VALIDATION OF A HACCP PROGRAM FOR THE PRODUCTION OF ARTISAN FERMENTED DRY CURED PORK PRODUCTS

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DEDICATION

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CHAPTER ONE

LITERATURE REVIEW

1.0 Artisan Meat Products:

1.1 Definition

'Artisan'is defined in the 1913 Webster's dictionary defines it as: "One trained to manual dexterity in some mechanic art or trade; and handicraftsman; a mechanic" (Webster's, 1913). Or defined on the online Merriam-Webster dictionary as: "a person who is skilled at making things by hand" (Merriam-Webster, 2015).

The term 'artisan', as used in modern terms, has other connotations, suggesting the consideration of production volume and even moral implications. Anything not mass produced could be termed as artisan. In the food community, artisan also conjures a notion of high quality, locally made, or even moral superiority, particularly on animal well-being and 'nose-to-tail' use of animals. Though recently, the term has been used as a common marketing term by large producers and big business to connote quality (Ruhlman, 2010).

1.2 Background

Prior to agriculture and farming around 8000-10000 B.C., primitive man developed domesticated farm animals. Harvesting created the issue of preserving the excess food for future times of scarcity. Northern European climates could utilize cold storage, where as more temperate climates like the Mediterranean could use drying. The application of fire resulted in methods such as smoking and cooking. Salting as a preservation method may have originated when primitive man rubbed marine salt on meat or fish or used a sea

water brine. Dry-cured meat production most likely originated in the Mediterranean, as the climate is favorable for drying and ripening, where as northern Europe used smoking (Toldra, 2002).

Charcuterie is the name of the branch of cooking that utilizes the fundamental methods of curing and preservation. Pre-dating any notion of 'culinary arts', these methods were deployed by, or even predating, the earliest of civilizations by necessity, as survival depended on economy and frugality. Cooking, likely discovered by accident, may have actually came out of the need for preserved food stuffs, dating to the ancestors of *Homo sapiens*. Preservation of food by salting, drying, smoking and cooking has been practiced by virtually every culture, helping nomadic populations store surplus food, and eventually leading to more settled societies. The relevance of charcuterie in the modern era with technologies such as freezing, refrigeration, and modified atmosphere packaging, is less about survival issues, but more about taste and pleasure (Ruhlman, 2005).

Artisans in Italy still adhere to the same methods that have always been used in the preparation of salt-cured meats, notably hams, of which the best are produced in six denominazione di origine controllata, or specific regions which adhere to very specific manufacturing practices. While industrialized food production across Europe became the norm after the Second World War, traditional methods are still practiced, which are costly in labor and time, even though cured, cooked, refrigerated hams exist in supermarkets. Additionally, governments concerned with food safety risks, such as salmonella and botulism, as well as the public eating habits of too much fat and salt, have stifled the culinary tradition. Though this tradition has been practiced and perfected before any scientific rationale could explain the underlying chemical and microbial processes which render the preserved products safe. The notion of terrior, or essence of the land, can create distinctly different cured hams, though they are all cured with salt, sugar and spice. Regional differences in yeasts and molds, temperature, humidity, and storage conditions as well as the diet and care of pigs, all influence the flavor and texture of the ham. This heterogeneity is celebrated in cured meats (The Economist, 2006).

1.3 Why the resurgence of popularity in U.S.

While the science behind the tradition of preserving meat through fermentation was not studied until recent times, the methods of production had gradually changed over time. In modern times, production has increased, consolidated, and new technology has reduced processing time. Popularity of the newer, lower-cost products have increased, but the nature of the product has required more refrigeration and advanced packaging because of the sweeter cure used, higher moisture content, and less salty, more mild flavors (Campbell-Platt, 1995).

Because this modern trend has produced fewer products with similar flavor profiles, there has seen a small, but growing interest toward eating foods from lesser-known parts of the world. In addition, there has been a renewed interest in traditionally fermented meat products that adhere to a slower, less accelerated production process (Campbell-Platt, 1995).

While fermented foods have been considered healthy foods throughout history by certain societies, a renewed interest in fermented foods has increased in modern times. Fermented foods are recognized as being easier to digest, with probiotic and bacteriocin properties, and therapeutic properties on the immune system (Zeuthen, 1995).

Settanii and Moschetti (2014) state that over the past few years, the increase demand for traditional food products has greatly increased, and this poses a contradiction for the consumer. The production of safer foods with a longer shelf-life because of innovative food production technologies has decreased the risk of illness associated with food consumption. However, new life-style choices by consumers, demanding organic foods with reduced levels of chemical preservatives has led to a re-discovery of traditional food products. Additionally, there is a perception that products produced traditionally are considered more ‘natural,’ and in-turn, of higher quality and legitimacy. This has created conflicting challenges for food producers, since consumers have become more

demanding and recent governmental requirements have become more rigorous. The more rigorous hygienic standards and production criteria, to satisfy the increasing demand, has strongly affected the relationship between the production environment and product characteristics. The move from artisanal, small scale production to highly automated large scale production has influenced the entire agri-food chain, impairing the local character or terroir of the product that is usually associated with it (Settani & Moschetti, 2014).

A hot restaurant industry trend according to a 2011 Mintel Group publication is the “farm to fork” connection between the source of the food and the diners/restaurants who consume/serve it (Mintel Group, 2011). Additionally, the view of chefs as farmers and butchers has caught on. Where taking consumers’ desire of demonstrably fresh and expertly prepared foods to their logical end, chefs are becoming farmers, are butchering animals themselves and extending in-house artisanship to making salumi and other gourmet foods (Mintel Group, 2010).

2.0 Fermented/Aged Meat Products

The factors leading to preserved meat include the lactic acid produced in meat post mortem, the lactic acid produced by the fermenting microbial starter cultures, the salt and its water activity lowering properties, the nitrite formed from nitrate, and the water loss during aging and storage. Additionally, industry consortiums have best manufacturing practices that recommend fermentation temperature-time criteria to prevent the growth of pathogens, such as *Staphylococcus aureus* (Lawrie, 1995).

2.1 Fermentation of Meat

Fermentation is the anaerobic transformation of carbohydrates by microorganisms into metabolites, such as organic acids (formic, acetic, propionic or lactic), alcohols, and gases. The metabolites of one microbe may give a competitive advantage over other microbes present by either preventing their growth or injuring the competing microbes themselves. Non-pathogenic microbes in food can inhibit pathogenic microbial growth, thus preventing them from producing toxins or spoiling the food. To produce preserved food, the microorganisms, the environment, and the nature of the food need to be assessed or controlled (Lawrie, 1995).

While traditional fermentation was initiated by the indigenous microorganisms, modern fermented meat is inoculated with pure strain cultures, in precisely controlled environments (Lawrie, 1995).

2.2 Aging of Meat

During the aging step, water is removed as vapor. The water loss causes a reduction in microbial activity because of the loss of available water for maintaining osmotic pressure. Additionally, as the mass and volume decreases, the texture will harden, and a development of aroma compounds will occur (Zukal & Incze, 2010).

Water vapor will leave the surface of a product only if the water activity of the surface is higher than that of the relative humidity of the air around the product. Drying rate depends on drying surface dimensions, the difference between the water activity and the humidity of the air, which is the driving force, and the characteristics of the porous outer layers (casing, mold). As the water vapor leaves the surface of the product, the moisture content of the air increases. This causes the relative vapor content to be higher, because evaporation cools down the product and environment. To continue to drive the moisture loss, the ambient air has to become drier and warmer. This is achieved by powerful environmental control systems (Zukal & Incze, 2010).

Sufficient water availability for microbial metabolism may determine growth rates. As solutes such as salt and sugar increase in concentration, the ability of microbes to maintain their osmotic pressure becomes more difficult. Water vapor of a solution decreases as the concentration of solutes increases. This is described as water activity (a_w), which is the ratio of the water vapor of the medium to that of pure water at the same temperature. Because water activity and microbial growth are closely related, the manipulation of water activity can influence the growth rates of microbes. A water activity range of 1.0-0.75 permits bacterial growth, which is why fresh meat, with a water activity of 0.99, spoils quickly. In curing, the use of salt and sugar decreases the water vapor pressure of the medium, thus making microbial growth more difficult. Halophilic bacteria are salt-tolerant, thus thrive in such environments. These bacteria also demonstrate the ability to reduce nitrate and nitrite to nitric oxide, giving cured meats their pink hue (Lawrie, 1995).

2.3 Effect of Meat Composition on Fermentation and Aging

Meat Composition:

Post mortem compositional changes during storage are caused by biochemical, chemical and microbiological processes. Intrinsic enzymes within the meat and are mainly desirable, while intrinsic chemical reactions are not. Changes produced by microbes that

have been introduced to the meat pose the most risk to consumer health and eating quality. Proteolytic enzymes post mortem are temperature sensitive, with increased activity at higher temperatures. These proteases are responsible for increased tenderness and flavor. Non-enzymatic chemical reactions denature proteins, and release the water within them. The extent of water loss is mostly dictated by the pH of the meat. A normal meat pH is around 5.5, which is near the isoelectric point (pI), which leads to significant water loss. A high pH, above 6.0, has low water loss (exudation), resulting in a near neutral pH and high water content. This can cause microbial susceptibility. A low pH, below 5.5, results in PSE (pale, soft, exudative), which is excessively watery (<6% drip loss) and prone to denaturation. The water is present between muscle fibers, as apposed to within the muscle fibers. Upon death, proteins release water, and the degradation of these proteins, as well as fats and carbohydrates, create a metabolite rich medium for a wide range of microbial growth, namely bacteria, yeasts and molds. These microbes produce enzymes which free amino acids and then metabolize them. Glycogen can be metabolized to acetic and butyric acids. Additionally, pathogens may produce toxins, thus adulterating the meat, and becoming dangerous for human consumption (Lawrie, 1995).

Microbial Spoilage/Contamination:

In vivo sources of contamination not detectable by veterinary inspectors include Salmonella, Listeria, and E. coli. Post mortem contamination via the animal's blood is unlikely. Contamination by the bacteria in the gastrointestinal tract is a source if it is ruptured during slaughter. Soil and fecal matter may be present on the feet and skin. The environmental sources at the slaughter plant include the water used, instruments, surfaces, personnel, and airborne particulates. Cooling carcasses prevents most bacteria from growth, and 90% of those that are viable at chill temperatures, are most likely harmless psychrotrophs. Psychrotrophs such as Listeria can grow at refrigeration temperatures. Contamination by *Salmonella* spp., *E. coli*, *Staphylococcus aureus*, and *Clostridium* species, can occur at all stages post mortem if proper cooling and handling

techniques are not followed. Hazard analysis: critical control points', or HACCP, provides effective control measures, which help with the "identification of hazards, understanding the risks they present, identifying the locations or stages at which control could be exercised (critical control points), and selecting the options for such control." Mesophilic (10-40 °C) bacteria, present in reservoirs such as lymph nodes, may grow if proper refrigeration and chilling immediately post mortem is not achieved. Temperatures below -1.5 °C reduce the number of bacteria by removing water in the form of ice (Lawrie, 1995).

Microbial growth is closely related to the pH of the medium. Optimal pH for bacteria is approximately a pH 7. Below pH 4 and above pH 9 exhibits no growth. Anaerobic glycolysis produces lactic acid immediately after death, resulting in a pH 5.5 to 7.0. Because available glycogen in muscles determines the pH drop, the lower the glycogen levels at the time of death due to stress, starvation, rough handling, etc, will result in a higher pH, thus promoting more rapid spoilage. In addition, a lower pH of 5.5 increases nitrite concentration, thus decreasing the amount needed to control pathogens (Lawrie, 1995).

3.0 Types of Dry-Cured Fermented Meat: Whole Muscle vs. Comminuted (Salami)

Whole pieces of meat, either constituting a whole muscle or groups of muscles, such as a ham, coppa (shoulder), lonza (loin) or smaller pieces such as jerky, are cured and dried. Salami is a comminuted type of fermented meat, which contains small pieces of chopped or ground meat from one species or more. There are also fermented products that are made from bones or offal, but these are much less common, and not commercially made.

Whole-Muscle:

Whole-meat products are produced around the world, with hams being the most common. The traditional European method requires the surface of the meat to be rubbed with salt and potassium/sodium nitrate. Starter cultures, spices and sugar may be added as well. This cure can range for weeks to months. The cure is applied and the meat is kept cool (5-10 °C) for 10-15 days, then it is allowed to rest for another 14-50 days. This is followed by a drying-aging period of several weeks or months at elevated temperatures (18-35 °C). A cool smoke may be applied. The methods vary from region to region to account for temperature and humidity differences. The lack of a cook step in this type of ham raises the risk of *Trichinella spiralis*. Additionally, *Staphylococcus aureus* and enterotoxinogenic bacteria have been found to contaminate dry-cured hams, requiring good hygiene practices of personnel. Other species, such as beef, lamb, goat, etc. are often fermented as well, with different microbiological concerns and production methods (Camplbell-Platt, 1995).

While dry-cured hams are the most well-known whole muscle product, loins (lonza) and shoulders (coppa) are also dry-cured. The process is very similar, but each stage is shorter due to the reduced size of the muscle group or muscle being cured and dried. One major difference is that these smaller pieces are stuffed into large casings, so that they can be hung to ripen/dry.

Salami:

Comminuted, or chopped meat, often referred to as sausage, is common around the world. These sausages are not always fermented, as there are raw sausages that must be cooked before consumption. Fermented sausages are produced by chopping pork, (or beef or lamb) into small pieces, and mixed with salt, nitrate/nitrite, sugar, spices and seasonings. This mixture is then stuffed into animal intestines or synthetic casings. Traditionally, inoculation with microflora from previous batches of fermented sausage, known as 'back-slopping', was used, while today a cocktail of cultures for various functional properties, such as lactic acid producing *Lactobacillus brevis, Lactobacillus plantarum,* and *Pediococcus* spp., and nitrate reducing bacteria such as *Micrococcus* spp. or non-pathogenic *Staphylococcus* spp. The lactic acid produced by the bacteria drops the pH and the hydrogen-peroxide is reduced by the catalase enzyme produced by the nitrate-reducing bacteria (Campbell-Platt, 1995).

3.1 Manufacture of Dry-Cured Whole Muscles

To elaborate on the above discussion, the steps required to produce a flavorful and safe product are deliberate. The raw material is of the upmost importance, as the entire process depends on the quality of the hams. After slaughter, it is important to refrigerate the hams throughout to 2-4 °C. The pH is important to the safety and quality of the hams. A high pH at 24 h postmortem, known as dark, firm and dry (DFD), is prone to microbiological contamination and high water binding due to its neutral pH. This is caused by the depletion of glycogen stores prior to death, ultimately limiting lactic acid production Hams that are pale, soft, and exudative (PSE), have a lower pH 24 h postmortem (pH 5.2-5.4), which is due to rapid and excessive lactic acid production and higher temperatures postmortem. This causes a lower water binding capacity, because the muscles are near their isoelectric point. Losing moisture rapidly may result in an excessively dry surface later in the process where microbes could penetrate and spoil the ham. The ideal pH at 24 h postmortem is 5.6-6.1. Before the salting step, some of the skin

is removed to facilitate salt penetration, as skin and fat are barriers to salt diffusion (Toldra & Aristoy, 2010; Toldra, 2002).

