IOWA STATE UNIVERSITY
Digital Repository

Graduate Theses and Dissertations

Iowa State University Capstones, Theses and Dissertations

2010

Investigating the microbiological safety of uncured no nitrate or nitrite added processed meat products

Armitra Lavette Jackson
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd

 Part of the Animal Sciences Commons

Recommended Citation
Jackson, Armitra Lavette, "Investigating the microbiological safety of uncured no nitrate or nitrite added processed meat products" (2010). *Graduate Theses and Dissertations*. 11214.
https://lib.dr.iastate.edu/etd/11214

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Investigating the microbiological safety of uncured no nitrate or nitrite added processed meat products

by

Armitra Lavette Jackson

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Meat Science

Program of Study Committee:
Joseph Sebranek, Major Professor
James Dickson
Aubrey Mendonca
Kenneth Prusa
Brad Skaar

Iowa State University

Ames, Iowa

2010

Copyright © Armitra Lavette Jackson, 2010. All rights reserved.

DEDICATION

This work is dedicated to Candice Batts, who unexpectedly passed away in 2008 before completing her doctoral degree in the Department of Agricultural and Biosystems Engineering at Iowa State University. This one is for you. This work is also dedicated to my Aunt Marie Doss. You were one of my biggest cheerleaders. I will you more than words can express. Your loving spirit lives on.

TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION 1
Dissertation Organization 3

CHAPTER 2. LITERATURE REVIEW 4
History of Nitrites and Nitrates 4
Discovery 4
United States Regulations 4
Bacon: A Unique Case 5
Microbiological Role of Nitrate/Nitrite in Meat Products 6
Human Exposure to Nitrate/Nitrite 6
Endogenous Sources 7
Exogenous Sources 7
Concerns Associated With Nitrite and Nitrates 8
Relationship to Cancer 8
Infant Methemoglobinaemia 10
Conventional Curing of Processed Meats 11
Growth of Natural and Organic Market 12
Consumer Trends 12
Available Products 15
Organic and Natural Food Recalls 16
Natural and Organic Processed Meats 18
Definitions 18
Ingredients 21
Processing 23
Challenges Associated with Natural and Organic “Naturally Cured” Meats 26
Regulatory 26
Manufacturing 27
Marketing 28
Safety 29
Quality 29
Foodborne Illness in the United States 32
Burden of Illness Pyramid 33
Safety of Meat in the National School Lunch Program 34
Food Safety in U.S. Airports 36
Clostridia 36
Clostridium perfringens 37
Characteristics of the Bacterium 37
Factors That Affect Growth 37
Foodborne Outbreaks 38
Prevention and Control 41
Clostridium botulinum 42
Characteristics of the Bacterium 42
Factors That Affect Growth and Toxin Production in Food 43

Foodborne Botulism in the United States 44
C. botulinum in Meat Products 46
Detection of BoNT and Enumeration of *C. botulinum* 47
Infant Botulism 48
C. perfringens vs. *C. botulinun* 49
BOTOX 50
Treatment and Control 52
Summary of Literature 53
References 55

CHAPTER 3. SURVIVAL AND GROWTH OF *CLOSTRIDIUM PERFRINGENS* ON COMMERCIAL NO-NITRATE-OR-NITRITE ADDED (NATURAL AND ORGANIC) FRANKFURTERS, BACON AND HAM 66
Abstract 66
Introduction 67
Materials and Methods 69
Bacterial strain and spore suspension 69
Sampling preparation and inoculation 69
Microbiological analysis 72
Data analysis 72
Results and Discussion 73
Acknowledgments 77
References 77

CHAPTER 4. USE OF NATURAL INGREDIENTS TO CONTROL GROWTH OF *CLOSTRIDIUM PERFRINGENS* ON FRANKFURTERS AND HAM 84
Abstract 84
Introduction 85
Materials and Methods 88
Bacterial strain and spore suspension 88
Manufacture of frankfurters 88
Manufacture of hams 91
Sample inoculation 92
Microbiological analysis 93
Analytical analysis 94
Data analysis 95
Results and Discussion 95
Acknowledgments 100
References 100

CHAPTER 5. USE OF NATURAL INGREDIENTS TO CONTROL GROWTH OF *CLOSTRIDIUM BOTULINUM* ON FRANKFURTERS AND HAM 108
Abstract 108
Introduction 109
Materials and Methods 112

Bacterial strain and spore suspension 112
Product manufacture and inoculation of frankfurters and hams 112
C. botulinum microbiological analysis 114
Analytical analysis 114
Results and Discussion 114
Acknowledgments 117
References 117

CHAPTER 6. GENERAL CONCLUSIONS 122

ACKNOWLEDGEMENTS 124

CHAPTER 1. GENERAL INTRODUCTION

For the past few years, natural and organic foods have become extremely popular among consumers. Because of the perceived health benefits of natural and organic products, consumers are willing to pay more for this unique group of products. The meat industry has recognized this trend and has begun to manufacture products that simulate conventionally cured meat products, but without the direct addition of sodium nitrite. Manufacture of these products without direct addition of nitrite is necessary to qualify the products as natural or organic because nitrite is a preservative. The main concern with these processed meats marketed as natural and organic is that they do not contain formulated sodium nitrite ($NaNO_2^-$) in concentrations known to be highly effective in inhibiting the growth of many foodborne pathogens. This is because preservatives are not permitted in natural and organic products. As a result, these products contain natural sources of nitrite/nitrate (e.g., celery powder, celery juice and sea salt).

Sodium nitrite has been used for centuries in one form or another. Because sodium nitrite is a multifunctional ingredient in cured meat, it has a well-documented history of playing a major role in cured color development, flavor enhancement and possessing antimicrobial and antioxidant characteristics. For this reason, sodium nitrite is often referred to as a “magic” ingredient. This substance has a long-standing history of effectively inhibiting foodborne pathogens such as *Clostridium botulinum*. To date, there is no known replacement for this substance.

For many years, the use of sodium nitrite in the manufacture of cured meats has been under severe scrutiny. The media has played a major role in the shadow

that has been cast on this substance. There have been many concerns that ingesting nitrite via cured meats can result in cancer. This continues to be an issue despite the fact research has proven there is no link between meats cured with sodium nitrite and cancer. Consequently, the meat industry has spent many years defending its decision to use and continue the use of sodium nitrite in processed cured meats.

Processed meats with the direct addition of sodium nitrite have a long history of being safe for human consumption. However, the microbiological safety of cured meats manufactured to simulate conventionally cured meats, but without the direction addition of sodium nitrite, is not well understood. An earlier study of the potential for *Clostridium perfringens* growth in commercially available natural/organic frankfurters illustrated that there is wide variation in the potential for pathogen growth among the commercially available natural and organic frankfurters, meaning that the bacterial safety of these products is not well controlled. Therefore, the first overall objective of this study was to quantify the potential for *C. perfringens* growth in commercially available processed meats manufactured to simulate conventionally cured products, but without the direct addition of nitrite or nitrate and in products to which no nitrite/nitrate source was used. Natural and organic processed meats may require additional protective measures in order to consistently provide the same level of safety from bacterial pathogens that is achieved by conventionally cured meat products. Therefore, the second overall objective of the study was to identify and test ingredients that might improve product safety properties without altering the unique natural/organic status of these products.

Dissertation Organization

This dissertation is organized into six chapters. The first chapter is a general introduction. The second chapter is a general literature review that contains information relevant to this research project. Chapter 3 is a manuscript entitled "Survival and growth of *Clostridium perfringens* on commercial no-nitrate-or-nitrite added (natural and organic) frankfurters, bacon and ham." Chapter 4 is a manuscript entitled "Use of natural ingredients to control growth of *Clostridium perfringens* on frankfurters and ham." Chapter 5 is entitled "Use of natural ingredients to control growth of *Clostridium botulinum* on frankfurters and ham." All tables and graphs in the papers appear at the end of each respective paper. The sixth chapter gives a general summary of this research.

CHAPTER 2. LITERATURE REVIEW

History of Nitrates and Nitrites

Discovery

The act of curing meat unintentionally unfolded many centuries ago before man even knew the significance and greatness of this process. It has been suggested by many that the curing process was initially performed from salt contaminated with nitrate (saltpeter) *(73)*. Before the invention of refrigeration, preserving meat and fish with the use of a salt solution was well recognized and accepted. The objective of this was to preserve foods in times of scarcity *(73)*. Because salt has a direct effect on water activity (e.g., drying effect), the salting of meat prevented spoilage and controlled microbial contamination. Today, meat curing is an intentional process. Sodium nitrite is a unique non-meat ingredient in that it is involved with cured meat color and cured meat flavor development and possesses antimicrobial and antioxidant characteristics. Through its antimicrobial and antioxidant properties, nitrite is able to extend the shelf life of meat products. Because of the many effects nitrite has in meat systems, this substance is commonly referred to as the “magic” ingredient.

United States Regulations

Meat products manufactured in the United States are heavily regulated by the United States Department of Agriculture (USDA) – Food Safety and Inspection Service (FSIS). The amount of ingoing sodium or potassium nitrite in comminuted products manufactured in the United States is 156 ppm *(111)*. This is based on the green weight of the meat block (which is different in other countries). According to

regulations, dry cured products are restricted to 625 ppm of ingoing sodium nitrite or potassium nitrite. Immersion cured and massaged or pumped products are limited to 200 ppm ingoing sodium nitrite or potassium nitrite. According to the USDA, a minimum of 120 ppm of ingoing sodium nitrite is required for all cured “Keep Refrigerated” products. The only instance in which the rule is not in effect is when “the establishment can demonstrate that safety is assured by some other preservation process, such as thermal processing, pH or moisture control” *(111)*. Neither nitrate nor nitrite is permitted in baby or toddler food. Nevertheless, the food is safe for consumption due to the sterilization processing to which all baby food is subjected.

Bacon: A Unique Case

Due to potential nitrosamine formation in bacon, USDA-FSIS outlined different regulations for this product. For pumped and/or massaged bacon with the skin off, 120 ppm of ingoing sodium nitrite or 148 ppm of potassium nitrite is required in addition to 550 ppm of ingoing sodium ascorbate or sodium erythorbate *(111)*. A maximum of 120 ppm sodium nitrite or 148 ppm of potassium nitrite can be included in immersion cured bacon without the skin. To adjust for variables in pumping and draining procedures, plus or minus 20% of the target concentration is allowed at the point of injection. Dry cured bacon without the skin can contain 200 ppm of sodium nitrite or 246 ppm of potassium nitrite. It is important to note that nitrate is not allowed in any cured bacon product. Concern for nitrosamine formation from nitrites became of great issue in the 1970’s and almost totally terminated the use of nitrite as a curing agent *(87)*.

Microbiological Role of Nitrate/Nitrite in Meat Products

Despite the controversy surrounding the safety of nitrite, this substance is widely recognized as a highly effective antimicrobial agent. However, it is worth noting that this has not always been the case. Until the 1940's, it was believed that nitrite's only function in meat was color development and flavor. It was nitrate that was thought to be the antimicrobial agent. Through many studies, it became evident in the 1950's that it was nitrite that possessed the antimicrobial characteristic *(108)*. Today, nitrate is only recognized as a means by which nitrite is produced. Nitrite is effective against gram-positive and gram-negative bacteria, but not effective against yeasts and molds *(108)*. The exact mode of action by which inhibition occurs is unclear. Nitrite is even more effective when used with other agents. The inhibitory effects of nitrite are increased by the addition of NaCl and acidic conditions *(97)* and also by refrigeration and anaerobic packaging conditions *(15, 97)* . Before the use of nitrite in meat products, botulinum outbreaks were common. It is widely accepted that minimum residual nitrite levels of 40 to 80 ppm are needed to inhibit growth of *C. botulinum (53)*. Since 1899, there have been only seven outbreaks in the United States and Canada involving *C. botulinum* in which temperature abuse and underprocessing were the problems *(76)*.

Human Exposure to Nitrate/Nitrite

Although sometimes done subconsciously, all humans consume nitrate and nitrite on a regular basis via products other than processed meats. Nitrite is found in blood plasma and can be from an endogenous or exogenous source.

Endogenous Sources

Nitrite aids in normal bodily functions needed for survival. For example, nitric oxide, which is synthesized in humans, plays an important role in immune response, control of blood pressure and brain function *(9)*. Nitric oxide has also been referred to as a natural metabolite that is necessary for survival and function of the human biological system *(94)*. For example, it is widely accepted that nitrite is reduced to nitric oxide in the stomach where it is instrumental in destroying swallowed pathogens that can cause gastroenteritis in humans *(78)*. Nitrite can be found in human saliva and sweat. The majority of ingested nitrite is secreted through the urine *(16)*.

Exogenous Sources

Nitrate and nitrite can also be present in blood plasma from exogenous sources. Nitrates and nitrites are found in vegetables and fruits from incorporation through the nitrogen cycle in the soil and water. Vegetables account for approximately 80% of nitrate consumed via food and 10–15% of nitrate consumed via water *(9)*. Saliva is the largest source of ingested nitrite, accounting for 92.8% *(17)*. So if nitrite were as dangerous as critics say that it is, individuals would need to take much caution in the natural process of swallowing. Walker *(120)* compiled a summary table of nitrate levels from root vegetables (beets, carrots, potatoes, radish and turnips) from previous studies by other authors. His review reported that beets, radish and turnips had the highest nitrite levels, with concentrations around 1,000–2,000 ppm. In that same review, Walker also summarized nitrate levels from leafy vegetables (cabbage, chicory, leek, lettuce, spinach) from previous studies by other

authors. Spinach and lettuce were found to have the highest concentration of nitrate. A search of the literature showed that nitrate levels in vegetables are variable. This held true even when the same vegetables were evaluated. The National Academy of Science found the following to be true for dietary intake of nitrite: 39% was a result of cured meat consumption, 34% was from baked good and cereals and 16% was from vegetable *(65)*.

Concerns Associated with Nitrite and Nitrates

Cured meats have been a staple in the American diet for many years and have a long-standing history of being safe for consumption. These products include formulated sodium nitrite, thus providing the cured color, flavor and safety that individuals have come to expect from conventionally cured products. For many years, the safety of these products has been questioned. For an equal number of years, the meat industry has defended the safety of cured meats through sound research. There are two main concerns associated with nitrate and nitrite: cancer and infant methaemoglobinaemia.

Relationship to Cancer

Despite the scientific research that confirms the safety of meat products manufactured with nitrite, there are still those who continue to challenge the safety of these products. There were many epidemiological studies that attempted to link nitrite to cancer. Peters et al. *(74)* conducted a study that linked hot dog consumption with child leukemia. Sarasua and Savitz *(86)* conducted a study in which they linked childhood cancer to the consumption of cured and broiled meat. However, many of these studies are based on epidemiology without biological

support. Recently, there was an effort by the Cancer Project (formed by a group of Washington, D.C.–based physicians) to eliminate processed meats from school lunch meals *(45)*. This was another attempt to scare schools and parents into eliminating processed meats from the diets of children. Along the same lines, the Cancer Project is also proposing that cancer warning labels be placed on hot dog packages. According to the *Los Angeles Times (42)*, the group is suing companies such as Oscar Mayer (owned by Kraft Foods), Sara Lee, Marathon Enterprises and Hebrew National (owned by ConAgra) in an effort to require the companies to place "cancer-risk warning on labels on hot dog packages sold in New Jersey." As many in the scientific research world have done time and time again, it is important to note that processed meats have a scientifically proven positive reputation of being safe and nutritious for human consumption. In addition, the use of nitrite in processed meats is heavily regulated by USDA. As a result, human consumption is safe at the levels in which nitrite is included in the formulation of processed meats. Shapley, in an article that discussed nitrate and nitrite levels in fruits and vegetables *(89)*, stated that it's not the nitrate or the nitrite that is the problem in processed meats. It's actually the fat, calories and sodium that comes in the package that consumers should be concerned about. It is a known fact that humans actually ingest higher levels of nitrate through the consumption of water, fruits and vegetables than from cured meats. Nevertheless, consumers are strongly encouraged to consume fruits and vegetables for health benefits.

Infant Methemoglobinaemia

Infant methemoglobinaemia, also referred to as “blue baby syndrome”, is typically a problem when infants consume water contaminated with large amounts of nitrates from private wells. Methemoglobinaemia occurs when the iron contained within the hemoglobin molecule is oxidized from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state *(67)*. Infant methemoglobinaemia is extremely rare. In Wisconsin in 1992, an infant developed infant methemoglobinaemia after consuming well water contaminated with nitrate was used to prepare formula *(18)*. At the onset of infant methemoglobinaemia, the infant’s skin color will become a bluish grey color *(49)*. If not treated in a timely manner, death can occur. Wells typically become contaminated with nitrates through fertilizers or sewage. Although public drinking water systems are heavily regulated by the U.S. Environmental Protection Agency (EPA), private wells are not as regulated. The Safe Drinking Water Act, passed in 1974 by Congress, sets maximum contaminant levels for 90 contaminants in drinking water. Water is considered contaminated when the levels of nitrate (measured as nitrogen) are above 10 mg/L *(117)*. A study found that In Iowa, nitrate levels for approximately 2% of wells in the state were greater than 10 mg/L, which is dangerous for infants to consume *(51)*. As a result, water with levels greater than 10 mg/L should not be used in the preparation of infant formula. If a contaminant is found to be above its maximum contaminant level, treatments such as ion exchange, reverse osmosis and electrodialysis can be used to bring it under its maximum contaminant level *(116)*.

However, a study was conducted that found infant methemoglobinaemia could be caused by gastrointestinal inflammation and the overabundance of nitric oxide and not just by contaminated well water *(11)*. A search of the literature did not identify any cases in which infant methemoglobinaemia occurred as a result of consuming food, particularly cured meats. Some research suggests that ascorbic acid and vitamin K in food may serve as a protective mechanism for these types of food *(31)*.

Conventional Curing of Processed Meats

The conventional curing process is primarily defined by the direct addition of sodium nitrite. The term “meat curing” is well recognized as the addition of nitrate or nitrite, salt, sugar and spices with the purpose of extending the shelf life of meat by preservation. The most obvious effect of sodium nitrite in cured meat is color. However, this multifunctional ingredient also possesses flavor, antioxidant and antimicrobial properties. Other ingredients, such as salt, sodium lactate, dextrose, sodium phosphate, sodium diacetate and spices, are used in the processing of conventionally cured meats. Using the direct addition of sodium nitrite affords the processor the luxury of knowing exactly how much is going into the product as well as affecting the concentration of residual nitrite. This, in turn, assures the processor that he or she is adhering to USDA regulations on the use of nitrite in cured meat products. Equally important is the fact that the processors are assured the levels known to inhibit the growth of foodborne pathogens such as *Clostridium botulinum* are being reached. Despite the ongoing controversy surrounding conventionally cured meats, research continues to prove the safety of these products.

Growth of Natural and Organic Market

Consumer Trends

There has been a steady interest among consumers in foods labeled as natural and organic. Between 1997 and 2008, there was a $17 billion increase in the sale of organic food products *(27)*. In hard economic times, consumers tend to find ways to save money. However, approximately 40% of consumers who purchase organic food products stated they would not change their habits because of the recession whereas 3% of consumers have discontinued the purchase of organic products *(83)*. In fact, while shopping for different brands of naturally cured meat products for the study described in Chapter 3, it was not uncommon to visit a retailer only to find the shelves empty. This is a testament to the confidence consumers have in these products. Many studies have shown that consumers continually choose to purchase organic and natural food products because of the perceived heath benefits when compared to their conventional counterparts. This was addressed in the *2009 U.S. Families' Organic Attitudes & Belief Study (72)*, which reported that 55% of parents purchase organic products because of the belief that these products are healthier. Also, consumers have become infatuated with the idea of consuming preservative–free foods. Many other studies have reported that consumers favor organic and natural products because of concerns related to the use of pesticides, antibiotics, freshness, genetically modified foods and chemical additives *(13, 26, 90)*. These perceived health benefits, however, have not been positively substantiated by scientific research.

Because there is such a positive connotation with the words “natural” and “organic,” consumers are willing to pay more for this unique group of products. Meatingplace Magazine *(40)* reported that consumers are willing to pay as much as a 265% premium for these products. Another study found that consumers are willing to pay premiums that range from 10–40% *(121)*. In the past, retailers that sold natural and organic products were few in number. These stores were primarily small family-owned businesses. Today, natural and organic retailers can be seen in many regions across the United States and the world. According to the Whole Foods Market website, for example, Whole Foods Market currently operates 291 stores in the United States, the United Kingdom and Canada. This is compared to 181 stores in 2005 *(75)*. In 2005, Wild Oats Market, Inc., a chain similar to Whole Foods, operated 110 stores in the United States and Canada *(75)*. Trader Joe’s Co. Inc., another natural and organic store, began as a convenience store in 1958. According to Trader Joe’s website, the chain currently operates 338 stores and will open two stores in the coming months. This is compared to over 200 stores in 2005 *(75)*. Despite the current state of the economy, the number of natural and organic retailers continues to grow significantly. For example, Sprouts Farmers Market, headquartered in Phoenix, Arizona, recently made the announcement that they will open 12 additional stores *(8)*. The Organic Trade Association announced that in the midst of hard economic times, organic food sales increased by 15.8% in 2008 to reach $22.9 billion *(71)*. The increasing number of natural and organic retailers speaks volumes about the confidence consumers have in these products. Even though the momentum for organic and natural retailers is positive, some retailers

have experienced losses. For example, Whole Foods Market experienced a drop in stock price from a 52-week high of $42.78 per share to a low of $7.04 per share *(40)*. The time frame in which this drop occurred was between October 2007 and November of 2008, so it is possible this loss could be in response to the recession.

To better learn about the attitudes and behaviors among U.S. families as they relate to the purchase of organic food products, the Organic Trade Association partnered with KIWI Magazine to conduct a study entitled, *2009 U.S. Families' Organic Attitudes & Belief Study (72)*. This study found that 32% of those surveyed were "newly organic" (meaning they started purchasing organic products within the last 2 years), 20% were "experienced organic" (meaning that their first purchase of organic products was up to 5 years ago), 21% were "seasoned organic" (meaning they have purchased organic products for a period longer than 5 years and, in some cases, longer than 15 years) and 27% were "non-buyers" (meaning they had never purchased organic products). The "non-buyers" cited reasons such as high price, lack of interest and lack of knowledge about organic products for choosing not to make purchases. Overall, approximately 73% of the U.S. families surveyed revealed that they had purchased at least one organic product.

Due to the high demand for natural and organic products, food processors are catering to this increasing market by making these products more readily available to consumers. Publix, a conventional grocery store chain, has made a special effort to increase sales of natural and organic products by placing tan-colored tags on shelves to bring attention to environmental-friendly, organic and natural products that are minimally processed and contain no artificial flavors, colors or preservatives

(103). According to the Mintel Global New Products Database (GNPD), 23% of food and drink items introduced to the market were marketed as natural in 2008, representing a 9% increase in sales from 2007 *(101)*. In addition, new food and drink items marketed as natural accounted for one-third of new product launches. This was an increase of 16% from 2007.

Nevertheless, the Organic Trade Association in *The Past, Present and Future of the Organic Industry (70)* forecast that in the year 2025 the following (among other things) will be true: (1) it will be common for the average U.S. household to possess at least one organic food product or organic non-food product, (2) organic products will be available everywhere and (3) the organic industry will continue with its steady growth rate over the next 20 years.

Available Products

The meat industry has certainly followed the same trend as other food products as it relates to natural and organic products. According to meatingplace.com daily news, meat companies are routinely launching new organic and natural products. Kahiki Foods, a producer of Asian frozen foods, introduced a line of all-natural chicken. The chicken contains no MSG or artificial additives *(100)*. Baseball legend Nolan Ryan recently introduced Nolan Ryan's All-Natural Smoked Beef Sausage. The product contains no MSG, gluten or fillers *(46)*. Pizza Hut, an icon in American cuisine, recently made a shift from using conventional ingredients to natural ingredients throughout its menu. The new ingredients include pepperoni with no added nitrates or nitrite, no artificial flavors or preservatives and 100% real beef without the addition of fillers *(102)*. Campbell's Soup Company recently

premiered its all-natural Select Harvest soup line. The soups do not contain artificial ingredients *(79)*. Many companies have chosen to use celebrity appeal to market products. For example, Tyson Foods Inc., in partnership with Food Network host Robin Miller, plans to launch its 100% All Natural Chicken Nuggets and Patties. The products contain no preservatives or artificial ingredients and is minimally processed *(48)*. Actress Julia Louis-Dreyfus will appear in advertisements promoting ConAgra Food's addition of a new line of all-natural frozen entrees to its well-known Healthy Choice Brand. The entrees contain no artificial colors, flavors or preservatives *(29)*. The above list is by no means exhaustive. The list of natural and organic meat products will continue on its upward swing if consumer demand dictates such.

Organic and Natural Food Recalls

Organic and natural foods have not been excluded from recalls due to contamination by foodborne pathogens or other agents. Whole Foods Market recently recalled its organic raw hazelnuts due to contamination with *Salmonella (118)*. In Germany, animal feed fed to animals was contaminated with the carcinogenic herbicide nitrofen, thus leading to the contamination of organic meat products *(90)*. The depot in which the corn feed was stored was previously used to store nitrofen. An investigation found that the depot was not properly cleaned before it was used to store the animal feed *(110)*. Although a food recall was not issued, this incident speaks to the fact that organic products encounter problems also. In October 2009, Fairbanks Farm recalled 540,000 lbs of ground meat due to *E. coli O157:H7* contamination and a related outbreak of illnesses. This outbreak resulted in 2 deaths and 24 illnesses *(84)*. Approximately 1% of the recalled product was

supplied to Trader Joe's Butcher Shop. As stated on the chain's website, Trader Joe's contended that none of the illnesses reported were a result of meat purchased at their stores.

In August 2008, Whole Foods Market, due to possible *E. coli O157:H7* contamination, voluntarily recalled fresh ground beef that had been sold in 23 states and Canada. Whole Foods Market contended that the meat in question came from Coleman Natural Beef *(35)*. As a result of this incident, Whole Foods Market announced that they would revise the supplier approval procedures currently used by the retailer *(21)*. In 2006, there was a multi-state outbreak of *E. coli O157:H7* in spinach that was organically grown *(10)*. In another incident, according to the FSIS, Lucy Enterprises, Inc. recalled 13,776 lbs of frozen ground chicken products due to consumer complaints of foreign materials (plastic and bone material). Some of this product in question was sold in Trader Joe's Butcher Shop. This was a Class I Recall with a high health risk *(60)*. Although this incident did not contain product contaminated with foodborne pathogens, it still calls attention to the fact that natural and organic foods are not excluded from concern about contaminating materials. Incidents like the aforementioned show that natural and organic retailers are not invincible as it relates to products being contaminated with foodborne pathogens or other contaminants. It is clear that natural and organic retailers are likely to experience the same food safety concerns as conventional stores.

