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INVESTIGATING THE SURVIVAL OF MICROORGANISMS IN FERMENTED AND DRIED MEAT PRODUCTS CURED WITH VARIOUS SOURCES OF NITRITE

A Thesis in

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Abstract

Salami is a Ready-to-Eat (RTE), cured, fermented, and dried meat product traditionally processed without a thermal lethality step. Previous challenge studies have validated the safety of these products when manufactured with the regulated maximum amount (156 ppm) of in-going sodium nitrite to the raw meat batter. Sodium nitrite is a multifunctional ingredient used in cured, processed meats for its quality (i.e., color, flavor, and antioxidant activity) and food safety attributions to the products. Though most acknowledged as an inhibitor against the germination of *Clostridium* spp. spores, sodium nitrite has also been documented as an inhibitor against *Escherichia coli* O157:H7 (EC), *Listeria monocytogenes* (LM), and *Salmonella* spp. (S). Despite the vast benefits of nitrite addition to processed meat products, several organizations and agencies have correlated consumption of the ingredient with risk factors for many human cancers. Additionally, consumer demand for clean label products has increased, which has resulted in an increase in the production of meat products manufactured with alternative, natural curing agents. Natural curing agents are sourced from fruits or vegetables that are naturally high and variable in nitrate content. Due to the antimicrobial efficacy of nitrite, concerns have been raised for the safety of meat products manufactured with natural curing alternatives. While there is research that investigates this concern in RTE meat products like deli turkey or boneless ham, there is no published literature that validates these natural alternatives in a RTE, fermented, cured, and dried product manufactured without a thermal lethality step. The primary objective of this study was to determine the fate of EC, LM, and S throughout fermentation, drying, and storage of salami manufactured without a heat treatment and with various sources of nitrite.

Preliminary experimentation was conducted prior to beginning two challenge studies. There were two trials in the preliminary experiment. The objective of the first study was to

determine the ratio of surrogate organism inocula (mL), *E. coli* O157:H12 and *L. innocua*, to pork to achieve at least a 6 $\log_{10}$ CFU/g concentration when organisms were inoculated together and separately. The first trial found that a 6 $\log_{10}$ CFU/g inoculation is achieved when inoculum is added as 1 mL to every 453.6 g of pork. After the completion of trial 1, it was determined necessary to minimize the amount of ingoing liquid from the inoculum while still achieving high levels of pathogen inoculation in the raw meat batter. Therefore, the inoculum was centrifuged to achieve concentrated amounts of the pathogens in the form of a pellet that would be redistributed with a minimum amount of Buffered Peptone Water (BPW) in the raw pork batter. It was unknown, however, what the minimum amount of BPW would be. Therefore, the objective of the second trial was to determine the minimum amount of BPW needed to effectively distribute pellets of mixed cultures of three strains each of EC, LM and S to effectively disperse the pathogen pellets throughout raw, ground pork to achieve at least a 6 $\log_{10}$ CFU/g inoculation of each organism. The results from trial two demonstrated that resuspending the pathogen pellets with 0.1 mL BPW for every 453.6 g of pork achieved at least a 6 $\log_{10}$ log CFU/g inoculation in the meat system.

The first challenge study determined the fate of surrogate organisms, *E. coli* O157:H12 and *L. innocua*, throughout fermentation and drying of salami manufactured with various sources of commercially available nitrite. Three replications of three treatments were evaluated in this study. Treatments were positive control (purified 6.25% $NaNO_2$; PC), celery powder (CP), and Swiss chard (SC). All treatments were formulated to 156 ppm ingoing nitrite, according to manufacturer recommendations. Surrogate inocula were prepared by individually inoculating single, isolated colonies of the surrogates into 14 mL Tryptic Soy Broth (TSB) which was incubated for 24 h at 36ºC. To prepare salami, pork shoulder butts (IMPS 406) were deboned,

cubed (2.54 cm x 2.54 cm), ground (~5mm), vacuum packaged as batches, and stored at ~4ºC overnight until salami manufacturing the following morning. During salami manufacturing, ground pork was combined with surrogate inocula, dry ingredients according to treatment, and a starter culture (Safepro® B-LC 007 starter culture; CHR Hansen; Hoersholm, Denmark) that was suspended in DI water. After distribution of inocula and additional ingredients, raw batter was stuffed into 55 mm fibrous casings, fermented (pH < 5.0) at 24-26ºC and then dried to a target water activity (a_w) of ≤ 0.88. Individual salami (n=9) were sampled on days (D) 0, 1, 2, 3, 7, 14, and 28 for surrogate survival, pH, and a_w (N=216). Unique comparisons between sampling days within treatment were analyzed using a General Linear Model procedure (SAS OnDemand Version 9.4). Comparisons across treatments within organism on the same day were analyzed using a mixed model procedure in SAS. Treatment, pH, a_w, and treatment by sampling day interaction were included in the model as fixed effects. A significance level of $P < 0.05$ was used to determine significant differences in all analyses.

Using the procedures described above, salami achieved a pH of 4.73 ± 0.17 by D3 of fermentation. Neither *E. coli* O157:H12 nor *L. innocua*, were significantly impacted by pH ($p > 0.05$). Salami never achieved the target a_w. Ultimate a_w of all salami treatments was 0.90 ± 0.4 on D28. *L. innocua* was found to be significantly impacted by a_w ($p = 0.0319$), whereas *E. coli* O157:H12 was not ($p = 0.0678$). Treatment group had a significant impact on both organisms ($p < 0.0001$). Total reductions from D0 to D28 of *E. coli* O157:H12 in PC, CP, and SC were 0.52, 1.76, and 0.93 $\log_{10}$ CFU/g respectively. Total reductions from D0 to D28 of *L. innocua* in PC, CP, and SC were 0.52, 1.94, and 1.51 $\log_{10}$ CFU/g, respectively.

The second challenge study determined the fate of three strains each of EC, LM, and S in RTE salami manufactured with various sources of nitrite and without a heat treatment. EC

isolates EDL933 (ATCC 43895; ground beef outbreak) Sakai, and PA-2 (Hartzell, et al., 2011), LM serotypes Scott A, 1/2a isolate FSL R2-603 (deli meats outbreak) and 4b isolate H3396 (hot dog outbreak), and S serovars Typhimurium (ATCC 14028; chicken organs), Montevideo isolate SMvo13, and Derby (ATCC 7378; human isolate) were identified for use in this study. Twenty-four hour cultures of each organism were prepared by individually inoculating single, isolated colonies of each pathogen strain into 25 mL of TSB in duplicate which were incubated for 24h at 36ºC. After incubation, cultures were centrifuged (~20ºC for 5 minutes, at 11,000 x g). The pathogen pellets were resuspended within strain with 2.5 mL BPW to be distributed in the raw pork.

Three replications of four treatment groups were evaluated in this study: negative control (no nitrite source; NC), PC, SC, and Prosur® Natpre T-10 (dried fruit extract; T-10). The ingoing salt content of NC was adjusted to meet that of PC. PC and SC were formulated to 156 ppm ingoing nitrite. SC and T-10 were utilized according to manufacturer recommendations. Salami were prepared as previously described in the first challenge study. Upon achieving the target water activity, salami were vacuum packaged and stored at ambient temperatures (20 ± 0.003ºC). Salami were evaluated in triplicated for pathogen survival, pH, and a_w on days 1, 2, 3, 7, 14, 21, 28, 35, 42, 49, and 118 (n=9; N=432). Results were analyzed using the same procedures as described for the first challenge study.

The fixed effects of pH and a_w did not have a significant impact on any of the resulting pathogen population differences between treatments($p > 0.05$). All salami treatments achieved a pH < 5.0 after the first 24h of fermentation. Additionally, all salami treatments achieved a a_w of ≤ 0.88 by the third week of manufacturing. Treatment had a significant impact on all pathogen populations during the study ($p < 0.0001$). Reductions of EC between D0 (raw batter) and D21

(when salami met the target a_w) were 1.33, 2.61, 0.78, and 2.14 $\log_{10}$ CFU/g for NC, PC, SC, and T-10, respectively. LM reductions between D0 and D21 were 1.06, 2.35, 2.57, and 1.19 $\log_{10}$ CFU/g for NC, PC, SC, and T-10, respectively. S reductions between D0 and D21 were 0.58, 2.17, 2.3, and 0.73 $\log_{10}$ CFU/g for NC, PC, SC, and T-10, respectively. All treatments achieved at least a 5 $\log_{10}$ reduction of EC and S by D118. NC was the only treatment group to not achieve a 5 $\log_{10}$ reduction of LM (4.55 $\log_{10}$ CFU/g) by D118.

This research was the first to evaluate pathogen survival in non-heat treated salami manufactured with various sources of nitrite and during extended, reduced oxygen, ambient storage. If processors use purified sodium nitrite or Swiss chard at 156 ppm nitrite, they may use this research as scientific validation of a 2 $\log_{10}$ CFU/g reduction of LM and S in pork salami, in combination with a raw material sampling plan, Hazard Analysis and Critical Control Point plan, and Good Manufacturing Practices according to the Blue Ribbon Task Force Option #5.

Table of Contents

List of Tables

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Chapter 1
Literature Review

Cured, Fermented, and Dried Meat Products

History

Fresh meat is a nutritious and highly sought-after food source; however, the product's major challenge is that it is perishable, which was a battle for humans prior to emergence of meat processing and preservation technologies (Leroy et al., 2013). Early developments of meat processing were based on the concept of inhibiting or deterring microbial decomposition in fresh products to preserve the food during warm seasons (Pearson & Gillett, 2012). It is understood that meat processing likely began before the dawn of civilization when primitive humans discovered that salt is an effective preservative when applying the ingredient to the surface of meat, but the exact origins of this process is unknown (Pearson & Gillett, 2012). Meat curing likely arose from this salting process due to nitrate inclusion as an impurity in the salt (Hierro et al., 2015).

Southern European countries located on or near the Mediterranean Sea are likely the origins of dry-cured meat production, because of their ideal climates (i.e., mild winters and modest amount of rainfall) for the natural drying and ripening of meat (Toldrá, 2002). Modern dry-cured and fermented sausages were likely invented around 1730 in Italy and adopted by German countries around 1780s. As Europeans migrated to the Americas, dry-cured meat manufacturing practices did as well (Toldrá, 2002).

Roman butchers were said to mince beef and pork, blend them with salt and spices, and place them into a room to dry. Organisms, likely what we now know as *Kocuria* and *Staphylococci*, were added to this meat blend accidentally from environmental contamination and led to fermentation. This sausage manufacturing practice spread through the Roman empire and the art of producing fermented products was later, further spread throughout the rest of

Europe (Zeuthen, 2007).

Batches of meat were purposefully and naturally contaminated with microorganisms through the practice of "backslopping" or "mother batching" meat from one batch of sausage to the next. This process is recognized as the first starter cultures in fermented meat products. As industrial production of fermented products increased, starter cultures were standardized to increase the rate of productivity and create more consistent products in both safety and quality (Zeuthen, 2007).

Salami

The word "salami" derived from the Medieval Latin word "salumen", meaning "salted stuffs" or, less likely, the Cypriot city, Salamis (Leroy et al., 2013; Troldrá, 2007). Salami products are typically ground lean pork, beef, or both that is typically mixed with curing agents, salt, carbohydrates (e.g., dextrose or other sugars for fermentation), spices and flavorings, and starter cultures (Toldrá, 2002; Maddock, 2015). The resulting batter is then stuffed into casings that may be natural or synthetic. Natural casings are inconsistently shaped and can be difficult to work with but are much more traditional than synthetic. Synthetic casings include fibrous or restructured collagen, which are permeable to smoke and the evaporation of both water and gas. Synthetic casings hold advantages over natural casings like uniformity, consistency, variety, and machinability (Toldrá, 2002). After stuffing, the product is hung into a specific room or chamber to ferment and dry. Environmental parameters (i.e., temperature, relative humidity, and air velocity) of these rooms and chamber can typically be controlled throughout fermentation and drying. Final pH and water activity (a_w) values from fermentation and drying, respectively, can vary depending on the desired product (Toldrá, 2002).

Dry and semi-dry sausages are possibly the largest category of dried-meat products, especially in the United States (USDA-FSIS, 2016). Production of salami in the United States differs from that in traditional European practices (Toldrá, 2002; Maddock, 2015). Traditional European products do not have a heat treatment, whereas products manufactured in the United States commonly have a thermal lethality treatment. Additionally, the United States has a much faster fermentation step, higher a_w, and shorter production time, than traditional European products (Schwing and Neidhardt, 2007).

Hurdle Technology

The purposeful combination of multiple preservative factors in food to maintain the microbial safety and the sensory and nutritional quality of food is referred to as "hurdle technology" (Leistner, 1985; Leistner and Gorris, 1995; Leistner, 2000). In theory, the hurdles applied should inhibit any potential microorganisms that may contaminate the food (Leistner, 1995). Though there are many potential hurdles that may be applied, the most significant are temperature control (high or low), a_w, pH, redox potential (Eh), additional ingredients such as preservatives (e.g., nitrate, nitrite, salt), and competitive microorganisms (e.g., naturally occurring, or additional lactic acid producing bacteria).

Salami can be safely produced without a thermal lethality step because of the hurdles achieved throughout manufacturing. Raw, Ready-To-Eat (RTE) salami are typically fermented by the addition of a starter culture containing lactic acid producing bacteria and other microorganisms that can produce antimicrobial microbial (bacteriocins) and lower the pH and Eh of the product (Leistner, 1995). Nitrite is also added to salami products, providing an antimicrobial and preservative effect to the products. The role of nitrite in food safety is

discussed later in this literature review. Throughout ripening, however, pH and Eh will increase, and nitrite concentrations will deplete within the product (Leistner, 1995). A_w, the availability of water for chemical and biochemical reactions in a foodstuff (Zeece, 2020), continues to decrease as pH and Eh increase and water not bound to proteins or other ingredients (i.e., free water) in the salami is evaporated during ripening (Leistner, 1995). Simultaneously, salt concentrations increasing in the product as water is lost adds an additional hurdle to microbe growth. Once achieving the aimed a_w for the specific operation, salami may be vacuum packaged, eliminating oxygen availability to microbes.

Concerted hurdles in a product should lead to optimal microbial stability and employ a synergistic effect with larger preservative intensity than only one hurdle may provide (Leistner, 2000). McKinney et al. (2019) demonstrated the efficacy of applying multiple hurdles during salami manufacturing in a challenge study. The study showed that a $5\log_{10}$ reduction of *Listeria monocytogenes* (LM) and *Salmonella* (S) can be achieved with by-products from lactic acid producing bacteria, low pH and a_w, and reduced oxygen conditions during storage. Similar results were displayed by Watson, Cutter, and Campbell (2021) in a duck salami when the raw meat materials were treated with a 2.5% Beefxide solution prior to manufacturing.

Nitrite as an Ingredient in Processed Meats

History

Meat curing likely arose in the deserts of Asia where nitrate was applied onto meat via impurities from salt (Hierro et al., 2015; Blikered and Kolari, 1975). Long before the Christian era, China and India gathered a form of the salt contaminate called “saltpeter”, “nitre”, or what we now know to be sodium or potassium nitrate from the walls of caves (Binkered and Kolari, 1975); . The reddening effect by nitrates from the salt was first mentioned during the late Roman

times. By the medieval era, the reddening effect on meat was attributed to salt and saltpeter (Keeton, 2017). The role of nitrite in the formation of cured meat pigment and flavor became further understood in the late 19th and early 20th centuries. As the art of curing progressed, meat curing became understood as the addition of salt, sugar, spices, nitrate, or nitrite to meat for preservation and flavor enhancement (Pegg and Shahidi, 2004). Until the 1940's, nitrite was only acknowledged as a benefit to cured meat quality, then data appeared to support the significant antimicrobial effect of nitrite (Tompkin, 2005).

Utilization of nitrate was allowed by the Meat Inspection Act of 1906. After experimentation in the 1920s, rules were created for the application of nitrite in meat. On January 19, 1923, the Bureau of Animal Industry of the USDA was the first to give permission for the direct use of nitrite to meat processors (Binkered and Kolari, 1975). The USDA issued a rule in 1931 that curing solutions containing both nitrate and nitrite must be limited to 156 ppm nitrite and 1716 ppm nitrate ingoing to the product (Keeton, 2017). The Wholesome Meat Act (1967) mandated that curing processes be approved to comply with state and federal regulations (Toldrá, 2002). In 1970, the use of nitrates and nitrites in combination was regulated not to exceed 200 ppm nitrite in the finished product under the Meat Inspection Regulations, USDA Consumer Marketing Service (Bikered and Kolari, 1975).