The hams are rubbed with sodium chloride (30-50 g/kg) and sodium/potassium nitrate (2187 ppm). The nitrate is not a preservative, but rather a precursor to nitrite. Nitrate is reduced by the enzyme nitrate reductase, a bacterial enzyme present in the natural flora (or in a starter culture) of the ham, which acts as a time release capsule, to nitrite. Nitrite is very necessary, as it is very effective against *C. botulinum*, the bacteria responsible for botulism. The use of sodium chloride is necessary to reduce the a_w and to prevent the growth of spoilage microbes. It also is important for the flavor of the ham. Salting takes place under refrigeration, and the length is determined by ham size, but typically 14-21 days (Toldra & Aristoy, 2010; Toldra, 2002). Reynolds *et* al. demonstrated that a higher salt content (8%) did not reduce moisture or a_w more rapidly than a lower salt content (5.51%) (Reynolds *et al*., 2001).

The next step is the resting step, where the hams are rinsed to remove the excess salt, and placed on shelving to rest. This step is necessary for salt equalization, where the complete homogeneous distribution of the salt and nitrate/nitrite throughout the ham can take place. The length of this step (14-48 days) is determined by a variety of factors, including ham size, ratio of lean surface to mass, pH, intramuscular fat (a barrier to salt diffusion) and temperature of the resting room (Toldra & Aristoy, 2010; Toldra, 2002).

The following step is a smoking step, which is typical of a few ham types, such as speck. Smoking is done for its characteristic flavor, but more importantly, it protects the surface from the growth of molds and yeasts, due to the bactericide and bacteriostatic smoke compounds present (Toldra & Aristoy, 2010).

The ripening/drying stage has two main objectives: 1) to dry the hams to approximately 32% weight loss, and 2) to provide time for flavor development enzymatic reactions to occur. The air speed, temperature, and relative humidity are crucial factors that influence the rate of water diffusion from the interior of the ham and its subsequent rate of evaporation from the surface. These rates must be synchronized, or the surface will

dehydrate and prevent further water diffusion to the surface. A hard, dry surface is susceptible to microbial spoilage, and the interior would be prone to incomplete drying. The higher temperature enhances proteolytic and lipolytic enzymatic reactions. Proteolysis directly contributes to textural properties by breaking down myofibrillar proteins of the muscle structure, as well as the generation of peptides and free amino acids. These influence taste and are also the substrates for further flavor reactions. Lipolysis also directly influences flavor and the formation of flavor precursors. The generation of free fatty acids effects flavor, and the production of free polyunsaturated fatty acids are substrates for oxidative reactions to ham-specific volatile aromatic compounds. Lipolysis also prevents rancid odors and fat yellowing by breaking down triglycerides. Proteolysis and lipolysis is only possible due to the low microbial counts inside the ham. Because of the pH, salt content, and low a_w, the growth and enzymatic actions of spoilage bacteria are limited, thereby allowing textural, aromatic, and flavor reactions to occur. The ripening/drying stage can last from 3-36 months, but 6-9 months is typical for an acceptable quality (Toldra & Aristoy, 2010; Toldra, 2002).

It is important to note that the pH of the finished ham will be higher than the pH at the start of the process. Proteolysis generates free amino acids which will act as a buffer. Reynolds *et al*. 2001 stated that it is unlikely that pH alone is a major factor in the microbial stability of hams (Reynolds *et al.*, 2001).

3.2 Manufacture of Dry-Fermented Sausages

"For the manufacture of typical dry fermented sausages, lean meat (60-70%) and fatty tissue (30-40%) are comminuted, mixed with about 2.4-3% salt, curing agents, some sugar, spices and, in many cases, starter cultures. The mix is placed with inclusion of as little oxygen as possible, into vapour-permeable casings and subjected to a fermentation and drying process. Control of temperature and relative humidity is essential for the production of dry sausages. Ripening chambers with precisely adjustable temperature and humidity are expensive, particularly if they are equipped with smoke generators and

smoke combustion devices. This is the main reason why today only a small proportion of fermented dry sausages are manufactured on an artisanal scale…" (Lucke, 1994)

Sausage Fermentation

Meat as a substrate for microorganisms - … Lean meat contains considerable amounts of peptides and amino acids but only small amounts of glucose and glucose-6-phosphate. The content of these fermentable sugars, as well as the content of lactic acid and the pH, depend on the glycogen content of the muscle at slaughter and my vary considerably. As a rule, meat with a pH above 5.9 contains too little lactate and sugar for a safe fermentation; it binds water tightly and provides better conditions for growth of acid-labile bacteria (Lucke, 1994).

The solid-substrate fermentation inherent to meat means bacteria grow in micro-colonies, and therefore starter cultures need to out-compete the indigenous microflora of the raw material without the use of a kill-step (cooking), which would change the appearance of the meat. The comminuted meat quickly becomes anaerobic in the interior, resulting in obligate aerobe growth to the surface (Lucke, 1994).

For example, Lucke (1994) discusses the importance of pathogen control during sausage fermentation. An initial ripening temperature above 20 °C and lactic acid fermentation lowering the pH to about 5.3 is important for the control of S. aureus. Additionally, the American Meat Institute specified the maximum time allowed in 'good manufacturing practice for fermented dry or semi-dry sausage' to reach pH 5.3. (e.g., 80 h at 24 °C). If nitrate was used instead of nitrite, the addition of nitrate reducing starters also contributed to the suppression of S. aureus. In unsmoked sausages, the molds colonizing the surface raise the pH, and since the antimicrobial smoke constituents are absent, the risk of S. aureus is raised (Lucke, 1994). However, in unsmoked sausages, if the fermentation temperature is low enough, or the rate of acid production fast enough, S. aureus is reliably suppressed (Metaxopoulos *et al.*, 1981).

Genuine salamis are made with little sugar. The reduced carbohydrate level allow only a moderate pH reduction and are therefore traditionally ripened at lower temperatures. When such sausages were fermented at 23 °C, addition of lactic acid bacteria (Lactobacillus plantarum, Lactobacillus sake, Lactobacillus curvatus or Pediococcus pentosaceus) markedly contributed to the inhibition of S. aureus. The rapid production of lactic acid decreased the pH to values below 5.3, and no evidence for the involvement of any other control mechanism was found. At lower fermentation temperatures, acid formation became a less important factor. Additionally, initial a_w values below 0.955 even favored the growth of S. aureus because it slowed down acid production. Nitrite had only a minor inhibitory effect (Lucke, 1994).

Salt and nitrite play an important role in suppression of salmonellae early in the fermentation when the pH is still above 5.3 (Lucke, 1994). Schillinger and Lucke (1989) found that sausages formulated with only 2% salt and 40-70 mg/kg sodium nitrite added (about half the usual amount) can be safely produced if the pH is lowered to 5.3 within 1-2 days at about 20 °C (Schillinger & Lucke, 1989). This, however, was only feasible with very rapid acid producers such as Lactobacillus sake or by the use of acidulants such as glucono-delta-lactone. Strains of lactic acid bacteria differed somewhat in their effect on salmonellae; these differences, however, were found to be largely, if not entirely due to the formation of lactic acids from fermentable sugars (glucose, glucose-6-phosphate, ribose) originally present in the meat (Lucke, 1994).

4.0 Hurdle Technology Concept

4.1 Definition

The hurdles of most importance in food preservation are temperature (high or low), water activity, pH, preservatives (e.g. nitrite), and competitive microorganisms (e.g. lactic acid bacteria). During food preservation, the three most important physiological responses of microorganisms are their homeostasis, metabolic exhaustion, and stress reactions, which are the target of hurdle technology. Homeostasis disruption, by preservation factors or hurdles in the food system, within microorganisms is the key for food preservation. By disrupting pH in the food system, homeostasis is unstable, preventing microbe growth or causing death, before it is repaired or re-established. Microorganisms "in stable hurdle-technology foods strain every possible repair mechanisms for their homeostasis to overcome the hostile environment, by doing this they completely use up their energy and die, if they become metabolically exhausted" (Leistner, 2000). This autosterilization in hurdle-technology food systems are microbiologically stable and continue to become more safe, particularly at ambient temperature. Leistner (2000) states that survival of salmonellae in fermented sausages during the ripening process will die more quickly if stored at ambient temperatures, and if stored under refrigeration, they will survive longer and pose a possible risk of foodborne illness. Bacteria under stress, such as starvation, temperature, pH, and a_w, may become more resistant or more virulent. The reactions to the stress involve production of protective stress shock proteins. Synthesis of these proteins could be more difficult if multiple stresses are received simultaneously. The consumption of energy to produce elevated levels or several types of these protective stress shock proteins could lead to metabolic exhaustion (Leistner, 2000).

Leistner (2000) suspects that multiple hurdles not only have an additive effect, but a synergistic effect on microbial stability. Targeting multiple aspects of microbial cell

function (cell membrane, DNA, enzyme systems, pH, a_w) would disrupt homeostasis, causing the need of repair of homeostasis at the same time as production of stress shock proteins. Leistner (2000) states that "this could mean that it is more effective to employ different preservative factors (hurdles) of small intensity than one preservative factor of larger intensity, because different preservative factors might have a synergistic effect." "The novel and ambitious goal for an optimal food preservation is the multitarget preservation of foods, in which intelligently applied gentle hurdles will have a synergistic effect" (Leistner, 2000).

4.2 Hurdles in Dry-Cured Whole Muscle Production

The main hurdle in hams is the water activity, though it has a slow effectiveness because of the slow salt penetration and drying times (Lund *et al*., 2000). Leistner (1987) suggests careful transport and slaughtering of hogs to minimize bacterial counts, with the rapid chilling to < 4 °C in 24 h to inhibit rapid growth of bacteria in the interior of the hams. The starting pH of the ham should be ≤5.8, to eliminate high water binding which would pose a microbiological risk. Curing temperatures should be kept below 5 °C until the a_w is <0.96 (>4.5% salt content). Ripening and aging may proceed at 20-25 °C to increase enzyme ripening, flavor development, and moisture loss. The final salt content should be 5-7%, with a a_w <0.92. The pH may rise due to the generation of free amino acids as a result of proteolysis (Leistner, 1987).

4.3 Hurdles in Fermented Sausage Production

A sequence of hurdles in fermented sausage production leads to stable and safe products. The first hurdle is the addition of nitrite at the onset of fermentation, as this inhibits salmonellae present in the raw sausage. The nitrite is broken down during the beginning of fermentation, and is only effective for a short time (Leistner, 1995).

The next hurdle is the redox potential (E_h), which is high following the exposure to oxygen during chopping. The E_h is reduced by aerophilic bacteria present in the sausage, inhibiting spoilage bacteria, and more importantly, giving lactic acid bacteria a selective advantage (Leistner, 1995).

After the redox potential is reduced, competitive flora becomes the most important hurdle during fermentation. The lactic acid bacteria suppress pathogenic microorganisms by reducing the pH and possibly through the production of bacteriocins (Leistner, 1995).

The pH value is the next important hurdle in raw sausage, particularly with quick-ripened products with higher a_w. How rapid and how far the pH drops is affected by the amount of fermentable sugar available and the fermentation temperature. During the ripening/aging of salami, the pH will rise, but that is of less concern because other hurdles have been met by that time. For salami with a final pH >5.4, the a_w is more important than pH (Leistner, 1995).

The a_w value continues to fall during the ripening/aging period, and thereby becoming and increasingly important hurdle. The quickness of and how far the drop of a_w is influenced by recipe formulation, ripening temperature, but most importantly by the relative air humidity in the ripening chamber relative to ripening time (Leistner, 1995).

The sequence of these hurdles results in a stable, safe product, because spoilage and pathogenic bacteria are inhibited, while lactic acid bacteria are given a selective advantage and are relatively unaffected by the hurdles (Leistner, 1995).

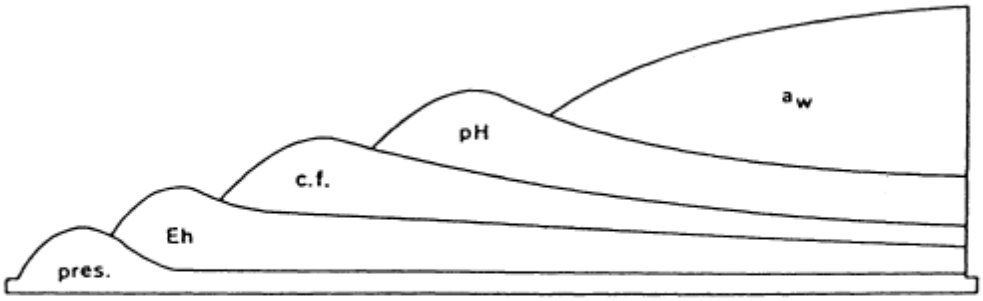

Figure 7.1 Sequence of hurdles in raw sausage. preso = preservation; Eh = redox potential; c.f. = competitive flora; pH = acidity; a_w = water activity.

Figure 1: Sequence of Hurdles in Raw Sausage (Leistner, 1995).

5.0 Risk Assessment of Artisan Pork Products

5.1 Definition

Microbiological risk assessment (MRA) is implemented for application of microbiological food safety knowledge in a logical, systematic, consistent and transparent way to assess food safety risks. It attempts to determine the chance that a certain food will cause illness, who will be affected, and what those effects will be. It offers a scientific basis for the management and control of the microbiological risks posed by foods (Adams & Mitchell, 2002).

Implementation of the MRA consists of a series of steps, starting with a statement of purpose for the assessment itself. The next step is hazard identification, in which it is necessary to decide which hazardous organisms will be of concern in the product. This is followed by an exposure assessment, which assesses the food as a vehicle for the intake of a particular pathogen by estimating the level of pathogen or toxin in the food. In contrast to exposure assessment, the hazard characterization step determines the effect the hazard will have on people, and is characterized by frequency, nature, severity and duration of illness caused by the presence of the hazard in the food. The last step is risk characterization, where information gathered in the previous steps is used to produce and estimate of the probability of illness and its severity in a given population (Adams & Mitchell, 2002).

The risk assessment should be applied over the entire process, starting at the farm, in the slaughter facility, at the processing plant, at retail/foodservice, and finally to the consumer.

5.2 FSIS *Salmonella* Compliance Guidelines

FSIS *Salmonella* Compliance Guidelines for Small and Very Small Meat and Poultry Establishments that Produce Ready-to-Eat (RTE) Products

Regardless of the lethality process used, all establishments that produce RTE meat and poultry products must provide supporting documentation that there process for their RTE products achieves the required or recommended reduction of *Salmonella*. This supporting documentation must be provided as part of an establishment's hazard analysis decision-making documents and validation data must be included in its HACCP records (USDA, 2012).

It is particularly important that, as part of initial validation, the establishment identifies all of the critical operational parameters (e.g., pH, water activity, humidity, pressure, etc.) identified in the scientific supporting documentation that may influence the effectiveness of the process. During the initial validation period, the establishment should verify that it is able to implement all the critical operational parameters as used in the scientific support to ensure that the process can be successfully implemented in its own system (USDA, 2012).

There are no regulatory requirements regarding the level of reduction of *Salmonella* in RTE dried, fermented sausage. However, FSIS considers that a 5-$\log_{10}$ reduction of *Salmonella* in meat products … would produce a product safe for consumption. This guidance is based on FSIS's "Risk Assessment of the Impact of Lethality Standards on Salmonellosis from Ready-to-Eat Meat and Poultry Products (USDA, 2012).

Other than guidelines posted in conjunction with the proposed rule "Performance Standards for the Production of Processed Meat and Poultry Products" in 2001 and the Blue Ribbon Task Force recommendations on dry, fermented sausages in the 1990's, FSIS has not published any guidance on this topic. Establishments would have to obtain supporting documentation for their process from published studies, a processing authority, or challenge studies (USDA, 2012).

