EVALUATION OF VEG STABLE ™ 504 CELERY JUICE POWDER FOR USE IN PROCESSED MEAT AND POULTRY AS A NITRITE REPLACER

By

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To my family and friends- I could not have finished without your support.

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EVALUATION OF VEG STABLE ™ 504 CELERY JUICE POWDER FOR USE IN PROCESSED MEAT AND POULTRY AS NITRITE REPLACER

By

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The objectives of this research were to determine the composition of Veg Stable™ 504 celery juice powder containing pre-generated nitrite (CJPPN) and monitor its shelf life for six months; to determine the effectiveness of different levels of CJPPN against *Clostridium botulinum* in a turkey bologna system formulated with synthetic sodium nitrite (also referred to as modern cure or prague powder); to evaluate the effect of holding time, prior to heat processing, on the color, pH, and residual nitrite of beef frankfurters using different levels of the CJPPN; and to ascertain the effects of CJPPN on objective color, pH, quality attributes and consumer acceptance and microbiology of vacuum packaged turkey bologna over an extended storage time.

C. botulinum spores tested on a bologna type emulsion product containing either a combination of 0.20% Veg Stable™ 504, and 0.20% Veg Stable™ 515 (cherry powder used to replace synthetic sodium erythorbate), and modern cure (commercially available nitrite) combined with sodium erythorbate showed 1- 3 log reduction over 48 hours, while a 3 log increase was observed for 0.20% Veg Stable™504 without exhibition of any spoilage or off odor in the meat product. Modern cure used alone or in

conjunction with sodium erythorbate revealed a 2 log reduction over 48 hours in RCM (Reinforced Clostridial Media), while 156 ppm Veg Stable™ 504, and 156 ppm Veg Stable™ 504 *and* 469 ppm Veg Stable™ 515 experienced a 3 log reduction. RCM broth did not support the growth of *C. botulinum*. Over 48 hours, 156 ppm Veg Stable™ 504 and 156 ppm Veg Stable™ 504 and 469 ppm Veg Stable™ 515 experienced 1 log decrease regarding *C. botulinum* spore counts when compared to modern cure alone and with ascorbic acid.

The usage of different levels of CJPPN to evaluate the effect of holding time on the color, pH, and residual nitrite in beef frankfurters showed some promising results. All treatments had similar ($P > 0.05$) pH, L* and b* values. The a* values for the control (modern cure) were higher ($P < 0.05$) than 0.20% celery powder as holding time increased. The different treatments of Veg Stable™ 504 (0.20%, 0.30%, and 0.40%) were shown to be comparable to traditionally cured frankfurters, in regards to L*, b*, and pH values. The a* values for the traditionally cured beef frankfurter was overall redder than the frankfurters containing celery juice powder. The control had significantly higher levels of residual nitrite ($P < 0.05$) when compared to 0.20, 0.30, 0.40 % celery juice powder. Less residual nitrite was present in the beef frankfurters manufactured using different levels of Veg Stable 504™ at 0.20, 0.30, and 0.40%. As the celery juice powder usage level increased from 0.20% to 0.40%, the more residual nitrite concentration also increased, but remained significantly less ($P < 0.05$) than the control (modern cure).

The consumer sensory panel evaluated turkey bologna manufactured with 0.20% Stable™ 504, 0.20% Stable™ 504 + 0.20% Veg Stable™ 515, 156 ppm Stable™ 504 +

469 ppm Veg Stable™ 515, and 156 ppm Prague powder + 550 ppm of sodium erythorbate. The panelists reported that the turkey bologna manufactured with celery powder juice containing the pre-generated sodium nitrite had a comparable appearance, aroma, texture, and flavor as the products cured with synthetic sodium nitrite ($P > 0.05$). The exception was the product that contained ten times the recommended usage level (156 ppm Stable™ 504 + 469 ppm Veg Stable™ 515), and was not well liked by the consumer panelists. In addition, no pathogenic microorganisms were detected. Veg Stable™ 504 could successfully be used for naturally cured meat products if the levels of nitrite are increased (use as preservative).

CHAPTER 1
INTRODUCTION

Consumers have been making more health conscious food choices over the years. The meat industry has taken advantage of this opportunity and tried to satisfy its consumers. A variety of products that include claims such as uncured, natural, organic, no preservatives, minimally processed are offered to consumers at the retail level. Products offered range from fresh to processed food products, and consumers have been flooded and confused by the different claims.

Treating meat with chemical compounds such as salt, nitrate/ nitrite, spices, and phosphates has resulted in cured meat products. Curing is a method of preservation that dated back to the twelfth centuries B.C., with salt as the main ingredient. Desirable changes in the products were noticed and attributed to nitrite in the 19th century. In addition to being recognized as an important ingredient in the curing process, nitrite has four main functions: stabilization of the color of the lean tissues, characteristic flavor of cured meat, retardation of fat oxidation, and inhibition of several pathogenic and spoilage organisms (such as Clostridium, Bacillus, Pseudomonas, and Salmonella).

The use of nitrite in cured meat has become controversial because of potential nitrosamine formation from nitrite, which are suspected of being carcinogens. After much research was conducted on nitrosamines and carcinogenicity of nitrites, no correlations were found between the consumption of cured meat products by humans and cancer.

Consumers are still distrustful of nitrites, leading to the development of alternative methods to cure meat. New sources of nitrate/nitrite have been found, and used in meat products to satisfy consumers' needs for natural, no preservative added items. There

are cured meat products available at the retail level labeled “uncured, no nitrates/nitrates added” that have some of the nitrite cured products characteristics. The United States Department of Agriculture (USDA) allows meat products which conventionally contain nitrates or nitrites to be manufactured without nitrates or nitrites. Consequently, these specific meat products need to be labeled as “Uncured” following the common, descriptive name with disclaimers including “No Nitrate or Nitrite Added” (Code of Federal Regulations Title 9, Part 317.17 and 319.2 (CFR, 2010).

Uncured products are separated into two categories: uncured with no intention of replacing nitrate or nitrite (uncured) and uncured with the intention of replacing nitrate or nitrite (naturally cured). Despite the fact that “natural curing” is not officially defined by the USDA, it refers to curing that result from the microbial conversion of nitrates to nitrites with similar characteristics to the direct addition of synthetic nitrite. Ingredients containing nitrate or nitrite (such as sea salts, green plants, and vegetables) are needed to manufacture such products. Some vegetable sources containing significant concentrations of nitrates (celery powder specifically), in combination with nitrate reducing starter culture are used for the production of naturally cured meat products.

Quality attributes and consumer acceptance of uncured, no nitrate/nitrite added commercial hams, bacons, and frankfurters were evaluated and compared to meat products cured with synthetic nitrite. Hams were comparable in color, residual nitrate/nitrite, total and cured pigments, lipid oxidation, and accepted by consumers. Frankfurters showed some variation in the amount of curing reactions, and a slight difference regarding consumer sensory attributes (Sindelar et al., 2007).

When using vegetable juice powder in meat products, nitrate-reducing microorganisms must be utilized. Even though the pre-generated nitrite from celery juice powder should be similar to the synthetic nitrite, little is known on how its products compare to the traditional nitrite added products.

As a result, incubation time is needed to allow the curing to occur, which consumes time. The hypothesis for this project is that there are no differences between products cured with commercially available sodium nitrite (modern cure) and uncured products with the intention of replacing sodium nitrite, using celery juice powder containing pre-generated nitrite (CJPPN). Regardless of its source, nitrite should chemically react the same manner to cure meat products.

Consequently the objectives of this research were to determine the composition of CJPPN and monitor its shelf life for six months; to determine the effectiveness of different levels of CJPPN against *Clostridium botulinum* in a turkey bologna system formulated with synthetic sodium nitrite (also referred to as modern cure or prague powder); to evaluate the effect of holding time, prior to heat processing, on the color, pH, and residual nitrite of beef frankfurters using different levels of the CJPPN; and to ascertain the effects of CJPPN on objective color, pH, quality attributes and consumer acceptance and microbiology of vacuum packaged turkey bologna over an extended storage time.

CHAPTER 2
REVIEW OF LITERATURE

History of Curing

Curing meat is defined as the addition of nitrite and or nitrate and salt (NaCl) to meat at different stages of preparation for processing (Honikel, 2007). This particular technique is very ancient and has been used for centuries. The preservation of fish using salt can be traced back to 3500 B.C. Salt containing nitrates was used in Homer's Odyssey around eight century B.C. for meat preservation (Kramlich et al., 1973; Pearson and Tauber, 1984). Meat products dried with salt were inconsistent in their quality and appearance. It was observed that a certain type of salt was at the origin of the change in the desired meat color. The appealing and preservative characteristics of saltpeter or potassium nitrate were understood in the late 19th century, with the change in color attributed to nitrate impurities. Reduction activities occurring in the muscle tissues postmortem were responsible for the conversion from nitrate to nitrite, resulting in curing (Pegg and Shahidi, 2004). The reduction of nitrite to nitric oxide and nitrous acid was found by Haldane (1901) and Hoagland (1908) to be cured meat red color. In 1925, the United States Department of Agriculture (USDA) authorized the use of sodium nitrite for meat curing after extensive research. This critical ingredient was found to have several functions, and included color stabilization, characteristic color and flavor, and antimicrobial properties against pathogenic (*Clostridium botulinum* spores) and spoilage microbes (Pearson and Tauber, 1984; Aberle et al., 2001; Russell et al., 2003).

Chemistry of Meat Color

Color is one of the most important factors consumers consider when purchasing meat products (Shahidi, 1998; Aberle et al., 2001). Myoglobin, an atom composed of a

complex of pyrrole rings, is the red pigment that is at the origin of meat's color. In the last few decades, extensive research has been conducted on nitrite and meat pigments, mainly myoglobin. This particular pigment has the ability to lose or gain electrons and also bind to different chemicals. Several factors such as species, maturity or age, muscle physical activity, and environment affect myoglobin (which constitutes 80 to 90% of the total red meat pigments) concentrations in muscles (Forrest et al., 1975). Meat color varies from purplish- red for freshly cut beef to a light gray color for faded pork. Oxymyoglobin (oxygen combined to myoglobin) and myoglobin when oxidized, lose an electron, resulting in metmyoglobin (a brown color). Myoglobin is readily converted to oxymyoglobin (oxidation), or to metmyoglobin (oxidation). The conversion of metmyoglobin back to myoglobin or oxymyoglobin through oxidation is more difficult. Besides oxygen, other chemicals such as nitric oxide can also bind to myoglobin or metmyoglobin. Nitrososomyoglobin, a red pigment (the result of nitric oxide binding to myoglobin or metmyoglobin), is unstable but is rendered stable by heat (Rust, 1980). Cured meat pigment is named nitrosohemochrome, and is pink in color.

Cassens et al. (1979) found that when nitrite is in a meat mixture, it oxidizes myoglobin to metmyoglobin (a grayish brown color). Nitric oxide and nitrous acid are formed as the nitrite from sodium nitrite interacts with water to form nitric oxide myoglobin. Meat pigments (specifically myoglobin) react with nitrite to form an unstable pink color (Russell and Gould, 2003). After application of a heat treatment (cooking), this pigment is stable and is known as nitrosylhemochrome (cured pigment), which is light, temperature, and oxygen sensitive (Hornsey, 1956; Rust, 1980; O'Boyle et al., 1990; Boles and Pegg, 2005).

Meat Curing

Chemical reactions occurring during curing of meat was detailed by Haldane (1901), demonstrating the result of red-ox reactions and NO-myoglobin attributed the characteristic red colour (Honikel, 2008). The reactant was found to be nitrite anion with nitrous acid (HNO2) or nitric oxide (NO) reacting with the myoglobin (Honikel, 2008).

Curing reaction

Nitrate	$\longrightarrow$	Nitrite (reduction by starter culture)
$KNO_2 + H^+$	$\rightleftharpoons$	$HNO_2 + K^+$
$2HNO_2$	$\rightleftharpoons$	$N_2O^3 + H_2O$
N_2O_3	$\rightleftharpoons$	$NO + NO_2$
NO + myoglobin	$\longrightarrow$	NO-myoglobin

Figure 2-1. Scheme of the proposal of Hoagland (1910, 1914) for the action of nitrate in cured meat products

Adapted from Honikel (2008)

Honikel, K. (2008). The use and control of nitrate and nitrite for the processing of meat products. Meat Science, 78, 68-76.

Synthetic and Natural Nitrite

Nitrogen sources are the atmosphere (78% by volume), saltpeter (the minerals KNO_3) and Chile saltpeter ($NaNO_3$). Nitrogen (N_2) can be obtained by liquefaction of air followed by a fractional distillation. NH_3 production can be converted to NH_4^+ through neutralization, and through oxidation to nitrite, nitrates and HNO_3 (Schweitzer and Pesterfield, 2010). Nitrogen is the source to the formation of oxides such as NO, N_2O, NO_2, N_2O_3. Nitrogen oxide (NO) is a colorless gas, while NO_2 is a brown gas. Commercially, the production of NO occurs at high temperature with the oxidation of

NH3 and a Pt (Platinum) catalyst (Schweitzer and Pesterfield, 2010). NO quickly combines with O_2 to form NO_2.

Nitrous acid salts' are manufactured by heating nitrates with C (Carbon) or Fe (Iron) (reducing agent). The bubbling of a blend comprising NO and NO_2 into a solution of the hydroxide can be done by the nitrites of alkali metals. HNO_2 colorless, weak and unstable acid can be quickly separate into NO and NO_2 (Schweitzer and Pesterfield, 2010).

Nitrites are typically created by using C (Carbon), Fe (Iron) or Pb (Lead) and the reduction of nitrates at elevated temperatures (Greenwood and Earnshaw, 1998). Industrially, impure $NaNO_2$ is manufactured by absorbing "nitrous fumes" in aqueous alkali or carbonate solutions and then recrystallizing the product (Greenwood and Earnshaw, 1998).

$$NO + NO_2 + 2NaOH \text{ (or } Na_2CO_3) \longrightarrow 2NaNO_2 + H_2O \text{ (or } CO_2)$$

Figure 2-2. Manufacturing of sodium nitrite, adapted from Greenwood and Earnshaw

Greenwood, N.N.; and A. Earnshaw. 1998. Chemistry of the Elements (2nd Edition).. Elsevier.http://knovel.com/web/portal/browse/display?_EXT_KNOVEL_DISPLAY_bookid=402&VerticalID=0. Accessed July 27, 2010.

Choice of Celery Powder

Natural sodium nitrite differs from synthetic sodium nitrite in the manner they are produced. To obtain natural source of nitrites, vegetable sources are used. Celery has been most commonly used because of its mild flavor and color. Sindelar et al. (2010) stated that for natural curing, many natural sources of nitrates are available, with the most common used being celery juice or celery juice powder. In addition of it availability, it has a reduced impact on the sensory characteristics of the manufactured meat products.

Celery juice powder is produced by harvesting celery which are washed, chopped, and pureed. Physical separation is used to remove solids and the liquid concentrate filtered. During the whole process, no solvents are involved. For the production of celery juice powder containing nitrates, the fermentation process is not needed until a meat system is available. In order to manufacture celery juice powder containing pre-generated nitrite, the fermentation is crucial after obtaining the liquid concentrate (Florida Food Product Inc., 2008). Strains of *Staphylococcus carnosus* are used, while temperature, aeration, agitation, and pH are carefully monitored. Following fermentation, the liquid concentrate is pasteurized in order to kill the starter culture, thus stopping the process. The celery juice containing nitrites is evaporated, blended and standardized with sea salt before being vacuum dried to maintain the active ingredient, milled and vacuum packaged (Florida Food Product Inc., 2008).

Nitrites and Cured Meat

Although earlier work found *Salmonella* and *Lactobacillus* species to be more resistant to nitrite, a variety of other bacteria were affected (Adams and Moss, 2008). Russell and Gould (2003) reported that besides *Clostridium botulinum*, nitrite inhibits the growth of *Escherichia coli*, Achromobacter, Enterobacter, Flavobacterium, Micrococcus and Pseudomonas species at allowed levels in products. Nitrite mechanism of action is still not very well understood due to interactions of pH, salt content, nitrate or nitrite, and processing technique (Adams and Moss, 2008). Adams and Moss (2008) stated that nitrous acid was at the origin of lowering pH, consequently causing bacterial inhibition. Several factors such as processing conditions, formulation and product temperature affect the safety of products as they relate to *Clostridium botulinum.* At temperatures below 8°C, cooked meats are affected at first by non-proteolytic strains of *C. botulinum.*

This growth can be prevented at pH < 5.0 with an aqueous salt content greater or equal to 5%, and with a water activity at or below 0.97 (Bell et al., 2000). Proteolytic strains may appear if temperature abuse occurs, and is over 10°C.

Nitrite was found to have several functions, which included color stabilization, characteristic color and flavor, and antimicrobial properties against pathogenic (*Clostridium botulinum* spores) and spoilage microbes (Pearson and Tauber, 1984; Aberle et al., 2001; Russell et al., 2003). Even though all the characteristics of nitrite are of great importance, the most noticeable aspect is its characteristic pink color and flavor. Nitrite decreased over time with a more rapid decline occurring with low pH, and or with increasing temperature (Kim and Foegeding, 1993).

Sofos et al. (1979a) tested a frankfurter emulsion containing mechanically deboned chicken meat, and found that 20 and 40 ppm of nitrite had no effect on botulinal growth and toxin production. A nitrite level of 156 ppm seemed to hinder the production of toxin, with less inhibition recorded when compared to other findings. The effectiveness of 0 and 80 ppm of nitrite was evaluated in chicken, beef and pork by Sofos et al. (1979b). Although the botulinal toxin was retarded in pork considerably, no effect was noticed for chicken and beef. In addition, meat-soy mixture containing 156 ppm of sodium nitrite significantly delayed botulinal toxin growth. A meat-soy mixture was more effective in delaying toxin than all meat mixtures. *C. botulinum* spores germination rate was not affected by the addition of 0, 20, 40 and 156 ppm in a chicken frank formulation. Sofos et al. (1979c) concluded that nitrite effectiveness was in delaying the development of the cells after germination, and before toxin production (Tompkin, 1993). Sodium nitrite at 0, 100, and 200 ppm was evaluated in the spoilage

of a vacuum-packed bologna type sausage. Nielsen (1983a) found Enterobacteriaceae and *Moraxella* and *Moraxella* like organisms to be progressively inhibited with increasing concentration of sodium nitrite, and or with decreasing temperatures (2, 5, 10, and 20°C). To a certain level, Gram positive cocci, yeasts, and lactic acid bacteria were inhibited. Consequently, lactic acid bacteria became the dominant spoilage bacteria in the vacuum packed products. Nielsen (1983b) found that lactic acid bacteria were affected by nitrite in the studies on vacuum packed cooked pork loin. Consequently, vacuum packaging cured meat in low oxygen permeable packaging with low temperature storage helps maintain a longer shelf life (Tompkin, 1993).

Functions of Nitrite and Factors Affecting Residual Nitrite

Nitrite is a very unstable compound which depletes rapidly once incorporated into the meat during curing. Honikel (2007) stated that it is not surprising to find nitrate in products when only nitrite has been added; this can be explained by the oxidation of nitrite to nitrate.

Curing color is developed through several reactions. In the presence of nitrate, a nitrate reductase is needed to reduce nitrate (NO_3^-) to nitrite (NO_2^-). Addition of nitrite into a meat system causes the oxidation of myoglobin to metmyoglobin (brown color) (Cassens et al., 1979). In acidic conditions, nitrous acid (HNO_2) is formed. The addition of reductants produces nitric oxide (NO) which binds to metmyoglobin, resulting into nitrosylmyoglobin. In the presence of heat, the characteristic cured color (Nitrosylhemochrome) is formed. Factors such as pH, storage conditions, processing temperatures, reducing agents, meat to water ratio have been recognized as influence on the residual nitrite level. Sebranek (1974) found nitrite levels to decrease with low pH in water solutions maintained at room temperature.

Residual nitrite constitutes the nitrite level present in a meat system after processing. Since the 1980s, issues regarding residual nitrite have been scientifically debated. Studies were conducted to provide a better understanding of how the curing reaction occurred, its stability and presence in the final product. The studies of red blood cells by Shahidi and Pegg (1992) using different levels of reducing agents (ascorbate or erythorbate) added or not, revealed the following results: without reducing agents present, cured meat color failed to develop. In addition, the cooked cured meat pigment was not stable in the presence of oxygen and light. Pigments remain stable for extended periods when positive pressure with NO (Nitric oxide) was used. Nitric oxide was the most important product resulting from the added nitrate or nitrite. Cured meat commonly contains ascorbic acid or sodium erythorbate which act as reducing agents.

Regulations and Hazards

The usage of nitrite and or nitrate in cured meat products was regulated in the early 20th century in the United States (US) by the US Department of Agriculture in 1925 (USDA, 1925). Allowable amounts of nitrate and nitrite were set for the purpose of curing meat, and under the rules residual nitrites would be 200 ppm or less for nitrite and /or nitrate in the finished products (Russell et al., 2003). Because of advances in technology, the USDA assembled a scientific panel for the specific review of nitrite in curing of meat in 1973. Following recommendations by the panel, allowable nitrite in bacon was reduced to120 ppm of sodium nitrite or 148 ppm (parts per million) of potassium nitrite was recommended for use in bacon, and the amount of reducing agent (sodium erythorbate, or ascorbate) for use as an additive was restricted to a maximum amount of with 550 ppm maximum level of sodium erythorbate, or ascorbate(USDA, 1978a). This regulation was accompanied by the Nitrosamine Monitoring Program for

bacon, which is still valid today (USDA, 1978b). Restricted ingredients (nitrates, nitrites, phosphates, ascorbates, corn syrup) can be used in specific amounts depending on the process (curing, pumping, and smoking) in meat and poultry products. According to USDA FSIS (United States Department of Agriculture Food Safety Inspection Service) Directive 7620.3 (Processing Calculations Inspector's Handbook), the restricted ingredients are expressed in ounces (oz) or pounds (lb) per pounds of the meat/poultry or gallons of pickle solution. Nitrite is a very toxic product when compared to nitrate, and as a general rule, 10 times more toxic (Honikel, 2007). Hill (1996) stated that methaemoglobin (Met-Hb) formation from oxyhaemoglobin is the source of nitrite toxicity. Met-Hb has a lower ability to bind with oxygen, making the process of oxygenation, and deoxygenation in the transport from the lungs to the tissues difficult. At 10% or less, the presence of Met-Hb is not noticeable, but above 10%, it is the cause of cyanosis which may lead to coma and subsequently death. Excess nitrite and nitrate can be toxic because they are converted to nitric oxide. Therefore the amounts used must be based on the meat block (meat and/or poultry products or byproducts) in the formulation, not the finished product (USDA FSIS Directive 7620.3). The USDA FSIS (Food Safety and Inspection Service) Directive 7620.3 set standards for limits of sodium and potassium nitrate and nitrite in different types of cured meat products. The method of curing determines the maximum ingoing amount of nitrite and nitrate following regulations. The basis of nitrite concentration is based on the green weight of the meat block. Table 2-1 displays the present levels and regulations according to the USDA FSIS Processing Calculations Inspectors Handbook (1995). For communited meat, 156 parts per million ingoing was allowed for sodium nitrite and Potassium nitrite. Sodium

nitrate and potassium nitrate are higher and at 1718 parts per million. The USDA requires a minimum of 120 ppm of ingoing nitrite in all injected and immersion cured bacon, and this was based on research reviewed after the bacon standard was set. Even though there is no minimum requirement for ingoing nitrite level for cured products that are shelf stable, 40 ppm is needed for some preservative effect and cured meat color. Table 2-1 shows the current regulations according to the USDA FSIS Processing Calculations Inspectors Handbook (USDA FSIS, 1995).

