2013

The effect of pH and nitrite concentration on the antimicrobial impact of celery juice compared with sodium nitrite on Listeria monocytogenes

Ashley Horsch
Iowa State University

The effect of pH and nitrite concentration on the antimicrobial impact of celery juice compared with sodium nitrite on *Listeria monocytogenes*

by

Ashley Marie Horsch

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Co-Majors: Meat Science; Food Science and Technology

Program of Study Committee:

Joseph G. Sebranek, Co-Major Professor
Lester A. Wilson, Co-Major Professor
James S. Dickson

Iowa State University

Ames, Iowa

2013

TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

Recently, the natural and organic markets have exploded in popularity amongst consumers and have driven meat processors to develop preservative free products. Because of concerns regarding the formation of carcinogenic compounds called nitrosamines, nitrite is one of the preservatives not allowed in natural and organic meat products. Nitrite is particularly important in cured meat products because of the unique flavor, color, and inhibition of the pathogen *Clostridum botulinum*. In order to create a product without synthetic nitrite and still provide the same unique characteristics listed above, processors have turned to natural compounds such as celery juice.

Celery juice, along with other vegetables, contain high amounts of nitrate. With the appropriate conditions and starter cultures, the nitrate can be converted to nitrite. Currently, manufacturers of the nitrate-rich celery juice have developed a product that pre-converts the nitrate to nitrite. This allows the processers to skip the incubation step and directly add the pre-converted concentrate to the meat block. However, with inclusion percentages of the celery juice ranging between 0.2-0.4%, increased pathogen growth (*C.perfrigens & Listeria monocytogenes*) has been observed when compared to traditionally cured products.

L. monocytogenes has recently been a prominent concern to the food industry because of its ability to survive refrigeration temperatures and contaminate ready-to-eat foods. Since consumers do not necessarily always heat treat ready-to-eat foods, they easily can fall victim to this organism if the product is contaminated. The reason this organism is highly scrutinized, is the fact that a large percentage of the individuals who contract listeriosis, result in death. With continued recalls within the meat industry, more research is needed to better understand the effects that naturally cured products have on *L. monocytogenes*.

Thesis Organization

This thesis is organized into four chapters. The first chapter encompasses a general introduction to the main topics discussed in the thesis. The second chapter contains a literature review on pertinent topics related to the research within the thesis. The third chapter entails the manuscript entitled "The effect of pH and nitrite concentration on the antimicrobial impact of celery juice compared with sodium nitrite on *Listeria monocytogenes* on restructured ham." The fourth chapter is a general summary of the research.

CHAPTER 2. LITERATURE REVIEW

History of Nitrite and Nitrate

The exact discovery of curing by whom is unknown to this day, but considerable history has shed light on the approximate period of time curing practices were in use (Pegg & Shahidi, 2000). Preceding the curing process was the use of salt as a meat preservative dating back to 1600 BC in the Jewish Kingdom, China, Babylonia, and Samaria (Jensen, 1953). There they learned that covering meat in salt extended the shelf-life significantly compared to meat without a coating of salt (Pegg & Shahidi, 2000). The meat was able to maintain its quality due to the effect of salt decreasing the available water, thus limiting microbe growth. Along with the drying effect of salt came an unappealing gray color seen in the meat (Pegg & Shahidi, 2000). It quickly became apparent when using particular types of salt, the development of a reddish color was observed, thus eliminating the gray color outcome. This was due to the fact that the source of salt contained "saltpeter" (potassium nitrate) or what we call sodium nitrate today (Binkerd & Kolari, 1975). Instead of having a salt source that was strictly salt, these sources were adulterated with nitrate. When introduced to a meat system the nitrate would be reduced to nitrite, which resulted in a red color, unique flavor, and extended shelf life associated with cured meats.

Functions of Nitrite and Nitrate

Color

The typical red color found within cured meat products is due mainly to nitrite and not nitrate. Both Kisskalt (1899) and Lehmann (1899) gave evidence in their studies that nitrite was indeed responsible for creating the red color found in processed meats. Two years later, a scientist by the name of Haldane studied the cause of the unique red color in cured

meats and determined that the pigment nitrosylhemoglobin (NOHb) was converted to nitrosylhemochromogen during the heating process (Haldane, 1901). This conversion was determined to be solely responsible for giving the red appearance in cooked cured meats. Hoagland reconfirmed this in his studies and went on to explain that the reduction of nitrate to nitrite is critical for forming NOHb (Hoagland, 1908). The chemistry behind this reaction reduces nitrate to nitrite, which in turn creates nitric oxide. The nitric oxide then attaches itself to the heme of the hemoglobin (cooked cured meats) or myoglobin (uncooked cured meats) to create the red color seen in the final product (Cassens et al., 1979; Pegg & Shahidi, 2000). Based on this information, nitrite began to be added directly to meat blocks instead of nitrate in the early 1900's (Pegg & Shahidi, 2000).

Flavor

Lipid oxidation contributes greatly to meat flavor deterioration (MFD) and warmed-over flavor (WOF) found in meats (Shahidi, 1992). The formation of tasteless primary products from lipid oxidation, such as hydroperoxides, forms secondary products through their degradation such as aldehydes, acids, alkanes, alkenes, esters, etc (Shahidi, 1992). Aldehydes in particular are responsible for MFD and WOF (Shahidi, 1992; Toldra et al., 2009). It is common knowledge that unsaturated fatty acids have increased susceptibility to lipid oxidation, and with increased amounts of these fatty acids in meat, faster rates of degradation are found. In a study by Cross and Ziegler (1965), they found that when nitrite was used in the formulation there were decreased amounts of aldehyde formation, which indicated nitrite was an effective antioxidant. Along with decreased aldehyde formation, studies have shown that lower concentration of esters are found in nitrate/nitrite added

products (Flores et al., 1998). Barbieri et al. (1992) and Parolari (1996) denoted the large ester formation in Italian ham was due to a lack of nitrate/nitrite added to the formulation. Like aldehydes, esters contribute to the aged meat flavor, which if present in large amounts, can create undesirable flavors (Barbieri et al., 1992). Because nitrite retards lipid oxidation, it contributes to the cured meat flavor by retarding WOF and MFD (rancidity) from occurring. However, many researchers have suggested that it is not only nitrite that produces the cured flavor but a combination of nitrite and other volatiles produced from the complex environment of meat (Toldra et al., 2009). Since the meat system is so multifaceted, the exact compound responsible for the cured flavor remains unknown.

Lipid oxidation

It is well known that lipid oxidation is one of the main contributors to deterioration in meat and poultry products. Nitrite far exceeds any other antioxidant in delaying the onset of rancidity and warmed over flavors. In 1980, researchers compared prominent antioxidants [butylated hydroxytoluene (BHT) and citric acid] to varying degrees (50 ppm , 200 ppm, 500 ppm) of nitrite treatments (McDonald et al., 1980). They found the reduction in thiobarbituric acid (TBA) to be superior to the other antioxidants at any concentration of nitrite. Other studies indicated that at low concentrations (as low as 20 ppm) nitrite was still significantly effective at reducing TBA values (Morrissey & Tichivangana, 1985; Al-Shuibi & Al-Abdullah, 2002). Sebranek (2009) suggested that the effect of nitrite was due to its ability to create nitric oxide, which then would bind itself to the heme group and create nitriso- and nitrosyl- compounds that had antioxidant capabilities. Since, nitrite is so effective, the United States Department of Agriculture (USDA) has prohibited the use of synthetic

antioxidants in cured products (Sindelar & Milkowski, 2011). One exception to the rule is dry and semidry sausages.

Microbiological implications

Clostridium botulinum

Nitrite not only contributes to meat color and flavor characteristics, but also provides antimicrobial capabilities within meat products. When it comes to gram-positive and gram-negative bacteria, nitrite is an effective antimicrobial, however, yeasts and molds are unaffected by nitrites' presence (Tompkin, 2005). Most commonly associated as nitrite's antimicrobial target is *Clostridium botulinum*. This organism is of particular importance because of the harmful toxins it produces and when those are ingested by unknowing consumers, detrimental symptoms such as nausea, vomiting, paralysis of muscles, double vision are typical, and in severe cases, death occurs (Pegg & Shahidi, 2000). Low incidence of *C. botulinum* toxin production in cured meats is largely due to the addition of nitrite to these meat systems. Speculations of the exact mechanism which allows nitrite to inhibit *C. botulinum* are as follows: 1) formation of substance derived by nitrite reactions with meat compounds, 2) nitrite is an oxidant or reductant to intracellular enzymes, 3) nitrite interrupts *C. botulium* metabolism by making less iron available, and 4) nitrite reacts with cell membranes which minimizes transport of substances essential for *C. botulinum* metabolism (Sofos et al., 1979; Benedict, 1980). However, after reviewing many studies, conclusions on the exact mechanism of how nitrite inhibits *C. botulinum* is still inconclusive. To put the importance of nitrite in preventing *C. botulinum* in prospective, an article that was printed in 2001 stated that since 1899 (when direct nitrite use increased) there were 51 home-processed

meat outbreaks, and of those 51, 43 were from uncured meat products (Archer, 2001; Pierson & Smooth, 1982).

Clostridium perfringens

Clostridium perfringens is a spore-forming, gram-positive organism (Montville & Matthews, 2005). When inadequate heating or cooling occurs, spores are formed, which may result in illness when consumed. The spores cause diarrhea and cramp-type symptoms by attaching themselves to the villi within the intestinal tract (Montville & Matthews, 2005). *C. perfringens* is especially problematic within foodservice type operations because of the large amounts of food that are prepared. The problem arises when improper cooling or heating of the product occurs and causes the food product to fall into the dangerous temperature range of 50°C to 15°C (for cooling) (Labbe, 1989) or into the optimal temperature range for *C. perfringens* growth, 43-45°C (Taormina & Dorsa, 2004). The amount of spores ingested determines the severity of the symptoms.

In order to control the growth of the harmful spores produced from *C. perfringens*, the U.S. Food Safety and Inspection Service (FSIS) created stabilization guidelines for processors to follow (USDA, 1999). According to the guidelines, "all ready-to-eat meat and poultry products must reach an internal temperature between 54.4°C and 26.7°C within 1.5 hours and reach an internal temperature between 26.7°C and 4.4°C within an additional 5 hours after being thermally processed (6.5 hours total cooling time)" (USDA, 1999). If the products contain a minimum of 100 ppm nitrite, "the internal temperature must be between 54.4°C and 26.7°C within 5 hours and be between 26.7°C and 7.2°C in an additional 10 hours of being thermally processed (15 hours total cooling time)" (USDA, 1999). Another

means to control the spore formation of *C. perfringens* is to add nitrite to the meat or poultry product. It has been proven in multiple experiments that nitrite effectively inhibits *C. perfringens* (Perigo & Roberts, 1968; Sauter et al., 1977). More recent work has suggested that nitrite blocks the sulfhydryl sites within *C. perfringens*, which explains why nitrite is able to administer it's bacteriostatic effect on the organism (Tompkin, 2005).

Listeria monocytogenes

Along with *C. perfringens* inhibition, nitrite has been found to control *Listeria monocytogenes* growth (Duffy et al., 1994; Ngutter & Donnelly, 2003). *Listeria monocytogenes* has become a hot topic of concern for meat processors recently due to its ability to withstand an adverse environment like refrigeration temperatures as well as contamination of ready-to-eat meats (Lungu et al., 2009). The inhibition and control of this organism has become the primary and emerging focus within the industry and academia. As a result, the focus of the work in this thesis is on *L. monocytogenes* in "uncured, no nitrate or nitrite added" processed meats.

Listeria monocytogenes is a gram positive organism that also facilitates facultative anaerobic and non-spore forming characteristics (Wagner & McLauchlin, 2008; Lungu et al., 2009). It was first discovered in 1926 in the United Kingdom within laboratory rodents (Murray et al., 1926). In 1936, the implications of this bacterium became evident when its infection, listeriosis, caused abortions in pregnant women and meningitis in adults (Gray & Killinger, 1966). Populations that are immunocompromised such as pregnant women, children, and the elderly are especially prone to listeriosis (Liu, 2008). Upon contracting listeriosis 20-30% of the cases result in death (Doganay, 2003). Its ability to withstand non-

optimal environments, such as refrigeration temperatures, makes this organism very challenging to control in food processing plants. Ready-to Eat (RTE) meats are of particular concern because these products do not require additional heat treatment by the consumers once purchased from the grocery store. These meats have already been heat treated by the manufacturer, but cross-contamination of slicers or temperature abuse, can reintroduce *L. monocytogenes* that might have otherwise been killed at the thermal processing step (Reij & Den Aantrekker, 2004). Not only is listeriosis a public health problem, but there can also be devastating economical outcomes for the vitality of meat manufacturers upon its outbreak.

Growth factors

Listeria monocytogenes is very problematic to food processers for many reasons. First, its psychrophillic nature allows it to grow at refrigeration temperatures (Wagner & McLauchlin, 2008). It can also survive as low as 0˚C and as high as 45˚C, but prefers a temperature range comprised of 30-37˚C (Liu, 2008). Optimal pH for this organism is 7.1 and can range from 3.0-9.6 (Lungu et al., 2009). RTE meats are of particular concern because of their high water activity (Aw>0.92) and *L. monocytogenes'* capability of surviving salt concentrations up to 10% (Wagner & McLauchlin, 2008). For optimal growth *L. monocytogenes* needs to consume riboflavin, thiamine, thioctic acid, amino acids and carbohydrates (mainly glucose) (Liu, 2008; Lungu et al., 2009). Since, meat is comprised of many of these nutrients, it is evident why *L. monocytogenes* can thrive in meat products.

Outbreaks

The reality of the dangers *L. monocytogenes* imposes on the human population are well known. In 1998, 100 cases of listeriosis were caused by contamination of hot dogs

within the United States (Evans et al., 2004). In the same year, another outbreak occurred with frankfurters, and of 108 cases, it caused 14 deaths and 4 miscarriages/stillbirths (Mead et al., 2006). Between the years 1996-2000, 60% of all recalls were implemented due to *L. monocytogenes* adulteration (Wong et al., 2000). Even with increased control and preventative measures taken in previous years, the cantaloupe outbreak in 2011 reminded us we still have obstacles to overcome in both the food and meat industry. A total of 146 cases, 40 deaths, and 1 miscarriage occurred in 28 states (CDC, 2011). With increasing incidences manufacturers as well as consumers have expressed their concern and because of this, the prevention of listeriosis has become a prominent priority.