Food Safety

Nitrite is also known to effectively control the growth of spoilage and pathogenic organisms in processed meat products (Sindelar and Milkowski, 2011). Though best acknowledged to inhibit *Clostridium spp.* germination and toxin production, nitrite has been shown to also inhibit LM, *Escherichia coli* O157:H7 (EC), *Salmonella spp.*(S), *Staphylococcus*

aureus, and *Bacillus cereus* in meat products (Milkowski, et al., 2010). The mechanisms by which nitrite serves as an antimicrobial are not well understood and may differ depending on the target organism (Tompkin, 2005). It is thought that nitrite inhibits pathogens by blocking essential compounds for growth and survival (e.g., oxygen, glucose, and other metabolic enzymes), penetrating the cell membrane and disturbing the cell's ability to divide, and has bacteriostatic activity against pathogens (Yarbrough, et al., 1980; Buchanan et al., 1988; Duffy et al., 1994; Työppönen et al., 2002). Furthermore, the effectiveness of nitrite as an antimicrobial is dependent on other intrinsic factors (e.g., residual nitrite levels, low pH values, salt concentration, reductants, iron content, other antimicrobial agents, etc.) of the meat product. The role of nitrite as an antimicrobial in all cured meat products is important, however, the inclusion of this ingredient is especially critical in RTE products produced without a thermal lethality step, like salami.

Yarbrough, Rake, and Eagon (1980) concluded multiple mechanisms by which nitrite may inhibit EC and a wide range of bacteria. Their listed mechanisms include ideas that center around nitrite blocking essentials (e.g., oxygen uptake, glucose catabolism, and metabolic enzymes) for EC growth and survival. Further, it has been shown that nitrite is able to disturb bacterial enzyme function by penetrating the cell membrane and disturbing cell growth (Työppönen, Petäja, and Mattila-Sandholm, 2002).

Nitrite has been shown to reduce the growth rate, increase the lag time, and have bacteriostatic activity against LM. These impacts are especially influenced by the interaction of nitrite with pH, a_w, and ascorbate (Buchanan et al., 1988; Duffy et al., 1994). Later, a similar study confirmed that LM growth is influenced by ingoing and residual nitrite concentrations in cooked, cured pork sausage (King, 2016). Significant LM reductions by the addition of nitrite

has been presented through modeling. Such models permit formulations of cured meat products that will not support the growth of LM (Milkowski et al., 2010). The USDA-FSIS has done extensive research to develop prediction models showing how the inclusion of nitrite inhibits the growth of EC, LM, and S (USDA, 2006).

Hospital et al. (2014) found that by the end of ripening fermented sausages, those manufactured without nitrate or nitrite favored growth of S with 2-2.5 $\log_{10}$ CFU/g greater populations of the pathogen than with the cured counterparts. Their research displayed the effectiveness of nitrite as a pathogen hurdle when used in combination with other hurdles like pH and a_w to control S. Otherwise, there is limited research supporting a significant inhibition of S by nitrite inclusion (Rice and Pierson, 1982; Lamas et al., 2016).

Health Concerns

An increasing amount of literature and the American Institute for Cancer Research (AICR) associates cured processed meat consumption with an increased risk of some cancers (AICR, 2021). The AICR states that there is convincing evidence of increased risk for colorectal cancer and limited suggestive evidence of increased risk for lung, pancreatic, stomach, esophageal, and nasopharyngeal cancers because of processed meat consumption. Based on sufficient evidence, the International Agency for Research on Cancer (IARC), an agency part of the World Health Organization (WHO), classified processed meats as carcinogenic to humans, and the report concluded that a 50g portion of processed meat increased the risk of colorectal cancer by 18% (IARC-WHO, 2015).

The risk of cancer from processed meats is partially attributed to the nitrites added to some meat products that may potentially react with amines, organic derivatives of ammonia

(NH_3) in which one hydrogen atom has been replaced by at least one carbon group (Moss, Smith, and Tavernier, 1995). The reaction between nitrite and an amine forms nitrosamines (Cassens, Lee, and Buege, 1978). Nitrosamines are a large group of compounds that have been displayed as carcinogenic in all species of animals they have been tested in and, despite direct causal evidence, have been named as a suspected human carcinogen (Brown, 1999). Firm evidence for dietary nitrates and nitrites as causes of cancer in humans is lacking (Milkowski et al., 2010).

A review of the epidemiologic evidence for cancer risk from dietary nitrates, nitrites, and N-nitroso compounds (Eichholzer and Gutzwiller, 1998) found that epidemiologic evidence for the carcinogenic potential from these compounds is inconclusive in regard to stomach, brain, esophageal, and nasopharyngeal cancers. A study supported this epidemiological evidence by showing that there was no direct linkage in patterns in national cured meat consumption and brain or nervous system cancer (Murphy, Sadler, and Blot, 1998). They found that cured meat consumption had decreased significantly between the 1970s and the 1990s while the incidence of brain and nervous system cancer in adults had risen. This claim also was supported by research done by the U.S. National Toxicology Program (NTP) in which groups of 50 male and female rats and mice were exposed to drinking water with 0, 750, 1,500, or 3,000 ppm sodium nitrite for 2 years to determine the toxic and carcinogenic potential of the molecule (NTP, 2001). There was no evidence of carcinogenic activity in male or female rats and for male mice for any of the sodium nitrite concentrations. There was ambiguous evidence, however, of carcinogenic activity in female mice based on the positive trend of squamous cell papilloma or carcinoma of the forestomach.

Despite health concerns associated with nitrite, research conducted since the mid-1980's has shown that dietary nitrite plays a significant, positive role in human health. Nitrite is thought

to improve cardiovascular health by increasing nitric oxide availability in the vascular system, opening blood vessels (vasodilation) and therefore reducing blood pressure, and inhibiting platelet aggregation which helps prevent and treat cardiovascular disease (Machha and Schechter, 2011). Furthermore, Science Magazine acknowledged nitric oxide, the resulting compound from nitrite addition in processed meats, as, "The Molecule of the Year" (Koshland, 1992). The article called the molecule, "extraordinarily beneficial" due to its attributions to reducing blood pressure, inhibiting invaders of the immune response, enhancing long-term memory, and assistance in male sexual impotence.

Naturally Cured Meat Products

History

The development of naturally cured meat products began in response to previously discussed health concerns associated with nitrite, specifically in processed meats (Sebranek et al., 2012). To address health concerns surrounding cured, processed meats, processors in the 1960's manufactured uncured products, without any nitrite source included, as substitutes for products that would traditionally include nitrite (Sebranek et al., 2012). This action prompted the USDA in 1979 to establish the labeling requirement that, providing comparable size, flavor, consistency, and general appearance, "Any product… for which there is a standard in this part and to which nitrate or nitrite is permitted or required to be added, may be prepared without nitrate or nitrite and labeled…"Uncured" in the same size and style of lettering as the rest of such standard name…" (9 CFR § 319.2).

These truly uncured products did not share the same quality, safety, or shelf-life of their cured counterparts, and until the 1990's, there was little further development of "uncured

products" (Sindelar & Milkowski, 2011; Sebranek et al., 2012). In the 1990's, the inclusion of nitrate and nitrate reducing bacteria, a concept that had been used since the 1950's, was applied to nitrates sourced from fruits or vegetables with naturally high nitrate contents (e.g., celery or Swiss chard). The bacterial reduction of nitrate to nitrite required an incubation, or fermentation, step during product manufacturing. This addition step dramatically increased production time and decreased throughput of some commercial products. Therefore, pre-reduced natural curing agents were developed by introducing the nitrate reducing bacteria and the nitrate source prior to processed meat manufacturing (Krause, et al., 2011; Sebranek et al., 2012). Products manufactured with natural curing agents have been shown to still have residual nitrate and nitrite and nitrite content (Sindelar et al., 2007a); Despite the residual content of natural curing agents, meat products manufactured with natural sources of nitrite fall under 9 CFR § 319.2 and are required to be labeled "Uncured" and "No Nitrates/Nitrites Added".

Current Consumer Trends

The variables consumers use to decide which foods to purchase, and to repeat a purchase, are complex and not homogenous across all consumers (Grant et al., 2021). Food labels provide food manufacturers a tool to communicate key qualities of a product to consumers that can assist in purchasing decisions. "Clean label" food products have dramatically increased in popularity over the past decade (Asioli et al., 2017). The term, "clean label", broadly, is the expectation that the food product is 'clean' by assumption or by inferences that a consumer may make based on textual or visual claims on the packaging or label. Strictly, clean label is defined as the ingredient information is found 'clean' on inspection based off ingredients being short, simple, and perceived to be non-artificial or not chemical sounding (Asioli et al., 2017). The clean label

category encompasses processed meat products that utilize natural alternatives to sodium nitrite.

Consumers in general prefer a clean label food product and they are willing to pay premiums for this label (Grant et al., 2021). Specifically, in a survey of 1,300 consumers throughout Europe, North America, and Asia-Pacific, 76% of respondents said they would be more likely to buy a product that had easily recognizable and trusted ingredients (Bizzozero, 2017). Typical consumers' attitudes toward processed meat indicated that they view the products as "unhealthy" (Tobin et al., 2014). The perception of processed meats being unhealthy, combined with their increased preference for clean label products, has increased the amount of naturally cured processed meat products available at the retail level. An analysis shows that the naturally cured segment of processed meats is expected to have the highest revenue-based compound annual growth rate (4.3%) from 2021 to 2028 of all meat products (Grand Review Research, 2021). Additionally, the alternative formulations segment was ranked second in the article, "Top 10 Food Trends of 2021" published by the Institute of Food Technologists (2021). Coincidingly, charcuterie products have increased in popularity since the COVID-19 pandemic (Schouten and Loria, 2021; Hormel Foods Corporation, 2021; Cohan, 2023). The combination of consumer-led "clean label" trends and increase in charcuterie product popularity has led to an observed increase in the availability of naturally cured charcuterie products, like salami.

Challenges and Concerns

As previously discussed, purified nitrite sources serve a critical role in the safety of cured processed meat products, especially those that are RTE and manufactured without a thermal lethality step. The observed increase in naturally cured meat products has not come without the concern for the efficacy of pathogen inhibition. This concern is due to the variability of nitrite

levels in alternative curing agents (Sebranek and Bacus, 2012) and potentially insufficient utilization levels of ingoing or residual nitrites (Sindelar et al., 2007b).

Though the amounts of naturally occurring nitrate in plants are high, they are highly variable and dependent on agricultural practices, growing season, and genetic factors (Kalaycıoğlu & Erim, 2019). Therefore, variability in nitrite content must be considered during cured meat product manufacturing as the quality, and more importantly, safety of the products are dependent on the amount of ingoing nitrate and nitrite to the product (Sebranek and Bacus, 2007). Lower concentrations (~40 ppm) of ingoing nitrite to a meat product are required to observe typical cured meat quality characteristics than that required to achieve adequate product safety in comminuted meat products (≥120 ppm; USDA, 1995). Because processors may achieve desired quality attributes relatively easily in products using a reduced natural nitrite formulation, they may, therefore, continue with a reduced utilization rate (40-50 ppm; Sebranek and Bacus, 2007).

Formulations with reduced amounts of ingoing nitrite from natural nitrite sources have raised the question of their ability to inhibit pathogens (Rivera et al., 2019). In a survey of commercially available frankfurters, Schrader (2018) found that uncured products significantly supported more growth of LM than the products manufactured with purified nitrites. Another study of commercially available products (frankfurters, hams, and bacon) observed significantly more pathogen growth in naturally cured products than those manufactured with purified sources (Sullivan, 2011). The inhibition of LM by cultured celery powder with or without the addition of a cultured sugar-vinegar blend was evaluated (Golden et al., 2014). This research concluded that the concentration of ingoing nitrite, rather than the source, is the primary factor for increasing the safety of RTE meat products. These examples provide evidence that the question of pathogen

inhibition by naturally cured products has been investigated. There remains concern with, and a knowledge gap of, the ability of pathogen inhibition in non-thermally treated, RTE, fermented and dried processed meat products manufactured with natural, or alternative, curing agents.

Escherichia coli O157:H7

History

It can only be speculated how long *E. coli* O157:H7 (EC) has been causing human illness. The earliest probable cases of EC infections were in 1974 and '75, when patients experienced symptoms similar to those associated with hemorrhagic colitis and Hemolytic Uremic Syndrome (HUS; Law, 2000) However, the pathogen was not recognized until 1982 when it was identified as the causative agent in two outbreaks of hemorrhagic colitis (Griffin and Tauxe, 1991). Soon after, EC was associated with the production of Shiga toxins (O'Brien, et al., 1983) and identified with the development of HUS in children (Karmali, et al., 1983). Research in this era led to the classification of the enterohemorrhagic *E. coli* (EHEC) and Shiga Toxin-Producing *E. coli* (STEC) groups.

During the 1990's, EC was identified as the causative agent for two large outbreaks that impacted meat inspection and regulations worldwide. In 1993, ground beef contaminated with EC was undercooked and served to Jack-in-the Box consumers. This contamination resulted in a multi-state outbreak with over 600 illnesses, either bloody diarrhea or HUS, and four children dead (CDC, 1993). After this incident, Hazard Analysis and Critical Control Point (HACCP) programs became mandated for meat and poultry products sold in the United States. EC was also considered to be an as "adulterant" in raw ground beef products, and a zero fecal tolerance for beef carcasses was announced (Murano, Cross, and Riggs, 2018). In 1994, another EC outbreak

linked to commercially distributed dry-cured salami infected 20 case patients with a median age of seven (CDC, 1995) This outbreak led the United States Department of Agriculture and Food Safety and Inspection Service (USDA-FSIS) to develop guidelines that require a 5-log reduction of EC and other EHEC during dry-cured sausage manufacturing (Incze, 1998).

Characteristics

EC is a Gram-negative bacillus and a facultative anaerobe (Meuller and Tainter, 2022). Unlike 93% of other *E. coli* organisms, EC is unable to ferment sorbitol within 24h; this provides a differentiating marker for the identification of EC against other *E. coli* organisms (Harris, 1990). Although heat sensitive, EC can survive in chilled or frozen conditions for weeks to years on food products, like meat (Dykes, 2004).

Optimal growth of EC has been determined at a temperature of 37ºC, and the organism has been shown to grow poorly below 8-10ºC and above 44-45ºC (Getty, et al., 2000; Dykes, 2004). Additionally, it has been shown that at least 99.99% of EC cells will be inactivated in ground beef patties cooked to an internal temperature of 68.3ºC for 40s. Furthermore, EC cells are particularly more acid resistant than other *E. coli* serotypes as it can survive at pH levels as low as 3.6. This trait may influence thermal and salt resistance during fermented sausage manufacturing (Getty, et al., 2000). EC is salt resistant, having been shown to survive in-vitro salt ranges typically used in fermented sausages (2-3.5% NaCl; Getty, 2000). Hinkens et al. (1996) and Riordan et al. (1998) demonstrated the survivability of EC in pepperoni products containing a salt content of 4.4% and 4.8%, respectively. Lastly, the minimum and maximum a_w for EC growth is 0.95 and between 0.990-0.995, respectively (Sperber, 1983).

The *E. coli* species is divided into serogroups based on the lipopolysaccharide somatic

(O) antigen (e.g., O157 and O111). Serogroups are further divided into serotypes based on flagella (H) antigens (e.g., H7 and H12; Dykes, 2004). Characteristics that set EC aside from generic *E. coli* organisms are its genetic determinants for numerous virulence factors (Dykes, 2004). These determinants include genes responsible for attachment to host cells (attachment and effacement genes), host cell death (verotoxin genes), and host cell lysis (hemolysin genes).

Foodborne Illnesses Caused by *E. coli* O157:H7

Since being identified as a human pathogen, EC has become one of the most significant foodborne pathogens mainly due to the severity of symptoms associated with its infection and its low infectious dose (Dykes, 2004). Symptoms can vary for each infected individual (CDC, 2021a). More severe and complicated symptoms of EC infection have been observed in children younger than five-years-old, and in those older than 65 years old (Dykes, 2004). Clinical manifestations of EC infections range in severity from asymptomatic carriage to abdominal cramping, diarrhea, hemorrhagic colitis, HUS, thrombocytopenia purpura (TTP), or death (Griffin and Tauxe, 1991; Mead and Griffin, 1998; Dykes, 2004).

Hemorrhagic colitis resulting from EC infection can be characterized by abdominal cramping, grossly bloody diarrhea, little to no fever, and evidence of colonic mucosal edema, erosion, or hemorrhage (Griffin and Tauxe, 1991). Symptoms arise three to four days after EC ingestion and begin with abdominal cramps and non-bloody diarrhea. Over 70% of patients have reported the diarrhea to evolve to diarrhea with varying amounts of blood (MacDonald et al., 1996; Slutsker et al., 1997).