Table 2-1. Maximum ingoing nitrite and nitrate limits (in ppm) for meat and poultry products.

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

Adapted from the USDA FSIS Processing Calculations Inspector's Handbook (FSIS Directive 7620.3).

United States Department of Agriculture. 1995. Processing inspector's calculations handbook. Food Safety and Inspection Service Directive 7620.3. Washington, DC. http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf, Accessed July 27, 2010.

Modern cure mix or Prague powder #1 contains 6.25% of sodium nitrite and 93.75% of salt, and is used to cure meat products. Nitrite is not used in a pure form, but in compounds or mixes following regulations. It is crucial to note that it is extremely important to follow regulations and guidelines regarding calculations for nitrite amounts

(cure mix). Nitrite contents from a natural source such as a vegetable powder may not be pure (100%). For food safety purposes, sufficient nitrite compound must be added to the product being processed, but not more than the regulation allows.

Calculation for Nitrite Used in Pure Form;

$$\frac{\text{lb nitrite} \times 1{,}000{,}000}{\text{green weight of meat block}} = \text{ppm}$$

Calculation for Nitrite Used in Curing Compounds or Mixes

$$\frac{\text{lb cure mix} \times \%\ \text{nitrite in mix} \times 1{,}000{,}000}{\text{green weight of meat block}} = \text{ppm}$$

Figure 2-3. Curing agent calculation

Adapted for the USDA Processing Inspector's Calculations Handbook, 1995

United States Department of Agriculture. 1995. Processing inspector's calculations handbook. Food Safety and Inspection Service Directive 7620.3. Washington, DC. http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf, Accessed July 27, 2010.

Vacuum-Packaged Cooked Cured Meat

Packaging is one of the essential components in the meat distribution chain that allows a smooth transition from the farm to the table with an increased shelf-life. A safe quality food product is needed at the consumer level with the least cost (Mauer et al., 2004). Robertson (1993) and Soroka (1999) defined food packaging as "a coordinated industrial and marketing system for enclosing products in a container to meet containment, protection, preservation, distribution, identification, communication, and convenience needs" (Mauer et al., 2004). Packaging allows meat products (fresh and processed) to be preserved longer at the retail level and marketed. Without this

protection, most of the products would spoil in the supply chain, or before they reach consumers.

Vacuum packing meat consists of inserting a meat product into a gas impermeable pouch, removing air with a vacuum packaging apparatus, and sealing the pouch with the aid of a heat sealer. There are varieties of plastic bags available with different barrier properties in order to meet product specificities.

Packaging is designed to protect the product it contains, prevent additional microbial contamination, prolong shelf life, and maintain quality from the processor to the consumer. Vacuum packaging to increase shelf-life of meat to 12 weeks in chilled storage or 20 weeks at super chill (-1 ± 0.5°C) storage (James, 2000). Shelf-life is influenced by factors such as temperature, species, Good Manufacturing Practices, and the status of the meat at packaging. Packaging cannot improve a deteriorating product. Packaging has been used for several years to help preserve the characteristics of meat. Muscle foods are characterized by quality attributes such as color (most noticeable), flavor, tenderness, juiciness, and texture (Xiong et al. 1999). Consumers mostly judge products on appearance, which will be negatively affected if not properly packaged. Meat products need to be moved quickly (air, land, sea) and effectively through the distribution chain in order to protect against stresses such as vibrations, shocks, compression, water or moisture loss, oxygen, light, microbial, physical and chemical contaminations. Packaging is a coordinated system to prepare food products for transport, distribution, storage, retailing and consumption (Soroka, 1999). Its purpose is to protect, extend shelf-life, provide convenience and communicate information. According to Rourke (2005), manufacturers of cooked meat products select packaging

for two major reasons: preservation of product quality (appearance, flavor, odor and texture) and inhibition of microbial growth.

Clostridium botulinum

Clostridium botulinum is a ubiquitous organism found in both terrestrial and aquatic environments all around the world. There are different strains which are classified into seven types, in regards to the ht specificity of the neurotoxin: A, B, C, D, E, F, and G. Because toxin type was not enough for classification, Bergey's Manual divided the strains into four groups. Group I organisms are proteolytic and include type A, B, and F. Group II organisms are nonproteolytic and include type E, B, and F. Group III organisms of generally non proteolytic type C or D, and Goup IV organisms are proteolytic, but their spores are rarely seen in cultures.

The first description of *Clostridium botulinum* emerged in blood sausages, with sausage meaning botulus in Latin (Riemann, 1969).One of the first outbreaks named " Sausage poisoning" was reported in Germany by Kerner (1815) affected 230 people. It was linked to smoked blood sausage that was not reheated before consumption. The causative organism was not known at the time, and therefore not isolated. A major outbreak involving home cured ham occurred in Belgium. After isolating the toxin from the food that was responsible for the outbreak in 1895, Van Ermengem named the organism Bacillus botulinus. Its symptoms were found to be similar to those of the blood sausage poisoning. A separate genus, Clostridium was given to this bacillus after it was found to be anaerobic (Hauschild and Dodds, 1993). Nitrite is the primary anticlostridial agent in cured meat, and is thought to induce germination of clostridial spores, but inhibit cellular growth.

Hustad et al. (1973) studied various levels of nitrite and nitrate, 400 ppm of sodium ascorbate, 2.8% of fermentable carbohydrate, and 4.52% of brine. 50 ppm of sodium nitrite used in 55 franks temperature abused at 27°C for over 4 weeks only resulted in 2 toxic samples. Sodium nitrite used at 150 ppm did not become toxic while nitrate showed variable results. A rapid decrease in pH and high brine were attributed to sodium nitrite inhibitory effect. Bowen and Deibel (1974) and Bowen et al. (1974) designed a study in which different levels of ascorbate (9 ppm, 105 ppm, and 655 ppm) were evaluated for their effect on the antibotulinal effect of nitrite (0 to 150 ppm), in the absence of nitrate. The brine level in the franks was 4.83% and the samples were toxic at 50 ppm of sodium nitrite at all levels of sodium ascorbate. One sample became toxic at 100 ppm of sodium nitrite and no contaminated sample was detected at 150 ppm of sodium nitrite. Assuming alike pH responses, the above two studies suggested that the combination of brine and nitrite level (50- 100 ppm) slowed botulinal growth until lactic acid flora lowered the pH to an inhibitory level (Tompkin, 1993).

Nitrite effectiveness was variable among the diverse laboratories and cured meat products tested, with factors such as pH during abuse, brine level, residual nitrite, level of viable botulinal spores and vegetative cells, temperature abuse, reducing agents, available “iron”, type of meat, ingredients, thermal process, growth of competitive flora, type and level of phosphate (Tompkin, 1993). Aberle et al. (2001) reported that between 40 to 80 ppm of residual nitrite is needed to prevent the formation of *Clostridium botulinum* spores in meat products.

The key reference (temperature) for psychrotrophic *C. botulinum* minimum growth was found to be 3.3°C; toxins were produced in certain foods within 7 days at 15°C, and

12 days at 8°C (Bell et al., 2000). In vacuum packed or modified atmosphere packaged foods growth of this organism should not occur at storage temperature 3°C or less. But at storage temperatures between 3° C to 8°C (chill temperatures), shelf life should not be more than 10 days if no additional control factors are used.

Lücke and Roberts (1993) suggested that salt concentration, pH and temperature affected the growth of *C. botulinum*, even with debates over the importance of ingoing and residual nitrite.

The growth of non-proteolytic strains of *C. botulinum* in cooked uncured meats was studied. Meng and Genigeorgis (1994) found the growth and toxin production of type B and E blend in cooked uncured chicken and beef. Toxins were detected at 4, 8, and 12°C after 90 days, after initial inoculation of 10^4 spores into 3 grams of meat. They also found that inoculums sizes had an effect on the formation of toxins, more than the variation of salt concentration (Meng and Genigeorgis, 1993). Consequently a suggestion was made to have a shelf life of no more than 15 days for cooked, chilled meat products that contained no added preservatives such as nitrite and in which the salt concentration was less than 3.50%.

Cooked cured meats preserved with nitrite were found to be more inhibitory to *C. botulinum*, with most research being conducted with proteolytic strains. Non-proteolytic *C. botulinum* has been used under temperature abuse conditions at greater than 10°C. Bell et al. (2000) stated that “Nitrite does not completely prevent the growth of *C. botulinum* but merely extends the lag time and reduces the rate of growth”. Pivnick and Bird (1965) researched the growth of type E in chilled foods under abuse conditions, and demonstrated a relation between the spores load and the potential for growth:

10,000 spores per gram of meat resulted in toxic products after a smaller incubation period at 10 and 15°C. *C. botulinum* type E showed growth potential in many commercial products at abused temperatures (> 10°C), but no toxin formation was detected at 5°C during 8 weeks. It was also shown that the product condition (micro environment) was more of a factor than the type of packaging and the gaseous atmosphere. Tompkin (1978, 1979) found high ascorbate /isoascorbate levels (> 400 ppm) to deplete the amount of nitrite, and consequently its inhibitory effect.

Spoilage of Vacuum-packaged Cooked Cured Meat

General Characteristics

Meat and meat products are very perishable. Deterioration begins soon after exsanguination, resulting in microbial, chemical and physical changes. The initial microbial load plays a role in the determination of the food product's shelf-life. Shelf life is in turn defined as point during storage at which a product is deemed unacceptable due to appearance, off odor and or off flavor (Borch et al., 1996a,b). Three major microorganisms found in meat are fungi, mold, and bacteria (being a major source of contamination). The molds and yeast growing on meat are aerobic. Bacteria thriving in meat could be aerobic, anaerobic or facultative. During refrigerated storage some genera of bacteria found on meat and poultry are mainly *Pseudomonas, Moraxella, Psychrobacter* and *Acinetobacter* on the surface, followed by *Aeromonas, Shewanella, Micrococcus, Lactobacillus, Streptococcus, Leuconostoc, Pedicoccus, Flavobacterium*, and *Proteus* (Aberle et al., 2001). Some additional organisms of concern associated with meat in general are *Salmonella, Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Staphylococcus aureus* (Romans et al., 1994).

Spoilage from Lactic Acid Bacteria (LAB)

Vacuum packaged cured meat products such as bologna offered at the retail level have generally long shelf-lives (60 to 90 days). Handling, particularly slicing may contaminate and consequently increase the level of bacteria present. Collins-Thompson and Lopez (1981) found certain LAB to be at both the origin of nitrite depletion (estimation at 30%) and the deterioration of cured meat. Spoilage of refrigerated vacuum packaged cooked cured meat can be mostly attributed to homofermentative lactobacilli and leuconostocs which are LAB (Yang and Ray, 1994; Korkeala et al., 1997). Developments of off-odors, off- flavors occur as bacterial levels reach 10^7 to 10^9 colony forming units (CFU) per gram (Borch et al., 1996; Korkeala et al., 1997). Cassens (1994a) found that the LAB degraded carbohydrates (primary substrate) and then proteins; undesirable end products creations due to proteolysis are synonyms of spoilage. Cassens (1994b) stated that LAB in addition to competing against other microorganisms for dominance and nutrients, inhibit psychrotrophic Enterobacteriaceae, *B. thermosphacta*, *Salmonella* and *Staphylococcus aureus* through lactic acid production. Besides survival in acidic pH environment (Egan, 1983), LAB are not sensitive to nitrites (Korkeala et al., 1992), low temperatures, anaerobic conditions, to 3-5% salt (Egan, 1983; Schillinger and Lücke, 1987a), and are present on vacuum packaged cooked cured meats (Holley, 1997a,b).

Nielsen (1983a) found LAB to constitute the prevalent microbial flora in vacuum packaged products, when studying the effect of various levels (0, 100, 200 ppm) of sodium nitrite in sliced vacuum packaged bologna sausage; a combination of increasing levels of sodium nitrite, added to decreasing storage temperature (2- 20^{o}C) contributed to the inhibition of microorganisms such as *B. thermosphacta*, Enterobacteriaceae,

Moraxella and *Moraxella* like organisms. Silla and Simonsen (1985) found similar results: LAB were also the prevalent microbes in the study of four cured vacuum packaged and in modified atmosphere cured meat when spoiled. Nitrite concentration and packaging were correlated to spoilage. Anaerobic conditions with the usage of nitrite were found to affect two species of *L. plantarum*; in contrast, aerobic conditions rendered the bacteria resistant at 50 ppm usage level (Collins-Thompson and Thomson, 1986).

Vacuum and gas packaged meat products could be susceptible to spoilage by Lactobacillus spp. and Leuconostoc spp., creating loose packaging during storage (Ray and Bhunia, 2008). Factors such as pH, processing method, chemical composition, temperature, and storage conditions influence the presence of bacteria on cured meat.. Most cured meats flora is constituted by lactic acid bacteria. Lactic acid bacteria have the ability to grow under packaging conditions and are fermentative and able to utilize the carbohydrates available in the meat matrix (Russell and Gould, 2003; Ray and Bhunia, 2008).

With lactic acid as the end product, LAB are made of non spore forming and Gram positive bacteria that ferment sugar (glucose). Egan (1983) classified LAB into two categories according to the carbohydrate metabolism: homolactic or heterolactic. Homolactic fermentation is defined as a lactic acid fermentation in which sugars (lactose, glucose, and pentose) is converted into lactic acid exclusively. Heterolactic ferementation involves the conversion of sugars into several compounds such as lactic acid, carbon dioxide, ethanol, acetic acid. Different types of sugars (mannose, galactose, and fructose) with specific pathways can be used.

Application of Natural Ingredients as Nitrite Replacers

New Generation of Meat Products.

Bryan (2006) stated that in the 1970s there was a public outcry regarding nitrate and nitrite usage in cured meat, and its end product (N-nitrosamine) was suspected of being carcinogenic to humans. Regardless of nitrite's multiple functions (color stabilization, characteristic color and flavor, and antimicrobial properties against pathogenic (*Clostridium botulinum* spores) and spoilage microbes) (Pearson and Tauber, 1984; Aberle et al., 2001; Russell et al., 2003) demands are being made by consumers for less usage of chemical preservatives. Issues were raised regarding the formation of N-nitrosamines (suspected carcinogens) in cured meat. Researchers have been and are still working on developing alternative methods to cure meat, to match all the attributes of nitrite without generating any carcinogenic compounds. According to Romans et al. (1994), testing has been performed on more than 700 products with the objective of finding a compound with similar characteristics to nitrite, without any success. Celery juice or powder, in addition to being natural may be used as an alternative replacement for sodium nitrite. In addition, there has been a push by consumers for healthy, green and palatable food products (Food Marketing Institute, 2008). According to FMI's U.S. *Grocery Shopper Trends* (2008), there has been a 10% increase in natural and organic foods sales in retail stores.

Natural Products

Food preservation systems are moving toward the use of natural ingredients and antimicrobials to satisfy consumers who are demanding less chemical preservatives in their foods. Spices have different functions with flavor, taste, aroma, and texture being primary. Usage as preservative, antimicrobial and antioxidant constitute the secondary

functions. Used as medicine by the Greeks, and seasoning by the Romans, celery had a long history before its arrival in North America. *Apium graveolens L. (celery)* from the Apiaceae or Umbelliferae family, is a biennial plant originating from the Mediterranean, Middle East, Asia, and was grown wild (Encyclopædia Britannica. 2009).

Nitrates in Vegetables

Nitrogen originated from the atmosphere and is present on earth in nitrogenous compounds such as ammonia. Different pathways which include microorganisms, plants, humans, and atmospheric conditions such as lightning can contribute to nitrogen fixation. Ammonia may be absorbed by plants through their roots, but its degradation results in the presence of nitrates through bacterial oxidation (Hill, 1996). This process is called nitrification. Plants are able to absorb and concentrate nitrate. Consequently some plants including celery are rich in nitrates. Nitrates are unequally distributed in a plant, with certain parts having an accumulation. In ascending order, nitrates are found in the floral part, fruit or grain, leaves, roots, and petioles or stems (Brady, 1984). In 1972, The Committee on Nitrate Accumulation added broccoli, celery, lettuce, kale, mustard greens, and collards to nitrate accumulators' list. Factors that may affect the concentration of nitrate in vegetables are variable, but include climate, soil, species, agricultural practices and storage conditions.

Natural Curing

The USDA Food Standards and Labeling Policy Book (USDA, 2005) details the guidelines to be followed in order for a meat product to be labeled "natural". It pertains to the ingredients, not to the meat itself. The term "Natural" as defined by the USDA is in regards to" products that do not contain any artificial flavoring, coloring ingredients, or chemical preservatives (as defined in 21 CFR 101.22), or any other artificial or synthetic

ingredients; and the product and its ingredient are not more than minimally processed". The term "natural curing" is not officially defined by the USDA, but refers to products that are cured in similar ways as the conventional products: the only difference is concerning the origin of the ingredients used, and the intention or not of replacing nitrate/ nitrite in the product. Natural ingredients with elevated concentration of nitrate are used in combination with nitrate-reducing starter culture (such as *Staphylococcus carnosus*) to produce nitrite while following meat processing steps. Ingredients such as sea salt, turbinado sugar and celery powder are used to fulfill consumers' needs, and to manufacture products that have similar characteristics when compared to the conventionally cured products. Vegetable sources such as beets, radishes, turnip greens contain elevated amounts of nitrates.

Celery juice powder is one of the ingredients currently used as a natural source of nitrate/ nitrite to naturally cure meat products. The National Academy of Sciences (1972) found that nitrate levels in plants are influenced by environmental stresses such as drought and pest damage. Walker (1990) reported nitrate levels in celery (3,151 ppm), turnip greens (9,040 ppm), beets (3,288 ppm), spinach (2,470 ppm), and melon (4,932). Most recently, Sindelar et al. (2007) found 27,462 ppm of nitrate in celery juice powder. The levels of nitrate in the celery powder seem not to be as stable as the synthetically formulated sodium nitrite which contains 6.25% nitrite. This wide variability in ppm may cause a potential problem in meat products: too little for preservation or sufficient levels to cause a health hazard. Standardization protocols may need to be in place in order to ensure the same quality of product after manufacture. The need to

preserve the naturally cured meat products against *Clostridium botulinum* spores is great.

In order to manufacture natural cured meat equivalent to conventional cured meat, natural substitutes for all the ingredients used in the conventional products are needed. Additives with reducing properties are often added to meat, during the processing of cured meat. Sodium ascorbate, ascorbic acid, or sodium erythorbate are commonly used. Regulations permit the use of ascorbic acid and sodium ascorbate in cured meat at maximum levels of 469 ppm and 547ppm, respectively (USDA, 1995). Ranken (1974) determined that sodium erythorbate had similar characteristics and properties as ascorbic acid, except for the Vitamin C property.

Acerola cherry powder which contains ascorbic acid, is known as *Malpighia emarginata* DC. or *Malpighia punicifolia* L. (acerola trees) originated from the Caribbean islands, Central America, and the Amazonian region. The fruit is red and oval and is called acerola fruit or West Indian Cherry. The fruit really resembles cherries, and is red, with three segments containing stones with Vitamin C content of 1,677.6 ppm of edible portion of the raw fruit (Johnson, 2003). Itoo et al. (1990) reported that unripe fruits are very rich in ascorbic acid (> 3.00%) in green fruits with a decreasing content during ripening. Acid content is dependent on the species of tree (Jackson, 1963). The most important characteristic of this fruit is its high content of ascorbic acid or vitamin C (Pino and Marbot, 2001). Vitamin C contents were 4.80, 1.90 and 0.97 g/100g for the concentrated immature, the immature, and the mature acerola juices, respectively (Righetto et al., 2005). Natural curing can be accomplished with a natural source of

ascorbic acid (cherry powder) combined with the celery juice powder (nitrate/nitrite source).