Prevention and Control

Pre-requisite programs

As previously discussed, avoiding outbreaks is crucial, and to do so requires the proper execution of prevention protocols. Pre-requisite programs such as Good Manufacturing Practices (GMP) and Standard Sanitation Operating Procedures (SSOP) are effective means of reducing contamination (Robbins & McSwane, 1994). Since, poor personnel hygiene is one of the most common causes of food-borne illness infections, these programs offer efficient control steps by implementing proper sanitation techniques for food handlers (hand washing, etc.) (Robbins & McSwane, 1994). Along with excellent personnel hygiene practices, sanitation of equipment is another essential component to the success of the pre-requisite programs. RTE meats are especially vulnerable to recontamination from slicers, knives, peeling and other food contact surfaces after they have exited the thermal processing step (Reij & Den Aantrekker, 2004). Since 1971, manufactures have instituted

HACCP (Hazard Analysis and Critical Control Points) programs as a means to control food borne pathogens (DHEW, 1971). To regulate the meat industry even further, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) implemented a zero-tolerance policy for *L. monocytogenes* in 1980 (USDA, 2003). Even with all these preventative programs installed, a combination of programs and new intervention methods, such as natural nitrite sources, are needed to fully inhibit *L. monocytogenes*.

Organic acid salts

Recently, an increased demand for products deemed "natural" or "organic" have led producers to look into effective natural antimicrobials as alternatives to commonly used synthetic counterparts. Within meat products, it has been demonstrated by numerous studies that organic acids provide antilisterial effects (Mbandi & Shelef, 2002; Porto, et al., 2002; Lu et al., 2005). Within those studies, dipping and inclusion within the meat batter have been prominent methodologies of incorporating the organic salts. Various forms of diacetate and lactate are the most commonly used organic salts within Ready-To-Eat (RTE) meat products (Theron & Lues, 2007). Both diacetate and lactate have a synergistic effect on *L. monocytogenes* when in combination with each other or another organic salt (Samelis et al., 2005; Thompson et al., 2008). Thompson et al. (2008) found that sodium diacetate is more effective in combination with sodium lactate then when either is alone. The industry commonly incorporates lactates between 1.5% and 3.0% which then can be added by itself or in combination with sodium diacetate at 0.125% to 0.25% (Thompson et al., 2008; Tompkin, 2002). Although, organic acid salts have shown to be very effective in reducing *L. monocytogenes* growth, they lack the ability to provide initial lethality to the organism

(Porto-Fett et al., 2010). It has been demonstated by Porto-Fett, et al. (2010) that in addition to potassium lactate and sodium diacetate, lauric arginate applied after peeling provided an effective solution that delivered both suppression and initial lethality of listeria on frankfurters. Through the use of organic salts, concern has been continuing to mount, because of the possibility that acid-tolerant foodborne pathogens could occur (Quintavalla & Vicini, 2002). By adding the organic acid salts, the pH lowers, which in turn could give rise to the acid tolerance response (ATR) by the microorganism (Theron & Lues, 2007). In some cases the organism would then become resistant to heat (Ryu et al., 1999), osmosis, and salt (Leyer & Johnson, 1993), which is of great concern to the processor.

Nitrite

It is common knowledge that nitrite is an effective antimicrobial in regards to *C. botulinum*, but it is also effective against *L. monocytogenes* as well. Many studies have determined that the addition of nitrite does in fact reduce *L. monocytogenes* growth (Buchanan et al., 1989; McClure et al, 1991; Schlyter et al., 1993). Duffy et al. (1994) determined that when sodium nitrite was combined with sodium ascorbate, the *L. monocytogenes* growth was significantly reduced by increasing the concentration of residual nitrite. Residual nitrite has been shown to effect the growth of *L. monocytogenes*. Without enough ingoing nitrite added to the meat product, the residual nitrite concentration is not sufficient to protect against this ambiguous organism. Numerous studies have indicated that low concentrations of ingoing nitrite (e.g. 30 ppm) are inadequate (Buchanan et al., 1989; McClure et al., 1991; Schlyter et al., 1993). While reviewing the studies it became evident that pH directly affected nitrite's listericidal ability. The growth of *L. monocytogenes* for the

treatment combinations of nitrite and pH 5.3 or below were not detected in the McClure et al. (1991) study, thus rendering this combination superior. Nitrite treatments with a pH of 6.0 or above failed to inhibit or suppress growth (McClure et al., 1991). Other factors such as, vacuum packaging, high salt (NaCl) concentrations, and low refrigeration temperatures all contribute to enhancing nitrites effect on *L. monocytogenes* (Tompkin, 1983).

Regulations of Nitrite and Nitrate

The method used in the curing process dictates the maximum allowable ingoing nitrite and nitrate amounts. For comminuted product (bologna, salami, etc.), 156 parts per million (ppm) is the maximum sodium nitrite addition based on the green weight of the meat block (USDA, 1995). When using nitrate in these products the maximum quantity is 1718 ppm (USDA, 1995). For immersion and massaged curing, 200 ppm sodium nitrite is the maximum allowed concentration and when using nitrate, the maximum concentration is 700 ppm. Dry curing limits are 625 ppm and 2187 ppm for nitrite and nitrate, which are based on the green weight of the product. The nitrite or nitrate would be applied directly to the surface of the meat product (country ham, prosciutto, etc.) and dried for an extended period of time. It is important to keep in mind that the United States Department of Agriculture (USDA) has mandated that all products considered cured and labeled “Keep Refrigerated” must have a minimum of 120 ppm ingoing nitrite. However, if the processor can verify an effective alternative to providing food safety through a different preservation process (thermal processing, pH control, moisture control), they are allowed to fall below 120 ppm (USDA, 1995).

Since the discovery of nitrosamine formation in bacon and its link to cancer, bacon has unique nitrite inclusion limits. For pumped and/or massaged bacon without the skin, 120 ppm ingoing sodium nitrite is required. However, USDA requires that 550 ppm of sodium ascorbate or sodium erythorbate to be included to minimize the amount of residual nitrite produced by the cure, thus minimizing the nitrosamine production. For immersion cured bacon without skin, the maximum ingoing nitrite concentration is 120 ppm. Dry cured bacon without skin allows up to 200 ppm of nitrite that can be added during the process. When the skin is present in either pumped/messaged, immersed, or dry cured bacon, the maximum limits of ingoing nitrite and sodium erythorbate or sodium ascorbate need to be adjusted according to a 10% reduction (USDA, 1995). The 10% reduction is based on the skin comprising approximately 10% of the pork bellies weight. Since, the skin barely absorbs any nitrite or curing accelerators the reduction must be made to represent the actual weight of meat that is retaining nitrite and the accelerators. USDA has prohibited any use of nitrate in bacon products due to the risk of increased nitrosamine formation (USDA, 1995).

Health Benefits of Nitrites and Nitrates

Nitrites and nitrates are commonly deemed synthetic by nature and are not considered natural substances. This misconception has largely been fueled by epidemiological studies indicating that all dietary nitrites and nitrates cause cancer. However, the general public is unaware of the fact that fruits and leafy green vegetables contain nitrate. The high amount of vegetables consumed in the Mediterranean diet has been thought to have contributed to the reduced incidence of health diseases, such as cardiovascular disease. (Lundberg et al., 2006; Hord et al., 2009). When compared to the average Western diet, the Mediterranean diet

contains up to 20 times more nitrite and nitrate (Garg, 2006). When the vegetables are consumed, bacteria in saliva reduces nitrate to nitrite. From here, nitrite gets converted to nitric oxide by the acidic environment of the stomach, and is the molecule responsible for many health benefits (McKnight et al., 1997). Nitric oxide homeostasis in the body has been shown to be key in avoiding diseases and maintaining optimal health. As humans age, the ability to produce nitric oxide begins to decrease and a nitric oxide deficiency occurs (Parthasarathy & Bryan, 2012). Since nitric oxide plays an important role in maintaining optimal blood pressure levels and aids in controlling the blood flow within the cardiac muscle (Bryan & Hord, 2010), it is evident why older individuals need an increase in dietary nitrite/nitrate. Studies have shown that nitrate supplementation have reduced the risk of hypertension, atherosclerosis, heart failure, and thrombosis (Lundberg et al., 2009; Bryan & Loscalzo, 2011). Along with cardiovascular improvement, nitrate consumption has also been proven to improve physical endurance. By increasing oxygen circulation, nitrate supplementation demonstrated its effect on enhancing physical performance in various studies (Larson et al., 2010; Lansley et al., 2011; Murphy et al., 2012). With the mounting research in favor of increased performance, athletes have begun to supplement themselves before exercise to increase the amount of nitrate available, which will aid the body in its need of oxygen. Even though there are many positive health outcomes to supplementing nitrate, caution should be taken to avoid toxicity. Nitrate, even at higher doses, is nontoxic, because only a small fraction of it is converted to nitrite (Lundberg et al., 2011). However, nitrates' reduced form, nitrite, is very toxic at low concentrations (100-200 mg/kg) (Lundberg et al., 2011). A runner was reported to have taken sodium nitrite before exercise, and mistakenly

thought that the substance was nitrate (Lundberg et al., 2011). In doing so he developed nitrite toxicity symptoms which are associated with the condition methemoglobinemia (Lundberg et al., 2011).

Health Concerns Associated With Nitrites and Nitrates

During the 1950's the first reports of carcinogenic nitrosamine formation were discovered. Nitrite was being used to preserve fish meal which was the primary feed source within mink farms. The farmers started to notice that the mink developed unusually high numbers of tumors while on this diet. It was soon discovered through a rat model experiment, that the nitrites that were added to the fish meal, were reacting with the free amines and forming the carcinogenic compounds, nitrosamines, which contributed to the tumor development seen in the mink (Barnes & Magee, 1954; Magee & Barnes, 1956). Fish are a primary example of how meat contributes to nitrosamine formation. Because of its high amounts of free amines, this meat system is highly susceptible to producing nitrosamines. Within other meat systems (cured meats) secondary amines react with nitrite, which also creates carcinogenic nitrosamines that were seen in the fish meal. It wasn't until 1970 when a report entitled "Nitrosamines as Enviromental Carcinogens" was published that widespread public concern emerged (Lijinsky & Epstein, 1970). The authors reported that either secondary amines or nitrites must be eliminated to remove the risk of cancer formation via nitrosamine consumption. Extensive investigations regarding nitrosamines were conducted in order to determine which laws should be put into place to reduce the risk of nitrosamine formation. It was unveiled that specific conditions are required to produce nitrosamines, which include: secondary amines, presence of nitrite, neutral pH, product temperatures

reaching above 130˚C (Sindelar & Milkowski, 2012). Bacon became the primary cured meat product of concern, because of the high temperatures it was subjected to during the most commonly used cooking preparation; frying. To reduce the nitrosamine formation, in 1978 regulations mandated that a maximum of 120 parts per million (ppm) of nitrite and a minimum of 550 ppm sodium ascorbate or sodium erythorbate be added to bacon in order to reduce the amount of nitrite within the product (Sindelar & Milkowski, 2012). Along with the bacon regulations, all cured products were subjected to maximum levels of nitrite that could be added to the product that would maintain a relative low risk of nitrosamine consumption.

"Uncured" Processed Meats

Recent curing alternative

Recently, in the last few years the organic and natural markets have exploded in popularity and have resulted in opportunities for meat processors to increase their earnings. Consumers looking for alternatives to highly "processed" foods have driven the market towards food products of natural and organic origin. Their concerns center around issues of pesticides, hormones, antibiotics, and chemical additives (Devcich et al., 2007). The prominent chemical additive of concern is nitrite/nitrate. With past research claiming that nitrites are hazardous compounds that cause an array of harmful health issues (e.g. cancer) has the public on defense about its use (Barnes & Magee, 1954; Lijinsky & Epstein, 1970). Even though many studies have shown beneficial effects of dietary nitrite/nitrate (McKnight et al., 1997; Lundberg, et al., 2009; Bryan & Loscalzo, 2011; Parthasarathy & Bryan, 2012) and USDA making extensive efforts to reduce ingoing nitrite and nitrate concentrations

(USDA, 1995), the overall perception of consumers is still negative. In order to call a product organic or natural, it must not contain sodium nitrite. To continue to produce a product that has the same characteristics of a conventionally cured product, manufacturers began to find alternatives to sodium nitrite that are deemed "natural" and "organic." Juices derived from celery juice, lettuce, carrot, spinach, and beets contain detectable amounts of nitrate that can be added to meat products and still produce the same characteristics (color, food safety, flavor) typically seen in conventionally cured systems (National Academy of Sciences, 1981; Sebranek, 2006). Beets have high nitrate concentrations, but due to its high pigment concentration, USDA does not permit it in natural products because it is defined as a "coloring agent" (Sebranek & Bacus, 2007). Celery is the most commonly used source of nitrate because it has very little vegetable pigment and a mild flavor, thus limiting the impact on the final meat product (Sebranek & Bacus, 2007).

Labeling

Currently, there are two categories of uncured no-nitrate/nitrite-added meat products. The first is the product that is truly "uncured," which contains absolutely no nitrate or nitrite, and there was no intention by the manufacturer to add it during the process (Sindelar et al., 2007a). Without the addition of nitrate or nitrite the products create negative quality attributes, which negatively effects the consumers perception and acceptability of the product (Hustad et al., 1973; Brown et al., 1974; Froehlich et al., 1983). In addition, the absence of nitrate or nitrite also impacts the microbiological quality, and in turn reduces shelf life significantly. The second category includes products that had a source of nitrate or nitrite intentionally added during processing (Sindelar et al., 2007a). These products mimic the

characteristics of flavor, color, aroma, and sensory normally scene in conventionally cured meat products (Sindelar et al., 2007a). According to USDA (2010a; 2010b), the following must be included when labeling an "uncured" product:

> "Any product, such as frankfurters and corned beef, for which there is a standard in this part and to which nitrate or nitrite is permitted or required to be added, may be prepared without nitrate or nitrite and labeled with such standard name when immediately preceded with the term ''Uncured'' in the same size and style of lettering as the rest of such standard name: *Provided,* That the product is found by the Administrator to be similar in size, flavor, consistency, and general appearance to such product as commonly prepared with nitrate and nitrite…."
>
> "……which contain no nitrate or nitrite shall bear the statement ''No Nitrate or Nitrite Added.'' This statement shall be adjacent to the product name in lettering of easily readable style and at least one-half the size of the product name."
>
> "……the statement ''Not Preserved—Keep Refrigerated Below 40 °F. At All Times'' unless they have been thermally processed to Fo 3 or more; they have been fermented or pickled to pH of 4.6 or less; or they have been dried to a water activity of 0.92 or less."

Most processers produce uncured products to provide meat that is of "natural" origin. To create a product of natural origin the processors must follow regulations set by the USDA (2005):

> "(1) the product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and (2) the product and its ingredients are not more than minimally processed. Minimal processing may include: (a) those traditional processes used to make food edible or to preserve it or to make it safe for human consumption, e.g., smoking, roasting, freezing, drying, and fermenting, or (b) those physical processes which do not fundamentally alter the raw product and/or which only separate a whole, intact food into component parts."

It is important to remember that nitrate/nitrite is considered a chemical preservative and is not allowed in "natural" labeled products. To maintain the incorporation of nitrate and nitrite in natural products processors turned to compounds like celery powder, which naturally

contained nitrate. With the help of current processes they were able to create nitrite by adding starter cultures to stimulate the conversion from nitrate to nitrite. Other chemical preservatives such as phosphates, antioxidants (e.g. BHT), and sodium lactates are also prohibited from use in natural products (Sindelar et al., 2010).