Complications with EC infections are observed to occur in 10% of cases and can usually be indicated by clinical features (Dykes, 2004). About 3-7% of cases evolve to HUS, a disease

characterized by blood in the urine, which often leads to kidney failure (Tortora, Funke, and Case, 2019). Approximately 5% of HUS patients die, and those who survive may require kidney dialysis or transplants. Although TTP is related to HUS, fewer patients develop this disease (Dykes, 2004). TTP is a rare and life-threatening blood disorder, in which blood flow to major organs (e.g., brain, kidneys, and heart) is blocked by blood clots in small vessels throughout the body (NIH, 2022). If left untreated, TTP has an acute mortality rate of about 90%.

Outbreaks

Infections and outbreaks of EC have been a nationally reportable disease since 1994, as designated by the Council of State and Territorial Epidemiologists (Council of State and Territorial Epidemiologists, 1994) . Since then, reports of infection have increased by 211% from 1994 to 2000, possibly due to the improvement of surveillance, greater awareness of EC among the public and healthcare workers, and a true increase in infections (USDA, 2001). EC infections may also be underreported, due to the lack of surveillance of non-bloody diarrhea for EC. Accounting for discrepancies in reporting, Mead et al. (1999) estimated that there are 73,480 EC infections annually and 85% (~63,000 cases) are a result of foodborne transmission.

Although acknowledged as a safe product due to the presence of multiple pathogen hurdles including lowered pH and a_w and the inclusion of nitrite and salt, dry-cured, fermented salami has been identified as the source of EC transmission in foodborne outbreaks. Twenty laboratory-confirmed cases of EC infections in Washington and California were linked to the consumption of contaminated dry-cured salami in 1994 (CDC, 1995). This outbreak caused three hospitalizations, including a two-year-old who developed HUS. The severity of the outbreak led the USDA-FSIS to form a proposed rule for a 5-$\log_{10}$ reduction of EC in dry and semidry

products in 1995 (USDA-FSIS, 2001). A subsequent study investigated the production of dry fermented salami because of the outbreak and concluded that EC could survive the current manufacturing practices as well as be transmitted through the product (Tilden et al., 1996).

Listeria monocytogenes

History

LM was first described by Murray et al. (1926), who originally called the organism, "*Bacterium monocytogenes*" when the researchers isolated the pathogen from an epidemic disease in a laboratory breeding unit of rabbits and guinea pigs. Pirie isolated an identical organism to Murray et al. from gerbil livers and named it *Listerella hepatolytica* (Gray and Killlinger, 1966). Later, at the Third International Congress for Microbiology, the Committee on Nomenclature was made aware that the name *Listrella* had already been given to a mycetozoan, leading Pirie to propose *Listeria* as the name for the genus (Pirie, 1940). LM became recognized as a foodborne pathogen and a cause of listeriosis in the 1980's when numerous listeriosis infections were associated with the consumption of contaminated food (Franciosa et al., 2001).

Characteristics

LM is a bacillus shaped, non-spore-forming, catalase positive, and Gram-positive microorganism. LM cells are motile at temperatures between 20-25ºC and demonstrate a characteristic tumbling motility with peritrichous flagella; however, motility of LM is limited at 37ºC (Buncic and Avery, 2004). The meat industry is especially concerned with LM due to its environmental ubiquity, common presence in food processing plants, endurance to various pathogen stressors typically used to inhibit pathogen growth in processing environments (i.e.,

sanitizers, pH, and temperature), and ability to form biofilms on product manufacturing surfaces (Buncic and Avery, 2004). LM resistance to pH can be dependent on other intrinsic factors of the food product. Survival in hard salami in refrigerated storage for 12 weeks at pH levels between 4.3 – 4.5 has been demonstrated (Johnson et al., 1998). Growth of LM can occur at temperatures between 4 and 45ºC, but 30-37ºC has been observed to be optimal (Low and Donachie, 1997). Growth can occur in aerobic and microaerobic conditions and is absent in anaerobic conditions. The application and ingoing concentration of sodium nitrite in processed meat products can limit the growth potential of LM and increase the efficacy of other antimicrobials on LM inhibition (Duffy, Vanderline, and Grau, 1994; King, et al., 2015).

LM is the only species in the *Listeria* genus that has been identified as a human pathogen (Goin, Mengaud, and Cossart, 1994). There are currently 13 serotypes of LM that can be distinguished: ½a, ½b, ½c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 5, 6a, and 6b. Serotyping of LM is done based on somatic (O) and flagellin (H) antigen. Although distribution of these serotypes can differ geographically, three serotypes have been implicated in over 90% of all LM infections: 1/2a, 1/2b, and 4b (Buncic and Avery, 2004). Pathogenicity may differ among LM isolates, but it is thought that 4b may be particularly adapted to human host tissues as it is the most frequently identified as the cause of foodborne outbreaks.

Listeriosis

LM causes a severe and invasive disease called listeriosis. Listeriosis can have several clinical forms in humans. Consumption of LM can cause acute, self-limited, febrile gastroenteritis in healthy individuals. Commonly reported symptoms of LM caused gastroenteritis include fever, erythromelalgia, headaches, and gastrointestinal symptoms (e.g.,

abdominal pain, diarrhea, nausea, or vomiting (Ooi and Lorber, 2005) Incubation periods and symptom durations are typically less than 24h and between one to three days, respectively (CDC, 2022). LM caused gastroenteritis is rarely diagnosed, however, as more than 90% of healthy adults possess immunity.

After the consumption of LM, the pathogen binds to epithelial host cells. After its attachment to host cells, LM promotes its uptake in a process facilitated by bacteria surface proteins. A pore forming toxin and bacterial phospholipases promote the rupture of the cell wall, which permit LM to enter the cytoplasm of the cell. LM replicates efficiently in the cytoplasm and begins an infection cycle of replicating then invading neighboring cells (Pizarro-Cerdá et al., 2012).

The majority of clinical listeriosis cases occur in immunocompromised individuals (FDA, 2020). Pregnant women have a 12-fold increased risk of contracting listeriosis after the consumption of contaminated food compared to the non-immunocompromised population (Hof, 2003). Maternal listeriosis, itself, can be asymptomatic or can express itself in nonspecific, flu-like symptoms. However, this illness may transmit to the fetus via the placenta and cause abortion or still birth (Buncic and Avery, 2004). If the unborn fetus does not die *in utero*, the baby may be born with a severe systematic infection of neonatal listeriosis that is manifested as meningitis and sepsis (Zou et. al., 2020). In adults who are not pregnant but are otherwise immunocompromised (i.e., those who are undergoing immunosuppressive therapy, elderly, or who have cancer, diabetes, or other health conditions) the most common clinical expressions of listeriosis are meningitis and meningoencephalitis (mortality rate of 20-50%) and sepsis (Buncic and Avery, 2004). Treatment of listeriosis in the immunocompromised can be as long as 3 to 6 weeks with the remaining risk of relapses caused by surviving LM cells within the host.

Outbreaks

Listeriosis is a rare, but serious, infection. The disease has a high mortality rate of 20-30% (FDA, 2020) and the highest hospitalization rate (99%) among the foodborne diseases (Scallan et al., 2011). LM remains the most isolated pathogen in the USDA-FSIS fermented sausage monitoring program (USDA-FSIS, 2001) and is frequently the causative agent in outbreaks related to deli meats and cheeses. In late January 2023, USDA-FSIS recalled over 69,000 pounds of ready-to-eat charcuterie products that were adulterated with LM because of contaminated product-contact-surfaces. A multistate outbreak of listeriosis infected and hospitalized twelve individuals, and resulted in one death (CDC, 2021b). Eleven of the infected reported eating prepackaged, Italian-style meats like salami, mortadella, and prosciutto. The world's largest listeriosis outbreak in history was in South Africa from 2017 – 2018. Bologna was identified as the source of this outbreak that had a record breaking 982 cases of listeriosis and 189 deaths (Baumgaertner, 2018).

***Salmonella* spp.**

History

In the 1880's, S serovar Typhi was the first reported observation and isolation of an organism in the *Salmonella* genus. (Dawoud et al., 2017). Later in the 1880's, Theobald Smith was the first to isolate S from swine, which he thought was the cause of hog cholera, hence the original name of the species, "*Salmonella choleraesuis"* (Schultz, 2008). The first laboratory-confirmed case linking S infections to the consumption of contaminated food was in Germany, 1888. Raw beef sourced from a dying cow had infected at least 50 individuals and resulted in one death of a 29-year-old man. The organism, now known as S Typhimurium, was isolated from the

spleen and blood of the man who died during the outbreak as well as the raw ground beef (Tauxe, 1990). Salmonellosis, the infection caused by S, was named a reportable disease to the CDC in 1943. The National *Salmonella* Surveillance System was established in 1963. The surveillance system was developed to assist in S epidemiology and depends on the voluntary reporting of S isolates by state health departments and federal agencies (Tauxe, 1990).

Characteristics

S is a bacillus shaped, Gram-negative organism that is in the Enterobacteriaceae family. The pathogen can grow well in aerobic and anaerobic conditions and grows optimally at 37ºC and at a pH of 7.0 (Gast et al., 2020). S growth can occur in temperatures ranges between 5 – 45ºC and in a pH range of ~ 4.0 – 9.0. The intestinal tracts of animals are frequently the source of this pathogen, making meat products particularly at-risk of S contamination (Tortora, Funke, and Case, 2019).

The *Salmonella* genus contains only two species, *S. enterica* and *S. bongori.* S is further divided into subspecies then serovars. *S. enterica* subspecies *enterica* is one of six *S. enterica* subspecies and contains more than 2500 serovars pathogenic to warm-blooded animals (Issenhuth-JeanJean et al., 2014; Tortora, Funke, and Case, 2019). The Kauffman-White scheme may be used to differentiate serovars by designating numbers and letters to an organism that correspond to the antigens on the capsule, cell wall, and flagella of that organism, which are denoted as K, O, and H, respectively (Tortora, Funke, and Case, 2019). S subspecies *enterica* serovars Typhimurium and Enteritidis are the two most isolated serovars from humans (Hendriksen et al., 2011). S has been divided into host- and non-host-adapted serovars, or those who can or cannot induce clinical signs of salmonellosis in the host, respectively (Nielsen, 2004)

Salmonellosis

Consumption food contaminated with S may lead to an infection called salmonellosis. Salmonellosis symptoms most frequently include potentially bloody diarrhea, fever, and stomach cramps. Some infected individuals may experience nausea, vomiting, or headaches (CDC, 2019). After an incubation time of 12 to 72h, an inflammatory response to the multiplication of S results in the onset of symptoms (CDC, 2013). Mortality rate is low, ~1%, is typically higher in infants and the elderly. Death is usually due to septic shock (Tortora, Funke, and Case, 2019). Severity of symptoms and incubation time may vary and is dependent on the amount of S cells consumed. The infectious dose is usually large and estimated to be at least 1000 cells of S.

Upon consumption of the pathogen, S enters an epithelial cell, multiples in a vesicle within epithelial cells and in mucosal cells (Tortora, Funke, and Case, 2019). Occasionally, S can cross the epithelial cell membrane, enter the lymphatic system and bloodstream, and eventually affect many organs (Tortora, Funke, and Case, 2019).

It is estimated that non-typhoidal S causes 11% (~1 million) of foodborne illnesses in the United States annually, making it the second most causative agent of illnesses to norovirus (Scallen et al., 2011). S is the leading cause of hospitalizations and deaths due to the consumption of contaminated food in the United States, however. The pathogen contributed 39% to the estimated 55,961 hospitalizations and 28% of the estimated 1,351 deaths caused by foodborne illnesses annually (Scallen et al., 2011).

Outbreaks

S contamination of fermented and semi-dry or dry sausages is uncommon due to S inhibiting intrinsic factors in these products (e.g., low pH and a_w). There have been outbreaks of

salmonellosis associated with the consumption of fermented, semi-dry or dry sausages contaminated with S, however. Twenty-six confirmed cases of salmonellosis were associated with the consumption of Lebanon bologna, a traditional, fermented, semi-dry beef sausage, in October of 1995 (Sauer et al., 1997). The Lebanon bologna was said to be contaminated with S Typhimurium and was the first documented outbreak of S from fermented sausages. In a multistate investigation of S Montevideo infections, 272 cases across 44 states were associated with the consumption of Ready-to-Eat (RTE) salami from July 2009 to April 2010 (Gieraltowski et al., 2013). The pathogen was isolated during environmental sampling of the RTE salami manufacturing plant and sealed containers of black and red pepper. This outbreak highlighted the potential for RTE product contamination by non-meat ingredients and the ability of S survival throughout RTE salami manufacturing.

More recently, two separate S outbreaks were associated with RTE, fermented, and dried meat products manufactured with natural sources of nitrite. From August to September of 2021, the CDC and USDA-FSIS investigated 40 S illnesses across 17 states and confirmed that an "uncured" antipasto Italian-style meat product was the outbreak food source (USDA-FSIS, 2021a). Of the infected, twelve were hospitalized (CDC, 2021c). USDA-FSIS also investigated the establishment for possible deviations in their manufacturing processes that may have caused the outbreak and found that the establishment used a reduced salt formulation and lacked a scientific validation for achieving a 5 $\log_{10}$ reduction of S in their products (USDA-FSIS, 2022a). The CDC and USDA-FSIS conducted another multistate S outbreak investigation later that same year (October to December). Thirty-four salmonellosis infections, including seven hospitalizations, across 10 states were associated with uncured salami stick consumption (CDC, 2021d). The USDA-FSIS concluded that the product manufacturer did not have sufficient

evidence to support the fermentation and drying interventions to adequately control S (USDA-FSIS, 2022a). As a result of the two outbreaks, the USDA-FSIS is working with the National Advisory Committee on Meat and Poultry Inspection to determine how to address knowledge gaps and validate the safety of RTE, fermented, and dried products (USDA-FSIS, 2022).

United States Regulations

Dry and Semidry Meat Products

There are no, current standards of identity for dry- and semidry- fermented meat products (Hunt and Boyle, 2007). However, there are regulatory definitions and specifications to differentiate the two products. Unless another Moisture Protein Ratio (MPR) is specified, dry sausages must have an MPR of 1.9:1 or lower and semidry, shelf-stable sausages must have an MPR of 3.1:1 or less and pH of 5.0 or less. Salami is a dry product and should have a MPR of 1.9:1 (USDA, 2005).

USDA-FSIS requires that fermented meat products have a written Hazard Analysis and Critical Control Point (HACCP) plan including all decision-making and supporting documents used in the development of the plan. Traditionally prepared salami without a thermal lethality step falls under the HACCP category of non-heat-treated, shelf-stable, meat and poultry products. This category applies to products that are further processed by curing, fermenting, or drying and are not required to be frozen or refrigerated (USDA-FSIS, 2020).

On May 5, 2023, the USDA-FSIS released a new guidance document that provides information to processors on the safe production of RTE fermented, salt-cured, and dried products (USDA-FSIS, 2023). The guidance document was written to address small and very small processors' questions regarding RTE, fermented, salt-cured, and dried products.

Additionally, the new publication gives an overview of the RTE, fermented, salt-cured and dried product category, biological hazards associated with these products, pathogen lethality options for processors, and existing scientific support processors may use to validate their processing procedures.

Nitrates and Nitrites

Either sodium or potassium nitrite may be used in cured products and regulatory limitations are the same for both. Calculations for ingoing parts per million (ppm) of both curing agents should be formulated based on green weight, or raw weight, of the meat, rather than the final weight of the product (USDA-FSIS, 1995). Sodium or potassium nitrite should be used at ¼ oz per 100 lb (156 ppm) of chopped meat, meat byproduct, or poultry product (9 CFR § 424.21(c)). Additionally, the use of nitrite or the combination of nitrate and nitrite should not result in over 200 ppm nitrite in the finished food product.

The labels of products manufactured with sodium nitrate or nitrite, potassium nitrate or nitrite, or those that have been salted for preservation are not permitted to be labeled as "fresh" (9 CFR § 317.8). Further, any substance mixed with another substance to cure a product (e.g., salt, sugar, potassium nitrite, sodium nitrite, etc.) must be identified in the ingredient statement of the product (9 CFR § 317.17(a)).

All cured products labeled "keep refrigerated" are required to contain a minimum of 120 ppm ingoing nitrite except for products from establishments that can demonstrate that product safety is controlled through other measures including hurdles like pH, a_w, and thermal processing (USDA-FSIS, 1995). Products that have been processed to ensure shelf stability do not have a minimum regulatory limit of nitrite. Shelf stable products may be those that have undergone a complete thermal process or have an adequate control of pathogens with hurdle technologies like

pH, a_w, or a combination of both. Forty ppm nitrite is helpful, however, for preservation and product quality purposes (USDA-FSIS, 1995).

Natural Curing Agents

Natural sources of nitrite are not approved as curing agents, as detailed in 9 CFR § 424.21 (c). Rather, natural sources are approved for use as antimicrobials and flavorings and are considered safe and suitable for use in meat and poultry products according to the USDA-FSIS Directive 7120.1 "Safe and Suitable Ingredients used in the Production of Meat and Poultry Products" (USDA-FSIS, 2022b). Natural nitrite sources should be formulated based upon the support for the minimum amount of nitrate or nitrite and minimum times for fermentation, aging, and curing.