CHAPTER 3
EVALUATION OF ANTIMICROBIAL PROPERTIES OF CELERY JUICE POWDER CONTAINING PRE-GENERATED NITRITE ON *CLOSTRIDIUM BOTULINUM* IN DIFFERENT CONDITIONS

Introduction

Nitrite has unique characteristics in cured meat products, which have not been reproduced by other ingredients or chemicals through years of research. In recent years, natural and organic foods which incorporate processed meat segments have experienced a rapid growth (Sebranek and Bacus, 2007). There has been an increasing demand by consumers for more usage of natural antimicrobials as preservatives to produce cured meat products. Because the direct addition of nitrite to natural or organic processed meat is forbidden, alternative methods were needed as substitutes to produce products displaying similar characteristics of nitrite-cured meat (Sebranek and Bacus, 2007). A product containing a naturally occurring source of nitrates is used in combination with particular microorganisms to reduce the nitrates to nitrite (Bacus, 2006). National Academy of Sciences (1981) has found vegetables such as celery, lettuce, and beets to contain nitrate concentrations as elevated as 1500 ppm to 2800 ppm. Sebranek (2006)'s analysis of commercially available carrot, celery, beet and spinach vegetable juices revealed 171, 2114, 2273, and 3227 ppm of nitrate, respectively. In recent years, vegetable juice powders containing nitrates, and pre-converted vegetable juice powders containing nitrites, have become commercially available to manufacture uncured meat and poultry products. Currently, there are no regulations for the use of vegetable juice powders by the United States Department of Agriculture. With this thriving market, consistency in the quality of the meat products is necessary. Because of its reactivity, nitrite produced through natural processes is

variable. Synthetic nitrite has been proven over the years to be a strong antimicrobial agent. Sodium nitrite used in combination with sodium chloride is mostly responsible for the control of *C. botulinum* in cured meat (Hauschild, 1980). Limited work regarding the antimicrobial activity of celery powder as a source of nitrite has been published. It is necessary to insure a minimum amount of nitrite (from pre-converted juice powders) in products for safety purposes. *Clostridium botulinum* is ubiquitous to the environment and is Gram-positive spore-forming anaerobic bacteria. Its' species are composed of four groups which can form a neurotoxin. *Clostridium botulinum* group I (proteolytic) and *C. botulinum* group II (nonproteolytic) have been implicated in of the majority of foodborne botulism. With proteolytic *C. botulinum* being a mesophile, and non-proteolytic *C. botulinum* being a psychrotroph (Peck, 2006), most safety risks in chilled foods can be attributed to nonproteolytic *C. botulinum* (Lindstro¨m, Kiviniemi, and Korkeala, 2006a; Peck, 2006; Peck and Stringer, 2005) which gets nutrients from carbohydrates. Extending shelf life through vacuum packaging is common in the food industry, with the main objective of excluding oxygen. Through this process, oxidative reactions and growth of spoilage, aerobic microorganisms are controlled. In contrast, a favorable environment is created for anaerobic bacteria (Peck 1997; Peck *and* Stringer 2005).

This study was carried out to assess the effectiveness of vegetable juice powder in combination with a natural source of ascorbic acid as an anti-clostridial agent in different conditions (broth, agar, and meat systems) with the aim of evaluating the survival and growth of *Clostridium botulinum*.

Materials and Methods

Sample Preparation

Turkey bologna products were formulated and manufactured at the University of Florida, containing 0.20% Veg Stable™ 504,0.20% Veg Stable 504™ and 0.20% Veg Stable™ 515, 156 ppm modern cure and 547 ppm sodium erythorbate, 156 ppm modern cure, and 156 ppm Veg Stable™ 504. Ground turkey meat was purchased from a local supermarket, and used to make turkey bologna emulsion type products with only the source of nitrite added. One and a half pounds of the emulsion was formulated for each treatment, with each time having triplicate samples. The meat batter was filled in FoodSaver® Pint Bag (in duplicate), cooked in a water bath (99°C) to an internal target temperature of 74°C. The product was then removed from the water bath and chilled in ice water for 30 minutes to stop the cooking process. The cooked products were sliced, and vacuum packaged in FoodSaver® bags, and stored at 4 ± 1°C cooler before being shipped for analyses 24 hours later. Samples of the products were shipped overnight to Deibel Laboratories (Madison, WI) to determine anti-botulinum properties of CJPPN alone and in combination with the cherry powder extract, when compared to the conventional modern cure. Meat samples were inoculated with approximately 1,000 spores per grams of the *C. botulinum* cocktail by injecting the organisms with a syringe through a septum to preserve the integrity of the packaging. Samples were inoculated with 0.5 ml. of the diluted cocktail. Samples were diluted in Buffered peptone water and blended to determine counts. Meat samples were tested at 0 (immediately after the addition of the *C. botulinum* cocktail), 24, 48 and 72 hours. Counts were done in SFP agar which was incubated up to 48 hours under anaerobic conditions at 30°C.

Products

The CJPPN with or without Cherry juice powder was evaluated in Reinforced Clostridial broth or in vacuum packed turkey bologna products. Non-proteolytic and proteolytic strains of *Clostridium botulinum.*were employed in the inoculum cocktail.

Preparation of Inoculum

Upon arrival at Deibel Laboratories, the turkey bologna samples were inoculated with approximately 1,000 spores per gram of the *C. botulinum* cocktail by injecting the spores with a syringe through a septum to preserve the integrity of the packaging. Samples were inoculated with 0.5 ml. of the diluted cocktail. Samples were diluted in Buffered peptone water and blended to determine counts. The sample homogenate was spread on SFP agar which was incubated up to 48 hours under anaerobic conditions at 30 ° C.

Rcm broth

Flasks containing 100ml of RCM Broth were made according to manufacturer's directions, and sterilized by autoclaving. RCM broth is a general growth media used for the cultivation of *C. botulinum*. The proper concentration of the products to be tested was added to the broth. The flasks were then inoculated with the *C. botulinum* spore cocktail at approximately 1,000 spores per ml. The *C. botulinum* spore cocktail was heat shocked at 80°C for 10 minutes before being added to the RCM Broths. All flasks were held under anaerobic conditions and analyses performed at 0, 24, and 48 hours.

Experiments

A total of four experiments were conducted. Experiment one consisted of testing recommended levels of CJPPN (0.20%), alone or with a natural source of ascorbic acid (Cherry powder at 0.20%), and a level to match the level permitted by federal

regulations for commercially available sodium nitrite (modern cure) in RCM (Reinforced Clostridial Media) broth. The test tubes were incubated anaerobically at 32.5°C ± 2.5°C for up to 48 hours. After incubation, growth was evident by the appearance of turbidity. Subcultures were made from the previously incubated tubes, plated onto Shahidi-Ferguson Perfringens Agar (SFP agar), and incubate under anaerobic conditions at 32.5°C ± 2.5°C for 48 hours for the detection of clostridia.

Experiment two consisted of testing recommended levels of celery juice powder containing pre-generated (0.20%), alone or with a natural source of ascorbic acid (Cherry powder at 0.20%) in a meat system, with modern cure and sodium erythorbate as a control. Meat samples were inoculated with *C.botulinum* cocktail by injecting the organisms with a syringe through a septum to preserve the integrity of the packaging. Samples were inoculated with 0.5 ml. of the diluted cocktail and incubated anaerobically at 32.5°C ± 2.5°C for up to 48 hours. After incubation, subcultures were made from the meat samples using Buffered peptone water. Counts were done on SFP agar plates which were incubated under anaerobic conditions at 32.5°C ± 2.5°C for 48 hours for the detection of clostridia.

Experiment three comprised a negative control containing 0.20% of celery juice powder containing Pre-generated Nitrite, alone and in combination with 0.20% of Cherry juice powder, Celery juice powder at legal limit comparable to modern cure, and modern cure as a control and enumerated in Shahidi-Ferguson Perfringens Agar (SFP agar**).** Meat samples were inoculated with *C.botulinum* cocktail by injecting the organisms with a syringe through a septum to preserve the integrity of the packaging. Samples were inoculated with 0.5 ml. of the diluted cocktail and incubated anaerobically at 32.5°C ±

2.5°C for up to 72 hours. After incubation, subcultures were made from the meat samples using Buffered peptone water. Counts were done on SFP agar plates which were incubated under anaerobic conditions at 32.5°C ± 2.5°C for 48 hours for the detection of clostridia.

Experiment four was conducted in RCM broth with a negative control, modern cure at its legal limit with or without sodium erythorbate, celery juice powder containing pre-generated (156 ppm), alone or with a natural source of ascorbic acid (Cherry powder at 469 ppm). *C. botulinum* spore cocktail was heat shocked at 80 degrees C. for 10 minutes before being added to the 100 ml flask containing sterilized RCM Broth. All flasks were The test tubes were incubated anaerobically at 32.5°C ± 2.5°C for up to 48 hours. Subcultures were made from the previously incubated tubes, plated onto Shahidi-Ferguson Perfringens Agar (SFP agar), and incubate under anaerobic conditions at 32.5°C ± 2.5°C for 48 hours for the detection of clostridia.

Media and spore inoculum.

The *C. botulinum* spore inoculums used were a composite of five type A (69A, 77A, 90A, 56A, and 62A) and four type B (53B, 13983B, 113B, and Lamanna B) strains. The cocktail contains both proteolytic and non-proteolytic strains.

Results and Discussion

The effect of Veg Stable™ 504 (celery juice powder containing pre-generated nitrite) used alone or with Veg Stable™ 515 (Cherry juice powder, source of ascorbic acid) on *C. botulinum* in RCM broth is shown in Table 3-1. After an initial inoculation of 3 log, a 4.50 to 5.00 log increase in *C. botulinum* was observed over 48 hours for all treatments, except for the treatment containing celery juice powder and cherry powder at equivalent of legal limits. RCM broth appeared to support the growth of *C. botulinum*

very well. Gas and off odor was present in all treatments with the exception of the latter which was normal, and experienced almost a 3 log decrease in RCM broth over 48 hours. A combination of 156 ppm of Veg Stable™ 504 and Veg Stable™ 515 reduced the growth of *C. botulinum* over 48 hours. *C. botulinum* spores were tested on a bologna type emulsion product (Table 3-2) with 0.20% Veg Stable™504, a combination of Veg Stable™ 504, and Veg Stable™ 515, and modern cure (commercially available nitrite) combined with sodium erythorbate over 48 hours. A 3 log increase was observed after 48 hours for 0.20% Veg Stable™504, without exhibition of any spoilage or off odor in the meat product. The treatment containing a combination of Veg Stable™ 504 and Veg Stable™ 515 remained stable for spore counts, about 3 log CFU/g over the test period. Commercially available nitrite (modern cure) which has been known for its anti-clostridial effect, combined with sodium erythorbate showed a 3 log decrease over 48 hours.

Table 3-3 illustrates *C. botulinum* spore counts in bologna type emulsion products with a negative control Veg Stable™ 504 (no *C. botulinum* inoculation), 0.20%, and 156 ppm of Veg Stable™ 504, and a combination of 0.20% Veg Stable™ 504 and 0.20% Veg Stable™ 515, and only modern cure (without sodium erythorbate). The negative control which showed a 5 log increase while all the other treatments had 4- to 5 log increase over 72 hours. Meat products containing only nitrite and 0.20% Veg Stable™ 504 (no *C. botulinum* inoculation) exhibited and off-odor with presence of liquid. Table 3-4 displays the effect of the RCM broth (Control), 156 ppm Modern cure, 156 ppm Modern cure *and* 547 ppm sodium erythorbate, 156 ppm Veg Stable™ 504, and 156 ppm Veg Stable™ 504 *and* 469 ppm Veg Stable™ 515 on *C. botulinum* spores over 48

hours. All treatments presented normal appearances. The inoculated control was stable over the time specified previously. Modern cure used alone or in conjunction with sodium erythorbate revealed a 2 log reduction over 48 hours, while 156 ppm Veg Stable™ 504, and 156 ppm Veg Stable™ 504 *and* 469 ppm Veg Stable™ 515 experienced a 3 log reduction.

Tompkin (2005) has thoroughly documented the antibotulinal role of nitrites in cured meats. Nitrite has been shown to prevent growth of *Clostridum botulinum* and *Listeria monocytogenes*, but the mechanism of action has not been clarified (Tompkin, 2005; Sebranek and Bacus 2007). Because nitrite is the active ingredient in the celery juice powder, it is expected to be effective against *C. botulinum*. In addition to RCM broth supporting the growth of this microorganism (Table 3-1), the different levels of Veg Stable™ 504 alone or in combination with Veg Stable™ 515 showed 4 log increases over 48 hours. The combination of 156 ppm Veg Stable™ 504 and 469 ppm Veg Stable™ 515 resulted in a slightly less than 3 log reductions. In order to better comprehend these results, the celery juice powder was analyzed (Figure 3-1), and revealed interesting results. Nitrite was less than 1% of the powder. The celery juice powder contained an average of approximately 85% dry matter which was proteins, fibers, carbohydrates and minerals. In contrast with modern cure which is composed of 6.25% nitrite and 93.75% salt, celery juice powder may be needed in greater amount to match the same effectiveness. Because the RCM was not a meat system, a bologna type emulsion product was used to test Veg Stable™ 504, alone or in combination with Veg Stable™ 515, against modern cure. Modern cure was proven, as stated in the literature, to have anticlostridial properties which were evident by a 3 log reduction in *C.*

botulinum (Table 3-2). The samples were inoculated at 5 log: for comparison purposes, inoculation of samples at 3 log with *C. botulinum* spores would have resulted in less than 1 log CFU/gram of spores. These results are supported by researchers who determined that the growth of *C. botulinum* is not supported by products containing a significant amount of initial nitrite (Christiansen et al. 1973; Hustad et al. 1973; Roberts et al. 1981a,b,c). Veg Stable™ 504 used at 0.20% was not as effective as its synthetic counterpart (modern cure) because it experienced a 3 log increase in *C. botulinum* over the same amount of time. The increase may be due to low content of nitrite. If the powder contains 1% or less of the active ingredient, and only 0.20% is used, less nitrite is present. In contrast, research conducted by Shahidi and Ferguson (1971) did not support the statement about nitrite. The researchers reported that all treatments experienced an increase over 72 hours in a bologna type emulsion product. Furthermore, bacterial spore counts were performed on SFP Agar which incorporates the supplements to increase the selectivity of the medium. Shahidi and Ferguson (1971) laboratory study on SFP agar concluded that SFP agar promotes the luxuriant growth of *C. perfringens*. Even though it is inhibitory to many species of bacteria found in food, this media is not inhibitory to various strains of *C. perfringens*. Growth in the negative control containing Veg Stable™ 504 with no inoculation of *C. botulinum* in the meat sample, suggest the presence of spores in the powder. Typical growth for *C. botulinum* was shown; it may or may not be *C. botulinum*. This explanation would be plausible since celery juice powder originated from the celery plant which could have been contaminated from the ground. It was necessary to investigate the effectiveness of Veg Stable™ 504 as it compared to modern cure with or without sodium erythorbate. In this

particular case, the RCM broth did not support the growth of *C. botulinum*. Over 48 hours, 156 ppm Veg Stable™ 504 and 156 ppm Veg Stable™ 504 and 469 ppm Veg Stable™ 515 experienced 1 log decrease regarding *C. botulinum* spore counts when compared to modern cure alone and with ascorbic acid.

Conclusion

Nitrite is effective as an antibotulinal compound used to prevent food poisoning (botulism) (Romans et al., 2001). Wolff and Wasserman (1972) found this compound able to inhibit the division of *C. botulinum* vegetative cells while the residual amount prevented its growth. Depending on the environment, residual nitrite may play a key role as an antibotulinal agent in meats in combination with pH (Tompkin, 2005). Literature suggests that the inhibition factor from nitrite relies on pH. Nitric oxide is the active substance, which can be generated through the use of ascorbate to reduce nitrite, (Wirth, 1985; Gibson,1986). A shortfall of this study relates to the pH not being measured during the incubation and test times.

In summary, results of this study showed some promising outcomes for the antibotulinal effect of celery juice powder containing pre-generated nitrite At 156 ppm in a broth, celery juice powder shows antimicrobial activities. Additional studies need to be designed to test different combinations of the two powders over longer shelf life, and in a meat system environment.

Table 3-1. *Clostridium. botulinum* spore counts in Reinforced Clostridium broth with different levels of Veg Stable™ 504 (pre-generated nitrite) and Veg Stable™ 515 (ascorbic acid) and no turkey bologna mixture added

	Time (h)		
	0	24	48
Treatments*	Log CFU/ g		
1	3.34	7.78	8.49
2	3.60	7.85	8.18
3	4.95	3.60	2.00
4	3.38	8.72	7.95

*1= RCM broth (Control); 2= 0.20% Veg Stable™ 504; 3= 156 ppm Veg Stable™ 504 and 469 ppm Veg Stable ™ 515; 4= 0.20% Veg Stable™ 504 and 0.20% Veg Stable™ 515

Table 3-2. *Clostridium. botulinum* spore counts in turkey bologna emulsion with different levels of Veg Stable™ 504 (pre-generated nitrite) and Veg Stable™ 515 (ascorbic acid)

	Time (h)		
	0	24	48
Treatments*	Log CFU/ g		
1	3.08	2.60	6.00
2	3.08	2.30	3.00
3	5.20	3.20	2.43

*1= 0.20% Veg Stable™ 504; 2= 0.20% Veg Stable 504™ and 0.20% Veg Stable™ 515; 3= modern cure and sodium erythorbate

Table 3-3. *Clostridium. botulinum* spore counts in turkey bologna type emulsion with different levels of Veg Stable™ 504 (pre-generated nitrite), and Veg Stable™ 515 (ascorbic acid) cultured on Shahidi-Ferguson Perfringens agar

	Time (h)			
	0	24	48	72
Treatments*	Log CFU/ g			
1	2.36	2.30	6.15	6.28
2	2.41	1.81	5.51	6.06
3	2.30	5.56	6.40	7.23
4	2.30	5.15	6.40	7.27
5	<1.00	3.08	6.40	6.40

*1= Control (modern cure); 2=156 ppm Veg Stable™ 504; 3=0.20% Veg Stable™ 504; 4= 0.20% Veg Stable™ 504 and 0.20% Veg Stable™ 515; 5=Negative Control Veg Stable™ 504

Table 3-4. *Clostridium. botulinum* spore counts in Reinforced Clostridium broth with different levels of Veg Stable™ 504 (pre-generated nitrite) and Veg Stable™ 515 (ascorbic acid) and no turkey bologna mixture added *

	Time (h)		
Treatments*	0	24	48
	Log CFU/ g		
1	1.00	1.00	1.00
2	4.96	3.70	3.00
3	4.97	2.95	2.95
4	5.00	3.88	2.00
5	4.95	3.60	2.00

*1= RCM broth (Control); 2= 156 ppm modern cure; 3= 156 ppm modern cure and 547 ppm sodium erythorbate; 4= 156 ppm Veg Stable™ 504; 5=156 ppm Veg Stable™ 504 and 469 ppm Veg Stable™ 515

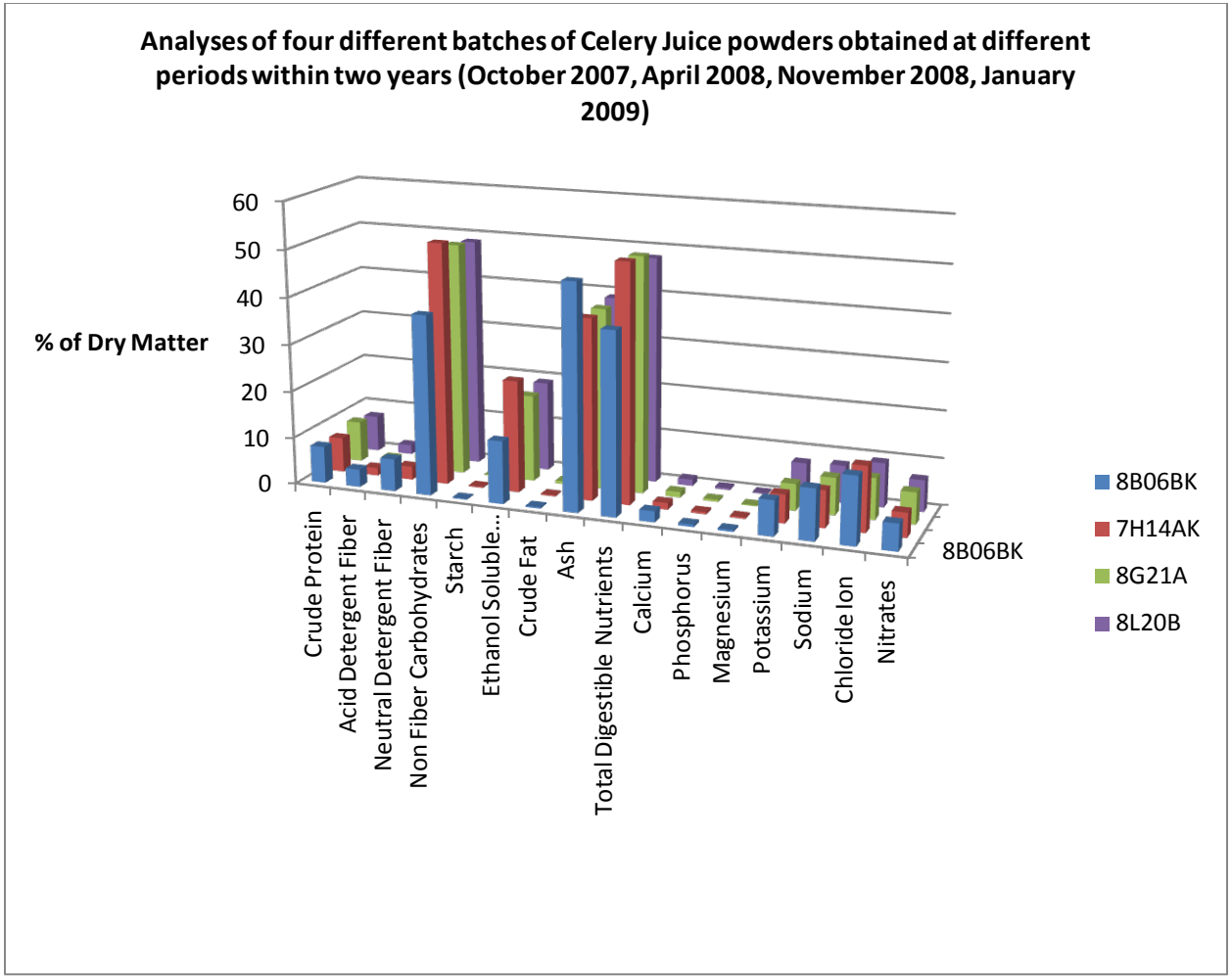

Figure 3-1. Analyses of four different batches of Celery juice powders obtained at different periods within two years (October 2007, April 2008, November 2008, January 2009) as % of Dry matter