Curing process

During the manufacture of uncured products it is essential to have the proper amount of ingoing nitrate/nitrite. Manufacturers of the celery powder have suggested inclusion percentages based on the weight of the entire batch. 0.2%-0.4% is what is most commonly used when integrating celery juice into the meat product. Sindelar et al. (2007a) found that 0.4% celery juice in frankfurters did not emit negative sensory (aroma, flavor) characteristics that would be associated with the vegetable additive. However, when they incorporated the celery juice into a ham product at 0.35%, the sensory panelists were able to detect vegetable flavor and aroma of the celery powder (Sindelar et al., 2007b). Based on these results it is important to keep the type of product you are producing in mind when determining the percentage of celery powder to use. An equilibrium of enough ingoing nitrate/nitrite and vegetable off flavors must be maintained with each process. When using celery powder in its nitrate form, processing of that meat product must include an incubation step. The nitrate within the celery powder is converted to nitrite using starter cultures, such as *Staphylococcus carnosus* (Sindelar et al., 2010). Temperature and time become crucial for optimal conversion within the product. The optimal temperature for nitrite reductase activity is when the internal temperature of the product reaches between 90-100˚F. The incubation step can last anywhere between 1-2 hours, depending on the diameter of the product (Sindelar et al.,

2010). When producing a frankfurter the incubation period is 120 minutes compared to 90 minutes for smoked sausage (Sindelar et al., 2010). Since the frankfurter has a smaller diameter, more time must be given to the product to convert the nitrate to nitrite before it is thermally processed. Since, the diameter is small, the amount of time it takes to reach the ultimate cooking temperature is much shorter than a larger diameter product (smoked sausage). If not given enough time, the starter culture will be killed before it completes an adequate conversion; thus reducing the nitrite present.

Processors became disgruntled by having to wait 1-2 hours for the conversion to take place. So, the producers of the celery powder developed a pre-converted nitrite product. Now, instead of waiting for the incubation step to be completed the processors are able to add the pre-converted celery powder directly to the meat, and are immediately able to thermally process after preparation. The developers of the pre-converted nitrite created a process that allowed them to conduct the incubation step in their facilities and manufacture the converted powder as a one-step addition similar to conventional nitrite's inclusion. By doing so, the accuracy of ingoing nitrite was improved and celery powder products had higher ingoing nitrite concentrations than previously seen.

Challenges

A major controversy pertaining to uncured meat products is how they are labeled. As stated previously, USDA mandates that any uncured product must state that no nitrates or nitrites are added. To many in the meat industry, this is a false statement and is misleading to the consumers. They believe that they are not consuming nitrates/nitrites, when in fact they are. The only difference is that the nitrates and nitrites are coming from natural forms found

in vegetables. With the increasing demand of this product category, there has been a rise in concern with the misleading information on the label. Currently, USDA is considering revising uncured labeling to provide a more accurate representation of these products.

Perhaps even more importantly than labeling issues are concerns pertaining to the safety of uncured processed meats. Even though there are similarities to overall product qualities between no-nitrate/nitrite-added uncured and conventionally cured products, questions have been raised about whether or not the no-nitrate/nitrite-added products do in fact provide the same microbiological safety as conventionally cured products. Studies have found that the no-nitrate/nitrite-added uncured products were subpar in reducing microbiological growth when compared to conventional treatments (Sindelar, 2006; Wanless et al., 2010; Schrader, 2010; Sullivan et al., 2012). This is attributed to lower ingoing nitrite then conventional treatments, which contributes to lower residual nitrite concentrations (Sindelar et al., 2010). Without enough residual nitrite, it creates an environment suitable for microbiological invasion, thus reducing the shelf life and safety of the product. Current pre-converted celery juice powders contain 10,000-15,000 ppm nitrite (Sindelar et al., 2010). Only 1% of celery juice powder is nitrite (Djeri, 2010), compared to conventional cure that is 67% nitrite. In order to have the same effectiveness, more celery juice powder must be added to the formulation. However, by increasing the amount added, the concern for increased "vegetable" flavor arises, which is perceived negatively by consumers. Recently, the manufactures of celery juice powders have developed processes that allow them to increase the nitrite concentrations without increasing the vegetable off-flavor.

Other possible reasons for the increase in microbial growth could be attributed to the composition of celery juice. Since it is a concentrate, many different components exist within the powder. Djeri (2010) analyzed the celery juice powder and indicated that 85% was dry matter (proteins, fibers, carbohydrates, minerals). Any one of these components could react either in a positive or negative way towards the nitric oxide formation. These components could also contribute to the high pH associated with celery juice. Typically, a pH range of 8.5-10 is seen with celery juice powders. It is important to note that nitrite's effectiveness relies heavily on pH. According to Tarr (1941), a pH at or above 7 inhibits nitrites' microbiological effectiveness. The lower the pH, the more reactive nitrite becomes and produces more nitric oxide, which is demonstrated in the following equation.

$$NO_2 + H^+ \longrightarrow 2HNO_2 \longrightarrow N_2O_3 + H_2O \longrightarrow N_2O_3 \longrightarrow NO_2 + NO$$

Increasing the amount of nitric oxide produced allows it to be used for microbiological inhibition of *Clostridium botulinum* and suppression of *Listeria monocytogenes* growth (Perigo & Roberts, 1968; (McClure et al, 1991). A decrease in pH by as little as 0.2 pH units, can cause the rate of the curing reaction to double (Sebranek, 1979). Previous research conducted in our laboratory has observed higher pHs within the final meat product when celery juice was the primary treatment (Myers, 2012). This has potential to significantly alter the effectiveness of nitrite as an antimicrobial agent. Reduced antimicrobial effectiveness is of particular concern relative to *L. monocytogenes*, because this organism has been shown to be prevalent in the environment and can easily contaminate ready-to-eat processed meats. Consequently, the objective of this thesis was to compare the celery juice concentrate to conventional nitrite using the same nitrite concentrations, and evaluate whether the other

components present in celery juice affect the impact of nitrite on *L. monocytogenes*. Because of the well-known impact of pH on nitrite reactions, pH was included as a variable in assessing the effects of celery juice and conventional nitrite concentrations on *L. monocytogenes* growth.

References

Al-Shuibi, A.N. and Al-Abdullah B.M. (2002). Substitution of nitrite by sorbate and the effect on properties of mortadella. *Meat Science*, 62, 473-478.

Archer, D.L. (2001). Nitrite and the impact of advisory groups. *Food Technology*, 55(3), 26.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M., Mamgia A. (1992). Flavour compounds of dry-cured ham. *Journal of Agriculture & Food Chemistry*, 40, 2389-2394.

Barnes, J.M., Magee, P.N. (1954). Some toxic properties of dimethylnitrosamine. *British Journal of Industrial Medicine*, 11, 167-174.

Benedict, R.C. (1980). Biochemical basis for nitrite inhibition of Clostridium botulinum in cured meat. *Journal of Food Protection*, 43, 877-891.

Binkerd, E.F. and Kolari, O.E. (1975). The history and use of nitrate and nitrite in the curing Of meat. *Food and Cosmetic Toxicology*, 13, 655-661.

Brown, C.L., Hedrick, H.B., Bailey, M.E. (1974). Characteristics of cured ham as influenced by levels of sodium nitrite and sodium ascorbate. *Journal of Food Science*, 39, 977-979.

Bryan, N.S., & Loscalzo, J. (2011). Nitrite and nitrate in human health and disease. In A. Bendich (Ed.), Nutrition and health. New York: Humana Press.

Bryan, N.S., & Hord, N.G. (2010). Dietary nitrates and nitrites: the physiological context for potential health benefits in: N.S. Bryan (Ed.), Food, Nutrition and the nitric oxide pathway: Biochemistry and bioactivity, DESTech Publications, Inc., Lancaster, PA, 59-77.

Buchanan, R.L. (1989). Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. *Journal of Food Protection*, 52(12), 844-851.

Cassens, R.G., Greaser, M.L., Ito, T., Lee, M. (1979). Reaction of nitrite in meat. *Food Technology*, 33, 46-57.

CDC. (2011). Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms, Colorado. Accessed December 8, 2011. Accessed December 8, 2011. http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html

Cross, C.K. and Ziegler, P. (1965). A comparison of the volatile fractions from cured and uncured meat. *Journal of Food Science*, 30, 610-614.

Devcich, D.A., Pedersen, I.K., Petrie, K.J. (2007). You are what you eat: Modern health worries and the acceptance of natural and synthetic additives in functional foods. *Appetite*, 48,333-337.

DHEW. (1971). Proceedings of the 1971 national conference on food protection. U.S. Department of Health, Education and Welfare, Public Health Service, Washingtion, D.C.

Djeri, N. (2010). Evaluation of vegstable 504 celery juice powder for use in processed meat and poultry as a nitrite replacer. Ph.D. Thesis. University of Florida, Gainesville, FL.

Doganay, M. (2003). Listeriosis: clinical presentation. *FEMS Immunology and Medical Microbiology*, 35, 173-175.

Duffy, L.L., Vanderlinde, P.B., Grau, F.H. (1994). Growth of Listeria monocytogenes on vacuum-packed cooked meats: effects of pH, aw, nitrite and ascorbate. *International Journal of Food Microbiology*, 23, 377-390.

Evans, M.R., Swaminathan, B., Graves, L.M., Altermann, E., Klaenhammer, T.R., Fink, R.C., Kernodle, S., Kathariou, S. (2004). Genetic markers unique to Listeria monocytogenes serotype 4b differentiate epidemic clone II (hot dog outbreak strains) from other lineages. *Applied and Environmental Microbiology*, 70, 2383.

Flores, M., Spanier, A.M., Toldra F. (1998). Flavour analysis of dry-cured ham. In Flavor of Meat, Meat Products and Seafoods, Second Edition, ed. F. Shahidi. Blackie Academic and Professional, London, UK, pp320-341.

Froehlich, D.A., Gullet E.A., Usborne W.R. (1983). Effect of nitrite and salt on the color, flavor and overall acceptability of ham. *Journal of Food Science*, 48, 152-154.

Garg, H. (2006). Diet and cardiovascular disease: The role of antioxidants and nitrite. School of Medicine, Boston University, Boston, 1-54.

Gray, M.L. and Killinger A.H. (1966). Listeria monocytogenes and listeric infection.

Bacteriological Reviews, 30, 309.

Haldane, J. (1901). The red color of salted meat. *Journal of Hygiene*, Cambridge, 1, 115.

Hoagland, R. (1908). The action of saltpeter upon the color of meat. United States Department of Agriculture, Bureau of Animal Industry, 25th Ann. Rept. P. 301. Washington, DC.

Hord, N.G., Tang, Y., Bryan N.S. (2009). Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *American Journal of Clinical Nutrition*, 90, 1-10.

Hustad, G.O., Cerveny, J.H., Trenk, H., Deibel, R.H., Kautter, D.A., Fazio, T., Johnston, R.W., Kolari, O.E. (1973). Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. *Applied Microbiology*, 26, 22-26.

Jensen, L.B. (1953). Early preparations of foods. In man's foods. Nutrition and environments in food gathering times and food producing times. The Garrad Press, Champaign, IL, pp. 159-170.

Kisskalt, K. (1899). Beitrage zur Kenntnis der Ursachen des Rotwerdens des Fleisches beim Kochen, nebst einigen Versuchen uber die Wirkung der schwefligen Saure auf die Fleischfarbe. *Archiv Fur Hygiene Und Bakteriologie*, 35, 11.

Labbe, R. G. (1989). *Clostridium perfringens*, p. 191–234. *In* M. P. Doyle (ed.), Foodborne Bacterial pathogens. Marcel Dekker, New York.

Lansley, K.E., Winyard, P.G., Bailey S.J., Vanhatalo, A., Wilkerson, D.P., Blackwell, J.R., Gilchrist, M., Benjamin, N., Jones, A.M. (2011). Acute dietary nitrate supplementation improves cycling time trial performance. *Medicine and science in sports*, 43(6), 1125-1131.

Larsen, F.J., Weitzberg, E., Lundberg, J.O., Ekblom, B. (2010). Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. *Free radical and biology medicine*, 48(2), 342-347.

Lehmann, K.B. (1899). Uber das Haemorrhodin, ein neues weitverbreitetes Blutfarbstoffderivat. Sber. phys.-med. Ges. Wurzh, 4, 57.

Leyer, G.J., Johnson, E.A. (1993). Acid adaptation induces cross-protection against Environmental stresses in Salmonella typhimurium. *Applied Environmental Microbiology*, 56, 1842-1847.

Lijinsky, W., & Epstein, S.S. (1970). Nitrosamines are environmental carcinogens. Nature 225, 21. Liu, D. 2008. Epidemiology, pp. 27-59. In Liu, D. (ed.), Handbook of *Listeria monocytogenes*. Taylor & Francis Group, LLC, Boca Raton, FL.

Liu, D. (2008). Epidemiology, pp. 27-59. In Liu, D. (ed.), Handbook of *Listeria monocytogenes*. Taylor & Francis Group, LLC, Boca Raton, FL.

Lu, Z., Sebranek, J.G., Dickson, J.S., Mendonca, A.F., Bailey, T.B. (2005). Inhibitory effects of organic salts for control of *Listeria monocytogenes* on frankfurters. *Journal of Food Protection*, 68(3), 499-506.

Lundberg, J.O., Feelisch, M., Bjorne, H., Jansson, E.A., Weitzberg, E. (2006). Cardioprotective effects of vegetables: is nitrate the answer?. *Nitric oxide*, 15, 359-362.

Lundberg, J.O., Gladwin, M.T., Ahluwalia, A., Benjamin, N., Bryan, N.S., Butler, A., Cabrales, P., Fago, A., Feelisch, M, Ford, P.C., Freeman, B.A., Frenneaux, M., Friedman, J., Kelm, M., Kevil, C.G., Kim-Sharpiro, D.B., Kozlov, A.V., Lancaster, J.R. Jr., Lefer, D.J., McColl, K., McCurry, K., Patel, R.P., Petersson, J., Rassaf, T., Reutov, V.P., Richter-Addo, G.B., Schechter, A., Shiva, S., Tsuchiva, K., van Faassen, E.E., Webb, A.J., Zuckerbraun, B.S., Zweier, J.L., Weitzberg, E. (2009). Nitrate and nitrite in biology, nutrition and therapeutics. *Nature Chemical Biology*, 5(12), 865-869.

Lundberg, J.O., Larsen, F.J., Weitzberg, E. (2011). Supplementation with nitrate and nitrite salts in exercise: a word of caution. *Journal of applied physiology*, 111, 616-617.

Lungu, B., Ricke, S.C., Johnson, M.G. (2009). Growth, survival, proliferation and pathogenesis of *Listeria monocytogenes* under low oxygen or anaerobic conditions: A review. *Anaerobe*, 15, 7-17.

Magee, P.N., Barnes, J.M. (1956). The production of malignant primary hepatic tumours in the rat by feeding dimethyinitrosamine. *British Journal of Cancer*, 10(1), 114-122.

Mbandi, E., Shelef, L.A. (2002). Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and Salmonella spp. in beef bologna. *International Journal of Food Microbiology*, 76, 191-198.