Additionally, any product that is required to be labeled by a common or usual name (e.g., hot dogs, bacon, pepperoni, etc.) and is normally required to be processed with nitrate or nitrite may be prepared without these additional ingredients and labeled "uncured" with the caveat that products labeled "uncured" must be found to be similar in size, flavor, consistency, and general appearance to their counterparts manufactured with purified nitrites (9 CFR § 317.17 (b)). Furthermore, products containing no direct addition of nitrate or nitrites should bear the statement, "no nitrate or nitrite added" which is qualified by the statement, "except for those naturally occurring in [insert name of natural source of nitrite]." These statements must be adjacent to, and in the same lettering and size as, the product name. Additionally, the statement "Not preserved – Keep Refrigerated Below 40ºF At All Times" must be featured on the label and at least half the font size as product name. Products that have been thermally processed, fermented, or pickled to a pH less than 4.6, or have been dried to a a_w of 0.92 or less may be

exempt from including this statement (9 CFR § 317.17 (c2)).

E. coli O157:H7 and *Salmonella* spp. in RTE Fermented and Dried Meat Products

A 5-$\log_{10}$ reduction of EC and S is recommended by the USDA-FSIS in fermented (USDA-FSIS, 2001) and shelf-stable, RTE products (USDA-FSIS, 2021b). The USDA-FSIS Proposed Rule of Performance Standards for the Production of Processed Meat and Poultry Products (2001) offers four options to either achieve a 5-$\log_{10}$ reduction of EC or demonstrate the control for its presence in finished products: 1) Apply the cooking treatment in either 9 CFR § 318.17 or 9 CFR § 318.23, 2) apply a validated integrated heat treatment of equal lethality, 3) test product using ICMSF lot acceptance criteria, or 4) apply a validated 5-$\log_{10}$ relative reduction or process that results in less than 1 cell of EC per 100 grams of finished product. It has not been demonstrated that a 5-$\log_{10}$ reduction of EC will result in the same reduction of S, therefore, fermented RTE products must be validated for both EC and S if it contains beef (USDA-FSIS, 2001). No viable EC or S organisms can be present in the finished product.

Listeria monocytogenes in Fermented and Dried, RTE Meat Products

LM has not been linked to outbreaks related to contaminated fermented sausage consumption; however, it is the most isolated pathogen in the USDA-FSIS monitoring program for fermented sausages. USDA-FSIS has not proposed LM as a reference organism for fermented sausages but would consider the final product adulterated if discovered to be contaminated with LM (USDA-FSIS, 2001).

A rule has been mandated by the USDA-FSIS that requires manufacturers of RTE

products to employ effective measures against LM and comply with one of three alternatives provided in 9 CFR § 430.4. Alternative 1 is the application of a post-lethality treatment that reduces or eliminates microorganisms present on the product *and* apply an antimicrobial agent or processes that controls LM growth. Alternative 2 is using *either* a post-lethality treatment *or* an antimicrobial agent or process that controls LM growth. Companies choosing alternatives 1 or 2 must validate their post lethality treatment for LM control and include it in their HACCP plan. Additionally, the antimicrobial agent or process must be validated for its control of LM and recorded in either the company's HACCP or Sanitation Standard Operating Procedure. Alternative 3 is the dependence on sanitation measures only to control LM. Processors who choose alternative 3 are likely to be subjected to more frequent testing by the USDA-FSIS than those who choose alternatives 1 or 2.

Statement of the Problem

Health concerns associated with nitrite consumption from processed meats initially drove consumers away from cured products manufactured with purified nitrites. Consumers now have become invested in knowing where their food comes from and increasingly prefer foods manufactured with minimal ingoing ingredients that are easily recognizable on the ingredient statement, and more natural-seeming products (Román, et al., 2017; IFT, 2018; Grant, et al., 2021). These consumer preferences have been observed to coincide with the increase in production and availability of naturally cured meat products. Natural curing agents are often sourced from a fruit or vegetable that is high in nitrate content; however, these curing agents may have inconsistent amounts of nitrate/nitrite and may impact the ingoing nitrite content in meat products. Research has shown that although lower amounts (~40 ppm), of ingoing nitrite content

can achieve desired cured product qualities such as color, flavor, and antioxidant activity, higher amounts (120 ppm), are required to inhibit pathogen growth and spore germination (USDA-FSIS, 1995). This finding may encourage processors to lower the amount of curing agent applied in their products to save money while still achieving desired quality attributes. Pathogen inhibition is critical in processed meat products, and inhibition by nitrite is especially crucial in products manufactured without a thermal lethality step, such as salami. Although the application of natural curing agents in other products like deli ham, turkey, bacon and frankfurters has been thoroughly investigated (Sullivan, et al., 2012; Golden, et al., 2014; Weyker, et al., 2016), there is a gap in scientific literature that investigates the antimicrobial efficacy of natural sources of nitrite in raw, RTE, fermented and dried meat products. To fill this gap, a challenge study using relevant pathogenic bacteria incorporated into raw, RTE, fermented, and dried products can be conducted to determine the antimicrobial efficacy of various nitrite sources.

Objectives

This study aims to determine the safety of RTE, fermented, and dried salami manufactured without a thermal lethality step and with various sources of commercially available nitrite. The salami will be manufactured according to typical industry manufacturing procedures for raw, RTE salami. Curing agents will be formulated to 156 ppm ingoing nitrite according to manufacturer recommendations on a raw meat weight basis. High populations of relevant surrogate and pathogenic organisms will be inoculated into ground pork shoulder butts. The inoculated pork will be mixed with a seasoning blend and starter culture, stuffed into fibrous casings, and hung into a salami cabinet for fermentation and drying. After achieving the target a_w, the salami will be vacuum sealed and stored at ambient temperatures. Comparing organism

populations between treatments throughout fermentation, drying, and storage will help determine the antimicrobial efficacy of each curing agent during the production of raw RTE salami. This project intends to provide meat processors with scientific support for decision-making while developing a HACCP plan for products similar to those investigated in this study.

References

American Institute for Cancer Research (AICR). 2021. "Processed Meat (Sausages, Ham, Bacon, Hot Dogs, Salami)." American Institute for Cancer Research. March 31, 2021. https://www.aicr.org/cancer-prevention/food-facts/processed-meat/.

Asioli, D., J. Aschemann-Witzel, V. Caputo, R. Vecchio, A. Annunziata, T. Næs, and P.Varela. 2017. "Making Sense of the 'Clean Label' Trends: A Review of Consumer Food Choice Behavior and Discussion of Industry Implications." *Food Research International* 99 (1): 58–71. https://doi.org/10.1016/j.foodres.2017.07.022.

Baumgaertner, E. 2018. "Bologna Blamed in Worst Listeria Outbreak in History." *The New York Times*, March 30, 2018. https://www.nytimes.com/2018/03/30/health/bologna-listeria-south-africa.html.

Binkerd, E.F, and O.E Kolari. 1975. "The History and Use of Nitrate and Nitrite in the Curing of Meat." *Food and Cosmetics Toxicology* 13 (6): 655–61. https://doi.org/10.1016/0015-6264(75)90157-1.

Bizzozero, Judie. 2017. "75% of Consumers Will Pay Extra for Clean Label Ingredients." Food & Beverage Insider. March 15, 2017. https://www.foodbeverageinsider.com/market-trends-analysis/75-consumers-will-pay-extra-clean-label-ingredients.

Brown, J.L. 1999. "N-Nitrosamines." *Occupational Medicine* 14 (4): 839–48. pmid: 10495488.

Buchanan, R. L., Heidi G. Stahl, and Richard C. Whiting. 1989. "Effects and Interactions of Temperature, PH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the Growth of *Listeria monocytogenes*." *Journal of Food Protection* 52 (12): 1–7. https://doi.org/10.4315/0362-028X-52.12.844.

Buncic, S, and S. M. Avery. 2004. "*Listeria monocytogenes*." In *Encyclopedia of Meat Sciences*, edited by Werner K Jensen, Carrick Devine, and Michael Dikeman. Elsevier Ltd.

Cassens, R. G., T. Ito, M. Lee, and D. Buege. 1978. "The Use of Nitrite in Meat." BioScience 28 (10): 633–37. https://doi.org/10.2307/1307393.

Centers for Disease Control and Prevention (CDC). 1993. "Update: Multistate Outbreak of *Escherichia coli* O157:H7 Infections from Hamburgers -- Western United States, 1992-1993." www.cdc.gov. April 16, 1993. https://www.cdc.gov/mmwr/preview/mmwrhtml/00020219.htm.

Centers for Disease Control and Prevention (CDC). 1995. "*Escherichia coli* O157:H7 Outbreak Linked to Commercially Distributed Dry-Cured Salami -- Washington and California, 1994." www.cdc.gov. March 10, 1995. https://www.cdc.gov/mmwr/preview/mmwrhtml/00036467.htm.

Centers for Disease Control and Prevention (CDC). 2013. "Infection with *Salmonella*." https://www.cdc.gov/training/SIC_CaseStudy/Infection_*Salmonella*_ptversion.pdf.

Centers for Disease Control and Prevention (CDC). 2019. "Symptoms." Centers for Disease Control and Prevention. 2019. https://www.cdc.gov/*Salmonella*/general/*Salmonella*-symptoms.html.

Centers for Disease Control and Prevention (CDC). 2021a. "Symptoms." E. Coli. 2021. https://www.cdc.gov/ecoli/ecoli-symptoms.html.

Centers for Disease Control and Prevention (CDC). 2021b. "Outbreak of Listeria Infections Linked to Deli Meats | Outbreak of Listeria Infections Linked to Deli Meats | October 2020 | Listeria | CDC." www.cdc.gov. January 2021. https://www.cdc.gov/listeria/outbreaks/delimeat-10-20/index.html.

Centers for Disease Control and Prevention (CDC). 2021c. “CDC: *Salmonella* Outbreaks Linked to Italian-Style Meats.” Centers for Disease Control and Prevention. August 31, 2021. https://www.cdc.gov/*Salmonella*/italian-style-meat-08-21/index.html.

Centers for Disease Control and Prevention (CDC). 2021d. “CDC: *Salmonella* Outbreak Linked to Salami Sticks.” Centers for Disease Control and Prevention. October 23, 2021. https://www.cdc.gov/*Salmonella*/i45-10-21/index.html.

Centers for Disease Control and Prevention (CDC). 2022. “Learn about the Symptoms of Listeria.” Centers for Disease Control and Prevention. May 3, 2022. https://www.cdc.gov/listeria/symptoms.html#:~:text=Symptoms%20of%20intestinal%20illness%20usually.

Cohan, D. J. 2023. “Toward a Social Psychology of Charcuterie Boards | Psychology Today.” Www.psychologytoday.com. Psychology Today. March 27, 2023. https://www.psychologytoday.com/us/blog/social-lights/202303/toward-a-social-psychology-of-charcuterie-boards.

Council of State and Territorial Epidemiologists. 1993. “Position Statement Archive | Council of State and Territorial Epidemiologists (CSTE).” www.cste.org. 1993. https://www.cste.org/page/PositionStatements.

Dawoud, Turki M, Z. Shi, Y. M Kwon, and S. C Ricke. 2017. “Chapter 7: Overview of Salmonellosis and Food-Borne *Salmonella*: Historical and Current Perspectives.” Essay. In Producing Safe Eggs: Microbial Ecology of *Salmonella*, 113–38. Elsevier.

Duffy, L.L., P.B. Vanderline, and F.H. Grau. 1994. “Growth of *Listeria monocytogenes* on Vacuum-Packed Cooked Meats: Effects of PH, Aw, Nitrite and Ascorbate.” *International Journal of Food Microbiology* 23 (3-4): 377–90. https://doi.org/Growth%20of%20Listeria%20monocytogenes%20on%20vacuum-

packed%20cooked%20meats:%20effects%20of%20pH,%20aw,%20nitrite%20and%20ascorbate.

Dykes, G.A. 2004. "*Escherichia coli* O157:H7." In *Encyclopedia of Meat Sciences*, edited by Werner K. Jensen, Carrick Devine, and Michael Dikeman, 781–86. Elsevier Ltd. https://doi.org/10.1016/b0-12-464970-x/00052-0.

Eichholzer, M., and F. Gutzwiller. 1998. "Dietary Nitrates, Nitrites, and N-Nitroso Compounds and Cancer Risk: A Review of the Epidemiologic Evidence." *Nutrition Reviews* 56 (4): 95–105. https://doi.org/10.1111/j.1753-4887.1998.tb01721.x.

Franciosa, G., S. Tartaro, C. Wedell-Neergaard, and P. Aureli. 2001. "Characterization of *Listeria monocytogenes* Strains Involved in Invasive and Noninvasive Listeriosis Outbreaks by PCR-Based Fingerprinting Techniques." *Applied and Environmental Microbiology* 67 (4): 1793–99. https://doi.org/10.1128/aem.67.4.1793-1799.2001.

Gast, K., and R. E. Porter. 2020. "*Salmonella* Infections." Chapter In Diseases of Poultry, 14th ed., I:719–53. Hoboken, NJ: Wiley Blackwell.

Gieraltowski, L., E. Julian, J. Pringle, K. Macdonald, D. Quilliam, N. Marsden-Haug, L. Saathoff-Huber, et al. 2013. "Nationwide Outbreak of *Salmonella* Montevideo Infections Associated with Contaminated Imported Black and Red Pepper: Warehouse Membership Cards Provide Critical Clues to Identify the Source." Epidemiology and Infection 141 (6): 1244–52. https://doi.org/10.1017/S0950268812001859.

Golden, M. C., L. M. McDonnell, V. Sheehan, J. J. Sindelar, and K. A. Glass. 2014. "Inhibition of *Listeria monocytogenes* in Deli-Style Turkey Breast Formulated with Cultured Celery Powder and/or Cultured Sugar-Vinegar Blend during Storage at 4°C." Journal of Food Protection 77 (10): 1787–93. https://doi.org/10.4315/0362-028X.JFP-14-059.

Gouin, E., J. Mengaud, and P. Cossart. 1994. "The Virulence Gene Cluster of *Listeria monocytogenes* Is Also Present in Listeria Ivanovii, an Animal Pathogen, and Listeria Seeligeri, a Nonpathogenic Species." *Infection and Immunity* 62 (8): 3550–53. https://doi.org/10.1128/iai.62.8.3550-3553.1994.

Grand View Research. 2021. "GVR Report CoverNorth America Processed Meat Market Size, Share & Trends Report North America Processed Meat Market Size, Share & Trends Analysis Report by Meat Type (Poultry, Beef, Pork, Mutton), by Type (Cured, Uncured), by Product (Chilled, Frozen, Canned, Dry & Fermented), and Segment Forecasts, 2021 - 2028." https://www.grandviewresearch.com/industry-analysis/north-america-processed-meat-market.

Grant, K. R., R. K. Gallardo, and J. J. McCluskey. 2021. "Consumer Preferences for Foods with Clean Labels and New Food Technologies." *Agribusiness* 37 (4). https://doi.org/10.1002/agr.21705.

Gray, M. L., and A. H. Killinger. 1966. "*Listeria monocytogenes* and Listeric Infections." *Bacteriological Reviews* 30 (2): 309–82. https://doi.org/10.1128/br.30.2.309-382.1966.

Griffin, P. M., and R. V. Tauxe. 1991. "The Epidemiology of Infections Caused by *Escherichia coli* O157: H7, Other Enterohemorrhagic E. Coli, and the Associated Hemolytic Uremic Syndrome." *Epidemiologic Reviews* 13 (1): 60–98. https://doi.org/10.1093/oxfordjournals.epirev.a036079.

Hendriksen, R. S., A. R. Vieira, S. Karlsmose, D. M. A. Lo Fo Wong, A. B. Jensen, H. C. Wegener, and F. M. Aarestrup. 2011. "Global Monitoring of *Salmonella* Serovar Distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: Results of Quality Assured Laboratories from 2001 to 2007." *Foodborne Pathogens and Disease* 8 (8): 887–900. https://doi.org/10.1089/fpd.2010.0787.

Hierro, E., M. Fernández, L. de la Hoz, and J. Ordóñez. 2015. “Mediterranean Products.” In *Handbook of Fermented Meat and Poultry* , 301–12. Chichester, West Sussex, Uk ; Hoboken, Nj: Wiley, Blackwell.

Hinkens, J. C., N. G. Faith, T. D. Lorang, P. Bailey, D. R. Buege, C. W. Kaspar, and J. B. Luchansky. 1996. “Validation of Pepperoni Processes for Control of *Escherichia coli* O157:H7.” *Journal of Food Protection* 59 (12): 1260–66. https://doi.org/10.4315/0362-028x-59.12.1260.

Hof, H. 2003. “History and Epidemiology of Listeriosis.” *FEMS Immunology & Medical Microbiology* 35 (3): 199–202. https://doi.org/10.1016/s0928-8244(02)00471-6.