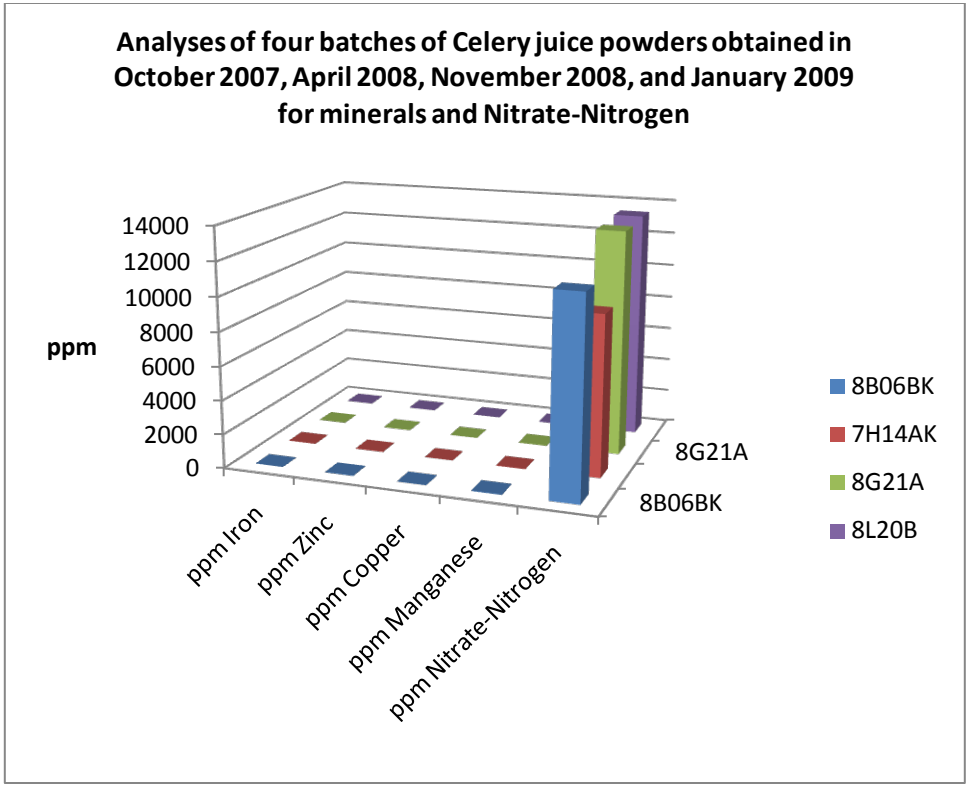

Figure 3-2. Analyses of four batches of Celery juice powders obtained in October 2007, April 2008, November 2008, January 2009 for minerals and Nitrate-Nitrogen in parts per million (ppm)

CHAPTER 4
EFFECT OF HOLDING TIME ON COLOR, PH AND RESIDUAL NITRITE OF BEEF FRANKFURTERS FORMULATED WITH EITHER CELERY POWDER EXTRACT WITH PRE-GENERATED NITRITE OR COMMERCIALLY AVAILABLE SODIUM NITRITE

Introduction

Consumers are demanding Natural *and* Organic food products and are willing to pay significant premiums. The U.S. market for organic food and beverages has grown from $1 billion in 1990 to an estimated $20 billion in 2007, with a projected growth up to $23 billion in 2008. Organic food sales are predicted to grow yearly about 18 percent between 2007 and 2010 (Organic Trade Association Manufacturer Survey, 2007). In order to satisfy this growing demand, meat processors have marketed natural and organic products. Natural meat products are defined by 21 CFR 101.22 as not containing any artificial flavor or flavoring , color ingredient, or chemical preservative, any artificial or synthetic ingredient, with the product and its ingredients minimally processed (USDA Food Standards and Labeling Policy Book, 2005). Nitrite is a unique ingredient used to cure meat for which there seems to be no substitute. To date, no replacement for nitrite has been discovered that reproduces its characteristic cured meat aroma and flavor of the meat products (Gray et al., 1981). According to Shahidi and Pegg (1992) no single compound can demonstrate all the distinctive characteristics of nitrite. Because nitrite is a preservative, direct addition of nitrite to natural or organic processed meats is not permitted. As a result, different processes are needed to produce natural or organic processed meats that have similar characteristics of nitrite-cured meat (Sebranek and Bacus, 2007). To naturally cure meat products without direct addition of nitrite (chemical preservative), alternative sources such as celery juice concentrate, celery juice powder, sea salt, and evaporated cane juice are utilized.

Vegetables are well known to contain significant amounts of nitrate. Celery, lettuce, and beets, for example, have been reported to contain concentrations as high as 1,500 – 2,800 ppm (NAS, 1981). Similar nitrate levels in several vegetables have also been reported (Walker, 1990; Santamaría et al., 1999; Fujihara et al., 2001).

Uncured, natural and organic processed meat products can be produced by either no addition of nitrate/ nitrite, or by indirect incorporation of nitrate through vegetable sources (Sindelar et al., 2007a). Sindelar et al. (2007a) analyzed commercial celery juice powder and found nitrate content to be around 27,500 ppm (2.75%).

A natural curing process currently consists of naturally occurring nitrates which are reduced to nitrite in the meat products by specific microorganisms (Bacus, 2006). Celery juice and its powder are commonly utilized in combination with lactic acid starter culture to produce naturally cured meat products. With the use of nitrate, incubation time is required to allow the conversion to nitrite by starter cultures such as *Kocuria varians*, *Staphylococcus xylosus*, and *Staphylococcus carnosus* (Sebranek and Bacus, 2007). Incubation time was necessary in small diameter products such as sausages. This is in contrast with large diameter products like hams which have sufficient time for nitrate to nitrite conversion due to the slow temperature increase and long heat process (Sindelar et al., 2007 b,c).With natural and organic processed meats constituting a niche market, there are few regulations for naturally cured, organic products. Regulations are well defined for organic foods, but there has not been a final ruling regarding uncured, and natural products. In order for consumers to make informed decisions and be less confused, Sebranek and Bacus (2007) suggested that there was a need for improved labeling regulations.

Meat processors are now provided with an innovative vegetable juice powder containing pre-generated nitrite (natural source).This creative celery powder containing pre-generated nitrite is currently available on the market. However, very little is known about its shelf life, and effectiveness as a replacement for synthetic nitrite in cured meats.

The objective of this study was to evaluate the shelf life, and the role of holding time on the color, pH, and residual nitrite of beef frankfurters formulated with celery powder containing pre-generated nitrite (CP) at different levels when compared to modern cure (MC).

Materials and Methods

Shelf Life Evaluation

The evaluation of the shelf-life stability of the celery juice powder consisted of obtaining two freshly manufactured batches of products from a manufacturer. One of the batches was separated into 25 gram samples (in duplicate) which were vacuum packaged with a commercially available FoodSaver® Pro Sport Model Vacuum Packager (Jarden Corporation, Rye, N.Y.) equipped with FoodSaver® rolls material. This packaging material was composed of two different sides: one was uneven, while the other side was smooth. The specifications for the FoodSaver® bags are 164.232 cc/m_2/24 hrs @ 23ºC on the rough side, and 0.334 cc/m_2/24 hrs @ 23ºC on the smooth side. The products were stored in a 4 ± 1^{o}C walk-in cooler for a total of six months. Samples (in duplicate) were sent to an approved research laboratory (ABC Research Corporation) for nitrite analyses. Concurrently, samples were also analyzed in duplicate at the University of Florida Meat Science Research laboratory using the AOAC method 973.31 (AOAC, 1995).

Experimental Design

In order to evaluate the effectiveness of celery juice powder, a freshly processed batch was obtained from a local processor. This natural source of nitrite was used to manufacture beef frankfurters, and compare them against commercially available sodium nitrite (modern cure). Ground beef was purchased fresh from a local grocery store in Gainesville, Florida. Four beef frankfurter products were formulated using either 0.20%, 0.30%, 0.40% CP, or MC (control) at the University of Florida's Meat Laboratory Kitchen. To simulate processing conditions in a pilot plant, the experiment was conducted in a 4 ± 1^{o}C walk-in cooler. Holding time was defined as the setting time from stuffing to cooking. Analyses were performed at various holding times in minutes: 20, 40, 60, 80, 100, and 120. The different products were formulated with only salt and the source of nitrite. Three independent replications with different purchase dates for the meat were analyzed after processing for objective color values (Hunter L*, a*, b*), pH and residual nitrite.

Processing

The ground beef was placed in mixer with 1.5% sodium chloride to extract salt-soluble proteins and blended for 4 minutes using Krups food processor set on speed 3 (Krups Type 700, Household Food Processor, Germany). After blending, the source of nitrite was added, and the meat product was then mixed for 2 minutes. Finally, the product was stuffed into approximately 3.8 cm diameter cellulose casings (Jumbo hotdog casing, Visko Teepak, Mariehamn, Finland) using a stuffer (The Sausage Maker Inc., Buffalo, N.Y., U.S.A.). The product was cooked in a 90-99°C water bath (180 Series, Precision Scientific Inc., Chicago, IL, U.S.A.) with a thermometer (Extech 421307 Type K Dual Input by Extech Instrument Corporation, Waltham, MA, U.S.A.)

inserted in the geographic center for a target internal temperature of 74°C . At the end of the cooking process, the product was removed and cooled in a 3 ± 1°C ice water bath to stop the process, and until approximate room temperature was achieved (20°C). After 15 minutes, the cooked product was transferred in 4 ± 1°C walk in cooler overnight, until analyzed.

Chemical Analysis

For all chemical analyses, samples were ground in an electric mini food chopper (Braun Multiquick Model 4185 MR430 HC by Procter *and* Gamble, Cincinnati, OH, U.S.A.).

Color Measurement

A HunterLab MiniScan XE (HunterLab, Reston, VA, U.S.A.) with 2.54 cm aperture, illuminant A and 10° standard observer was used to determine: lightness (L*), redness (a*, red-green), and yellowness (b*, yellow-blue) on the inner surface of frankfurter slices. Calibration was performed using a black and white tile before each usage. Readings were performed in duplicate in 4 ± 1^{o}C walk-in cooler.

pH Analysis

The pH analysis of the chopped beef frankfurter was performed using a pH meter (Accumet basic AB15, Fisher Scientific, Pittsburgh, PA 15238) which was calibrated with buffers 4.0 and 7.0. Ten grams of chopped meat product were placed in a beaker, and 90 ml of deionized water was added. Duplicate pH measurements were taken.

Nitrite Analysis for Celery Powder

Nitrite was determined with modifications to the AOAC method 973.31 (AOAC, 1995). Five grams of finely comminuted sample were weighed into a 100 ml beaker. 50 ml of water heated to 80°C was added. All lumps were broken up with a glass rod, and

the mixture transferred to a 500 ml volumetric flask. Hot water was used to wash the beaker and all the washing was transferred to the flask. Enough hot water was then added to bring the volume up to 300 ml. The flask was then placed in a steam bath for 2 hours with occasional shaking. After the 2 hours, the flask was removed and cooled to room temperature, and then filled to volume with distilled, de-ionized water (DDW). Approximately 45 ml of sample was then transferred to a 50 ml volumetric flask. Under a fume hood, 2.5 ml sulfanilamide reagent (0.5g sulfanilamide in 150 ml 15% acetic acid) was added. After 5 minutes, 2.5 ml NED reagent (0.2 g N-(1-naphthyl) ethylenediamine 2HCl in 150 ml 15% acetic acid) were added and filled to volume with sample. Color was allowed to develop for 15 minutes. Solution was transferred to a spectrophotometer cuvette and absorbance was measured at 540 nm against a blank of 45 ml DDW, 2.5 ml sulfanilamide reagent, and 2.5 ml NED reagent. Nitrite was determined by comparing sample reading with a standard curve as described in the official method. All nitrite assays were done in duplicate.

Residual Nitrite Content

The residual nitrite content was determined in 5 g samples using the nitrite/nitrate colour-assay (Cat. No. 11 756 281 001) according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany) for meat and meat products.

The principle of the Nitrite/Nitrate colorimetric method (Phototmetric endpoint determination) consists of reducing nitrate to nitrite by reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase (NR). The nitrite formed reacts with sulfanilamide and N-(1-naphthyl)-ethylene-diamine dihydrochloride to give a red-violet diazo dye.

$$Nitrate + NADPH + H \xrightarrow{+NR} Nitrite + NADP + + H_2O$$

$$Nitrite + sulfanilamide + N-(1-naphthyl)-ethylenediamine \rightarrow diazo\ dye$$

Figure 4-1. Principle of the Nitrite/Nitrate colorimetric method (Phototmetric endpoint determination), adapted from Roche Applied Science

Nitrite/Nitrate, colorimetric method. Phototmetric endpoint determination. Roche GmbH, Roche Applied Science, Mannheim, Germany. http://www.roche-applied-science.com/pack-insert/1746081a.pdf. Accessed July 27, 2010.

The diazo dye is measured on the basis of its absorbance in the visible range at 540 nm (Hg 546 nm).The result is calculated from the calibration curves constructed using the standard solutions. The change in absorbance obtained for the sodium nitrite and potassium nitrate standard solutions is plotted on the y-axis against the corresponding nitrite or nitrate concentrations in mg/l on the x-axis.

$^{\Delta A}$nitrite = $(A_2\text{-}A_1)$nitrite - $(A_2\text{-}A_1)$ Blank nitrite

$^{\Delta A}$nitrite + nitrate = $(A_2\text{-}A_1)$nitrite + nitrate - $(A_2\text{-}A_1)$ Blank nitrite + nitrate

$^{\Delta A}$nitrate = $^{\Delta A}$nitrite + nitate - $^{\Delta A}$nitrite

Figure 4-2. Calculation of Nitrite/Nitrate change in absorbance (Adapted from Roche Applied Science

Nitrite/Nitrate, colorimetric method. Phototmetric endpoint determination. Roche GmbH, Roche Applied Science, Mannheim, Germany. http://www.roche-applied-science.com/pack-insert/1746081a.pdf. Accessed July 27, 2010.

Nitrite and nitrate concentrations in the samples are determined from the calibration curves using the change in absorbance measured. A multiplication by the dilution factor was needed if samples were diluted during preparation.

When analyzing solid or semisolid samples that had to be weighed out, the result was calculated with respect to the mass of sample.

$$\text{Content}_{\text{nitrite}} = \frac{C_{\text{nitrite}} \text{ X } 1000}{\text{Mass}_{\text{Sample}} \text{ in g/l sample solution}} \text{ (mg nitrite/kg sample)}$$

$$\text{Content}_{\text{nitrate}} = \frac{C_{\text{nitrate}} \text{ X } 1000}{\text{Mass}_{\text{Sample}} \text{ in g/l sample solution}} \text{ (mg nitrate/kg sample)}$$

Figure 4-3. Calculation of Nitrite/Nitrate content in solid or semisolid samples

The results are determined as sodium nitrite and potassium nitrate.

Conversion factor from $NaNO_2$ to nitrite (NO_2^-) is 46.006 : 68.995 = 0.667

Conversion factor from KNO3 to nitrate (NO_3^-) 62.005 : 101.11 = 0.613

Figure 4-4. Conversion factors for sodium nitrite and potassium nitrite

Nitrite/Nitrate, colorimetric method. Phototmetric endpoint determination. Roche GmbH, Roche Applied Science, Mannheim, Germany. http://www.roche-applied-science.com/pack-insert/1746081a.pdf. Accessed July 27, 2010.

The residual nitrite content was calculated using a calibration curve. As the residual nitrite content has to be labeled as sodium nitrite ($NaNO_2$), the nitrite content determined in the assay had to be converted into the sodium nitrite equivalent.

Statistical Analysis

The experimental design was a randomized block design with four treatments in three blocks. Statistical analysis was performed with SAS (Statistical Analysis Systems, version 9.1, Cary, NC). Since measurement times were correlated over time, a repeated measures statistical analysis with the “Proc Glimmix” procedure of SAS was used to analyze results with sampling time (Time), Treatment, and the Time by Treatment interaction being the main fixed effects in the model. The interaction Repetition (block) by treatment was included in the random effects term. When the interaction Treatment*Time was significant for a given response variable, the LSMEANS differences were used to compare the treatments at each sampling time.

Result and Discussion

The shelf life of a batch of celery juice powder is shown in Figure 5-1. The level of nitrite decreased over the six months storage time. The results obtained were confirmed by an approved laboratory. Nitrite has been known as a very reactive chemical which has been affected in this case by exposure to light and oxidation. This result suggests the fact that the celery powder juice needs to be both protected against light and oxygen.

Objective Color Analyses

L* values for Lightness (Table 4-1) were similar ($P > 0.05$) for all treatments at all holding times. The values varied between 41.68 and 48.42 for all treatments. L* values averaged respectively 45.73, 44.17, 46.44, and 46.53 for treatments 1, 2, 3 and 4 through time. There were no significant differences between the treatments and through time. Increasing the level of celery juice powder and holding time had no significant effect ($P > 0.05$) on L* values.

a* values for Redness (Table 4-2) varied from 3.63 to 7.11. Treatment 1 (0.20% Veg Stable™ 504) had a significantly lower a* value ($p < 0.05$) than treatment 4 (modern cure) for time 20. No significant differences were observed between treatments ($P > 0.05$)for times 20, 40 and 100. Overall, Modern cure a* values were higher ($P < 0.05$) than 0.20% CP as holding time increased. Modern cure which contained more nitrite resulted in a redder cured color.

b* values for Yellowness (Table 4-3) were similar for all treatments at all holding times (20- 120 minutes). The values were between the ranges of 13.26 and 23.95. There were no significant differences ($P > 0.05$) between treatments and through time.

Increasing the level of celery juice powder did not affect the yellowness of the different frankfurter products.

pH and Residual Nitrite Analyses

pH values (Table 4-4) showed no significant difference ($P > 0.05$) among treatments through time. pH values varied from 5.65 to 5.83 for all treatments.

Residual nitrite was significantly higher ($P < 0.05$) for MC when compared to 0.20%, 0.30%, and 0.40% Celery juice powder (Table 4-5). Residual nitrite values ranged from 3.50 to 41.24 ppm. The data revealed that as the usage level of celery juice powder increased, the residual nitrite concentration increased in the beef frankfurters. The data revealed that beef frankfurters manufactured with MC or CP were not affected by the holding times.

Sindelar et al. (2007a) studied the quality attributes and consumer acceptance of uncured, no-nitrate/ nitrite-added commercial hams, bacons, and frankfurters. Four commercial uncured, no nitrate/nitrite added frankfurter products were purchased in addition to a control (nitrite added). The control sample showed a higher L* value, which translates into a lighter product. No differences ($P > 0.05$) were observed regarding a* values.

Baseler (2009) conducted experiments which evaluated the characteristics for nitrite-added and no-nitrite-or-nitrate-added Canadian style bacon. One of the experiments used natural cure with preformed nitrite in celery powder, which is the same as celery powder containing pre-generated nitrite. Boneless pork loins were purchased frozen, then were ground. Treatments applied comprised control, control with sodium ascorbate, natural cure with preformed nitrite in celery powder, natural cure, natural cure with cherry powder, and natural cure with lemon powder. Products

containing the natural cure with preformed nitrite in celery powder were not significantly different ($P > 0.05$) from the naturally cured products, but had significantly lower residual nitrite than the control over a 7 week storage time ($P < 0.05$). Low amounts of nitrite were detected throughout the experiment. The control, control with sodium ascorbate, natural cure with preformed nitrite in celery powder products had significantly lower b* values ($P < 0.05$) than natural cure natural cure with cherry powder, and natural cure with lemon powder; these values ranged from 5.59 to 7.01. Baseler (2009) also found residual nitrite in the Canadian-style bacon to be 6.99 ppm when preformed nitrite in celery juice powder was used. In addition 8.09 ppm in residual nitrite was measured when natural cure was used in combination with cherry powder.

Nitrite incorporated in meat for curing purposes can be detected in the final product as residual nitrite. Literature has shown that reactions occur and among other things, pigments combine added nitrite. Nitrite depletion is rapid, and affected by factors such as storage, temperature. Cassens (1997) stated that after completion of the manufacturing process, not more than 10–20% of the nitrite originally added is analytically detectable. Immediately after processing, about 50-70% of the ingoing nitrite can be analyzed for in the product. Thermal processing resulted in a loss of 20-80% (Cassens et al., 1978). Kudryashow (2003) found early loss of nitrite to be around 65% independently from the ingoing concentration, follow by a drop of one third of the latter. Dederer (2006) using nitrite in German cured meat products analyzed from 1996 to 2001 to show disappearance of nitrite until 60 days. Also, the higher the pH value, the slower the disappearance of nitrite. The lower amount of residual nitrite present in the four products is supported by literature. In addition the ingoing nitrite amount in celery

juice powder are respectively 20, 30, and 40 ppm for 0.20%, 0.30%, and 0.40% Veg Stable™ 504. The assumption in this case is that 10,000 ppm of nitrite was present in the pre-converted celery juice powder. Preliminary data resulting from the analysis of the powder and its shelf life showed a decrease of nitrite content over time. Literature also has shown nitrite to be a very reactive and unstable compound.

Kulchaiyawat (2009) evaluated the quality of a dozen organic, natural, uncured, and traditionally cured bacon products available at the retail level. Variability were found to exist between the traditionally cured products and the organic, natural and uncured ones. Lower levels of residual nitrites in the non traditionally cured products were attributed to the incorporation of lower amounts of ingoing nitrite. Variability were found to exist in the lean part of the products for a* and b* values for all the products ($P < 0.05$) when they were compared to the traditionally cured products. The differences could also be attributed to the difference in muscles.

Conclusions

Celery powder containing pre-generated nitrite was used in a beef frankfurter product with the intention of replacing nitrite and simulating curing characteristics. The objective of this study was to evaluate the role of holding time on the color, pH, and residual nitrite of frankfurters formulated with celery powder or modern cure, stuffed into casings and allowed to set at 4 ± 1°C prior to cooking. All treatments had similar ($P > 0.05$) pH, L* and b* values. The a* values for Modern cure were higher ($P < 0.05$) than 0.20% celery powder as holding time increased. Residual Nitrite was higher ($P < 0.05$) for beef frankfurters with MC when compared to beef frankfurters with 0.20%, 0.30%, and 0.40% celery powder. The different treatments of Veg Stable™ 504 (0.20%, 0.30%, and 0.40%) were shown to be comparable to traditionally cured frankfurters, in regards

to L*, b*, and pH values. The a* values for the traditionally cured beef frankfurter was overall redder than the frankfurters containing celery juice powder. Due to similarities regarding color, pH, and residual nitrites, no additional time would be necessary for frankfurters prepared with the celery powder prior to the cooking process. “Incubation” or holding time seems not to be necessary when utilizing celery juice powder containing pre-generated nitrite.