McClure, P.J., Kelly, T.M., Roberts, T.A. (1991). The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 14(1), 77-91.

McDonald, B., Gray, J.I., Gibbins, L.N. (1980). Role of nitrite in cured meat flavor: Antioxidant role of nitrite. *Journal of Food Science*, 45, 893-897.

McKnight, G.M., Smith, L.M., Drummond, R.S., Duncan, C.W., Golden, M., Benjamin, N. (1997). Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. *Gut*, 40, 211-214.

Mead, P.S., Dunne, E.F., Graves, L., Wiedmann, M., Patrick, M., Hunter, S., Salehi, E., Mostashari, F., Craig, A., Mshar, P., Bannermann, T., Sauders, B.D., Hayes, P., Dewitt, W., Sparling, P., Griffin, P., Morse, D., Slutsker, L., Swaminathan, B., Listeria Outbreak Working Group. (2006). Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiology and Infection*, 134, 744.

Montville, T. J., and Matthews, K. R. (2005). *Clostridium perfringens.* p. 221- 231. *In*, Food microbiology-an introduction. ASM Press, Washington, DC.

Morrissey, P.A., and Tichivangana, J.Z. (1985). The antioxidant activities of nitrite and nitrosylmyoglobin in cooked meats. *Meat Science*, 14:175-190.

Murphy, M., Eliot, K., Heuertz, R.M., Weiss, E. (2012). Whole beetroot consumption acutely Improves running performance. *Journal of the academy of nutrition and dietetics*,112(4), 548-552.

Murray, E.G.D., Webb, R.A., Swann, M.B.R. (1926). A disease of rabbits characterized by a large mononuclear leukocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. Journal of Pathology and Bacteriology, 29, 407-439.

Myers, K. M. (2012). Evaluation of high hydrostatic pressure, meat species, and ingredients to control *Listeria monocytogenes* in ready-to-eat meats. Ph.D. Thesis. Iowa State University, Ames, IA.

National Academy of Sciences. (1981). The health effects of nitrate, nitrite and n-nitroso compounds. Washington, DC: National Academy Press.

Ngutter, C.M. and Donnelly, C.W. (2003). Nitrite-induced injury of *Listeria monocytogenes* and the effect of selective versus nonselective recovery procedures on its isolation from frankfurters. *Journal of Food Protection*. 66:2252-2257.

Parthasarathy, D.K., & Bryan, N.S. (2012). Sodium nitrite: The “cure” for nitric oxide insufficiency. *Meat Science*, 92, 274-279.

Parolari, G. (1996). Achievements, needs and perspectives in dry-cured ham technology: the example of Parma ham. *Food Science and Technology International*, 2, 69-78.

Pegg R.B, Shahidi, F. (2000). Nitrite curing of meat: The N-Nitrosamine problem and nitrite alternatives. Food & Nutrition Press, Inc. Trumbull, CT.

Perigo, J. A., and Roberts, T. A. (1968). Inhibition of clostridia by nitrite. *Journal of Food Technology*, 3, 91-94.

Pierson, M.D., Smooth, L.A. (1982). Nitrite, nitrite alternatives, and the control of *Clostridium botulinum* in cures meats. *CRC Critical Reviews in Food Science & Nutrition*, 17, 141-187.

Porto, A.C.S., Franco, B.M.G., Sant'Anna, E.S, Call, J.E., Piva, A., Luchansky, J.B. (2002). Viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially-prepared with and without 2.0 o 3.0% added potassium lactate, during extended storage at 4 and 10˚C. *Journal of Food Protection*, 65(2), 308-315.

Porto-Fett, A.C.S., Campano, S.G., Smith, J.L., Oser, A., Shoyer, B., Call, J.E., Luchansky, J.B. (2010).Control of *Listeria monocytogenes* on commercially-produced frankfurters prepared with and without potassium lactate and sodium diacetate and surface treated with lauric arginate using the Sprayed Lethality in Container (SLIC) delivery method. *Meat Science*, 85, 312-318.

Quintavalla, S., Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science*, 62, 373-380.

Reij, M.W., Den Aantrekker, E.D., and ILSI Europe Risk Analysis in Microbiology Task Force. (2004). Recontamination as a source of pathogens in processed foods. *International Journal of Food Microbiology*, 91, 1-11.

Robbins, M. and McSwane, D. (1994). Sanitation doesn't cost, it pays: is it true and can we prove it?. *Journal of Environmental Health*, 57, 14-20.

Ryu, J.H., Deng, Y., Beuchat, L.R. (1999). Survival of *Escherichia coli 0157:H7* in dried beef powder as affected by water activity, sodium chloride content and temperature. *Food Microbiology*, 16, 309-316.

Samelis, J., Bedie, G.K., Sofos, J.N., Belk, K.E., Scanga, J.A., Smith, G.C. (2005). Combinations of nisin with organic acids or salts to control *Listeria monocytogenes* on sliced pork bologna stored at 4˚C in vacuum packages. *Lebensmittel-Ebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, 38, 21-28.

Sauter, E. A., Kemp, J. D., Langlois, B. E. (1977). Effect of nitrite and erythorbate on recovery of *Clostridium perfringens* spores in cured pork. *Journal of Food Science*, 42, 1678-167.

Schlyter, J.H., Glass, K.A., Loeffelholz, J., Degnan, A.J., Luchansky, J.B. (1993). The effects

of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. *International Journal of Food Microbiology*, 19(4), 271-281.

Schrader, K.D. (2010). Investigating the control of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added meat products. Ph.D. Thesis. Iowa State University, Ames, IA.

Sebranek, J.G. (1979). Advances in the technology of nitrite use and consideration of alternatives. *Food Technology*, 33(7), 58-62, 93.

Sebranek, J.G. (2009). Basic curing ingredients. Pages 1-24 in Ingredients in Meat Products. R.Tarte, ed. Springer Science+Buisness Media LLC, New York, NY.

Sebranek, J.G., Bacus, J. (2007). Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality, and safety issues. American Meat Science Association White paper series, No. 1.

Sebranek, J.G. (2006). Unpublished data. Iowa State University, Ames, IA.

Shahidi, F. (1992). Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free Compositions. In Lipid Oxidation in Food, ed. A.J. St. Angelo. ACS Symposium Series 500. *Journal of the American Chemical Society*, Washington, DC, 161-182.

Sindelar, J.J. (2006). Investigating uncured no-nitrate-or-nitrite-added processed meat products. Ph.D. Thesis. Iowa State University, Ames, IA.

Sindelar, J.J., Cordray, J.C., Olson, D.G., Sebranek, J.G., Love, J.A. (2007a). Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *Journal of Food Science*, 72(8), S551-S559.

Sindelar, J.J., Cordray, J.C., Sebranek, J.G., Love, J.A., Ahn, D.U. (2007b). Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *Journal of Food Science*, 72 (6): S388-S395.

Sindelar, J.J. & Milkowski, A.L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide*, 26, 259-266.

Sindelar, J.J, and Milkowski, A.L. (2011). Sodium nitrite in processed meat and poultry meats: A review of curing and examining the risk/benefit of its use. American Meat Science Association. AMSA white paper series, No. 3.

Sindelar, J.J., Sebranek, J.G., Bacus, J.N. (2010). Uncured, natural and organic processed

meat products. American Meat Science Association. Meat processing technology series.

Sofos, J.N., Busta, F.F., Allen, C.E. (1979). Botulism control by nitrite and sorbate in cured meats: A review. *Journal of Food Protection*, 42, 739-770.

Sullivan, G.A., Jackson-Davis A.L., Schrader, K.D., Xi, Y., Kulchaiyawat C., Sebranek J.G., Dickson J.S. (2012). Survey of naturally and conventionally cured commercial frankfurters, ham, and bacon for physio-chemical characteristics that affect bacterial growth. *Meat Science*, 92(4), 808-815.

Taormina, P.J. & Dorsa, W.J. (2004). Growth Potential of *Clostridium perfringens* during cooling of cooked meats. *Journal of Food Protection*, 67, 1537-1547.

Tarr, H.L.A. (1941). The action of nitrites on bacteria. *Journal of the Fisheries Research Board of Canada*, 5(3), 265-275.

Theron, M.M., Lues, J.F.R. (2007). Organic acids and meat preservation: A review. *Food Reviews International*, 23, 141-158.

Thompson, R.L., Carpenter, C.E., Martini, S., Broadbent, J.R. (2008). Control of *Listeria monocytogenes* in ready-to-eat meats containing sodium levulinate, sodium lactate, or a combination of sodium lactate and sodium diacetate. *Journal of Food Science*, 78(5), M239-M244.

Toldra, F., Aristoy, M-C., Flores, M. (2009). Relevance of nitrate and nitrite in dry-cured ham and their effects on aroma development. *Grasas Y Aceites*, 60(3), 291-296.

Tompkin, R.B. (2002). Control of *Listeria monocytogenes* in the food processing environment. *Journal of Food Protection*, 65(4), 709-725.

Tompkin, R.B. (1983). Nitrite. In: A.L. Branen and P.M. Davidson, (Eds.) Antimicrobials in food. Marcel and Dekker, New York, NY, pp. 205-256.

Tompkin, R.B. (2005). Nitrite. p. 169-236. *In* P.M. Davidson, J.N. Sofos, and A.L. Branen (ed.), Antimicrobials in food. Taylor & Francis, Boca Raton, FL. <http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf>. Accessed 12.28.12.

United States Department of Agriculture (USDA). (1999). Compliance guidelines for cooling heat-treated meat and poultry products (stabilization). Food Safety and Inspection Service. Available at: www.fsis.usda.gov.

United States Department of Agriculture (USDA). (2003). Control of *Listeria monocytogenes*

in ready-to-eat meat and poultry products; final rule. *Federal Register*, 68, 34208-34254.

United States Departmart of Agriulture (USDA). (2005). Food Standards and Labeling Policy Book.http://www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy_Book_082005.pdf. Accessed Jan. 22, 2013.

United States Department of Agriculture (USDA). (2010a). Interpretation and statement of labeling policy for cured products; special labeling requirements concerning nitrate and nitrite. Code of Federal Regulations, 9 CFR 317.7.

United States Department of Agriculture (USDA). (1995). Processing Inspector's Calculations Handbook (FSIS Directive 7620.3).

United States Departement of Agriculture (USDA). (2010b). Products and nitrates and nitrites. Code of Federal Regulations, 9 CFR 319.2.

Wagner, M. & McLauchlin, J. (2008). Biology, pp. 3-25. In Liu, D. (ed.), Handbook of *Listeria monocytogenes*. Taylor & Francis Group, LLC, Boca Raton, FL.

Wanless, B., McDonnell L., Glass K. (2010). Effect of nitrite and natural antimicrobials on *Clostridium botulinum* toxigenesis in model frankfurter and ham products. Abstract in Institute of Food Technologists Annual Meeting. Chicago, IL: Institute of Food Technologist.

Wong, S., Street, D., Delgado, S.I., Klontz, K.C. (2000). Recalls of foods and cosmetics due to microbial contamination reported to the U.S Food and Drug Administration. *Journal of Food Protection*, 63, 1113.

CHAPTER 3. The effect of pH and nitrite concentration on the antimicrobial impact of celery juice compared with sodium nitrite on *Listeria monocytogenes*

A paper to be submitted to *Meat Science*

A.M. Horsch[a,b], J.G. Sebranek[a,b,*], J.S. Dickson[b], S.E. Niebuhr[b], E.M. Larson[b], N.A. Lavieri[b], B.L. Ruther[b], L.A. Wilson[a]

[a]Department of Food Science and Human Nutrition; [b]Department of Animal Science, Iowa State University, Ames, IA 50011

Abstract

Increasing consumer concerns of harmful preservatives have intensified consumers' demand for natural and organic alternatives. In response to this demand, uncured or no-nitrate-or-nitrite-added meat products which utilize celery juice concentrates as an alternative to sodium nitrite, have emerged on the market to replace conventional nitrite sources. The objective of this study was to evaluate the effect of celery juice pH for the impact of nitrite on *L. monocytogenes* growth. In addition, equal concentrations of nitrite in celery juice and conventional nitrite were evaluated to determine the impact of nitrite concentration from these sources on *L. monocytogenes* growth. These objectives were assessed using both a broth and ham system. Celery juice (CJ) was less effective than the conventional nitrite in the broth study at 100 ppm nitrite concentration but in the ham experiment the CJ treatments at both 100 and 200 ppm resulted in similar growth of *L. monocytogenes* ($p>0.05$) compared to their counterparts 100 and 200 ppm sodium nitrite. Adjusting the pH of the celery juice proved to be more effective at suppressing *L. monocytogenes* growth at 200 ppm than 100 ppm in the ham. No differences in growth ($p>0.05$) were found between the unadjusted 100

ppm celery juice (pH~9.2) and adjusted 100 ppm celery juice (pH~6.0) in either the broth or ham study. Color measurements of the ham indicated that all the CJ treatments were darker (lower L*) and more yellow (higher b*) than the sodium nitrite treatments. As concentration increased within the CJ treatments the L* became significantly lower ($p<0.05$) and b* values became significantly ($p<0.05$) greater. Overall, similar redness (a*) values were seen in both the CJ and sodium nitrite treatments. Residual nitrite concentrations were similar for both the 100 and 200 ppm treatments in the ham study, except for the adjusted (pH~ 6.3) 200 ppm CJ treatment which had significantly less ($p<0.05$) residual nitrite than the unadjusted (pH~6.6) 200 ppm CJ and 200 ppm sodium nitrite treatments.

Introduction

For centuries nitrate and nitrite have been used extensively in preserving meat products. Accidental discovery of these curing agents probably occurred during the traditional salting of meat dating back to 1600 BC (Jenson, 1953). Specific types of salt that were adulterated with nitrate developed a reddish color, which lead to what is commonly seen in cured meats today (Pegg & Shahidi, 2000). Other characteristics such as distinct flavors, decreased lipid oxidation, and inhibition of bacteria growth also contribute to the uniqueness of cured products (Sindelar & Milkowski, 2011).

However, concerns emerged in the 1950's relating to the safety of nitrate and nitrite inclusion in meat products. Studies indicated that free amines in herring meal were reacting with nitrite to form carcinogenic compounds called nitrosamines (Barnes & Magee, 1954; Magee & Barnes, 1956). In response to the nitrosamine concern, the United States Department of Agriculture (USDA) enforced maximum inclusion concentrations of nitrite in

all cured meat products that are still effective today (USDA, 1995). These maximum levels are strictly adhered to and have reduced the risk of nitrosamine production (Sindelar & Milkowski, 2012). Recently, new research has indicated that nitric oxide homeostasis in the body is critical for maintaining optimal blood pressure levels and controlling the blood flow of cardiac muscles (Bryan & Hord, 2010). This research, along with others, has clearly shown that dietary nitrate can be beneficial to an individual's overall health; especially for aging adults (McKnight et al., 1997; Parthasarathy & Bryan, 2012). Thus, nitrite in food is currently viewed by many in a much more positive light.