Hormel Foods Corporation. 2021. “Pandemic Proves Charcuterie Popularity.” Hormel Foods. January 15, 2021. https://www.hormelfoods.com/newsroom/news/pandemic-proves-charcuterie-popularity/.

Hospital, X. F., E. Hierro, and M. Fernández. 2012. “Survival of Listeria Innocua in Dry Fermented Sausages and Changes in the Typical Microbiota and Volatile Profile as Affected by the Concentration of Nitrate and Nitrite.” *International Journal of Food Microbiology* 153 (3): 395–401. https://doi.org/10.1016/j.ijfoodmicro.2011.11.032.

Hunt, M. C. , and E. Boyle. 2007. “Chapter 25– International Standards: USA.” In *Handbook of Fermented Meant and Poultry*. Blackwell Publishing, Ltd.

Incze, K. 1998. “Dry Fermented Sausages.” *Meat Science* 49 (January): S169–77. https://doi.org/10.1016/s0309-1740(98)90046-8.

Institute of Food Technologists (IFT). 2018. “What Is Clean Label.” *Brain Food- the Official Blog of IFT* (blog). 2018. https://www.ift.org/news-and-publications/blog/2018/november/what-is-clean-label.

Issenhuth-Jeanjean, S., P. Roggentin, M. Mikoleit, M. Guibourdenche, E. de Pinna, S. Nair, P. I. Fields, and F. Weill. 2014. "Supplement 2008–2010 (No. 48) to the White–Kauffmann–Le Minor Scheme." Research in Microbiology 165 (7): 526–30. https://doi.org/10.1016/j.resmic.2014.07.004.

Johnson, J. L., M. P. Doyle, R. G. Cassens, and J. L. Schoeni. 1988. "Fate of *Listeria monocytogenes* in Tissues of Experimentally Infected Cattle and in Hard Salami." *Applied and Environmental Microbiology* 54 (2): 497–501. https://doi.org/10.1128/aem.54.2.497-501.1988.

Kalaycıoğlu, Z., and F. B. Erim. 2019. "Nitrate and Nitrites in Foods: Worldwide Regional Distribution in View of Their Risks and Benefits." *Journal of Agricultural and Food Chemistry* 67 (26): 7205–22. https://doi.org/10.1021/acs.jafc.9b01194.

Karmali, M. A., M.Petric, B. T. Steele, and C. Lim. 1983. "Sporadic Cases of Haemolytic-Uraemic Syndrome Associated with Faecal Cytotoxin and Cytotoxin-Productin *Escherichia coli* in stools." *The Lancet* 321 (8325): 619–20. https://doi.org/10.1016/s0140-6736(83)91795-6.

Keeton, J. T. 2017. "History of Nitrite and Nitrate in Food." In *Nitrite and Nitrate in Human Health and Disease*, edited by Nathan S. Bryan and Joseph Loscalzo, 85–97. Springer Nature. https://doi.org/10.1007/978-3-319-46189-2.

King, A. M., K. A. Glass, A. L. Milkowski, D. L. Seman, and J. J. Sindelar. 2016. "Modeling the Impact of Ingoing Sodium Nitrite, Sodium Ascorbate, and Residual Nitrite Concentrations on Growth Parameters of *Listeria monocytogenes* in Cooked, Cured Pork Sausage." *Journal of Food Protection* 79 (2): 184–93. https://doi.org/10.4315/0362-028x.jfp-15-322.

Koshland, D. E. 1992. "The Molecule of the Year." Science 258 (5090): 1861–1861. https://doi.org/10.1126/science.1470903.

Krause, B.L., J.G. Sebranek, R.E. Rust, and A. Mendonca. 2011. “Incubation of Curing Brines for the Production of Ready-To-Eat, Uncured, No-Nitrite-Or-Nitrate-Added, Ground, Cooked and Sliced Ham.” *Meat Science* 89 (4): 507–13. https://doi.org/10.1016/j.meatsci.2011.05.018.

Lamas, A., J. Miranda, B. Vázquez, A. Cepeda, and C. Franco. 2016. “An Evaluation of Alternatives to Nitrites and Sulfites to Inhibit the Growth of *Salmonella* Enterica and *Listeria monocytogenes* in Meat Products.” *Foods* 5 (4): 74. https://doi.org/10.3390/foods5040074.

Law, D.. 2000. “The History and Evolution of *Escherichia coli* O157 and Other Shiga Toxin-Producing E. Coli.” *World Journal of Microbiology and Biotechnology* 16: 701–9. https://doi.org/10.1023/a:1008927820535.

Leistner, L.. 1985. “Hurdle Technology Applied to Meat Products of the Shelf Stable Product and Intermediate Moisture Food Types.” *Properties of Water in Foods*, 309–29. https://doi.org/10.1007/978-94-009-5103-7_19.

Leistner, L.. 2000. “Basic Aspects of Food Preservation by Hurdle Technology.” *International Journal of Food Microbiology* 55 (1-3): 181–86. https://doi.org/10.1016/s0168-1605(00)00161-6.

Leistner, L., and L. G.M. Gorris. 1995. “Food Preservation by Hurdle Technology.” *Trends in Food Science & Technology* 6 (2): 41–46. https://doi.org/10.1016/s0924-2244(00)88941-4.

Leroy, F., A.Geyzen, M. Janssens, L. De Vuyst, and P. Scholliers. 2013. “Meat Fermentation at the Crossroads of Innovation and Tradition: A Historical Outlook.” *Trends in Food Science & Technology* 31 (2): 130–37. https://doi.org/10.1016/j.tifs.2013.03.008.

Low, J.C., and W. Donachie. 1997. “A Review of *Listeria monocytogenes* and Listeriosis.” *The Veterinary Journal* 153 (1): 9–29. https://doi.org/10.1016/s1090-0233(97)80005-6.

MacDonald, I. A. R., I. M. Gould, and J. Curnow. 1996. “Epidemiology of Infection due to *Escherichia coli* O157: A 3-Year Prospective Study.” *Epidemiology and Infection* 116 (3): 279–84. https://doi.org/10.1017/s0950268800052584.

Machha, A., and A. N. Schechter. 2011. “Dietary Nitrite and Nitrate: A Review of Potential Mechanisms of Cardiovascular Benefits.” *European Journal of Nutrition* 50 (5): 293–303. https://doi.org/10.1007/s00394-011-0192-5.

Maddock, R. 2015. “US Products- Dry Sausage.” In *Handbook of Fermented Meat and Poultry*, 295–98. Chichester, West Sussex, Uk ; Hoboken, Nj: Wiley, Blackwell.

McKinney, S., C. Cutter, and J. Campbell. 2017. “Pathogen Reductions during Traditional Fermentation and Drying of Pork Salamis.” *Meat and Muscle Biology* 1 (3): 129–29. https://doi.org/10.22175/rmc2017.122.

Mead, P. S., and P. M. Griffin. 1998. “*Escherichia coli* O157:H7.” *The Lancet* 352 (9135): 1207–12. https://doi.org/10.1016/S0140-6736(98)01267-7.

Mead, P. S., L. Slutsker, P. M. Griffin, and R. V. Tauxe. 1999. “Food-Related Illness and Death in the United States.” *Emerging Infectious Diseases* 5 (6): 841–42. https://doi.org/10.3201/eid0506.990625.

Milkowski, A., H. K. Garg, J. R. Coughlin, and N.S. Bryan. 2010. “Nutritional Epidemiology in the Context of Nitric Oxide Biology: A Risk–Benefit Evaluation for Dietary Nitrite and Nitrate.” *Nitric Oxide* 22 (2): 110–19. https://doi.org/10.1016/j.niox.2009.08.004.

Moss, G.P., S. Smith, and D. Tavernier. 2019. “Glossary of Class Names of Organic Compounds and Reactivity Intermediates Based on Structure (IUPAC Recommendations 1995).” *Pure and Applied Chemistry* 67 (8-9): 1307–75. https://doi.org/10.1351/pac199567081307.

Mueller, Matthew, and Christopher R. Tainter. 2023. “*Escherichia coli*.” *Www.ncbi.nlm.nih.gov*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK564298/?report=printable.

Murano, E. A., H. R. Cross, and Penny K Riggs. 2018. “The Outbreak That Changed Meat and Poultry Inspection Systems Worldwide.” *Animal Frontiers: The Review Magazine of Animal Agriculture* 8 (4): 4–8. https://doi.org/10.1093/af/vfy017.

Murphy, R. S., C. J. Sadler, and W. J. Blot. 1998. “Trends in Cured Meat Consumption in Relation to Childhood and Adult Brain Cancer in the United States.” *Food Control* 9 (5): 299–305. https://doi.org/10.1016/s0956-7135(98)00020-6.

Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. “A Disease of Rabbits Characterised by a Large Mononuclear Leucocytosis, Caused by a Hitherto Undescribed BacillusBacterium Monocytogenes (N.sp.).” *The Journal of Pathology and Bacteriology* 29 (4): 407–39. https://doi.org/10.1002/path.1700290409.

National Institutes of Health (NIH), and National Heart, Lung, and Blood Institute. 2022. “Thrombotic Thrombocytopenic Purpura (TTP) | NHLBI, NIH.” Nih.gov. March 24, 2022. https://www.nhlbi.nih.gov/health/thrombotic-thrombocytopenic-purpura.

Nielsen, B. 2004. “*Salmonella* Spp.” In *Encyclopedia of Meat Science*, edited by Werner K. Jensen, Carrick Devine, and Michael Dikeman. Elsevier Ltd.

O'Brien, A. D, T. A Lively, M. E Chen, S. W Rothman, and S. B Formal. 1983. "*Escherichia coli* 0157:H7 strains associated with haemorrhagic colitis in the united states produce a shigella dysenteriae 1 (shiga) like cytotoxin." *The Lancet* 321 (8326): 702. https://doi.org/10.1016/s0140-6736(83)91987-6.

Ooi, S. T., and B. Lorber. 2005. "Gastroenteritis due to *Listeria monocytogenes*." *Clinical Infectious Diseases* 40 (9): 1327–32. https://doi.org/10.1086/429324.

Pearson, A.M., and T.A. Gillett. 2012. *Processed Meats*. 3rd ed. Springer.

Pegg, R. B., and F. Shahidi. 2004. *Nitrite Curing of Meat*. John Wiley & Sons.

Pirie, J. H. 1940. "The Genus Listerella Pirie." *Science* 91 (2364): 383–83. https://doi.org/10.1126/science.91.2364.383.

Pizarro-Cerdá, J., A. Kühbacher, and P. Cossart. 2012. "Entry of *Listeria monocytogenes* in Mammalian Epithelial Cells: An Updated View". *Cold Spring Harbor Laboratory Press*. https://doi.org.10.1101/cshperspect.a010009.

Rice, K. M., And M. D. Pierson. 1982. "Inhibition of *Salmonella* by Sodium Nitrite and Potassium Sorbate in Frankfurters." *Journal of Food Science* 47 (5): 1615–17. https://doi.org/10.1111/j.1365-2621.1982.tb04995.x.

Riordan, D. C. R., G. Duffy, J. J. Sheridan, B., S. Eblen, R. C. Whiting, I. S. Blair, and D. A. Mcdowell. 1998. "Survival of *Escherichia coli* O157:H7 during the Manufacture of Pepperoni." *Journal of Food Protection* 61 (2): 146–51. https://doi.org/10.4315/0362-028x-61.2.146.

Rivera, N., M. Bunning, and J. Martin. 2019. "Uncured-Labeled Meat Products Produced Using Plant-Derived Nitrates and Nitrites: Chemistry, Safety, and Regulatory Considerations."

Journal of Agricultural and Food Chemistry 67 (29): 8074–84. https://doi.org/10.1021/acs.jafc.9b01826.

Román, S., L. M. Sánchez-Siles, and M. Siegrist. 2017. "The Importance of Food Naturalness for Consumers: Results of a Systematic Review." Trends in Food Science & Technology 67 (September): 44–57. https://doi.org/10.1016/j.tifs.2017.06.010.

Sauer, C. J., J. Majkowski, S. Green, and R. Eckel. 1997. "Foodborne Illness Outbreak Associated with a Semi-Dry Fermented Sausage Product." Journal of Food Protection 60 (12): 1612–17. https://doi.org/10.4315/0362-028X-60.12.1612.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. "Foodborne Illness Acquired in the United States—Major Pathogens." *Emerging Infectious Diseases* 17 (1): 7–15. https://doi.org/10.3201/eid1701.p11101.

Schouten, R., and K.Loria. 2021. "Slideshow: Pandemic Propels Popularity of Charcuterie." Www.foodbusinessnews.net. January 20, 2021. https://www.foodbusinessnews.net/articles/17730-slideshow-pandemic-propels-popularity-of-charcuterie.

Schrader, K. 2018. "Investigating the Control of *Listeria monocytogenes* on Uncured, No-Nitrateor-Nitrite-Added Meat Products." PhD Dissertation, Iowa State University.

Schultz, M. 2008. "Photo Quiz." Centers for Disease Control and Prevention. December 2008. https://wwwnc.cdc.gov/eid/article/14/12/08-1188_article.

Schwing, J., and R. Neidhardt. 2007. "North European Products." In *Handbook of Fermented Meat and Poultry*, 349–57. Ames, Iowa: Blackwell Publishing Professional.

Sebranek, J. G., A. L. Jackson-Davis, K. L. Myers, and N. A. Lavieri. 2012. "Beyond Celery and Starter Culture: Advances in Natural/Organic Curing Processes in the United States." Meat Science 92 (3): 267–73. https://doi.org/10.1016/j.meatsci.2012.03.002.

Sebranek, J. G., and J. N. Bacus. 2007. "Cured Meat Products without Direct Addition of Nitrate or Nitrite: What Are the Issues?" *Meat Science* 77 (1): 136–47. https://doi.org/10.1016/j.meatsci.2007.03.025.

Sindelar, J. J., J. C. Cordray, D. G. Olson, J. G. Sebranek, and J. A. Love. 2007A. "Investigating Quality Attributes and Consumer Acceptance of Uncured, No-Nitrate/Nitrite-Added Commercial Hams, Bacons, and Frankfurters." Journal of Food Science 72 (8). https://doi.org/10.1111/j.1750-3841.2007.00486.x.

Sindelar, J.J., J.C. Cordray, J.G. Sebranek, J.A. Love, and D.U. Ahn. 2007b. "Effects of Varying Levels of Vegetable Juice Powder and Incubation Time on Color, Residual Nitrate and Nitrite, Pigment, PH, and Trained Sensory Attributes of Ready-To-Eat Uncured Ham." *Journal of Food Science* 72 (6): S388–95. https://doi.org/10.1111/j.1750-3841.2007.00404.x.

Sindelar, J. J., and A. L. Milkowski. 2011. "Sodium Nitrite in Processed Meat and Poultry Meats: A Review of Curing and Examining the Risk/Benefit of Its Use." American Meat Science Association.

Slutsker, L., A. A. Ries, K. D. Greene, J. G. Wells, L. Hutwagner, and P. M. Griffin. 1997. "*Escherichia coli* O157: H7 Diarrhea in the United States: Clinical and Epidemiologic Features." *Annals of Internal Medicine* 126 (7): 505. https://doi.org/10.7326/0003-4819-126-7-199704010-00002.

Sperber, W. H. 1983. "Influence of Water Activity on Foodborne Bacteria — a Review1." *Journal of Food Protection* 46 (2): 142–50. https://doi.org/10.4315/0362-028x-46.2.142.

Sullivan, G.. 2011. “Naturally Cured Meats: Quality, Safety, and Chemistry.” PhD Dissertation, Iowa State University.

Sullivan, G. A., A. L. Jackson-Davis, S. E. Niebuhr, Y. Xi, K. D. Schrader, J. G. Sebranek, and J. S. Dickson. 2012. “Inhibition of *Listeria monocytogenes* Using Natural Antimicrobials in No-Nitrate-Or-Nitrite-Added Ham.” *Journal of Food Protection* 75 (6): 1071–76. https://doi.org/10.4315/0362-028x.jfp-11-511.

Tauxe, R. V. 1991. “*Salmonella*: A Postmodern Pathogen.” Journal of Food Protection 54 (7): 563–68. https://doi.org/10.4315/0362-028X-54.7.563

Tilden, J., W. Young, A. M. McNamara, C. Custer, B. Boesel, M. A. Lambert-Fair, J. Majkowski, et al. 1996. “A New Route of Transmission for *Escherichia coli*: Infection from Dry Fermented Salami.” *American Journal of Public Health* 86 (8): 1142–45. https://doi.org/10.2105/ajph.86.8_pt_1.1142.

Tobin, B. D., Maurice G. O’Sullivan, R. Hamill, and J. P. Kerry. 2014. “European Consumer Attitudes on the Associated Health Benefits of Neutraceutical-Containing Processed Meats Using Co-Enzyme Q10 as a Sample Functional Ingredient.” *Meat Science* 97 (2): 207–13. https://doi.org/10.1016/j.meatsci.2014.01.010.