Natural and Organic Processed Meats

Definitions

Organic and natural processed meats fall under two different categories as it relates to USDA regulations. It is important to note that natural is not equivalent to organic. However, they are somewhat similar in the respect that neither one can be manufactured with formulated sodium nitrite or nitrate or potassium nitrite or nitrate. The USDA clearly outlines the regulations for foods. The Code of Federal Regulations 9 CFR 319.2 *(22)* states:

> Any product, such as frankfurters and corn beef, for which there is a standard in this part and to which nitrate or nitrite is permitted or required to be added, may be prepared without nitrate or nitrite and labeled with such standard name when immediately preceded with the term “Uncured'” in the same size and style of lettering as the rest of such standard name: *Provided*, That the product is found by the Administrator to be similar in size, flavor, consistency, and general appearance to such products as commonly prepared with nitrate and nitrite: *And providing further*, That labeling for such product complies with the provisions of Sec. 317.17(C) of this subchapter”.

The USDA Food Standards and Labeling Policy Book *(112)* defines what can be labeled as “natural.” The policy states:

> The product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and the product and its ingredients are not more than minimally processed. Minimal processing may include: (a)

> those traditional processes used to make food edible or to preserve it or to make it safe for human consumption, e.g., smoking, roasting, freezing, drying, and fermenting, or (b) those physical processes which do not fundamentally alter the raw product and/or which only separate a whole, intact food into component parts, e.g., grinding meat, separating eggs into albumen and yolk, and pressing fruits to produce juices *(112)*.

In addition, the policy also states:

> All products claiming to be natural or a natural food should be accompanied by a brief statement which explains what is meant by the term natural, i.e., that the product is a natural food because it contains no artificial ingredients and is only minimally processed. This statement should appear directly beneath or beside all natural claims or, if elsewhere on the principal display panel; an asterisk should be used to tie the explanation to the claim *(112)*.

The USDA definition of “natural” has experienced controversy since its inception. This controversy is most likely due to the fact that there are so many views on what should be deemed “natural.” One example is the issue of sodium lactate (from a corn source). In the initial rule, sodium lactate was considered a natural flavoring. This created a great deal of concern for many because sodium lactate is widely recognized as an antimicrobial, which directly interferes with the policy of “no preservatives” *(112)*. This confusion resulted in USDA issuing the following in the 2005 USDA Food Standards and Labeling Policy Book:

> “Sugar and natural flavorings from oleoresins or extractives” are acceptable for “all natural” claims. The other text, including the reference to “sodium

lactate (from a corn source)" has been removed from the guidance on "natural claims."

At the writing of this literature review, USDA was seeking comments on the "natural" claims labeling. Comments were to be submitted by November 13, 2009 *(36)*.

The USDA Organic Foods Production Act (OFPA) governs the labeling of products as organic. The National List of Allowed and Prohibited Substances clearly outlines which ingredients are approved and forbidden in products labeled as organic *(66)*. According to the USDA's Labeling and Marketing Information *(114)*, allowance is based on the percentage of organic ingredients in a particular product and falls into one of three categories. Products labeled as "100 percent organic" must contain only organically produced ingredients and may bear the USDA seal on the label. Products labeled as "organic" must contain at least 95% of organically produced products and may bear the USDA seal on the label. Products labeled as "made with organic ingredients" must consist of at least 70% organically produced ingredients and are allowed to display the statement "made with organic ingredients" on the label. There are stiff penalties for misuse of the organic labels.

Steps must be followed in order for a conventional production system to become an organic and natural production system. According to the Organic Certification Fact Sheet *(113)*, the producer must first identify a suitable certifier. The producer is responsible for securing a certifier based on his/her experience and knowledge in organic farming. Next, the producer must submit an application to the certifier. The certifier then reviews the application to ensure it is complete. Assuming all goes well with the review, an organic inspector inspects the farm. If the producer

is in compliance with regulations relating to organic farming, the certifier will grant certification to the producer. This certification is in effect until termination (e.g., voluntary or through review). Organic farms are inspected annually to ensure compliance with regulations. Unannounced inspections are allowed. With that said, a study was conducted to evaluate the prevalence of *Escherichia coli O157:H7* in organically and naturally raised cattle. The study found that the chances of *E. coli O157:H7* being present at a natural and organic cattle production system are the same as that of a conventional cattle production system *(80)*. Studies that yield results of this type cause many to question whether or not it is worth becoming an organic producer.

Ingredients

Because there is such a push from consumers to create “clean labels” on ingredient statements, there is a tendency for processors to use more natural ingredients. This trend has resulted in sodium nitrite being unacceptable for use. Many unique ingredients are used in the processing of naturally cured meats. A review of product ingredient statements by Sebranek and Bacus *(87)* found that sea salt was the most common denominator on product labels of natural and organic processed meats. Although sea salt is a potential source of nitrate and thus, nitrite, it has been suggested that sea salt contains very low concentrations of nitrate. A review of product ingredient statements by Sebranek and Bacus *(87)* found that turbinado sugar (raw sugar) was the second most common denominator on product labels of natural and organic processed meats. Because turbinado sugar is completely natural and contains no chemicals, its popular use in natural and organic

products is logical. It must be noted that research suggests that raw sugar is not a significant source of nitrate or nitrite *(88)*. Natural flavorings are also included and are defined as those ingredients whose primary contribution to the food is flavor, that contribute no nutritional value and are not derived from animals. Celery is a commonly used natural source of nitrate.

A natural source of nitrate must be used for natural and organic processed meats since formulated sodium nitrite is not permitted in products labeled as natural or organic. Sindelar et al. *(92, 93)* demonstrated that vegetable concentrates are a good source of nitrate. Celery juice and celery powder are a desirable nitrate source in processed meat products because the mild flavor profile does not interfere with the flavor of the meat and also because the low pigment content of celery does not manifest itself in the meat product *(88)*. However, a starter culture is needed in the natural curing of meat to reduce the nitrate to nitrite. Starter cultures are commercially available to meat processors. For example, Chr Hansen manufactures CS-299 Bactoferm, which uses *Staphylococcus carnosus* as the reducing bacterial agent. A newer version of this starter culture, CS-300 Bactoferm, is now available. This culture uses a blend of two bacterial strains (*Staphylococcus carnosus* and *Staphylococcus carnosus* subspecies *utitilis*) to reduce nitrate to nitrite. The advantage of CS-300 is that the conversion process is faster due to the two bacterial strains present *(41)*. It is also important to note that starter cultures may also serve as a flavor enhancer and may aid in color stability. Evaporated cane juice is used as a substitution to refined sugar. Although both evaporated cane juice and refined

sugar are both derived from sugar cane, it is widely accepted that evaporated cane juice is not subjected to the same degree of processing as is refined sugar.

Processing

The goal of the natural curing process is to manufacture products in a manner in which they exhibit the same characteristics consumers have come to expect from conventionally cured processed meats. These characteristics include color, flavor and safety. The process of naturally curing meat involves a nitrate source and also a bacterial component that is capable of reducing the nitrate to nitrite. The process of naturally cured meats is similar to that of meats manufactured with the direct addition of nitrite. The main difference between the conventional and natural curing process is that the natural curing process involves the addition of nitrate to the meat with the addition of a bacterial reducing starter culture capable of converting nitrate to nitrite. Because of this, an “incubation” period is added in the smokehouse schedule to allow the successful conversion of nitrate to nitrite. The “incubation” step for natural frankfurters and bacon is approximately 1 hour at 110ºF (42ºC) and 110–115ºF (42–46ºC), respectively. More incubation time may be needed for bacon because of the thin diameter. If ham products are relatively small, the heating process may also require adjustment. On the other hand, if the ham pieces are relatively large in diameter, no incubation period is needed because of the slow temperature increase that occurs during heat processing of large diameter products *(87)*. Product formulations and processing procedures for natural hot dogs, hams and bacons are listed in Table 1.

Table 1. Natural Hot Dog, Natural Ham and Natural Bacon Formulation and Process

Natural Hot Dog

Formulation

Pork 72's	52.60%
Beef 50's	22.70
Water	20.30
Sea salt	1.28
Natural Hot Dog Seasoning (Cane sugar, natural flavors, sea salt,celery powder, onion powder, garlic powder, oleoresin paprika)	3.10
Starter culture	0.02

Process

1) Grind meats through a 3/16-inch (4.8 mm) plate.
2) Mix starter culture with water totaling 0.50% of the total batch.
3) Mix/chop lean meats, adding in order, salt, ½ of the water, fatty meats, seasoning, and remaining water.
4) Add diluted starter culture.
5) Continue mixing/chopping until the meat blend temperature researches 50–54ºF (10–12ºC).
6) Emulsify to 62–64ºF (17–18ºC).
7) Stuff and link.
8) Place on smokehouse rack and process using the smokehouse schedule.
    a. 110ºF (42ºC) 60 minutes
    b. 140ºF (60ºC) 20 minutes
    c. 155ºF (68ºC) 30 minutes
    d. 175ºF (79ºC) 30 minutes
    e. 185ºF/30% RH to 165ºF (73ºC) internal temperature
    f. Shower

Natural Ham

Formulation

Water	79.135%
Natural Ham Seasoning (Sea salt, cane sugar, celery powder)	20.820
Starter culture	0.045

Process

1) Bone and trim pork, inside and outside pork rounds.
2) Dissolve and mix natural ham seasoning and starter culture into water prior to use.
3) Inject meat to 132% of green weight with the prepared pickle.
4) Macerate injected muscles on each side.

Table 1. (continued)

5) Tumble/massage under vacuum for a total of 5 hours. Tumble with 1/3 interval active and 2/3 interval inactive (10 minutes on and 20 minutes off).
6) Stuff hams into pre-smoked netted casings.
7) Place hams in vacuum packager and evacuate (without packaging materials) to remove air.
8) Place hams on cook rack.
9) Smoke hams to internal temperature of 158ºF (70ºC) using the following smokehouse process.
10) Chill in cooler overnight (8 – 10 hours).
11) Remove netting before vacuum packaging.

Smokehouse Schedule

Dry Bulb (ºF)	Wet Bulb (ºF)	RH%	Time (minutes)
165 (74ºC)	115 (46ºC)	22	45
165 (74ºC)	115 (46ºC)	22	15
165 (74ºC)	115 (46ºC)	22	30
165 (74ºC)	115 (46ºC)	22	60
175 (79ºC)	155 (68ºC)	59	30
180 (82ºC)	180 (82ºC)	100	Core temperature 158ºF (70ºC) Estimated 2 hours

Natural Bacon

Formulation

Water	66.38%
Sea salt	22.00
Cane sugar	10.40
Celery powder	1.20
Starter culture	0.02

Process

1) Trim pork bellies.
2) Prepare pickle prior to use.
3) Dissolve the following in water: sea salt, cane sugar, celery powder, and starter culture.
4) Pump pork bellies to 115% of green weight.
5) Place the pumped pork bellies on bacon hooks and smokehouse process.
6) Chill and slice.

Smokehouse Schedule

Dry Bulb (ºF)	Wet Bulb (ºF)	RH%	Time (minutes)
110 (42ºC)	92 (35ºC)	70	75
145 (63ºC)	–	–	60

Table 1. (continued)

145 (63ºC)	–	–	15
134 (57ºC)	–	–	90
140 (60ºC)	120 (49ºC)	55	Core temperature 128ºF (53ºC) Estimated180 minutes

Reprinted from Sebranek et al. *(87)*

Challenges Associated with Natural and Organic "Naturally Cured" Meats

Sebranek and Bacus *(87, 88)* appropriately divided the issues associated with products without the direct addition of nitrate and nitrite into five categories. These categories of concern include regulatory, manufacturing, marketing, safety and quality.

Regulatory

Although the rules governing organic products are well defined, those regulating natural products are not as clear. One of the issues with the regulation of naturally cured products is that the regulation is based on processor "intent" or "function", which can be difficult to implement *(12)*. In addition, some portions of the regulations that govern the processing of naturally cured meats conflict with ingredients that are allowed in natural products. For example, spice oleoresins are permitted in natural products and are considered natural flavorings *(112)* despite the fact that oleoresins are subjected to a chemical process that results in extraction of the spice and, further, are known to preserve food *(12)*. This practice plays on the emotions of consumers. As a result, a petition was submitted in October 2006 to USDA claiming the act of allowing synthetic ingredients in products labeled as

natural was inconsistent with properties characteristic of this unique group of products. Prior to August 2005, a product qualified as natural if it was “minimally processed” and contained “no artificial ingredients” *(87)*. However, this definition allowed for much confusion and processor interpretation.

Manufacturing

The controversy surrounding naturally cured meats stems from the need to distinguish between a chemical preservative and a natural preservative. This controversy exists despite the likeness of the two ingredients from different sources *(12)*. Because formulated sodium nitrite is not permitted in natural and organic meat products, there is a growing trend to use natural sources of nitrate and nitrite in cured meat products. Substantial variability in composition of natural sources of nitrate and nitrite can make it difficult for processors to ensure that the desired quantity of nitrite is consistently present in organic and natural products. Because of the sheer nature of a product occurring in nature, variability is inevitable. Meat products cured with the direct addition of nitrite are restricted to 156 ppm of nitrite added, and bacon is limited to 120 ppm. Sebranek and Bacus *(88)* reported less ingoing nitrate (40–60 ppm) in naturally cured products when compared to that of products with the direct addition of sodium nitrite (156 ppm for most products and 120 ppm for bacon). The authors reported similar residual nitrate levels in the two products and also similar color and color stability. The restricted use of ingredients such as phosphates, erythorbate and synthetic antioxidants most likely also contributes to the reduced shelf life of naturally cured products *(87)*.

There are many factors that are likely to contribute to the variation in the nitrate content in batches of natural sources, such as celery powder. Variety of celery seed is a contributing factor in the concentration of nitrate. Depending on the region, different types of soil could be used to plant the celery seed. Since celery is grown all over the United States, climate could play an important role in nitrate concentrations. Variation in nitrite derived can also be of issue in instances in which the processor is carrying out his/her own microbial conversions. In this case, the incubation step must be at the appropriate time and temperature so that bacterial conversion will result in the desired concentration of nitrite. Also in this situation, residual nitrite concentrations are of concern because of the formation of carcinogenic nitrosamines. This variability in the nitrate and nitrite concentrations makes these organic and natural products more susceptible to safety concerns.

Marketing

The way in which food products are marketed affects the success of the product in the marketplace. As a result, companies set aside a substantial amount of money for these efforts. The problem with the marketing of naturally cured products is the confusion it can create for the consumer. The phrase “no nitrate or nitrite added” appears on the product label of meat products that are naturally cured despite the use of a curing process. To a consumer, the phrase insinuates that the product contains no nitrates or nitrites. In reality, these products do include nitrates and nitrite from natural sources. Sebranek and Bacus *(88)* suggested the best way to provide consumers with accurate information is to remove the statement “no nitrate or nitrite added” and “uncured” from the label and use a term such as

“naturally-cured” instead. In addition, Bacus *(12)* suggested the terms “Naturally preserved with _____” or “Naturally cured with _____” will reflect the true nature of the product.

Safety

The safety issues of meats manufactured to simulate conventionally cured meats using natural sources of nitrate and nitrite can be divided into two categories: first, nitrite has a well documented history of possessing antimicrobial characteristics and second; residual nitrite concentrations play a role in carcinogenic nitrosamines *(88)*. Because the direct addition of sodium nitrite has a well documented history of controlling foodborne pathogen such as toxin formation in *Clostridium botulinum*, the exclusion of this important ingredient subjects naturally cured products to safety concerns. In addition, residual nitrite concentrations are also important in the formation of nitrosamines. To ensure the safety of naturally cured products, special care must be taken to ensure ingoing and residual nitrite concentrations are at the level they should be. The effects of nitrite are enhanced by factors such as pH, salt and thermal processing *(108)*.

Quality

Color is usually considered an important quality aspect of cured meats because it has a direct affect on the likelihood of a consumer making an initial and subsequent purchase of the product. The bright pinkish color is signature and most recognized for cured meats. Cured meats possess this characteristic because of the effects of sodium nitrite. This can be simulated in meats manufactured with the intention to replace nitrite. However, this characteristic is absent in those products

manufactured without nitrite. These products tend to possess an unattractive brownish color. Sebranek et al. *(87)* reported that 40–50 ppm of ingoing nitrite would be sufficient for stable color development.

After the consumer has made the initial purchase of the product due to its attractive pinkish color, the product must have a flavor that is satisfying to the consumer and similar to that of conventionally cured products. Sindelar et al. *(91)* found that meat attributes, including residual nitrite and lipid oxidation, were more variable in natural and organic products when compared to their conventional counterparts. In a study that investigated quality attributes and consumer acceptability of naturally cured and uncured products, Sindelar at al. *(92)* found that one out of four commercially available naturally cured and uncured brands of bacon had a flavor profile similar to that of the nitrite-added control. Two out of four brands of commercially available naturally cured hams possessed a flavor profile similar to the nitrite control whereas none of the commercially available franks possessed a flavor profile similar to the nitrite added control. This variability in naturally cured products must be addressed to ensure consumers have a flavor experience similar to conventionally cured products. As with color, 40–50 ppm of ingoing nitrite is sufficient to result in the cured meat flavor consumers have come to expect *(87)*.

Many questions have been raised concerning the nutritional value of organically produced food compared to that of conventionally produced food. The findings have been inconsistent across the board. Bourn et al. *(13)* reported that these types of studies are normally conducted by first purchasing the product in a retailer as consumers would. Because factors such as maturity at harvest and

freshness cannot be controlled, these studies could have confounding factors that could affect the nutritional value results. One reason for the variability in nutritional value could possibly be attributed to the variations in the experimental designs aimed to investigate this. Bourn et al. *(13)* compared five studies that evaluated the nutritionally value and general quality of organic and conventional foods purchased from retailers. Experimental design differed among the following studies. Anon *(7)* evaluated tomatoes, green beans, silverbeet and capsicum carotene and reported that vitamin C levels were similar in both organic and conventional food and mineral levels were much higher in the organic products evaluated. Conklin and Thompson *(23)* evaluated tomatoes, potatoes, sweet peppers, carrots, apples, grapes and leaf and iceberg lettuce and found that visual quality was the same in both organic and conventional products. Pither and Hall *(77)* evaluated apples, green cabbage, carrots, tomatoes and potatoes and found a higher level of vitamin C in organic apples and a higher potassium level in organic carrots. Vitamin C levels were higher in conventional carrots when compared to organic carrots. Smith *(95)* evaluated apples, potatoes, baby food, pears and sweet corn and found higher levels of Ca, Mg, Fe, Mn and Na in all organic products except baby foods and no differences in K, Cu and Zn levels. Stopes et al. *(99)* evaluated nitrate levels in organic and conventional cabbage, carrots, beetroot, bean sprouts, potatoes and lettuce and reported a range of values but no consistent difference between the organic and conventional products.

Foodborne Illness in the United States

Product contamination and foodborne illness due to microbial contamination of food by pathogenic bacteria such as *Listeria monocytogenes, Campylobactor jejuni, Escherichia coli O157:H7* and others continues to be a serious public heath concern not only in the United States, but also around the world. At the writing of this dissertation, there were a total of 13 active federal cases of product recalls in the United States that involved the potential presence of foodborne pathogens *(33)*. Using the Foodborne Illness Cost Calculator, the USDA Economic Research Services (ERS) estimated that in 2008 alone, outbreaks involving Salmonella from all sources had a cost of approximately $2,646,750,437. Outbreaks involving Shiga toxin-producing *E. coli O157* (STEC O157) cost approximately $478,061,302. Due to the underreporting of foodborne illnesses and failure to identify causative agents, determining the cost of foodborne illness due to pathogenic bacteria can be extremely difficult.

Foodborne illnesses seem to happen despite the fact that there are many regulations in place that are designed to protect the food supply from pathogenic bacteria contamination. Mead et al. *(59)* reported that there are nearly 76 million cases of foodborne illness reported in the United States. That same report *(59)* concluded that foodborne illness surveillance can be affected by the underreporting of illnesses, transmission of foodborne pathogens via water and person-to-person and also unidentified pathogens causing illness.

Burden of Illness Pyramid

The Burden of Illness Pyramid was created by the Centers for Disease Control and Prevention (CDC) in an effort to present a clean picture of the reporting of foodborne illnesses (Figure 1). The pyramid illustrates events that must happen in order for like illnesses in a population to be registered in surveillance. As a result, any step in the pyramid that is missed will result in the illness not being registered in surveillance. The Foodborne Diseases Active Surveillance Network (FoodNet), in turn, collects data at each step on the pyramid. At the bottom of the pyramid, the general population is exposed to the organism. Next, an individual exposed to the organism becomes ill. After the symptoms of the illness become bothersome and severe, the individual seeks care. This step is the beginning of underreporting

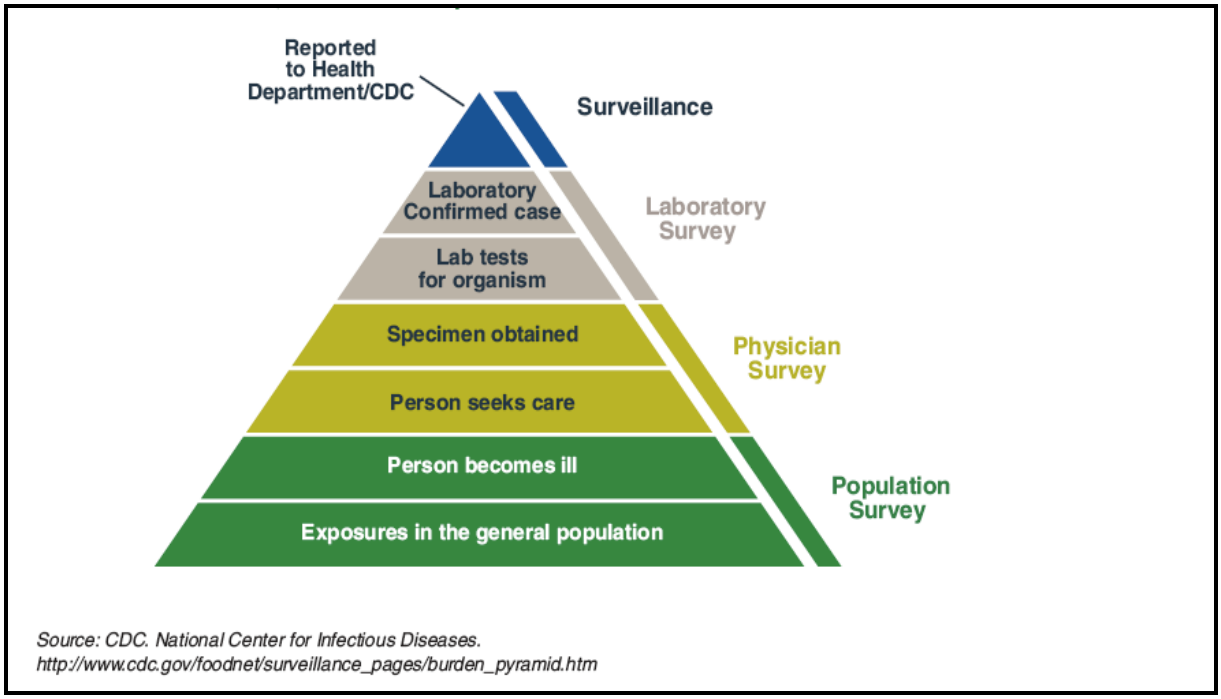

Figure 1. Burden of Illness Pyramid.

because many individuals choose to ignore symptoms, thus refusing to seek care. If the individual decides to seek care, the next step in the pyramid would involve the collection of a specimen, which can include a bacterial stool culture. Next, a laboratory test is performed that would identify the causative organism. Once the causative organism is confirmed, the incident then becomes a laboratory confirmed case. This information is reported to the health department and the CDC *(6)*. Although most of the general population will fully recover from these incidents, individuals who are immuno-compromised (e.g., AIDS, diabetic, pregnant and cancer patients) will face more serious complications and even death in some instances. Most foodborne pathogens generally have more of an effect on the younger and older populations. Although foodborne illnesses happen quite often, typically the incidences highlighted in the media involve either a large multi-state outbreak, child death(s) or the company involved is well known.

Safety of Meat in the National School Lunch Program

According to MeatPoultry.com *(84)*, there is currently an investigation into whether or not there is a high risk of *E.coli* contaminating ground beef in the school lunch programs. The article mentioned that Rep. George Miller of California, who initiated the effort, is concerned that contaminated food may be purchased for school-meal programs. The fact that children are more likely to be affected by foodborne illnesses due to an underdeveloped immune system may also be a factor for this effort. However, an investigation of USDA records showed that, through testing, the agency has been successful in preventing contaminated ground beef from entering schools in the United States *(47)*.

There have been other occasions in which the safety of meat purchased by USDA for the National School Lunch Program has been questioned. In August 2009, Beef Packers, Inc. recalled approximately 825,769 pounds of ground beef product due to *Salmonella* contamination *(32)*. It was later discovered that ground beef supplied to school lunch programs by the same company was not included in the recall. The USDA–Agricultural Marketing Service (AMS) cited three reasons why product from the schools was not included in the recall: (1) tests performed on the ground beef were negative for foodborne pathogens, (2) the beef used to make school products was kept separate from commercial beef, so any contamination in commercial beef should not carry over to the beef produced for consumption in schools and (3) the recall committee had no evidence that anyone fell ill from beef produced on the days products for schools were made *(64)*. An article in *USA Today* argued that school lunch programs received meat that would not meet the safety or quality standards of many fast–food chains. Nevertheless, USDA contends that meat served in the school lunch program meets or exceeds standards of commercial product *(30)*. In addition, Sen. Kirsten of New York sent a letter to USDA urging the agency to begin testing “every single batch of beef” that will be consumed by children in U.S. schools. The senator is asking USDA to cancel contracts with those companies that continue to disregard USDA food safety policies *(85)*. With that said, Daniels et al. *(25)* found that between the years of 1973 and 1997, there were 604 foodborne outbreaks in schools in which beef, poultry, Mexican-style food salad products and dairy products (with the exception of ice cream) were the most commonly implicated products. While all the recent attention points to the concern of

the safety of ground beef in the school lunch program, this study shows that other foods are likely to cause foodborne illness in U.S. schools.