Regardless, consumers are apprehensive about the use of chemical preservatives, such as nitrate and nitrite, and this is driving consumers to seek alternative food products in natural and organic markets. In doing so, organic sales alone have risen from $1 billion in 1990 to $26.7 billion in 2010 (Organic Trade Association, 2011). To meet the needs of these consumers meat manufactures have created "no-nitrate-or-nitrite-added" or "uncured" labeled meat products that qualify to be labeled as natural or organic. In order to produce a product with the same characteristics seen in a conventionally cured product, manufacturers began using vegetable juice alternatives that contained high concentrations of nitrate. This allows the manufacturers to comply with the natural and organic labeling regulations (USDA, 2005). Celery juice concentrate is prominently used by the meat industry for this purpose because it has very little vegetable pigment and a mild flavor which minimizes the "vegetable" flavor sometimes perceived in the final meat product (Sebranek & Bacus, 2007). Originally, celery juice powder was first available in its nitrate form. Before processing, the celery juice powders would have to undergo a time-consuming incubation step where a

nitrate-reducing starter culture would be added to reduce nitrate to nitrite. Further developments created a pre-converted celery juice containing nitrite that eliminated the wait time of the incubation step and allowed direct addition during processing. Current pre-converted celery juice powders contain 10,000-15,000 ppm nitrite and are the most commonly used celery juice powder used today (Sindelar et al., 2010).

Listeria monocytogenes has become a hot topic of concern for meat processors recently due to its contamination of ready-to-eat meats and ability to withstand an adverse environment like refrigeration temperatures (Lungu et al., 2009). In 1936, the implications of this bacterium first became evident when its infection, listeriosis, caused abortions in pregnant women and meningitis in adults (Gray & Killinger, 1966). Populations that are immunocompromised such as pregnant women, children, and the elderly are especially prone to listeriosis (Liu, 2008). Even though this organism is not the most prevalent of the foodborne pathogens (Scallan et al., 2011), it has devastating consequences, since 20-30% of those contracting listeriosis result in death (Doganay, 2003). Schrader (2010) analyzed eight commercial brands of no-nitrate-or-nitrite-added frankfurters and found that five were less effective in reducing *L. monocytogenes* growth compared to conventionally cured brands. Myers (2012) also observed an increase in growth of *L. monocytogenes* on the no-nitrate-or-nitrite-added products and speculated that it could be attributed to the elevated pH observed in these products. Typically, celery juice concentrate has a pH ranging from 8.5-10 and may impact meat product pH as a result. It is important to note that nitrite's effectiveness relies heavily on pH (Tompkin, 2005). According to Tarr (1941), a pH at or above 7 inhibits nitrites' microbiological effectiveness. By reducing the pH, more nitric oxide is produced and

results in an increase in *L. monocytogenes* suppression (McClure et al, 1991). Reduced antimicrobial effectiveness is of particular concern relative to *L. monocytogenes,* because this organism has been shown to be prevalent in the environment and can easily contaminate ready-to-eat processed meats. Consequently, the objective of this study was to evaluate the impact of pH on the effectiveness of nitrite in celery juice for the suppression of *L. monocytogenes* growth on restructured ham products. In addition, the celery juice concentrate was compared to conventional nitrite using the same nitrite concentrations to evaluate whether the various components present in the celery juice affect the impact of nitrite on *L. monocytogenes*.

Materials and Methods

Broth Study

Broth preparation

Trypticase soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton, Dickson and Company, Sparks, MD., U.S.A.) was chosen for its neutral pH (~ 7.2) and its ability to support *Listeria monocytogenes* growth. Two groups of TSBYE were made. One received a pH adjustment using 1M hydrochloric acid to reduce the pH of the broth to 5.8. The pH of 5.8 was chosen because it best represents a typical meat system pH. The other group did not receive a pH adjustment (pH = ~ 7.2). These broths were then used to prepare experimental treatments for incubation with *L. monocytogenes* (Table 1).

Sample preparation

Two controls were created for each TSBYE adjusted group (Unadjusted = ~7.4, Adjusted = ~ 5.8) by adding distilled water as a treatment. The pre-converted celery juice

(VegStable 504, Florida Food Products, Eustis, FL) treatments consisted of two 100 ppm treatments, one unadjusted for pH prior to use and one adjusted (Unadjusted pH = ~9.2, Adjusted pH = ~6.0). The celery juice concentrate was added to distilled water to obtain 100 ppm nitrite concentration. 10 grams of citric acid (Fisher Scientific, Waltham, MA) was mixed with 90 ml of distilled water to obtain a 10% solution and then was added accordingly to reduce the pH of the adjusted celery juice treatment to ~6.0. Two ml of each treatment, along with 2 ml of the *L. monocytogenes* inoculum were added to 16 ml of each corresponding TSBYE treatment. Treatments were stored in dark conditions and held at 10°C.

Table 1
Broth study treatment descriptions.

Treatment	Description
A	Unadjusted control (unadjusted TSBYE + H_2O)
[a]B	Adjusted control (adjusted TSBYE + H_2O)
C	Unadjusted TSBYE + unadjusted 100 ppm celery juice
*[a]D	Adjusted TSBYE + adjusted 100 ppm celery juice
[a]E	Adjusted TSBYE + 100 ppm sodium nitrite
[a]F	Adjusted TSBYE + 200 ppm sodium nitrite

*Citric acid used to adjust pH of celery juice to 6.0.
[a]Hydrochloric acid used to adjust TSBYE pH to 5.8

Inoculum preparation and sample inoculation

5 strains of *Listeria monocytogenes* (Scott A, H7969, H7764, H7769, H7762) were obtained from the Food Safety Research Laboratory (FSRL) at Iowa State University. Each strain received a minimum of two consecutive 24 hour transfers into TSBYE and were incubated at 35°C. After 48 hours all 5 strains were homogenized together to create a cocktail (~10^9 cells per ml). The cocktail was diluted using 0.1% peptone water (Difco, Becton

Dickinson, Sparks, MD) to obtain 10^4 cells per ml. 2 ml of the diluted cocktail were added to each treatment.

Microbiological analysis

Appropriate ten-fold dilutions from each homogenized experimental treatment were made. From each treatment's designated dilutions, 0.1 ml was surface plated in duplicate onto Modified Oxford Medium supplemented with Modified Oxford Antimicrobial Supplement (MOX) (Difco, Becton Dickinson, Sparks, MD) on days 0, 2, 4, 6, 8, 10, and 12. Inoculated plates were incubated at 35°C for 48 hours. After 48 hours inoculated plates were counted.

pH determination

pH analysis was conducted by directly inserting the pH electrode (Fisher Scientific, Accumet 15, Waltham, MA) into the broth for each treatment. The pH meter was calibrated using phosphate buffers 4.0 and 7.0. Measurements were taken on days 0, 2, 4, 6, 8, 10, and 12.

Ham Study

Product manufacture

Seven treatments (Table 2) were produced to determine if pH and concentration of nitrite impacted the growth of *Listeria monocytogenes* in natural and conventional cured ham products. Two replications were conducted. Pre-converted celery juice (VegStable 504, Florida Food Products, Eustis, FL) was used as the natural source of nitrite. 10% solution of citric acid (Fisher Scientific, Waltham, MA) was added to celery juice for treatments 3 and 5 to lower the celery juice pH to approximately 6.

Table 2
Ham study treatment formulations.

Treatment*	Code	Ham Insides (kg)	Water (kg)	Salt (kg)	Sugar (kg)	VegStable 504 (g)	Sodium nitrite (g)	Sodium nitrite (ppm)[b]
1	Control	9.09	1.83	0.24	0.14	-	-	-
2	Unadj 100 ppm CJ	9.09	1.83	0.24	0.14	75.6	-	100
3[a]	Adj 100 ppm CJ	9.09	1.83	0.24	0.14	75.6	-	100
4	Unadj 200 ppm CJ	9.09	1.83	0.24	0.14	151.2	-	200
5[a]	Adj 200 ppm CJ	9.09	1.83	0.24	0.14	151.2	-	200
6	100 ppm $NaNO_2$	9.09	1.83	0.24	0.14	-	1.13	100
7	200 ppm $NaNO_2$	9.09	1.83	0.24	0.14	-	2.27	200

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.
[a]Treatments with addition of citric acid to obtain a pH of 6 in the celery juice.
[b]Total batch weight basis.
All treatments were based on a total of 11.3 kg.

Hams were produced at the Iowa State University (ISU) Meat Laboratory. Pork inside ham muscles (semimembranosus) were received fresh from a local processor and held at 0°C. The ham muscles were course-ground (Biro MFG Co., Model 7.5 424852, Marblehead, Ohio, U.S.A.) using a 9.52 mm plate. Non-meat ingredients were added to a vacuum paddle mixer (Fotosa, SA., Barcelona, Spain) along with the ham muscles according to the formulations found in Table 2. It should be noted that USDA sodium nitrite limits are based on the meat block weight, but to correspond with the concentrations used in the broth experiment, sodium nitrite was formulated on a total batch weight basis for this experiment. No phosphates were included because they are not permitted ingredients for natural and organic labeled meat products. After mixing for 2 minutes, the meat mixture was reground

through a 6.35 mm plate and stuffed into a 50 mm diameter impermeable plastic casing (Nalobar APM 45, Kalle USA, Gurnee, IL) using a vacuum stuffer (Risco vacuum stuffer, Model 1040C, Stoughton Mass., U.S.A.). Impermeable casings were used to minimize the transfer of nitrogen oxide gases during thermal processing. Treatments were then placed into a single truck smokehouse (Thermal Processor, Maruer-Atmos, Reichenau, Germany) for thermal processing. All products reached an internal temperature of 73.9°C. Products were then transported to a 0°C cooler overnight to stabilize. The next day each treatment was sliced (Bizerba, SE 12 D, Piscataway, NJ., USA) into 11 mm thick portions weighing approximately 25 g $\pm$ 0.5 g. For microbiology analysis, individual slices were placed in each bag (Cryovac Sealed Air Corporation, B2470, Duncan, SC) with an oxygen transmission rate of 3-6 cc at 40°F (m^2, 24 hrs atm @ 40°F, 0% RD) and a water vapor transmission rate of 0.5-0.6 g at 100°F (100% RD, 100 in^2, 24 hrs) and vacuumed packaged (UV 2100, Multivac, Inc., Kansas City, MO). For chemical analysis, two 25 gram slices were placed together into one bag (Cryovac Sealed Air Corporation, B2470, Duncan, SC) and vacuum packaged. The microbiology samples were transported to the Food Safety Research Laboratory (FSRL) and stored at 4°C in a dark cooler in the Meat Laboratory. Samples for chemical analysis were transported to a separate 4°C dark storage cooler.

Inoculum preparation

5 strains of *Listeria monocytogenes* (Scott A, H7969, H7764, H7769, H7762) were obtained from the FSRL at Iowa State University. Strains were individually grown in trypticase soy broth containing 0.6% yeast extract (TSBYE) (Difco, Becton, Dickson and Company, Sparks, MD., U.S.A.) and underwent two 24 hour transfers at 35˚C. All 5

transferred stains were combined to create a 50 ml cocktail (~10^9 cells per ml). From this cocktail dilutions were made using 0.1% buffered peptone water (Difco, Becton Dickson and Company, Sparks, MD., U.S.A.) to obtain a target inoculation of 10^4 cells per gram.

Sample inoculation

The packages containing the ham slices were aseptically opened and surface inoculated with 0.25 ml of the *L. monocytogenes* cocktail to obtain target 10^4 cells per gram for each slice of ham. Ham slices were then repackaged using the FSRL vacuum packager (Multivac, Model A-300/52, Kansas City, Mo., USA) and stored in a dark cooler at 4°C.

Microbiological analysis

On days 0, 3, 7, 10, 14, 21, 28, and 35, one inoculated 25 g sample from each treatment was aseptically removed from its packaging and placed into a 7.5 inch x 12 inch WhirlPak™ filter bag (VWR International, Radnor, PA) along with 99 ml of buffered peptone water (Difco, Becton Dickinson, Sparks, MD). It was then homogenized (Stomacher 400, Seward Medical, London, UK) on the normal setting for 60 seconds. Following homogenization, appropriate ten-fold serial dilutions were made using 0.1% buffered peptone water. Designated dilutions of 0.1 ml were surface plated in duplicate on MOX (Difco, Becton Dickinson, Sparks, MD). Inoculated plates were incubated at 35°C for 48 hours. Immediately following incubation the inoculated plates were counted.

pH determination

The pH meter (Inlab Solids Pro probe; MultiSeven pH meter, 92 Metler Toledo Inc, Columbus, OH) was calibrated using 4.0, 7.0, and 10.0 phosphate buffers. A 9:1 water: slurry

was used to determine the pH of the ham samples on days 0, 3, 7, 10, 14, 21, 28, and 35. All measurements were done in duplicate.

Color analysis

Color was analyzed using the HunterLab LabScan XE spectrocolorimeter (HunterLab, Reston, VA). A port size of 3 cm and a viewing area of 2.54 cm were used along with Illuminant A and 10° standard observer. The instrument was standardized by covering the white standard (X= 80.45, Y= 85.37, Z= 90.79) with saran wrap (SC Johnson & Sons, Racine, WI) to account for the saran wrap used on the samples while taking measurements. Four measurements (CIE L*, a*, and b*) were taken randomly for each treatment on days 0, 3, 7, 10, 14, 21, 28, and 35.

Residual nitrite

Samples from color analysis were then ground and homogenized using a food processor (KitchenAid, Model KFP715, St Joseph, MI). Residual nitrite was determined according to AOAC method 973.31 (AOAC, 1990c) on days 0, 3, 7, 10, 14, 21, 28, and 35 and expressed as sodium nitrite. All measurements were done in duplicate.

Water activity

Samples were analyzed with AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, Wash., U.S.A.) on day 0. The 0.76 and 1.00 standards were used to calibrate the instrument. All measurements were conducted in duplicate.

Proximate analysis

Moisture (AOAC, 1990b), crude protein (AOAC, 1993), and crude fat (AOAC, 1990a) were analyzed in duplicate for each treatment on day 0.

Statistical analysis

For the broth and ham experiments, statistical analysis was conducted using a randomized complete block design including replication, treatment, day and treatment x day in the model as fixed block effects. Measurements were analyzed using the statement proc glimmix with the Statistical Analysis System (SAS 9.2, SAS Institute Inc., Cary, NC, 2008). Due to the significant interaction between treatment and day, treatment means were compared for each day resulting in all pairwise comparisons calculations. Tukey multiple comparison adjustment was used to determine the pairwise comparisons. For moisture, fat, protein and water activity in the ham study, the proc glm statement was used to determine differences amongst means. In both experiments, significant differences were denoted with a $p<0.05$.