The FSIS compliance guidelines for *Salmonella* list five options for dried and semi-dried fermented sausages. The five options include the use of a heat process, the use of a validated 5-$\log_{10}$ inactivation treatment, conduct a "hold and test" program for finished product, propose other approaches to ensure a 5-$\log_{10}$ reduction of *Salmonella*, or the use of a combination of raw batter testing and at least a 2-$\log_{10}$ reduction. The guideline noted that this option may provide less assurance of product safety, because a 2-$\log_{10}$ reduction may not be sufficient for contamination levels. Though it did concede that if the establishment had controls in place to assure contamination levels are low on incoming product, that the option would be considered viable (USDA, 2012).

5.3 FSIS Risk Assessment Model

FSIS categorizes salami as a moderate risk.

The Food Safety and Inspection Service (FSIS) lethality guidelines for *Salmonella* in Ready-to-Eat (RTE) are based on the 2005 "Risk Assessment of the Impact of Lethality Standards on Salmonellosis from Ready-to-Eat Meat and Poultry Products." FSIS is responsible for ensuring the safety of the nation's commercial supply of meat, poultry and egg products. The agency proposed specific lethality levels for RTE products that processors would have to meet. The 'required lethality' is based on the probability of survival of *Salmonella spp.* that may be present in raw materials. The public health risk corresponding to the consumption of RTE meat products is influenced by the probability of survival of *Salmonella spp.* The risk assessment addresses various lethality levels and the resulting level of public health risk.

FSIS proposed "a minimum lethality performance standard of 6.5-log reduction of *Salmonella* in meat for all categories (cooked, fermented, salt-cured, dried)." The risk assessment addresses the public health impact of 5.0-log reduction versus a 6.5-log reduction. The risk to public health associated with the consumption of RTE meat products is estimated by the level of contamination of raw materials, the required lethality

standard, the extent of compliance with the standard, thermal processing safety factors, the storage of the product and potential growth of the surviving organisms, the frequency and extent of consumer re-heating, and the amount of consumption of the product. An analysis software tool was developed to take into account these factors and the "sparse data available for the estimation of risk associated with RTE products," while accommodating the high level of uncertainty and variability.

The risk assessment acknowledges many limitations and uncertainties. Pathogen burden in raw materials is uncertain for current production, and the data used is from the FSIS Microbiological Baseline Surveys. Uncertainty exists in the extent of growth of pathogen populations, particularly because of the broad categories in which RTE products have been placed, which creates uncertainty in the growth rates, storage conditions, and in estimating the maximum population density. Production volume estimates of specific RTE meats products is limited and therefore estimates of population health risks is more uncertain. The risk assessment acknowledges that given the possibility of levels of uncertainty by orders of magnitude, that "the relative ranking (or attribution of total risk) among products should not be considered robust.

The risk assessment assigns salami to the Fermented or Direct Acidified, Uncooked, Shelf-stable (FUSS). The *primary control mechanism* for the FUSS category is fermentation, which is viewed as less controllable and less predictable than compared to thermal processing. The *role of formulation in lethality* for FUSS products is characterized as critical for the achievement of lethality, as the risk is "due to process variability and due to variability in processors' ability to understand, predict and ensure a prescribed degree of lethality." The *margin of safety* for FUSS products was characterized as small, due to variance in cooking because of quality and yield considerations. Additionally, the study cites that the nature of contamination in comminuted products reduces the margin of safety because of the distribution of pathogens and being further from the source of heat. The final product characterization, that assigns products to risk categories and risk factors, is the *re-growth of pathogens*. This risk is characterized as the allowance of the resuscitation and re-growth of

pathogens in a RTE product, and FUSS product risk for re-growth of pathogens is characterized as controlled, unlike cooked products.

Raw material pathogen burden is the primary factor in estimating the impact of lethality standards. FSIS based raw material quality on the FSIS Baseline Microbiological Surveys from 1992-1997. The raw material assigned to FUSS was 50% ground beef and 50% ground pork. The *Salmonella* pathogen burden estimates for ground beef are approximately half a 0.5-log greater than for ground pork. Additionally, the pathogen burden estimates for intact pork are approximately 2-log lower than that of ground pork.

While FSIS considers fermented sausages as a moderate risk product, Mataragas, Skandamis, and Drosinos (2008) developed risk profiles for various combinations of hazard/food product based on data found in the literature and the Risk Ranger program. The authors found that the risk posed by *L. monocytogenes* and *Salmonella* spp. for the preserved meats such as fermented sausages was moderate for high-risk population and low for the rest (Mataragas *et al*., 2015).

The FSIS Pathogen Reduction/HACCP rule published in July, 1996 combines the concepts of Hazard Analysis and Critical Control Point systems with the FSIS requirement for written Sanitation Standard Operating Procedures (SSOPs). In order to avoid confusion, it is important to have a clear understanding of how the HACCP concepts relate to SSOPs as well as the relationship between the SSOPs, Good Manufacturing Practices (GMPs), and HACCP plans that many companies already have in place (AMIF, 1997).

6.0 Hazard Analysis Critical Control Point (HACCP)

6.1 Definition

Hazard Analysis Critical Control Point system is considered worldwide to be essential to an effective and rational proactive methodology, which must be integrated into every step of the food chain, from primary production to final consumption, in order to assure food safety (Fraqueza & Barreto, 2010). Food safety is understood in terms of biological, chemical and physical agents that may be present throughout the food chain and cause the final product to be adulterated. These hazards, if not controlled, are likely to cause illness or injury to consumers. The probability of these hazards to occur in food and cause food-borne illness is known as risk (Fraqueza & Barreto, 2010).

Hazard analysis consists of two distinct parts: the first is hazard identification and the second is hazard evaluation (risk assessment of that hazard at each process step until the point at which the hazard is controlled). If the correct significant hazards aren't identified, then the HACCP plan that is developed cannot possibly be valid (Fraqueza & Barreto, 2010).

Critical Control Points (CCPs) need to be identified in materials and process steps to control the hazards. For each CCP, critical limits must be defined. The critical limits are associated with the preventive measures that control hazards and are measurable (Fraqueza & Barreto, 2010). Additionally, tolerance deviations must be defined. Critical Control Points must be monitored, and any deviation from the set critical limits must have a corresponding corrective action to restore control of the hazard. Plan compliance must be demonstrated with verification procedures. Documentation and operational records must be kept for monitoring procedures, corrective actions, and verification, to demonstrate system effectiveness. The plan must be updated when the process changes or as required by new legislation. If hazard analysis indicate products share equivalent

potential hazards, risks, CCP's and critical limits, then it is unnecessary for each product to have separate HACCP plans (Fraqueza & Barreto, 2010).

At each processing step, biological, chemical, and/or physical hazards must be analyzed to determine if it is reasonably likely for them to occur. Potential hazards are usually based on experimental and epidemiological data and literature references. Any processing step with a reasonably likely hazard to occur must consider any possible control measure than can be applied. A specific hazard may require more than one control measure, and one control measure may control more than one hazard. Once the probability of occurrence and severity of a hazard is assessed, the risk determines if the hazard will be addressed. "The justification for why a hazard is or is not reasonably likely to occur based on epidemiological data or referenced works is important and can also be viewed as a form of validation" (Fraqueza & Barreto, 2010). Additionally, by following Good Manufacturing Practices (GMPs) and having them as part of the HACCP plan can reduce the occurrence of hazards or justify the unlikelihood of the hazard to occur.

The CCP consists of two basic elements: the control system itself that implements the control measures, and the monitoring system. Control systems are usually practices/procedures that, when not done correctly, are the leading causes of food-borne illness outbreaks… Effective monitoring systems of these practices that control hazards are crucial to the safety of the product (Fraqueza & Barreto, 2010).

Critical limits are specified for controlling the preventive measures at each CCP identified, which will define if a product is safe or unsafe. A critical limit is a maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard. Additionally, critical limits are established based on published data (scientific literature, in-house and supplier specifications, regulatory guidelines), experimental data, expert advice, and mathematical modeling (Fraqueza & Barreto, 2010). These must be established for monitoring actions as well as verification procedures. Validation of critical limits is essential in an industry, confirming that control

measures at critical points are capable of controlling the identified hazards (Fraqueza & Barreto, 2010).

Monitoring procedures should be objective methods, such as measurement of temperature, pH, a_w, relative humidity, and microbiological tests. If there is a deviation from a CCP's critical limit, a mandatory corrective action should be acted upon quickly. Corrective actions can include processes adjustment, correction of temperature, time, microbiological assessment, etc. These actions could lead to product release, rejection and destruction, or product reworking. Analyzing the cause of deviation from the critical limit is necessary to prevent further deviations (Fraqueza & Barreto, 2010).

Verification of the HACCP system must periodically be tested to ensure control measures and monitoring procedures at CCPs are working effectively. Verification actions include sampling raw materials and end products and performing microbiological tests, equipment calibration, and environmental sampling (Fraqueza & Barreto, 2010).

HACCP plan data must be archived and available for consultation. These records can provide evidence that preventive measures are effective to eliminate or reduce hazards to acceptable level in the HACCP plan at CCPs, GMP, or other preventive measures are used for HACCP plan validation (Fraqueza & Barreto, 2010).

Quantitative risk assessment is a valuable tool to validate critical limits at critical control points (Fraqueza & Barreto, 2010).

6.2 Critical Control Points (CCPs) of Dry-Cured Whole Muscle Products

The Critical Control Points of the manufacturing process for dry-cured hams proposed by Toldra (2002) to control the growth of pathogens are 1) receiving raw hams below 7 °C, and 2) a pH ≤ 5.8 at the beginning of processing to mitigate microbiological risk during later stages (Hoornstra *et al*., 2001).

Reynolds *et al.* validated a dry cured ham process, which demonstrated 5.5, 5.5, and 4.0 log reductions in populations of *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes*, respectively, by day 69 of the curing process. A a_w of 0.923 and 0.908 at day 69 and day 120, respectively, with a salt content of 8% in the finished product, a pH of 5.5, and storage temperature of 20 °C, resulted in revention or limit proliferation of staphylococci (Toldra, 2002). These reductions in a_w values closely paralleled a_w reduction values using less salt in a study by Huerta (1988), resulting in a a_w value of 0.909 at day 125 with a final salt content of 5.51% (Reynolds *et al*, 2001).

6.3 Critical Control Points (CCPs) of Dry Fermented Sausages

The Critical Control Points of the manufacturing process for dry fermented sausages proposed by Lucke (2000) to control the growth of *Salmonella enterica, Staphylococcus aureus* and *Listeria monocytogenes* are 1) a carcass pH ≤ 5.8, 2) a a_w during grinding/mixing of batter between 0.955-0.965, 3) a batter sugar content of 0.3-0.5%, 4) the use of a starter culture, 5) a fermentation temperature of 18-22 °C and a pH ≤ 5.3 at about 3 days of fermentation, and 6) an aging temperature of 10-15 °C to achieve a $a_w \leq$ 0.90 (Huerta *et al.*, 1988).

The crucial pathogen reduction factors are pH and a_w, which are dependent on fermentation temperature. A pH reduction with fermentation temperatures ≤ 20 °C is less important to pathogen reduction than a_w reduction. The pH reduction for inhibition of pathogens is most important for fermentation temperatures > 20 °C (Lucke, 2000). A rapid pH decrease during fermentation (48 h) at ≤ 20 °C, a slow moisture lose, and a small a_w drop did not achieve a rapid pathogen reduction (Gounadaki *et al.*, 2005).

Mataragas et al (2015) demonstrated differences in *Salmonella enterica* total inactivation in short and long aging Italian style salami. Both short and long maturated fermented sausages reached the first decimal (1-log) reduction of *Salmonella enterica* in the same time period and all the pH drops reached approximately 5.0 or less within approximately

48 h. The overall pathogen reduction was higher in the longer aged fermented sausages (40 days) than the shorter aged style (20 days). The difference in inactivation was attributed to the a_w (long maturation period). It was also noted that the reduction rate of a_w was very slow and for greater total inactivation of pathogens, a higher rate of dehydration would be required. Mataragas et al (2015) cites the importance of a_w values relative to the CCPs in the salami production process for pathogen control. Their study had higher a_w values during the grinding and mixing of the batter, as well as in the finished product, than the recommended limits as published by Lucke (2000).

7.0 Good Manufacturing Practices (GMPs)

The American Meat Institute Foundation (AMIF) released the "Good Manufacturing Practices for Fermented Dry & Semi-Dry Sausage Production" in 1997. GMPs are programs that comprise the basic, universal steps and procedures that control operating conditions within establishments and ensure favorable conditions for the production of safe food. HACCP systems relate to hazards within a specific process. GMPs are the control factors that relate to the entire operation and are not process-specific. GMPs include such programs as pest control, recall procedures, construction/maintenance and sanitation (Mataragas *et al.*, 2015).

In order to avoid confusion, it is important to have a clear understanding of how the HACCP concepts relate to Sanitation Standard Operating Procedures (SSOPs) as well as the relationship between the SSOPs, GMPs, and HACCP plans that many companies already have in place (AMIF, 1997).

7.1 AMIF Process Control Points

pH Control and Time-Temperature Control for Fermentation are the process control points needed to achieve properly fermented and acidulated sausages, which prevent the growth of pathogenic microbes. A pH of 5.3 or lower must be reached, either as a result of lactic acid bacteria or by direct acidulation. To ensure a proper pH drop occurs, pH measurements must be taken and documented for each lot of product. The pH drop may be achieved by incorporating commercially prepared culture into the chopped or ground meat, or a "less acceptable" procedure is the use of a "mother" batch of inoculum, which must be ensured to produce the rapid pH drop. Reliance on naturally present lactic acid bacteria in the raw meat should not be used (AMIF, 1997).

Direct acidulation by the incorporation of USDA-approved organic acids, such as citric or lactic acid, may be used.

The Time-Temperature Control for Fermentation process control point ensures a process reaches pH 5.3 in sufficient time. The degree-hour is a calculated limit based on the temperature and the time it takes a product to reach pH 5.3. Degrees are measured as the excess over 60F (the critical temperature at which staphylococcal growth effectively begins.) Degree-Hours is the product of time in hours at a particular temperature and the "degrees." Degree-Hours is calculated for each temperature used during fermentation. The limitation of the number of degree-hours depends upon the highest temperature in the fermentation process prior to the time that a pH of 5.3 or less is attained. Processes attaining less than 90F prior to reaching pH 5.3 are limited to 1200 degree-hours, processes exceeding 89F prior to reaching pH 5.3 are limited to 1000 degree-hours, and processes exceeding 100F prior to reaching pH 5.3 are limited to 900 degree-hours. If a variable temperature process is used, each temperature/time step is calculated for its contribution to the degree-hours, with the limit set by the highest temperature used during the process (AMIF, 1997).

7.2 AMIF Sanitation Requirements

FSIS requires the development and implementation of Sanitation Standard Operating Procedures (SSOPs) in federally inspected facilities, which are written by the establishment and its adherence will demonstrate knowledge of and commitment to sanitation. The SSOPs are applied to all areas and functions of an establishment, in which the procedures must address and maintain, on a daily basis, the prevention of direct product contamination/adulteration. This is achieved by maintaining facilities and equipment, having well trained employees, and having a well designed process flow, which are fundamental to an effective cleaning and sanitation program to address the sources of contaminating microorganisms (AMIF, 1997).