Food Safety in U.S. Airports

Air travel is a popular mode of transportation as it allows passengers to reach destination relatively quickly. Consequently, individuals will consume food in airport restaurants due to its convenience. A *USA Today* review *(123)* of 800 restaurants at 10 airports revealed disturbing results involving food safety violations. A review at the Seattle–Tacoma International Airport found that 42% of restaurants (57 restaurants reviewed) had at least one “critical” violation. The same results were reported for 77% of restaurants (35 restaurants reviewed) reviewed at Reagan National Airport. The report also found that many grab-and-go coolers failed to maintain appropriate temperatures that would protect food from pathogenic growth. Chicken in salad at Fuddruckers in Detroit Metro Airport was reported to be 60ºF in the cooler. The article reported that in August 2009 in Cibo Bistro & Wine Bar at Reagan National Airport, a worker handled bread and raw chicken while failing to change gloves. Rat droppings were found at Atlanta’s airport on 12 different occasions from October 2008 through March 2009. These findings clearly demonstrate the need for better monitoring of food establishments in U.S. airports.

Clostridia

Clostridia are a genus of anaerobic, gram positive, spore-forming organisms that are widely dispersed in the environment. This genus comprises of over 100 species *(58)*. *Clostridium perfringens* and *Clostridium botulinum* are two organisms that are associated with foodborne illness.

Clostridium perfringens

Characteristics of the Bacterium

Clostridium perfringens was first recognized as a causative agent of foodborne disease in the 1940's by McClung *(57)*. However, McLauchlin et al. credited Hobbs for the recognition of *C. perfringens* as a cause of foodborne illness *(58)*. It is worth noting that this organism is cited as *Clostridium welchii* in UK literature. However, the name *C. perfringens* is used more often because this species name was first used to characterize the organism *(58)*. *C. perfringens* is a sprore-forming, gram-positive pathogenic bacterium whose spores are the cause of contamination leading to Type A foodborne illnesses *(52)*. *C. perfringens* cells can exist in the vegetative and spore state. Vegetative cells are heat sensitive and can be deactivated at 75ºC. Spores, however, are extremely heat resistant and can survive very high and very low temperatures. *C. perfringens* is an opportunistic organism in that it is ubiquitous in the environment. This pathogen can be found in the soil (10^3–10^4/g), intestinal tract of animals (10^3–10^6/g) and in approximately 50% of raw and frozen meat products *(58)*.

Factors That Affect Growth

There are many factors that can affect the growth, or lack thereof, of *C. perfringens*. Scientists have extensively studied the effects of temperature on *C. perfringens* growth. Growth of *C. perfringens* is optimal between 43ºC and 45ºC, and the growth range lies between 15ºC and 50ºC. Strain morphology, pH and growth medium used are also factors that affect temperature needed for optimum growth *(52)*. Walker et al. *(81)* conducted a study to investigate the growth of six *C.*

perfringens strains below, within and above the widely accepted optimum range. Four of the strains failed to produce vegetative growth at temperatures between 5ºC and 15ºC. Spore germination was observed in all strains between the temperatures of 32ºC and 40ºC. When sodium chloride was used as the solvent to adjust a_w, *C. perfringens* was able to grow at an a_w of 0.97 while cell death was reported at 0.95 *(105)*. pH values between 6.0 and 7.0 are optimal for the growth of *C. perfringens,* with cell growth inhibited at pH 5.5 and cell death occurring in days at pH 5 *(122)*. This makes meat and poultry products possible vehicles for growth. *C. perfringens* spores are extremely hardy and can survive harsh temperatures. Strong et al. *(104)* reported that spores counts remained high for 30 days at -17ºC in frozen gravy.

As discussed previously in this work, nitrite has a proven record of inhibiting many foodborne pathogens such as *C. perfringens*. The bacteriostatic properties of sodium nitrite on *C. perfriengens* are well documented. Sodium nitrite in combination with sodium chloride effectively inhibits the growth of *C. perfringens*. Sodium nitrite also has the ability to protect the product in the event of temperature abuse (as will discussed in Chapters 3 and 4 of this dissertation). Nitrite is thought to inhibit the growth of *C. perfringens* by blocking of sulfhydryl sites on sites in the bacterial cells of the pathogen *(108)*. Without the addition of sodium nitrite, the chances of contamination and foodborne illness increase greatly.

Foodborne Outbreaks

The United States has experienced many foodborne outbreaks in which *C. perfringens* has been the implicated pathogen. Because *C. perfringens* has the ability to produce only 7 of the 20 amino acids necessary for successful growth, high

protein food items (e.g., poultry and meats) are the most implicated foods related to illness *(63)*. Toxicoinfections of *C. perfringens* occur when an individual consumes a food that is contaminated with vegetative cells of *C. perfringens*. Although acids in the stomach kill most vegetative cells, some of the cells survive and continue to the small intestine. Sporulation of the cells begins, thus producing enterotoxins. The spores attach to the intestinal villi, which causes diarrhea and cramps *(63)*. A diagram detailing the pathogenesis of *C. perfringens* food poisoning can be found in Figure 2. Ingestion of preformed toxins is extremely uncommon. Between the years

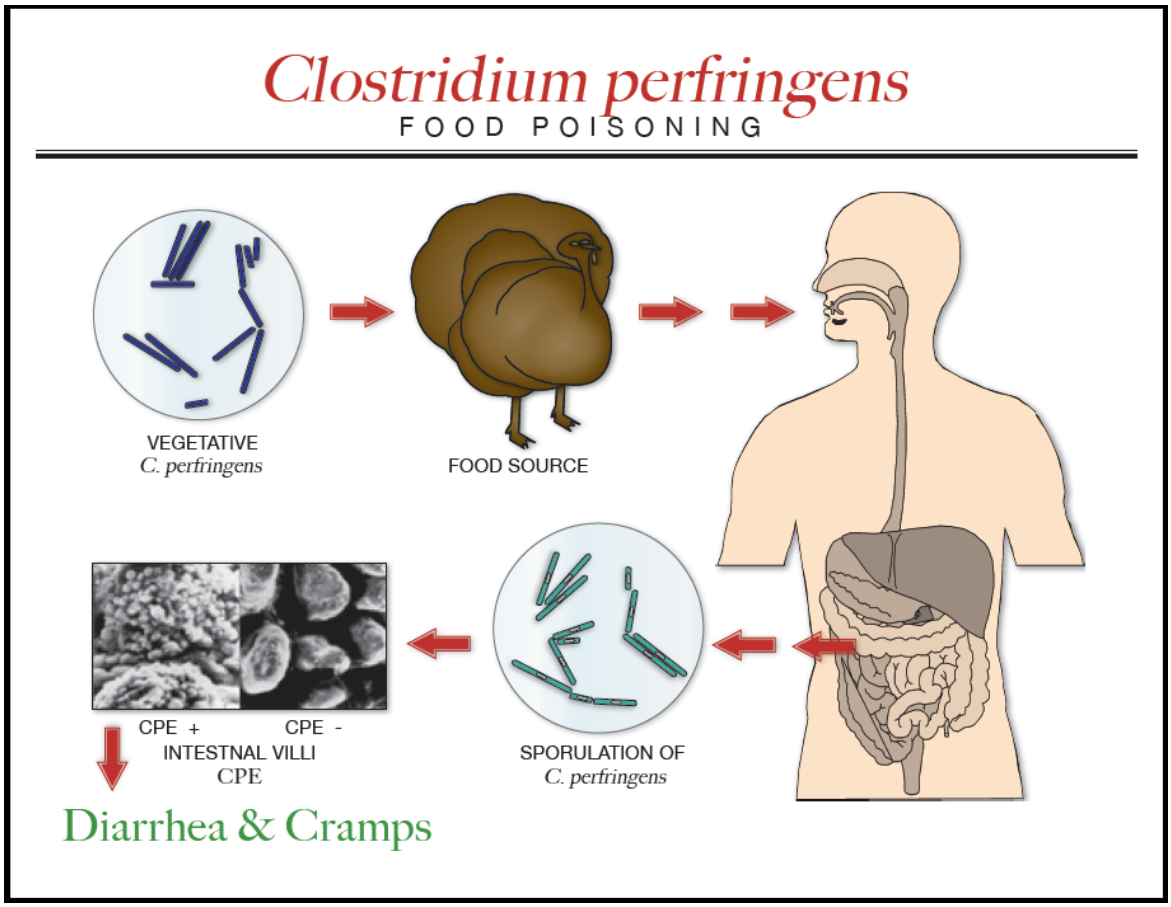

Figure 2. Pathogenesis of *C. perfringens* food poisoning. A diagram adapted by A. Jackson with permission from McClane *(56)*.

of 1993 and 1997, there were 40 cases of foodborne outbreaks involving *C. perfringens (63)*. According to Mead et al. in a 1999 report *(59)*, there have been nearly 250,000 cases of foodborne illness involving *C. perfringens*. The first documented large-scale outbreak was reported in Leicester, England in 1943 when school children became ill after consuming gravy contaminated with *C. perfringens (50)*. In 1990, an outbreak occurred in Michigan when minestrone soup was slowly cooled and briefly reheated after being prepared two days earlier *(82)*. In 1998, many individuals became ill after consuming food at a luncheon. An investigation revealed that the in-home caterer was unlicensed and did not have the proper equipment to properly cool and heat food. Although the presence of *C. perfringens* was not confirmed through laboratory tests, all the evidence pointed to *C. perfringens* as the implicated organism *(3)*. In 2008, 100 inmates in a Wisconsin county jail became ill after eating a casserole dish that contained beef and ground turkey *(20)*. *C. perfringens* enterotoxin was isolated in the stools of six ill inmates. After analyzing a portion of the leftover casserole, 43,000 CFU/g of *C. perfringens* were isolated from the dish. An investigation found that ingredients used in the casserole had been prepared and improperly stored.

Improper heating and cooling of foods contributes to foodborne illnesses associated with *C. perfringens*. Because of this, USDA-FSIS regulations prohibit the internal temperature of meat products to remain between 54.4ºC and 26.7ºC for over 1.5 hour nor between 26.7ºC and 4.4ºC for more than 5 hours during the cooling process *(115)*. Outbreaks of *C. perfringens* are common in institutional settings such as hospitals, nursing homes and schools because food is often prepared well in

advance and allowed to remain in items such as steam tables until consumed. *C. perfringens* will grow if the food is not chilled at the proper temperature. Subsequently, *C. perfringens* is often referred to as the “cafeteria” bug because large volumes of food are prepared and left for long periods of time on steam tables, thus increasing the chance of improper cooling and heating.

Due to common symptoms of diarrhea, nausea and vomiting, foodborne illnesses caused by *C. perfringens* usually go unreported. Reporting usually happens only when large numbers of people become sick around the same time period. A foodborne *C. perfringens* outbreak is typically confirmed when the enterotoxin is isolated from the stool of many individuals who display symptoms of the illness. However, the results are more conclusive if the stool sample is taken close to the onset of symptoms typical of *C. perfringens (63)*. Montville et al. *(63)* reported that *C. perfringens* toxicoinfections are typically self-limiting because the diarrhea actually aids in expelling spores from the body and also the toxins attach to the oldest intestinal cells which are quickly replaced by new cells during normal body processes.

Prevention and Control

The most obvious way to control *C. perfringens* in food is to ensure temperature and time control by making sure the food product is cooled quickly after the cooking process. The product should also be heated properly, thus destroying any vegetative cells that could be present. Perhaps Hobbs *(43)* stated it best in 1953 when he said, “Outbreaks of this kind should be prevented by cooking meat

immediately before consumption, or if this is impossible, by cooling the meat rapidly and keeping it refrigerated until it is required for use.”

Clostridium botulinum

Characteristics of the Bacterium

Clostridium botulinum is an anaerobic, gram-positive spore-forming bacterium that has been recognized as a foodborne disease for over 1000 years. However, it was Emilie Pierre Marie van Ermengem who isolated and described the cause of the illness and found that the toxin was produced by the bacterium *Bacillus botulinus* (which is now known as *C. botulinum*) *(119)*. The term “botulism” is derived from the Latin word *botulus*, which means sausage *(97)*. *C. botulinum* produces botulinum toxin (BoNT), which is the most toxic substance known to man. The toxins produced are divided into seven types and assigned letters A–G. Foodborne, wound and intestinal botulism are associated with types A, B, E and, in rare instances, F. Botulism in animals is caused by types C and D; type G has not been linked to a disease *(62)*. *C. botulinum* is also divided into groups based on their physical differences. Group I contains type A and proteolytic strains of types B and F. Group II contains all type E strains and nonproteolytic strains of types B and F. Group III encompasses types C and D. Group IV contains type G. Foodborne illness involving *C. botulinum* is caused by the ingestion of preformed toxins. Because of the toxicity of this organism and the potential for use as a weapon of mass destruction, the U.S. government heavily monitors its use. Because *C. botulinum* spores can be found in the environment, foods can become contaminated during the harvesting processing.

Factors That Affect Growth and Toxin Production in Food

Factors such as temperature, a_w, NaCl, added preservatives, pH, redox potential and competing microorganisms all have an effect on the growth of *C. botulinum* in foods. However, it is important to note that each of these factors is more effective when used in combination with one another. This is commonly known as the hurdle effect. Grouping and characteristics of strains of *C. botulinum* are listed in Table 2. Types A and B can tolerate up to 10% NaCl whereas type E strain growth is inhibited by a_w of 0.975 with 5% NaCl *(109)*. It is important to note that NaCl is considered to be one of the most important factors used to inhibit growth of *C. botulinum* as it significantly decreases a_w. While it is well accepted and documented that *C. botulinum* cannot grow in the presence of oxygen, there have been reports of this organism thriving in the presence of oxygen because when some foods are heated, the dissolved oxygen is driven out, thus creating an oxygen-free environment *(62)*. Although natural or liquid smoke has the ability to inhibit *C.*

Table 2. Grouping and characteristics of *C. botulinum* strains. Adapted from Johnson *(44)*.

	Toxin Group			
Characteristic	**I**	**II**	**III**	**IV**
Neurotoxin type(s)	A, B, F	B, E, F	C, D	G
Minimum temp (°C) for growth	10	3	15	12
Optimum temp (° C) for growth	35–40	18–25	35–40	35–40
Maximum temp (° C) for growth	48	45	NA[a]	45
Minimum pH for growth	4.6	5.0	NA	NA
Minimum a_w for growth	0.94	0.97	NA	NA
Inhibitory NaCl concentrations (%)	10	5	3	>3
$D_{100°C}$ of spores (min)	~25	<0.1	NA	NA
$D_{121°C}$ of spores (min)	0.21	<0.005		

[a]NA: Insufficient data available.

botulinum in fish, this is not the case for meats. In addition, spores of *C. botulinum* are extremely resistant to irradiation *(62)*. Temperature is also an important factor and used to control growth of *C. botulinum* in foods. *C. botulinum* is able to grow at low and high temperatures. Glass et al. *(37)* reported growth as low as 3.3℃. *C. botulium* stains are also capable of growing in the range of 45℃ to 50℃ *(44)*. This temperature range falls within the danger zone. This stresses the fact that temperature abuse can lead to the production of *C. botulinum* in foods.

Foodborne Botulism in the United States

It is well recognized that botulism was a significant problem in ham and sausage during the 19th century. Foodborne botulism occurs when food contaminated with *C. botulinum* toxins are consumed, passed through the stomach and absorbed from the small intestine. The toxins then react with the target organs (end-plates or synapses of parasympatic nerves) and the release of acetylcholine (ACh) at the neuromuscular junction and synapses is blocked *(69)*. ACh is extremely important in muscle contraction because it is used as a neurotransmitter by some neurons at the neuromuscular junction *(34)*. During this process, the *C. botulinum* toxins bind to the nerve ending at the nerve-muscle junction *(2)*. A diagram adapted from Oguma et al. *(69)* detailing the pathogenesis of *C. botulinum* food poisoning can be found in Figure 3. Between the years of 1950 and 1996, there were 1,087 cases of foodborne botulism in the United States *(62)*. Adams and Moss *(2)* listed four common features that are distinguishable in outbreaks of botulism: (1) “The food has been contaminated at source or during processing with spores or vegetative cells of *C. botulism”*; (2) “The food receives some treatment that restricts the

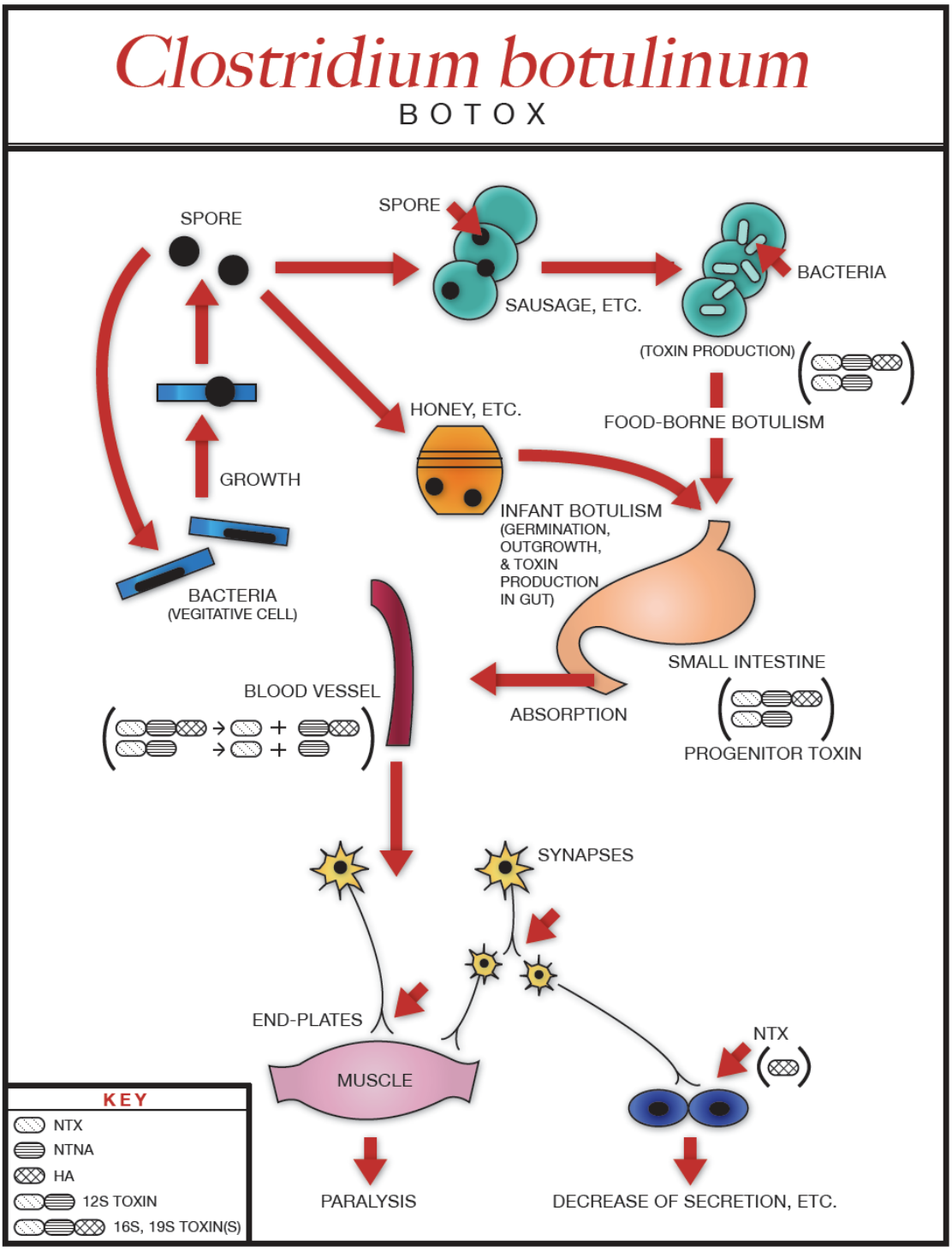

Figure 3. Pathogenesis of *C. botulinum* food poisoning. Adapted by A. Jackson from Oguma et al. *(69)*.

competitive microflora and, in normal circumstances, should also control *C. botulinum"*; (3) "Conditions in the food (temperature, pH, E_h, a_w) are suitable for the growth of *C. botulinum"* and (4) "The food is consumed cold or after a mild heat treatment insufficient to inactivate toxin." It is recognized that foodborne botulism occurs in clustered regions *(28)*. Foodborne botulism can occur at any age and include symptoms of double vision, blurred vision, drooping eyes, difficulty swallowing, muscle weakness, and paralysis of breathing muscles *(19)*.

C. botulinum **in Meat Products**

Research has proven that the incidence of *C. botulinum* in fresh meats is very low. Greenberg et al. *(38)* reported that only 1 sample out of 2,358 raw meat samples (chicken, beef, pork) was found positive for *C. botulinum* type C. Taclindo et al. *(106)* conducted a study in which "vulnerable" foods (e.g., foods that are found in refrigerated cases in retailers and consumed with little or no heating) were examined for *C. botulinum* contamination. Of the 73 samples of luncheon meat evaluated, only 1 sample was positive for *C. botulinum*. In the same study, an evaluation of 17 sausage samples yielded no positive samples. Abrahamson et al. *(1)* determined the prevalence of *C. botulinum* toxin in semi-preserved meat products to support the few studies that have demonstrated the low prevalence of *C. botulinum* in meats (e.g., cooked ham, smoked turkey, smoked chicken, bologna). Only 6 samples of 372 were found to contain *C. botulinum* toxin. Five of the samples (cooked ham) contained type A toxin and one sample (smoked turkey) contained type B toxin.

In most instances, *C. botulinum* is often mentioned when nitrite is discussed. This is because it was the fear of *C. botulinum* that started the intentional use of sodium nitrite in meat products. By the 1970s, it was generally accepted that as more sodium nitrite is added to a meat system, the more inhibition of *C. botulinum* growth and toxin production is achieved *(73)*. Many scientists have demonstrated this fact. Pierson et al. *(76)* reported results of a study in which Greenberg inoculated canned ham samples with 100 spores/g at different nitrite levels (0, 50, 100, 150, 200, 300, 400, 500 ppm). No growth was observed in the canned ham that received between 200 and 500 ppm of nitrite. However, it is also accepted that when the spore concentration of *C. botulinum* increases, the effects of nitrite can decrease *(73)*. Pierson et al. reported results of a study in which Greenberg inoculated canned ham samples with 10,000 spores/g at different nitrite levels (0, 50, 100, 150, 200, 300, 400, 500 ppm). *C. botulinum* toxin was detected in canned ham samples containing all nitrite levels with the exception of 500 ppm *(76)*. Many mechanisms are involved in the inhibition of *C. botulinum* by nitrite. It is thought that nitrite inhibits *C. botulinum* by a reaction between ferredoxin and nitric oxide with the germinated cell *(108)*. In addition, nitrite inhibits the growth of *C. botulinum* in a heated system by preventing vegetative cell growth from spores and also inhibiting cell division of any vegetative cells should they be present *(76)*. The effectiveness of nitrite in inhibiting the growth of *C. botulinum* is widely accepted.

Detection of BoNT and Enumeration of *C. botulinum*

The mouse assay is currently the only standard and accepted method for the detection of BoNT. In this assay, mice are injected intraperitoneally with sample

treatments and observed for 48 hours for signs of botulism *(98)*. This method, however, is extremely expensive and time consuming. Neurotoxin gene (bot)-specific polymerase chain reaction (PCR) has been used to confirm BoNT in feces and suspected foods. The disadvantage to this method is that only one of seven toxins can be detected at a time *(55)*. However, Lindström et al. *(54)* reported that a multiplex PCR allows more than one toxin to be detected at a time.

The most probable number (MPN) technique has been recommended for the estimation of *C. botulinum* count. However, because of the low levels of *C. botulinum* spores in fresh meats, Greenberg et al. *(39)* advised against using the MPN technique for routine enumeration of botulinal spores as the chances of a spore entering a tube is extremely low and unlikely. The authors also cited the large amount of incubation space needed and the large amount of glassware as other reasons for not using the MPN method.

Infant Botulism

Scientists in the California Department of Public Health discovered infant botulism in the 1970's *(62)*. Midura et al. *(61)* found that infant botulism was most often caused by type *C. botulinum* groups A and B. Infant botulism has been historically associated with the consumption of honey. Nevas et al. reported that *C. botulinum* was found through the entire honey production chain *(68)*. Because *C. botulinum* spores are not destroyed during the production process, they can survive for long periods of time in honey *(96)*. Heating honey to temperatures that would destroy *C. botulinum* spores would negatively affect the taste of honey and destroy its structure *(68)*. In this case, there was evidence that more than one *C. botulinum*

strain was present in the product. In California, approximately 30% of infant botulism cases have involved the consumption of honey *(44)*. Because of this, the American Academy of Pediatrics advises against the consumption of this product by infants *(62)*. However, in 2005, a case of infant botulism with a link to infant formula milk powder was reported *(14)*. In contrast to foodborne botulism, infant botulism is common in children 6 months old or younger. Between the years of 1978 and 2001, there were six reported cases of infant botulism in the United Kingdom *(58)*. Because infants do not have protective intestinal microbiota that colonize the gut and prevent the growth of botulism, the *C. botulinum* spores can germinate and produce toxins. This toxin production is performed in vivo *(69)*. A diagram detailing the pathogenesis *C. botulinum* in infant botulism food poisoning can be found in Figure 3. Because this can also be the case for adults who are immuno-compromised, infant botulism is also known as intestinal botulism *(62)*. BabyBIG (Botulism Immune Globulin Intravenous) is a drug created under the Orphan Drug Act of 1983 to treat infant botulism in the United States. This drug is administered only to children under the age of one. It is important to note that the term “orphan” refers to a drug created to treat diseases that affect fewer than 200,000 individuals. This drug consists of botulism antitoxin antibodies derived from humans. The California Department of Public Health’s Infant Botulism Treatment and Prevention Program maintains a supply of antitoxin against infant botulism *(19)*.

C. perfringens* vs. *C. botulinum

Because *C. perfringens* and *C. botulinum* belong to the same genus, they are alike in many respects. Both organisms are anaerobic, gram positive, spore-forming

organisms that are widely dispersed in the environment. In addition, both organisms can exist in the vegetative and spore state. Spores from each organism are highly heat resistant, can tolerate high NaCl and can survive in low temperature environments. Sodium nitrite has been proven to effectively inhibit the growth of both pathogens in meat products. Both pathogens pass through the stomach to the small intestine to inflict harm in its host.

In some respects, the two organisms are different. Unlike *C. perfringens*, *C. botulinum* foodborne illness results from the consumption of preformed toxins in contaminated food *(58)*. They are also different in their mode of action and how they affect the human body. *C. perfringens* spores attach to the intestinal villi, which results in diarrhea and cramps. *C. botulinum* spores block the release of Ach from synaptic vesicles at nerve terminals *(62)*, causing paralysis of the muscles.