Results and Discussion

Broth Study

Listeria monocytogenes growth and pH

Table 3 and Fig. 1 illustrate the differences between treatments found for growth of *L. monocytogenes* in broth over the 12 day period. On days 0 and 2 there were no significant differences ($p>0.05$) amongst treatments. As expected the unadjusted control (pH~7.3) and adjusted control (pH~6.1) broth treatments had similar ($p>0.05$) growth throughout the entire study and resulted in greater growth ($p<0.05$) than all other treatments for days 4-12. This confirms that the addition of nitrite regardless of the source (celery juice or sodium nitrite) significantly affects the growth of *L. monocytogenes*. No differences ($p>0.05$) in growth were found between treatment C (unadjusted TSBYE + unadjusted 100 ppm CJ, pH 7.6) and D

(adjusted TSBYE + adjusted 100 ppm CJ, pH 6.2). In addition, these treatments also had statistically different ($p<0.05$) pH's, where treatment D maintained a lower pH (6.20 – 6.44) than treatment C (7.60 – 6.95) throughout the entire study (Table 4). Because, the pH's are different, this experiment suggests that there is no difference in the antimicrobial effect of nitrite against *L. monocytogenes* within this pH range of 6.2 – 7.6. No differences ($p>0.05$) between treatment D (adjusted TSBYE + adjusted 100 ppm celery juice) and treatment E (adjusted TSBYE + 100 ppm sodium nitrite, pH 6.10 – 6.11) were observed between days 0 and 8. On day 10 and 12, significantly higher numbers of *L. monocytogenes* were observed for the celery juice treatment (treatment D) compared to the sodium nitrite treatment (treatment E). Because the pH's of treatments D and E do not differ (Table 4), it appears that, when compared in broth, the celery juice may be less effective than sodium nitrite at the same nitrite concentration. In this experiment, sodium nitrite at both 100 ppm (treatment E) and 200 ppm (treatment F) were superior to the other treatments for suppressing *L. monocytogenes* growth. Treatment F (200 ppm sodium nitrite) had the lowest growth compared to all other treatments on days 8-12, again confirming that nitrite concentration affects the antimicrobial impact of nitrite against *L. monocytogenes*.

Table 3

Least square means for the interaction of treatment and day for *Listeria monocytogenes*[1] growth in broth study

Treatment*	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
A	4.00[a]	4.60[a]	6.10[a]	7.35[a]	8.75[a]	9.75[a]	10.15[a]
B	3.90[a]	4.45[a]	5.85[a]	7.15[a]	8.35[a]	9.30[a]	9.65[a]
C	4.00[a]	4.10[a]	4.60[b]	6.05[b]	7.25[b]	8.05[b]	8.45[b]
D	3.95[a]	4.15[a]	4.90[b]	5.60[b]	6.65[bc]	7.60[b]	8.20[b]
E	3.95[a]	4.00[a]	4.65[b]	5.25[bc]	5.85[c]	6.55[c]	7.10[c]
F	3.90[a]	3.95[a]	4.20[b]	4.50[c]	4.75[d]	4.95[d]	5.25[d]

SEM[2] = 0.303

*Treatments: A, unadjusted TSBYE + distilled H_2O (unadjusted control); B, adjusted TSBYE + distilled H_2O (adjusted control); C, unadjusted TSBYE + unadjusted 100 ppm celery juice; D, adjusted TSBYE + adjusted 100 ppm celery juice; E, adjusted TSBYE + 100 ppm sodium nitrite; F, adjusted TSBYE + 200 ppm sodium nitrite.

[1]*Listeria monocytogenes* growth recorded as log CFU/ml.

[2]SEM = standard error of the means.

[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

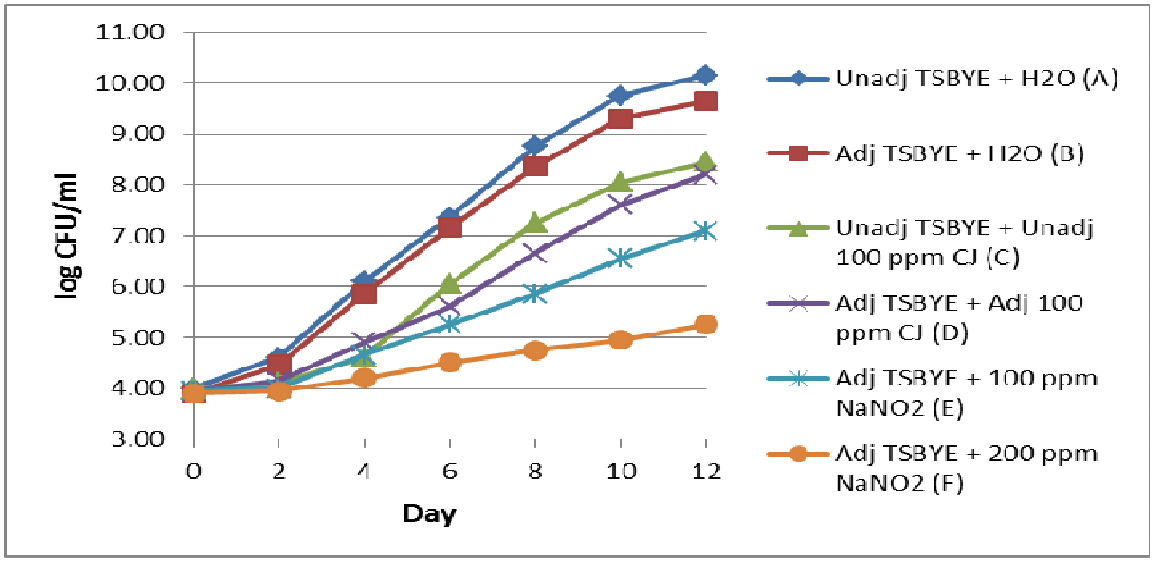

Fig. 1. Least square means of *L. monocytogenes* (log CFU/ml) growth amongst broth treatments after 10^4 log CFU/ml inoculation held at 10˚C for 12 days.

Throughout the 12 days of this experiment, both sodium nitrite (treatments E & F) treatments had statistically similar ($p>0.05$) pH's. This demonstrates that the concentrations of sodium nitrite used in this experiment did not affect the pH of the broth environment for adjusted TSBYE. However, as shown in table 4, treatment C (unadjusted TSBYE + unadjusted 100 ppm celery juice) had a higher pH ($p<0.05$) than all other treatments including treatment A (unadjusted TSBYE control) on days 4-12, which suggests that the growth of the microorganisms in the broth may have decreased the pH in the unadjusted TSBYE without added nitrite. While not statistically different from treatment A at days 0-2, it is noteworthy that treatment C had a numerically higher pH compared to all other treatments on each day.

Table 4

Least square means for the interaction of treatment and day for pH in broth study.

Treatment*	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
A	7.32^{a}	7.32^{a}	7.31^{b}	7.22^{b}	7.10^{b}	6.78^{b}	6.06^{bc}
B	6.10^{b}	6.12^{b}	6.12^{c}	6.10^{c}	6.17^{c}	5.86^{d}	5.65^{c}
C	7.60^{a}	7.72^{a}	7.79^{a}	7.81^{a}	7.80^{a}	7.54^{a}	6.95^{a}
D	6.20^{b}	6.30^{b}	6.38^{c}	6.41^{c}	6.38^{c}	6.36^{bc}	6.44^{b}
E	6.10^{b}	6.12^{b}	6.13^{c}	6.12^{c}	6.11^{c}	6.05^{cd}	6.02^{bc}
F	6.09^{b}	6.12^{b}	6.13^{c}	6.12^{c}	6.12^{c}	6.11^{cd}	6.11^{b}
SEM[1] = 0.150							

*Treatments: A, unadjusted TSBYE + distilled H_2O (unadjusted control); B, adjusted TSBYE + distilled H_2O (adjusted control); C, unadjusted TSBYE + unadjusted 100 ppm celery juice; D, adjusted TSBYE + adjusted 100 ppm celery juice; E, adjusted TSBYE + 100 ppm sodium nitrite; F, adjusted TSBYE + 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

Ham Study

Listeria monocytogenes growth and pH

Table 5 and Fig. 2 show the least square means of *L. monocytogenes* growth for all treatments on each day. Significant differences ($p>0.05$) amongst treatments were not

detected until day 7. As expected, the control (no nitrite source) had significantly ($p<0.05$) greater numbers of *L. monocytogenes* than all other treatments for days 10-35.

Table 5
Least square means for the interaction of treatment and day on *Listeria monocytogenes*[1] growth in ham study

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	3.60^{a}	3.75^{a}	4.55^{a}	5.30^{a}	6.00^{a}	7.80^{a}	8.45^{a}	9.25^{a}
2	3.75^{a}	3.60^{a}	3.75^{b}	4.30^{b}	4.70^{b}	5.85^{b}	6.45^{b}	7.15^{b}
3	3.75^{a}	3.65^{a}	3.90^{ab}	4.10^{b}	4.55^{b}	5.30^{bc}	6.05^{bc}	6.70^{bc}
4	3.80^{a}	3.80^{a}	3.75^{b}	4.05^{b}	4.20^{bc}	5.10^{c}	5.70^{c}	6.65^{bc}
5	3.55^{a}	3.70^{a}	3.65^{b}	3.75^{b}	3.70^{c}	4.20^{d}	4.75^{d}	5.50^{d}
6	3.80^{a}	3.75^{a}	3.90^{ab}	4.05^{b}	4.30^{bc}	5.10^{c}	6.30^{bc}	7.05^{b}
7	3.55^{a}	3.70^{a}	3.85^{ab}	4.20^{b}	4.20^{bc}	4.85^{cd}	5.70^{c}	6.00^{cd}
SEM[2] = 0.369								

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.
[1]*Listeria monocytogenes* growth recorded as log CFU/g.
[2]SEM = standard error of the means.
[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

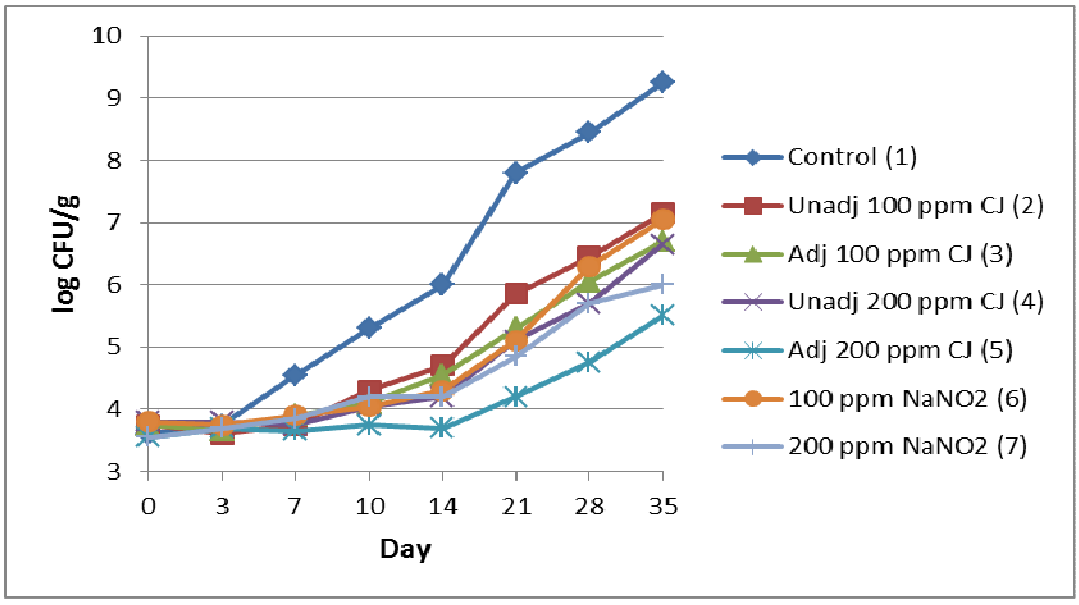

Fig. 2. Least square means of *L. monocytogenes* (log CFU/g) growth amongst ham treatments after 10^4 log CFU/g inoculation held at 4˚C for 35 days.

Other researchers (Duffy et al., 1994; Ngutter & Donnelly, 2003) have shown that nitrite is effective in suppressing *L. monocytogenes* growth in meat products. No differences ($p>0.05$) in growth were observed between the Unadj 100 ppm CJ (treatment 2) and Adj 100 ppm CJ (treatment 3). On days 0, 3, 7, 14, 21, and 35, the Adj 100 ppm CJ (treatment 3) had a significantly lower pH ($p<0.05$) than the Unadj 100 ppm CJ (treatment 2) (Table 6). Even though the pH's were different for the majority of the experiment, the microbiology data indicates that there was no difference in the antimicrobial effect within the pH range observed with the 100 ppm celery juice treatments. Similar results for microbial growth were also noted in the broth experiment. On days 21-35, the Adj 200 ppm CJ (treatment 5) had significantly ($p<0.05$) lower *L. monocytogenes* growth than the Uadj 200 ppm CJ (treatment 4). The pH differences ($p<0.05$) were significant for the duration of the experiment between the Unadj 200 ppm CJ and Adj 200 ppm CJ treatments where the Adj 200 ppm CJ treatment maintained a lower pH (Table 6). Since, the concentration of nitrite for both of these treatments was the same, the pH difference may have affected the microbial growth differences observed at 200 ppm in this experiment. Looking back at the adjusted and unadjusted 100 ppm celery juice treatments (Table 5) where there were no differences in *L. monocytogenes* growth, it is interesting to note that the unadjusted and adjusted 200 ppm celery juice treatments were indeed different ($p<0.05$). This suggests that both pH and concentration of celery juice may have affected the product pH and the subsequent *L. monocytogenes* growth as observed in this experiment.

During the 21 & 28 day time period, the Unadj 100 ppm CJ (treatment 2) resulted in significantly ($p<0.05$) higher numbers of *L. monocytogenes* (Table 5) than the Unadj 200

ppm CJ (treatment 4), but at the end of the study (day 35) both treatments had similar ($p>0.05$) populations. During the entire study, the Unadj 200 ppm CJ treatment maintained a higher pH ($p<0.05$) than the Unadj 100 ppm CJ (Table 6).

Table 6

Least square means for the interaction of treatment and day on pH in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	6.14^{de}	6.14^{de}	6.14^{de}	6.11^{e}	6.12^{d}	6.13^{d}	6.08^{d}	6.12^{d}
2	6.46^{b}	6.45^{b}	6.43^{b}	6.42^{b}	6.44^{b}	6.44^{b}	6.42^{b}	6.28^{bc}
3	6.28^{cd}	6.28^{cd}	6.28^{cd}	6.27^{bcd}	6.27^{cd}	6.28^{c}	6.28^{bc}	6.10^{d}
4	6.68^{a}	6.65^{a}	6.65^{a}	6.63^{a}	6.64^{a}	6.65^{a}	6.65^{a}	6.64^{a}
5	6.35^{bc}	6.37^{bc}	6.36^{bc}	6.36^{bc}	6.36^{bc}	6.37^{bc}	6.37^{b}	6.37^{b}
6	6.11^{e}	6.13^{e}	6.12^{e}	6.13^{de}	6.14^{d}	6.12^{d}	6.12^{d}	6.16^{cd}
7	6.24^{ce}	6.24^{ce}	6.23^{ce}	6.23^{ce}	6.23^{cd}	6.24^{cd}	6.22^{cd}	6.13^{cd}

$SEM^{1} = 0.051$

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

[a-e]Means in same column that have different superscripts are significantly different ($p<0.05$).