Facilities should have easily cleaned smooth surfaces (ceilings, walls, floors), operative drains, and well-designed, repairable equipment, all of which should be routinely cleaned, sanitized, and be moisture-free (no standing water) (AMIF, 1997).

Cleaning aids, such as mops, squeegees, brooms, and hoses, should also be be cleaned and sanitized themselves. They should be stored in sanitizing solution or off the floor. Use disposable wipes and discard scouring pads daily (AMIF, 1997).

Plant management and supervision personnel must set the example of the importance for food quality and safety themselves and educated employees on correct food handling practices and cleaning/sanitizing procedures. Personnel hygiene is of the upmost importance. Outerwear should be changed at least daily and footwear should be waterproof that can be cleaned and sanitized. Disposable gloves and aprons should be used in RTE areas (AMIF, 1997).

7.3 AMIF Production and Process Controls

Refrigeration should have adequate capacity and capability to maintain sufficient air circulation, temperature, humidity, etc. They must be cleaned and sanitized regularly (AMIF, 1997).

Cross contamination should be eliminated by establishing traffic patterns of personnel, meat, meat containers, ingredients, etc, between raw and finished product areas. If employees must work in both raw and RTE areas, they must change outerwear, wash hands, change gloves, and clean and sanitize footwear (or change footwear). Using color coded hats, frocks, and footwear in raw and RTE areas facilitate the prevention of cross contamination (AMIF, 1997).

7.4 Critical Operations

Critical operations represent the ideal environment or actions for cross contamination. These include equipment wash areas, which should be separate, designated areas for raw and RTE. Slicing and packaging equipment should be kept separate or completely

disassembled, cleaned, and sanitized, prior to moving from raw to RTE areas. All food contact surfaces must be cleaned and sanitized daily (AMIF, 1997).

8.0 Objectives of Current Study

8.1 Chapter 2

In chapter 2, we will demonstrate the validity of the production process for fermented and aged salami. Data will validate the CCPs in the HACCP plan and will be reinforced with previous research.

8.2 Chapter 3

In chapter 3, we will demonstrate the validity of the production process for dry-cured whole muscle pork products. Data will validate the CCPs in the HACCP plan and will be reinforced with previous research.

CHAPTER TWO

Fermented Aged Salami

1.0 Introduction

The development of a science-based process control system for food safety, which promotes systematic prevention of biological, chemical, and physical hazards, is required by the United States Department of Agriculture in all meat processing facilities (USDA, FSIS, 1998). This system, known as Hazard Analysis Critical Control Point (HACCP) is a plan that is specific to the facility and their products and processes. The identification of potential food safety hazards, that can cause adverse health effects when present at an unacceptable level, must be identified throughout the entire process.

The fermentation and aging of salami has been used for thousands of years, and the products are having a resurgence in popularity in recent times in the United States. The purpose of this study was to validate an artisan fermentation and dry-curing process in the production facility to demonstrate that it meets the FSIS pathogen reduction requirements.

The fermentation process determines the extent of survival of food-borne pathogens and therefore the safety of fermented meat products. The process is based on manufacturing conditions such as pH, water activity, time-temperature profile during fermentation, and relative humidity (Mataragas *et al.*, 2015).

While there have been validation studies for fermented and aged sausages, the process, formulation and aging parameters used in this study are unique, requiring validation. The formulation used in this study use potassium nitrate instead of sodium nitrite. The environmental parameters during fermentation are unique as well. This study was done to evaluate the fermentation and aging process for its efficacy against *E. faecium*, a known surrogate for *Salmonella* spp. and frequently used for in-plant validation studies, as well as the overall microbiological safety of the process.

2.0 Material and Methods

2.1 Sausage preparation and inoculation of surrogate

2.1.1 Carcasses

Receiving: The processor received thirty half-carcass hogs each week during the trial period. The pasture-raised hogs were obtained from four farms and slaughtered at either of two USDA inspected facilities. Carcasses were received each Friday and required to have an internal temperature measured in the ham of <40 °F.

Preparation: The carcasses were "singed and scraped" to remove any hair that may be present. Following the "singe and scrape" process, carcass halves, inside and out, were thoroughly rinsed with 50 ppm ECA solution (ElectroChemical Activation water, Zap Water Technologies, Inc., Richfield, MN). The carcasses were hung and stored in a <40 °F cooler over the weekend (72-96 h).

Breaking: Carcass breaking occurred 72-96 h after receiving. The carcass breaking room temperature was kept ≤50 °F. The carcass halves were broken into specific muscle groups for whole muscle cures, salami lean, trim, fats, and secondary cuts, which were separated and stored in totes. These totes were stored in a cooler at 34-36 °F.

2.1.2 Cure Ingredients and Processing

The pork used in the salami was 75% lean trim and 25% fat back. The lean and fat were partially frozen prior to grinding. The grind was produced in a Fatosa bowl chopper. Toward the end of the grind, the bacterial strain was added and thoroughly mixed in, followed by the marinade and cure mixture. The marinade and cure mixture consisted of 2.5% sodium chloride, 0.5% sugar, 1718 ppm (0.1718%) potassium nitrate per FSIS Processing Inspectors Calculation Handbook (USDA, FSIS, 1995), wine, spices, and Danisco Texel SA-301 starter culture. Danisco Texel SA-301 starter culture consists of

Lactobacillus sakei, Staphylococcus carnosus, Staphylococcus xylosus, and a dextrose carrier.

The following salami products were the inoculated products, which were representative of each casing size used and one smoked salami: the Vecchio in beef middle, the Chuck Fred in beef middle and smoked, the Pork Queen in hog middle, the Extra Vecchio in hog bung, and the Big Chets in beef bung. The casings are from Natural Casing Co., Peshtigo, WI.

The salami batter was stuffed into casings using a vacuum stuffer. The casings were tied into approximately 12 ounce links.

2.1.3 Fermentation and Aging

The links were fermented for 72 hours at 70-78 °F and ≥ 80% humidity. The room was then progressively cooled to 60 °F during the next 72 hours. The Chuck Fred was cold smoked for one hour prior to being placed in the aging room.

Aging took place in a 55-58 °F room with ≤ 60% humidity. Aging times varied based on salami size and casing type. The Vecchio and Chuck Fred aged for 21 days, the Pork Queen aged for 49-56 days, the Extra Vecchio aged for 52 to 56 days and the Big Chet's aged for 84 days.

2.2 Microbiological and Physiochemical Analysis

Carcass Swabs

During the three weeks of production in which the validation study was performed, the split carcasses were swabbed for the detection of *Salmonella spp.* These swabs were performed at three different points in time prior to the processing of the carcasses. After the singe and scrape step, half the carcasses from each farms represented in the delivery from one slaughter facility, were swabbed using Paradigm Diagnostics Inc. (PDX)

SecurSwab surface swabs or Enviro Swabs (3M, St. Paul, MN). Swabbed surfaces included the skin side of the split carcass (trotters, face, belly, belly-ham crease, tail, shoulder and ham) as well as the cut side along the spine and face. The carcasses were then sprayed inside and out with an ECA (electrolyzed water) solution containing 50 ppm free available chlorine. The same carcasses were then swabbed again after the rinse. The same carcasses were swabbed after 72 hours in the carcass cooler. The swabs were transferred to the University of Minnesota the same day in a cooler. Paradigm Diagnostics Inc. Salmonella Indicator Broth (PDX-SIB, AOAC 071102) was added to the swab tube. Tubes were analyzed after incubation at 37 °C for 48 hours. Presence of *Salmonella spp.* is indicated by a color change in the broth. Any suspect samples were streaked on xylose lysine deoxycholate agar (XLD agar) for confirmation.

Bacterial Strain

The salami batter was inoculated with *Enterococcus faecium* NRRL B-2354(ARS Culture Collection) (ATCC8459). The inoculum was grown in Tryptic Soy Broth (TSB), (Acumedia, Neogen) for 24 h at 37 °C. The 10^9 overnight culture was used to inoculate the meat batter of each salami product to a level of 10^7 CFU/g.

pH and a_w

The pH and a_w were taken at each sampling point. The first pH meter used was a Hanna Instruments Portable HACCP Compliant pH/temperature Meter for Meat (Hanna Instruments HI 99163). The meter was calibrated before each use and was temperature compensated. The meter was replaced twenty five days into the trial after highly inaccurate measurements. Ryan Cox, Dept. of Animal Science, University of Minnesota, consulted and suggested the use of a Testo meter.

The replacement meter was a Testo 205 pH/Temperature meter by Testo Pty Ltd. The Testo 205 is temperature compensated with an accuracy of ±0.02 pH. The meter was two point calibrated prior to each use. The meat processing industry is the primary application for the meter. Upon replacement, pH measurements were made of all products from all trials and recorded. Additional pH trials were conducted the following three weeks, recording the pH at each sampling time as the previous trials for each product.

The a_w was taken at each sampling point. The Aqualab Pa_wkit water activity meter by Decagon Devices, Inc. was used for all trials. A two point calibration occurred at least once a week using the standards that accompanied the meter. The meter had an accuracy of ±0.02. Salami a_w samples were taken from the grind or at the microbiology sample site. The sample extended from the surface into the middle of the product by 1.5 cm.

Salami Microbiology Sampling

The sampling schedule for the salami products included post inoculation, post cure addition, after fermentation, and during aging. The Vecchio and Chuck Fred were sampled every week during aging, the Pork Queen and Extra Vecchio were sampled every two weeks during aging and Big Chet's were sampled every month. Samples were randomly taken from the inoculated salami.

The samples were cut from Extra Vecchio and Big Chet, while whole Vecchio, Chuck Fred, and Pork Queen salami were taken, and were placed in Whirl Pac bags and refrigerated prior to being transferred, in a cooler with ice packs, to the department of Food Science at the University of Minnesota. The samples were processed the same day.

Approximately one to three gram samples were stomached in Buffered Peptone Water (Fischer Scientific) for 30 seconds, and serially diluted from 10^1 to 10^7. Dilutions were spread plated on m-enterococcus agar (m-EA: Acumedia, Neogen Corporation). The plates and incubated at 37 ± 2 °C for 48 hours. Plates were counted at 48 hours with a backlit fluorescent counter. The medium is selective that allows direct count of

enterococci without the need for confirmation. The selective agent is Sodium Azide, which suppresses Gram-negative organism growth. *Enterococcus faecium* colonies appeared circular, smooth and red to maroon in color. The red color is formed by the reduction of the dye Triphenyl Tetrazolium Chloride (TTC) within the bacterial cell, producing the insoluble red colored formazan (Neogen, 2015). The m-EA had a detection limit of 10 CFU/g.

End Product Testing

Third party end-product testing was conducted to determine microbiological safety and product composition. Salmonella (PCR: AOAC-PTM #010803), Listeria (PCR: AOAC-PTM #090701), and Staphylococcus aureus (Petrifilm: AOAC #2003.07) testing was conducted by Market Fresh Labs, Minneapolis, MN. Product composition was conducted by RTECH Laboratories, Land O'Lakes, St. Paul, MN. Analytical tests were protein content (Kjeldahl), moisture content (forced air, 100C, 16.5 hrs), salt content (as NaCl by direct titration), and a sugar content profile (HPLC).

***Trichinella spirialis* Testing**

The Bio-Rad *Trichinella spiralis* Antibody Test Kit (manufactured by SafePath Laboratories, LLC, Lisence # 430) is a USDA approved ELISA test as part of the National Trichinae Certification Program (USDA, 2015). The test kit antigen, which was purified from larvae of infected pigs, has a high degree of specificity for *T.spiralis* and can be qualitatively determine antibodies in swine sera, plasma, whole blood, or tissue fluid (Biorad, 2015). The principle of the procedure is the binding of an excretory-secretory antigen to the *T. spiralis* antibody in the sample, which is followed by a binding of the secondary antibody anti-swine IgG peroxidase complex (enzyme conjugate). A chromogen is added and reacts in the presence of the enzyme complex and peroxide,

producing a blue color. The reaction ends once a stop solution is added, and a positive sample is indicated by a color change to yellow (Biorad, 2014).

The four farms represented and the number of pigs tested for *Trichina spirialis* in the study are Hidden Stream Farm LLC, Elgin, MN (10 pigs); Moo Oink Cluck Farm, Somerset, WI (4 pigs); Pork & Plants LLC, Altura, MN (6 pigs); YKer Acre, Wrenshall, MN (8 pigs). The blood samples were collected in 0.4% sodium citrate at the time of slaughter and were frozen immediately to -20 °C. The frozen blood was transferred to the University of Minnesota for analysis.

The samples were thawed immediately before analysis and the micro-well plate were allowed to adjust to room temperature for thirty minutes in the packaging to prevent water condensation. The dilution buffer concentrate was diluted to 1:20 with deionized water. The blood was diluted to 1:100 using the dilution buffer in plastic pcr tubes. 100 µl of negative control was added to a well and 100 µl of positive control was added to a second well. 100 µl of each diluted sample were placed into wells. The samples were incubated for 10 minutes at room temperature. The contents of the wells were shaken out and washed 3 times with the wash buffer. Two drops (100 µl) of enzyme conjugate was added to each well and incubated for 10 minutes at room temperature. Again, the wells contents were shaken out and washed 3 times with the wash buffer. Each well was washed with DI water. One drop (50 µl) of substrate A (chromogen tetramethylbenzidine) and one drop (50 µl) of substrate B (citric acid and peroxide) were added to each well. The wells were lightly tapped to mix the substrates and allowed to incubate for 10 minutes at room temperature. Two drops (100 µl) of stop solution (1 M phosphoric acid) were added to each well. The wells were placed on white paper and read visually for a color change. The positive and negative controls were run concurrently with the assays, and confirmed the assay viability.

Environmental Monitoring Program

A *Listeria monocytogenes* monitoring program consisted of weekly environmental surface swabbing and analysis in the packaging room of the Ready-to-Eat (RTE) area of the processing plant. The surfaces swabbed were the vacuum packaging machine, table, cutting board, knife, gloved hands, and floor drain. Swabs were labeled and placed into refrigeration. The swab tubes were transferred to the University of Minnesota in a cooler and processed the same day.

The swab tubes (SecurSwab, Paradigm Diagnostics, Inc. or Enviro Swab, 3M) were filled with a selective enrichment media containing a metabolic indicator and incubated at 37 °C for 48 hours. The PDX-LIB (Paradigm Diagnostics, Inc, AOAC-RI approved, Certificate 040501) indicator broth turns black when *Listeria spp.* is present. The broth contains antibiotics that inhibit most non-*Listeria* microorganisms, growth enhancers for injured *Listeria* and indictor compounds in which the β-glucosidase enzyme act upon, changing the broth from yellow to black.

3.0 Results

3.1 Microbiological Analysis

Carcass Swab: All swabs prior to, immediately after and 72 hours after the 50 ppm anolyte rinse were negative for *Salmonella spp*. for all farms. Swabbing continued for approximately 6 more weeks, with negative results.

***Trichinella spiralis* test results:** The blood samples from all 28 hogs were negative for the *Trichinella spiralis* antibody and the positive and negative controls confirmed the viability of the assays.

Listeria Monitoring Program Results: Weekly swabs were taken at the following RTE area sites: vacuum packaging machine; packaging table, cutting board, knife, gloved hands, and floor drain. The environmental sampling occurred during the three trial weeks and continued for another three. All swabs for all weeks were negative for *Listeria spp.*

Third-Party Microbial End-Product Test Results: All salami products in the validation study were tested for the following pathogens by MarketFresh, LLC laboratories: *Staphylococcus aureus, Listeria* spp., and *Salmonella* spp. All salami came back negative for *Listeria* spp., *Salmonella* spp., and <10 for *Staphylococcus aureus*.