BOTOX

Although botulinum is the most toxic substance known to man, it can be used to treat neuromuscular diseases that can cause muscles to be more active than normal. In order for this toxin to be used, it must be diluted to extremely low concentrations. In 2002, BOTOX (derived from type A botulinal toxin) was approved by the United States Food and Drug Administration (FDA) for the treatment of cervical dystonia, which results in the head being twisted in abnormal positions due to involuntary contractions of the neck and muscle shoulders *(62)*. Potential side effects related to this treatment are swallowing and breathing difficulties *(5)*. This use of BOTOX is usually used as an alternative to surgery.

In addition to medical uses, there are those who choose to use BOTOX for cosmetic reasons. There are many who choose to use this substance to “erase” the traces of age by removing facial wrinkle lines. Injecting BOTOX under the skin near the wrinkle causes flaccid paralysis, thus removing the unwanted wrinkle *(62)*. Even though the results of BOTOX can result in a younger looking being, there are risks associated with its use. There have been reported cases of hypersensitivity reactions, such as urticaria, soft tissue edema, dyspnea and anaphylaxis *(5)*. Nevertheless, there are those individuals who question why someone would want to inject a toxin into one’s body for cosmetic purposes. A 16th century Swiss chemist may have given the best response to this question when he simply stated, “The dose makes the poison” *(107)*.

When discussing BOTOX, it is imperative to mention Eric Johnson and Edward Schantz, both of who are experts in botulinum toxin at the Food Research Institute at the University of Wisconsin–Madison. According to the Fall 2008 issue of *On Wisconsin (4)*, in 1979, Schantz had been providing a batch of botulinal toxin he developed (named 79-11) to San Francisco ophthalmologist Alan Scott. This toxin was an important ingredient in an eye medicine (called Oculinum) that was being developed by Scott. Because Johnson and Scott thought Oculinum would be successful, they appealed to the Wisconsin Alumni Research Foundation (WARF) on behalf of University of Wisconsin–Madison researchers to secure a patent for 79-11. Because the market for botulinum toxin was, at the time, virtually nonexistent and the idea of injecting a toxin in human was unthinkable in the 1970’s, the patent application was denied. Scott sold Oculinum to the pharmaceutical company

Allergan in 1988. After the FDA approved botulinum batch 79-11 for the treatment of crossed eyes and uncontrolled blinking, Allergan renamed the drug BOTOX. At this point, Schantz and Johnson no longer owned the rights to batch 79-11. Soon after, the drug was discovered to treat other medical problems, such as pediatric cerebral palsy and cervical dystonia. The discovery that BOTOX could be used to remove wrinkles appealed to many. The report also stated that in 2007, Allergan's profit in the sale of BOTOX was $1.2 billion. Because the patent application was denied to Johnson and Schantz by WARF, neither the University of Wisconsin–Madison nor WARF saw any of the profits. In 2006, Johnson and other researchers developed a purer batch of botulinal toxin. Unlike for batch 79-11, WARF did apply for the patent and the foundation licensed the product to Mentor Company (supplier of medical products) who renamed it PurTox. In 2008, the first set of clinical trials was completed. Mentor Company will be allowed to file for a Biologics License Application for FDA approval *(24)*. The main difference between BOTOX (formally 79-11) and PurTox is that PurTox will work faster and last longer due to its purity. According to Johnson, FDA approval of PurTox would not only mean money for the University of Wisconsin–Madison, it would also restore credit to those who were responsible for the development of botulinal toxin use in medicine and cosmetics *(4)*.

Treatment and Control

Because botulism is typically a problem in anaerobic environments, such as those present in canned vegetables and meat, care must be taken to ensure these canned products are subjected to temperatures sufficient and proven to destroy *C. botulinum.* Because *C. botulinum* is widespread in the environment, contamination of

foods through harvest, formulation and processing is possible. Thus, care must be taken to protect these food products. Consequently, several preventive measures can prevent the formation of botulinal neurotoxins in food: “(i) avoid[ing] contamination of food by spores; (ii) inactivating spores that are present in food; (iii) preventing spores from germination and vegetative cell growth resulting in botulinal neurotoxins formation; and (iv) inactivation of botulinal neurotoxins in food” *(44)*. Infant botulism can be prevented by restricting honey from the diets of children under the age of one since honey is not subjected to high enough temperatures that will kill the organism *(58)*. Individuals who become infected with botulism are given a dose of equine antitoxin as soon as possible because the antitoxin works on free toxins that have not bound to the nerve endings *(58)*. Because antibiotics can encourage toxin production, it is not recommended for treatment in children or adults. CDC maintains a supply of antitoxin *(19)*.

Summary of Literature

The safety of the food supply is a topic that continues to be of concern around the world. Given all the recent food recalls of conventional, natural and organic products, one cannot help but to think about this serious issue. In an effort to protect food from contamination by pathogenic bacteria, many foods contain ingredients that possess antimicrobial characteristics. Sodium nitrite, for example, is included (in part) in the formulation of many processed meats to prevent growth of pathogens such as *C. botulinum*. Because many epidemiological studies have reported connections between the consumption of processed meats and cancer, many feel this group of food is unsafe for human consumption. Despite the efforts of many to

deem processed meats unsafe for consumption due to the addition of sodium nitrite, there is a well documented history of the safety of conventionally cured meats. This has been proven time and time again through sound scientific research. However, consumer fascination with “preservative-free” foods has led to the production of meats products that contain natural sources of nitrate/nitrite. The microbiological safety of uncured, no nitrate/nitrite-added meat products is not well understood. Research is needed in this area to ensure naturally cured meats possess the same safety characteristics consumers have come to expect from their conventional counterparts. Therefore, the objective of the first phase of this study was to quantify the potential for *Clostridium perfringens* growth in commercial processed meats manufactured without the direct addition of nitrite/nitrate. The results are expected to document the relative likelihood of pathogen growth on “cured” meats manufactured without the direct addition of nitrate or nitrite. Because different brands will be chosen, it is expected that pathogen growth will be variable. The development of supplemental treatments to increase the level and consistency of antimicrobial protection in these products is important to provide consumers with the degree of safety that they have come to expect from conventionally cured meats. Therefore, the objective of this study was to identify and test ingredients that might improve product safety without altering the unique natural/organic status of these products.

References

1. Abrahamsson, K., and H. Riemann. 1971. Prevalence of *Clostridium botulinum* in semipreserved meat products*. Appl. Microbiol.* 21:543-544.

2. Adams, M. R., and M. O. Moss. 2008. Bacterial agents of foodborne illness. Royal Society of Chemistry, Cambridge, UK.

3. Aghoghovbia, T. 2008. Outbreak of foodborne illness associated with a catered lunch-Shawnee County. Available at: http://www.kdheks.gov/epi/download/SN_Nov08_Preliminaryreport2.pdf. Accessed November 15, 2009.

4. Allen, J. 2008. Warf's new wrinkle*.* p. 39-43. Fall 2008 *On Wisconsin.* Wisconsin Alumni Association., Madison, Wisconsin.

5. Allergan, P. I. Botox cosmetics. Available at: http://www.allergan.com/assets/pdf/botox_cosmetic_pi.pdf. Accessed January 7, 2010.

6. Angulo, F., A. Voetsch, D. Vugia, J. Hadler, M. Farley, C. Hedberg, P. Cieslak, D. Morse, D. Dwyer, and D. Swerdlow. 1998. Determing the burden of human illness from foodborne diseases: CDC's emerging infectious disease program foodborne disease active surveillance network (foodnet)*. Veterinary Clinic of North America: Food Animal Practice*. 14:165-172.

7. Anon. 2000. Organic food is far more nutritious*. Newsletter of the National Association of Sustainable Agriculture Austrialia (NASAA)*. February: 10.

8. Anonymous. 2009. What comes naturally*.* p. 34-35. *Meatingplace* Magazine (February 2009). Marketing and Technology Group, Chicago, IL.

9. Archer, D. L. 2002. Evidence that ingested nitrate and nitrite are beneficial to health *J. Food Prot.* 65:872-875.

10. Avery, A., and D. Avery. 2007. Organic spinach really is to blame*.* Feedstuffs 19 March 2007, vol. 79, p. 9. The Miller Publishing Co, Minnetonka, MN.

11. Avery, A. A. 1999. Infantile methemoglobinemia: Reexamining the role of drinking water nitrates*. Environ. Health Perspect.* 107:583-586.

12. Bacus, J. N. 2007. Navigating the processed meats labeling maze. Available at: http://members.ift.org/NR/rdonlyres/29475E6D-84C4-437A-A43E-250E6AA0FC61/0/1107processedMeatLabel.pdf. Accessed November 28, 2009.

13. Bourn, D., and J. Prescott. 2002. A comparision of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Crit. Rev. Food Sci. Nutr.* 42:1-34.

14. Brett, M. M., J. McLauchlin, A. Harris, S. O'Brien, N. Black, R. J. Forsyth, D. Roberts, and F. J. Bolton. 2005. A case of infant botulism with a possible link to infant formula milk powder: Evidence for the presence of more than one strain of *Clostridum botulinum* in clinical specimens and food. *J. Med. Microbiol.* 54:769-776.

15. Buchanan, R. L., H. G. Stahl, and R. C. Whiting. 1989. Effects and interactions of temperature, pH, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes. J. Food Prot.* 52:844-851.

16. Cassens, R. G. 1997. Composition and safety of cured meats in the USA. *Food Chem.* 59:561-566.

17. Cassens, R. G. 1997. Residual nitrite in cured meat. *Food Tech.* 51:53-55.

18. Centers for Disease Control and Prevention. 1993. Methemoglobinemia in an infant-Wisconsin, 1992. *MMWR*. 42:217-219.

19. Centers for Disease Control and Prevention. 2006. Facts about botulism. Available at: http://www.bt.cdc.gov/agent/botulism/factsheet.asp. Accessed January 6, 2010.

20. Centers for Disease Control and Prevention. 2009. *Clostridium perfringens* infection among inmates at a county jail --- Wisconsin, August 2008. *MMWR*. 58:138-141.

21. Clifford, S. 2008. Recall leads Whole Foods to a change. Available at: http://www.nytimes.com/2008/08/12/business/12food.html. Accessed November 9, 2009.

22. Code of Federal Regulations. 2006. Animal and animal products. 9 CFR 317.17, 9 CFR 319.2. U.S. Government Printing Office. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_06/9cfrv2_06.html#301. Accessed December 31, 2009.

23. Conklin, N. C., and G. Thompson. 1993. Produce quality in organic and conventional produce: Is there a difference? *Agribusiness*. 9:295-307.

24. Cosmetic Surgery Review. 2008. Purtox trials underway-the new face of botox? Available at: http://cosmeticsurgerytoday.wordpress.com/2008/10/30/purtox-trials-underway-the-new-face-of-botox/. Accessed January 7, 2010.

25. Daniels, N. A., L. Mackinnon, S. M. Rowe, N. H. Bean, P. M. Griggin, and P. S. Mead. 2002. Foodborne disease outbreaks in United States schools. *The Pediatric Infectious Disease Journal*. 21:623-628.

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

27. Dimitri, C., L. Oberholtzer, and U.S. Department of Agriculture. 2009. Marketing U.S. organic foods-recent trends from farms to consumers. Economic information bulletin 58. Available at: http://www.ers.usda.gov/Publications/EIB58/EIB58.pdf. Accessed, November 28, 2009.

28. Dodds, K. L. 1993. *Clostridium botulinum* in foods. p. 53-68. *In* A.H.W. Hauschild, and K.L. Dodds (ed.), *Clostridium botulinum*: Ecology and control in foods. Marcel Dekker, New York, NY.

29. Editors (Meatingplace). 2009. Healthy Choice revamps brand, launches all-natural frozen meals. Available at: http://www.poultryplace.com/MembersOnly/webNews/details.aspx?item=11886. Accessed November 3, 2009.

30. Eisler, P., B. Morrison, and A. DeBarros. 2009. Fast-food standards for meat top those for school lunches. Available at: http://www.usatoday.com/news/education/2009-12-08-school-lunch-standards_N.htm. Accessed December 22, 2009.

31. Flier, L. J., Jr., C. U. Lowe, L. A. Barness, R. B. Goldbloom, F. P. Heald, M. A. Holliday, R. W. Miller, D. O'Brien, G. M. Owen, H. A. Pearson, C. R. Scriver, J. William B. Weil, O. L. Kine, J. Cravioto, and C. Whitten. 1970. Infant methemoglobinemia: The role of dietary nitrate. *Pediatrics*. 46:475-478.

32. Food Safety and Inspection Service. 2009. California firm recalls ground beef products due to possible *Salmonella* contamination. Available at: http://www.fsis.usda.gov/News/Recall_041_2009_Release/index.asp. Accessed December 23, 2009.

33. Food Safety and Inspection Service. 2009. Current recalls and alerts. Available at: http://www.fsis.usda.gov/Fsis_Recalls/Open_Federal_Cases/index.asp?src_location=Content&src_page=FSISRecalls. Accessed December 1, 2009, 2009.

34. Fox, S. I. 2002. Human physiology. McGraw-Hill Companies, Inc., New York, NY.

35. Gabbett, R. J. 2008. Whole Foods recalls ground beef in 23 states and Canada (updated). Available at: http://www.poultryplace.com/MembersOnly/webNews/details.aspx?item=9351. Accessed August 11, 2008.

36. Gabbett, R. J. 2009. USDA seeks comment on natural claim labeling. Available at: http://www.meatingplace.com/MembersOnly/webNews/details.aspx?item=13703. Accessed November 3, 2009.

37. Glass, K. A., and E. A. Johnson. 2002. Formulating low acid foods for botulinal safety. p. 323-350. *In* V.K. Juneja, and J.N. Sofos (ed.), Control of foodborne microorganisms. Marcel Dekker, New York, NY.

38. Greenberg, A., R. B. Tompkin, B. O. Bladel, R. S. Kittaka, and A. Anellis. 1966. Incidence of mesophilic *Clostridium* spores in raw pork, beef and chicken in processing plants in the United States and Canada. *Appl. Microbiol.* 14:789-793.

39. Greenberg, R. A., B. O. Bladel, and W. J. Zingelmann. 1966. Use of the anaerobic pouch in isolating *Clostridium botulinum* spores from fresh meat. *Appl. Microbiol.* 14:223-228.

40. Gregerson, J. 2009. "Clean" slate. p. 59-60. Meatingplace Magazine. Marketing and Technology Group, Chicago, IL.

41. Halliday, J. 2009. New culture to speed up curing meat naturally. Available at: http://www.foodnavigator.com/Financial-Industry/New-culture-to-speed-up-curing-meat-naturally. Accessed December 10, 2009.

42. Hirsch, J. 2009. Hot dogs should carry a warning label, lawsuit says. Available at: http://articles.latimes.com/2009/jul/23/business/fi-hot-dog23. Accessed November 25, 2009.

43. Hobbs, B. C., M. E. Smith, C. L. Oakley, G. H. Warrack, and J. C. Cruickshank. 1953. *Clostridium welchii* food poisoning. *J. Hyg. (Lond).* 51:75-101.

44. Johnson, E. A. 2007. *Clostridium botulinum.* p. 401-421. *In* M. Doyle, P., and L. Beuchat, R. (ed.), Food microbiology-fundamentals and frontiers. ASM Press, Washington, DC.

45. Johnston, T. 2008. AMI blasts group effort to take processed meats out of schools. Available at: http://www.poultryplace.com/MembersOnly/webNews/details.aspx?item=9343. Accessed November 4, 2009.

46. Johnston, T. 2009. Nolan Ryan's all-natural beef launches smoked beef sausage. Available at:

http://www.poultryplace.com/MembersOnly/webNews/details.aspx?item=13640. Accessed November 3, 2009.

47. Johnston, T. 2009. USDA data confirms safety of ground beef at schools. Available at: http://www.meatingplace.com/MembersOnly/webNews/details.aspx?item=14667. Accessed November 25, 2009.

48. Klingele, L. 2008. Tyson rolls out new all-natural chicken nuggets, patties. Available at: http://www.poultryplace.com/MembersOnly/webNews/details.aspx?item=10209. Accessed November 3, 2009.

49. Knobeloch, L., B. Salna, A. Hogan, J. Postle, and H. Anderson. 2000. Blue babies and nitrate-contaminated well water*. Environ. Health Perspect.* 108:675-678.

50. Knox, R., and E. K. MacDonald. 1943. Outbreaks of foodborne poisoning in certain Leicester institutions*. Medical Officer*. 69:21-22.

51. Kross, B. C., G. R. Hallberg, D. R. Bruner, K. Cherryholmes, and J. K. Johnson. 1993. The nitrate contamination of private well water in Iowa*. Am. J. Public Health*. 83:270-272.

52. Labbe, R. 1989. *Clostridium perfringens*. p. 191-234. *In* M.P. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, Inc., NY.

53. Lawrie, R. A. 1998. Lawrie's meat science. Woodhead Publishing Limited, Cambridge, England.

54. Lindström, M., R. Keto, A. Markkula, M. Nevas, S. Hielm, and H. Korkeala. 2001. Multiplex PCR assay for detection and identificaton of *Clostridium botulinum* types A, B, E, and F in food and fecal material. *Appl. Environ. Microbiol.* 67:5694-5699.

55. Lindström, M., M. Nevas, and H. Korkeala. 2006. Detection of *Clostridium botulinum* by multiplex PCR in foods and feces*. In* C.C. Adley (ed.), Foodborne pathogens: Methods and protocols Humana Press, Totowa, NJ.

56. McClane, B. A. 1992. *Clostridium perfringens* enterotoxin: Structure, action and detection*. J. Food Saf.* 12:237-252.

57. McClung, L. S. 1945. Human food poisoning due to growth of *Clostridium perfringens* (*C. welchii*) in freshly cooked chicken: Preliminary note*. J. Bacteriol.* 50:229-231.

58. McLauchlin, J., and K. A. Grant. 2007. *Clostridium botulinum* and *Clostridium perfringens.* p. 41-78. *In* S. Simjee (ed.), Foodborne diseases. Humana Press, Totowa, NJ.

59. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illnesses and death in the United States*. Emerg. Infect. Dis.* 5:607-625.

60. Metz, E. 2009. Washington firm recalls frozen ground chicken products that may contain foreign materials. Available at: http://origin-www.fsis.usda.gov/News_&_Events/Recall_008_2009_Release/index.asp. Accessed November 25, 2009.

61. Midura, T. F., and S. S. Arnon. 1976. Infant botulism: Identification of *Clostridium botulinum* and its toxin in faeces*. Lancet*. 2:934-936.

62. Montville, T. J., and K. R. Matthews. 2005. *Clostridium botulinum*. p. 203-220. *In*, Food microbiology-an introduction. ASM Press, Washington, DC.

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

64. Morrison, B., P. Eisler, and A. DeBarros. 2009. Why a recall of tainted beef didn't include school lunches. Available at: http://www.usatoday.com/news/education/2009-12-01-beef-recall-lunches_N.htm. Accessed December 5, 2009.

65. National Academy of Science. 1981. The health effects of nitrate, nitrite, and n-nitroso compounds*.* National Academy Press, Washington, DC.

66. National Organic Program. 2008. National list of allowed and prohibited substances. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5068682&acct=nopgeninfo. Accessed January 9, 2010.

67. Nelson, K. A., and M. A. Hostetler. 2003. An infant with methemoglobinemia*. Hosp. Physician*. 62:31-38.

68. Nevas, M., M. Lindström, A. Hörman, R. Keto-Timonen, and H. Korkeala. 2006. Contamination routes of *Clostridium botulinum* in the honey production environment. *Environ. Microbiol.* 8:1085-1094.

69. Oguma, K., Y. Fujinaga, K. Inoue, and K. Yokoto. 2000. Mechanisms of pathogensis and toxin synthesis in *Clostridium botulinum. In* J.W. Cary, J.E. Linz, and D. Bhatnagar (ed.), Microbial foodborne diseases-mechanisms of pathogensis and toxin synthesis Technomic Publishing Co., Inc., Lancaster, PA.

70. Organic Trade Association. 2005. The past, present and future of the organic industry: A retrospective of the first 20 years, a look at the current state of organic and forecasting the next 20 years. Available at: http://www.ota.com/pics/documents/Forecasting2005.pdf. Accessed November 25, 2009.

71. Organic Trade Association. 2009. U.S. organic sales grow by a whopping 17.1 percent in 2008. Available at: http://www.organicnewsroom.com/2009/05/us_organic_sales_grow_by_a_who.html. Accessed November 4, 2009.

72. Organic Trade Association, and Kiwi. 2009. 2009 U.S. families' organic attitudes & beliefs study. Available at: http://www.ota.com/organic/www.ota.com/pics/documents/01b_FinalOTA-KiwiExecutiveSummary.pdf. Accessed November 14, 2009.

73. Pegg, R. B., and F. Shahidi. 2000. Nitrite curing of meat: The n-nitrosamine problem and nitrite alternatives. Food and Nutrition Press, Inc., Trumbull, CT.

74. Peters, J. M., S. Preston-Martin, S. J. London, J. D. Bowman, J. D. Buckley, and D. C. Thomas. 1994. Processed meats and risk of childhood leukemia (California, USA). *Cancer Causes Control*. 5:195-202.

75. Petrak, L. 2005. Retailers go natural. Available at: http://www.meatanddeliretailer.com/Archives_Davinci?article=602. Accessed November 25, 2009.

76. Pierson, M. D., and L. A. Smoot. 1982. Nitrite, nitrite alternatives, and the control of *Clostridium botulinum* in cured meats. *Crit. Rev. Food Sci. Nutr.* 17:141-187.

77. Pither, R., and M. N. Hall. 1990. Analytical survey of the nutritional composition of organically grown fruit and vegetables. *Technical Memorandum, Campden Food & Drink Research Association*. No. 597.

78. Powlson, D. S., T. M. Addiscott, N. Benjamin, K. G. Cassman, T. M. de Kok, H. van Grinsven, L'hirondel Jean-Louis, A. A. Avery, and C. van Kessel. 2008. When does nitrate become a risk for humans? *J. Environ. Qual.* 37:291-295.

79. Proctor, H. 2009. Campbell debuts 12 new all-natural of Select Harvest soups. Available at: http://www.poultryplace.com/MembersOnly/webNews/details.aspx?item=13769. Accessed November 3, 2009.

80. Reinstein, S., J. T. Fox, X. Shi, J. M. Alam, D. G. Renter, and T. G. Nagaraja. 2009. Prevalence of *Escherichia coli O157:H7* in organically and naturally raised beef cattle*. Appl. Environ. Microbiol.* 75:5421-5423.

81. Rey, C. R., H. W. Walker, and P. L. Rohrbaugh. 1975. The influence of temperature on growth, sporulation, and heat resistance of spores of six strains of *Clostridium perfringens*. *J. Milk Food Technol.* 38:461-465.

82. Roach, R. L., and D. G. Sienko. 1992. *Clostridium perfringens* outbreak associated with minestrone soup*. Am. J. Epidemiol.* 136:1288-1291.

83. Salvage, B. 2009. Most consumers sticking with organic option. Available at: http://www.meatpoultry.com/news/daily_enews.asp?ArticleID=108118. Accessed December 23, 2009.

84. Salvage, B. 2009. Probe of *E. coli* and school lunches urged by lawmakers. Available at: http://www.meatpoultry.com/feature_stories.asp?ArticleID=107318. Accessed November 13, 2009.

85. Salvage, B. 2009. U.S. Senator wants tougher tests for school lunch beef. Available at: http://www.meatpoultry.com/news/daily_enews.asp?ArticleID=108010. Accessed December 16, 2009.

86. Sarasua, S., and D. A. Savitz. 1994. Cured and broiled meat consumption in relation to childhood cancer: Denver, Colorado (United States)*. Cancer Causes Control*. 5:141-148.

87. Sebranek, J., and J. Bacus. March 2007. Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality and safety issues*. American Meat Sci. Assoc.* 1:1-15.

88. Sebranek, J. G., and J. N. Bacus. 2007. Cured meat products without direct addition of nitrate or nitrite: What are the issues? *Meat Sci.* 77:136-147.

89. Shapley, D. 2007. Nitrite's nice, but forgo the hot dog. Available at: http://www.thedailygreen.com/healthy-eating/eat-safe/nitrite-nitrate-47123102. Accessed December 7, 2009.

90. Siderer, Y., A. Maquet, and E. Anklam. 2005. Need for research to support consumer confidence in the growing organic food market*. Trends Food Sci. Technol.* 16:332-343.

91. Sindelar, J., J. C. Cordray, and J. G. Sebranek. 2008. Consumer acceptance of commercial natural and organic processed meats*.* A.S. Leaflet R2258 Iowa State University Animal Industry Report.

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

93. Sindelar, J. J., J. C. Cordray, J. G. Sebranek, J. A. Love, and D. U. Ahn. 2007. Effects of vegetable juice powder concentration and storage time on some chemical and sensory quality attributes of uncured, emulsified cooked sausage*. J. Food Sci.* 72:S324-S332.

94. Sindelar, J. J., and T. A. Houser. 2009. Alternative curing systems*.* p. 379-405. *In* R. Tarte (ed.), Ingredients in meat products Springer, New York, NY.

95. Smith, B. L. 1993. Organic foods vs supermarket foods: Elemental levels*. J. Appl. Nutr.* 45:35-39.

96. Snowden, J. A., and D. O. Cliver. 1996. Microorganisms in honey*. Int. J. Food Microbiol.* 31:1-26.

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

98. Solomon, H. M., and T. Lilly, Jr. 2001. Bacteriological analytical manual online: *Clostridium botulinum*. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070879.htm#authors. Accessed January 6, 2010.

99. Stopes, C., L. Woodward, G. Forde, and H. Vogtmann. 1988. The nitrate content of vegetable and salad crops offered to the consumer as from organic or conventional production systems*. Biol. Agric. Hort.* 5:215-221.

100. Storck, A. B. 2009. Kahiki introduces bagged all-natural chicken in four flavors. Available at: http://www.meatingplace.com/MembersOnly/webNews/details.aspx?item=11370. Accessed November 3, 2009.

101. Storck, A. B. 2009. "Natural" claims on the rise among new products: Mintel. Available at: http://www.meatingplace.com/MembersOnly/webNews/details.aspx?item=11057. Accessed October 24, 2009.

102. Storck, A. B. 2009. Pizza hut debuts natural ingredients menu-wide Available at: http://www.meatingplace.com/MembersOnly/webNews/details.aspx?item=11049. Accessed November 3, 2009.

103. Storck, A. B. 2009. Publix uses shelf tags to highlight natural, organic, earth friendly products. Available at:

http://www.meatingplace.com/MembersOnly/webNews/details.aspx?item=13553&pf=false. Accessed November 3, 2009.

104. Strong, D. H., and J. C. Canada. 1964. Survival of *Clostridium perfringens* in frozen chicken gravy*. J. Food Sci. 2*9:479-482.