This difference also suggests that the increase in concentration of celery juice may affect the product pH. On days 14-35, the Adj 200 ppm CJ treatment had significantly lower ($p<0.05$) numbers of *L. monocytogenes* growth than that of the Adj 100 ppm CJ treatment (Table 5). This supports the previous observations that nitrite concentration impacts *L. monocytogenes* growth. In addition, 100 ppm $NaNO_2$ resulted in significantly greater populations of *L. monocytogenes* on day 35 compared to 200 ppm $NaNO_2$, which reiterates the impact of nitrite concentration on *L. monocytogenes* growth. On all days except day 14, Unadj 100 ppm CJ, Adj 100 ppm CJ, and 100 ppm sodium nitrite were statistically similar ($p>0.05$). Ultimately, these treatments at the end of the experiment, reached the same population,

which suggests that, at 100 ppm nitrite, celery juice is just as effective as sodium nitrite in reducing *L. monocytogenes* growth when used at that concentration. Previous studies (Schrader, 2010; Jackson et al., 2011) have shown that typical usage levels of celery juice (0.2-0.4% if the batch weight) resulted in 20-60 ppm of ingoing nitrite and have been less effective in reducing *L. monocytogenes* and *Clostridium perfringens* growth than the traditional sodium nitrite ingoing concentrations of 120-156 ppm. The subpar performance of the celery juice has been attributed to the low ingoing nitrite concentrations by numerous other researchers. However, celery juice concentrations used in commercial products have remained low because of the undesirable vegetable flavor perceived at higher concentrations. Sindelar et al. (2007) reported that the concentration of 0.35% celery juice elicited a higher negative response from panelists when compared to a lower concentration of 0.20%. In addition, the Adj 200 ppm celery juice (treatment 5) in this study was statistically similar ($p>0.05$) to 200 ppm $NaNO_2$ (treatment 7) for suppression of *L. monocytogenes* growth on all days except day 28 ($p<0.05$), which supports the previous observations that equal nitrite concentrations elicits a similar antimicrobial impact on *L. monocytogenes*. Because, the Unadj 200 ppm CJ (treatment 4) was different ($p<0.05$) than the Adj 200 ppm CJ (treatment 5), the results suggest that the pH adjustment in treatment 5 (Adj 200 ppm CJ) affected the antimicrobial impact of the celery juice. The Adj 200 ppm CJ (treatment 5) also suppressed growth ($p<0.05$) more effectively than all other treatments except treatment 7 (200 ppm $NaNO_2$) on days 21 and 35. The results from this experiment suggest that at higher concentrations of celery juice, the antimicrobial impact of pH of the celery juice is more prominent, probably due to the pH effect on a greater nitrite concentration. It is likely that

more nitrite in the celery juice when combined with more acidic conditions, increases the impact of the antimicrobial activity of nitrite.

Color analysis

Results for the L* color analysis of the hams across the 35 day experiment are shown in Table 7. On day 0, the control and 100 ppm $NaNO_2$ treatments were similar ($p>0.05$), while all other treatments exhibited significant differences ($p<0.05$) in lightness. All celery juice treatments were darker ($p<0.05$) than both the $NaNO_2$ treatments throughout the entire study. On all days, significant differences ($p<0.05$) were evident between the 100 ppm CJ treatments and 200 ppm CJ treatments. Results indicated that as the concentration of the celery juice increased, there was an increase in darkness (lower L*). This also matches the visual perception seen during the study.

Differences in a* measurements are shown in Table 8. As expected, the control had significantly less ($p<0.05$) redness than all other treatments throughout the 35 day study. On day 0, treatments 2, 4, 5, and 6 (Unadj 100 ppm CJ, Unadj 200 ppm CJ, Adj 200 ppm CJ, and 100 ppm $NaNO_2$. respectively) had statistically similar ($p>0.05$) redness values, while on the same day, 200 ppm $NaNO_2$ (treatment 7) was significantly redder ($p<0.05$) than all other treatments. Both 100 ppm CJ treatments (treatment 2 and 3) had statistically similar redness ($p>0.05$) as 100 ppm $NaNO_2$ (treatment 6) on days 3-35. In addition, both 200 ppm CJ (treatments 4 and 5) had statistically similar redness ($p>0.05$) as the 200 ppm $NaNO_2$ (treatment 7) on days 7-35. The similarities within each concentration for both the natural and conventional nitrite sources demonstrate that celery juice produced the same amount of redness as traditional nitrite for the majority of the storage time in this study.

Table 7

Least square means for the interaction of treatment and day on L* in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	70.67^{a}	70.39^{a}	70.21^{b}	70.30^{ab}	70.33^{a}	70.64^{a}	70.67^{a}	69.76^{ab}
2	67.33^{d}	67.08^{c}	67.75^{c}	66.99^{c}	66.76^{d}	67.14^{c}	67.24^{c}	66.57^{d}
3	68.88^{c}	67.75^{c}	67.35^{c}	67.68^{c}	67.58^{c}	67.40^{c}	67.34^{c}	67.62^{c}
4	65.11^{f}	64.41^{e}	64.11^{e}	64.24^{d}	64.09^{f}	64.29^{e}	63.78^{e}	63.86^{f}
5	66.49^{e}	65.60^{d}	65.71^{d}	64.99^{d}	65.16^{e}	65.36^{d}	65.49^{d}	65.39^{e}
6	71.45^{a}	70.83^{a}	71.08^{a}	70.65^{a}	70.28^{a}	70.57^{a}	70.51^{ab}	70.01^{a}
7	69.78^{b}	69.35^{b}	69.75^{b}	69.84^{b}	69.33^{b}	69.02^{b}	69.86^{b}	69.15^{b}

SEM[1] = 0.281

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

L* = lightness on scale of 0-100.

[a-f]Means in same column that have different superscripts are significantly different ($p<0.05$).

Table 8

Least square means for the interaction of treatment and day on a* in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	12.14^{d}	10.99^{d}	10.83^{c}	10.71^{c}	10.49^{c}	10.14^{c}	9.94^{b}	10.20^{d}
2	16.50^{b}	16.80^{ac}	16.24^{b}	16.87^{b}	16.93^{ab}	16.56^{b}	16.65^{a}	16.72^{bc}
3	16.00^{c}	16.64^{bc}	16.77^{a}	16.94^{b}	16.77^{b}	16.93^{ab}	17.01^{a}	16.57^{c}
4	16.32^{bc}	16.64^{bc}	16.97^{a}	17.04^{b}	17.05^{ab}	16.89^{ab}	16.88^{a}	16.99^{ac}
5	16.53^{b}	17.06^{ab}	16.89^{a}	17.51^{a}	17.36^{a}	17.24^{a}	16.84^{a}	17.10^{ab}
6	16.56^{b}	16.56^{c}	16.54^{ab}	16.95^{b}	16.96^{ab}	16.78^{b}	16.73^{a}	16.93^{ac}
7	17.05^{a}	17.21^{a}	16.94^{a}	17.10^{ab}	17.24^{a}	17.29^{a}	17.01^{a}	17.28^{a}

SEM[1] = 0.164

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

a* = redness on scale of 0-100.

[a-d]Means in same column that have different superscripts are significantly different ($p<0.05$).

The yellowness (b*) measurements (Table 9), indicated that the celery juice treatments were significantly more ($p<0.05$) yellow than the conventional nitrite treatments

and the control. Within the celery juice treatments, both the 100 ppm CJ (treatments 2 and 3) had significantly less ($p<0.05$) yellow than the 200 ppm CJ (treatments 4 and 5). This suggests that as the concentration of celery juice increased, there was an increase in yellowness in the final ham product. This is most likely due to the particulates of the plant-derived concentrate that includes plant pigments. During days 3-10, the Adj 200 ppm CJ (treatment 5) was more yellow ($p<0.05$) than the Unadj 200 ppm CJ (treatment 4), but started and ended the study with similar yellow ($p>0.05$) values. In this case, the results suggest that the pH adjustment of the 200 ppm CJ may have impacted the yellowness in the final product at certain time periods. Both $NaNO_2$ treatments elicited the lowest ($p<0.05$) amount of yellowness throughout the entire study when compared to the rest of the treatments.

Table 9
Least square means for the interaction of treatment and day on b* in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	13.40^{d}	13.55^{d}	13.77^{d}	13.85^{d}	13.85^{c}	13.79^{c}	13.76^{c}	13.90^{c}
2	14.06^{c}	14.22^{c}	13.72^{d}	14.18^{cd}	14.39^{b}	14.21^{b}	14.36^{b}	14.29^{bc}
3	14.48^{b}	14.61^{c}	14.44^{c}	14.52^{c}	14.61^{b}	14.46^{b}	14.54^{b}	14.41^{b}
4	17.09^{a}	17.01^{b}	17.07^{b}	17.23^{b}	17.16^{a}	17.18^{a}	17.32^{a}	16.78^{a}
5	16.98^{a}	17.55^{a}	17.52^{a}	17.82^{a}	17.49^{a}	17.54^{a}	17.21^{a}	17.17^{a}
6	11.40^{e}	11.08^{e}	11.24^{e}	11.35^{e}	11.26^{d}	11.30^{d}	11.24^{d}	11.18^{d}
7	11.42^{e}	11.21^{e}	11.04^{e}	11.25^{e}	11.41^{d}	11.41^{d}	11.19^{d}	11.37^{d}
SEM[1] = 0.145								

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.
[1]SEM = standard error of the means.
b* = yellowness on scale of 0-100
[a-e]Means in same column that have different superscripts are significantly different ($p<0.05$).

Residual nitrite

Residual nitrite concentrations for all treatments throughout the shelf life of the ham products are represented in Table 10 and Fig. 3. As expected, the control treatment had

essentially no residual nitrite and was significantly lower ($p<0.05$) than all other treatments. Because it has been suggested that nitric oxide, which is derived from nitrite, may provide an inhibitory effect against microorganisms (Tompkin, 2005); it is no surprise that the control treatment had both low residual nitrite concentrations and high numbers of *L. monocytogenes*. As shown in Table 10, the Adj 200 ppm CJ (treatment 5) had significantly less residual nitrite ($p<0.05$) than that of the Unadj 200 ppm CJ (treatment 4) on all days except day 7. It has been shown that reduced pH speeds up the curing reaction (creates more nitric oxide) and as a result, less residual nitrite can be expected (Cassens et al., 1978). This allows more nitric oxide to become available to act as an antimicrobial. However, when comparing the Unadj 100 ppm CJ and Adj 100 ppm CJ treatments, there was no significant difference ($p>0.05$) found between the residual nitrite concentrations (Table 10). These findings correspond to no differences found between the *L. monocytogenes* growth for these treatments, which could imply that at lower concentrations of celery juice (and nitrite) the pH impact on nitrite effectiveness is less. Unadj 100 ppm CJ, Adj 100 ppm CJ, and 100 ppm NaNO2 treatments all had significantly less residual nitrite ($p<0.05$) than the 200 ppm nitrite treatments (Table 10), which demonstrates that, as the concentration of ingoing nitrite increases, the residual nitrite amounts also increase accordingly. Xi et al. (2011) found the same trend when studying different ingoing sodium nitrite concentrations. Overall, the residual nitrite concentrations decreased gradually during the 35 day storage period. Others have also reported a gradual decline of residual nitrite throughout the shelf life of meat products (Jantawat et al., 1993; Myers et al., 2013). Significantly higher concentrations of residual nitrite ($p<0.05$) were found in the Unadj 200 ppm CJ treatment versus the 200 ppm

$NaNO_2$ treatment (Table 10). Similar results were shown in Myers et al. (2013). Those authors commented that it was unusual to have higher concentrations of residual nitrite that corresponded with increased growth of *L. monocytogenes*. They speculated that the celery juice may have provided beneficial nutrients to *L. monocytogenes* since 97.75% of the celery juice used in the experiment was composed of organic and inorganic constituents.

Table 10

Least square means for the interaction of treatment and day on residual nitrite[1] in ham study.

Treatment*	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 35
1	3.69^{d}	3.56^{d}	2.51^{d}	2.63^{d}	1.68^{e}	2.49^{d}	2.85^{d}	3.32^{d}
2	71.45^{c}	69.24^{c}	67.59^{c}	64.72^{c}	63.69^{c}	57.36^{c}	56.81^{c}	51.50^{c}
3	69.72^{c}	65.18^{c}	60.67^{c}	55.52^{c}	55.25^{cd}	51.09^{c}	49.16^{c}	40.42^{c}
4	151.09^{a}	143.20^{a}	128.89^{a}	123.71^{a}	123.93^{a}	118.38^{a}	115.19^{a}	106.94^{a}
5	133.23^{b}	122.65^{b}	115.68^{ab}	105.15^{b}	103.99^{b}	95.15^{b}	87.67^{b}	79.67^{b}
6	61.56^{c}	62.45^{c}	56.95^{c}	52.34^{c}	50.05^{d}	46.20^{c}	43.93^{c}	39.62^{c}
7	122.08^{b}	114.66^{b}	107.68^{b}	97.04^{b}	95.28^{b}	88.31^{b}	81.15^{b}	71.11^{b}

SEM[2] =4.69

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]Residual nitrite reported as ppm.

[2]SEM = standard error of the means.

[a-e]Means in same column that have different superscripts are significantly different ($p<0.05$).

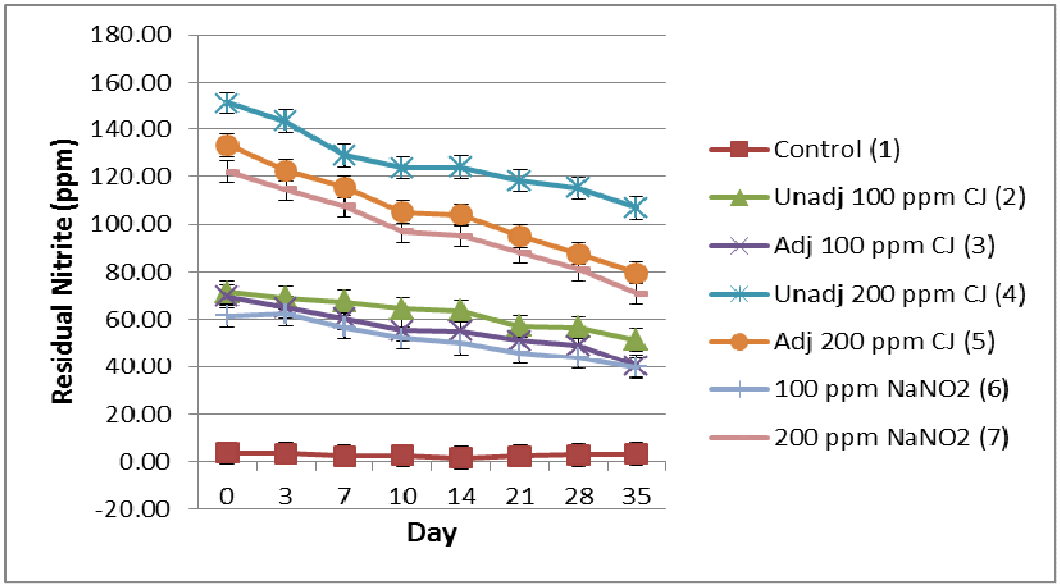

Fig. 3. Least square means of residual nitrite (ppm) for the ham study treatments after 10^4 log CFU/g inoculation held at 4˚C for 35 days.