The data revealed that measurements taken at different holding times for frankfurters prepared with Modern cure and celery powder were similar. Even though the celery powders were obtained at 10,000 ppm of nitrite, packaging and storage conditions affected the levels. Further analysis of the powder suggested the presence of various compounds (fibers, sugars, minerals) that may have an effect on the system, since traditional cure only contains sodium chloride and nitrite. In addition sensory analysis performed by Sindelar (2007) found 0.20% of vegetable juice containing a starter culture to be comparable to the conventionally cured ham product. At 0.35%, there were differences in sensory properties. In the case of preformed nitrite in the celery juice powder, the conversion from nitrate to nitrite utilizes a starter culture. The difference resides at the conversion step. During the fermentation step, a starter culture is added in the tank while temperature, aeration, agitation and pH are being carefully monitored. Consequently, comparisons could be made between products using pre-generated nitrite and products wherein a starter culture was added to the meat system during processing and required incubation time. Future research will need to evaluate quality attributes, microflora of celery powder containing pre-generated nitrite at the recommended level and levels equivalent to the modern cure through storage time.

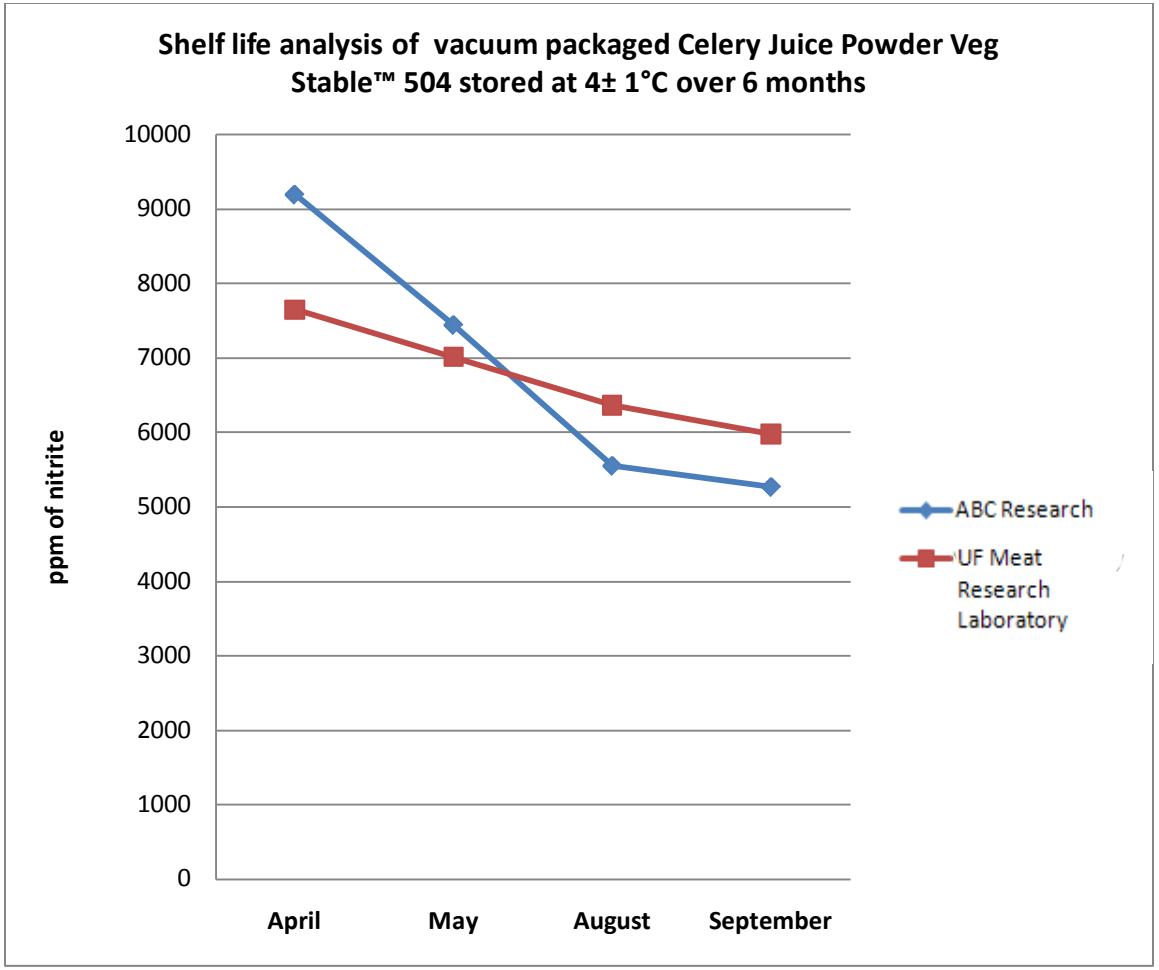

Figure 4-5. Shelf life analysis of vacuum packaged Celery Juice Powder Veg Stable™ 504 stored at 4 ± 1°C over 6 months

Table 4-1. Mean L* values (Lightness) for beef frankfurters containing celery powder and modern cure

	Holding time (min)					
Treatments*	20	40	60	80	100	120
1	45.75	47.02	45.26	46.75	45.88	43.73
2	44.23	41.68	45.85	47.23	44.11	41.93
3	46.45	46.34	46.96	47.66	46.01	45.22
4	47.97	46.24	43.66	45.72	48.42	47.17

*1 = 0.20% Veg Stable™ 504; 2 = 0.30% Veg Stable™ 504; 3 = 0.40% Veg Stable™ 504; 4 = Modern cure

Table 4-2. Mean a* values (Redness) for beef frankfurters containing celery powder and modern cure

	Holding time (min)					
Treatments*	20	40	60	80	100	120
1	3.77^{b}	3.72	3.95^{b}	3.63^{b}	4.38	4.16^{b}
2	4.03^{ab}	3.92	3.82^{b}	4.77^{ab}	4.53	3.92^{b}
3	5.74^{ab}	4.93	4.69^{b}	4.30^{b}	4.09	4.45^{b}
4	5.91^{a}	5.71	7.06^{a}	6.67^{a}	5.68	7.11^{a}

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.30% Veg Stable™ 504; 3 = 0.40% Veg Stable™ 504; 4 = Modern cure

Table 4-3. Mean b* values (Yellowness) for beef frankfurters containing celery powder and modern cure

	Holding time (min)					
Treatments*	20	40	60	80	100	120
1	14.45	14.36	14.24	14.44	23.95	13.55
2	14.07	13.80	14.21	14.11	13.56	13.29
3	13.98	14.40	14.33	14.67	14.20	14.41
4	14.26	13.82	13.38	13.67	13.47	13.26

*1 = 0.20% Veg Stable™ 504; 2 = 0.30% Veg Stable™ 504; 3 = 0.40% Veg Stable™ 504; 4 = Modern cure

Table 4-4. Mean pH values for beef frankfurters containing celery powder and modern cure

	Holding Time (min)					
Treatments*	20	40	60	80	100	120
1	5.81	5.79	5.80	5.78	5.76	5.75
2	5.79	5.82	5.81	5.82	5.65	5.80
3	5.80	5.82	5.83	5.79	5.67	5.69
4	5.70	5.74	5.76	5.76	5.75	5.74

*1 = 0.20% Veg Stable™ 504; 2 = 0.30% Veg Stable™ 504; 3 = 0.40% Veg Stable™ 504; 4 = Modern cure

Table 4-5. Mean Residual Nitrite values for beef frankfurters containing celery powder and modern cure

	Holding time (min)					
Treatments*	20	40	60	80	100	120
1	5.02^{b}	3.93^{b}	4.62^{b}	3.50^{b}	3.99^{b}	3.66^{b}
2	5.69^{b}	6.37^{b}	7.51^{b}	6.23^{b}	5.16^{b}	5.67^{b}
3	8.42^{b}	11.03^{b}	10.13^{b}	8.55^{b}	8.47^{b}	7.11^{b}
4	41.24^{a}	35.23^{a}	35.87^{a}	35.29^{a}	37.98^{a}	37.30^{a}

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)

*1 = 0.20% Veg Stable™ 504; 2 = 0.30% Veg Stable™ 504; 3 = 0.40% Veg Stable™ 504; 4 = Modern cure

CHAPTER 5
EVALUATION OF SLICED VACUUM PACKAGED TURKEY BOLOGNA CONTAINING DIFFERENT LEVELS OF CELERY JUICE POWDER STORED AT 4 ± 1°C FOR 10 WEEKS UNDER RETAIL DISPLAY LIGHT

Introduction

A significant amount of natural and organic products are being consumed due to demand by consumers. According to the Organic Trade Association (2007), organic food sales are predicted to grow annually about 18% from 2007 to 2010. Meat processors have entered this particular segment by using a natural source of nitrite to cure meat products. Because nitrite is a preservative, direct addition of nitrite to natural or organic processed meats is not permitted. As a result, different processes are needed to produce natural or organic processed meats that have similar characteristics of nitrite-cured meat (Sebranek and Bacus, 2007). Alternative sources of nitrate such as celery juice concentrate, celery juice powder, sea salt, and evaporated cane juice are utilized in naturally cured meat products instead of direct addition of synthetic nitrite.

Vegetables are well known to contain significant amounts of nitrate. Celery, lettuce, and beets, for example, have been reported to contain concentrations as high as 1,500 –2,800 ppm (National Academy of Sciences, 1981). Uncured, natural and organic processed meat products can be produced by either no addition of nitrate/ nitrite, or by indirect incorporation of nitrate through vegetable sources (Sindelar et al., 2007a). A natural curing process utilized consists of using naturally occurring nitrates which are reduced to nitrite in the meat products by specific microorganisms (Bacus, 2006). Celery juice and its powder which contain nitrates, are commonly utilized in combination with lactic acid starter culture to produce naturally cured meat products. With the use of nitrate, incubation time is required to allow the conversion to nitrite by

starter cultures such as *Kocuria varians*, *Staphylococcus xylosus*, and *Staphylococcus carnosus* (Sebranek and Bacus, 2007). With natural and organic processed meats constituting a niche market, there are few regulations for naturally cured, organic products. Meat processors have provided manufacturers with a vegetable juice powder containing pre-generated nitrite (natural source of nitrite).This new celery powder containing pre-generated nitrite simulating synthetic nitrite is currently available on the market. This powder is being used and seems to be effective.

Krause (2009) determined that the maximum ingoing level of nitrite in pre-converted celery juice powder to be 60 ppm in a ham product. This level was significantly less than the USDA FSIS maximum allowable limit of 200 ppm. The low initial levels were thought to be a food safety concern, regarding the growth of *C. botulinum* (Krause, 2009). Due to the high reactivity of the nitrite molecule, nitrite concentration may not be stable, and consequently lead to safety concerns. Establishments of standards are needed for consistency. It is crucial to evaluate the effect of pre-converted celery powder used at different levels on microorganisms of public health concern (*Listeria monocytogenes*, Salmonella, *Staphylococus aureus*), Lactic acid bacteria (LAB), anaerobic bacteria, aerobic bacteria, and psychrotrophs.

The objective of this study was to evaluate the shelf life, consumer acceptance, color, pH, TBARS and microbiological characteristics on turkey bologna formulated with different levels of Celery juice powder containing pre-generated nitrite (CP) when compared to modern cure (MC).

Materials and Methods

Product Manufacturing

Four 11 kg batches of turkey bologna were prepared under commercial conditions using the formulation outlined in Tables 5-1 and 5-2. Turkey thigh meat (7,945 grams per batch) was ground through a1.27 cm plate using Biro AFMG -52 Mixer Grinder (Marblehead, OH, U.S.A.). Turkey thigh meat, half of the ice, all the salt, and the appropriate source of nitrite (synthetic or natural), and source of ascorbic acid or sodium erythorbate (synthetic or natural) were chopped in Killia TK 20 1 2000S Bowl Chopper (Neumünster, Germany) to 7°C for protein extraction. The remaining water/ice and seasonings were then added, and chopping was continued until the batter reached 10°C. Four different batches of products were manufactured containing 0.20% Stable™ 504, 0.20% Stable™ 504 + 0.20% Veg Stable™ 515, 156 ppm Stable™ 504 + 469 ppm Veg Stable™ 515, and 156 ppm Prague powder + 550 ppm of sodium erythorbate. Each emulsion product (treatment) was stuffed into 11.43 cm diameter fibrous casing (Teepak, LLC, Lisle, U.S.A.) using Hollymatic Stuffer 55S (Countryside, IL, U.S.A.). Each batch was labeled with smoke house tags and processed in the smokehouse (model TR2-1700, Vortron, Inc., Beloit, WI) using a program detailed in Table 5-3. The smoked products were left on the rack for fifteen minutes before being transferred into the designated cooked meat cooler, to be cooled to 4°C. The fibrous casing was peeled and the log of meat sliced on Hobart 2612 Meat slicer (Troy, OH, U.S.A.). The bologna products were sliced into approximately 0.32-cm-thick slices (about 25 g per slice) and vacuum packaged (250 grams per package) using Cryovac barrier bags (B4770, Oxygen Transmission Rate: 3-6 cm^3/m^2/24 hr atm @ 40°F, 0%RH) and Multivac C 500 (Multivac Inc., Kansas City, MO) labeled, and stored for 10 weeks in a 4 ± 1°C walk-in

cooler on a shelf. Simulating a display environment, the products were displayed with GE T8 Linear Fluorescent lamps (General Electric, Fairfield, CT) that emitted 94 foot candle with 24 hours a day lighting schedule. The products were removed as needed for analysis. The packages were re-arranged periodically to ensure even exposure.

Proximate Composition

Proximate analysis was performed on all formulations in duplicate for the two trials. Duplicate samples per treatment were analyzed for moisture using the oven drying technique (method 985.14 AOAC, 2000), ash using the muffle oven technique (method 920.153 AOAC, 2000), fat (method 960.39 AOAC, 2000) and protein by difference.

Color Measurement

Colour measurements (L*, a*, b*) were performed using a HunterLab MiniScan_ XE Plus Spectrophotometer 45/0 LAV, 2.54-cm-diameter aperture, 10° standard observer (Hunter Associates Laboratory, Inc., Reston, VA, USA). Measurements consisted of readings taken at three different positions on the top slice from four packages from each treatment after removal of the plastic bag. The same readings were done on the second slice (directly below the slice exposed to light). Three measurements on two meat slices for a total of six measurements for each treatment were taken.

pH Determination

The pH analysis of the ground turkey bologna was performed using a pH meter (Accumet Basic AB15, Fisher Scientific, Pittsburgh, PA 15238, Model No. AB15, Serial No.AB81210535). pH was determined by blending the samples with distilled, de-ionized water in a 1:9 ratio. Duplicated pH measurements were taken for each treatment.

TBARS Analysis

Lipid oxidation was measured by the modified 2-thiobarbituric acid reactive substances (TBARS) test as described for cured meats (Zipser and Watts 1962). TBARS values were reported as mg of malonaldehyde equivalents/kg of meat sample. Each treatment had measurements made in duplicate.

Consumer Sensory Panel

In order to evaluate the turkey bologna samples and the purchase intent by consumers, an untrained consumer panel consisting of 75 individuals was conducted using the sensory facilities at the Food Science and Human Nutrition Department at the University of Florida. The booths were equipped with a computerized system (Compusense five, Compusense®, Ontario, Canada) in a room illuminated with white light. All the instructions were given in detail on the computer for the panelists. There were pass-through hatches to provide samples to panelists. The Compusense five program, a sensory and consumer research data collection program from Compusense®, was used during the consumer panel. During evaluation, the panelists were situated in private booths under incandescent /fluorescent light. The turkey bologna slices were cut into four pieces of approximately 3 cm x 3 cm from the center and were served cold. The turkey bologna samples were served randomly to the panelists. Each panelist was given crackers, water, a number (panelist number) to input in the computer, and instructed to lift the hatch' door completely once finished. The panelists were instructed to evaluate the four products for appearance, aroma, overall acceptability, texture, and flavor. A 9-point hedonic scale was used where 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like or dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely.

Purchase intent was evaluated on the following scale: 1=definitely would not, 2=probably would not, 3=might or might not, 4=probably would, 5=definitely would. In addition the consumers were also asked to rank the samples for preference. The panelists were also asked to identify their sex, age group, how often he/she consumed bologna, and whether he/ she would buy the products. The data were collected automatically at the end of the sensory session and printed for analysis.

Microbiological Analyses

The turkey bologna samples were analyzed for *Staphylococcus aureus, Salmonella, Listeria monocytogenes,* total anaerobes, total psychrotrophs, lactic acid bacteria and total aerobes. All media (Difco Laboratories, Detroit, MI 48232-7058) and materials used for the cultivation and maintenance of the bacteria were purchased from Fisher Scientific (Pittsburgh, PA 15238).Twenty-five grams of turkey bologna from each formulation were placed in sterile 18 x30 cm Fisherbrand stomacher bags (400 ml, Fisher Scientific, Pittsburgh, PA 15238) along with 225 ml of sterile 0.1% peptone water (Cat. No. DF01897-17-4).The stomacher bags were massaged by hand for two minutes to loosen any surface bacteria. One ml of the sample rinse was transferred to a test tube containing nine ml of sterile 0.1% peptone water from which 10^{-1} to 10^{-6} serial dilutions were prepared for each treatment. 1µl from the dilutions was pipetted and spread (using a glass hockey stick which was flame sterilized before spreading) onto the plates. 0.1 ml aliquot of each dilutionwas spread onto Xylose Lysine Desoxycholate Agar (XLD, Cat. No. DF0788-17-9) for *Salmonella* colonies, Plate Count Agar (PCA, Cat. No. DF0479-17-3) for total psychrotrophs counts, Anaerobic Agar (Cat. No. DF0536-17-4) for total anaerobes, Tryptic Soy Agar (TSA,Cat. No. DF0369-17-6) for total aerobes, Oxford Agar (Cat. No. DF0225-17-0) for *Listeria monocytogenes*, Oxford

mediaSupplement (Cat. No. DF0214-60-9), Remel Mannitol Salt Agar (Cat. No. 453902) for *Staphylococcus aureus*, APT Agar (Cat. No. DF0654-17-0) for lactic acid bacteria, and AnaeroGen™ 3.5L packets from Remel (Cat. No. 6535) were used in plastic anaerobic jars for the generation of anaerobic conditions. All plates were done in duplicate. The PCA plates were stored at 7°C for 10 days. The TSA plates, Mannitol Salt Agar, Modified Oxford Agar plates, MacConkey Sorbitol Agar plates, and APT plates were incubated for 48 hrs at 35 ± 1°C. The XLD, and Anaerobic Agar plates were stored for 24 ± 2 hrs at 37 ± 1°C. After incubation, suspected colony forming units (CFU) from each plate were counted, recorded and averaged.

Statistics

Statistical Analysis Software's (SAS Institute Inc., version 9.1, Cary, NC, U.S.A.) Proc GLM with a Tukey's studentized range test was used to separate means and determine least significant differences with $\alpha = 0.05$.The model accounted for repetition and analyzed for week, treatment, and week x treatment interactions. Measurements were deemed significant if $P < 0.05$ for Tukey's studentized range test.

Results and Discussion

Proximate Composition

Table 5-4 illustrates the proximate analyses for the different formulations of bologna. The moisture, treatment 3 was significantly higher ($P < 0.05$) at 72.28%. Fat content was 5.58 % while ash was around 2.78% with no significant differences between the treatments ($P > 0.05$). The protein content for treatment 3 was significantly lower ($P < 0.05$) all the other treatments. All the treatments were treated the same except the fact that the source of nitrite was different. Treatment 3 had 156 ppm of natural source of nitrite and 469 ppm of natural source of ascorbic acid. The differences

may be attributed to the presence of crude protein present in the celery juice powder (Figure 3-1). The same assumptions may be true for the cherry juice powder, although no analysis was conducted in this study for cherry powder.

Thiobarbituric Acid Reactive Substances (TBARS)

Table 5-5 shows the TBARS values for the turkey bologna products over 10 weeks storage time. In general except for turkey bologna treated with 0.20% Veg Stable™ 504 plus 0.20% Veg Stable™ 515, all TBARS were similar through 10 weeks. TBARS for treatment 2 were higher ($P < 0.05$) at week 5 when compared to weeks 7, 8 and 9. The values recorded for week 1 were however similar.

pH Analysis

In general, the pH values decreased as storage time increased (Table 5-6). At weeks 6 and through weeks 10, all pH values for all treatments were lower ($P < 0.05$) when compared to pH values on week 0. pH values varied from 6.30 to 5.06. For week 1, except for treatment 3 that showed a lower value, there were no differences ($P > 0.05$) between treatments. Weeks 2 to 4, and 6-10 did not show any significant differences between the treatments. Treatment 3 and 4 during week 5 had a lower pH value than treatment 1, but were not different from treatment 2. For treatment 1, pH values decreased from 6.3 to 5.12 through the 10 week storage time. Week1, 2 and 5 were not different ($P > 0.05$). From week 2 to 6 there were similarities in pH values. Week 3 to 10 were not different ($P > 0.05$) and varied from 5.53 to 5.512. Treatment 2 pH values changed from 6.10 to 5.29. No differences were noted between weeks 1 to 5, and 8; in addition weeks 3 to 10 were not different. For treatment 3, weeks 1 and 2 were not different, and weeks 3 to 10 were similar. pH values varied between 6.21 to 5.07. For treatment 4, the pH values measured were between 6.35 to 5.06 through the 10

weeks storage time. Weeks 1 and 2 were not different ($P > 0.05$) from each other, but had higher values than weeks 3 through 10. Week 4 pH value was significantly lower ($P < 0.05$) than all the weeks during the shelf life study. The decrease in pH could be attributed to microbes which were producing by-products such as acetic acid, lactic acid. In addition, the composition of the celery juice powder and the cherry juice powder may have provided substrate for microorganisms. This is supported by Pexera et al. (2002) who suggested that a decrease in pH in meat to depend on the availability of fermentable carbohydrates. Cooked meat pH value initially will not affect microbial growth, but during storage the pH changes from 6.5 to 5.0 due to Lactic Acid Bacteria (LAB) (Dykes et al. 1991; Borch et a.l, 1996).