105. Strong, D. H., E. F. Foster, and C. L. Duncan. 1970. Influence of water activity on the growth of *Clostridium perfringens. Appl. Microbiol.* 19:980-987.

106. Taclindo, C. T., T. Midura, G. S. Nygaard, and B. H.L. 1967. Examination of prepared foods in plastic packages for *Clostridium botulinum. Appl. Microbiol.* 15:426-430.

107. The Center for Consumer Freedom. 2006. The dose makes the poison. Available at: http://www.consumerfreedom.com/article_detail.cfm/a/176-the-dose-makes-the-poison. Accessed January 7, 2010.

108. Tompkin, R. B. 2005. Nitrite*.* p. 169-236. *In* P.M. Davidson, J.N. Sofos, and A.L. Branen (ed.), Antimicrobials in food. Taylor & Francis, Boca Raton, FL.

109. Tompkin, R. B., and L. N. Christiansen. 1976. *Clostridium botulinum.* p. 156-169. *In* M.P. Defigueirdeo, and D.F. Splittorstoesser (ed.), Food microbiology: Public health and spoilage aspects. The AVI Publishing Company, Inc., Westport, CT.

110. Tuffs, A. 2002. Organic meat products contaminated with carcinogenic herbicide*. Br. Med. J.* 324:1416.

111. U.S. Department of Agriculture. 1995. Processing inspectors' calculations handbook. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf. Accessed December 8, 2009.

112. U.S. Department of Agriculture. 2005. Food standards and labeling policy book*.* p://www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy_Book_082005.pdf.

113. U.S. Department of Agriculture. 2008. National organic program: Certification. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3004346&acct=nopgeninfo. Accessed December 31, 2009.

114. U.S. Department of Agriculture. 2008. Organic labeling and marketing information. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3004446&acct=nopgeninfo. Accessed November 13, 2009.

115. U.S. Department of Agriculture, and Food Safety and Inspection Service. 1999. Compliance guidelines for cooling heat-treated meat and poultry products (stabilization). Available at: http://www.fsis.usda.gov/. Accessed January 6, 2010.

116. U.S. Environmental and Protection Agency. 2006. Consumer fact sheet on nitrates/nitrites. Available at: http://www.epa.gov/safewater/contaminants/dw_contamfs/nitrates.html. Accessed January 1, 2010.

117. U.S. Environmental and Protection Agency. 2009. Drinking water contaminants. Available at: http://www.epa.gov/safewater/contaminants/index.html. Accessed January 1, 2010.

118. U.S. Food and Drug Administration. 2009. Whole Foods Market recalls bulk hazelnuts. Available at: http://www.fda.gov/Safety/Recalls/ucm195060.htm. Accessed December 23, 2009.

119. van Ermengem, E. P. 1979. A new anaerobic bacillus and its relation to botulism (originally published as "Ueber einen neuen anaeroben bacillus und seine bezieh- ungen zum botulismus" In zeitschrift fiir hygiene und infektionskrankheiten 26: 1-56, 1897)*. Rev of Infect Dis.* 1:701-719.

120. Walker, R. 1975. Naturally occuring nitrate/nitrite in foods*. J. Sci. Food Agric.* 26:1735-1742.

121. Winter, C. K., and S. F. Davis. 2006. Organic foods*. J. Food Sci.* 71:R117-R124.

122. Wrigley, D. M. 2001. *Clostridium perfringens.* p. 139-168. *In* Y.H. Hui, M.D. Pierson, and R.J. Gorham (ed.), Foodborne disease handbook, vol. 1: Bacterial pathogens. Marcel Dekker, Inc., New York.

123. Young, A. 2009. Safety of food at airports spotty. Available at: http://www.usatoday.com/travel/flights/2009-12-23-airport-food-safety_N.htm. Accessed December 30, 2009.

CHAPTER 3. SURVIVAL AND GROWTH OF *CLOSTRIDIUM PERFRINGENS* ON COMMERCIAL NO-NITRATE-OR-NITRITE ADDED (NATURAL AND ORGANIC) FRANKFURTERS, BACON AND HAM

A paper to be submitted to the Journal of Food Protection

Armitra L. Jackson[1], Gary Sullivan[1], Joseph G. Sebranek[1], James S. Dickson[1]

Abstract

The popularity of “preservative-free” foods among consumers has stimulated rapid growth of processed meats manufactured without sodium nitrite. The objective of this study was to quantify the potential for *Clostridium perfringens* growth in commercially available processed meats manufactured without the direct addition of nitrite or nitrate. Commercial brands of uncured, no-nitrate or nitrite-added frankfurters (10), bacon (9) and ham (7) were obtained from retail stores and challenged with a three-strain inoculation (5 $\log_{10}$ CFU/g) of *C. perfringens*. Reduced inhibition ($P<0.05$) was observed in seven brands of frankfurters, six brands of hams and four brands of bacon when compared to each respective sodium nitrite-added control. These products also demonstrated a wide variation in growth response. These results indicate that commercially available natural/organic cured meats have more potential for growth of this pathogen than do conventionally cured products. Natural and organic processed meats may require additional protective measures in order to consistently provide the same level of safety from bacterial pathogens that is achieved by conventionally cured meat products.

[1]Iowa State University, Ames, Iowa 50011, USA

Introduction

In the past decade, consumers have become increasingly interested in consuming foods that are “preservative free” and foods they perceive to be “healthier”. Therefore, consumers tend to associate the inclusion of ingredients such as sodium nitrite in cured meat products as undesirable and unhealthy. Consumers hold this belief despite the fact that cured meats are not considered a significant source of nitrite. Vegetables account for approximately 75–80% of nitrate consumed via food *(1, 2)* and 10–15% of nitrate consumed via water *(2)*. However, the perception that processed meats are unhealthy due to nitrite and nitrate has made processed meats marketed as “natural” and “organic” extremely attractive to health-conscious consumers. To that end, consumers are willing to pay premium prices for these products with the belief they are consuming a product that is safer than its conventional counterpart *(21)*. Due to the increased consumer demand for “natural” and “preservative-free” food products, a significant number of meat processors have begun to manufacture processed meats without formulated nitrite (e.g., sodium nitrite). Because nitrite is considered a chemical preservative by the United States Department of Agriculture–Food Safety Inspection Service (USDA–FSIS), it is not permitted in natural and organic meat products. In an effort to replace the properties introduced to cured meats by sodium nitrite, there is currently a new trend in the meat industry to utilize natural sources of nitrite or nitrate for processed meats that normally include nitrite as an added ingredient. This approach is being utilized because nitrite is irreplaceable in terms of its effect on color, flavor and other properties of cured meats. These “naturally cured” products are manufactured to

simulate traditionally cured meat products, but without the direct addition of nitrite. Manufacture of these products without the direct addition of nitrite is necessary to qualify the products as natural or organic because nitrite is a preservative. The major concern with these products is that they do not contain nitrate or nitrite in concentrations that are comparable to conventional products *(18)* and that are known to be highly effective in inhibiting the growth of *Clostridium perfringens* and many other foodborne pathogens. Further, neither the extent of the likely increased hazard nor appropriate counter measures for maintaining safety have been determined. Processed meats that are conventionally cured with the direct addition of sodium nitrite have a long history of safety relative to human consumption, but the microbiological safety of “naturally cured” meats manufactured without the direction addition of sodium nitrite is not well understood. As a result of reduced nitrite concentrations, these products are likely to be more susceptible to foodborne pathogens.

C. perfringens is a spore-forming, gram-positive pathogenic bacterium responsible for Type A foodborne illnesses *(9)*. Between the years of 1983 and 1992, there were nearly 250,000 cases of foodborne illness involving *C. perfringens (12).* Improper heating and cooling of foods contributes to foodborne illnesses associated with *C. perfringens*. Vegetative cells are heat sensitive and can be deactivated at 75℃ *(9)*. Spores, however, are extremely heat resistant and can survive very high and low temperatures. *C. perfringens* is an opportunistic organism in that it is ubiquitous in the environment. This pathogen can be found in the soil (10^3–10^4/g), in the intestinal tract of animals (10^3–10^6/g) and in approximately 50%

of raw and frozen meat products *(11)*. The effect of nitrite on *C. perfringens* is well documented *(5, 13, 15)*.

The objective of this study was to quantify the potential for *C. perfringens* growth in commercially available processed meats manufactured to simulate traditionally cured products, but without the direct addition of nitrite or nitrate, relative to products to which no nitrite/nitrate source was used, and relative to conventionally cured products. These products will be referred to as “truly natural”, “naturally cured” and “conventionally cured,” respectively, in this report.

Materials and Methods

Bacterial strain and spore suspension. *C. perfringens* strains ATCC 10258, 3124 and 12917 were obtained from the Food Safety Research Laboratory (FSRL) at Iowa State University. The organism was cultured in fluid thioglycollate medium and sporulation was induced in Duncan-Strong sporulation medium *(6)* as described by Juneja et al. *(8)*. The spore crop was harvested by centrifugation (9,500 x g, 10 min., 4℃) and then resuspended in phy siological saline (0.85% wt/vol sodium chloride). The three-strain cocktail was combined and vortexed just prior to inoculation.

Sampling preparation and inoculation. For each of the three replications, commercial brands of frankfurters, bacon and ham with the same sell–by date were purchased at grocery stores located in the Midwest region of the United States and from online stores. Products purchased from online distributors were shipped under refrigeration overnight to the Meat Laboratory at Iowa State University and were received during normal business hours. When the product arrived, product

temperature was measured and recorded to ensure temperature abuse had not occurred during shipping. Products were stored at 4℃ at the FSRL at Iowa State University and were utilized within one week of purchase. Each product was assigned a letter code.

According to the ingredient statements, seven frankfurter brands (denoted A and D–I, K) were naturally cured and manufactured using sea salt or celery juice powder as ingredients. Brands B and C were truly uncured and appeared to be manufactured without the intention to replace sodium nitrite or nitrate. Brands J and L were conventionally cured products used as the controls to demonstrate typical inhibition of *C. perfringens* in conventionally cured frankfurters. All frankfurter brands were 100% beef with the exception of brand C, in which all turkey was used. Nine bacon brands (denoted A-F and H-J) were naturally cured using sea salt or celery juice to simulate the typical curing process. Brand G, which contained ingredients typical for bacon processing, was used as the conventionally cured control. Seven ham brands (denoted A–F and H) were naturally cured using sea salt or celery juice. Brands G and I–K were fully–cooked conventionally cured hams and used as controls. Brands G and I were labeled as containing natural juices; brands J and K were water-added hams. Brand J was labeled as “96% fat free.” All product (frankfurters, bacon and ham) controls contained ingredients typically used in conventional product manufacture, such as salt, dextrose, potassium lactate, sodium phosphates and sodium erythorbate in addition to sodium nitrite. The number of controls used for frankfurters, bacon and ham (2, 1 and 4 respectively) was based on product availability from local retailers.

For each product, a 25-gram sample was placed in a 5 X 16 in vacuum package bag (Cryovac Packaging, Duncan, S.C., USA) and inoculated with 0.1 ml of the spore suspension to give a final spore concentration of 5-log spores/g. Frankfurters were inoculated under a biological safety cabinet (Nuaire, Model NU-425-400, Plymouth, Minn., USA) using a 1 cc needle (Difco, Becton Dickinson, Sparks, Md., USA) to inject the suspension into the interior of the frankfurters. A different needle was used for each sample. Bacon and ham samples were surface–inoculated. After packages were sealed under vacuum (Multivac, Model A-300/52, Kansas City, Mo., USA), all samples were heat shocked in a water bath (NESLAB Instruments, Inc., Newington, N.H., USA, RTE-211) to an internal temperature of 75℃ for 20 minutes to ensure that all vegetative cell s were inactivated and spores were activated *(9)*. A thermometer was used in a similar non-inoculated sample to monitor temperature during the heat shocking process. Following the heat shocking process, all products were chilled according to the USDA–FSIS guidelines for *C. perfringens* control in cured meats, which states that the cooked meat product's maximum internal temperature should remain between 130℉ (54.4℃) and 80℉ (26.6℃) for no more than 1.5 hr and between 80℉ (26.6℃) and 40℉ (4.4℃) for no more than 5 hr *(19)*. After the product reached an internal temperature of 4.4ºC, the product was stored in containers at room temperature (20℃) in the FSRL. Sampling was conducted on day 0, 1, 2, 4, 6, 8, 10 following inoculation for frankfurters and day 0, 1, 2, 6, 8, 10 following inoculation for bacon and ham. These sampling days were determined by results from preliminary studies.

Microbiological analysis. On the appropriate day, one package for each treatment was collected and opened aseptically. Sampling was achieved by blending each 25-gram sample with 225 ml of 0.1% peptone water in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, Wis., USA). Each sample was stomached for 30 s in the laboratory blender (Stomacher 400, Seward Medical, London, UK). All blended samples were maintained on an ice slush. Appropriate dilutions were plated with a glass rod in duplicate on perfringens agar with Tryptose Sulphite Cycloserine (TSC) and egg yolk emulsion (Oxoid, Basingstroke, UK) *(10)*. Agar plates were incubated at 35℃ in anaerobic jars with Gas Pak palladi um catalyst envelopes (Oxoid.) for 24 h. In an effort to ensure the anaerobic jars were functioning properly, anaerobic indicators were included in each jar.

Data analysis. Three independent replicate experiments were performed for each of the frankfurters, bacon and ham. Viable *C. perfringens* populations were determined by calculating the log value of bacterial counts on duplicate plates for each sample that was analyzed. A F-test was performed to confirm that there was a difference among brands. In the pairwise comparisons of the means, Tukey's Honestly Significant Difference (HSD) procedure was used to adjust for the multiple comparisons when testing for a significant difference between means of brands within a particular product. Significant levels were determined at $P<0.05$. Data were analyzed using the PROC GLM (general linear models) procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, N.C., USA).

Results and Discussion

Figures 1 to 3 illustrate the growth of *C. perfringens* over time on different brands of commercially available frankfurters, bacon and ham, respectively. Table 1 shows means and standard errors for growth of *C. perfringens* for all sampling days for each brand. In Figure 1, controls for the frankfurters (J and L) exhibited growth by inoculated *C. perfringens* that was not significantly ($P<0.05$) different from each other. As shown in Figure 1 and Table 1, overall growth was greater for all sampling days ($P<0.05$) in naturally cured frankfurter brands A, B, C, D, E, F and G when compared to conventionally cured controls J and L. Brands K, H and I were naturally cured products that exhibited no significantly greater ($P<0.05$) growth by inoculated *C. perfringens* than that of controls J and L. Brand K contained potassium lactate according to the ingredients statement, and this ingredient is recognized as a bacterial inhibitor. Brands H and I did not include lactate, and it is not clear whether brands H and I may have contained other ingredients that may have aided in the inhibition of *C. perfringens*.

As shown in Figure 2 and Table 1, greater overall growth for all sampling days ($P<0.05$) was observed in naturally cured bacon brands A, B, C and D when compared to control G. Brands E, F, H, I and J were also naturally cured, but exhibited no significantly greater ($P<0.05$) growth by inoculated *C. perfringens* than that of control G. Brand J contained sodium lactate, a significant antimicrobial, according to the ingredients statement on the package, but it is not clear why brands E, F, H and I did not demonstrate growth different from the control.

As shown in Figure 3 and Table 1, greater overall growth for all sampling days ($P<0.05$) was observed in naturally cured ham brands A, C, D, E and F relative to control G. When compared to controls I, J and K, greater growth for all sampling days ($P<0.05$) was also observed in brand B, in addition to the other naturally cured brands. Control G, however, resulted in more rapid growth than the other three controls (I, J, K).

The increased growth of *C. perfringens* in 7 of 10 naturally cured frankfurters, 4 of 9 naturally cured bacon and 6 of 7 naturally cured ham samples relative to their respective controls is most likely due to the fact that the traditionally cured controls were cured with conventional concentrations of sodium nitrite, thus resulting in increased inhibition. The naturally cured brands used natural sources of nitrite or nitrate and it may be that the variation in antimicrobial effectiveness could be due to variable concentrations of nitrite produced as a result of the natural curing process. It is also interesting to note the variation in the inhibitory response of *C. perfringens* among the nitrite-added ham controls. For example, brand G increased from log 3 to log 5 after 10 days, whereas brand K declined to log 1. Control brands G and K were significantly different ($P<0.05$) in terms of *C. perfringens* growth. Thus, even the conventionally cured products demonstrated variation, which may be due to the concentration of other ingredients used by various manufacturers.

Residual nitrate (ppm) and nitrite (ppm) were determined for the commercially available frankfurters, bacon and ham (Table 2). It is important to remember that these analyses were conducted on retail products well after the formulation and processing dates, and that residual nitrite at retail is not a good indicator of the

amount of nitrite added or formed in the product. This is particularly true given the variability of other ingredients and process treatments by different manufacturers. Nitrite concentrations, for example, for the frankfurters range from less than 1 ppm to over 65 ppm (Table 2). The only meaningful conclusion that can be made from the commercial product residual nitrite and nitrate data is that residual nitrite and nitrate was indeed present in all naturally cured and conventionally cured products. Drawing conclusions from the nitrite and nitrate data is extremely difficult because nitrite is a highly reactive chemical in a meat system and is sensitive to the concentrations of other ingredients and processing conditions, as well as time and temperature of storage. Sindelar et al. *(17)* also reported similar variation in a study in which commercially available frankfurters, bacon and ham were analyzed for nitrate and nitrite levels. In addition, Cassens et al. *(4)* reported that less than 50% of the nitrite originally included in the product formulation can be analytically detected after the heating process is completed and this subsequent loss of nitrite continues during product storage (such as in distribution and retail). Sauter et al. *(15)* reported similar results in a study that found that only 50–60% of the initial nitrite remained after 24 hours of curing. This explains why the amount of nitrite detected in commercially available processed meat product is typically much lower than the amount of nitrite included in the product formulation.

These results indicate that many of the commercial natural and organic cured meats have more potential for pathogen growth than do conventionally cured products of the same type. In the event of microbial contamination and product temperature abuse, the commercial brands of frankfurters, ham and bacon that

exhibited significantly greater growth of *C. perfringens* than that of the nitrite-added control have the potential to result in foodborne illnesses and product recalls. It is important to note that organic and natural foods have not been excluded from recalls due to contamination by foodborne pathogens. Recently, raw organic hazelnuts were recalled due to contamination with *Salmonella (20)*. In August 2008, natural fresh ground beef was recalled in 23 states and Canada due to possible *E. coli* O157:H7 contamination *(7)*. In 2006, there was a multi-state outbreak of *E. coli* O157:H7 in spinach that was organically grown *(3)*. Reinstein et al. *(14)* reported that, despite popular belief, the prevalence of *E. coli* O157:H7 for naturally and organically raised cattle was the same.

It is evident from Figures 1 to 3 that there is considerable variation in the potential for *C. perfringens* growth among the commercially available natural/organic frankfurters, bacon and ham, meaning that the bacterial safety of these products is not well understood or well controlled. Schrader et al. *(16)* found similar variations in commercial frankfurters inoculated with *Listeria monocytogenes*. Sindelar et al. *(17)* also found wide variations in cured meat pigment concentrations as well as nitrite concentrations among commercial naturally cured frankfurters, indicating that there is also a wide variation in the amount of curing reaction occurring during the manufacture of naturally cured products. Consequently, development of supplemental treatments to increase the impact and consistency of antimicrobial protection in these products is important to provide consumers with the degree of safety that they have come to expect from conventionally cured processed meats. This is particularly true because the natural and organic processed meats that have

natural sources of nitrate or nitrite added resemble conventionally cured meats in all obvious respects. Consequently, consumers will expect these products to have the same safety and shelf life characteristics as conventionally cured meats and are likely to handle them in similar fashion as conventionally cured meat products when more stringent food safety handling precautions may be warranted.

Acknowledgments

This project was supported by the National Integrated Food Safety Initiative (Grant no. 2006-51110-03609) of the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, the National Pork Board (Grant no. 06-008) and the Iowa State University Food Safety Consortium.

References

1. Adams, M. R., and M. O. Moss. 2008. The microbiology of food preservation. Royal Society of Chemistry, Cambridge, UK.

2. Archer, D. L. 2002. Evidence that ingested nitrate and nitrite are beneficial to health. *J. Food Prot.* 65:872-875.

3. Avery, A., and D. Avery. 2007. Organic spinach really is to blame. Feedstuffs 19 March 2007, vol. 79, p. 9. The Miller Publishing Co., Minnetonka, MN.

4. Cassens, R. G., M. L. Greaser, T. Ito, and M. Lee. 1979. Reactions of nitrite in meat. *Food Tech.* 33:46-57.

5. Davidson, P. M., and T. M. Taylor. 2007. Chemical preservatives and natural antimicrobial compunds. p. 713-745. *In* M.P. Doyle, and L.R. Beuchat (ed.), Food microbiology: Fundamentals and frontiers. ASM Press, Washington, DC.

6. Duncan, C. L., and D. H. Strong. 1968. Improved medium for sporulation of *Clostridium perfringens*. *Appl. Microbiol.* 16:82-89.

7. Gabbett, R. J. 2008. Whole foods recalls ground beef in 23 states and Canada (updated). Available at: http://www.poultryplace.com/MembersOnly/webNews/details.aspx?item=9351. Accessed November 6, 2009.

8. Juneja, V. K., J. E. Call, and A. J. Miller. 1993. Evaluation of methylxanthines and related compounds to enhance *clostridum perfringens* sporulation using a modified duncan and strong medium. *J. Rapid Methods Autom. Microbiol.* 2:203-218.

9. Labbe, R. 1989. *Clostridium perfringens*. p. 191-234. *In* M.P. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, Inc., New York, NY.

10. Labbe, R. G., and V. Juneja, K. 2002. *Clostridium perfringens.* p. 119-126. *In* D.O. Cliver, and H.P. Riemann (ed.), Foodborne diseases. Academic Press, San Diego, CA.

11. McLauchlin, J., and K. A. Grant. 2007. *Clostridium botulinum* and *clostridum perfringens*. p. 41-78. *In* S. Simjee (ed.), Foodborne diseases. Humana Press, Totowa, NJ.

12. Mead, P. S., L. Slutsher, V. Dietz, L. F. McCaig, J. Bresee, S., C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illnesses and death in the United States. *Emerg Infect Dis.* 5:607-625.

13. Perigo, J. A., and T. A. Roberts. 1968. Inhibition of clostridia by nitrite. *J Food Technol.* 3:91-94.

14. Reinstein, S., J. T. Fox, X. Shi, J. M. Alam, D. G. Renter, and T. G. Nagaraja. 2009. Prevalence of *Escherichia coli* O157:H7 in organically and naturally raised beef cattle. *Appl. Environ. Microbiol.* 75:5421-5423.

15. Sauter, E. A., J. Kemp, D., and B. E. Langlois. 1977. Effect of nitrite and erythorbate on recovery of *Clostridium perfringens* spores in cured pork. *J. Food Sci.* 42:1678-1679.

16. Schrader, K. D., J. C. Cordray, J. G. Sebranek, J. S. Dickson, and A. F. Mendonca. 2009. Control of *Listeria monocytogenes* on no-nitrate-or-nitrite added (natural and organic) frankfurters. A.S. Leaflet R2386 Iowa State University Animal Industry Report.

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

18. Sindelar, J. J., J. C. Cordray, J. G. Sebranek, J. A. Love, and D. U. Ahn. 2007. Effects of vegetable juice powder concentration and storage time on some

chemical and sensory quality attributes of uncured, emulsified cooked sausage. *J. Food Sci.* 72:S324-S332.

19. U.S. Department of Agriculture. 1999. Compliance guidelines for cooling heat-treated meat and poultry products (stabilization). Available at: http://origin-www.fsis.usda.gov/OPPDE/rdad/FRPubs/95-033F/95-033F_Appendix%20B.htm. Accessed March 10, 2010.

20. U.S. Food and Drug Administration. 2009. Whole foods market recalls bulk hazelnuts. Available at: http://www.fda.gov/Safety/Recalls/ucm195060.htm. Accessed December 23, 2009.

21. Winter, C. K., and S. F. Davis. 2006. Organic foods. *J. Food Sci.* 71:R117-R124.

Table 1. Means and standard errors for growth of *C. perfringens* for all sampling days in naturally cured, truly uncured and conventionally cured commercially available frankfurters, bacon and ham during storage at room temperature.

	Product[a]		
Brand	Frankfurters[b]	Bacon[c]	Ham[d]
A	5.29 ± 0.87^{ef}	5.27 ± 0.67^{e}	5.77 ± 0.67^{e}
B	6.10 ± 1.02^{e}	5.28 ± 0.77^{e}	4.60 ± 0.77^{efg}
C	5.47 ± 0.96^{ef}	4.80 ± 0.82^{e}	4.95 ± 0.78^{ef}
D	5.33 ± 0.81^{ef}	4.10 ± 0.69^{ef}	5.27 ± 0.66^{e}
E	4.00 ± 0.90^{fgh}	2.72 ± 0.46^{fg}	4.58 ± 0.73^{efg}
F	4.46 ± 0.93^{ghi}	2.73 ± 0.56^{fg}	5.02 ± 0.77^{e}
G	4.56 ± 0.92^{efg}	2.28 ± 0.56^{fg}	3.78 ± 0.45^{gh}
H	2.56 ± 0.46^{hij}	2.49 ± 0.35^{fg}	3.68 ± 0.46^{fgh}
I	2.28 ± 0.31^{ij}	1.81 ± 0.35^{g}	2.55 ± 0.35^{hi}
J	2.00 ± 0.33^{j}	1.18 ± 0.29^{g}	2.40 ± 0.34^{hi}
K	1.43 ± 0.32^{j}	—	1.58 ± 0.32^{i}
L	1.50 ± 0.18^{j}	—	—

Note. Counts are expressed as the mean (± standard error) log counts from each brand. Within each column, means followed by the same letter are not significantly different (P>0.05). Highlighted brands denote conventionally cured controls for each product.

[a]Products A–L represent different brands of commercial uncured, no-nitrate/nitrite-added frankfurters, bacon and ham products.

[b]Frankfurter brands A and D–I, K were naturally cured, brands B and C were truly uncured and brands J and L were conventionally cured controls.

[c]Bacon brands A–F and H–J were naturally cured and brand G was conventionally cured control.

[d]Ham brands A-F and H were naturally cured and brands G, I–K were conventionally cured controls.

Table 2. Mean residual nitrate and residual nitrite for naturally cured and conventionally cured commercially available frankfurters, bacon and ham.

