<th>Trait</th>
<th>vacuum package</th>
<th></th>
<th></th>
<th></th>
<th>p-value</th>
<th></th>
<th></th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
<td>2%</td>
<td>4%</td>
<td>6%</td>
<td></td>
<td>0%</td>
<td>2%</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>$L^*$</td>
<td>41.5</td>
<td>38.3</td>
<td>41.4</td>
<td>37.3</td>
<td>&lt;.0001</td>
<td>41.6</td>
<td>38.3</td>
<td>42.4</td>
<td>37.3</td>
</tr>
<tr>
<td>$a^*$</td>
<td>15.4</td>
<td>15.9</td>
<td>14.2</td>
<td>15.2</td>
<td>0.6309</td>
<td>15.4</td>
<td>15.9</td>
<td>13.8</td>
<td>15.2</td>
</tr>
<tr>
<td>$b^*$</td>
<td>16.3</td>
<td>16.0</td>
<td>15.1</td>
<td>15.0</td>
<td>0.0064</td>
<td>16.3</td>
<td>16.0</td>
<td>14.9</td>
<td>15.0</td>
</tr>
</tbody>
</table>
Table 8. Average pH measurement of ground beef in the meat system at varying levels of sodium lactate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
<th>MSE^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (percentage)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.64</td>
<td>0.061352</td>
</tr>
<tr>
<td>2</td>
<td>5.75</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.76</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.83</td>
<td></td>
</tr>
</tbody>
</table>

^aMean Standard Error
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>COW</td>
<td>Control-overwrap</td>
</tr>
<tr>
<td>CVP</td>
<td>Control-vacuum package</td>
</tr>
<tr>
<td>CLOW</td>
<td>Control + lactate-overwrap</td>
</tr>
<tr>
<td>CLVP</td>
<td>Control + lactate-vacuum package</td>
</tr>
<tr>
<td>LOW</td>
<td>Lactate, nitrite, erythorbate-overwrap</td>
</tr>
<tr>
<td>LVP</td>
<td>Lactate, nitrite, erythorbate-vacuum package</td>
</tr>
<tr>
<td>NLOW</td>
<td>Nitrite, erythorbate-overwrap</td>
</tr>
<tr>
<td>NLVP</td>
<td>Nitrite, erythorbate-vacuum package</td>
</tr>
</tbody>
</table>
CHAPTER 3. GENERAL CONCLUSIONS

The results from our study support our hypothesis that the addition of lactate to meat will generate NADH through LDH activity and reduce metmyoglobin to deoxymyoglobin, and subsequently, the resulting greater concentration of reduced myoglobin will react with nitrite to produce more nitric oxide, in turn accelerating the curing reaction. A reduction of nitrite was observed in both a model system and a meat system.

In the model system, we measured metmyoglobin reduction as facilitated by 8 different reaction components. The objective was to determine the needed components without which, there would be no reducing activity. The components evaluated for the reaction included equine metmyoglobin (MMb), sodium lactate, sodium nitrite solution, nicotinamide adenine dinucleotide (NAD$^+$), 2,6-dichloroindophenol (DCIP), L-lactate dehydrogenase (LDH) and phenazine methosulfate (PMS). The model system resulted in measureable metmyoglobin reducing activity when all the reaction components were included, as well as when nitrite was excluded. Exclusion of lactate, nicotinamide adenine dinucleotide (NAD), L-lactate dehydrogenase (LDH), and phenazine methosulfate (PMS) resulted in no activity. These results indicate that with or without nitrite, increased metmyoglobin reduction occurred, which implies that lactate facilitates reformation of the reduced pigment, either deoxymyoglobin without nitrite or nitric oxide myoglobin in the presence of nitrite.

Measurement of residual nitrite on the model system supported the hypothesis that the presence of more reduced myoglobin would facilitate reduction of nitrite to nitric oxide. The amount of residual nitrite (ppm) in the model system was decreased when
all reaction components were included. When lactate, NAD, LDH and PMS were excluded from the system, there was very little change or a slight increase in residual nitrite. Results from both parts of the model system experiment supported our hypothesis that the nitrite curing reaction is accelerated by addition of lactate, resulting in less residual nitrite due to lactate facilitated reduction of metmyoglobin.

A similar experiment was conducted using a meat system, to determine if the hypothesis would hold true in a meat mixture as well and the results were similar to that of the model system. The amount of residual nitrite decreased with an increasing amount of sodium lactate. However, no difference in the redness (a*) was observed suggesting that the additional nitric oxide produced by reduction of nitrite may have become involved in other reactions in the meat mixture. Lactate has become a common component of meat products for improved bacterial control and is also recognized as a means of improving color of fresh meat by facilitating reduction of metmyoglobin. This study suggests that the reduction of metmyoglobin also plays a role in cured meats because the reduced myoglobin subsequently increases the depletion of nitrite, probably forming nitric oxide. This is significant to the meat industry for potentially accelerating the curing reaction and for reducing residual nitrite. Therefore, this research indicates that the addition of sodium lactate has an impact on nitrite reactions in cured meat by facilitating the reduction of nitrite.

Additional, detailed research should be conducted to clarify the concentration of lactate that would be the most beneficial. Also, more research should be done to determine the effects of lactate on cured color and residual nitrite during extended storage of cured meats.
ACKNOWLEDGEMENTS

I would like to start by thanking Dr. Joe Sebranek, my major professor for giving me the opportunity, to not only learn and gain valuable experiences in the meat industry, but also for pushing me beyond my limits and making me a stronger person. Thank you for your guidance and support throughout my research project. The skills and knowledge gained will remain with me always and will be beneficial to me as I start my career. I would also like to thank Dr. Dennis Olson for serving on my committee and answering any questions that I had throughout the process. I would also like to thank Dr. Leo Timms for serving on my committee and being open for learning a whole new science. Your constant encouragement and positive attitude was always appreciated and helped me get through the long days.

I am grateful for all the help I have received in order to complete my research. I would like to extend a big thank you to Marcia King-Brink and Elaine Larsen for helping me learn laboratory techniques and procedures. Without you two, I would have been lost in the lab and it was great to know that there would always a friendly smile waiting on me when I walked into the lab.

Furthermore, I would like to recognize the Iowa State University Meat Laboratory staff and student employees for all the assistance provided to me throughout my research project. Randy Petersohn, Steve Bryant, Jeff Mitchell, Vail Olson, Mike Holtzbauer, and Deb Michel, thank you all for your support.

I would also like to acknowledge my fellow grad students Brian Krause, Adam Krause, Gary Sullivan, Jonathon Campbell, and Xi Yuan for not only your help in the lab, but for all of your support, friendship and encouragement. I would like to extend a
huge thank you to Armitra Jackson for your constant support and friendship. Whenever I needed someone to talk to you were there and that means more to me than you will ever know! Thank you to Mark Anderson for helping me with my statistics.

Most importantly I would like to thank my family who has always been my biggest fans and strongest supporters. Ashley, thank you for always being honest with me and making me a tougher person! Erica, thank you for always being someone that I could turn to when I needed someone to listen and making me feel better about anything. Mom and Dad, without your constant love, support, encouragement and faith in me, without you two I could not have completed my Master’s Degree. Michael, thank you for your love and support, you make each day brighter and happier!