Objective Color Analyses

L* values measured directly on the first slice exposed to light (Table 5-7) ranged from 66.96 to 56.21. No differences ($P > 0.05$) were noted between the four treatments for week1. At the exception of weeks 5 and 8, treatment 3 L* values were significantly ($P < 0.05$) lower than values for treatment 1, 2 and 4. Treatment 1 L* values' varied from 63.04 to 66.69 and slightly increased over the 10 weeks. Treatment 2 L* values for week 1 was lower than all other weeks. For weeks 2 to 10, there was an increase overall. L* values for treatment 3 changed from 59.05 to 56.21 and at the exception of week 1, there was no differences overall. Treatment 4 values ranged from 62.53 to 66.22 and with a lower value on week1 and weeks 2, 5, 7 and 8 showed similarities. L* values for the second slice directly below the one exposed to light ranged from 56.08 to 65.73 during the shelf life study. Except for week 1 that had a significantly lower L* value ($P < 0.05$) than the other weeks, weeks 2 to 10 were not significantly different from each other ($P > 0.05$) for treatment 1 and 2. Treatment 3 L* values' varied between

56.08 and 64.88, and were lower than all the other treatments, through the 10 weeks storage time. L* values for treatment 4 did not significantly increase over time ($P > 0.05$) and changed between 62.16 and 64.87. Overall, treatment 3 had the lowest L* values through time.

a* values (Table 5-8) fluctuated from 4.75 to 11.89. Treatment 3 revealed a redder color through time overall, at the exception of week 8. Treatment 1 showed the lowest a* values through the 10 weeks storage time, with values ranging from 4.75 to 8.58. With variation in a* values form 5.40 to 9.76, no differences were found between weeks 2 to 5. Weeks 1, 8, and 10 were similar with an average a* value of 5.82. Treatment 3 a* values' ranged from 8.32 to 11.89 and weeks 1 to 2, 4 to 5, and 9 to 10 were not different ($P > 0.05$). Week 8 displayed the lowest value. Overall, a* values for treatment 3 were higher than the values for treatment 4. Treatment 4 displayed a* values that ranged from 6.24 to 10.05. Weeks 2 to 7 and 10 were not different ($P > 0.05$). The redness of treatment 4 products was close to the values of treatment 3. The second slice a* values were different between treatments. Treatment 1 values varied from 7.04 to 10.74, and were significantly lower on week 1 and 10 ($P < 0.05$). Values from weeks 2 to 6 were not significantly different from each other and increased overall. Treatment 2 values ranged between 6.99 and 11.60. Weeks 2 to 6 were significantly different from the other weeks and were higher. The lower a* value was recorded for week 7. During the 10 weeks storage time, treatment 4 a* values varied between 8.53 and 11.88, and was significantly higher for weeks 2 to 6. Weeks 1 and 7 to 9 were not different ($P > 0.05$) from each other. There was an overall decrease over time.

b* values ranged from 20.09 to 29.69 (Table 5-9) for the slices that were directly exposed to light. Treatment 3 was overall significantly different from all the other treatments. Treatment 1, 2, and 4 had similar b* values, with treatment 4 having lower values and being less yellow than the other samples. With treatment 1 values' ranging from 21.38 to 23.84, weeks 1, 2, 9 were significantly different ($P < 0.05$) from each other. Weeks 3 to 8 and 10 were not different from each other ($P > 0.05$). Overall, b* values did not change over the 10 weeks storage time. Treatment 2 b* values' varied from 20.40 to 23.31 and at the exception of week 8, it decreased slightly. Treatment 3 values translated into an increase in yellowness with b* values between 27.48 and 29.69. For treatment 4, b* values ranged from 20.09 to 22.37 and did not change overall. Except for weeks 7 and 8 (which were elevated) and week 10 (which had the lowest value), samples in treatment 4 were not as yellow as the samples in treatment 3.b* values on the second slice directly below the first slice exposed to light ranged from 17.77 to 30.27. Treatment 1 had b*values between 19.53 and 22.04 and they were not different ($P > 0.05$) for week 1 to7. Weeks 8 to 10, 1 and 3 to 7 were not different ($P > 0.05$) from each other. Except for week 5 in which the color was darker, b* values were not different for treatment2. Through the 10 weeks, week5 (treatment 3) had the lowest b* value (20.26) while week 2 had the highest (30.27). Weeks 1 to 4, 6 and 8 to 10 were not different ($P > 0.05$) from each other. In addition, weeks 6 to 10 were not different from each other ($P > 0.05$). b* values ranged between 17.77 and 20.32 for treatment 4. Weeks 9 and 10 were different ($P < 0.05$) from all other treatments. Overall, through time treatment 3 had a significantly higher value than the other treatments and was lighter. Cooking greatly affects the color of meat color. Denatured metmyoglobin or

cooked meat color resulted in a lighter color for cooked chicken thigh meat. L* value increased from 45.50 to 67.40 (almost 50 %), b* value changed from 6.2 to 16.7, and a* value decreased slightly (Barbut, 2002). Nitrite added to turkey ham produced cured meat color, nitrosohemochrome which is stable once the pigment is formed and cooked. Exposure to light can cause color fading and result in the oxidation of the meat pigment which forms oxidized porphyrin.

Cooked product packaged in transparent packaging material is more affected. Meat color is also more damaged by fluorescent light (with high proportion of ultraviolet light) than incandescent light at the same light intensity. For example, chicken frankfurters had the following color measurements: L* value was 53.17, a* 20.03, and b* 20.34 on the outside (Barbut, 2002). On the inside of the frankfurter, the values measured were 55.22, 19.83, and 22.19 respectively for L*, a*, and b*. When the chicken frankfurters (outside) were exposed to ultraviolet light, L*, a*, and b* were respectively 60.00, 16.21, and 18.43. The chicken frankfurters became lighter (more white), less red, and less yellow. This is comparable to the bologna slices that were exposed to light directly and indirectly (second slice), only for the a* values. The bologna slices exposed to light were less red than the slices that were located directly below that first slice. Partial oxidation of the meat pigment may be attributed to the decrease in the redness of the turkey bologna slices.

Microbiology

No *Staphylococcus aureus*, *Salmonella*, *Listeria monocytogenes* microorganisms were detected during the storage period (Tables not shown). Aerobic counts (Table 5-10) varied between 4.51 and 6.16 and there were no differences through time for treatment 1. Treatment 2 and 3 did not show any differences through time ($P > 0.05$)

and bacterial counts ranged from 4.05 and 3.70 to 6.11 and 5.42 respectively. Aerobic counts for week 1 were different ($P < 0.05$) from week 4, which had the lowest values. This could be attributed to the oxygen being present in the package and the increase in surface area of the bologna slices that were layered in the packages. During the first trial the packages of turkey bologna containing the celery juice powder with or without cherry powder swell, hinting to gas production by the microorganisms present (Barbut, 2002).

Anaerobic bacteria counts (Table 5-11) varied from 2.99 to 5.72 through the shelf life study. Treatment 1 averaged around 4 log CFU/ gram and were not different through time overall ($P > 0.05$). Treatment 2 varied between 4.48 and 5.68 and weeks did not show any significant differences through time ($P > 0.05$). Treatment 3 had the lowest counts of anaerobic bacteria and ranged from 2.99 to 3.76. Treatment 4 was overall not significantly different through time, and varied between 4.21 and 5.72 log CFU /gram.

As storage time increased, Lactic Acid bacteria (Table 5-12) increased for all treatments except for treatment 3. Bacterial counts varied between 2.15 and 6.85, with treatment 4 with modern cure showing significantly lower counts through time overall. Treatment 1 had up to 6 log CFU/gram when a natural source of nitrite alone was used at 0.20%. When combined with a natural source of ascorbic acid (0.20%), no decrease was noticed in the bacteria counts. Usage of 156 ppm equivalent of natural nitrite and 469 ppm of natural ascorbic acid resulted in a slight decrease in bacterial counts over time. This decrease was not significantly different ($P > 0.05$).

The spoilage of refrigerated vacuum cooked meat was found to be associated with the presence of LAB, which constituted the major microbial group (Blickstad and Molin,

1983; Borch et al. 1996; Holley, 1997; Samelis and Georgiadou, 2000). Product composition and the flora greatly affected the species of lactic acid bacteria present. The species can range from pure to mixed culture (Korkeala and Ma¨ kela¨ , 1989).The decrease in pH observed in this study, could be attributed to the presence of LAB. During the first replication, gas formation and a decrease in pH values were recorded. Even though the specific species were not analyzed for, these findings are supported by Borch et al. 1996. Seman et al.(2002) and Cerveny et al. (2009) found that spoilage are caused by LAB when cured products are packaged anaerobically in impermeable films. Those bacteria included *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella*, and microorganisms from the genus *Brochothrix*.

The bacteria present may have produced lactic acid from glucose, along with the by products carbon dioxide and ethanol. Off-flavours, discoloration, gas formation, slime production and low pH were attributed to LAB spoilage of cooled meat products. *Leuconostoc mesenteroides* subsp. *mesenteroides* also has been also found to cause spoilage in vacuum packaged Vienna sausages (Dykes et al. 1994). *Carnobacterium viridans* (gram-positive, non-spore forming rod) has also been known to cause spoilage of vacuum packaged bologna. It is able to grow at refrigerated temperatures and is a facultative anaerobic (Holley et al., 2002). The presence of anaerobic bacteria in the turkey bologna may also be related to the presence of *Carnobacterium viridians*. Formulation, storage temperature, and packaging conditions will govern the type of spoilage that occurs. The addition of herbs, spices, and other ingredients after cooking can also be a significant source of spoilage microorganisms if these ingredients are not properly handled (Cerveny et al., 2009). Neilson and Zeuthen (1985) studied the

microflora of cooked bologna-type sausage and found that vacuum packaging resulted in the normal microflora restricting *Salmonella* and *Yersinia enterocolitica* but not *Staphylococcus aureus*. *Clostridium perfringens* and all pathogens were found to be inhibited by LAB, with greater effect when storage temperature was lower (Barbut, 2002).

Psychrotrophic bacteria counts are outlined in Table 5-13. Overall no differences were observed through time for treatments 1 and 2. The bacterial counts varied from 4.75 to 6.34 and 4.49 to 6.15 respectively. Except for week 1 that showed a lower count (3.7 to 4.96), no differences were revealed during storage ($P > 0.05$). The log CFU /gram ranged from 4.96 to 6.11. Weeks 1 and 2 were the exception for treatment 4, with the lowest counts. The bacteria counts varied from 3.47 to 6.09.

Consumer sensory panel showed promising results (Table 5-14). The panelists (53.3% males and 46.7% females) were administered demographic questions to better comprehend their purchasing decisions. The age categories of the participants included < 18 to 24 (76.80%), 25 to 34 (15.50%), 35 to 44 (1.25%), 45 to 54 (3.75%), and > 55 (2.70%). They were also asked how often they did consume turkey bologna, and 34.70% had never consumed turkey bologna, and 52% of the panelists had eaten it between once a month and less than once a year. Treatment 3 which was composed of 156 ppm Stable™ 504 and 469 ppm Veg Stable™ 515 was scored significantly lower than all the other treatments ($P < 0.05$) in appearance, aroma, texture, overall acceptability and purchase intent. For flavor, treatments 1, 2 and 3 were not different from each other ($P > 0.05$) with treatment 4 being significantly different from treatment 3. Treatments 1, 2 and 4 were also similar. Consumer panelists preferred the flavor of

the control and treatments 1 and 2. They disliked treatment 3, but were not able to distinguish the characteristic flavor or aroma.

Sindelar (2007a) found that in hams, panelists detected an atypical vegetable aroma and flavor at 0.35% in celery juice powder used in combination with a starter culture. The findings in this study are supported by Sindelar (2007a) in which celery juice powder used at 0.20% was comparable to a sodium nitrite added ham product regarding all sensory attributes. Using a celery juice powder containing pre-generated nitrite may be convenient, but may affect some of the sensory attributes in the final products. Consumers preferred the products that contained the least amounts of celery powder alone, or in combination with ascorbic acid. In addition, the spices used may also have contributed to masking the celery aroma and flavor. Consumers were not able to detect “celery” flavor even in the product that contained the highest amount of celery juice powder (treatment 3).

Conclusion

The results obtained from this study show promising results for Veg Stable™ 504 as a natural source of nitrite for use in meat curing. Overall, the color of the products containing celery juice powder, alone or in combination with a natural source of ascorbic acid (Veg Stable™ 515) were similar in color. The combination of 156 ppm of celery juice powder and 469 ppm of cherry juice powder showed a redder color through objective measurement. This specific product resulted in a darker and more yellow cured meat product which could be attributed to the tan colored celery juice powder, and the orange colored cherry juice powder. The decrease of pH over the ten weeks storage time were related to the growth of lactic acid bacteria in an anaerobic environment. Their growth did not reach the spoilage point (10^7-10^9). The cured meat

product did not develop off-odor and rancidity. No pathogenic microorganisms were detected throughout the storage time. All products including the control were deemed acceptable by the consumers. The combination of 156 ppm of celery juice powder and 469 ppm of cherry juice powder was less liked, although consumers could not detect the celery flavor. The ability to concentrate the amount of nitrite in the celery powder would be beneficial. Less celery juice powder will be needed to achieve the level of 156 ppm. In addition, a purification method will also be needed to eliminate the crude proteins, minerals, carbohydrates from the celery juice powder. More research need to be performed using Veg Stable™ 504 to study the accumulation of nitrates in the plant and the effect of the different processes on the nitrite levels.

Table 5-1. Turkey bologna formulation containing different levels of Veg Stable™ 504, Veg Stable™ 515, and modern cure

Formula Ingredients (grams)	1	2	3	4
Turkey Thigh	7,945	7,945	7,945	7,945
Water/Ice	1,986.25	1,986.25	1,986.25	1,986.25
Salt	198.63	198.63	198.63	198.63
Corn Syrup Solids	158.90	158.90	158.90	158.90
Seasonings	158.90	158.90	158.90	158.90
Modern cure[a]	------	------	------	19.83
SETB[b]	------	------	------	4.36
Veg Stable™ 504 (Celery powder)	15.89	15.89	828.93	------
Veg Stable™ 515 (Cherry powder)	------	15.89	18.04	------

[a] Modern cure, 6.25% Sodium Nitrite
[b] Sodium erythorbate
Veg Stable™ 504 (8544 ppm)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate

Table 5-2. Turkey bologna seasoning formulation

Spices	Grams/ 7,945 grams
Ground White Pepper	29.75
Ground Coriander	29.75
Ground Nutmeg	9.92
Ground Ginger	9.92
Ground Paprika	9.92
Garlic Powder	9.92

Table 5-3. Smokehouse cooking schedule for Turkey bologna

Stage	Dry Bulb	Wet Bulb	Relative Humidity	Time	Smoke
1	110	90	46	30 min	OFF
2	140	112	46	2.0 hrs	ON
3	160	130	46	4.0 hrs	OFF
4	200	170	50	To 165°F internal temperature	
5	Cold shower			5 minutes	OFF

Table 5-4. Chemical composition of the different formulations of bologna

Formulation*	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
1	70.33^{c}	5.72	20.11^{a}	2.83
2	71.27^{b}	5.50	19.61^{a}	2.61
3	72.28^{a}	5.58	18.35^{b}	2.78
4	70.79^{bc}	5.85	19.48^{a}	2.87
SEM	2.04	0.21	2.11	0.06

$^{a-c}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
SEM =Standard error of the means

Table 5-5. Least square means for the interactions of treatment combined storage time for Thiobarbituric acid reactive substances (TBARS) in turkey bologna

	Storage time (wk)									
Treatments*	1	2	3	4	5	6	7	8	9	10
1	1.17	1.67	1.66	1.02	1.28	1.18	1.57	1.78	1.96	1.17
2	1.94^{wx}	1.71^{wx}	1.40^{wx}	2.05^{wx}	2.40^{w}	1.96^{wx}	1.01^{x}	1.11^{x}	1.21^{x}	1.74^{wx}
3	1.45^{wx}	1.15^{x}	1.89^{wx}	1.59^{wx}	2.30^{w}	1.99^{wx}	1.97^{wx}	1.49^{wx}	1.58^{wx}	1.44^{wx}
4	1.95	1.42	1.74	1.27	1.56	1.33	1.28	1.30	1.94	1.51
SEM	0.55	0.38	0.43	0.27	0.28	0.21	0.28	0.20	0.22	0.37

$^{w-x}$ means in same row with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
TBARS value reported as mg malonaldehyde/kg of sample
SEM =Standard error of the means

Table 5-6. Least square means for the interactions of treatment combined storage time for pH in turkey bologna

Treatments*	Storage time (wk)									
	1	2	3	4	5	6	7	8	9	10
1	$6.30^{a,w}$	6.21^{wx}	5.53^{xy}	5.56^{xy}	$5.72^{a,wxy}$	5.12^{y}	5.41^{y}	5.46^{y}	5.42^{y}	5.52^{xy}
2	$6.08^{a,w}$	6.10^{w}	5.64^{wx}	5.60^{wx}	$5.67^{ab,wx}$	5.29^{x}	5.45^{x}	5.66^{wx}	5.44^{x}	5.38^{x}
3	$5.82^{a,wx}$	6.21^{w}	5.39^{xy}	5.07^{y}	$5.37^{b,xy}$	5.12^{y}	5.16^{y}	5.14^{y}	5.13^{y}	5.14^{y}
4	$6.23^{a,wx}$	6.35^{w}	5.34^{yz}	5.06^{z}	$5.39^{b,yz}$	5.72^{xy}	5.58^{yz}	5.25^{yz}	5.54^{yz}	5.32^{yz}
SEM	0.08	0.17	0.25	0.18	0.08	0.24	0.16	0.18	0.22	0.24

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{w-z}$ means in same row with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
SEM =Standard error of the means

Table 5-7. Least square means for the interactions of treatment combined storage time for L* values in direct contact with the slice exposed to light and of 2nd slice directly below the one exposed to light in turkey bologna

	Storage time (wk)									
Treatments	1	2	3	4	5	6	7	8	9	10
1*	63.04^{z}	64.69^{a,wxy}	65.69^{a,uvw}	64.46^{a,xy}	66.30^{a,uv}	66.28^{a,uv}	64.29^{a,y}	66.69^{a,u}	65.36^{a,vwx}	66.13^{a,uv}
2*	61.88^{y}	63.74^{a,wx}	64.43^{a,vw}	65.10^{a,vw}	64.72^{b,vw}	65.64^{a,uv}	62.56^{a,xy}	64.57^{ab,vw}	65.72^{a,uv}	66.96^{a,u}
3*	56.21^{w}	58.17^{b,uv}	57.59^{b,uvw}	57.94^{b,uv}	58.49^{c,uv}	57.59^{b,uvw}	57.74^{b,uv}	59.05^{c,u}	58.97^{b,uv}	57.46^{b,vw}
4*	62.53^{y}	63.73^{a,wxy}	64.99^{a,uvw}	64.99^{a,uvw}	64.60^{b,uvwx}	65.24^{a,uvw}	64.17^{a,vwxy}	63.01^{b,xy}	66.22^{a,u}	65.81^{a,uv}
SEM	0.34	0.49	0.50	0.59	0.31	0.55	0.52	0.83	0.50	0.42
1**	62.68^{a,v}	64.64^{a,uv}	65.33^{a,u}	65.53^{a,u}	65.36^{u}	64.38^{a,uv}	64.96^{a,u}	65.23^{a,u}	65.57^{a,u}	65.73^{a,u}
2**	62.12^{a,w}	64.56^{a,u}	64.74^{a,u}	64.11^{ab,uv}	64.88^{u}	64.77^{a,u}	64.11^{a,uv}	65.51^{ab,vw}	63.61^{b,uvw}	63.91^{b,uv}
3**	56.08^{b,z}	57.65^{c,xy}	56.99^{b,yz}	58.23^{c,wxy}	64.88^{u}	59.93^{b,v}	58.62^{b,vwx}	59.25^{c,vw}	57.94c^{,wxy}	58.87^{c,vwx}
4**	61.99^{a,v}	63.09^{b,uv}	63.84^{a,uv}	62.17^{b,v}	64.79^{u}	64.65^{a,u}	63.53^{a,uv}	62.16^{b,v}	64.87^{ab,u}	64.68^{ab,u}
SEM	0.31	0.24	0.52	1.06	0.85	0.66	0.43	0.81	0.55	0.36

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{v-z}$ means in same row with different superscripts are significantly different ($P < 0.05$)
1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
L* = lightness
L values in direct contact with the slice exposed to light in turkey bologna
**L* values of 2nd slice directly below the one exposed to light in turkey bologna
SEM =Standard error of the means

Table 5-8. Least square means for the interactions of treatment combined storage time for a* values in direct contact with the slice exposed to light and of 2nd slice directly below the one exposed to light in turkey bologna

	Storage time (wk)									
Treatments	1	2	3	4	5	6	7	8	9	10
1*	4.75^{c,x}	6.87^{c,uvw}	7.21^{c,uv}	8.58^{b,u}	6.86^{b,uvw}	6.17^{c,vwx}	5.20^{b,wx}	5.60^{vwx}	4.94^{c,wx}	5.03^{c,wx}
2*	5.56^{c,x}	9.76^{b,u}	9.19^{b,uv}	8.53^{b,uv}	8.98^{ab,uv}	7.64^{b,vw}	9.82^{a,u}	5.40^{x}	7.61^{b,vw}	6.51^{c,wx}
3*	10.76^{a,uvw}	11.15^{a,uvw}	11.49^{a,uv}	11.04^{a,uvw}	10.44^{a,vw}	11.89^{a,u}	10.17^{a,w}	8.32^{x}	10.52^{a,vw}	11.23^{a,uvw}
4*	7.90^{b,vw}	9.98^{b,u}	10.05^{ab,u}	9.16^{ab,uv}	8.79^{ab,uv}	8.82^{b,uv}	8.62^{a,uv}	6.24^{w}	8.02^{b,vw}	8.74^{b,uv}
SEM	0.42	0.23	0.61	0.71	0.79	0.41	0.46	0.92	0.54	0.46
1**	7.96^{c,wx}	9.43^{c,uvw}	9.86^{b,uv}	10.74^{u}	9.59^{b,uvw}	10.58^{u}	8.36^{bc,vwx}	8.72^{bc,vw}	8.66^{c,vwx}	7.04^{c,x}
2**	8.36^{bc,w}	11.28^{b,u}	11.31^{a,u}	11.49^{u}	11.60^{a,u}	11.29^{u}	6.99^{c,x}	8.21^{c,wx}	9.86^{b,v}	9.74^{b,v}
3**	10.02^{a,x}	12.83^{a,u}	12.14^{a,uv}	11.90^{v}	11.60^{a,vw}	11.82^{v}	11.55^{a,vw}	11.99^{a,uv}	10.78^{a,wx}	11.29^{a,vw}
4**	9.66^{ab,v}	11.55^{b,u}	11.51^{a,u}	11.88^{u}	11.07^{a,u}	11.37^{u}	9.53^{b,vw}	9.73^{b,v}	9.36^{bc,vw}	8.53^{b,w}
SEM	0.38	0.24	0.26	0.85	0.30	0.37	0.48	0.31	0.25	0.42