	Nitrate concentration(ppm)			Nitrite concentration(ppm)		
Product	Frankfurters[a]	Bacon[b]	Ham[c]	Frankfurters[a]	Bacon[b]	Hams[c]
A	30.69^{e}	14.37^{ef}	9.90^{e}	14.72^{g}	13.08^{g}	9.27^{fg}
B	4.70^{h}	14.27^{ef}	15.06^{fg}	0.62^{h}	4.56^{ef}	10.66^{gi}
C	18.79^{k}	25.14^{fg}	9.70^{e}	1.20^{h}	7.30^{efg}	11.13^{gi}
D	16.40^{k}	23.68^{fg}	12.30^{ef}	8.47^{e}	5.32^{efg}	7.14^{efk}
E	41.92^{fg}	41.25^{i}	4.71^{k}	3.34^{eh}	22.31^{i}	5.09^{k}
F	48.86^{g}	15.62^{ef}	13.24^{f}	65.69^{i}	6.16^{efg}	5.19^{k}
G	60.11^{i}	29.88^{g}	16.72^{go}	11.96^{g}	10.43^{fg}	12.64^{i}
H	35.59^{ef}	10.13^{e}	13.10^{f}	3.82^{eh}	3.39^{ef}	10.52^{gi}
I	36.34^{ef}	10.74^{e}	13.54^{f}	3.90^{eh}	4.92^{ef}	5.67^{ek}
J	42.30^{fg}	52.53^{i}	17.43^{gi}	6.83^{e}	8.63^{efg}	8.74^{efg}
K	37.11^{ef}	—	19.26^{i}	7.83^{e}	—	12.57^{i}
L	32.32^{ef}	—	—	5.73^{eh}	—	—

Note. Within each column, means followed by the same letter are not significantly different (P>0.05).

[a]Frankfurter brands A and D–I were naturally cured, brands B and C were truly uncured and brands J and K were conventionally cured.

[b]Bacon brands A-F and H–J were naturally cured and brand G was conventionally cured.

[c]Ham brands A–F and H were naturally cured and brands G, I–K were conventionally cured.

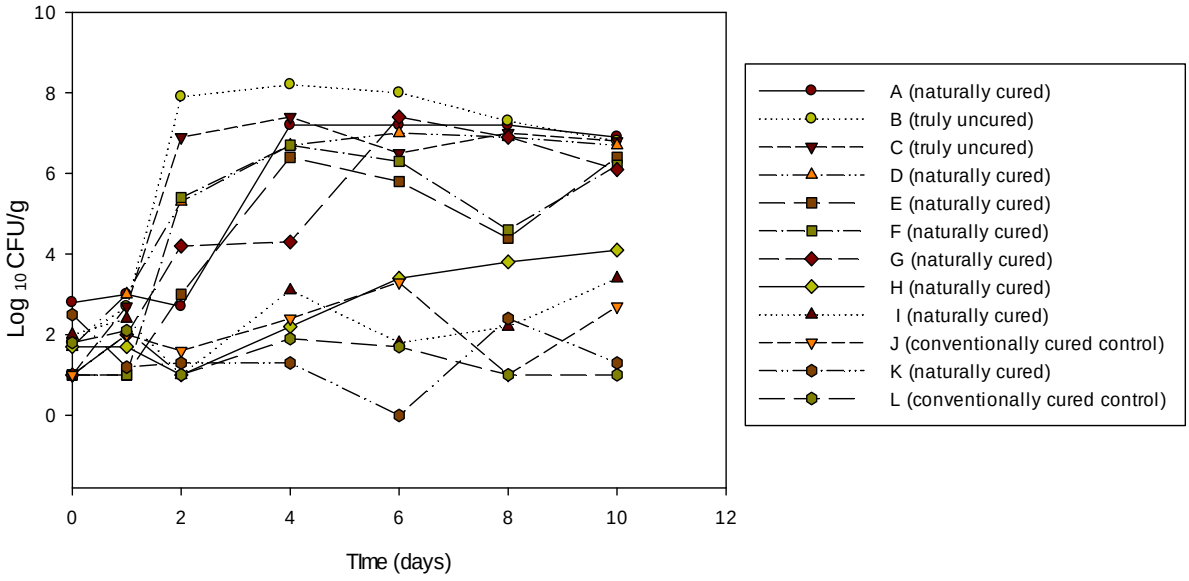

Figure 1. Growth of inoculated *Clostridium perfringens* in naturally cured, truly uncured and conventionally cured commercially available frankfurters during storage at room temperature.

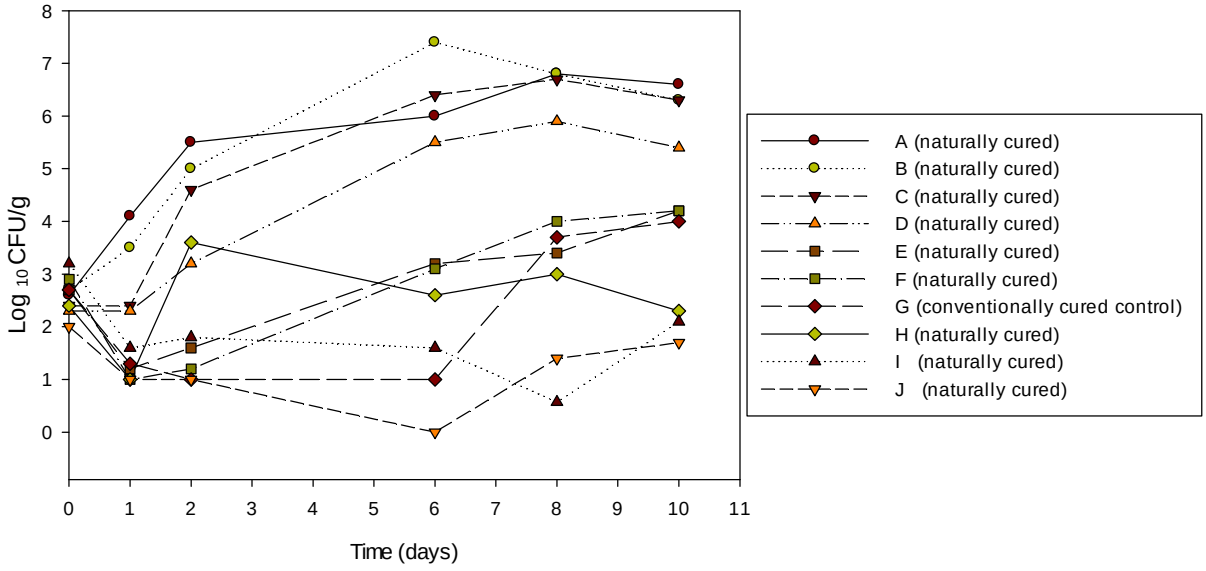

Figure 2. Growth of inoculated *Clostridium perfringens* in naturally cured and conventionally cured commercially available bacon during storage at room temperature.

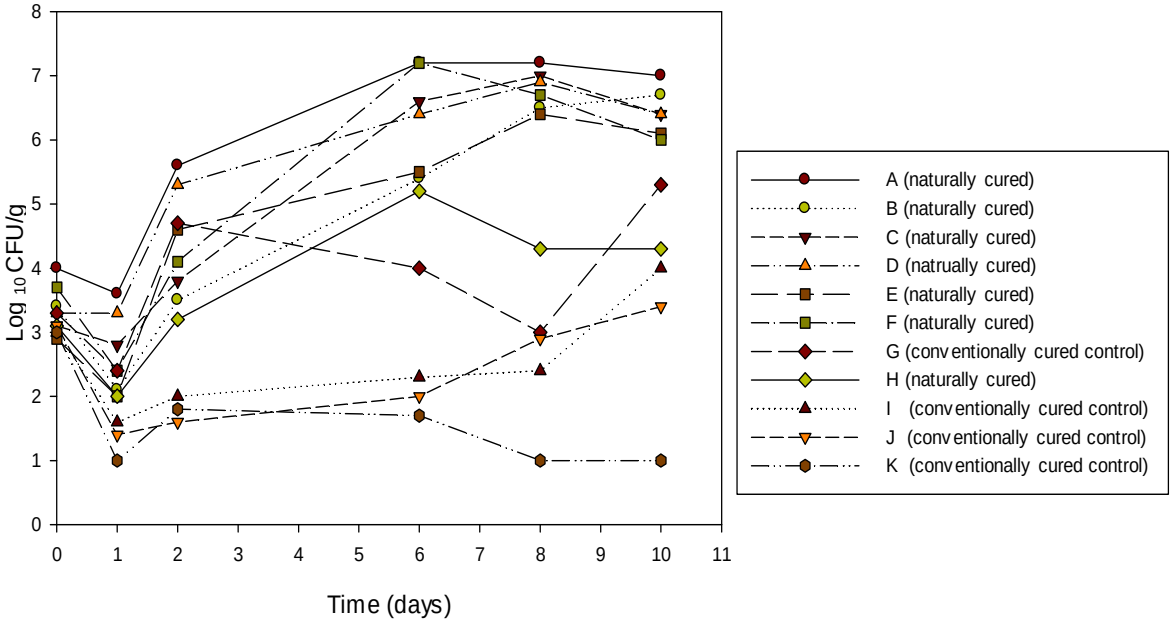

Figure 3. Growth of inoculated *Clostridium perfringens* in naturally cured and conventionally cured commercially available ham during storage at room temperature.

CHAPTER 4. USE OF NATURAL INGREDIENTS TO CONTROL GROWTH OF *CLOSTRIDIUM PERFRINGENS* ON NATURALLY CURED FRANKFURTERS AND HAM

A paper to be submitted to the Journal of Food Protection

Armitra L. Jackson[1], Gary Sullivan[1], Charlwit Kulchaiyawat[1], Joseph G. Sebranek[1], James S. Dickson[1]

Abstract

A major concern for meats marketed as natural/organic is that they do not contain nitrite in concentrations known to be effective for inhibiting the growth of foodborne pathogens. The development of supplemental treatments to increase the level and consistency of antimicrobial protection in these products is important to provide consumers with the degree of safety that they have come to expect from conventionally cured meats. Therefore, the objective of this study was to identify and test ingredients that might improve product safety without altering the unique natural/organic status of these products. Eight treatments of hams and frankfurters were prepared as follows: (1) uncured control (all typical ingredients except nitrite and nitrate), (2) conventionally cured control (erythorbate, nitrite, lactate/diacetate blend), (3) natural nitrate cure (with starter culture containing *Staphylococcus carnosus*), (4) natural nitrate cure (with culture and natural antimicrobial A containing vinegar, lemon and cherry powder blend), (5) natural nitrate cure (with culture and antimicrobial B containing cultured corn sugar and vinegar blend), (6) natural nitrite cure without additional antimicrobials, (7) natural nitrite cure with natural antimicrobial A and (8) natural nitrite cure with antimicrobial B. For the hams,

[1]Iowa State University, Ames, IA, USA.

treatments 3, 4, 5 and 8 did not have growth by inoculated *C. perfringens* that was significantly different ($P<0.05$) from that of the conventionally cured control. For frankfurters, treatments 4, 7 and 8 showed growth that was not significantly different when compared to the conventionally cured control. These results suggest that commercial natural/organic cured meats have more potential for pathogen growth than do conventionally cured products, but supplemental natural ingredients offer safety improvement.

Introduction

Clostridium perfringens is a spore-forming, gram-positive pathogenic bacterium that, in the case of food contamination, may lead to Type A foodborne illnesses *(7)*. Between the years of 1983 and 1992, there were nearly 250,000 cases of foodborne illness involving *C. perfringens (10)*. This pathogen can be found in the soil (10^3–10^4/g), in the intestinal tract of animals (10^3–10^6/g) and in approximately 50% of raw and frozen meat products *(9)*. Vegetative cells are heat sensitive and can be deactivated at 75℃ *(7)*. Spores, however, are extremely heat resistant and can survive very high and low temperatures. *C. perfringens* is an opportunistic organism in that it is ubiquitous in the environment *(7)*.

Cured processed meats have been a staple in the American diet for many years. These products have a long history of safety in terms of human consumption. Sodium nitrite is included in the ingredients, thus providing the cured color, flavor and microbiological safety that individuals have come to expect from conventionally cured products. Because of increased consumer interest in “preservative-free”

foods, “natural” and “organic” processed meats have become extremely popular. However, the direct addition of sodium nitrite is strictly prohibited in these products. The meat industry has recognized consumer interest in these products and has begun to manufacture products that simulate traditionally cured meat products, but without the direct addition of nitrite or nitrate. The continued long-standing but misguided concern regarding the perceived safety issues of sodium nitrite in processed meats relative to potential carcinogen formation has likely played a significant role in fueling the idea of using alternative curing systems. The goal of the natural curing process is to manufacture products in a manner that will result in the same characteristics consumers have come to expect from conventionally cured processed meats. The process of naturally curing meat involves a natural nitrate source and a bacterial culture that is capable of reducing the nitrate to nitrite. Natural curing of meat is similar to that of meats manufactured with the direct addition of nitrite. The main difference between the conventional and natural curing process is that the natural curing process involves the addition of nitrate to the meat with the addition of a bacterial reducing starter culture capable of converting nitrate to nitrite. Because of this, an “incubation” period is added in the smokehouse schedule to allow the successful conversion of nitrate to nitrite if a natural nitrate source is being used. The “incubation” step for natural frankfurters and bacon is approximately 1 hour at 110ºF (42ºC) and 110–115ºF (42–46ºC), respectively *(14)*. Less incubation time may be needed for bacon because of the thin diameter. If ham products are relatively small, the heating process may also require adjustment. On the other hand, if the ham pieces are relatively large in diameter, no incubation period is

needed because of the slow temperature increase that occurs during heat processing of large diameter products *(14)*. A second alternative for natural curing that has become available more recently is a natural nitrate source that has already been converted to nitrite by the supplier. Because a natural nitrite source involves the intentional pre-conversion of nitrate to nitrite, an incubation step of the meat product in which it is used is not needed because the pre-converted product already contains nitrite. The pre-converted system is convenient because unlike the natural nitrate source, the processor knows the quantity of nitrite to be added to the product, and the addition of starter culture is unnecessary.

A major concern for processed meats marketed as “natural” and “organic” is that they do not contain formulated sodium nitrite ($NaNO_2^-$) in concentrations known to be highly effective in inhibiting the growth of many foodborne pathogens. The ingoing nitrite is regulated at 156 ppm in most meat products cured with the direct addition of sodium nitrite and at 120 ppm for bacon. However, in naturally cured meat products, the ingoing nitrate concentration is significantly less, typically between 40 and 60 ppm *(14)*. Sindelar et al. *(15)* found residual nitrite concentrations for conventionally cured ham to be 29.67 ppm, whereas nitrite levels for naturally cured ham was between 4.91 and 9.23 ppm. Sodium nitrite has a long history of effectively inhibiting foodborne pathogens such as *Clostridium botulinum (2)*. This was further substantiated in a recent study with naturally cured model frankfurter and ham products *(17)*. To date, there is no known single replacement for this substance as a meat curing ingredient. A recent study conducted by Jackson et al. *(5)* found that most commercial no-nitrate-or-nitrite added frankfurters, bacon and

ham inoculated with *C. perfringens* resulted in faster growth of this pathogen than in conventionally cured products. Consequently, the development of supplemental treatments to increase the level and consistency of antimicrobial protection in these products is important to provide consumers with the degree of safety that they have come to expect from conventionally cured processed meats. Therefore, the objective of this study was to identify and test ingredients that might improve product safety properties without altering the unique natural/organic status of naturally cured processed meat products.

Materials and Methods

Bacterial strain and spore suspension. *C. perfringens* strains ATCC 10258, 3124 and 12917 were obtained from the Food Safety Research Laboratory (FSRL) at Iowa State University. The organism was cultured in fluid thioglycollate medium, and sporulation was induced in Duncan-Strong sporulation medium *(3)* as described by Juneja et al. *(6)*. The spore crop was harvested by centrifugation (9,500 x g, 10 min., 4℃) and then re-suspended in ph ysiological saline (0.85% wt/vol sodium chloride). The three strains were combined and vortexed just before inoculation took place.

Manufacture of frankfurters. Ready-to-eat frankfurters were manufactured with 80% lean fresh beef trimming and 50% lean fresh pork trimmings obtained from a local supplier at the Meat Laboratory at Iowa State University (ISU). The beef and pork trimmings were coarse-ground through a 4.8 mm plate. Fat content was measured using an Anyl Ray Fat Analyzer (Kartrig Pak, Model 316-48, Davenport, IA., USA) to formulate the final blend at 30% fat. The beef and pork were separated

into 8 batches weighing 6.80 kg each. Each treatment was assigned a letter code as listed in Table 1. All frankfurter treatments contained the base ingredients of 80/20 beef trim (6.80 kg), 50/50 pork trim (6.80 kg), natural frankfurter spice blend (243.23 g) (A.C. Legg Packing Co, Calera, AL., USA), water/ice (2.72 lbs), dextrose (2%) and salt (2.25%). Treatment A served as the uncured control and contained only the base ingredients of lean beef trim, fat pork trim, frankfurter spice blend, water/ice, dextrose and salt. Treatment B contained 0.45% natural nitrite cure (VegStable 504, Florida Food Products, Inc., Eustis, FL., USA) without additional antimicrobials. Treatment C contained 0.20% natural nitrate cure (VegStable 502, Florida Food Products) and 4.41 g of nitrate reducing starter culture containing *Staphlococcus carnosus* (CS-299 Bactoferm^{TM}, Chr. Hansen, Inc., Gainesville, FL., USA). Treatment D contained 0.20% natural nitrate cure (VegStable 502, Florida Food Products, Inc.), 4.41 g of nitrate reducing starter culture containing *Staphlococcus carnosus* (CS-299 Bactoferm^{TM}, Chr. Hansen, Inc.) and antimicrobial B, which consisted of cultured corn sugar and vinegar blend (Verdad 55, Purac America, Lincolnshire, IL., USA). Treatment E contained 0.20% natural nitrate cure (VegStable 502, Florida Food Products, Inc.), 4.41 g of nitrate reducing starter culture containing *Staphlococcus carnosus* (CS-299 Bactoferm^{TM}, Chr. Hansen, Inc.) and 1.4% antimicrobial A, which consisted of vinegar, lemon powder and cherry powder blend (VegStable 507, Florida Food Products, Inc.). Treatment F contained 0.45% natural nitrite cure (VegStable 504, Florida Food Products, Inc.) and antimicrobial B. Treatment G contained 0.45% natural nitrite cure (VegStable 504, Florida Food Products, Inc.) and 1.4% antimicrobial A. Treatment H served as the

conventionally cured control and contained 156 ppm sodium nitrite, 550 ppm sodium erythorbate and 2.5% lactate/diacetate blend (Purasal Opti.Form PD.4, Purac America) in addition to the base ingredients. All ingredients were added at concentrations recommended by the respective supplier.

The frankfurters were manufactured using a vacuum bowl cutter (Kramer & Grebe Model VSM65, Kramer & Grebe GmbH & Co. KG., Biendenkopf-Wallau, Germany). Salt, ½ water/ice, lean trim and seasoning were added to the bowl cutter. After initial mixing and chopping to 5℃, the remain ing water/ice was added with the fat trim and chopping continued until the meat blend temperature reached 55–60℉ (13–16℃). For the naturally cured products, the natu ral nitrate/nitrite cure and starter culture were added directly with the other non-meat ingredients. After chopping was complete, the meat batter was then transferred to a rotary vane vacuum-filling machine that contained a linking attachment (Risco vacuum stuffer, Model RS 4003-165, Stoughton, MA., USA). The batter was then stuffed into 28 mm impermeable casings. After stuffing, the frankfurters were placed in two separate single truck thermal processing ovens so that cooking could be completed at the same time for all treatments (Maurer, AG, Reichenau, Germany; Alkar Model MT EVD RSE 4, Alkar Engineering Corp., Lodi, WI., USA). Natural cure treatments with nitrate and starter culture (C, D and E) were placed on a different smokehouse truck than were treatments A, B, F, G and H to allow for an incubation step (1 hour at 100℉ (42℃)) to achieve nitrate-to-nitrite conversi on by the culture in these three treatments. Thermal processing was similar to that of conventionally cured frankfurters for all the treatments, with the exception of the incubation step.

Following processing, the frankfurters were placed in a cooler overnight at 0℃ to stabilize. The next day, the frankfurters were vacuum packaged (Multivac, Type AG800, Kansas City, MO., USA). Frankfurters were then transferred to the FSRL at ISU on the day after packaging to begin day 0 of the study.

Manufacture of hams. Hams were manufactured at the Meat Laboratory at ISU with pork inside ham muscles obtained from a local supplier. Each treatment was assigned a letter code (Table 1). The pork ham muscles were coarse-ground through a 9.53 mm. plate to maximize uniformity and separated into 8 batches weighing 18.14 kg each. The ham treatments contained the base ingredients of ground ham (18.14 kg), salt (0.50 kg), sugar (0.30 kg) and water (3.74 kg). Treatment A served as the uncured control and contained only the base ingredients of ground ham, salt, sugar and water. Treatment B contained 0.30% natural nitrite cure (VegStable 504, Florida Food Products, Inc.) without additional antimicrobials. Treatment C contained 0.30% natural nitrite cure and 0.70% antimicrobial A. Treatment D contained 0.30% natural nitrite cure and 3% antimicrobial B. Treatment E contained 0.30% natural nitrate cure (VegStable 502, Florida Food Products, Inc.) and 5 g of nitrate reducing starter culture containing *Staphlococcus carnosus* (CS-299 Bactoferm^{TM}, Chr. Hansen, Inc.). Treatment F served as the conventionally cured control and contained 156 ppm sodium nitrite, 9.98 g sodium erythorbate and 1.25 lbs lactate/diacetate blend (Purasal Opti.Form PD.4, Purac America) in addition to the base ingredients. Treatment G contained 0.30% natural nitrate cure, 5 g of nitrate reducing starter culture and 3% antimicrobial B. Treatment H contained 0.30% natural nitrate cure, 5 g of nitrate reducing starter culture and 0.70%

antimicrobial A. As for frankfurters, all ingredients were utilized at concentrations recommended by the supplier.

The ground ham was blended with ingredients using a double action mixer (Leland Southwest, Fort Worth, TX., USA). Non-meat ingredients were added and allowed to mix with the ground ham for 2 min. For the naturally cured products, the natural nitrate cure and starter culture or natural nitrite cure were added directly with the other non-meat ingredients (depending on treatments). The mixed product was then ground using a 6.35 mm. plate and transferred to a rotary vane vacuum-filling machine (Risco vacuum stuffer, Model RS 4003-165). The mixture was then stuffed into 35 mm impermeable casings. After stuffing, the products were placed in a single truck thermal processing oven (Maurer, AG). The treatments with nitrate and culture (C, D and E) were placed on a separate smokehouse truck than were treatments A, B, F, G and H to allow for an incubation step (2 hours at 110℉ (42℃)) to convert the nitrate to nitrite. Thermal processing for all treatments was similar to that of conventionally cured ham, with the exception of the incubation step. Following processing, the hams were placed in a 0℃ cooler overni ght to stabilize. The next day, the hams were sliced to 1.5 mm thick slices using a fully automatic slicing machine (Bizerba, Model A-500, Piscataway, NJ., USA) and vacuum packaged (Ulma Packaging, MINI Series, Ball Ground, GA, USA). Hams were then transferred to the FSRL at ISU to begin day 0 of the study.

Sample inoculation. While in the FSRL, 25-gram samples of each treatment of frankfurters and ham were placed in 5 X 16 in vacuum package bags (Cryovac Packaging, Duncan, SC., USA) and inoculated with 0.1 ml of the spore suspension

of *C. perfringens* to give a final spore concentration of 5-log spores/g. Frankfurters were inoculated under the biological safety cabinet (Nuaire, Model NU-425-400, Plymouth, MN., USA) using a 1 cc needle (Difco, Becton Dickinson, Sparks, MD., USA) to inject inoculum in the interior of the frankfurters. A different needle was used for each treatment. Ham was surface inoculated and massaged briefly to distribute the inoculum uniformly. After packages were sealed under vacuum (Multivac, Model A-300/52) all samples were heat shocked in a water bath (NESLAB Instruments, Inc., Newington, NH., USA, RTE-211) to an internal temperature of 75℃ for 20 min to ensure that all vegetative cells were inactivated and only spores remained *(7)*. A thermometer was used in a non-inoculated sample to monitor temperature during the heat shocking process. Following the heat shocking process, all product was chilled according to the U.S. Department of Agriculture (USDA) guidelines for *C. perfringens* control in cured meats, which states that the cooked meat product's maximum internal temperature should remain between 130℉ (54.4℃) and 80℉ (26.6℃) for no more than 1.5 hr and between 80℉ (26.6℃) and 40℉ (4.4℃) for no more than 5 hr *(16)*. After the product reached an internal temperature of 7.2℃, the product was stored at room temperature (20^{o}C) in the FSRL. Sampling was conducted on day 0, 1, 2, 4, 6, 8, 10 for frankfurters and day 0, 2, 4, 6, 8 for ham. These sampling days were determined by results from preliminary studies.

Microbiological analysis. On the appropriate day, one package for each treatment was collected and opened aseptically. Sampling was achieved by blending each 25-gram sample with 225 ml of 0.1% peptone water in a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, WI, USA). Each sample was stomached for

30 s in the laboratory blender (Stomacher 400, Seward Medical, London, UK). All blended samples were maintained on an ice slush. Appropriate dilutions were plated with a glass rod in duplicate on perfringens agar with Tryptose Sulphite Cycloserine and egg yolk emulsion (Oxoid, Basingstroke, UK) *(8)*. Agar plates were incubated at 35℃ in anaerobic jars with Gas Pak palladium catalyst en velopes (Oxoid) for 24 h. In an effort to ensure the anaerobic jars were functioning properly, anaerobic indicators were included in each jar.