3.2 *Enterococcus faecium* Reduction Results

The reduction of *Enterococcus faecium* in salami products Vecchio, Chuck Fred, Extra Vecchio, Pork Queen, and Big Chet's during the trial are shown below. The colony forming units per gram (CFU/g), the pH and water activity (a_w) are shown for each corresponding sample time. The original pH meter was not working correctly, and was replaced during the study. The asterisks (**) indicate the samples affected by the malfunctioning pH meter.

Vecchio Trials

Table 1: Vecchio processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.97	ND[c]	
Inoculated Salami Batter	7.78	**	
After Fermentation	6.71	**	
1 Week Aging	4.83	**	0.93
2 Weeks Aging	ND	5.22	0.92
3 Weeks Aging	4.23	5.15	0.80
Trial 2			
Entertococcus faecium Culture	9.02	ND[c]	ND
Inoculated Salami Batter	7.58	**	ND
After Fermentation	ND	**	0.93
1 Week Aging	5.26	5.08	0.88
2 Weeks Aging	5.52	5.26	0.90
3 Weeks Aging	4.57	5.23	ND
Trial 3			
Entertococcus faecium Culture	9.38	ND[c]	ND
Inoculated Salami Batter	7.40	**	ND
After Fermentation	6.65	**	0.95
1 Week Aging	6.56	4.78	0.93
2 Weeks Aging	5.11	5.13	0.92
3 Weeks Aging	5.92	5.04	0.80

[a] – Colony Forming Units per Gram
[b] – Water activity
[c] – No Data
** - Malfunctioning pH meter

Table 2: Log_{10} Reduction of *Enterococcus faecium* in Vecchio Salami Production.

Trial	Log_{10} Reductions at 3 Weeks Aging	a_w at 3 Weeks Aging
1	3.55	0.80
2	3.01	ND
3	1.48	0.80
Avg. $\pm$ SD	2.68 $\pm$ 1.07	0.80

Chuck Fred

Table 3: Chuck Fred processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Salami Batter	7.20	**	0.98
After Fermentation	6.11	**	0.92
After Smoking	5.81	**	0.92
1 Week Aging	5.00	**	0.87
2 Weeks Aging	4.21	5.14	0.92
3 Weeks Aging	3.79	5.31	0.87
Trial 2			
Entertococcus faecium Culture	9.02	ND[c]	ND
Inoculated Salami Batter	7.10	**	0.98
After Fermentation	5.57	**	0.93
After Smoking	5.66	**	ND
1 Week Aging	4.36	5.14	0.88
2 Weeks Aging	3.7	5.36	0.89
Trial 3			
Entertococcus faecium Culture	9.38	ND[c]	ND
Inoculated Salami Batter	7.83	**	0.99
After Fermentation	6.08	**	0.94
After Smoking	5.64	4.84	0.88
1 Week Aging	4.26	4.92	0.86
2 Weeks Aging	4.36	5.1	ND

[a] – Colony Forming Units per Gram
[b] – Water activity
[c] – No Data
** - Malfunctioning pH meter

Table 4: Log_{10} Reduction of *Enterococcus faecium* in Chuck Fred Salami Production.

Trial	Log_{10} Reductions at 2 weeks Aging	a_w at 2 Weeks Aging
1	2.99	0.92
2	3.40	0.89
3	3.47	ND
Avg. $\pm$ SD	3.29 $\pm$ 0.26	0.91

Pork Queen

Table 5: Pork Queen processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all four trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Salami Batter	7.23	**	ND
After Fermentation	6.85	**	0.93
1 Month Aging	5.74	5.04	ND
7.5 Weeks Aging	<100	5.29	0.88
Trial 2			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.02	ND[c]	ND
Inoculated Salami Batter	7.33	**	ND
After Fermentation	7.36	**	0.94
1 Month Aging	6.73	5.08	0.88
2 Months Aging	6.46	5.38	0.83
Trial 3			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.38	ND[c]	ND
Inoculated Salami Batter	7.56	**	ND
After Fermentation	5.99	4.99	0.96
1 Month Aging	4.34	5.26	0.92
7 Weeks Aging	<100	5.86	0.84

[a] – Colony Forming Units per Gram
[b] – Water activity
[c] – No Data
** - Malfunctioning pH meter

Trial 4			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	ND	ND[c]	ND
Inoculated Salami Batter	7.25	ND	ND
After Fermentation	6.53	5.02	0.96
1 Month Aging	5.12	ND	ND
2 Months Aging	<100	5.20	0.86

[a] – Colony Forming Units per Gram
[b] – Water activity
[c] – No Data
** - Malfunctioning pH meter

Table 6: Log_{10} Reduction of *Enterococcus faecium* in Pork Queen Salami Production.

Trial	Log_{10} Reduction at 2 Months Aging	a_w at 2 Months Aging
1	5.23	0.88
2	*0.87*	*0.83*
3	5.56	0.84
4	5.25	0.86
Avg. $\pm$ SD of Trials 1,3,4	5.35 $\pm$ 0.19	0.86 $\pm$ 0.02

Extra Vecchio

Table 7: Extra Vecchio processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Salami Batter	7.78	**	ND
After Fermentation	6.49	**	ND
1 Month Aging	4.81	5.30	0.93
7.5 Weeks Aging	<100	5.40	0.81
Trial 2			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.02	ND[c]	ND
Inoculated Salami Batter	7.58	**	ND
After Fermentation	6.34	**	0.96
1 Month Aging	5.91	5.30	0.94
2 Months Aging	<100	5.60	0.84
Trial 3			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.38	ND[c]	ND
Inoculated Salami Batter	8.08	**	ND
After Fermentation	6.53	**	0.96
1 Month Aging	4.34	5.36	0.92
2 Months Aging	<100	5.44	0.81

[a] – Colony Forming Units per Gram
[b] – Water activity
[c] – No Data
** - Malfunctioning pH meter

Table 8: Log_{10} Reduction of *Enterococcus faecium* in Extra Vecchio Salami Production.

Trial	Log_{10} Reductions at 2 Months Aging	a_w at 2 Months Aging
1	5.78	0.81
2	5.58	0.84
3	6.06	0.81
Avg. $\pm$ SD	5.81 $\pm$ 0.24	0.82 $\pm$ 0.02

Big Chet

Table 9: Big Chet processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Salami Batter	7.07	**	ND
After Fermentation	6.91	**	0.95
1 Month Aging	5.81	4.83	0.93
3 Months Aging	<100	5.13	0.90
Trial 2			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.02	ND[c]	ND
Inoculated Salami Batter	7.97	**	ND
After Fermentation	7.11	**	0.96
1 Month Aging	6.67	5.06	0.92
3 Months Aging	<100	5.21	0.91
Trial 3			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.38	ND[c]	ND
Inoculated Salami Batter	7.20	**	ND
After Fermentation	6.60	**	0.97
1 Month Aging	5.96	4.82	0.93
3 Months Aging	<100	5.03	0.89

[a] – Colony Forming Units per Gram
[b] – Water activity
[c] – No Data
** - Malfunctioning pH meter

Table 10: Log_{10} Reduction of *Enterococcus faecium* in Big Chet Salami Production.

Trial	Log_{10} Reductions at 3 Months Aging	a_w at 3 Months Aging
1	5.07	0.90
2	5.97	0.91
3	5.20	0.89
Avg. $\pm$ SD	5.41 $\pm$ 0.49	0.90 $\pm$ 0.01

3.3 Salami Moisture, Protein, and Moisture/Protein Ratios

Table 11: Vecchio Salami Moisture[a] and Protein[b] Content, and Moisture/Protein Ratios.

Trial	Moisture %	Protein %	Moisture/Protein Ratio
1	28.26	28.31	1.00
2	31.76	27.85	1.14
3	36.50	23.12	1.57

[a] – Forced air
[b] – Kjeldahl

Table 12: Chuck Fred Salami Moisture[a] and Protein[b] Content, and Moisture/Protein Ratios.

Trial	Moisture %	Protein %	Moisture/Protein Ratio
1	30.43	28.19	1.08
2	31.28	30.24	1.03
3	32.98	26.05	1.27

[a] – Forced air
[b] – Kjeldahl

Table 13: Pork Queen Salami Moisture[a] and Protein[b] Content, and Moisture/Protein Ratios.

Trial	Moisture %	Protein %	Moisture/Protein Ratio
1	37.91	23.21	1.63
2	36.32	22.68	1.60
3	33.76	24.58	1.37

[a] – Forced air
[b] – Kjeldahl

Table 14: Extra Vecchio Salami Moisture[a] and Protein[b] Content, and Moisture/Protein Ratios.

Trial	Moisture %	Protein %	Moisture/Protein Ratio
1	31.76	24.02	1.32
2	31.31	26.64	1.18
3	38.26	22.61	1.69

[a] – Forced air
[b] – Kjeldahl

Data not available for Big Chet trials.

3.4 Salami Additional pH Trials

Three additional pH trials were conducted for three consecutive weeks for the salami products using the new pH meter, as the original trial data is unreliable.

Table 15: Vecchio Additional pH Trials.

Processing Step	Trial 1	Trial 2	Trial 3
Raw Grind	5.58	5.83	5.74
24 h post marinade[a]	5.56	5.67	5.62
72 h of fermentation at 24 °C	4.95	5.30	5.16
1 week aging	5.21	5.39	5.30

[a] – Marinade contains salt, sugar, starter culture, spices, wine, garlic.

Table 16: Chuck Fred Additional pH Trials.

Processing Step	Trial 1	Trial 2	Trial 3
Raw Grind	5.8	5.81	5.68
24 h post marinade[a]	5.6	5.70	5.61
72 h of fermentation at 24 °C	4.92	4.97	5.34
1 week aging	5.11	5.38	5.37

[a] – Marinade contains salt, sugar, starter culture, spices, wine, garlic.

Table 17: Pork Queen Additional pH Trials.

Processing Step	Trial 1	Trial 2	Trial 3
Raw Grind	5.87	5.96	5.64
24 h post marinade[a]	5.55	5.62	5.62
72 h of fermentation at 24 °C	4.92	5.07	5.22
1 week aging	5.34	5.38	5.38

[a] – Marinade contains salt, sugar, starter culture, spices, wine, garlic.

Table 18: Extra Vecchio Additional pH Trials.

Processing Step	Trial 1	Trial 2	Trial 3
Raw Grind	5.95	5.88	ND[b]
24 h post marinade[a]	5.64	5.66	5.61
72 h of fermentation at 24 °C	4.97	5.30	5.05
1 week aging	5.01	5.20	5.16

[a] – Marinade contains salt, sugar, starter culture, spices, wine, garlic.
[b] – No Data

Table 19: Big Chet Additional pH Trials.

Processing Step	Trial 1	Trial 2	Trial 3
Raw Grind	5.78	5.82	ND[b]
24 h post marinade[a]	5.52	5.62	5.60
72 h of fermentation at 24 °C	4.86	5.30	5.20
1 week aging	5.14	5.17	5.22

[a] – Marinade contains salt, sugar, starter culture, spices, wine, garlic.
[b] – No Data

4.0 Salami Discussion

FSIS requires production facilities to validate their processes through the use of validation studies, GMPs, and industry experts.

FSIS has guidance for options for the control of *Salmonella* spp. The use of a heat treatment is not an option for the process used at the trial plant, as this is not the style of salami being made. Conducting a "hold and test" program of the finished product was not an option, as the expense was too great for a small producer making small batches of product. The use of a validated 5-$\log_{10}$ inactivation treatment was not possible, as there were no published studies demonstrating a 5-$\log_{10}$ reduction in dried or semi-dried fermented sausages that didn't use a heat treatment.

Salmonella spp. control had to be demonstrated for the process at the production facility. Control is achieved by using hurdle technology, which are meeting pH and water activity thresholds. To meet these thresholds, formulation, fermentation, and aging/drying were used as hurdles to achieve microbial stability and product safety. The formulation used by the processing plant specified an ingoing salt content of 2.5%, which is on the lower end of typical salt usage rate, though through moisture loss, the final salt content was in the range of 2.6-4.5%. Salt and sugar reduces a_w, and therefore provides a control mechanism. Fermentation reduces pH by the production of lactic acid, affecting bacteria homeostasis, thereby providing a control mechanism. The aging/drying step further reduced a_w to <0.92.

In addition, other hurdles contributing to the safety and reduced risk are incoming pathogen burden reduction, *Trichinella spiralis* testing, environmental monitoring, and raw batter sampling.

Mataragas et al. (2015) found that if the initial contamination of raw materials with *Listeria monocytogenes* or *Salmonella enterica* is high, then the final pathogen levels in Italian fermented sausages would be unacceptable. They concluded that pre-requisite programs and HACCP implementation are required to assure high quality incoming raw materials, proper personnel training, and limited recontamination of the products. If a

combination of low initial raw material contamination level, and consequently in the meat batter, and no recontamination, even a low non-thermal inactivation of the pathogens, may assure their safety (Mataragas *et al*., 2015).

The incoming pathogen burden of the raw material is an important factor in determining the risk of pathogens in the finished product. The negative results from the *Salmonella spp.* swabs at all three swab times on the carcass halves indicates that the incoming pathogen burden is undetectable. Additionally, this speaks to the pathogen reduction program used at the slaughter facilities and its effectiveness. Most importantly, the undetectable incoming pathogen burden limits the pathogen burden for the entire process. It is important to point to the fact that no *Salmonella spp.* growth occurred over the 72 hour window in which the carcasses were hung in the carcass cooler.

Though the risk of *Trichinella spiralis* is quite low in pork today, the risk increases for pigs that have free-range outdoor access, where exposure to rodents and wildlife is a possibility. Exposure to rodents or wildlife infected with trichinae, dead or alive, can transmit the parasite to the pig if it is chewed on or digested. The evaluation of farm management practices can determine the risk posed by free range pork. Limiting risk of exposure can be greatly reduced by following sound swine husbandry practices (Gamble, 2015). The blood tests conducted during the study had negative results for the 28 pigs tested, indicate that the supplying farms are Trichina free. Trichinae is usually associated with an entire farm and their husbandry practices. The absence of the Trichinae is important to the processes used at the salumi producer, as they do not freeze or heat-treat their meat. The processor has an ongoing testing program.

In order to reduce risk of contamination from the processing environment, we conducted *Listeria spp.* monitoring tests in the RTE area of the processing plant. The negative results for the detection of *Listeria spp.* in the processing facility's ready-to-eat area is an indication of appropriate cleaning practices as listed in the facility's SSOPs. *Listeria spp.* presence in ready-to-eat products has a zero tolerance policy. It is also an indicator of the general environment of the facility. The lack of detection in the weekly swab samples

speaks to the cleaning practices and the overall safety of the ready-to-eat products produced.

While control of the raw material is not transferred until the pig carcasses arrive at the processing facility, the HACCP plan dictates processes that ensure a high quality raw product. These include receiving temperatures of the carcasses and an electrolyzed water rinse of carcasses. Additionally, the slaughter facilities use an organic acid carcass rinse prior to carcass chilling, a pathogen control step. If the risk assessment is applied to the entire system, then part of the 5-$\log_{10}$ reduction required by FSIS should be attributed to the processes at the slaughter facility. Because the HACCP plan requires a raw material receiving temperature of < 45 °F, and *Salmonella* spp. demonstrates little to no growth below 7 °C (44.6 °F) (Tompkin, 1996), the risk of pathogen growth is controlled.