$^{a-c}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{u-z}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
a* = redness
a values in direct contact with the slice exposed to light in turkey bologna
**a* values of 2nd slice directly below the one exposed to light in turkey bologna
SEM =Standard error of the means

Table 5-9. Least square means for the interactions of treatment combined storage time for b* values in direct contact with the slice exposed to light and of 2nd slice directly below the one exposed to light in turkey bologna

	Storage time (wk)									
Treatments	1	2	3	4	5	6	7	8	9	10
1*	23.33^{b,uv}	23.84^{b,u}	22.63^{b,vw}	22.55^{b,vw}	22.23^{b,wx}	22.21^{b,wx}	22.95^{b,uvw}	22.94^{b,uvw}	21.38^{b,x}	22.04^{b,wx}
2*	22.79^{b,uv}	22.43^{bc,uvw}	22.25^{b,uvwx}	21.85^{b,vwx}	22.94^{b,uv}	22.69^{b,uv}	20.40^{c,y}	23.31^{b,u}	21.42^{b,wxy}	21.16^{c,xy}
3*	29.39^{a,u}	29.69^{a,u}	29.59^{a,u}	29.55^{a,u}	28.51^{a,uv}	26.93^{a,v}	29.14^{a,uv}	27.48^{a,uv}	28.01^{a,uv}	28.10^{a,uv}
4*	21.36^{c,uvwx}	21.85^{c,uvw}	20.68^{b,wx}	21.99^{b,uvw}	22.05^{b,uvw}	21.93^{b,uvw}	22.33^{b,uv}	22.37^{b,u}	20.74^{b,vwx}	20.09^{d,x}
SEM	0.34	0.47	0.70	0.41	0.68	0.89	0.41	0.51	0.32	0.24
1**	21.53^{b,uv}	22.04^{b,u}	21.35^{bc,uv}	21.08^{b,uvw}	21.30^{uv}	21.12^{b,uvw}	20.32^{uvw}	19.53^{c,w}	19.79^{b,vw}	19.79^{b,vw}
2**	21.13^{b,u}	21.41^{b,u}	21.46^{b,u}	20.43^{b,u}	16.93^{v}	21.29^{b,u}	21.51^{u}	20.76^{b,u}	19.08^{b,uv}	19.14^{bc,uv}
3**	28.45^{a,u}	30.27^{a,u}	29.31^{a,u}	28.99^{a,u}	20.26^{w}	27.22^{a,uv}	24.95^{v}	28.04^{a,uv}	26.92^{a,uv}	27.26^{a,uv}
4**	20.19^{b,uvw}	21.00^{b,uv}	20.32^{c,uvw}	21.02^{b,u}	19.94^{uvw}	19.72^{b,vw}	19.48^{w}	20.27^{bc,uvw}	17.81^{c,x}	17.77^{c,x}
SEM	0.41	0.36	0.29	0.76	1.70	0.77	1.76	0.34	0.24	0.41

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{v-z}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
b* = yellowness on a 0 to 100 white scale
b values in direct contact with the slice exposed to light in turkey bologna
**b* values of 2nd slice directly below the one exposed to light in turkey bologna
SEM =Standard error of the means

Table 5-10. Least square means for the interactions of treatment combined storage time for Aerobic counts in turkey bologna

	Storage time (wk)									
Treatments*	1	2	3	4	5	6	7	8	9	10
1	4.51^{a}	4.84^{a}	5.06	5.88	6.06^{a}	6.16	6.08^{a}	6.09^{a}	6.06^{a}	5.91
2	4.05^{b}	4.19^{b}	4.88	5.89	5.94^{a}	6.11	5.93^{a}	5.82^{ab}	5.71^{ab}	5.40
3	3.70^{c}	4.06^{b}	5.33	5.42	5.33^{ab}	5.37^{ab}	5.27^{ab}	5.16^{ab}	5.26^{ab}	5.18
4	3.00^{d,x}	3.29^{c,wx}	3.64^{wx}	5.10^{w}	4.96^{b,wx}	4.95^{b,wx}	4.83^{b,wx}	4.06^{b,wx}	3.99^{b,wx}	4.07^{wx}
SEM	0.12	0.23	0.87	1.67	1.69	1.76	1.70	1.52	1.37	1.32

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{v-z}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
Bacterial counts were reported in log CFU/ gram
SEM =Standard error of the means

Table 5-11. Least square means for the interactions of treatment combined storage time for Anaerobic counts in turkey bologna

	Storage time (wk)									
Treatments*	1	2	3	4	5	6	7	8	9	10
1	4.54^{wx}	4.57^{wx}	3.34^{x}	4.96^{w}	4.98^{w}	4.82^{w}	4.76^{wx}	4.68^{wx}	4.56^{wx}	4.62^{wx}
2	4.64	4.48	4.73	5.68	5.65	5.33	5.22	5.18	5.52	5.29
3	2.99	2.91	2.90	3.76	3.70	3.62	3.45	3.47	3.37	3.28
4	4.21^{x}	4.19^{x}	4.71^{wx}	5.72^{w}	5.63^{wx}	5.46^{wx}	5.45^{wx}	5.08^{wx}	5.14^{wx}	5.27^{wx}
SEM	1.25	1.18	1.37	1.87	1.85	1.70	1.63	1.58	1.45	1.38

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{v-z}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
Bacterial counts were reported in log CFU/ gram
SEM =Standard error of the means

Table 5-12. Least square means for the interactions of treatment combined storage time for Lactic acid bacteria counts in turkey bologna

	Storage time (wk)									
Treatments*	1	2	3	4	5	6	7	8	9	10
1	$4.61^{b,y}$	$4.74^{b,y}$	$5.27^{ab,xy}$	6.85^{w}	6.65^{w}	$6.29^{ab,wx}$	$6.36^{ab,w}$	$6.25^{ab,wx}$	$6.13^{ab,wx}$	$6.03^{ab,wx}$
2	$4.16^{b,y}$	$5.16^{a,xy}$	$5.84^{a,wx}$	6.72^{w}	6.82^{w}	$6.77^{a,w}$	$6.63^{a,w}$	$6.59^{a,w}$	$6.46^{a,wx}$	$6.36^{a,wx}$
3	$5.33^{a,wx}$	$5.06^{a,x}$	$5.84^{a,wx}$	6.06^{w}	6.05^{w}	$5.73^{b,wx}$	$5.64^{bc,wx}$	$5.77^{bc,wx}$	$5.58^{bc,wx}$	$5.51^{bc,wx}$
4	$2.15^{c,x}$	$3.81^{c,wx}$	$4.15^{b,w}$	5.34^{w}	5.09^{w}	$4.80^{c,w}$	$5.26^{c,w}$	$5.24^{c,w}$	$5.11^{c,w}$	$4.98^{c,w}$
SEM	0.15	0.08	0.58	1.28	1.16	0.82	0.72	0.64	0.47	0.45

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{v-z}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
Bacterial counts were reported in log CFU/ gram
SEM =Standard error of the means

Table 5-13. Least square means for the interactions of treatment combined storage time for Psychrotrophic counts in turkey bologna

	Storage time (wk)									
Treatments*	1	2	3	4	5	6	7	8	9	10
1	$4.77^{a,x}$	$4.75^{a,x}$	$5.09^{ab,wx}$	6.34^{w}	5.66^{wx}	5.83^{wx}	5.83^{wx}	$5.76^{ab,wx}$	5.64^{wx}	$5.57^{ab,wx}$
2	$4.60^{ab,wx}$	$4.49^{ab,x}$	$5.18^{ab,wx}$	6.04^{wx}	6.15^{w}	6.05^{wx}	5.76^{wx}	$5.42^{b,wx}$	5.92^{wx}	$5.77^{ab,wx}$
3	$4.96^{a,x}$	$5.21^{a,wx}$	$5.61^{a,wx}$	6.03^{w}	6.11^{w}	5.99^{w}	5.97^{w}	$5.96^{a,w}$	5.86^{wx}	$5.80^{a,wx}$
4	$3.71^{b,xy}$	$3.47^{b,y}$	$4.79^{b,wxy}$	5.95^{wx}	6.09^{w}	5.81^{wx}	5.48^{wxy}	$5.61^{ab,wxy}$	5.56^{wxy}	$5.17^{b,wxy}$
SEM	0.26	0.27	0.64	1.60	1.43	1.28	1.01	0.79	0.77	0.64

$^{a-b}$ means in same column with different superscripts are significantly different ($P < 0.05$)
$^{v-z}$ means in same column with different superscripts are significantly different ($P < 0.05$)
*1 = 0.20% Veg Stable™ 504; 2 = 0.20% Veg Stable™ 504 + 0.20% Veg Stable™ 515; 3 = 156 ppm Veg Stable™ 504 + 469 ppm Veg Stable™ 515; 4 = Modern cure + 547 ppm of Sodium erythorbate
Bacterial counts were reported in log CFU/ gram
SEM =Standard error of the means

Table 5-14. Least square means for the interactions of treatment combined storage time for sensory attributes of turkey bologna

	Sensory attributes∞					
Product*	Appearance	Aroma	Texture	Flavor	Overall Acceptability	Purchase Intent**
1	5.93[a]	6.15[a]	5.73[a]	5.87[ab]	6.00[a]	2.65[a]
2	5.99[a]	6.03[a]	5.69[a]	5.84[ab]	5.92[a]	2.69[a]
3	4.53[b]	5.41[b]	5.17[b]	5.33[b]	5.25[b]	2.31[b]
4	5.79[a]	6.03[a]	5.64[ab]	5.97[a]	5.95[a]	2.64[ab]
SEM×	0.14	0.13	0.13	0.15	0.12	0.09

[a -b] Means within the same column with different superscripts are different ($P < 0.05$)

*1= 0.20% Stable™ 504, 2= 0.20% Stable™ 504 + 0.20% Veg Stable™ 515, 3= 156 ppm Stable™ 504 + 469 ppm Veg Stable™ 515, and 4= 156 ppm Prague powder + 550 ppm of Sodium erythorbate (Control)

∞Sensory attributes = consumer panel scores using a 9 point hedonic scale where 1= dislike extremely, 5 = neither like nor dislike, 6= like slightly, 9= like extremely

**Purchase Intent: 1=definitely would not, 2=probably would not, 3=might or might not, 4= probably would

×SEM= standard error of the means for uncured with the intention of replacing nitrite and commercial nitrite added

CHAPTER 6
GENERAL CONCLUSION

The purpose of this study was to investigate celery juice powder Veg Stable 504™ and compare it to Prague powder #1 or modern cure which is currently used in cured meat products. Due to its instant curing effect, Veg Stable 504™ had the potential to satisfy both consumers and processors. Consumers requesting a natural source of nitrite will be pleased, and simultaneously cured meat manufacturers will save time and money. For example Veg Stable 501™ (natural source of nitrate) requires the use of a starter culture in combination with an incubation time to produce products similar to those cured with modern cure.

The hypothesis for this project was to prove that there were no differences between products cured with commercially available sodium nitrite (modern cure) and uncured products with the intention of replacing sodium nitrite, using CJPPN. After analysis, the composition of the CJPPN was found to contain crude protein, fibers, carbohydrates and minerals, in addition to the nitrite. In addition, nitrite which is a very reactive substance was found to be affected by the packaging material, light, and oxygen exposure. Moreover, the nitrite content decreased over time. Holding time did not have an effect on the color, pH, and residual nitrite. Veg Stable 504™ was found to be an instant curing ingredient that did not necessitate incubation time, nor a starter culture. The challenge test designed to test the effectiveness of Veg Stable 504™ against synthetic sodium nitrite using *Clostridium botulinum* in a meat system showed some mixed results. Depending on the medium some effect was shown. Solubility of the celery and cherry juice powders in the selected broth or media was also an issue. More research will need to be performed to thoroughly evaluate the safety of the natural cure.

The products manufactured with celery powder juice containing the pre-generated sodium nitrite had a comparable appearance, aroma, texture, and flavor to the products cured with synthetic sodium nitrite. The exception was the product that contained ten times the recommended usage level (156 ppm of celery juice powder and 469 ppm of cherry juice powder), and was not well liked by the consumer panelists. Less residual nitrite was present in the beef frankfurter products manufactured using different levels of Veg Stable 504™ at 0.20, 0.30, and 0.40%. The more celery juice powder was used, the more residual nitrite was present, but significantly less than the control (modern cure).

APPENDIX A
VEGETABLE JUICE POWDER PRODUCT INFORMATION (FLORIDA FOOD PRODUCTS, INC.)

FLORIDA FOOD PRODUCTS, INC.
2231 W. Hwy 44 • Post Office Box 1300 • Eustis, Florida USA 32727-1300
352/357-4141 • Fax 352/483-3192 • E-Mail: contact@floridafood.com

Allergens/Sensitizing Agents

The Florida Food Products VEG STABLE™ 504 contains the following, as indicated by checked responses:

ALLERGENS	Yes	No
Milk or Milk products (butter, buttermilk, casein, cream, sodium caseinate, curds, whey, cheese, yogurt, lactose, lactoglobulin, etc.)		x
Eggs or Egg products (mayonnaise, albumin, lecithin, etc.)		x
Soybeans or Soybean products		x
Peanuts or Peanut products		x
Tree Nuts (almonds, Brazil, cashew, pecan, walnut, pine nuts, hazelnut, macadamia, chestnuts, pistachio, etc.)		x
Wheat and Wheat products (bran, cereal extracts, bulgar, farina, wheat germ, graham flour, malt, gluten, starch, flour, semolina, etc.)		x
Grains (barley, corn, millet, oats, rice, rye, sorghum, etc.)		x
Fish or Seafood (any type and their products)		x
Shellfish (shrimp, prawns, crab, lobster, crawfish, oysters, clams, scallops, etc.)		x
Seeds or Seed products (cottonseed, poppy, sesame, sunflower, caraway seeds, etc.)		x
Celery	x	

SENSITIZING AGENTS		
FD&C Yellow #5 or #6		x
Sulfites		x
Monosodium Glutamate		x

Comments: _____

The information above is to the best of our knowledge, truthful, accurate and presented in good faith. However, no warranty or guarantee is implied or inferred.

Growers and Processors of Food and Cosmetic Ingredients
Visit our Web Site at: www.floridafood.com

FLORIDA FOOD PRODUCTS, INC.
2231 W. Hwy 44 • Post Office Box 1300 • Eustis, Florida, USA 32727-1300
352/357-4141 • Fax 352/483-3192 • E-Mail: contact@floridafood.com

Allergens/Sensitizing Agents

The Florida Food Products Veg Stable™ Cherry 515 contains the following, as indicated by checked responses:

ALLERGENS	Yes	No
Milk or Milk products (butter, buttermilk, casein, cream, sodium caseinate,		x
Eggs or Egg products (mayonnaise, albumin, lecithin, etc.)		x
Soybeans or Soybean products		x
Peanuts or Peanut products		x
Tree Nuts (almonds, Brazil, cashew, pecan, walnut, pine nuts, hazelnut,		x
Wheat and Wheat products (bran, cereal extracts, bulgar, farina, wheat germ,		x
Grains (barley, corn, millet, oats, rice, rye, sorghum, etc.)		x
Fish or Seafood (any type and their products)		x
Shellfish (shrimp, prawns, crab, lobster, crawfish, oysters, clams, scallops, etc.)		x
Seeds or Seed products (cottonseed, poppy, sesame, sunflower, caraway seeds,		x
Celery		x

SENSITIZING AGENTS		
FD&C Yellow #5 or #6		x
Sulfites		x
Monosodium Glutamate		x

Comments: _____

_____

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FLORIDA FOOD PRODUCTS, INC.

Product Specifications and Information

PRODUCT NAME –VEG STABLE™ CHERRY 515

INGREDIENT LISTING- Cherry powder & Organic evaporated cane juice

USE – Meats, dry soups, beverages, health supplements, cosmetics and seasoning blends.

DESCRIPTION – Veg Stable™ CHERRY is a dried powder derived from fresh cherries and organic evaporated cane juice. Anti-caking agents may be added.

USE RATE – 0.1 - 0.5%

GENERAL	Veg Stable™ CHERRY
APPEARANCE	Tan, free flowing powder
MOISTURE	≤5%
pH (3 % solution)	5.8-6.2
TOTAL PLATE COUNT	20,000 cfu/gm max.
YEAST & MOLD	100 cfu/gm max.
TOTAL COLIFORMS	Negative
PRESERVATIVES	None

PACKAGING - Available in 20 kg. vacuum-sealed foil bag-n-box.

SHELF STABILITY AND STORAGE CONDITIONS - Store in cool, dry area not to exceed 90°F. when properly stored, the recommended shelf life is two years.

AVAILABILITY – Veg Stable™ CHERRY is available year round from inventory. Advance notice for quantities above 5,000 lbs. is required.

PROCESSING SEASON - December - June

The technical information and suggestions for use contained herein are believed to be reliable, but they are not to be construed as warranties and no patent liability can be assumed. Specifications are subject to change based on raw material variations.

Revised: 9/25/07
Issued:2/1/07

Growers and Processors of Food and Cosmetic Ingredients
2231 W. Hwy 44 Eustis, FL 32727
(352) 357-4141

SP515

FLORIDA FOOD PRODUCTS, INC.
2231 W. Hwy 44 • Post Office Box 1300 • Eustis, Florida USA 32727-1300
352/357-4141 • Fax 352/483-3192 • E-Mail: contact@floridafood.com

PRODUCT NAME – Veg Stable™ 504

INGREDIENT DECLARATION – Celery powder (or natural flavors), sea salt.

USE – Meats, natural curing processes

DESCRIPTION – Veg Stable™ 504 is a water-soluble dried powder consisting of celery powder and sea salt. Veg Stable™ 504 is high in naturally occurring nitrites that are standardized with sea salt. Anti-caking (silicon dioxide) agents may be added.

GENERAL SPECIFICATIONS	Veg Stable™ 504
APPEARANCE	Tan to Brown free flowing powder
MOISTURE	≤5%
pH (5% solution)	8.5 - 10
TOTAL PLATE COUNT	20,000 cfu/gm
YEAST & MOLD	100 cfu/gm max.
TOTAL COLIFORMS	Negative
PRESERVATIVES	None

PACKAGING - Available in 44.1 lb. vacuum-sealed foil bag-n-box.

SHELF STABILITY AND STORAGE CONDITIONS - Store in a cool and dry area not exceeding 70°F. When properly stored vacuum sealed, the recommended shelf life is one year.

SUGGESTED USAGE - to 0.2 - 0.4% of gross weight

AVAILABILITY – Veg Stable™ 504 is available year round from inventory. Advance notice for quantities above 2,200 lbs. is required.

PROCESSING SEASON - December - June

The technical information and suggestions for use contained herein are believed to be reliable, but they are not to be construed as warranties and no patent liability can be assumed. Specifications are subject to change based on raw material variations.

MATERIAL SAFETY DATA SHEET

OSHA Standard 29 CFR 1910.1200

Manufacturer's Name:	Florida Food Products, Inc.
Address:	2231 W. Highway 44 Eustis, FL 32726
Phone:	(352) 357-4141
Fax:	(352) 483-3192

SECTION I: PRODUCT IDENTITY INFORMATION

Product Identity: Veg Stable™ #504

SECTION II: CHEMICAL COMPOSITION & DATA

CAS Number: Not Applicable (Mixture)

Chemical Name: Not Applicable

Description: Celery powder, sea salt, & silicon dioxide for anti-caking may be added.

SECTION III: HAZARD IDENTIFICATION

This product is a "mixture" of which there is no health hazard data available. Hazard communication standards require that such mixtures should be assumed to present the same health hazards as do hazardous components that constitute at least 1% of the mixture (0.1% for carcinogens).

Hazards of individual components may be altered by being part of a mixture.

Nuisance dust may cause irritation to the respiratory tract, eyes and skin causing sneezing. Repeated contact may cause allergic dermatitis.

MATERIAL SAFETY DATA SHEET
OSHA Standard 29 CFR 1910.1200

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SECTION IV: EMERGENCY & FIRST AID MEASURES

Eye Contact: Remove contact lenses, flush eyes with water for 10 minutes. If irritation persists, see a Physician.

Skin Contact: Remove contaminated clothing. Wash the affected area with soap and water. If irritation persists, see a Physician.

Ingestion: None usually needed.

Inhalation: Remove to fresh air. If symptoms persist, see a Physician.

SECTION V: FIRE FIGHTING MEASURES

Flash Point:	Not Applicable
Extinguishing Media:	Foam, CO_2, Dry Chemical
Special Procedures:	None
Unusual Hazards:	None

SECTION VI: ACCIDENTAL RELEASE & DISPOSAL MEASURES

Sweep or wipe up any spilled material for disposal. Wash the spill area with soap and water to remove all residual matter.

Dispose in accordance to Local, State, and Federal Regulations.

MATERIAL SAFETY DATA SHEET
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SECTION VII: HANDLING & STORAGE

Store in cool, dry area not to exceed 70°F. When properly stored vacuum sealed, the recommended shelf life is one year.

SECTION VIII: EXPOSURE CONTROLS & PERSONAL PROTECTION

Eye Protection:	Prevent eye contact; wear safety goggles or glasses.
Gloves:	Wear gloves
Respiratory:	Wear dust respirator
Ventilation:	Use in a well ventilated area
Other Equipment:	Eye wash & safety shower

SECTION IX: PHYSICAL & CHEMICAL PROPERTIES

Physical State:	Free Flowing Powder
Appearance:	Tan to Brown
Specific Gravity:	Not Applicable
Vapor Pressure:	No Data Available
Water Solubility:	Complete
pH(5% solution):	8.5 - 10

MATERIAL SAFETY DATA SHEET
OSHA Standard 29 CFR 1910.1200

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SECTION X: STABILITY & REACTIVITY

Stability: Stable

Incompatibility: Reducing agents & acids

Hazardous Combustion of Decomposition Products: Carbon Monoxide, Carbon Dioxide and Smoke.