Analytical analysis. Residual nitrite and moisture were determined by the AOAC methods *(1)*. pH was determined using a pH/ion meter (Accumet 950, Fisher Scientific Company, Pittsburg, PA) equipped with a probe (Hanna Instruments FC 200B, Fisher Scientific Company, Pittsburg, PA) that was calibrated with 4.0 and 7.0 phosphate buffer. Sample preparation and nitrate determination methods were modifications of Ahn and Maurer (1987). Five grams of meat product samples were weighed in a 50-ml test tube and homogenized with 20 ml of deionized distilled water (DDW) using a Polytron homogenizer (Type PT 10/35, Brinkman Instrument Inc., Westbury, NY, USA) for 10 s at high speed. The homogenate was heated for 1 h in 80℃ water bath. After cooling in cold water for 10 min, 2.5 ml of the homogenate was transferred to a disposable test tube (16 x 100 mm). Carrez II (dissolve 10.6g potassium ferrocyanide in 100ml DDW) and Carrez I (dissolve 23.8g zinc acetate in 50ml DDW, then add 3ml glacial acetic acid and dilute to 100ml with DDW) reagents were added (0.1 ml each) to precipitate proteins. The solution was diluted with 2.3 ml of DDW and mixed well. After precipitation, the supernatant was centrifuged at 10,000 rpm x g for 20 min and the clear upper layer was used for

nitrate measurement by high performance liquid chromatography (Agilent 1100 Series HPLC system, Agilent Technologies, Wilmington, DE, USA). The column used was Agilent Zorbax SAX (analytical 4.6 x 150mm, 5-micron) (Agilent, Wilmington, DE, USA) and the elution buffer was 15m*M* phosphate buffer, pH 2.35, with isocratic elution. Flow rate was 1.0 ml/min and sample injection volume was 25 µL. The wavelength of diode array detector used was 210 nm. The area of nitrate peak was used to calculate nitrate concentration (ppm) using nitrate standard curve. Duplicate samples were analyzed for residual nitrite, residual nitrate, moisture and pH.

Data analysis. Two independent replicate experiments were performed for the frankfurters and three independent replicate experiments were performed for ham samples. Viable *C. perfringens* populations were determined by calculating the log value of bacterial counts on duplicate plates for each sample that was analyzed. A F-test was performed to confirm that there was a difference among treatments. In the pairwise comparisons of the means, Tukey's Honestly Significant Difference (HSD) procedure was used to adjust for the multiple comparisons when testing for a significant difference between means of treatments within a particular product (e.g., frankfurters and hams). Significant levels were determined at $P<0.05$. Data were analyzed using the PROC GLM (general linear models) procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Figure 1 illustrates the effect of treatment on growth of *C. perfringens* over time from spore inocula in frankfurters manufactured with conventional or natural

nitrate/nitrite sources and natural antimicrobials. Mean growth of *C. perfringens* for all sampling days was significantly greater ($P<0.05$) in the uncured control (A) and naturally cured treatments B, C and D than in the nitrite-added control (H). Growth in treatments B and C can be attributed to the lack of additional antimicrobials in the formulation. Rapid growth in treatment A is the result of no nitrite present in any form (truly uncured). In addition, the exclusion of a natural antimicrobial from the formulation also played a role in this rapid growth. Interestingly, treatment D had significantly greater growth than that of the control even though the formulation contained a natural antimicrobial, initially indicating that the antimicrobial did not have effects comparable to nitrite in a conventionally cured product. Treatment D contained a residual nitrite concentration (10.21 ppm) that was significantly different ($P<0.05$) from treatments E, F and G (19.24 ppm, 34.39 ppm, 29.46 ppm, respectively), and all of these treatments contained a natural antimicrobial (Table 2). In addition, treatment D had a nitrate concentration (71.8 ppm) that was significantly greater ($P<0.05$) than treatments F and G (42.5 ppm, 52.75 ppm, respectively). This suggests less nitrate-to-nitrite conversion and may have contributed to significantly greater growth in treatment D than in the conventionally cured control, despite the presence of the natural antimicrobial. Treatments E, F and G showed no significantly greater ($P<0.05$) growth by inoculated *C. perfringens* than that of the nitrite-added control. This similarity to that of the conventionally cured control is likely due to the use of natural antimicrobials that contained vinegar and naturally occurring acids such as citric acid from lemon/lime powder and ascorbic acid from cherry powder. Vinegar, however, has more of an impact on pH than ascorbic acid *(4)*. Because

vinegar contains organic acids such as acetic acid, diacetate and acetates, the antimicrobial product is acidic, as expected for vinegar. Organic acids are known to have a more potent inhibitory impact on microbial growth than other weak acids of similar pH *(12)*. In addition, propionic acid is known to inhibit spore-forming bacteria at pH 6 *(12)*. In the event of temperature abuse, products with formulations similar to that of treatments E, F, G and H appear to offer improved protection from *C. perfringens*. Product with formulations similar to treatments A (truly uncured), B (natural nitrite source without antimicrobial), C (natural nitrate source with starter culture) and D (natural cure with antimicrobial B) would be at greater risk for a foodborne outbreak and/or product recall because these treatments resulted in significantly greater growth than that of the conventionally cured control (Table 3).

Figure 2 illustrates the effect of treatments on growth of *C. perfringens* over time from spore inocula in ham manufactured with conventional or natural nitrate/nitrite sources and natural antimicrobials. Growth was significantly faster ($P<0.05$) in the truly uncured treatment A and naturally cured treatments B and C when compared to the nitrite-added control F. Treatment A (truly uncured) had the greatest growth rate due to the lack of nitrite in any form. Treatment B was naturally cured, but did not contain a natural antimicrobial, thus allowing for faster growth of *C. perfringens*. However, it is unclear why naturally cured treatment C had faster growth than the control when natural antimicrobial A was used in the formulation. Treatment C had a pH (6.31) that was significantly higher ($P<0.05$) than treatments D and G (6.16, 6.10, respectively), all of which contained a natural antimicrobial (Table 4). In addition, treatment C also had a moisture content (73.57) which was

significantly (P<0.05) greater than treatments D, G and H (72.18, 71.94 and 72.53, respectively). These could be contributing factors for the significantly higher growth for treatment C when compared to the nitrite-added control. Residual nitrate and nitrite were not greatly different and do not appear to have played a role in this faster growth. Treatments E, H, G and D showed no significantly greater (P<0.05) growth by inoculated *C. perfringens* than that of the nitrite-added control (F). Treatments H, G and D contained natural antimicrobials, thus controlling the growth of *C. perfringens* as previously discussed for frankfurters. However, it is unclear why treatment E yielded results similar to the nitrite-added control when it did not contain an added natural antimicrobial. However, in the event of temperature abuse, in addition to the conventionally cured control (F), products with formulations similar to that of treatments D, G, H (all naturally cured with antimicrobials) and treatment E (naturally cured without antimicrobial) would be better protected than most naturally cured products of the same formulation, whereas products with formulations similar to A (truly uncured), naturally cured treatments B (without antimicrobial) and C (with antimicrobial) would have a greater probability of causing foodborne illness and could be subjected to a food recall (Table 5). A similar study with *L. monocytogenes* was recently conducted in hams that received the same treatments as this study *(11)*. In that study, treatments E, H, G, C and D showed no significantly greater (P<0.05) growth by inoculated *L. monocytogenes* than that of the nitrite-added control, effects very similar to those observed for *C. perfringens* in the present study.

The effectiveness of a variety of antimicrobials in traditionally cured meats is well documented. This study showed that some commercially available natural

ingredients and compounds offer potential to improve antimicrobial impact for naturally cured meat products. While the addition of natural antimicrobials appeared to improve control of *C. perfringens* in most cases, these products also demonstrated a considerable variation of inhibitory activity and, in general, were not as effective as conventional cures that included the maximum amount of added nitrite that is permitted. It is also fair to say that the observations of greater growth of *C. perfringens* in treatments that contained antimicrobial B (frankfurter product) and antimicrobial A (ham product) could be attributed to factors such as residual nitrite concentrations (in the case of the frankfurters), moisture content and pH (in the case of the ham), and not the antimicrobial itself. For example, utilizing a natural nitrite source for meat curing involves the intentional pre-conversion of nitrate to nitrite. Vegetable products that include pre-converted nitrite commercially available for meat processing contain up to 15,000 ppm of nitrite *(13)*. The advantage of this system is that processor knows the quantity of nitrite to be added to the product. However, when using a natural nitrate source, the quantity of nitrite is unknown because this is dependent on the incubation step. Although the vegetable products with natural nitrate that are commercially available for meat processing contain up to about 40,000 ppm of nitrate (more than that of the pre-converted nitrite product), there is always the concern of not achieving the level of nitrate-to-nitrite conversion. Further, the natural nitrite and nitrate vegetable product are limited to 0.3% of the product formulation due to vegetable-like flavors *(13)* resulting in 80-120 ppm nitrate.

Wanless et al. *(17)* found that the addition of the same antimicrobials used in the study described herein inhibited the production of botulinum toxin in frankfurters

and ham held at refrigeration and room temperatures. This suggests that the naturally cured products containing natural antimicrobials would also be protected to a greater extent from botulinum toxin production than the naturally cured product alone in the event of temperature abuse. Consequently the results of this study suggest a means of improving the safety of naturally cured processed meats, but additional measures may be required to achieve the same consistent level of safety that is provided by conventionally cured meat products.

Acknowledgments

This project was supported by the National Integrated Food Safety Initiative (Grant no. 2006-51110-03609) of the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, the National Pork Board (Grant no. 06-008) and the Iowa State University Food Safety Consortium.

References

1. Association of Official Analytical Chemists. 1990. Nitrites in cured meats. Official method 973.31. In: Official methods of analysis. 15th ed., Arlington, VA.

2. Christiansen, L. N. 1980. Factors influencing botulinal inhibition by nitrite. *Food Tech.* 34:237-239.

3. Duncan, C. L., and D. H. Strong. 1968. Improved medium for sporulation of *Clostridium perfringens*. *Appl. Microbio.* 16:82-89.

4. Gregerson, J. 2009. Tapping natural ingredients to create clean-label cured meats. Available at: http://www.meatingplace.com/MembersOnly/technology/details.aspx?item=10021. Accessed November 5, 2009.

5. Jackson, A. L., G. Sullivan, J. G. Sebranek, and J. S. Dickson. 2009. Survival and growth of *Clostridium perfringens* on commercial no-nitrate-or-nitrite added (natural and organic) frankfurters, bacon and ham. Unpublished data. Iowa State University, Ames, IA.

6. Juneja, V. K., J. E. Call, and A. J. Miller. 1993. Evaluation of methylxanthines and related compounds to enhance *Clostridium perfringens* sporulation using a modified duncan and strong medium. *J. Rapid Methods Autom. Microbiol.* 2:203-218.

7. Labbe, R. 1989. *Clostridium perfringens*. p. 191-234. *In* M.P. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, Inc., New York, NY.

8. Labbe, R. G., and V. K. Juneja. 2002. *Clostridium perfringens.* p. 119-126. *In* D. Cliver, O., and H. Riemann, P. (ed.), Foodborne diseases. Academic Press, San Diego, CA.

9. McLauchlin, J., and K. A. Grant. 2007. *Clostridium botulinum* and *clostridum perfringens*. p. 41-78. *In* S. Simjee (ed.), Foodborne diseases. Humana Press, Totowa, NJ.

10. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illnesses and death in the United States. *Emerg Infect Dis.* 5:607-625.

11. Niebuhr, S., G. Sullivan, A. L. Jackson, and J. G. Sebranek. 2009. Use of natural ingredients to control growth of *Listeria monocytogenes* on ham. Unpublished data Iowa State University, Ames, IA.

12. Russell, A. D. 2005. Mechanisms of action, resistance, and stress adaptation. p. 633-657. *In* P.M. Davidson, J.N. Sofos, and A.L. Branen (ed.), Antimicrobials in food. Taylor & Francis, Boco Raton, FL.

13. Sebranek, J. 2010. Understanding natural/organic curing systems. Available at: http://www.meatingplace.com/MembersOnly/technology/details.aspx?item=14429. Accessed, March 8, 2010.

14. Sebranek, J., and J. Bacus. March 2007. Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality and safety issues. *American Meat Sci. Assoc,*. 1:1-15.

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

16. U.S. Department of Agriculture. 1999. Compliance guidelines for cooling heat-treated meat and poultry products (stabilization). Available at: http://origin-

www.fsis.usda.gov/OPPDE/rdad/FRPubs/95-033F/95-033F_Appendix%20B.htm. Accessed March 10, 2010.

17. Wanless, B., L. McDonnell, and K. Glass. 2009. Effect of nitrite and natural antimicrobials on *Clostridium botulinum* toxigenesis in model frankfurter and ham products. Unpublished data. University of Wisconsin-Madison, Madison, WI.

Table 1. Treatment labels used for frankfurters and ham.

Label	**Frankfurters**
A	Truly uncured
B	Natural nitrite source
C	Natural nitrate source, starter culture
D	Natural nitrate source, starter culture, antimicrobial B
E	Natural nitrate source, starter culture, antimicrobial A
F	Natural nitrite source, antimicrobial B
G	Natural nitrite source, antimicrobial A
H	Conventionally cured control

Label	**Ham**
A	Truly uncured
B	Natural nitrite source
C	Natural nitrite source, antimicrobial A
D	Natural nitrite source, antimicrobial B
E	Natural nitrate source, starter culture
F	Conventionally cured control
G	Natural nitrate source, starter culture, antimicrobial B
H	Natural nitrate source, starter culture, antimicrobial A

Table 2. Mean residual nitrite and residual nitrate for frankfurter treatments.

Treatment	Residual Nitrite[a] (ppm)	Residual Nitrate[b] (ppm)
A	2.0^{c}	20.15^{d}
B	39.3^{e}	41.45^{fg}
C	38.50^{eh}	29.15^{dg}
D	10.21^{d}	71.8^{e}
E	19.24^{g}	61.7^{eh}
F	34.39^{h}	42.5^{f}
G	29.46^{f}	52.75^{fh}
H	39.91^{e}	49.8^{fh}

Note. Means with different superscripts within a given column differ by $P<0.05$.
[a]Residual nitrite and nitrite determination reported in ppm of sample
Treatments: (A) truly natural (B) natural nitrite source (C) natural nitrate source, starter culture (D) natural nitrate source, starter culture, clean label antimicrobial B (E) natural nitrate source, starter culture, natural antimicrobial A (F) natural nitrite source, clean label antimicrobial B (G) natural nitrite source, natural antimicrobial A (H) conventionally cured control

Table 3. Means and standard errors for growth of *Clostridium perfringens* for all sampling days in naturally cured, truly uncured and conventionally cured frankfurters during storage at room temperature.

Treatment[a]	Mean
A	5.77 ± 0.76^{b}
B	4.63 ± 0.80^{b}
C	2.84 ± 0.51^{c}
D	2.76 ± 0.24^{cd}
E	1.20 ± 0.32^{de}
F	1.49 ± 0.29^{de}
G	0.49 ± 0.24^{e}
H	0.12 ± 0.08^{e}

Note. Counts are expressed as the mean (± standard error) log counts from each brand. Within each column, means followed by the same letter are not significantly different (P>0.05).

[a]Treatments: (A) truly natural (B) natural nitrite source (C) natural nitrate source, starter culture (D) natural nitrate source, starter culture, antimicrobial B (E) natural nitrate source, starter culture, antimicrobial A (F) natural nitrite source, antimicrobial B (G) nitrite source, natural antimicrobial A (H) conventionally cured control

Table 4. Mean residual nitrate, nitrite, moisture and pH for ham treatments.

Treatment	Residual Nitrate[a] (ppm)	Residual Nitrite[b] (ppm)	Moisture	pH
A	0.48^{c}	2.59^{c}	74.37^{d}	6.14^{ef}
B	11.98^{eg}	25.59^{f}	73.72^{g}	6.20^{eg}
C	13.08^{eg}	25.41^{f}	73.57^{dg}	6.31^{d}
D	10.03^{ef}	22.73^{f}	72.18^{f}	6.16^{ef}
E	7.18^{f}	50.78^{d}	73.35^{eg}	6.19^{eg}
F	20.60^{d}	40.00^{e}	71.90^{f}	6.09^{f}
G	14.46^{g}	43.40^{eg}	71.94^{f}	6.10^{f}
H	10.81^{e}	48.50^{dg}	72.53^{ef}	6.25^{dg}

Means with different superscripts within a given column differ by P<0.05.

[a,b] Residual nitrate and nitrite determination reported in ppm of sample.

Treatments are as follows: (A) truly natural (B) natural nitrite source (C) natural nitrite source, antimicrobial A (D) natural nitrite source, antimicrobial B (E) natural nitrate source, starter culture (F) conventionally cured control (G) natural nitrate source, starter culture, antimicrobial B (H) natural nitrate source, starter culture, antimicrobial A.

Table 5. Means and standard errors for growth for *Clostridium perfringens* all sampling days of in naturally cured, truly uncured and conventionally cured ham during storage at room temperature.

Treatment[a]	Mean
A	6.84 ± 0.84[b]
B	6.20 ± 0.77[b]
C	4.60 ± 0.67[c]
D	3.16 ± 0.18[d]
E	2.62 ± 0.10[de]
F	1.78 ± 0.34[de]
G	1.70 ± 0.35[e]
H	1.62 ± 0.35[e]

Note. Counts are expressed as the mean (± standard error) log counts from each brand. Within each column, means followed by the same letter are not significantly different (P>0.05).

[a]Treatments are as follows: (A) truly natural (B) natural nitrite source (C) natural nitrite source, antimicrobial A (D) natural nitrite source, antimicrobial B (E) natural nitrate source, starter culture (F) conventionally cured control (G) natural nitrate source, starter culture, antimicrobial B (H) natural nitrate source, starter culture, antimicrobial A.

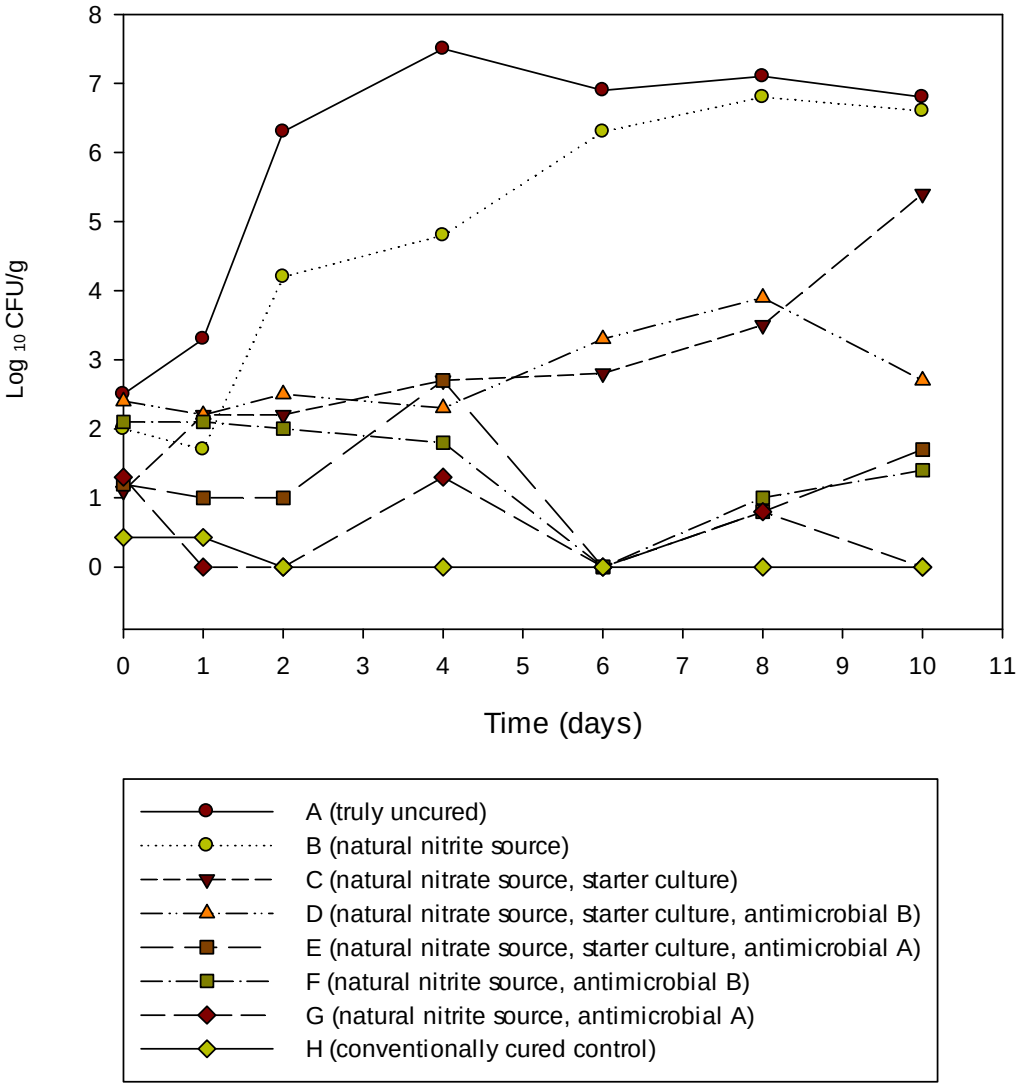

Figure 1. Effect of curing treatments and antimicrobial ingredients on growth of *C. perfringens* from spore inocula in frankfurters during storage at room temperature.

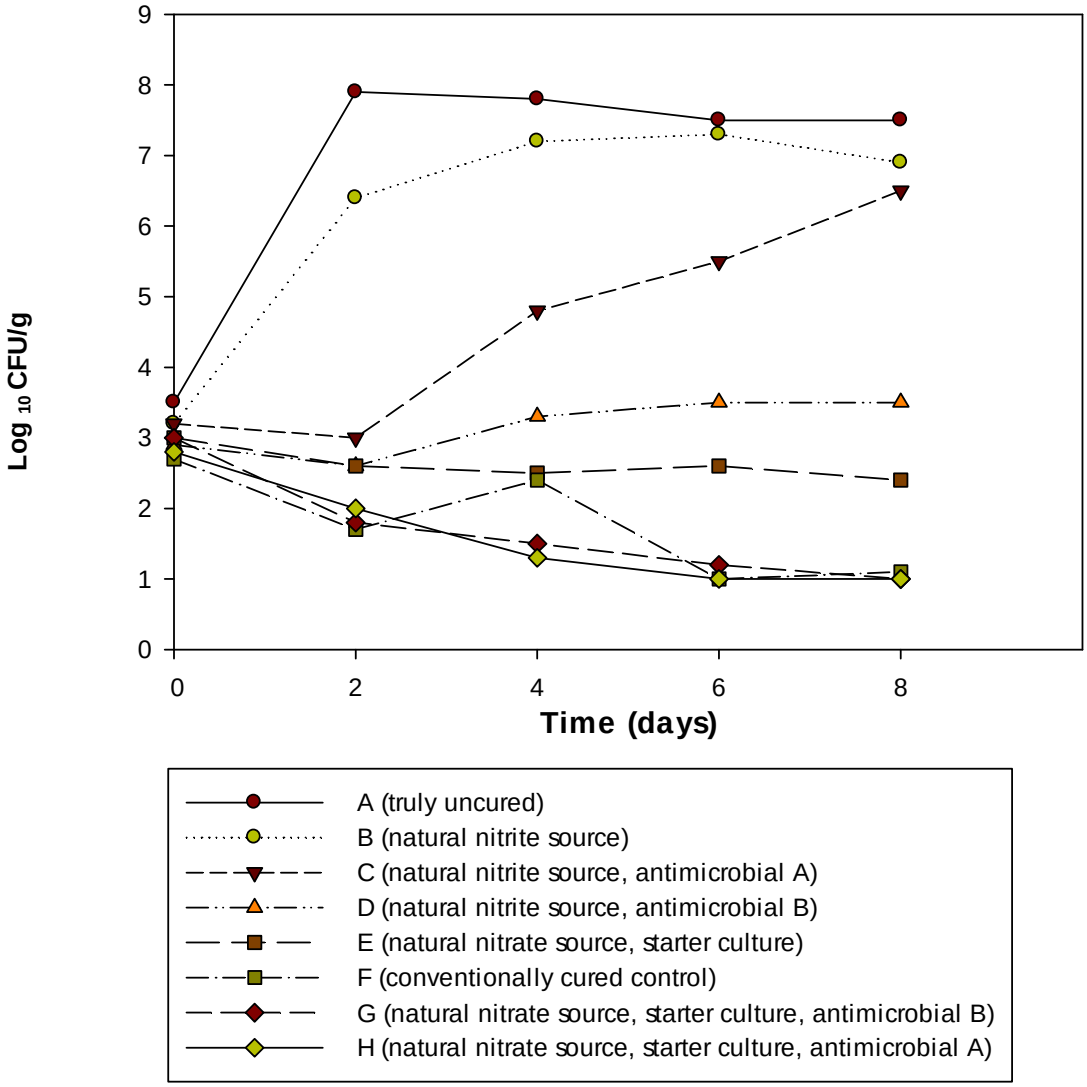

Figure 2. Effect of curing treatments and antimicrobial ingredients on growth of *C. perfringens* from spore inocula in ham during storage at room temperature.

CHAPTER 5. USE OF NATURAL INGREDIENTS TO CONTROL GROWTH AND TOXIN PRODUCTION BY *CLOSTRIDIUM BOTULINUM* ON FRANKFURTERS AND HAM

A paper prepared for submission to the Journal of Food Protection

Brandon Wanless[1], Lindsey McDonnell[1], Armitra Jackson[2], Joseph Sebranek[2], Kathleen Glass[1]

Abstract

Despite the evidence that nitrite in cured meats poses no hazard to human health, the marketing of “nitrite-free” cured meats has been of interest to consumers for quite some time. More recently this has evolved into a unique category of natural and organic processed meats that are manufactured with natural sources of nitrate and/or nitrite. However, these curing ingredients result in significantly less nitrite in these products than in conventionally cured products. The microbiological safety of “naturally cured” meats manufactured without the direct addition of sodium nitrite is not well understood or controlled. Therefore, the objective of this study was to assess the effectiveness of commercially available ingredients for improved safety of naturally cured processed meats by inoculation challenge with *Clostridium botulinum*. Five treatments each of frankfurters and ham with conventional or natural nitrite sources and natural antimicrobials were prepared: (A) truly uncured (B) conventionally cured (C) pre-converted nitrite w/o antimicrobial (D) pre-converted nitrite + lemon/cherry/vinegar blend (E) pre-converted nitrite + cultured corn sugar/vinegar blend. Conventional nitrite-added and pre-converted nitrite with the

[1]University of Wisconsin-Madison, Madison, WI, USA.
[2]Iowa State University, Ames, IA, USA.

addition of an antimicrobial had the greatest effect in terms of delaying toxin production. Truly uncured treatments had the shortest time to initial confirmed toxicity. These results confirm that commercial natural/organic cured meats have more potential for pathogen growth and toxin production by *C. botulinum* than do conventionally cured products, but other natural ingredients may offer safety improvement.