Toldrá, F.. 2002. *Dry-Cured Meat Products*. Connecticut, USA: Food & Nutrition Press, Inc.

Tompkin, R. Bruce. 2005. “Antimicrobials in Good.” In *Antimicrobials in Food*, edited by P. Michael Davidson, John N. Sofos, and A.L. Branen. Taylor & Francis.

Tortora, G.J, B. R. Funke, and C. L. Case. 2019. *Microbiology: An Introduction*. 13th ed. Boston: Pearson.

Työppönen, S., E. Petäjä, and T. Mattila-Sandholm. 2002. "Bioprotectives and Probiotics for Dry Sausages." *International Journal of Food Microbiology* 83 (3): 233–44. https://www.sciencedirect.com/science/article/pii/S0168160502003793?via%3Dihub.

U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS). 2006. "Pathogen Modeling Program (PMP) Online." Pathogen Modeling Program Online. 2006. https://pmp.errc.ars.usda.gov/default.aspx.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 1995. "Processing Inspectors' Calculations Handbook." https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/7620.3.pdf.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2001. "Performance Standards for the Production of Processed Meat and Poultry Products. Proposed Rule. Federal Register."

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2005. "Food Standards and Labeling Policy Book." August 2005.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2016. "Ready to Eat and Shelf Stable Products Process Familiarization." https://www.fsis.usda.gov/sites/default/files/media_file/2021-02/33_IM_RTE_SS_Process.pdf.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2020. "FSIS Product Categorization." https://www.fsis.usda.gov/sites/default/files/media_file/2020-08/Product-Categorization.pdf.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2021a. Fratelli Beretta USA, Inc. Recalls Ready-to-Eat Uncured Antipasto Meat Products Due to Possible *Salmonella* Contamination | Food Safety and Inspection Service. November 18,

2021. https://www.fsis.usda.gov/recalls-alerts/fratelli-beretta-usa-inc.-recalls-ready-eat-uncured-antipasto-meat-products-due.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2021b. "FSIS Cooking Guideline for Meat and Poultry Products (Revised Appendix A)." USDA-FSIS. fsis.usda.gov/sites/default/files/media_file/2021-12/Appendix-A.pdf.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2022a. *Salmonella* Outbreaks Linked to ItalianStyle Meats Outbreak Investigation After Action Review. Available at https://www.fsis.usda.gov/sites/default/files/media_file/2022-04/FSIS-After-Action-Review-2021-09_2022-01.pdf

US Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2022b. "Safe And Suitable Ingredients Used In The Production Of Meat, Poultry, And Egg Products." October 24, 2022. https://www.fsis.usda.gov/sites/default/files/media_file/2021-09/7120.1.pdf.

US Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2023. "FSIS Ready-to-Eat Fermented, Salt-Cured, and Dried Products Guideline." May 5, 2023. https://www.fsis.usda.gov/sites/default/files/media_file/documents/FSIS-GD-2023-0002.pdf

U.S. Department of Health and Human Services, and National Toxicology Program (NTP). 2001. "NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Nitrite." https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr495.pdf.

Watson, S. C., C. N. Cutter, and J. A. Campbell. 2021. "Fate of *Salmonella* Spp., *Listeria monocytogenes*, and Campylobacter Spp. During Fermentation and Drying of Duck Salami." *Food Protection Trends* 41 (4): 423. https://doi.org/10.4315/1541-9576-41.4.423.

Weyker, R. E., K. A. Glass, A. L. Milkowski, D. L. Seman, and J. J. Sindelar. 2016. "Controlling *Listeria monocytogenes* and Leuconostoc Mesenteroides in Uncured Deli-Style Turkey Breast Using a Clean Label Antimicrobial." Journal of Food Science 81 (3): M672–83. https://doi.org/10.1111/1750-3841.13232.

World Health Organization, International Agency for Research on Cancer (WHO-IARC). 2015. "IARC Monographs Evaluate Consumption of Red Meat and Processed Meat." IARC. https://www.iarc.who.int/wp-content/uploads/2018/07/pr240_E.pdf.

Yarbrough, J. M., J. B. Rake, and R. G. Eagon. 1980. "Bacterial Inhibitory Effects of Nitrite: Inhibition of Active Transport, but Not of Group Translocation, and of Intracellular Enzymes." *Applied and Environmental Microbiology* 39 (4): 831–34. https://doi.org/10.1128/aem.39.4.831-834.1980.

Zeece, M.. 2020. *Introduction to the Chemistry of Food*. Academic Press.

Zeuthen, P.. 2007. "A Historical Perspective of Meat Fermentation." In *Handbook of Fermented Meat and Poultry*. Ames, Iowa: Blackwell Publishing, Ltd.

Zou, M., J. Yang, C. Wiechers, and J. Huehn. 2020. "Acute Neonatal *Listeria monocytogenes* Infection Causes Long-Term, Organ-Specific Changes in Immune Cell Subset Composition." *European Journal of Microbiology and Immunology* 10 (2): 98–106. https://doi.org/10.1556/1886.2020.00007.

Chapter 2
Determining the Effect of Inoculum Volume and Preparation Method on Initial Bacteria Concentration in Ground Pork

Abstract

The effect of inoculum volume and preparation method on initial bacteria concentration in raw, ground pork was investigated. The research was conducted in two trials. Trial one applied surrogate organisms, *Listeria innocua* (LI) and *Escherichia coli* O157:H12 (EC), to determine the optimal ratio of overnight culture (mL) to pork (g) to achieve at least a 6 log10 CFU/g initial inoculation. Three overnight culture volumes, 0.1-, 0.5-, and 1.0-mL, of EC and LI were inoculated into 453.6 g ground pork together and separately. EC and LI did not achieve a 6 log10 CFU/g inoculation when inoculated in 0.1- and 0.5-mL increments. However, target inoculation populations of EC and LI were achieved when inoculated in 1.0mL increments, separately (EC, 7.76 $\log_{10}$ CFU/g; LI, 6.22 $\log_{10}$ CFU/g) and together (EC, 6.29 $\log_{10}$ CFU/g; LI, 6.72 $\log_{10}$ CFU/g). Trial two applied the knowledge gained in trial one and used centrifugation to obtain a concentrated pellet of EC O157:H7, *L. monocytogenes* (LM), and *Salmonella* spp. (S); this trial aimed to determine the volume (0.1mL, 0.5mL, and 1.0mL) of Buffered Peptone Water (BPW) needed to disperse the bacteria pellets in raw, ground pork effectively and achieve at least a 6 $\log_{10}$ initial inoculation. All BPW volumes applied achieved the target inoculation. There were no significant differences in EC or LM populations in the inoculated pork at any volume ($p > 0.05$). There were significant differences observed in S populations in inoculated pork when comparing 0.1mL to 0.5mL and 0.5mL to 1.0mL (0.1mL - 0.5mL, $p = 0.0250$; 0.5mL - 1.0mL, $p = 0.0497$). Based on these results, it is recommended to use 1.0mL inoculum per 453.6 g of pork and disperse centrifuged pellets with a minimum of 0.1mL BPW to achieve at least a 6 $\log_{10}$ CFU/g of each bacterium studied.

Introduction

Microbial challenge studies serve a valuable role in validating that a food manufacturing process meets compliance standards set for the product (NACMF, 2010). There are parameters that should be considered prior to conducting these studies, such as selection of appropriate pathogens or surrogates, level of ingoing inoculum, and method of inoculum preparation (IFT, 2003). Preliminary research is often conducted prior to performing a challenge studyto standardize these parameters and to ensure that they are appropriate for the food products or process being evaluated (NACMF, 2010).

The United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) requires a 5-log reduction of *Escherichia coli* O157:H7 (EC) and *Salmonella* (S) in fermented and dried sausage (USDA-FSIS, 2001; USDA-FSIS 2021). Additionally, any product that is ready-to-eat (RTE) without any additional preparation to achieve food safety by the consumer is considered adulterated if it contains or has come into direct contact with a food contact surface contaminated with *Listeria monocytogenes* (LM; 9 CFR §430.4). For these reasons, EC, LM, and S are appropriate organisms to select for a challenge study addressing fermented, dried, and RTE sausage products. As appropriate, these pathogenic organisms may be substituted by non-pathogenic surrogates. For example, *Listeria innocua* (LI) and generic, non-pathogenic strains of EC are commonly used as surrogate organisms for LM and EC, respectively (IFT, 2003).

This research was conducted to gain knowledge that would later be applied to a challenge study addressing the survival of EC, LM, and S in cured, fermented, and dried meat products. It is critical that studies addressing products with high target reductions (i.e., 5-log reduction of EC

and S in fermented and dried sausage) achieve an initial inoculation high enough to demonstrate the required reduction, quantify survivors, and display high levels of inactivation in each organism applied (IFT, 2003). Although applying multiple organisms and organism strains in the same study is preferred to minimize the number of studies and incorporate variability, there may be antagonistic effects across organisms. If this effect exists, inappropriate inhibition of an organism may be observed. Therefore, it is critical to screen target organisms for antagonism prior to beginning a challenge study (NACMF, 2010). Accordingly, the first objective of this preliminary work was to determine the ratio of non-pathogenic bacteria inocula (mL) to pork (g) to achieve at least a 6 $\log_{10}$ CFU/g inoculation when organisms were inoculated individually and together (*Trial 1*).

The target water activity of the cured, fermented, and dried sausage products in the sequential research will be less than or equal to 0.88. Therefore, minimizing the amount of ingoing inoculum volume critical to meet this parameter. Centrifuging overnight cultures is commonly used to minimize inoculum volume while maintaining target inoculation populations. Centrifuging creates a highly concentrated bacterial pellet which can be later resuspended in a smaller volume of liquid and then inoculated into a foodstuff (NACMF, 2010). However, it was unknown how to effectively distribute a bacterial pellet throughout a raw meat batter while minimizing the volume of ingoing liquid while maintaining high inoculation levels (> 6 LOG CFU/g). Therefore, using knowledge gained in trial 1, the second objective of this study was to determine the minimum volume of buffered peptone water (BPW) needed to effectively disperse a pellet of pathogenic bacteria throughout ground pork and achieve at least a 6 log CFU/g inoculation of each organism (*Trial 2*).

Materials and Methods

Inoculum Preparation

Trial 1- Non-pathogenic organisms, EC O157:H12 (PSU 7.11711; ground beef) and LI (PS002981; apple microbiome), were obtained from the Penn State *E. coli* Reference Center and the Pennsylvania State University Food Science Department culture collection, respectively. A single, isolated colony of each bacterium was transferred from Tryptic Soy Agar (TSA; Becton, Dickinson and Company; BD, Franklin Lakes, NJ) to 10mL of Tryptic Soy Broth (TSB; BD) and incubated at 36ºC for 24h. After incubation, overnight cultures of EC and LI were streaked for isolation onto Sorbitol MacConkey agar (SMAC; HiMedia Laboratories, LLC; HiMedia; Kelton, PA) and Modified Oxford agar (MOX; HiMedia) respectively. EC plates were incubated for 24h at 36ºC and LI plates were incubated for 48h at 28ºC, as required by sequential organism confirmation tests. Confirmation tests were performed with rapid latex slide agglutination tests after incubation (EC & LI: Microgen® Bioproducts; Hardy Diagnostics; Santa Maria, CA). Isolated colonies from the same selective media plates were inoculated into 10 mL of TSB and incubated at 36ºC for 24h to obtain bacterial cell populations of about 8 $\log_{10}$ CFU/mL (adapted from USDA-FSIS Microbiology Laboratory Guidebook, 2012).

Trial 2- EC O157:H7 isolates EDL933 (ATCC 43895; ground beef isolate), Sakai, and PA-2 **(**Hartzell, et al., 2011), LM serotypes Scott A, ½a isolate FSL R2-603 (deli meats outbreak), and serotype 4b isolate H3396 (hot dog outbreak), and S serovars Typhimurium (ATCC 14028, chicken organs), Montevideo isolate Smvo13, and Derby (ATCC 7378; human isolate) were received from the American Type Culture Collection (ATCC; Manassa, VA), The Microbiology Culture Collection at the Pennsylvania State University Department of Food Science, and the Centers for Disease Control and Prevention (CDC; Atlanta, GA). Frozen

cultures were transferred to TSB and aerobically incubated at 36ºC for 24h. After incubation, EC, LM, and S were streaked for isolation onto SMAC with Cefixime-Tellurite supplement (CT-SMAC; HiMedia), MOX, and Xylose Lysine Deoxycholate (XLD; Criterion™, Hardy Diagnostics), respectively. EC and S were incubated for 24h at 36ºC and LM plates were incubated at 28ºC for 48h according to confirmation test recommendations. Confirmation tests on isolated colonies of each pathogen strain were performed following incubation (EC, S, & LM: Microgen® Bioproducts; Hardy Diagnostics).

To prepare overnight cultures, a single, isolated colony of each pathogen strain was inoculated into 10 mL of TSB and incubated at 36ºC for 24h. After incubation, three- 1 mL aliquots of each pathogen strain were pipetted into individual, sterile test tubes and centrifuged at 20ºC for 5 minutes at approximately 11,000 x g (Avanti JLA-16.250; Beckman Coulter, Pasadena, CA). After centrifugation, the supernatant was disposed of, and the remaining culture pellets for each pathogen strain were resuspended, within organisms, with 0.1, 0.5, or 1.0 mL of BPW (BD). All pathogens were kept separately until later dispersal in ground pork, resulting in three tubes each of EC, LM, and S for each BPW volume.

Pork Inoculation and Microbial Analysis

Trial 1- Lean, ground pork was purchased from a local retailer and weighed into seven-453.6g batches. EC and LI overnight cultures (prepared as described above) were aseptically inoculated into a batch of pork as one of three volumes, 0.1, 0.5, or 1.0 mL. The organisms were inoculated individually, apart from an additional treatment, in which 1.0 mL of both organisms were added to the same 453.6g pork. Following inoculation, the pork was hand massaged for 15 minutes then allowed to rest for 30 minutes to distribute and ensure the attachment of the

bacteria to the meat surface.

Samples were collected immediately after the resting period. Sixty-gram pork samples from each treatment were diluted with 240 g of BPW (BD) to create a 1:5 dilution. The diluted pork samples were stomached at 230 rpm for 30 s (Stomacher® 400 Circulator; Seward Limited; West Sussex, UK), after which they were serially diluted into 9 mL BPW. Aliquots (0.1mL) of dilutions were spread plated in duplicate onto SMAC and MOX to determine the initial log CFU/g inoculation achieved by each volume of bacteria inoculated into the pork. Plates were incubated according to the USDA Microbiological Laboratory Guidebook (SMAC for 24h at 36ºC, and MOX for 48h at 36ºC; USDA-FSIS, 2022). After the appropriate incubation period, typical EC and LI colonies were counted.

Trial 2- Ground pork trim was received from the Pennsylvania State University Meats Laboratory (University Park, PA) and divided into three 453.6g batches. Each batch of pork was randomly assigned to one of the three volumes of BPW. Each resuspended pathogen, prepared as described above, was inoculated into its assigned batch of pork, and hand massaged, for 15 minutes. The massaged, inoculated pork was allowed to rest for 30 minutes for bacterial attachment to meat.

Sixty-gram samples were collected from each inoculated pork batch immediately after resting. To create a 1:5 dilution, 240g of BPW was added to the pork sample then stomached at 230 rpm for 30s. The resulting stomachate was then serially diluted into 9 mL BPW, from which 0.1 mL aliquots were spread plated onto CT-SMAC, MOX and XLD to determine the initial $\log_{10}$ CFU/g populations achieved by EC, LM, and S, respectively. Plates were incubated as detailed by the USDA Microbiological Laboratory Guidebook. Typical EC, LM, and S colonies were enumerated after each appropriate incubation time.

Statistical Analysis

Trials 1 & 2- Enumerated plate duplicates were averaged and transformed to $\log_{10}$ CFU/g. Statistics were analyzed using a general linear model procedure in Statistical Analysis Software (Version 9.4 OnDemand, SAS Institute Inc., Cary, NC). Unique comparisons were made between treatments within organisms in each treatment. A significance level of $P < 0.05$ was used. These experiments were executed in a single replicate.

Results

Trial 1- Target inoculation populations of EC and LI were achieved when inoculated in 1.0 mL increments, separately (EC, 7.76 $\log_{10}$ CFU/g; LI, 6.22 $\log_{10}$ CFU/g) and together (EC, 6.29 $\log_{10}$ CFU/g; LI, 6.72 $\log_{10}$ CFU/g). No significant differences were observed between the 0.1- and 0.5-mL inoculations of EC ($p = 0.1534$). EC populations increased by 1.88 $\log_{10}$ CFU/g between the 0.5- and 1.0-mL inoculations ($p = .008$) and decreased by 1.47 $\log_{10}$ CFU/g when inoculated with LI ($p = 0.0155$). LI increased by 0.53 and 0.46 $\log_{10}$ CFU/g when added in 0.5- and 1.0-mL increments, respectively (0.1–0.5 mL, $p = 0.0074$; 0.5–1.0 mL, $p = 0.0114$). Populations of LI increased significantly when inoculated with EC ($p = 0.0094$; Table 2.1).