Proximate analysis and Aw

The least square means of % moisture, % fat, % protein, and Aw are listed in Table 11. No differences ($p>0.05$) were observed for % moisture, % fat, and Aw between treatments. Protein differences ($p<0.05$) were observed between the Adj 200 ppm CJ and both the control and 200 ppm $NaNO_2$ treatments, and may have resulted from raw meat differences in the formulation between treatments or the addition of celery juice plus the citric acid. An explanation for the lower protein content in the Adj 200 ppm CJ treatment is not clear, but is unlikely to be of any practical significance since all other compositional properties did not differ among the treatments.

Table 11

Proximates and water activity measurements for all ham treatments on day 0.

Treatment*	Moisture (%)	Fat (%)	Protein (%)	Aw
1	75.37[a]	2.66[a]	18.85[a]	0.9791[a]
2	75.71[a]	1.98[a]	18.17[ab]	0.9778[a]
3	75.74[a]	1.87[a]	18.27[ab]	0.9768[a]
4	75.36[a]	1.79[a]	18.40[ab]	0.9749[a]
5	75.51[a]	2.19[a]	17.70[b]	0.9753[a]
6	75.64[a]	2.09[a]	18.33[ab]	0.9785[a]
7	75.41[a]	2.41[a]	18.46[a]	0.9781[a]
SEM[1]	0.213	0.214	0.161	0.0007

*Treatments: 1, no nitrite source added (Control); 2, unadjusted 100 ppm celery juice; 3, adjusted 100 ppm celery juice; 4, unadjusted 200 ppm celery juice; 5, adjusted 200 ppm celery juice; 6, 100 ppm sodium nitrite; 7, 200 ppm sodium nitrite.

[1]SEM = standard error of the means.

[a-b]Means in same column that have different superscripts are significantly different ($p<0.05$).

Conclusion

The broth experiment indicated that the pH adjustment that occurred between the two 100 ppm celery juice treatments (unadjusted TSBYE + unadjusted CJ and adjusted TSBYE + adjusted CJ) did not have an antimicrobial effect on *L. monocytogenes* growth. The same results were observed for the unadjusted and adjusted 100 ppm CJ treatments within the ham study. Differences in *L. monocytogenes* growth between the 100 ppm $NaNO_2$ and both the 100 ppm CJ treatments demonstrated that celery juice was less effective than conventional nitrite at the same nitrite concentration for suppressing *L. monocytogenes* in the broth system. However, the results from the ham experiment show that at equal concentrations of nitrite, celery juice was as effective as the sodium nitrite treatments in the meat product. Because the ham experiment represents the practical application of celery juice in the meat industry, it is a more realistic model compared to the broth system. At the same time, the broth experiment

suggested that the pH impact of celery juice concentrate can affect nitrite reactions and could be a consideration for some product applications.

As the concentration of the celery juice concentrate increased within the ham study, the pH of the ham product increased as well. When the pH adjustment was applied to the 200 ppm CJ, there was decreased *L. monocytogenes* growth and lower residual nitrite concentrations. Even though the pH adjustment had an impact on *L. monocytogenes* growth at 200 ppm, the Adj 100 ppm CJ did not show the same effect, which could be due to the lesser nitrite concentration. Similar residual nitrite concentrations and *L. monocytogenes* growth for the Unadj and Adj 100 ppm CJ treatments suggest that a larger pH reduction may need to be used at 100 ppm of nitrite in order to accelerate the nitric oxide production and therefore reduce *L. monocytogenes* growth. Particulates within the celery juice concentrate, such as fibers, sugars, and minerals (Djeri, 2010), could also hinder the reactivity of nitrite, depending on the chemical properties of these components.

The celery juice treatments also affected ham color and as the concentration was increased, the hams became darker (lower L*) and more yellow (higher b*) than conventional treatments. This is most likely due to the particulates (fibers, sugars, and minerals) that are present in the celery juice. Overall, the redness (a*) values were similar for both the celery juice and conventional treatments at equal nitrite concentrations.

Future research efforts on the use of celery juice concentrate as a meat curing agent for natural and organic processed meats should focus on developing a more concentrated form of celery juice that has increased nitrite concentration, lower pH and reduced vegetable off-flavors in order to increase the effectiveness of the ingoing nitrite. Even though this study

shows that celery juice was as effective as conventional nitrite in ham at equal nitrite concentrations, potential pH impact of the celery juice concentrate may be of significance for nitrite reactions in some applications. In addition, flavor strongly impacts consumer acceptability of meat products, and from previous research (Sindelar et al., 2007) sensory panel results indicated that celery juice concentrate can impart an undesirable flavor at high concentrations. This would be a concern for consumer products with concentrations of celery juice comparable to our study which used 0.67% (100 ppm) and 1.33% (200 ppm) to reach the desired nitrite concentrations.

References

AOAC, Association of Official Analytical Chemists. (1990a). Fat (crude) or ether extract in meat. Official method 960.39.*Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

AOAC, Association of Official Analytical Chemists. (1990b). Moisture in meat. Official method 950.46. *Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

AOAC, Association of Official Analytical Chemists. (1990c). Nitrites in cured meats. Official Method 973.31. *Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

AOAC, Association of Official Analytical Chemists. (1993). Crude protein in meat and meat products. Official method 992.15.*Official Methods of Analysis* (15th ed.). Arlington, VA: AOAC.

Barnes, J.M., Magee, P.N. (1954). Some toxic properties of dimethylnitrosamine. *British Journal of Industrial Medicine*, 11(3), 167-174.

Bryan, N.S., & Hord, N.G. (2010). Dietary nitrates and nitrites: the physiological context for potential health benefits in: N.S. Bryan (Ed.), Food, Nutrition and the nitric oxide pathway: Biochemistry and bioactivity, DESTech Publications, Inc., Lancaster, PA, 59-77.

Cassens, R.G., Ito, I., Lee, M., & Buege, D. (1978). The use of nitrite in meat. *Bioscience*, 28(10), 633-637.

Djeri, N. (2010). Evaluation of vegstable 504 celery juice powder for use in processed meat and poultry as a nitrite replacer. Ph.D. Thesis. University of Florida, Gainesville, FL.

Doganay, M. (2003). Listeriosis: clinical presentation. *FEMS Immunology and Medical Microbiology*, 35, 173-175.

Duffy, L.L., Vanderlinde, P.B., Grau, F.H. (1994). Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, aw, nitrite and ascorbate. *International Journal of Food Microbiology*, 23, 377-390.

Gray, M.L. and Killinger A.H. (1966). *Listeria monocytogenes* and listeric infection. *Bacterilogical Reviews*, 30, 309.

Jackson, A.L., Sullivan, G.A., Kulchaiyawat, C., Sebranek, J.G., Dickson, J.S. (2011). Survival and growth of *Clostridium perfringens* in commercial no-nitrate-or-nitrite-added (natural and organic) frankfurters, hams, and bacon. *Journal of Food Protection*, 74(3), 410-416.

Jantawat, P., Runglerdkriangkrai, J., Thunpithayakul, C., & Sanguandeekul, R. (1993). Effects of initial nitrite level, heating and storage on residual nitrite and spoilage of canned ham roll. *Journal of Food Quality*, 16(1), 1-11.

Jensen, L.B. (1953). Early preparations of foods. In man's foods. Nutrition and environments in food gathering times and food producing times. The Garrard Press, Champaign, IL, pp. 159-170.

Liu, D. (2008). Epidemiology, pp. 27-59. In Liu, D. (ed.), Handbook of *Listeria monocytogenes*. Taylor & Francis Group, LLC, Boca Raton, FL.

Lungu, B., Ricke, S.C., Johnson, M.G. (2009). Growth, survival, proliferation and pathogenesis of *Listeria monocytogenes* under low oxygen or anaerobic conditions: A review. *Anaerobe*, 15, 7-17.

Magee, P.N., Barnes, J.M. (1956). The production of malignant primary hepatic tumours in the rat by feeding dimethyinitrosamine. *British Journal of Cancer*, 10(1), 114-122.

McClure, P.J., Kelly, T.M., Roberts, T.A. (1991). The effects of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 14(1), 77-91.

McKnight, G.M., Smith, L.M., Drummond, R.S., Duncan, C.W., Golden, M., Benjamin, N. (1997). Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. *Gut*, 40 (2), 211-214.

Myers, K. M. (2012). Evaluation of high hydrostatic pressure, meat species, and ingredients to control *Listeria monocytogenes* in ready-to-eat meats. Ph.D. Thesis. Iowa State University, Ames, IA.

Myers, K.M., Cannon, J., Montoya, D., Dickson, J., Lonergan, S., Sebranek, J. (2013). Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. *Meat Science*, 94(1), 69-76.

Ngutter, C.M. and Donnelly, C.W. (2003). Nitrite-induced injury of *Listeria monocytogenes* and the effect of selective versus nonselective recovery procedures on its isolation from frankfurters. *Journal of Food Protection*. 66:2252-2257.

Organic Trade Association. (2011). Industry statistics and projected growth. Available at: http://www.ota.com/organic/mt/business.html. Accessed 20 February 2013.

Parthasarathy, D.K., & Bryan, N.S. (2012). Sodium nitrite: The "cure" for nitric oxide insufficiency. *Meat Science*, 92, 274-279.

Pegg R.B, Shahidi, F. (2000). Nitrite curing of meat: The N-Nitrosamine problem and nitrite alternatives. Food & Nutrition Press, Inc. Trumbull, CT.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., (2011). Foodborne illness acquired in the United States-major pathogens. *Emerging Infectious Diseases*, Vol 17(1). http://wwwnc.cdc.gov/eid/article/17/1/p1-1101_article.htm. Accessed 21 February 2013.

Schrader, K.D. (2010). Investigating the control of *Listeria monocytogenes* on uncured, no-nitrate-or-nitrite-added meat products. Ph.D. Thesis. Iowa State University, Ames, IA.

Sebranek, J.G., Bacus, J. (2007). Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality, and safety issues. American Meat Science Association White paper series, No. 1.

Sindelar, J.J., Cordray, J.C., Sebranek, J.G., Love, J.A., Ahn, D.U. (2007). Effects of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *Journal of Food Science*, 72(6), S388-S395.

Sindelar, J.J. and Milkowski, A.L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide*, 26, 259-266.

Sindelar, J.J, and Milkowski, A.L. (2011). Sodium nitrite in processed meat and poultry

meats: A review of curing and examining the risk/benefit of its use. American Meat Science Association. AMSA white paper series, No. 3.

Sindelar, J.J., Sebranek, J.G., Bacus, J.N. (2010). Uncured, natural and organic processed meat products. American Meat Science Association. Meat processing technology series.

Tarr, H.L.A. (1941). The action of nitrites on bacteria. *Journal of the Fisheries Research Board of Canada*, 5(3), 265-275.

Tompkin, R.B. (2005). Nitrite. p. 169-236. *In* P.M. Davidson, J.N. Sofos, and A.L. Branen (ed.), Antimicrobials in food. Taylor & Francis, Boca Raton, FL. <http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf>. Accessed 12.28.12.

USDA, United States Department of Agriculture. (2005). Food Standards and Labeling Policy Book. http://www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy_Book_082005.pdf. Accessed Jan. 22, 2013.

USDA, United States Department of Agriculture. (1995). Processing Inspector's Calculations Handbook (FSIS Directive 7620.3).

Xi, Y., Sullivan, G.A., Jackson, A.L., Zhou, G.H., Sebranek, J.G. (2011). Use of natural antimicrobials to improve the control of *Listeria monocytogenes* in a cured cooked meat model system. *Meat Science*, 88, 503-511.

CHAPTER 4. GENERAL CONLUSIONS

Natural and organic meat products have become increasingly popular to the general consumer for its ability to provide a preservative-free product. Nitrite is included in preservatives not allowed in meat products labeled natural or organic. To circumnavigate the legalities, manufactures have incorporated celery juice has the nitrite source in these products to obtain the same unique characteristics seen in conventionally cured meat products. However, by substituting conventional sodium nitrite with a celery juice concentrate, there has been less ingoing nitrite observed in the celery juice inclusion percentages used, which causes an increased risk of *Listeria monocytogenes* growth within these products. *L. monocytogenes* is of utmost concern to processors because upon its outbreak, a large percentage of infected individuals have fatal outcomes.

Although the literature indicates that celery juice included at typical levels of 0.2-0.4% has greater growth of *L. monocytogenes*, this study showed that at equal concentrations celery juice is just as effective as sodium nitrite in ham. In addition, when the pH adjustment was applied to the Adj 200 ppm CJ treatment, an increased antimicrobial effect was observed by reduced *L. monocytogenes* growth. However, for both the broth and ham study, the pH adjustment did not have an antimicrobial impact on *L. monocytogenes* when applied to 100 ppm CJ. Color analysis in the ham study indicated that as the concentration of the celery juice increased, the products became darker (lower L*) and more yellow (higher b*).

For future research, emphasis should be focused on developing a more nitrite concentrated form of celery juice that minimizes the vegetable flavor that is currently seen in higher concentrations of celery juice. Since the appearance of the celery juice treated hams

were darker and more yellow, sensory analysis regarding the flavor and color should be considered when developing a more nitrite concentrated celery juice powder.

ACKNOWLEDGEMENTS

First, I would like to thank my major professor, Dr. Joseph Sebranek, for his guidance and expertise over the last couple of years. Words cannot describe how appreciative I am of the opportunity to obtain my degree at Iowa State University. I fully appreciated his patience and understanding throughout the whole process. I am truly blessed to have had him as my major professor and will always be grateful. I would also like to thank Dr. Jim Dickson for lending his expertise when designing the experiment and answering my many microbiology questions. I also greatly appreciated the advice and guidance of my co-major professor, Dr. Lester Wilson. To all my committee members I would like to thank them for serving as members.

I would like to thank Steve Niebuhr, who assisted with the microbiology protocol and answered many questions I had about microbiology. I would also like to thank Elaine Larson for conducting the proximate and color analysis. Her patience and guidance in the laboratory was very much appreciated.

I also wanted to thank Nick Lavieri, Ben Ruther, and Jonathon Campbell (graduate students) who helped with the manufacture of the hams. Also, I would like to thank Alyssa Fitzpatrick and Jennifer Kirk (undergraduates) for their outstanding help in the chemistry and microbiology labs.

Lastly, I would like to thank my family for their support and understanding. Having the support to pursue Graduate School from my fiancé, Cullan, meant the world to me. Thank you to my parents, Gerry and Connie, for their unwavering love and encouragement.

I cannot begin to express how grateful I am to all of those mentioned above, because their help was pivotal to my success here at Iowa State University.