To determine the effectiveness of the processing procedures to control and/or eliminate pathogens, we conducted an in-plant validation. It was demonstrated that if the following CCPs were met during salami production, the risk of pathogen contamination in the finished product would be low, and the product would be considered safe for public consumption.

The following CCPs for the fermented aged salami were: 1) CCP#1 identified in the salami process was a chemical food safety hazard during the addition of potassium nitrate. There is potential for toxic effects of N-nitroso compounds, therefore the USDA regulates the amount of potassium nitrate used in curing (2.75 ounces/100 # meat or 1718 ppm). Potassium nitrate is used to control *C. botulinum*; 2) CCP#2 identified was a biological food safety hazard during fermentation. There is potential for the growth of pathogens (*Salmonella* spp., *S. aureus*) during fermentation because of the elevated temperature. and the use of starter cultures and salts/sugar to inhibit the growth of *Salmonella* spp. and *S. aureus* by reducing pH to 5.3 in the degree-hours allowed by the AMIF GMPs; 3) CCP#3 identified in the salami process was a biological food safety hazard during the drying/aging step. There is potential for the growth of pathogens (*Salmonella* spp., *L. monocytogenes, S. aureus*) and subsequent toxigenesis. This hazard

is addressed by controlling the room temperature and relative humidity to assure the desired a_w goal of ≤ 0.91 is achieved. The room parameters are addressed in the AMIF GMPs.

This study demonstrated a 5-log reduction for large caliber salami (Pork Queen, 5.35 $\pm$ 0.19 (excluding 2nd trial); Extra Vecchio, 5.81 $\pm$ 0.24; Big Chet, 5.41 $\pm$ 0.49), therefore meeting the requirements of the CCPs of pH $\leq$ 5.3 and $a_w \leq 0.91$. The small caliber salami (Vecchio, 2.68 $\pm$ 1.07; Chuck Fred, 3.29 $\pm$ 0.26) did not achieve a 5-log reduction.

Despite not achieving a 5-log reduction, the trial products underwent third-party end-product testing, and the results showed no presence of *Salmonella* spp., *Listeria* spp., <10 cfu/g *S. aureus*. All the trial salami had a M:P ratio between 1.70:1 and 1.0:1 (Tables 11-14), and all salami reached a a_w of 0.91 or below (Tables 1, 3, 5, 7, 9).

While the 5-log reduction was not able to be met in the three trials for the small caliber, short aged salami, it was demonstrated that an average 2.68-3.29-log reduction was achieved. This is similar to a process validation study conducted by a recognized processing authority and expert in pathogenic microbiology, Deibel Laboratories, Inc. It demonstrated that a 3.0-3.5-$\log_{10}$ reduction in *Salmonella* was achieved using similar parameters as our validation study. Deibel tested three batches of typical salami, using Danisco Texel starter cultures, fermented to a pH value of 5.3 or less in the degree-hours specified by AMIF's GMPs, dried to a Moisture/Protein ratio of ~ 1.9/1 or less, and reached a water activity of less than 0.920 (Deibel, 2013).

Neither the formulation nor the fermentation schedule (temperature, relative humidity) for the Deibel validation were disclosed, but the AMIF GMPs were met. The drying schedule for the salami was 25-31 days, a temperature range of 12-21 °C, and a relative humidity range of 68-84% (Deibel, 2013). These parameters parallel the parameters used in our validation study.

The Deibel validation study demonstrated a 2.59-3.02-$\log_{10}$ reduction of *Salmonella* immediately following fermentation, and a 2.75-3.09-$\log_{10}$ reduction by the mid-dry point (Deibel, 2013).

Deibel Laboritories' validation study demonstrated that by following AMIF GMPs for pH drop, achieving the Moisture/Protein Ratio as defined by the standard of identity for salami, and reaching a water activity of less than 0.92 by using the drying parameters described, a 3.02-3.58-$\log_{10}$ reduction of *Salmonella* was validated. By following the fermentation parameters only, a validated 2.59-3.02-$\log_{10}$ reduction of *Salmonella* was demonstrated. Our study used the same parameters, and demonstrated average reductions of 2.68-3.29-log reductions of *E. faecium* in small caliber salami. In addition, the process in this study also met the Moisture/Protein Ratio, a_w, and pH parameters.

The Vecchio trial that demonstrated a 1.48-log reduction and the Chuck Fred trial that demonstrated a 2.99-log reduction were the only outliers in the small caliber salami. This could be due to the short time in the aging room.

The water activity of Chuck Fred trial 1 at two weeks aging was 0.92, while the a_w after the first week was 0.87. This increase was most likely due to the sampling environment. The samples were taken out of the aging room and brought to an employee common room for the measurements. The higher humidity during the summer months and in the employee common room may have given higher a_w readings.

Potential explanations for not meeting the CCPs for the small caliber salami are 1) the less time spent in the aging room compared to large caliber in order to meet quality standards; 2) the casings did not allow the diffusion of moisture of the salami.

Therefore, raw batter testing was implemented during the process. An option under the FSIS *Salmonella* Compliance Guideline is the proposal of other approaches to ensure at least a 5-$\log_{10}$ reduction of *Salmonella*, where the sum of the treatments would result in the reduction. A raw batter testing program coupled with at least a 2-$\log_{10}$ reduction would be a viable option. This was the solution the plant decided to use.

Appropriateness of FSIS Risk Model

According to Adams and Mitchell (2002), FSIS required producers to validate that their processes achieve a 5-$\log_{10}$ reduction in viable numbers of *E. coli* O157:H7. This objective assumed a worst-case scenario based on the maximum level of *E. coli* O157:H7 detected on a beef carcass and a requirement that product contains less than one CFU per 100 g. The coincidence of such a series of improbable events (high level contamination/low infective dose) is unlikely, so products from any process meeting this standard would be considered safe. This approach considerably over-estimates risk (Adams & Mitchell, 2002).

Additionally, the reason a worst-case scenario is inappropriate is because our raw material pathogen burden was demonstrated to be undetectable, the implementation of Good Manufacturing Practices (GMPs) dictate proper pH and time/temperature parameters, proper training of employees eliminate cross-contamination and recontamination of finished product, Sanitation Standard Operating Procedures addressing environmental concerns, and overall facility maintenance is performed.

Water activity is not included as one of the primary control mechanisms, which is a major pathogen control mechanism in salami. Product composition is defined as 50% beef and 50% pork. The risk from the *role of formulation* cites processors' ability to understand, predict and ensure a prescribed degree of lethality is a poor assumption. There are measurements that can be made, just as temperature in a cooking process, that can be made for FUSS (Fermented or Direct acidified, Uncooked, Shelf-Stable) products, such as pH and a_w. The *margin of safety*, cited by the risk model, highlighted a variance in cooking (more cooking for consistency, less cooking for increased yield) for cooked products, though there is no cook step for FUSS, which would remove the desire to undercook for the purpose of increased yield. Additionally, because water activity *is* a control mechanism, there is no undercooking for yield purposes.

While comminuted products do pose more risks for pathogen distribution over whole muscle, there is no concern for undercooked product in FUSS products. FUSS risk for *re-growth of pathogens* is characterized as controlled, an ongoing product safety characteristic in which cooked products do not have.

The raw material assigned to FUSS in the risk model was 50% ground beef and 50% ground pork. The overall pathogen burden estimates for ground beef is approximately -0.3 CFU/g, while the model does not have an overall pathogen burden for ground pork. The model assigns the 50% ground beef/50% ground pork an overall pathogen burden of -0.6 CFU/g. Intact pork is assigned the pathogen burden of -2.7 CFU/g. Ground beef has a 0.5-log greater *Salmonella* pathogen burden than for ground pork. Additionally, the pathogen burden estimates for intact pork are approximately 2-log lower than that of ground pork.

The intact pork risk more resembles the risk for the salami products at the plant conducting the validation study, as the plant breaks down and grinds its own ground pork, and no beef is used in the products. The plant sprays the carcasses with a 50 ppm free available chlorine rinse prior to carcass breaking as an intervention. Additionally, since the baseline surveys were conducted in 1992-1997, HACCP has been implemented in slaughter facilities and improvements have been made in pathogen control, such as organic acid carcass rinses prior to chilling.

The broad assumptions made in the FSIS risk assessment for whole categories of product does not accurately portray the actual risk. It does not consider the water activity hurdle in pathogen control, the proper raw material burden, or the current intervention steps taken at slaughter and production facilities. Additionally, the addition of raw batter sampling identifies the initial contamination level, which is the largest determinant for product safety (Mataragas *et al.*, 2015). The processing plant conducting the process validation study has less risk than FSIS assumes.

The critical limits set forth in the HACCP plan have all been met and/or surpassed. The study validated the HACCP plan of the production process. By meeting the CCPs,

meeting the product identity standards, and the final product tests concluded the product was free of pathogens. The risk of consumption of dry fermented salami produced at this processing facility, using the CCPs outline, is lower than the FSIS assumed risk, and the products are safe.

The purpose of the risk assessment was to consider the microbial safety of the RTE fermented and aged salami products (Vecchio, Chuck Fred, Pork Queen, Extra Vecchio, Big Chets) produced by artisan techniques. Pathogens of concern are *Salmonella* spp., *Staphylococcus aureus, Listeria* spp., and *C. botulinum*. The quantitative risk to the consuming population in terms of infection or intoxication is outside the scope of this study, but the relative risk compared to the FSIS risk assessment study can give insight to the safety of the product.

While the pathogens of concern do pose a realistic hazard in raw salami, all of the pathogens can be controlled or eliminated through proper GMPs (pH, a_w, time-temperature, SSOPs, etc) and having appropriate CCPs and the corresponding critical limits. The extent of exposure to these microorganisms or their toxins at the time of consumption is important to determine. Realistic predictions of exposure can be made by considering reliable information regarding the levels of the particular pathogens in the raw materials, the effects on them of processing, storage, distribution, preparation by the consumer, and portion size.

This study demonstrated an undetectable amount of incoming *Salmonella* spp. on the carcasses, as well as a process that reduced a *Salmonella* surrogate by approximately 3-log for small caliber salami and at least 5-log for large caliber. The pathogen burden is also reduced because the facility receives intact split pig carcasses, which receive a chemical rinse at the facility, and are broken down and ground for use in the salami. End-product testing also demonstrated the microbial safety of the products. The on-going prevention of pathogen re-growth in these shelf-stable products and the unnecessary preparation by the consumer decrease risk of exposure to pathogens or toxins. Additionally, the products are consumed in small portion sizes.

The subsequent implementation of the raw batter testing for each batch of salami gives a quantitative raw material pathogen burden, which decreases risk, as the batch can then be tested at the end of processing.

The characterization of risk in quantitative terms is outside the scope of this assessment, but comparison of the exposure risk to pathogens in this study to that of the FSIS *Salmonella* Risk Assessment may be possible. The exposure risk is smaller at the facility in this study, which indicates that the products produced are safer to consume than FSIS assumes.

5.0 Conclusion

In discussion with FSIS officials during the initial phases of production, they suggest that validating the process at the production facility, and demonstrating a 5-$\log_{10}$ reduction would be the best study.

FSIS *Salmonella* guidelines recognizes that the achievement of a 5-$\log_{10}$ reduction of *Salmonella* in some fermented and dried products may be hard to demonstrate, and that "the guidance provides establishments with alternative lethality approaches within the guidelines, including utilizing good manufacturing practices and incoming product testing to support the safety of lower levels of lethality" (USDA, 2012).

Based on the results of this validation study, the process employed by this processing facility for the manufacture of fermented and dried salami: fermenting to a pH ≤5.3 with in the appropriate degree-hours, aging to a water activity of ≤0.91 and a Moisture/Protein ratio of ~1.9/1 or less, have been validated for pathogen reduction in salami. The study demonstrated approximately a 3- $\log_{10}$ reduction in the short aged salami, and at least 5-$\log_{10}$ reduction in the long aged salami.

CHAPTER THREE

Cured Aged Whole Muscle Pork Products

1.0 Whole Muscle Introduction

The development of a science-based process control system for food safety, which promotes systematic prevention of biological, chemical, and physical hazards, is required by the United States Department of Agriculture in all meat processing facilities (USDA, FSIS, 1998). This system, known as Hazard Analysis Critical Control Point (HACCP) is a plan that is specific to the facility and their products and processes. The identification of potential food safety hazards, that can cause adverse health effects when present at an unacceptable level, must be identified throughout the entire process.

The dry-curing of whole muscles has been used for thousands of years, and the products are having a resurgence in popularity in recent times. The purpose of this study was to validate an artisan dry-curing process in the production facility to demonstrate that it meets the FSIS pathogen reduction requirements.

Langlois and Kemp (1974) demonstrated that the initial contamination and the curing process affected microbial flora of dry cured hams (Langlois & Kemp, 1974). Huerta (1988) demonstrated a reduction in the final stages of the dry curing process of coliform populations (Huerta *et al.*, 1988). Reynolds *et al*. conducted a validation of a dry cured ham process to study the reduction of *E. coli* O157:H7, *L. monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp. because very little information was available (Reynolds *et al.*, 2001).

While there have been validation studies for dry cured hams, the process and formulation used in this study is unique, requiring validation. The formulation used in this study uses less salt as well as potassium nitrate instead of sodium nitrite. The salting time and salt equilibration time is shorter than those in other studies. This study was done to evaluate the dry curing process for its efficacy against *E. faecium*, a known surrogate for

Salmonella spp. and frequently used for in-plant validation studies, as well as the overall microbiological safety of the process.

2.0 Material and Methods

2.1 Whole Muscle preparation and inoculation of surrogate

2.1.1 Carcasses

Receiving: The processor received thirty half-carcass hogs each week during the trial period. The pasture-raised hogs were obtained from four farms and slaughtered at either of two USDA inspected facilities. Carcasses were received each Friday and required to have an internal temperature measured in the ham of <40 °F.

Preparation: The carcasses were "singed and scraped" to remove any hair that may be present. Following the "singe and scrape" process, carcass halves, inside and out, were thoroughly rinsed with 50 ppm ECA solution (ElectroChemical Activation water, Zap Water Technologies, Inc., Richfield, MN). The carcasses were hung and stored in a <40 °F cooler over the weekend (72-96 h).

Breaking: Carcass breaking occurred 72-96 h after receiving. The carcass breaking room temperature was kept ≤50 °F. The carcass halves were broken into specific muscle groups for whole muscle cures, salami lean, trim, fats, and secondary cuts, which were separated and stored in totes. These totes were stored in a cooler at 34-36 °F.

2.1.2 Cure Ingredients and Process

The meat used in the Coppa (shoulder and loin), Lonza (loin), Filetto (tenderloin), and Speck (boneless ham) were cured using 3.5% sodium chloride, ≤2187 ppm (0.2187%) potassium nitrate per FSIS Processing Inspectors Calculation Handbook (FSIS, 1995), 0.3%-0.5% sugar, spices, and Danisco Texel DCM-1 starter culture. Texel DCM-1 is a surface starter culture containing *Staphylococcus carnosus*, *Staphylococcus vitulinus*, and a dextrose carrier, which was applied at 10 g per 100 kg meat. The cure mix was

calculated for each specific batch of product types and applied by hand. The whole muscles were placed in totes and stored in ≤45 °F/7.2 °C for two weeks. The muscles were turned in the totes every two days. The Speck were placed on plastic shelving to rest for an additional two weeks prior to fermentation.