Trial 1 Results

Volume Added	*E. coli* O157:H12	*Listeria innocua*
0.1	5.32 ± 0.15^{a}	5.23 ± 0.04^{a}
0.5	5.88 ± 0.04^{a}	5.76 ± 0.16^{b}
1.0	7.76 ± 0.31^{c}	6.22 ± 0.01^{c}
1.0*	6.29 ± 0.11^{b}	6.72 ± 0.03^{d}

Table 2.1 Average populations ($\log_{10}$ CFU/g) of *E. coli* O157:H12 and *Listeria innocua* when inoculated at various volumes (mL) per 453.6 g ground pork. Different letters within organism signify statistical differences ($^{a\text{-}d}$) ($p<0.05$). "*" = organisms inoculated together.

Trial 2– All pathogens at all BPW dispersion volumes achieved and exceeded a 6-$\log_{10}$ CFU/g inoculation in 453.6 g of ground pork. None of the BPW volumes used with EC and LM pellet dispersion were significantly different ($p > 0.05$). Significant differences were observed, however, in S between all volumes of BPW apart from the 0.1 to 1.0 mL comparison (0.1-0.5, $p = 0.0250$; 0.5-1.0, $p = 0.0497$; 0.1-1.0, $p = 0.1998$) (Table 2.2).

Trial 2 Results

Volume Added	*E. coli* O157:H7	*L. monocytogenes*	*Salmonella* spp.
0.1	6.21± 0.16[a]	6.80± 0.15[a]	6.20± 0.25[a]
0.5	6.78± 0.06[a]	6.53± 0.00[a]	6.88± 0.21[c]
1.0	6.48± 0.29[a]	6.55± 0.01[a]	6.44± 0.06[b]

Table 2.2 Average populations ($\log_{10}$ CFU/g) of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. after centrifugation, dispersion with various volumes (mL) of BPW, and inoculation in 453.6 g ground pork. Different letters within organism signify statistical differences (a-c) ($p<0.05$).

Discussion

A primary component of an inactivation challenge study is quantifying the amount of bacteria reduction in the product, making high initial inoculation populations critical (IFT, 2003). The aimed inoculations in both trials of this research were based on the USDA-FSIS guidelines for a 5-log reduction of S and EC in RTE, shelf-stable, and fermented and dried meat products (USDA-FSIS, 2001; USDA-FSIS 2021). However, inoculum volumes and methods that achieved target inoculations (e.g., 1 mL of inoculum per 453.6 g pork) may be applied to challenge studies addressing food products outside of this category that also require high levels of reduction. Nevertheless, researchers should conduct their own preliminary research prior to a challenge study to standardize the inoculum in the specific food matrix being studied (NACMF,

2010).

Results from trial one demonstrated that inoculum should be added as 1 mL inoculum to every 453.6 g of pork to achieve a target inoculation of 6 to 7 $\log_{10}$ CFU/g. Applying this much liquid inoculum to a meat system with the intent of drying to a low water activity may result in the inability to dry the product sufficiently. The results from trial two, however, displayed the ability to apply the knowledge gained from trial one while minimizing the amount of ingoing liquid to the meat system. Although all BPW volumes achieved the target inoculation levels, the 0.5 mL BPW treatment achieved the highest inoculation levels for EC and S at 6.78 $\log_{10}$ CFU/g and 6.88 $\log_{10}$ CFU/g, respectively. Conversely, the 0.1 mL BPW treatment achieved the highest inoculation level for LM at 6.80 $\log_{10}$ CFU/g. To minimize the amount of liquid, it is recommended that 0.1 mL of BPW be used to disperse pellets of bacteria throughout a meat system.

This research was conducted in a single repetition of each treatment group in each trial. Typically, it is desirable to take samples that are least duplicate or triplicate for each sampling time (IFT, 2003). While not compared statistically, trial two achieved similar inoculation populations as trial one by applying the knowledge gained in the latter. The comparability of results in each trial displays the utility of the results in trial one. Additionally, none of the treatments in either trial in which organisms were inoculated in the same meat system showed evidence of antagonism. Nonetheless, completing multiple, independent repetitions and increasing the sample size of this research would add value to the results and ensure that the outcomes are reproducible.

References

Hartzell, A., C.Chen, C. Lewis, K.Liu, S. Reynolds, and E. G. Dudley. 2011. "*Escherichia coli*O157:H7 of Genotype Lineage-Specific Polymorphism Assay 211111 and Clade 8 Are Common Clinical Isolates within Pennsylvania." *Foodborne Pathogens and Disease* 8 (7): 763–68. https://doi.org/10.1089/fpd.2010.0762.

Institute of Food Technologists (IFT), and Food and Drug Administration (FDA). 2001. "Evaluation and Definition of Potentially Hazardous Foods." https://www.fda.gov/files/food/published/Evaluation-and-Definition-of-Potentially-Hazardous-Foods.pdf.

National Advisory Committee on Microbiological Criteria for Foods. 2010. "Parameters for Determining Inoculated Pack / Challenge." *Journal of Food Protection* 73 (1): 140–202.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2012. "Microbiology Laboratory Guidebook | Food Safety and Inspection Service." Usda.gov. 2012. https://www.fsis.usda.gov/news-events/publications/microbiology-laboratory-guidebook.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2001. "Performance Standards for the Production of Processed Meat and Poultry Products. Proposed Rule. Federal Register." 2001.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2021. "FSIS Cooking Guideline for Meat and Poultry Products (Revised Appendix A)." USDA-FSIS. fsis.usda.gov/sites/default/files/media_file/2021-12/Appendix-A.pdf.

Chapter 3
Survival of Surrogate Organisms During Fermentation and Drying of Salami Cured with Various Sources of Nitrite

Abstract

Nitrite is an ingredient added to processed meat products for its contributions to product quality (i.e., color, flavor, and antioxidant activity) and safety. Nitrite is well-documented as an inhibitor against pathogens like *Clostridium* spp., pathogenic *Escherichia coli, Listeria monocytogenes,* and *Salmonella* spp. Despite the benefits of nitrite addition in processed meat products, the ingredient has unfortunately been correlated with increased risk of cancers. Additionally, consumer interest in clean label products has increased. Therefore, an increase in the utilization of natural nitrite alternatives has been observed. Natural nitrite alternatives are sourced from fruit or vegetables that are high, but variable, in nitrate content. The variability of the natural nitrite alternatives has raised concerns of the efficacy of the ingredients as pathogen inhibitors in processed meat products. Although studies have investigated pathogen inhibition by natural nitrite alternatives, there is limited published work that investigates the efficacy of these ingredients in a fermented and dried product manufactured without a thermal lethality step. Therefore, a study was designed to determine the survival of two surrogate organisms, *E. coli* O157:H12 (EC) and *L. innocua* (LI), during fermentation and drying of salami.

Three treatments were conducted in three independent replications. Treatments were positive control (PC; 6.25% sodium nitrite), Swiss chard powder (SC), and celery powder (CP). All treatments were formulated to 156 ppm ingoing sodium nitrite based on manufacturer's recommendations. Salami were manufactured using ground (~5mm) pork shoulder butts (IMPS 406), which were inoculated with 14 mL each of EC and LI 24 h cultures. After distribution of the 24 h cultures in the ground pork, dry ingredients and a starter culture were mixed into the inoculated pork. The mixed meat batter was stuffed into 55 mm fibrous casings, hung into a commercial drying cabinet to ferment (pH < 5.0) and dry to a target water activity (a_w) of less

than or equal to 0.88. Three salami per treatment replication (n=9; N=216) were randomly evaluated for pH, a_w, and surrogate growth and survival on day (D) 0, 1, 2, 3, 7, 14, 21, and 28. Comparisons of surrogate populations between days within a treatment were made using a General Linear Model procedure in SAS. Comparisons between treatments on a day within a surrogate were made with a Mixed Model Procedure in SAS which included pH and a_w as fixed effects.

Salami in this experiment never achieved the target a_w. Furthermore, EC population differences between treatments were not significantly impacted by a_w, whereas LI population differences between treatments were. Neither LI nor EC population differences between treatments were significantly impacted by pH. SC and CP achieved greater total reductions of EC and LI by D28 than those in PC. Total reductions of EC and LI in PC were both 0.52 $\log_{10}$ CFU/g. SC achieved total reductions of 1.76 and 1.94 $\log_{10}$ CFU/g for EC and LI, respectively. Lastly, total reductions of EC and LI in CP were 0.93 and 1.51 $\log_{10}$ CFU/g respectively. More research is needed to determine the efficacy of natural nitrite alternatives on the inhibition of pathogenic organisms in fermented and cured salami manufactured without a thermal lethality step.

Introduction

Nitrates and nitrites are added to processed meat products as multifunctional ingredients that contribute to cured meat qualities such as color, flavor, and antioxidant activity (Sindelar and Milkowski, 2011). Additionally, nitrite is an effective antimicrobial against spoilage and pathogenic organisms in cured meat products. Although most acknowledged for inhibition of *Clostridium* species *spore germination,* growth and toxin production, nitrite has also been shown to inhibit *Listeria spp*., *Escherichia coli*, *Salmonella spp*., and others (Milkowski, et al., 2010). The impact of nitrite inclusion is significant in all cured, processed meat products; however, its role as an antimicrobial is especially critical in ready-to-eat (RTE) products that are processed without a thermal lethality step.

Salami is a type of cured, fermented, and dried meat product that is traditionally uncooked and consumed raw (Maddock, 2015). Products consumed raw, like salami, rely on salt, acidulation or other by-products produced during fermentation via starter cultures, drying, and the addition of nitrite as hurdles that inhibit pathogen growth (Feiner, 2006). Although uncommon, salami has been associated with outbreaks of foodborne pathogens. In 1994, 23 individuals were infected with *E. coli* O157:H7 after consuming raw, dry-cured salami (CDC, 1995). After this outbreak, the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) developed processing options to achieve a 5 $\log_{10}$ reduction of *E. coli* O157:H7 in fermented sausages (USDA-FSIS, 2001). More recently, there were two outbreaks of *Salmonella* spp. related to naturally cured, fermented, and dried meat products that were manufactured with Swiss chard (USDA-FSIS, 2021 & 2022). Together, these outbreaks resulted in 74 illnesses and 19 hospitalizations. Although fermented sausage products have yet to be associated with listeriosis outbreaks, *L. monocytogenes* is the most frequently isolated

pathogen in the USDA-FSIS monitoring program for fermented sausages (FSIS, 2001). These outbreaks and associations with foodborne pathogens demonstrate the cruciality of investigating the efficacy of the meat industry's manufacturing processes for controlling pathogens when producing raw, RTE meat products, especially when using alternative nitrite sources.

Researchers have investigated the adequacy of pathogen control in non-heat treated, RTE products' processing procedures when cured with purified nitrite. Research by Nightingale et al., (2006) suggested that the manufacturing process evaluated did not achieve the proposed lethality performance standards for *Salmonella* in Italian-style salami products, and reduced LM by less than 1.0 $\log_{10}$ CFU/g. Alternatively, another study investigated salami casing type and a 2.5% antimicrobial solution during manufacturing of salami produced without a thermal lethality step. This study validated the safety of raw, RTE salami with and without an antimicrobial treatment (McKinney et al., 2019). There remains a knowledge gap, however, on the adequacy of these processing procedures for pathogen control in products manufactured with alternative, or natural sources of nitrites.

The application of naturally sourced nitrites in processed meat products has been observed to increase with consumers' interest in, and willingness to pay for, clean-label foods (Bizzozero, 2017; Iqbal et al., 2021). Natural nitrites used in processed meat products are typically sourced from fruits or vegetables with high concentrations of nitrate (e.g., celery or Swiss chard), which can be reduced to nitrite before or during product manufacturing (Sebranek et al., 2012). However, depending on the fruit or vegetable source, growing season and practices, and genetic factors, concentrations of nitrate in these natural sources may be variable (Kalaycıoğlu and Erim, 2019). It has been shown that although low concentrations of ingoing nitrite can achieve cured meat quality attributes (~ 40 ppm), higher concentrations are required

(120 ppm) for all products labeled “keep refrigerated” to prevent pathogenic organism growth or toxin production (USDA, 1995). Because concentrations of nitrite may vary in natural sources, and the vast antimicrobial capacity of traditional nitrite in cured processed meats, there are concerns for the pathogen inhibition capability of naturally sourced nitrites in meat (Rivera, Bunning, & Martin, 2019; Sullivan et al., 2012a).

The efficacy of naturally sourced nitrites and other clean label antimicrobials on pathogen inhibition has been well investigated on RTE deli-style products, like ham and turkey. A survey of commercially available frankfurters found that over half of the naturally cured products supported significantly more *C. perfringens* and *L. monocytogenes* growth, possibly due to the concentrations of ingoing nitrite (Sullivan et al., 2012b). Another study demonstrated that the concentration, rather than the source, of ingoing nitrite to deli turkey is the most important factor when inhibiting *L. monocytogenes* (Golden et al., 2014). McDonnell, Glass, and Sindelar (2012) displayed the efficacy of nitrite for controlling *L. monocytogenes* when evaluating natural sources of antimicrobials in alternative-cured ham, uncured roast beef, and deli-style turkey breast. The findings from these studies, and numerous others, contribute to the knowledge of natural sources of nitrite as antimicrobials in RTE deli-style products. However, little work has been done to evaluate the survival of pathogens in non-heat treated, RTE, fermented and dried meat products. Therefore, the objective of this research was to investigate the survival of surrogate organisms, *E. coli* O157:H12 and *L. innocua*, during fermentation and drying of salami manufactured with various sources of nitrite according to typical, industry processing procedures.

Materials and Methods

Inoculum Preparation

Isolated colonies of *E. coli* O157:H12 (EC; PSU 7.11711; ground beef) and *L. innocua* (LI; PS002981; apple microbiome) were received on Tryptic Soy Agar (TSA; Becton, Dickinson and Company; BD; Franklin Lakes, NJ) from the *E. coli* Reference Center and Dr. Kovac's laboratory at the Pennsylvania State University Food Science Department, respectively. Isolated colonies were independently transferred from TSA to 10 mL of Tryptic Soy Broth (TSB; BD) and incubated for 24h at 36ºC. Following incubation, overnight cultures were streaked onto Sorbitol MacConkey Agar (SMAC; HiMedia, HiMedia Laboratories, LLC; Kelton, PA) and Modified Oxford agar (MOX; HiMedia) for EC and LI isolation, respectively. EC plates were incubated for 24h at 36ºC and LI plates were incubated for 48h at 28ºC. After the appropriate incubation periods, confirmation tests were performed on two isolated colonies per plate with rapid agglutination tests (EC & LI: Microgen® Bioproducts; Hardy Diagnostics; Santa Maria, CA).

Inocula for each treatment group were prepared by inoculating 14 mL TSB with a single, isolated colony of EC and LI, independently. The inoculated TSB tubes were incubated for 24h at 36ºC to obtain a cell concentration of approximately 8 $\log_{10}$ CFU/mL (adapted from the USDA-FSIS Microbiology Laboratory Guidebook, 2012).

Salami Manufacturing and Inoculation

Pork shoulder butts (Institutional Meat Product Specifications 406) and dry ingredients were received from a regional, wholesale suppliers for all three, independent replications. The pork shoulder butts were deboned and cubed into approximately 2.54 cm x 2.54 cm cubes and

ground (GMG 180A; Hollymatic Corp.; Countryside, IL) through an approximately 5 mm plate (400 Triumph 3/16" Holes #103421; Speco Inc.; Schiller Park, IL). Ground pork was then vacuum sealed in 50.8 cm x 71.2 cm bags of 3 mil thickness (Con Yeager Spice Company; Zelienople, PA) as 6.35 kg batches and stored at ~4ºC for less than 24h.

Previously ground pork was removed from the vacuum sealed bags, transferred to a hand mixer (Hakka 15-Liter Capacity Tank Stainless Steel Manual Meat Mixer; Hakka Brothers; Hayward, CA), and inoculated with 14 mL EC and LI inocula (as prepared above). Inocula were added prior to the inclusion of dry ingredients and manually mixed into the pork thoroughly to ensure bacteria distribution. Dry ingredients with one of three curing agents, sodium nitrite, celery powder (Florida Food Products, LLC; FFP; Eustis, FL) or Swiss chard powder (FFP), depending the treatment group, were added on a per weight basis to the inoculated meat block (Table 3.1). Curing agents were formulated to 156 ppm of ingoing nitrite based on the manufacturer's recommendations. After dry ingredient distribution, 3g Safepro® B-LC 007 starter culture (CHR Hansen; Hoersholm, Denmark) combined with 32mL DI water, was mixed into the meat batter.