MATERIAL SAFETY DATA SHEET

OSHA Standard 29 CFR 1910.1200

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SECTION I: PRODUCT IDENTIFICATION EMERGENCY INFORMATION

Trade Name: Veg Stable™ Cherry 515

Description: Dried cherry powder, and organic evaporated cane juice.

SECTION II: HAZARDOUS COMPONENTS OF MIXTURES

None

SECTION III: HEALTH INFORMATION AND PROTECTION

There are no records of human irritation.

SECTION IV: FIRE AND EXPLOSION DATA

There are no fire or explosion risks associated with the product.

SECTION V: SPILL CONTROL PROCEDURE

Wash down the drain where local ordinances permit.

SECTION VI: NOTES

None

MATERIAL SAFETY DATA SHEET

OSHA Standard 29 CFR 1910.1200

Manufacturer's Name:	Florida Food Products, Inc.
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Phone:	(352) 357-4141
Fax:	(352) 483-3192

SECTION VII: TYPICAL PHYSICAL AND CHEMICAL PROPERTIES

Specific Gravity:	N/A
Vapor Pressure:	N/A
Solids:	98.0% min.
Water Solubility:	Complete
Boiling Point:	Not applicable

SECTION VIII: REACTIVITY DATA

None

SECTION IX: STORAGE AND HANDLING

Store in a cool dry area.

SECTION X: HAZARDOUS CLASSIFICATION

None

APPENDIX B
CONSUMER SENSORY EVALUATION RESPONSES

Project: TURKEY BOLOGNA 10-09-2009

Question Number:8
Question Number:8
Question Type:Comment
Question Title:Using the keyboard located in the tray under the counter, please describe the differences, if any, between the samples (please be specific).
Design:T=4, K=4, B=120

Products

Products	Code	Name
1 - 858	1	Control
2 - 681	2	0.20% CP
3 - 562	3	0.20% CP + AA
4 - 482	4	Equiv 156ppm

Results

RegCode	Name	Session	Samp-Set	Sample	
RegCode	Name	Session	Samp-Set	Sample	
1	Panelist 1	1	1	1-858	sample 858 was OK in taste but texture needs to be improved
2	Panelist 2	1	2	1-858	i bit into a chunk of crunchy fat or something. that was not good. it also has that balogne taste that i generally do not like.
4	Panelist 4	1	4	1-858	It smelled and looked like an uncooked hotdog, whch i did not like. It tasted better than expected, but the texture was too rubbery.
6	Panelist 6	1	6	1-858	the meat was soft but the ends(at the circular part) was a little hard
8	Panelist 8	1	8	1-858	Not as porous as sample 681, liked a little more
9	Panelist 9	1	9	1-858	Tasted pretty good,and the texture was smooth but it didn't feel as slimy.
10	Panelist 10	1	10	1-858	tough skin, slightly rubbery texture
11	Panelist 11	1	11	1-858	tOUGH SKIN. lITTLE FLAVOUR
14	Panelist 14	1	14	1-858	tastes nasty and its bubbly and offpink appearance bothers me.
16	Panelist 16	1	16	1-858	this sample was soft which is good but not much flavor to it
17	Panelist 17	1	17	1-858	just ok
18	Panelist 18	1	18	1-858	Tastes like good bologna...
19	Panelist 19	1	19	1-858	it didnt have the best texture and could have had more flavor

21	Panelist 21	1	21	1-858	had very slight pepper spicy taste?
24	Panelist 24	1	24	1-858	The flavor was stronger, but the smell was not overwhelming. While I thought the big holes in the sample were not appealing, I still liked it. I wish I could have seen how these worked in a sandwich or something because I rarely eat sliced bologna by itself.
25	Panelist 25	1	25	1-858	I liked it had a nice smokey flavor
26	Panelist 26	1	26	1-858	i might be biased on these samples, because i eatthin sliced bologne if i do eat bologne.... the taste was alright, not bad, not great
28	Panelist 28	1	28	1-858	The texture of the meat versus the skin was too different.
29	Panelist 29	1	29	1-858	tasty
31	Panelist 31	1	31	1-858	I didn't like the texture, and the flavor wasn't the best.
32	Panelist 32	1	32	1-858	chewy and flavorless
33	Panelist 33	1	33	1-858	bland
34	Panelist 34	1	34	1-858	I didn't enjoy the taste or the texture of this product
35	Panelist 35	1	35	1-858	it felt greasy and the taste was incredibly weak
37	Panelist 37	1	37	1-858	In this case, the flavor was stronger than the aroma. I was pleasantly surprised by how flavorful this sample was!
38	Panelist 38	1	38	1-858	The taste was not good.
39	Panelist 39	1	39	1-858	rubbery texture, way too salty
40	Panelist 40	1	40	1-858	good smoky flavor, good color thickness, would probably buy
43	Panelist 43	1	43	1-858	Really good, texture and taste. I would give it a 9 if I liked bologna more.
46	Panelist 46	1	46	1-858	too tangey
47	Panelist 47	1	47	1-858	Felt weird.
48	Panelist 48	1	48	1-858	it was not an overpowering smell or taste
49	Panelist 49	1	49	1-858	I liked the taste and aroma of sample 858, however, the texture was a little chewy for me.
50	Panelist 50	1	50	1-858	it's too salty
56	Panelist 56	1	56	1-858	not as soft as i would like
57	Panelist 57	1	57	1-858	has a good texture, is not too soft or hard, just right
58	Panelist 58	1	58	1-858	dislike flavor
59	Panelist 59	1	59	1-858	tough skin, many holes
60	Panelist 60	1	60	1-858	pretty ordinary. nice flavor a bitmild. kinda tough on the outside.
61	Panelist 61	1	61	1-858	Did not taste very good.
62	Panelist 62	1	62	1-858	doesnt taste that good
63	Panelist 63	1	63	1-858	It has a better taste. Something added to this.
64	Panelist 64	1	64	1-858	It looked and tasted normal in comparison to the others.
66	Panelist 66	1	66	1-858	fllavoor ookk
67	Panelist 67	1	67	1-858	Goiod taste
68	Panelist 68	1	68	1-858	i like that it tasted like ham
69	Panelist 69	1	69	1-858	it reminds me of swiss cheese because it looked holey. but taste was acceptable
70	Panelist 70	1	70	1-858	The spice flavor complemented the flavor and texture of the meat very well in sample 858.
74	Panelist 74	1	74	1-858	I liked that it was not too salty because I really do not like excessive salt on meats. I liked the overall taste and it did not taste too fake.
75	Panelist 75	1	75	1-858	the bologna has a weird taste. It doesnt really taste much like turkey
1	Panelist 1	1	1	2-681	needs to more soft

2	Panelist 2	1	2	2-681	tastes the same.
4	Panelist 4	1	4	2-681	Had good flavor, tasted slightly spicy. Somewhat juicier than sample 858, which is good.
5	Panelist 5	1	5	2-681	soft
6	Panelist 6	1	6	2-681	the sample really tasted good. Overall felt good
8	Panelist 8	1	8	2-681	i'm not a big fan of bologna but it is satisfactory
9	Panelist 9	1	9	2-681	It tasted good enough but it just felt a tad odd.
10	Panelist 10	1	10	2-681	skin was a bit tough
11	Panelist 11	1	11	2-681	ArtificAL FLAVOUR, RUBBERY TEXTURE
12	Panelist 12	1	12	2-681	It tasted really good
14	Panelist 14	1	14	2-681	it is smooth and does not have a bad texture. it has a nice color in appearance. it tastes flavorful but not too bold and not spicy but not bland either.
15	Panelist 15	1	15	2-681	texture was pleasant
16	Panelist 16	1	16	2-681	the texture was softer than bologna i've eaten in the past
17	Panelist 17	1	17	2-681	good texture and flavor
18	Panelist 18	1	18	2-681	Tastes salty
19	Panelist 19	1	19	2-681	it just tasted like plain bologna, nothing special
21	Panelist 21	1	21	2-681	tasted like 482
22	Panelist 22	1	22	2-681	This sample had some strange hard chunks in it....I did not like that at all. While it looked pretty gppd, the taste did not meet expectations. It was somewhat bland??
24	Panelist 24	1	24	2-681	Its a little too firm. Maybe I am getting too used to eating bologna but it doesn't really have a striking taste when its in my mouth. The aftertaste is interesting.
25	Panelist 25	1	25	2-681	the outer skin is rough making it chewey, taistes ok
26	Panelist 26	1	26	2-681	there wasn't much of a aroma... it was very very light... i liked the taste for this one
28	Panelist 28	1	28	2-681	The flavor was too bland. Just tasted like salt.
29	Panelist 29	1	29	2-681	nice taste
31	Panelist 31	1	31	2-681	I liked the taste, but I did not like the texture at all.
33	Panelist 33	1	33	2-681	not a good flavor
34	Panelist 34	1	34	2-681	I like the taste of bologna, but I didn't like the hard edges
35	Panelist 35	1	35	2-681	the texture was somewhat umpleasant to me
36	Panelist 36	1	36	2-681	I liked the flavor and the smell
37	Panelist 37	1	37	2-681	this sample has a strange aftertaste, too salty
38	Panelist 38	1	38	2-681	I guess I just like the idea of this product
39	Panelist 39	1	39	2-681	rubbery texture, too salty
40	Panelist 40	1	40	2-681	softer texture than 562
43	Panelist 43	1	43	2-681	Taste was a little strange... maybe too peppery? Texture was also too rubbery.
46	Panelist 46	1	46	2-681	taste good not too chewey
47	Panelist 47	1	47	2-681	It was okay.
48	Panelist 48	1	48	2-681	did not really care for the taste
49	Panelist 49	1	49	2-681	I find that this sample was too salty.
53	Panelist 53	1	53	2-681	flavor was nice
56	Panelist 56	1	56	2-681	it was really hard
57	Panelist 57	1	57	2-681	average taste, not too good
59	Panelist 59	1	59	2-681	appearance holey
60	Panelist 60	1	60	2-681	very flavorable. strong flavor and it is really tasty. i actually really like it. but i haven't eaten today, so i'm really hungry. that might be an inflence. so if i was in the store and was really hungry i'd buy this for sure. and eat it all in one sitting. really delicious.

61	Panelist 61	1	61	2-681	It wasnt very remarkable, just allright
62	Panelist 62	1	62	2-681	best tasting and great texture
63	Panelist 63	1	63	2-681	It tastes thick. Don't enjoy the texture.
64	Panelist 64	1	64	2-681	I liked its appearance in comparison to the others cause of its lack of holes.
66	Panelist 66	1	66	2-681	tstte annd texture are ok
67	Panelist 67	1	67	2-681	Too thick, and dislike the strong aftertaste
69	Panelist 69	1	69	2-681	it was thinly sliced which made it more convenient for eating however it was a tad bit spicy
70	Panelist 70	1	70	2-681	Sample 681 had a very tasty combination of meat and spice flavors.
74	Panelist 74	1	74	2-681	This sample was very tasty, I liked the flavor.
75	Panelist 75	1	75	2-681	it was salty and it was very dark compared to the other samples.
1	Panelist 1	1	1	3-562	it didn,t had taste like the previous samples
2	Panelist 2	1	2	3-562	tastes pretty much the same. the texture is a little softer. i dont know whether i like this or not.
4	Panelist 4	1	4	3-562	Had a good texture, but not verymuch flavor or smell.
6	Panelist 6	1	6	3-562	the taste is good
8	Panelist 8	1	8	3-562	Can actually taste the turkey
9	Panelist 9	1	9	3-562	It tasted fine, but the flavor just didn't seem as distinct.
10	Panelist 10	1	10	3-562	good flavor, soft texture, skin still tough
11	Panelist 11	1	11	3-562	tOUGH SKIIN
12	Panelist 12	1	12	3-562	It tasted the same as the last i thought
14	Panelist 14	1	14	3-562	its aftertaste was fine but i really did not like the grainy texture and the bubbly appearance of it as well as its thickness and how it is off-pink in color
15	Panelist 15	1	15	3-562	the aroma was pleasant
16	Panelist 16	1	16	3-562	sample did not have much taste
17	Panelist 17	1	17	3-562	flavor and texture
18	Panelist 18	1	18	3-562	Taste was a bit too salty
19	Panelist 19	1	19	3-562	weird taste
21	Panelist 21	1	21	3-562	has a strange taste
22	Panelist 22	1	22	3-562	No strange chunks, weird smells, or funky color. It tasted very good.
24	Panelist 24	1	24	3-562	Again, I like the skin, but the turkey flavor comes out more in this sample. I would perfer it to taste more like bologna.
25	Panelist 25	1	25	3-562	skin is chewy,not that flavor full
26	Panelist 26	1	26	3-562	its taste was a bit on the safe side.. not too much flavor... but that's whatthe cheese and other ingredients are for in a sandwich
28	Panelist 28	1	28	3-562	The texture was the best of the four. It was less spongy and had much more meat-like qualities.
29	Panelist 29	1	29	3-562	same taste to it as 482 but more appelaing
31	Panelist 31	1	31	3-562	The texture was better, and so was the flavor. I think it tasted only slightly better than the other samples.
32	Panelist 32	1	32	3-562	a little better but still kinda gross meat
33	Panelist 33	1	33	3-562	better flavor than the rest
34	Panelist 34	1	34	3-562	This product seemed to be more bland than the others, and the texture of the edge combined with the texture of the meat isn't my favorite
35	Panelist 35	1	35	3-562	it had a weak flavor
36	Panelist 36	1	36	3-562	I liked the stronger flavor.
37	Panelist 37	1	37	3-562	the aroma of this sample was actually stronger than the flavor, so I was slightly disappointed by its taste. overall still very good

38	Panelist 38	1	38	3-562	I did not like the taste
39	Panelist 39	1	39	3-562	rubbery texture, smokey flavor
40	Panelist 40	1	40	3-562	chewy consistency - seems odd intexture
43	Panelist 43	1	43	3-562	Good, but spice flavor was a bit too strong.
46	Panelist 46	1	46	3-562	it was ok
47	Panelist 47	1	47	3-562	The texture was good.
48	Panelist 48	1	48	3-562	although looked and seemed to taste good, there is a slight after taste that could hinder my purchasing this sample more than once
49	Panelist 49	1	49	3-562	Sample 562 was too bland
53	Panelist 53	1	53	3-562	flavor was the best
56	Panelist 56	1	56	3-562	i like this one. would go well with bread. good mix of saltiness and flavor. soft texture.
57	Panelist 57	1	57	3-562	had a good taste, just right flavor, not too strong or weak
58	Panelist 58	1	58	3-562	dislike flavor
59	Panelist 59	1	59	3-562	too bland
60	Panelist 60	1	60	3-562	boring flavor. weird texture. strange afatertaste. kinda chewy. not a fan.
61	Panelist 61	1	61	3-562	it smelled odd.
62	Panelist 62	1	62	3-562	has a weird texture
63	Panelist 63	1	63	3-562	I doon't like what's around it but it tastes ok. No suprising after taste.
64	Panelist 64	1	64	3-562	I'm not really a fan of this type of meat yet it was alright if i was starving.
65	Panelist 65	1	65	3-562	smell is a bit strong
66	Panelist 66	1	66	3-562	i disliked the favor
67	Panelist 67	1	67	3-562	Very thick, but a lot of flavor
69	Panelist 69	1	69	3-562	the taste was very flavorfull... and it felt smooth
70	Panelist 70	1	70	3-562	The spice flavors did not come through with the meat flavor, resulting in a slightly bland-seeming taste.
74	Panelist 74	1	74	3-562	The taste was a little bland.
75	Panelist 75	1	75	3-562	i do not like the taste and it looks slimy in the bubble holes
1	Panelist 1	1	1	4-482	the prominient taste of sample was good
2	Panelist 2	1	2	4-482	it didn't pop when i ate it. that's good. the taste is still not my favorite.
4	Panelist 4	1	4	4-482	Had a dry, sponge-like appearance which was not desirable. Tasted and smelled very bland.
5	Panelist 5	1	5	4-482	spicy flavour
6	Panelist 6	1	6	4-482	the taste was okay. it was soft
7	Panelist 7	1	7	4-482	discoloration
8	Panelist 8	1	8	4-482	I don't like the darker color
9	Panelist 9	1	9	4-482	It tasted fine, seemed to have a little bit of spice to it maybe?
10	Panelist 10	1	10	4-482	rubbery texture, tough skin
11	Panelist 11	1	11	4-482	cOLOUR SEEMED GREY. tOUGH SKIN. tOO SALTY.
12	Panelist 12	1	12	4-482	tasted more ike salami to me
14	Panelist 14	1	14	4-482	texture is awful and it has an ugly brown appearance. it is spicy and i do not like that. it looks so processed and i dont like that.
16	Panelist 16	1	16	4-482	this sample had less flavor and was not as spicy as the others
17	Panelist 17	1	17	4-482	no flavor or aroma
18	Panelist 18	1	18	4-482	Better meat taste, not as salty
19	Panelist 19	1	19	4-482	the taste was pretty good in comparison to my

					experience with bologna
21	Panelist 21	1	21	4-482	skin had good tough texture
22	Panelist 22	1	22	4-482	It tasted very 'rubber'-like. The smell was okay, but that was about it.
23	Panelist 23	1	23	4-482	spongy
24	Panelist 24	1	24	4-482	I liked the chewy texture of the skin, the taste as a whole matched my expectations of regular bologna. It was a little salty.
25	Panelist 25	1	25	4-482	chewy and taisteless
26	Panelist 26	1	26	4-482	the color was appealing... a bit too dark
28	Panelist 28	1	28	4-482	Everything was pretty balanced, nothing too strong or too weird.
29	Panelist 29	1	29	4-482	did not look very appealing, tasted alright but had some extra sharpness to it
31	Panelist 31	1	31	4-482	I don't like the texture, and the flavor was not memorable.
32	Panelist 32	1	32	4-482	best texture, but still gross chewy meat
33	Panelist 33	1	33	4-482	bland
34	Panelist 34	1	34	4-482	I enjoyed the flavor of the product, but the texture around the edge was not as pleasant
35	Panelist 35	1	35	4-482	the flavor was weak until the very enf\d when you got a slight taste of spicyness the texture was very bouncy but slightly umpleasant
37	Panelist 37	1	37	4-482	This sample's flavor was very different from the rest---wasn't very stong, somewhat bland. Not good overall
38	Panelist 38	1	38	4-482	Can't stand the taste
39	Panelist 39	1	39	4-482	rubbery and gritty texture, flavor still bad but not as bad as others
40	Panelist 40	1	40	4-482	good taste, but odd tinge of brown color makes the appearance unappealing
43	Panelist 43	1	43	4-482	Taste was good, but too rubbery.
46	Panelist 46	1	46	4-482	a litte chewey
47	Panelist 47	1	47	4-482	The taste stood out in this sample.
48	Panelist 48	1	48	4-482	This sample seemed slightly rubbery in texture...it also was a darker color which puzzled me and would prevent me from making the purchase
49	Panelist 49	1	49	4-482	Too salty
53	Panelist 53	1	53	4-482	texture was a little more manageable
56	Panelist 56	1	56	4-482	not as explosive in flavor, but a good balance of saltiness and flavor.
57	Panelist 57	1	57	4-482	taste was a little rancid
58	Panelist 58	1	58	4-482	dislike flavor
59	Panelist 59	1	59	4-482	tough casing, many holes
60	Panelist 60	1	60	4-482	kinda fluffy texture. rally salty, too. but it tastes alright. it looks spoiled, it's brown.
61	Panelist 61	1	61	4-482	Acceptable. No strong odour .
62	Panelist 62	1	62	4-482	i like the aroma because it's not strong like the others
63	Panelist 63	1	63	4-482	Has a dark color.
64	Panelist 64	1	64	4-482	The appearance of this one really bothered me since it was darker and had more holes.
66	Panelist 66	1	66	4-482	flavor disliked
67	Panelist 67	1	67	4-482	Disliked the aftertaste
68	Panelist 68	1	68	4-482	too saltly
69	Panelist 69	1	69	4-482	the taste was average but the textue was too rubbery
70	Panelist 70	1	70	4-482	Sample 482 was a bit too salty for my preference, but I found it to be quite good otherwise.
74	Panelist 74	1	74	4-482	It was similar to the last sample.

75	Panelist 75	1	75	4-482	it was salty and was dark in comparison to the other bologna and it s,\melt different for the rest

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BIOGRAPHICAL SKETCH

Noufoh Djeri was born in Togo, West Africa in 1978 and is the oldest child of Amoye Djeri and Koffi Djeri. She attended Lycée Notre Dame de la Paix in Lille and Institut de Genech in Genech (France). She then pursued a Brevet de Technicien Superieur Sciences et Technologies des Aliments at Lycée Sainte Colette (Corbie, France). In 1999, she moved to the United States and attended Santa Fe College (Gainesville, Florida) where she obtained her Associate of Arts degree. In 2002, she transferred to the University of Florida (Gainesville, Florida) in the Department of Animal Sciences. She had the pleasure of meeting a great mentor (Dr. Sally K. Williams), who employed her part time and shared her vast knowledge in the field of Meat Science. She also had the delight of working for Dr. Jörg Bungert in the Department of Biochemistry and Molecular Biology as a laboratory technician, until completion of her graduate studies. After graduating with her bachelor's degree, her mentor and employer gave her the opportunity to pursue her graduate studies. Her master's degree was obtained in 2007 and focused on developing and evaluating value-added raw and pre-cooked vacuum packaged goat meat products. For her doctorate program, Noufoh Djeri broadened her horizons by enlisting in food and packaging sciences courses, with her research focusing on the use of celery juice powder containing pre-generated nitrite as a natural source of nitrite for cured meat products. She was awarded the Doctor of Philosophy degree on August 7, 2010 from the Department of Animal Sciences. Upon receiving her doctorate degree, Noufoh Djeri hoped to work for the Food and Agriculture Organization of the United Nations. Her objective in life was to be of help to people in the developing countries.