2.1.3 Fermentation and Aging

All whole muscles were fermented for 24 hours at 70-78 °F and ≥ 80% humidity. The Coppa, Lonza, and Filetto were stuffed into beef bung casings (Natural Casing Co., Peshtigo, WI) prior to fermentation. Speck underwent a one hour cold smoke prior to aging.

Aging took place in a 55-58 °F room with ≤ 60% humidity. Aging times varied based on muscle size. The Filetto aged for 14-21 days, the Lonza aged for 42-70 days, the Coppa aged for 50-70 days and the speck aged for approximately 150 days.

2.2 Microbiological and Physiochemical Analysis

Carcass Swabs

During the three weeks of production in which the validation study was performed, the split carcasses were swabbed for the detection of *Salmonella spp.* These swabs were performed at three different points in time prior to the processing of the carcasses. After the singe and scrape step, half the carcasses from each farms represented in the delivery from one slaughter facility, were swabbed using Paradigm Diagnostics Inc. (PDX) SecurSwab surface swabs or Enviro Swabs (3M, St. Paul, MN). Swabbed surfaces included the skin side of the split carcass (trotters, face, belly, belly-ham crease, tail, shoulder and ham) as well as the cut side along the spine and face. The carcasses were then sprayed inside and out with an ECA (electrolyzed water) solution containing 50 ppm free available chlorine. The same carcasses were then swabbed again after the rinse. The

same carcasses were swabbed after 72 hours in the carcass cooler. The swabs were transferred to the University of Minnesota the same day in a cooler. Paradigm Diagnostics Inc. Salmonella Indicator Broth (PDX-SIB, AOAC 071102) was added to the swab tube. Tubes were analyzed after incubation at 37 °C for 48 hours. Presence of *Salmonella spp.* is indicated by a color change in the broth. Any suspect samples were streaked on xylose lysine deoxycholate agar (XLD agar) for confirmation.

Bacterial Strain

The whole muscles were inoculated with *Enterococcus faecium* NRRL B-2354(ARS Culture Collection) (ATCC8459). The inoculum was grown in Tryptic Soy Broth (TSB), (Acumedia, Neogen) for 24 h at 37 °C. The 10^9 overnight culture were used to inoculate a 2 cm x 2 cm square with 0.2 mL during the first trial. The dip method was used for the second and third trials, where the muscles were dipped into the overnight culture and allowed to dry in the storage tote prior to having the cure applied. Three Coppa, three Lonza, and four Filetto were inoculated each week. The change to the dip method was a result of the difficulty in locating the scribed squares once the muscle was stuffed into the casing. The resulting level of E. *faecium* was 10^7 CFU/g.

pH and a_w

The pH and a_w were taken at each sampling point. The first pH meter used was a Hanna Instruments Portable HACCP Compliant pH/temperature Meter for Meat (Hanna Instruments HI 99163). The meter was calibrated before each use and was temperature compensated. The meter was replaced twenty five days into the trial after highly inaccurate measurements. Ryan Cox, Dept. of Animal Science, University of Minnesota, consulted and suggested the use of a Testo meter.

The replacement meter was a Testo 205 pH/Temperature meter by Testo Pty Ltd. The Testo 205 is temperature compensated with an accuracy of ±0.02 pH. The meter was two point calibrated prior to each use. The meat processing industry is the primary application for the meter. Upon replacement, pH measurements were made of all products from all trials and recorded.

The a_w was taken at each sampling point. The Aqualab Pa_wkit water activity meter by Decagon Devices, Inc. was used for all trials. A two point calibration occurred at least once a week using the standards that accompanied the meter. Whole muscle a_w samples were taken at the micro sample site, by slicing along one side of the removed 2 cm x 2 cm wall. The sample extended from the surface into the middle of the product by 1.5 cm. The meter had an accuracy of ±0.02.

Whole Muscle Microbiology Sampling

The sampling schedule for the whole muscle products included post inoculation, post cure addition, after two weeks in the curing totes, after fermentation, and during aging. The speck was also sampled after two weeks of rest prior to fermentation and after a one hour cold smoke post fermentation. Filetto was sampled every week, Coppa and Lonza were sampled every two weeks, and Speck were sampled every month. Samples were randomly taken from inoculated pieces of each product.

The samples were cut from the 2 cm x 2 cm scribed squares during the first trial, and 2 cm x 2 cm squares were cut from the dip method trials 2 and 3. Samples were placed in Whirl Pac bags and refrigerated prior to being transferred, in a cooler with ice packs, to the department of Food Science at the University of Minnesota. The samples were processed the same day.

Approximately one to three gram samples were stomached in Buffered Peptone Water (Fischer Scientific) for 30 seconds, and serially diluted from 10^1 to 10^7. Dilutions were spread plated on m-enterococcus agar (m-EA: Acumedia, Neogen Corporation). The

plates and incubated at 37 ± 2 °C for 48 hours. Plates were counted at 48 hours with a backlit fluorescent counter. The medium is selective that allows direct count of enterococci without the need for confirmation. The selective agent is Sodium Azide, which suppresses Gram-negative organism growth. *Enterococcus faecium* colonies appeared circular, smooth and red to maroon in color. The red color is formed by the reduction of the dye Triphenyl Tetrazolium Chloride (TTC) within the bacterial cell, producing the insoluble red colored formazan (Neogen, 2015). The m-EA had a detection limit of 10 CFU/g.

End Product Testing

Third party end-product testing was conducted to determine microbiological safety and product composition. Salmonella (PCR: AOAC-PTM #010803), Listeria (PCR: AOAC-PTM #090701), and Staphylococcus aureus (Petrifilm: AOAC #2003.07) testing was conducted by Market Fresh Labs, Minneapolis, MN. Product composition was conducted by RTECH Laboratories, Land O'Lakes, St. Paul, MN. Analytical tests were protein content (Kjeldahl), moisture content (forced air, 100C, 16.5 hrs), salt content (as NaCl by direct titration), and a sugar content profile (HPLC).

***Trichinella spirialis* Testing**

The Bio-Rad *Trichinella spiralis* Antibody Test Kit (manufactured by SafePath Laboratories, LLC, Lisence # 430) is a USDA approved ELISA test as part of the National Trichinae Certification Program (USDA, 2015). The test kit antigen, which was purified from larvae of infected pigs, has a high degree of specificity for *T.spiralis* and can be qualitatively determine antibodies in swine sera, plasma, whole blood, or tissue fluid (Biorad, 2015). The principle of the procedure is the binding of an excretory-secretory antigen to the *T. spiralis* antibody in the sample, which is followed by a binding of the secondary antibody anti-swine IgG peroxidase complex (enzyme conjugate). A

chromogen is added and reacts in the presence of the enzyme complex and peroxide, producing a blue color. The reaction ends once a stop solution is added, and a positive sample is indicated by a color change to yellow (Biorad, 2014).

The four farms represented and the number of pigs tested for *Trichina spirialis* in the study are Hidden Stream Farm LLC, Elgin, MN (10 pigs); Moo Oink Cluck Farm, Somerset, WI (4 pigs); Pork & Plants LLC, Altura, MN (6 pigs); YKer Acre, Wrenshall, MN (8 pigs). The blood samples were collected in 0.4% sodium citrate at the time of slaughter and were frozen immediately to -20 °C. The frozen blood was transferred to the University of Minnesota for analysis.

The samples were thawed immediately before analysis and the micro-well plate were allowed to adjust to room temperature for thirty minutes in the packaging to prevent water condensation. The dilution buffer concentrate was diluted to 1:20 with deionized water. The blood was diluted to 1:100 using the dilution buffer in plastic pcr tubes. 100 µl of negative control was added to a well and 100 µl of positive control was added to a second well. 100 µl of each diluted sample were placed into wells. The samples were incubated for 10 minutes at room temperature. The contents of the wells were shaken out and washed 3 times with the wash buffer. Two drops (100 µl) of enzyme conjugate was added to each well and incubated for 10 minutes at room temperature. Again, the wells contents were shaken out and washed 3 times with the wash buffer. Each well was washed with DI water. One drop (50 µl) of substrate A (chromogen tetramethylbenzidine) and one drop (50 µl) of substrate B (citric acid and peroxide) were added to each well. The wells were lightly tapped to mix the substrates and allowed to incubate for 10 minutes at room temperature. Two drops (100 µl) of stop solution (1 M phosphoric acid) were added to each well. The wells were placed on white paper and read visually for a color change. The positive and negative controls were run concurrently with the assays, and confirmed the assay viability.

Environmental Monitoring Program

A *Listeria monocytogenes* monitoring program consisted of weekly environmental surface swabbing and analysis in the packaging room of the Ready-to-Eat (RTE) area of the processing plant. The surfaces swabbed were the vacuum packaging machine, table, cutting board, knife, gloved hands, and floor drain. Swabs were labeled and placed into refrigeration. The swab tubes were transferred to the University of Minnesota in a cooler and processed the same day.

The swab tubes (SecurSwab, Paradigm Diagnostics, Inc. or Enviro Swab, 3M) were filled with a selective enrichment media containing a metabolic indicator and incubated at 37 °C for 48 hours. The PDX-LIB (Paradigm Diagnostics, Inc, AOAC-RI approved, Certificate 040501) indicator broth turns black when *Listeria spp.* is present. The broth contains antibiotics that inhibit most non-*Listeria* microorganisms, growth enhancers for injured *Listeria* and indictor compounds in which the β-glucosidase enzyme act upon, changing the broth from yellow to black.

3.0 Results

3.1 Microbiological Analysis

Carcass Swab: All swabs prior to, immediately after and 72 hours after the 50 ppm anolyte rinse were negative for *Salmonella spp*. for all farms. Swabbing continued for approximately 6 more weeks, with negative results.

***Trichinella spiralis* test results:** The blood samples from all 28 hogs were negative for the *Trichinella spiralis* antibody and the positive and negative controls confirmed the viability of the assays.

Listeria Monitoring Program Results: Weekly swabs were taken at the following RTE area sites: vacuum packaging machine; packaging table, cutting board, knife, gloved hands, and floor drain. The environmental sampling occurred during the three trial weeks and continued for another three. All swabs for all weeks were negative for *Listeria spp.*

Third-Party Microbial End-Product Test Results: All whole muscle products in the validation study were tested for the following pathogens by MarketFresh, LLC laboratories: *Staphylococcus aureus, Listeria* spp., and *Salmonella* spp. All whole muscle products came back negative for *Listeria* spp., *Salmonella* spp., and <10 for *Staphylococcus aureus*.

3.2 *Enterococcus faecium* Reduction Results

The reduction of *Enterococcus faecium* in the whole muscle products Coppa, Lonza, Filetto, and Speck during the trial are shown below. The colony forming units per gram (CFU/g), the pH and water activity (a_w) are shown for each corresponding sample time.

Additional Filetto Trials

Three additional trials for Filetto were conducted following the initial three trials. The entire weeks' tenderloins were inoculated, by dip method, as opposed to the three or four tenderloins each week in the first three trials. This was to ensure that the products would undergo the actual curing procedure that the rest of the tenderloins are subjected to. The same sampling schedule was followed as the initial trials.

Coppa Trials

Table 1: Coppa processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Muscle	7.35	5.6	ND
After 2 Weeks in Curing Bins	6.18	5.51	0.93
After Fermentation	5.94	5.17	0.95
4 Weeks Aging	4.51	5.7	0.94
6 Weeks Aging	< 100	5.72	0.91
Trial 2			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Entertococcus faecium Culture	9.02	ND[c]	ND
Inoculated Muscle	7.29	5.6	0.98
After 2 Weeks in Curing Bins	7.07	5.24	ND
After Fermentation	5.26	5.68	0.96
4 Weeks Aging	3.81	5.64	0.91
6 Weeks Aging	< 100	5.74	0.88
Trial 3			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Muscle	7.02	5.21	0.98
After 2 Weeks in Curing Bins	5.15	5.65	0.96
After Fermentation	4.41	5.61	0.96
4 Weeks Aging	<100	5.66	0.90
6 Weeks Aging	< 100	5.74	0.89

[a] – Colony Forming Units per gram
[b] – Water Activity
[c] – No Data

Table 2: Log_{10} Reduction of *Enterococcus faecium* in Coppa through 6 weeks aging.

Trial	Log_{10} Reductions at 6 weeks of Aging	a_w at 6 Weeks Aging
1	5.35	0.91
2	5.29	0.88
3	5.02	0.89
Ave. $\pm$ SD	5.22 $\pm$ 0.18	0.89 $\pm$ 0.02

Lonza Trials

Table 3: Lonza processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Muscle	7.06	ND	ND
After 2 Weeks in Curing Bins	5.86	5.26	0.92
After Fermentation	5.04	5.33	0.93
4 Weeks Aging	4.49	5.29	ND
6 Weeks Aging	< 100	5.46	0.90
Trial 2			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Entertococcus faecium Culture	9.02	ND[c]	ND
Inoculated Muscle	7.11	5.27	ND
After 2 Weeks in Curing Bins	7.1	5.27	0.96
After Fermentation	5.98	5.48	0.95
4 Weeks Aging	4.76	5.65	ND
6 Weeks Aging	< 100	5.87	0.86
Trial 3			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Entertococcus faecium Culture	9.97	ND[c]	ND
Inoculated Muscle	7.38	5.4	0.98
After 2 Weeks in Curing Bins	7.12	5.77	ND
After Fermentation	7.32	5.67	0.98
4 Weeks Aging	4.73	5.68	0.93
6 Weeks Aging	< 100	5.51	0.91

[a] – Colony Forming Units per gram
[b] – Water Activity
[c] – No Data

Table 4: Log_{10} Reduction of *Enterococcus faecium* in Lonza through 6 weeks of aging.

Trial	Log_{10} Reduction at 6 Weeks Aging	a_w at 6 Weeks Aging
1	5.06	0.90
2	5.11	0.86
3	5.38	0.91
Ave. $\pm$ SD	5.18 ± 0.17	0.89 ± 0.2

Filetto Trials

Table 5: Filetto processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Inoculated Muscle	7.62	ND[c]	ND
After 2 Weeks in Curing Bins	5.86	ND	0.94
After Fermentation	5.04	ND	0.93
1 Week Aging	ND	ND	0.95
2 Weeks Aging	ND	5.42	0.93
3 Weeks Aging	3.37	5.98	0.83
Trial 2			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Inoculated Muscle	7.33	ND[c]	0.96
After 2 Weeks in Curing Bins	6.27	ND	0.94
After Fermentation	5.64	ND	0.97
1 Week Aging	5.62	5.68	0.91
2 Weeks Aging	4.45	5.72	ND
3 Weeks Aging	ND	5.91	0.91
Trial 3			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Inoculated Muscle	7.13	ND[c]	0.98
After 2 Weeks in Curing Bins	6.81	5.64	0.96
After Fermentation	5.73	5.54	0.90
1 Week Aging	5.08	5.67	ND
2 Weeks Aging	5.71	5.7	ND
3 Weeks Aging	5.68	5.79	0.92

[a] – Colony Forming Units per gram
[b] – Water Activity
[c] – No Data

Filetto 2nd Validation

The data for the second validation of filetto is presented below. The log reductions are shown through two weeks of aging.