Introduction

Processed meats have been a staple in the American diet for many years. These products have a long history of being safe for consumption. Sodium nitrite is included in the ingredients, thus providing the cured color, flavor and safety that individuals have come to expect from conventionally cured products. Because of consumer interest in “preservative-free” foods, “natural” and “organic” processed meats have become extremely popular, at least in part because the direct addition of sodium nitrite is strictly prohibited in these products. The meat industry has recognized consumer interest in the demand for these products and has begun to manufacture products that simulate traditionally cured meat products, but without direct addition of nitrite. The approach is necessary because nitrite produces unique color, flavor and antimicrobial properties in cured meats that cannot be duplicated by any other single ingredient. The continued concern regarding the use of sodium nitrite in processed meats has likely played a significant role in fueling the idea of using alternative curing systems. Despite the scientific research that confirms the safety of meat products manufactured with nitrite, there are still those who continue to challenge the safety of these products. There were many epidemiological studies

that attempted to link nitrite to cancer. Peters et al. *(10)* conducted a study in which researchers linked hot dog consumption with child leukemia. Sarasua and Savitz *(11)* conducted a study in which they linked childhood cancer to the consumption of cured and broiled meat. However, many of these studies are based on epidemiology without biological support, thus making the linkage of processed meats to cancer suspect. Due to potential nitrosamine formation in bacon, USDA-FSIS has outlined very specific regulations for these products to minimize concentration and eliminate the risk of these carcinogens. It is important to note that vegetables account for approximately 80% of nitrate consumed via food and 10–15% of nitrate is consumed via water *(1)*. The National Academy of Science found the following to be true for dietary intake of nitrite: 39% was a result of cured meat consumption, 34% was from baked good and cereals and 16% was from vegetables *(9)*. Saliva, accounting for 92.8%, is the largest source of ingested nitrite *(3)*. In addition, nitrite aids in normal bodily functions needed for survival. For example, nitric oxide, which is synthesized in humans, plays an important role in immune response, control of blood pressure and brain function *(1)*. Nevertheless, there is a continuing perception among consumers that nitrite consumption is a health risk, and this perception has been a significant part of the interest in processed meats marketed with “no nitrite or nitrate added” as part of the product label.

The main concern with processed meats marketed as “natural” and “organic” is that they do not contain formulated sodium nitrite ($NaNO_2^-$) in concentrations known to be highly effective in inhibiting the growth of many foodborne pathogens, particularly *Clostridium botulinum*. These products contain natural sources of

nitrite/nitrate (e.g., celery powder, celery juice and sea salt). Due to the fact that these products are derived from nature, variability is inevitable. The ingoing nitrite is regulated at 156 ppm in most conventionally cured meat products and at 120 ppm for bacon in products cured with the direct addition of sodium nitrite. However, in naturally cured meat products, the ingoing nitrate and/or nitrite concentration is significantly less. In naturally cured meat products, the ingoing nitrate concentration is typically between 40 and 60 ppm *(13)*, which is less than that of conventionally cured products. Sindelar et al. *(14)* found residual nitrite levels for conventionally cured ham to be 29.67 ppm, whereas ham that was naturally cured was between 4.91 and 9.23 ppm. Sodium nitrite has a long-standing history of effectively inhibiting foodborne pathogens such as *C. botulinum (4)*. To date, there is no known replacement for this substance. Recent inoculation studies with *Clostridium perfringens* and *Listeria monocytogenes* conducted by Jackson et al. *(8)* and Schrader et al. *(12)* found that commercial no-nitrate-or-nitrite-added frankfurters, bacon and ham have more potential for growth of these pathogens than do conventionally cured products.

Clostridium botulinum is an anaerobic, gram-positive, spore-forming bacteria that has been recognized as a foodborne disease for over 1000 years. However, it was Emilie Pierre Marie van Ermengem who isolated and described the cause of the illness and found that the toxin was produced by the bacterium *Bacillus botulinus* (which is now known as *C. botulinum*) *(15)*. The toxins produced are divided into seven types and assigned letters A–G. Because *C. botulinum* spores can be found in the environment, foods can become contaminated during harvesting and

processing. Research has documented that the incidence of *C. botulinum* in fresh meats in very low. Greenberg et al. *(6)* reported that only 1 sample out of 2,358 raw meat samples (chicken, beef, pork) was found positive for *C. botulinum* Type C. However, even one *C. botulinum* outbreak can devastate an entire food industry because of the potency of the toxin produced and the high fatality rates for those affected. Therefore, the objective of this study was to assess the effectiveness of commercially available ingredients/processes for improved control of *C. botulinum* in naturally cured processed meats.

Materials and Methods

Bacterial strain and spore suspension. *C. botulinum* strains 56A, 62A, 69A, 90A, 17B, 112B, 213B, Alaska E and Beluga E were obtained from the University of Madison–Wisconsin Food Research Institute. The organism was cultured in Trypticase-Peptone-Glucose-Yeast extract with cooked meat media and transferred several times. The spore crop was harvested by centrifugation and heat shocked to destroy vegetative cells. In an attempt to remove additional debris, the spores were spun down once more. The spores were then frozen in de-ionized water.

Product manufacture and inoculation of frankfurters and hams. Three replications of frankfurter and ham product was manufactured at Iowa State University (ISU) and then transported to University of Wisconsin–Madison for *C. botulinum* assessment. All frankfurter treatments contained the base ingredients of 80/20 beef trim, 50/50 pork trim, natural frankfurter spice blend (A.C. Legg Packing Co, Calera, Ala., USA), water/ice, dextrose and salt, and all ham treatments

contained the base ingredients of ground ham, salt, sugar and water. All treatments were assigned a letter code. Treatment A will be referred to as “truly uncured” and contained only the base ingredients relative to each product. Treatment B served as the conventionally cured control and contained sodium nitrite, sodium erythorbate and lactate/diacetate blend (Purasal Opti.Form PD.4, Purac America, Lincolnshire, Ill., USA) in addition to the base ingredients. Treatment C contained natural nitrite cure (VegStable 504, Florida Food Products, Inc., Eustis, Fla., USA) without additional antimicrobials. Treatment D contained natural nitrite cure and a natural antimicrobial, which consisted of vinegar, lemon powder and cherry powder blend (VegStable 507, Florida Food Products, Inc.). Treatment E contained a natural nitrite cure and an antimicrobial, which consisted of cultured corn sugar and vinegar blend (Verdad 55, Purac America). A concise list of frankfurter and ham treatments is listed in Table 1.

For each treatment, meat preparation batter was inoculated with approximately 3-log spores/g. One hundred gram portions were then placed in boilable, gas-impermeable pouches, and vacuum packaged. Meat was processed to an internal temperature of 71℃ by submersion in 74℃ water to cook the product and heat-shock the spores, and then chilled on ice. Temperatures were monitored with a thermocouple inserted through a rubber septum into a control package. Treatments of each product were stored at three different temperatures in an effort to simulate: (a) refrigeration temperatures (4℃), (b) temperature abuse during delivery, storage or in the consumer’s refrigerator (10℃) and (c) an extreme case of temperature abuse, such as cooler or freezer failure (22℃).

***C. botulinum* microbiological analysis.** Botulinal spore counts were verified on duplicate samples for each treatment using the Most Probable Number method (MPN) in Trypticase-peptone-glucose-yeast extract (TPGY) tubes *(5)*. At each sampling time, samples were evaluated for gas production and changes in odor and appearance and were assayed for changes in spoilage bacteria (APT with bromcresol purple and DRCA) and for the presence of botulinal toxin using the standard mouse bioassay method *(5)* on trypsinized and untrypsinized samples. Sampling of a treatment for a given temperature was discontinued if toxin was confirmed in at least two of three samples on two consecutive sampling intervals. Duplicate trials for four formulations (four test formulations plus one positive control with traditional nitrite) for two product types (ham and frankfurter-type) were evaluated (total of 16 tests).

Analytical Analysis. Triplicate samples from each treatment were assayed for moisture, salt, pH, a_w and nitrite (stored at -70°C until analysis). Moisture and nitrite were determined using AOAC methods *(2)*. Salt was measured as Cl- using the Brinkmann Autotitrator. pH was determined using an Orion 8104 combination pH electrode and Accumet pH meter. A_w was measured using the Aqua Lab CX-2 Water Activity Meter.

Results and Discussion

Table 2 demonstrates the earliest confirmed toxicity for each treatment of frankfurters and ham. Toxin was detected in truly uncured frankfurters (A) at 9 weeks and 5 days when held at 4℃ and 22℃, respectively . Rapid growth in treatment A is the result of the exclusion of nitrite present in any form and the

exclusion of a natural antimicrobial from the formulation. Temperature (refrigeration vs. room temperature) also played an important role in the time to toxin detection. However, no toxin was detected in conventionally cured frankfurters (B) while stored at either 4℃ or 10℃. In addition, treatment B had the longest time to toxin detection when stored at 22℃. These results related to treatment B are due to the addition of formulated sodium nitrite and an antimicrobial (lactate/diacetate blend) that is typical of commercial conventionally cured products. *C. botulinum* toxin was detected at 5 days in treatments C and D at 22℃ whereas no toxin was detected in treatment E at the same temperature. In addition, no toxin was detected at 4℃ or 10℃ for treatments D and E of frankfurters. It appears that the cultured corn sugar/vinegar blend antimicrobial (treatment E) was better able to inhibit *C. botulinum* toxin at 22℃ than was the vinegar/lemon/cherry powder blend (treatment D) antimicrobial.

For truly uncured ham (treatment A), toxin was detected at 3 days and 2 weeks at 22℃ and 10℃, respectively. In contrast, no toxin was detected in the nitrite-added control stored at any temperature during the testing interval. The explanation for these results is the same as reported above for frankfurters inoculated with *C. botulinum*. Toxin was detected in the naturally cured ham that contained no antimicrobial (treatment C) at 5 days and 8 weeks at 22℃ and 10℃, respectively. *C. botulinum* toxin was detected at 5 days in the treatment containing the lemon/cherry/vinegar blend antimicrobial (treatment D) at 22℃ whereas no toxin was detected in the treatment containing the cultured corn sugar/vinegar blend (E) at the same temperature. No toxin was detected in treatments D and E at 10℃. The study involving the 4℃ samples is in progress (IP) with no toxin detected after 16

weeks. This study demonstrates that, in most cases, certain natural ingredients can delay botulinum toxin production in frankfurters and hams both at refrigeration and abuse temperatures. These results are similar to results obtained from a study that examined the same treatments but with *C. perfringens* inoculations instead *(7)*. In both studies, *C. perfringens* growth and *C. botulinum* toxin production was best controlled by the use of formulated sodium nitrite.

Analyzed nitrite concentrations for the conventionally cured frankfurter product (100.71 ppm) and conventionally cured ham product (143 ppm) initially after formulation are higher than that of the naturally cured products (Tables 3 and 4). This observation is similar to those reported by Sebranek et al. *(13)* and Sindelar et al. *(14)*. Moisture, salt, pH and A_w appeared to be similar for all frankfurter and ham treatments. However, moisture for ham treatment E seems to be a little lower than the other ham treatments.

This project studied two natural ingredient products, which could improve the safety of natural frankfurters and hams. These antimicrobial ingredients, including a natural vinegar/lemon/cherry powder blend and a natural cultured corn sugar/vinegar blend, delayed toxin production by several months at refrigeration temperatures and by several weeks when foods were stored at room temperature following inoculation with *C. botulinum*. This shows that naturally cured products containing natural antimicrobials would have greater protection from botulinum toxin production than would naturally cured products without the natural antimicrobials.

Acknowledgments

This project was supported by the National Integrated Food Safety Initiative (Grant no. 2006-51110-03609) of the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, the National Pork Board (Grant no. 06-008) and the Iowa State University Food Safety Consortium.

References

1. Archer, D. L. 2002. Evidence that ingested nitrate and nitrite are beneficial to health. *J. Food Prot.* 65:872-875.

2. Association of Official Analytical Chemists. 1990. Nitrites in cured meats. Official method 973.31. In: Official methods of analysis. 15th ed., Arlington, VA.

3. Cassens, R. G. 1997. Residual nitrite in cured meat. *Food Tech.* 51:53-55.

4. Christiansen, L. N. 1980. Factors influencing botulinal inhibition by nitrite. *Food Tech.* 34:237-239.

5. Food and Drug Administration. 2001. Bacteriological analytical manual online, U.S. Food and Drug Administration. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm. Accessed March 1, 2010.

6. Greenberg, A., R. B. Tompkin, B. O. Bladel, R. S. Kittaka, and A. Anellis. 1966. Incidence of mesophilic Clostridium spores in raw pork, beef and chicken in processing plants in the United States and Canada. *Appl. Microbio.* 14:789-793.

7. Jackson, A. L., G. Sullivan, C. Kulchaiyawat, J. G. Sebranek, and J. S. Dickson. 2009. Use of natural ingredients to control the growth of *Clostridium perfringens* on franfurters and ham. Unpublished data. Iowa State University, Ames, IA.

8. Jackson, A. L., G. Sullivan, J. G. Sebranek, and J. S. Dickson. 2009. Survival and growth of *Clostridium perfringens* on commercial no-nitrate-or-nitrite added (natural and organic) frankfurters, bacon and ham. Unpublished data. Iowa State University, Ames, IA.

9. National Academy of Science. 1981. The health effects of nitrate, nitrite, and n-nitroso compounds. National Academy Press, Washington, DC.

10. Peters, J. M., S. Preston-Martin, S. J. London, J. D. Bowman, J. D. Buckley, and D. C. Thomas. 1994. Processed meats and risk of childhood leukemia (California, USA). *Cancer Causes Control*. 5:195-202.

11. Sarasua, S., and D. A. Savitz. 1994. Cured and broiled meat consumption in relation to childhood cancer: Denver, Colorado (United States). *Cancer Causes Control*. 5:141-148.

12. Schrader, K. D., J. C. Cordray, J. G. Sebranek, J. S. Dickson, and A. F. Mendonca. 2009. Control of *Listeria monocytogenes* on no-nitrate-or-nitrite added (natural and organic) frankfurters. A.S. Leaflet R2386 Iowa State University Animal Industry Report.

13. Sebranek, J., and J. Bacus. March 2007. Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality and safety issues. *American Meat Sci. Assoc,*. 1:1-15.

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

15. van Ermengem, E. P. 1979. A new anaerobic bacillus and its relation to botulism (originally published as "Ueber einen neuen anaeroben bacillus und seine bezieh- ungen zum botulismus" In zeitschrift fiir hygiene und infektionskrankheiten 26: 1-56, 1897). *Rev of Infect Dis.* 1:701-719.

Table 1. Treatment labels used for frankfurter and ham.

Label	Treatments
A	Truly uncured
B	Conventionally cured control (including antimicrobial lactate/diacetate blend)
C	Natural nitrite source
D	Natural nitrite source + lemon/cherry/vinegar blend antimicrobial
E	Natural nitrite source + cultured corn sugar/vinegar blend antimicrobial

Table 2. Earliest confirmed *C. botulinum* toxicity in frankfurters and ham

	Frankfurters		
Treatment[a]	22℃ 28 days	10℃ 12 wks	4℃ 20 wks
A	5 days	2 wks	9 wks
B	14 days	None	None
C	5 days	8 wks	None
D	5 days	None	None
E	None	None	None
	Hams		
	22℃ 14 days	10℃ 12 wks	4℃ 12 wks
A	3 days	2 wks	none
B	None	None	none
C	5 days	8 wks	none
D	5 days	None	none
E	None	None	none

Note. Data courtesy of Brandon Wanless, University of Wisconsin–Madison.
[a](A) truly uncured (B) conventionally cured (C) pre-converted nitrite w/o antimicrobial (D) pre-converted nitrite + lemon/cherry/vinegar blend (E) pre-converted nitrite +cultured corn sugar/vinegar blend.

Table 3. Analyzed values of moisture[a], salt (NaCl)[b], pH[c], water activity[d] and nitrite[e] in pre-cooked frankfurter batters.

Formulation	% moisture	% NaCl	pH	A_w	Nitrite (ppm)
Truly natural (A)	58.74	1.747	5.62	0.9690	0.00
	58.60	1.729	5.63	0.9685	0.00
	58.80	1.694	5.64	0.9702	0.00
	Average ± S.D.	*58.71 ± 0.10*	*1.723 ± 0.027*	*5.63 ± 0.01*	*0.9692 ± 0.0007*	*0.00 ± 0.00*
Conventional cured control (B)	57.79	1.925	5.68	0.9730	86.32
	56.91	2.082	5.70	0.9664	105.76
	57.18	2.010	5.70	0.9681	110.04
	Average ± S.D.	*57.29 ± 0.45*	*2.006 ± 0.079*	*5.69 ± 0.01*	*0.9691 ± 0.0023*	*100.71 ± 12.64*
Pre-converted nitrite w/o antimicrobial (C)	56.61	2.387	5.73	0.9587	33.12
	57.07	2.333	5.72	0.9609	28.85
	57.11	2.381	5.72	0.9614	26.71
	Average ± S.D.	*56.93 ± 0.28*	*2.367 ± 0.030*	*5.72 ± 0.01*	*0.9603 ± 0.0015*	*29.56 ± 3.26*
Pre-converted nitrite + lemon/cherry/vinegar blend (D)	56.16	2.555	5.75	0.9555	30.98
	56.53	2.475	5.77	0.9566	26.71
	57.22	2.519	5.77	0.9575	33.12
	Average ± S.D.	*56.64 ± 0.54*	*2.516 ± 0.040*	*5.76 ± 0.01*	*0.9565 ± 0.0008*	*30.27 ± 3.26*
Pre-converted nitrite +cultured corn sugar/vinegar blend (E)	58.01	2.385	5.60	0.9537	24.57
	57.28	2.480	5.62	0.9562	26.71
	57.00	2.397	5.62	0.9557	28.85
	Average ± S.D.	*57.43 ± 0.42*	*2.421 ± 0.042*	*5.61 ± 0.01*	*0.9552 ± 0.0013*	*26.71 ± 2.14*

Note. Data courtesy of Brandon Wanless, University of Wisconsin-Madison.
[a]Vacuum oven method, 5 h, 100°C (AOAC 934.01, 1990).
[b]Measured as Cl-, Brinkmann autotitrator, silver nitrate titration.
[c]Measured using a Aqua Lab CX-2 water activity meter.
[d]pH taken of batter using Orion 8104 combination pH electrode and Accumet pH meter.
[e]method 973.31 (Nitrites in cured meats, colorimetric method); AOAC 15th edition, 1990.

Table 4. Analyzed values of moisture[a], salt (NaCl)[b], pH[c], water activity[d] and nitrite[e] in pre-cooked ham batters.

Formulation	% moisture	% NaCl	pH	A_w	Nitrite (ppm)
Truly natural (A)	75.95	2.456	5.56	0.9741	0.00
	76.07	2.412	5.59	0.9760	0.00
	75.83	2.379	5.67	0.9749	0.00
Average ± S.D.	*75.95 ± 0.12*	*2.416 ± 0.039*	*5.61 ± 0.06*	*0.9750 ± 0.0008*	*0.00 ± 0.00*
Conventional cured control (B)	73.29	2.136	5.61	0.9689	144.50
	72.31	2.142	5.57	0.9671	144.50
	73.75	2.116	5.65	0.9689	142.00
Average ± S.D.	*73.12 ± 0.74*	*2.131 ± 0.014*	*5.61 ± 0.04*	*0.9683 ± 0.0006*	*143.67 ± 1.44*
Pre-converted nitrite w/o antimicrobial (C)	75.78	2.378	5.66	0.9744	37.70
	75.46	2.227	5.60	0.9763	35.25
	75.04	2.220	5.61	0.9760	32.50
Average ± S.D.	*75.43 ± 0.37*	*2.275 ± 0.089*	*5.62 ± 0.03*	*0.9755 ± 0.0013*	*35.15 ± 2.60*
Pre-converted nitrite + lemon/cherry/vinegar blend (D)	74.18	2.263	5.80	0.9725	37.70
	74.84	2.276	5.87	0.9744	35.25
	74.28	2.206	5.85	0.9750	37.70
Average ± S.D.	*74.43 ± 0.36*	*2.248 ± 0.037*	*5.84 ± 0.04*	*0.9740 ± 0.0010*	*36.88 ± 1.41*
Pre-converted nitrite +cultured corn sugar/vinegar blend (E)	72.34	2.217	5.64	0.9688	32.50
	72.54	2.166	5.62	0.9682	32.50
	73.26	2.268	5.57	0.9694	35.25
Average ± S.D.	*72.71 ± 0.48*	*2.217 ± 0.051*	*5.61 ± 0.04*	*0.9688 ± 0.0003*	*33.42 ± 1.59*

Data courtesy of Brandon Wanless, University of Wisconsin–Madison.

[a]Vacuum oven method, 5 h, 100°C (AOAC 934.01, 1990).

[b]Measured as Cl-, Brinkmann autotitrator, silver nitrate titration.

[c]Measured using a Aqua Lab CX-2 water activity meter.

[d]pH taken of batter using Orion 8104 combination pH electrode and Accumet pH meter.

[e]method 973.31 (Nitrites in cured meats, colorimetric method); AOAC 15th edition, 1990.

[f]Samples were too old for accurate pH measurement to be taken.

CHAPTER 6. GENERAL CONCLUSIONS

Because of the long-standing controversy surrounding the safety of sodium nitrite as a non-meat ingredient, the idea of manufacturing cured meats without nitrate or nitrite has been a topic of discussion for many years. Because there is no known replacement for this “magic ingredient,” manufacturing products without its inclusion can be extremely challenging. As consumer demand for naturally cured products increases, processors must be sure that these products are safe for consumption. Because formulated sodium nitrite is not permitted in naturally cured and organic products, additional measures must be taken to ensure this unique group of products possess the same safety characteristics as their conventional counterparts. Without sodium nitrite, these products are more susceptible to contamination by foodborne pathogens. Foodborne pathogens such as *Clostridium perfringens and Clostridium botulinum* must be well controlled. Although there are very few cases of foodborne botulism in the United States, a single incident would devastate the meat industry. Prior to this research, microbiological hazards associated with the no-nitrate and no-nitrate-added products were not well documented or understood. In addition, there were no available manufacturing guidelines available to processors to assure safety.

Results from an evaluation of commercially available frankfurters, bacons and hams indicated that commercial natural and organic cured meats have more potential for pathogen growth than do conventionally cured products. In the event of product temperature abuse or microbial contamination, the commercial brands of frankfurters, hams and bacons that exhibited significantly greater growth than that of

the nitrite-added control would likely cause illness and result in a food recall. Consequently, the development of supplemental treatments to increase the level and consistency of antimicrobial protection in these products is important to provide consumers with the degree of safety that they have come to expect from conventionally cured processed meats. As a result, studies were conducted to validate the effectiveness of natural ingredients (a natural vinegar, lemon/cherry powder blend and a natural cultured corn sugar/vinegar blend) for improved safety by inoculation challenge with *C. perfringens* and *C. botulinum*. Results from these studies suggest that commercial natural/organic cured meats have more potential for growth from both pathogens than do conventionally cured products, but other natural ingredients offer safety improvement by slowing the growth of *C. perfringens*. In addition, these studies demonstrated that certain natural ingredients have the ability to inhibit botulinum toxin production in frankfurters and hams both at refrigeration and abuse temperatures and offer improved safety relative to this pathogen as well.

ACKNOWLEDGEMENTS

I would first like to acknowledge my heavenly father, as none of this would be remotely possible without him. He has given me the strength to press forward when mountains seemed unsurpassable. His grace has sustained me through not only this academic journey, but through life itself. For this, I am eternally thankful. For this and every aspect of my life, he will get the glory.

I would like to thank my major professor Dr. Joseph Sebranek. I am simply amazed that I can walk across the hall and ask questions to one of the most regarded meat scientists in the industry. I have learned so much from you over the years. Your support has meant the world to me. I would also like to thank my graduate committee members who have supported me through the doctoral program. I really do feel like I had the best graduate committee a graduate student could ask for. Those individuals are Dr. Brad Skaar, Dr. Kenneth Prusa, Dr. Aubrey Mendonca and Dr. James Dickson. To Brad Skaar, for nurturing my love for teaching by allowing me to serve as a teaching assistant and food safety guest lecturer each semester during my graduate school journey. To Dr. Dickson for giving me a chance and taking me on as your Master's graduate student in August 2003. This would be the beginning of my love for research. To Ken Prusa for having a sense of humor that allowed me to relax a bit and not take myself so seriously. To Dr. Mendonca for reminding me that I had what it took to succeed and taking the time to give me advice about life. It is my sincerest hope that other graduate students will get a chance to experience each of you as I have.

I would like to acknowledge the Meat Science graduate students (both current and those who have graduated), ISU Meat Laboratory, personnel of the ISU Analytical Laboratory, Brandon Wanless (graduate student at the University of Wisconsin-Madison), Dr. Doug Ahn's Laboratory and friends I've met while at Iowa State University.

I would like to give a special thanks to Steve Niebuhr, lab technician for the Food Safety Research Laboratory at Iowa State University. Your support and inspiring conversations have been instrumental to my success in graduate school. I am a better person for having met you and Vicky. I would also like to thank Thelma Harding in the Graduate College. Words cannot express how much I appreciate you and all that you have done to make life easier while in graduate school at Iowa State. You have supported my two sisters and myself from the time we set foot on campus. We are all grateful for having the rare opportunity to meet someone who we will *never* forget. I would like to acknowledgement Jeff Johnson, President at CEO of the Iowa State University Alumni Association. Thanks so much for your encouragement and support. Everyone needs supporters and you were definitely one! I look forward to being a part of the Iowa State University Alumni Association.

I would also like to acknowledge the University of Arkansas at Pine Bluff, for this is where I first developed my love for Animal Science. To all of my professors at that great institution- thank you so much for your support and guidance. To Juana Johnson and Dorothy Holt for serving as positive role models. To Drs. Carolyn Blakely and Jacqueline McCray, who were the first "Drs." that I knew. Thank you all

for serving as influential role models and teaching me what it meant to be classy and sophisticated all at once.

I would like to thank my family as we are very close to each other. I appreciate your support while I have been miles away from home pursing advanced degrees. My parents, Homer and Deluncie Jackson, have been incredibly supportive. Words simply cannot express the love I have for you. Your encouragement has made me the person I am today. To my sisters, Dimitra, Joy and Leah-I love you so much and cannot wait to see what life has waiting for you! I can feel the love you continually send my way. My God continue to bless and keep you. I would also like to acknowledge my grandmother, aunts, uncles and cousins for their support and love. To my young cousins-the sky is the limit for what you can achieve. Be persistent with any goals you desire and do not let anyone or anything stand in your way.

Last but not least, I would like to thank my fiancé, Dedrick Davis, who has been my cheerleader since the moment we started dating. When I decided to attend Iowa State University for graduate school, I had no idea that I would meet my soul mate. I truly believed that God strategically placed you and I in Ames, Iowa to meet and be in each other's lives forever. You are the reason I tell my Iowa friends, "Never say never! You may just meet your soul mate in Ames, Iowa". You have been my rock and support through graduate school. I cannot wait to see what lies ahead for us.

From my years in graduate school, there is one thing I know for sure…God is in control and will give you strength to endure when you have run out of yourself. Amen for that!