Table 6: Filetto Second Validation processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Inoculated Muscle	7.12	ND[c]	ND
After 2 Weeks in Curing Bins	6.23	5.53	ND
After Fermentation	5.11	5.56	0.95
1 Week Aging	0.51	5.43	0.94
2 Weeks Aging	< 100	ND	0.90
Trial 2			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Inoculated Muscle	6.93	5.6	0.98
After 2 Weeks in Curing Bins	7.07	5.24	ND[c]
After Fermentation	5.26	5.68	0.96
1 Week Aging	3.81	5.64	0.91
2 Weeks Aging	< 100	5.74	0.88
Trial 3			
Process Step	Log_{10} CFU/g	pH	a_w[b]
Inoculated Muscle	7.02	5.21	0.98
After 2 Weeks in Curing Bins	5.15	5.65	0.96
After Fermentation	4.41	5.61	0.96
1 Week Aging	<100	5.66	0.90
2 Weeks Aging	< 100	5.74	0.89

[a] – Colony Forming Units per gram
[b] – Water Activity
[c] – No Data

Table 7: *Enterococcus faecium* $\log_{10}$ reductions in Filetto 2nd Validation:

Trial	Log_{10} Reduction at 2 Weeks Aging	a_w at 2 Weeks Aging
1	5.12	0.90
2	4.93	0.88
3	5.02	0.89
Ave. $\pm$ SD	5.02 ± 0.09	0.89 ± 0.01

Speck Trials

Table 8: Speck processing steps and the corresponding log_{10} CFU/g[a] of *E. faecium*, pH, and a_w[b] of the samples taken for all three trials.

Trial 1		
Processing Step	Log_{10} CFU/g	a_w
Inoculated Raw	7.88	ND
After Rub	6.76	ND
After 2 weeks in bins	5.96	ND
After 2 weeks on shelves	5.37	ND
After Fermentation	4.66	ND
After Cold Smoke	4.51	ND
After 12 weeks aging	< 100	0.91
Trial 2		
Inoculated Raw	7.23	ND
After Rub	7.1	ND
After 2 weeks in bins	5.53	ND
After 2 weeks on shelves	5	ND
After Fermentation	4.7	ND
After Cold Smoke	4.35	ND
After 12 weeks aging	< 100	0.90
Trial 3		
Inoculated Raw	7.86	ND
After Rub	7.12	ND
After 2 weeks in bins	5.66	ND
After 2 weeks on shelves	5.42	ND
After Fermentation	4.33	ND
After Cold Smoke	4.52	ND
After 12 weeks aging	< 100	0.87

[a] – Colony Forming Units per gram
[b] – Water Activity
[c] – No Data

Table 9: *Enterococcus faecium* Log Reductions in Speck Trials

Trial	Log_{10} Reduction at12 Weeks Aging	a_w at 2 Weeks Aging
1	5.88	0.91
2	5.23	0.90
3	5.86	0.87
Ave. $\pm$ SD	5.66 $\pm$ 0.37	0.89 $\pm$ 0.02

4.0 Whole Muscle Discussion

FSIS requires production facilities to validate their processes through the use of validation studies, GMPs, and industry experts.

FSIS has guidance for options for the control of *Salmonella* spp. The use of a heat treatment is not an option for the process used at the trial plant, as this is not the style of dry-cured pork products being made. Conducting a "hold and test" program of the finished product was not an option, as the expense was too great for a small producer making small batches of product.

Though there are validation studies for dry-cured hams, the process still need to be validated, as all processes vary from plant to plant.

The use of hurdle technologies contributed to the safety and reduced risk; they include reduction of incoming pathogen burden, *Trichinella spiralis* testing, and environmental monitoring.

Salmonella spp. control had to be demonstrated for the process at the production facility. Raw material initial microbial load greatly influences the intensity of the hurdles needed to achieved product stability and safety. Control of all pathogens in cured whole muscle products is achieved by decreasing water activity by salting and drying. To achieve microbial stability, reduction in a_w is accomplished using hurdle technology. The first hurdle is keeping a low microbial count on and in the ham. This is accomplished by minimizing stress in the hogs prior to slaughter and for the carcasses to be chilled rapidly. The ideal pH at the time of curing is 5.6-5.8, as this facilitates salt penetration. The second hurdle is the curing temperature, which should be kept ≤5 °C, as this prevents growth of most pathogens. This temperature must be maintained until the salt and nitrate has penetrated and equilibrated throughout the muscle(s), thus lowering the a_w to approximately 0.96 and allowing the muscles to be transferred to dry at ambient temperatures.

The formulation used by the processing plant specified an ingoing salt content of 3.5%, which is on the lower end of typical salt usage rate (3-5%), though through moisture loss, the final salt content is higher. Salt and sugar reduces a_w, and therefore provides a control mechanism, interrupting homeostasis. The use of nitrate is an effective control for *C. botulinum*, thereby providing a control mechanism.

The incoming pathogen burden of the raw material is an important factor in determining the risk of pathogens in the finished product. The negative results from the *Salmonella spp.* swabs at all three swab times on the carcass halves indicates that the incoming pathogen burden is undetectable. Additionally, this speaks to the pathogen reduction program used at the slaughter facilities and its effectiveness. Most importantly, the undetectable incoming pathogen burden limits the pathogen burden for the entire process. It is important to point to the fact that no *Salmonella spp.* growth occurred over the 72 hour window in which the carcasses were hung in the carcass cooler.

Though the risk of *Trichinella spiralis* is quite low in pork now, the risk increases for pigs that have free-range outdoor access, where exposure to rodents and wildlife is a possibility. Exposure to rodents or wildlife infected with trichinae, dead or alive, can transmit the parasite to the pig if it is chewed on or digested. The evaluation of farm management practices can determine the risk posed by free range pork. Limiting risk of exposure can be greatly reduced by following sound swine husbandry practices (Gamble, 2015). The blood tests conducted during the study had negative results for the 28 pigs tested, indicate that the supplying farms are Trichina free. Trichinae is usually associated with an entire farm and their husbandry practices. The absence of the Trichinae is important to the processes used at the salumi producer, as they do not freeze or heat-treat their meat. The processor has an ongoing testing program.

In order to reduce risk of contamination from the processing environment, we conducted *Listeria spp.* monitoring tests in the RTE area of the processing plant. The negative results for the detection of *Listeria spp.* in the processing facility's ready-to-eat area is an indication of appropriate cleaning practices as listed in the facility's SSOPs. *Listeria spp.*

presence in ready-to-eat products has a zero tolerance policy. It is also an indicator of the general environment of the facility. The lack of detection in the weekly swab samples speaks to the cleaning practices and the overall safety of the ready-to-eat products produced.

While control of the raw material is not transferred until the pig carcasses arrive at the processing facility, the HACCP plan dictates processes that ensure a high quality raw product. These include receiving temperatures of the carcasses and an electrolyzed water rinse of carcasses. Additionally, the slaughter facilities use an organic acid carcass rinse prior to carcass chilling, a pathogen control step. If the risk assessment is applied to the entire system, then part of the 5-$\log_{10}$ reduction required by FSIS should be attributed to the processes at the slaughter facility. Because the HACCP plan requires a raw material receiving temperature of < 45 °F, and *Salmonella* spp. demonstrates little to no growth below 7 °C (44.6 °F) (Tompkin, 1996), the risk of pathogen growth is controlled.

To determine the effectiveness of the processing procedures to control and/or eliminate pathogens, we conducted an in-plant validation. It was demonstrated that if the following CCPs were met during the production of whole muscle cures, the risk of pathogen contamination in the finished product would be low, and the product would be considered safe for public consumption.

The following CCPs for dry-cured aged pork products were 1) CCP#1 identified in the whole muscle product process was a chemical food safety hazard during the addition of potassium nitrate. There is potential for toxic effects of N-nitroso compounds, therefore the USDA regulates the amount of potassium nitrate used in curing (2187 ppm); 2) CCP#2 identified in the whole muscle product process was a biological food safety hazard during the drying/aging step. There is potential for the growth of pathogens (*Salmonella* spp., *L. monocytogenes, S. aureus*) and subsequent toxigenesis. This hazard is addressed by controlling the room temperature and relative humidity to assure the desired a_w goal of ≤ 0.91 is achieved. The room parameters are addressed in the AMIF GMPs.

This study demonstrated a 5-log reduction for the whole muscle pork products (Coppa, 5.22 $\pm$ 0.18; Lonza, 5.18 $\pm$ 0.17; Filetto, 5.02 $\pm$ 0.09; Speck, 5.66 $\pm$ 0.37), therefore meeting the requirements of the CCP of a water activity of $\leq$0.91.

The second validation attempt of filetto produced approximately 5-log reductions in two of the trials and a 4.93-log reduction in one trial. The second trial raw material inoculation did not reach the 7-log level, though the reduction level was <100 like the rest of the trials. By inoculating the entire batch of tenderloins and then applying the cure, the actual process used demonstrated a substantial reduction. The previous validation attempt separated out three or four tenderloins, which were inoculated, and then cured in a separate tote. The two week curing process includes turning over the muscles every other day in the totes, which redistributes the salt and cure mix. By separating out the inoculated tenderloins from the rest of the process, they were not being re-coated in as much of the excess salt/cure mix as they would have had they been in the other tote.

The trial products underwent third-party end-product testing, and the results showed no presence of *Salmonella* spp., *Listeria* spp., <10 cfu/g *S. aureus*.

Reynolds *et al*. (2001) conducted a validation study of the dry curing process for hams inoculated with *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*, as it had not been previously been demonstrated to meet control requirements, though "it has long been held that the low water activity (a_w), high salt concentration and nitrite levels produce a safe, shelf stable, country cured ham product." Pathogen control needs to be demonstrated during the production of ready-to-eat products as required by the USDA, FSIS Pathogen Reduction: Hazard Analysis and Critical Control Point System (Reynolds *et al*., 2001).

Reynolds *et al.* (2001) demonstrated (using a cure containing nitrate, nitrite, salt and sugar) a water activity (a_w) of 0.923 at day 69, and 0.908 at day 120. Hams were dry aged from day 49 to day 69 in 29.4 °C to comply with USDA *Trichina* treatment requirements, and then placed in 20-24 °C through day 120. A 5.5-log reduction in *Salmonella* was demonstrated at day 69, and at day 120 *Salmonella* spp. was at undetectable levels. Hams

cured concurrently, using only salt and sugar, had no significant statistical difference in a_w change and reduction in *Salmonella*. Additionally in both cure mixes, *E. coli* O157:H7 showed a 4 to 5 log reduction by day 69 and to undetectable levels by day 120, *Listeria monocytogenes* showed a 4 log reduction by day 69 and to undetectable levels by day 120. *Staphylococcus aureus* demonstrated a 1.5 log decrease by day 90, and a subsequent increase of 0.5 log by day 120 in ambient storage temperatures. Of the samples tested, none were positive for the enterotoxin. Reynolds *et al.* notes that other studies indicate a variety of conditions which are required for *S. aureus* growth and enterotoxin production, including: chemical composition of the food, pH, a_w, other bacteria present, composition of the atmosphere, time and temperature of storage, other ingredients, and processing (Reynolds *et al.*, 2001).

Reynolds *et al.* (2001) explains that though water activity and pH conditions during the initial curing stages were optimal for *S. aureus* growth, the temperature was too low (4.4 °C). And the fact there was actually a decrease in population before the hams were hung in ambient aging conditions, which would be more optimal for growth, that other limiting environmental factors had occurred during the curing process, resulting in a lack of staphylococcal enterotoxin production.

Reynolds *et al.* concluded that rapid reduction of *Salmonella* spp. and *E. coli* O157:H7 demonstrates the effectiveness of a low a_w in reducing pathogenic populations in hams that reached 0.91 by day 120.

In this validation study, hams were cured for 14 day and underwent salt equalization for an additional 14 day, both at 4.4-7.2 °C, a fermentation step for 24 h at 23 °C, and aged to day 84 at 13.3-14.4 °C. The 0.91 a_w threshold suggested by Reynolds *et al.* and as indicated in the HACCP plan, was reached or surpassed by day 84. Additionally, the use of nitrate did not affect the reduction of pathogens in Reynolds *et al.* study, so the in-going potassium nitrate used in the study would not have a potential influence on the reduction of *Salmonella*. The results demonstrated an average 5.66 log reduction in *E.*

faecium by day 84, which was similar to the *Salmonella* reduction results obtained by Reynolds *et al*, though a a_w of 0.91 was reached 36 day quicker (84 day versus 120 day).

This study used 3.5% sodium chloride in-going, where as Reynolds *et al.* used 8.25% in-going sodium chloride, with a final salt content of 8%. The hams in the study closely resembles Italian speck, a lightly smoked prosciutto. A finished speck ham does not contain more that 5% sodium chloride, according to the Speck Alto Adige P.G.I.(Protected Geographical Indication) (Speck Alto Adige, 2015). Parma prosciutto (hams) typically use 3-5% in-going sodium chloride, with a final salt content of 6.0% and a a_w of 0.92. Of the most common dried hams in the world (Serrano, Iberian, Parma, San Daniele, Bayonne, and Country-style), Serrano has a typical salt content of 8.7%, with the rest at or below 6.5%, with Country-style being the lowest at 4.7% (Toldra, 2002). Huerta and others (1988) demonstrated similar water activity change to 0.909, of a Spanish style dried ham, by day 125, with a final salt content of 5.51% (Huerta *et al.*, 1988).

The purpose of the risk assessment was to consider the microbial safety of the RTE dry-cured whole muscle products (speck, lonza, coppa, filetto) produced by artisan techniques. Pathogens of concern are *Salmonella* spp., *Staphylococcus aureus*, *Listeria* spp., and *C. botulinum*. The quantitative risk to the consuming population in terms of infection or intoxication is outside the scope of this study, but the relative risk compared to the FSIS risk assessment study can give insight to the safety of the product.

While the pathogens of concern do pose a realistic hazard in raw whole muscle products, all of the pathogens can be controlled or eliminated through proper GMPs (pH, a_w, time-temperature, SSOPs, etc) and having appropriate CCPs and the corresponding critical limits in place. The extent of exposure to these microorganisms or their toxins at the time of consumption is important to determine. Realistic predictions of exposure can be made by considering reliable information regarding the levels of the particular pathogens in the raw materials, the effects on them of processing, storage, distribution, preparation by the consumer, and portion size.

This study demonstrated an undetectable amount of incoming *Salmonella* spp. on the carcasses, as well as a process that reduced a *Salmonella* surrogate by over 5-log. The pathogen burden is also reduced because the facility receives intact split pig carcasses, which receive a chemical rinse at the facility, and are broken down for use in the products. End-product testing also demonstrated the microbial safety of the products. The on-going prevention of pathogen re-growth in these shelf-stable products and the unnecessary preparation by the consumer decrease risk of exposure to pathogens or toxins. Additionally, the products are consumed in small portion sizes.

The characterization of risk in quantitative terms is outside the scope of this assessment, but comparison of the exposure risk to pathogens in this study to that of the FSIS *Salmonella* Risk Assessment may be possible. The exposure risk is smaller at the facility in this study, which indicates that the products produced are safer to consume than FSIS assumes.

5.0 Conclusion

In discussion with FSIS officials during the initial phases of production, they suggest that validating the process at the production facility, and demonstrating a 5-$\log_{10}$ reduction would be the best study.

Based on the results of this validation study, the process employed by the processing facility for the manufacture of dry cured whole muscle products: using nitrate at an ingoing rate of ≤2187 ppm and aging to a water activity of ≤0.91, have been validated for pathogen reduction in whole muscle products. The study demonstrated at least a 5-$\log_{10}$ reduction and the processes was accepted by the USDA.

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