Treatment Formulations

Ingredient	Positive Control	Celery Powder	Swiss Chard
Pork Shoulder Butt (IMPS 406A)	96.20%	95.38%	95.38%
Salt	2.31%	2.29%	2.29%
Dextrose	0.58%	0.57%	0.57%
White Pepper	0.25%	0.25%	0.25%
Starter Culture + DI Water	0.53%	0.53%	0.53%
Granulated Garlic	0.21%	0.21%	0.21%
Peppercorns	0.11%	0.10%	0.10%
Curing Salt (6.25% NaNO2)	0.24%		
Celery Powder		0.66%	
Swiss Chard			0.66%
Total	100%	100%	100%

Table 3.1 Treatment formulations in percentages of total formulation.

After mixing, the salami meat blends were hand stuffed (Model MF-15V; Walton's Incorporated; Wichita, KS) into 55 mm permeable, fibrous casings (Globe Casings; Carlstadt, NJ) to approximately 255 g. Raw salami were immediately hung in a drying cabinet (AS50; Impianti Condizionamento Salumifici; Camposanto, Modena, Italy) to begin fermentation**.** Table 3.2 demonstrates the drying cabinet inputs for temperature, relative humidity (RH) and time for each phase. Salamis were fermented to a target pH of 5.0 (~72hr) and dried to a target water activity (a_w) (of 0.88. Salamis were sprayed with distilled white vinegar on D7 to inhibit mold (5% acidity; Wegmans Food Markets, Inc.; Rochester, NY).

Drying Cabinet Program

Phase	Min. Temperature	Max. Temperature	Min. Relative Humidity	Max. Relative Humidity	Time
Static Cooling	6	8	0	0	5
Hot Drip	24	26	0	0	36
Drying	24	26	55	65	12
Drying	22	24	60	60	12
Drying	20	22	65	75	12
Drying	18	20	68	78	24
Drying	16	18	72	80	24
Drying	14	16	75	82	24
Seasoning	12	14	75	80	24
Seasoning	12	14	77	85	21

Table 3.2 Drying cabinet program. Temperatures are in ºC. Relative humidity is in percentages. Relative humidity set to 0% is the ambient humidity. Time is in hours.

Microbial Analysis

Three salamis (n=9; N=216) were randomly selected from each replication to be evaluated on days 0 (raw meat blend), 1, 2, 3 and every week until day 28. Casing was removed aseptically and 20 g samples from each salami were combined with 240 g of Buffered Peptone Water (BPW; BD) in a filtered stomacher bag (BagFilter P; Interscience Laboratories Inc.; St.-Normandy, France). The diluted salami samples were then stomached at 230 rpm for 30s (Stomacher® 400 Circulator; Seward Limited; West Sussex, UK). Resulting stomachate was serially diluted using 9 mL BPW blanks. The dilutions were plated in duplicate onto SMAC and MOX, and incubated (SMAC, 24h; MOX 48h) at 36ºC for the enumeration of typical EC and LI colonies, respectively. pH (Testo 206-pH2 pH Meter; Testo, Inc.; Sparta, NJ) and a_w (AquaLab Water Activity Meter, Series 4TE; Decagon Devices, Inc.; Pullman, WA) measurements were also taken on each salami at each sampling time.

Statistical Analysis

The design of this experiment was completely randomized. Bacterial populations were averaged on a day within treatment, converted to $\log_{10}$ CFU/g before any statistical analysis. Significant differences of mean bacteria populations between sampling days within treatments and organisms were analyzed using the General Linear Model procedure with unique comparisons (SAS OnDemand Version 9.4; SAS Institute Inc.; Cary, NC). Comparisons of treatments within organism on the same day, and the significance of fixed effects (treatment, pH, a_w, and treatment by sampling day interactions) were analyzed by a mixed model procedure using SAS. A significance level of $P < 0.05$ was used to determine significant differences in all statistical analyses.

Results

pH and a_w

All salami reached an average pH of 4.73 ± 0.17 by D3 of fermentation. pH values increased during all sampling days following fermentation. Neither EC nor LI population differences between treatments were significantly impacted by the pH (EC: $p = 0.3304$; LI: $p = 0.6605$).

LI populations in the salamis were found to be impacted by a_w ($p = 0.0319$); However, EC populations were not found to not be impacted by a_w ($p = 0.0678$). The a_w for all treatments remained above the lower limit for EC growth ($a_w = 0.95$) until D7, when it decreased to an average of 0.93 ± 0.03 in all treatments. Salamis did not reach the target a_w of 0.88 by the end of sampling. Ultimate, average a_w on D28 for salamis was 0.90 ± 0.4, which is less than the lower limit for LI growth ($a_w = 0.92$).

Bacteria

Treatment groups had a significant impact on EC ($p < 0.0001$) and LI ($p = < 0.0001$) survival. Sample day and the treatment by sample day interaction also had significant impacts on both EC and LI populations in the salami ($p < 0.05$). No D0 populations were significantly different across treatments for either organism (EC: table 3.3; LI table 3.4; $p > 0.05$). Additionally, none of the treatments achieved a 5 $\log_{10}$ CFU/g total reduction of EC or LI by D28.

EC populations between D0 and 28 were significantly different for all treatments ($p < 0.05$). Celery powder had the largest total reduction of EC with a 1.76 $\log_{10}$ CFU/g reduction; Swiss chard had a reduction of 0.93 $\log_{10}$ CFU/g, and the positive control had the smallest reduction of EC with a 0.52 $\log_{10}$ CFU/g reduction. Final EC counts in celery powder on D28 were significantly different from that in the positive control ($p = 0.0079$). Final Swiss chard EC counts on D28 were not significantly different when compared to the positive control ($p = 0.2541$) and celery powder ($p = 0.1518$).

LI populations experienced a slow, but significant, decline from D0 to D28 in the positive control ($p = 0.0007$), celery powder ($p < 0.0001$), and Swiss chard ($p < 0.0001$) treatment groups. Final LI $\log_{10}$ CFU/g populations on D28 for the positive control, celery powder, and Swiss chard treatments were 5.92 ± 0.07, 4.68 ± 0.05, and 4.94 ± 017, respectively. LI populations in the positive control on D28 were significantly different when compared to those of celery powder ($p < 0.0001$) and Swiss chard ($p < 0.0001$). As was observed with EC, total reduction of LI was the greatest in salami manufactured with celery powder with a 1.94 $\log_{10}$ reduction, and the lowest in the positive control with a 0.52 $\log_{10}$ CFU/g reduction. Swiss chard had a total reduction of 1.51 $\log_{10}$ CFU/g.

***E. coli* O157:H12 Results**

Sample Day	Positive Control	Celery Powder	Swiss Chard
0	$6.02 \pm 0.13^{A, a}$	$6.38 \pm 0.07^{A, a}$	$6.08 \pm 0.04^{A, a}$
1	$6.47 \pm 0.22^{A, a}$	$6.09 \pm 0.24^{AB, a}$	$6.01 \pm 0.24^{B, a}$
2	$6.43 \pm 0.11^{A, a}$	$6.04 \pm 0.12^{A, a}$	$6.26 \pm 0.24^{A, a}$
3	$6.57 \pm 0.22^{A, a}$	$5.86 \pm 0.12^{B, a}$	$5.99 \pm 0.16^{AB, a}$
7	$5.85 \pm 0.22^{A, b}$	$5.91 \pm 0.32^{A, a}$	$5.76 \pm 0.08^{A, a}$
14	$6.49 \pm 0.32^{A, c}$	$6.00 \pm 0.13^{B, a}$	$5.53 \pm 0.15^{B, a}$
21	$6.24 \pm 0.07^{A, c}$	$6.31 \pm 0.29^{A, a}$	$5.33 \pm 0.03^{B, a}$
28**	$5.50 \pm 0.18^{A, d}$	$4.62 \pm 0.25^{B, b}$	$5.15 \pm 0.03^{AB, a}$
Total Reduction	0.52	1.76	0.93

Table 3.3 Average *E. coli* O157:H12 populations ($\log_{10}$ CFU/g ± Standard Error (SE)) and total reductions. Different lowercase letters are significantly different from the concentration on the previous day within column. Different uppercase letters are significantly different within rows ($p < 0.05$). Total reduction is the difference between sampling days 0 and 28. ** The first replication was excluded from the calculations for sampling at day 28.

***L. innocua* Results**

Sample Day	Positive Control	Celery Powder	Swiss Chard
0	6.44 ± 0.04 A, a	6.62 ± 0.04 A, a	6.45 ± 0.03 A, a
1	5.90 ± 0.03 A, b	5.94 ± 0.19 A, b	5.89 ± 0.12 A, b
2	5.78 ± 0.18 A, b	5.38 ± 0.15 B, c	5.55 ± 0.11 b, AB
3	5.41 ± 0.06 A, c	5.28 ± 0.06 A, c	5.09 ± 0.13 A, c
7	5.22 ± 0.08 A, c	5.49 ± 0.16 A, b	4.77 ± 0.07 B, d
14	5.61 ± 0.18 A, b	5.04 ± 0.18 B, c	4.80 ± 0.05 B, d
21	5.75 ± 0.06 A, b	5.97 ± 0.18 A, b	4.53 ± 0.08 B, d
28**	5.92 ± 0.07 A, b	4.68 ± 0.05 B, d	4.94 ± 0.17 B, c
Total Reduction	0.52	1.94	1.51

Table 3.4 Average *L. innocua* populations ($\log_{10}$ CFU/g ± Standard Error (SE)) and total reductions. Different lowercase letters are significantly different from the concentration on the previous day within column. Different uppercase letters are significantly different within rows ($p < 0.05$). Total reduction is the difference between sampling days 0 and 28. ** The first replication was excluded from the calculations for sampling at day 28.

Discussion

Although none of the treatments achieved a 5 $\log_{10}$ CFU/g total reduction of EC or LI, a slight reduction was observed across all groups. Therefore, the growth of both organisms was able to be suppressed. Furthermore, the positive control had the least total reduction of both organisms among the three groups in the experiment. This finding may be due to potentially variable levels of nitrite in the alternative sources compared to the standardized value in the purified (Sebranek et al., 2012). There may also be other antimicrobial properties in the alternative nitrite sources, such as increased salt content.

Food preservatives, like nitrite, are best at inhibiting microbial growth when applied in combination with several other factors. This concept is often referred to as “hurdle technology”

(Leistner & Gorris, 1995). Combining hurdles like nitrite, salt, low pH, and low a_w typically provide sufficient microbial hurdles to produce a raw, RTE salami product safely. However, the salamis in this experiment were unable to achieve the target a_w of 0.88. The inability of the salamis to dry in the allotted time may have permitted increased survival of EC and LI. Improper airflow in the drying cabinet due to overcrowding the salmi may have prevented proper drying (Campbell, 2021). Moreover, the addition of the EC and LI overnight cultures may have added a significant amount of liquid. Further research should be conducted that applies to address concerns of drying cabinet space and additional liquids.

A greater reduction of LI than EC was observed among all treatments. This observation may be due to the production of pediocin, a bacteriocin known to have strong antagonistic properties against *Listeria* spp., by *Pediococcus acidilactici*, an organism included in the starter culture used to manufacture the salami (Nielsen, Dickson, and Crouse, 1990; Khorshidian, et al., 2021). The differences observed between these organisms may also be due to nitrite possibly being more effective against LI than EC (Rahman, 2007; Sebranek and Bacus, 2007).

In cases where pathogenic organisms should not be used for product or personnel safety, a widely accepted practice is to use non-pathogenic surrogates that have the similar characteristics to the pathogen (IFT, 2003). In the case of this research, two non-pathogenic organisms, *E. coli* O157:H12 and *L. innocua,* were appropriately used in place of their pathogenic counterparts *E. coli* O157:H7 and *L. monocytogenes*. However, it is recommended that further research be conducted to evaluate the survival of Shiga Toxin producing *E. coli*, *L. monocytogenes*, and *Salmonella* spp. during fermentation, drying, and extended shelf-storage of raw, RTE, dried meat products manufactured with various sources of nitrite.

References

Bizzozero, J.. 2017. “75% of Consumers Will Pay Extra for Clean Label Ingredients.” Food & Beverage Insider. March 15, 2017. https://www.foodbeverageinsider.com/market-trends-analysis/75-consumers-will-pay-extra-clean-label-ingredients.

Campbell, J. A. 2021. “Fermentation and Thermal Processing [PowerPoint Slides].” Penn State Extension Butcher School Apprenticeship Program. The Pennsylvania State University., March 2, 2021.

Centers for Disease Control and Prevention (CDC). 1995. “*Escherichia coli* O157:H7 Outbreak Linked to Commercially Distributed Dry-Cured Salami -- Washington and California, 1994.” Www.cdc.gov. March 10, 1995. https://www.cdc.gov/mmwr/preview/mmwrhtml/00036467.htm.

Feiner, G. . 2006. “Raw Fermented Salami.” *Meat Products Handbook*, 314–75. https://doi.org/10.1533/9781845691721.2.314.

Golden, M. C., L. M. McDonnell, V. Sheehan, J. J. Sindelar, and K. A. Glass. 2014. “Inhibition of *Listeria monocytogenes* in Deli-Style Turkey Breast Formulated with Cultured Celery Powder and/or Cultured Sugar-Vinegar Blend during Storage at 4°C.” Journal of Food Protection 77 (10): 1787–93. https://doi.org/10.4315/0362-028X.JFP-14-059.

Institute of Food Technologists (IFT), and Food and Drug Administration (FDA). 2001. “Evaluation and Definition of Potentially Hazardous Foods.” https://www.fda.gov/files/food/published/Evaluation-and-Definition-of-Potentially-Hazardous-Foods.pdf.

Iqbal, J., D. Yu, M.Zubair, M. I. Rasheed, H. M. U. Khizar, and M. Imran. 2021. “Health Consciousness, Food Safety Concern, and Consumer Purchase Intentions toward Organic Food: The Role of Consumer Involvement and Ecological Motives.” *SAGE Open* 11 (2): 215824402110157. https://doi.org/10.1177/21582440211015727.

Kalaycıoğlu, Z. and F. Bedia Erim. 2019. “Nitrate and Nitrites in Foods: Worldwide Regional Distribution in View of Their Risks and Benefits.” *Journal of Agricultural and Food Chemistry* 67 (26): 7205–22. https://doi.org/10.1021/acs.jafc.9b01194.

Khorshidian, N., E. Khanniri, M. Mohammadi, A. M. Mortazavian, and M. Yousefi. 2021. “Antibacterial Activity of Pediocin and Pediocin-Producing Bacteria against *Listeria monocytogenes* in Meat Products.” *Frontiers in Microbiology* 12 (September). https://doi.org/10.3389/fmicb.2021.709959.

Leistner, L., and L. G.M. Gorris. 1995. “Food Preservation by Hurdle Technology.” *Trends in Food Science & Technology* 6 (2): 41–46. https://doi.org/10.1016/s0924-2244(00)88941-4.

Maddock, R.. 2015. “US Products - Dry Sausage.” In *Handbook of Fermented Meat and Poultry*, 295–98. Chichester, West Sussex, Uk ; Hoboken, Nj: Wiley, Blackwell.

McDonnell, L. M., K. A. Glass, and J. J. Sindelar. 2013. “Identifying Ingredients That Delay Outgrowth of *Listeria monocytogenes* in Natural, Organic, and Clean-Label Ready-To-Eat Meat and Poultry Products.” *Journal of Food Protection* 76 (8): 1366–76. https://doi.org/10.4315/0362-028x.jfp-12-501.

McKinney, S., C. Cutter, and J. Campbell. 2019. “Pathogen Reductions during Traditional Fermentation and Drying of Pork Salamis.” *Food Protection Trends* 39 (1). https://www.foodprotection.org/files/food-protection-trends/jan-feb-19-mckinney.pdf.

Milkowski, A., H.K. Garg, J. R. Coughlin, and N. S. Bryan. 2010. “Nutritional Epidemiology in the Context of Nitric Oxide Biology: A Risk–Benefit Evaluation for Dietary Nitrite and Nitrate.” *Nitric Oxide* 22 (2): 110–19. https://doi.org/10.1016/j.niox.2009.08.004.

Nielsen, J. W., J. S. Dickson, and J. D. Crouse. 1990. “Use of a Bacteriocin Produced by Pediococcus Acidilactici to Inhibit *Listeria monocytogenes* Associated with Fresh Meat.” *Applied and Environmental Microbiology* 56 (7): 2142–45. https://doi.org/10.1128/aem.56.7.2142-2145.1990.

Nightingale, K.K., H. Thippareddi, R.K. Phebus, J.L. Marsden, and A.L. Nutsch. 2006. “Validation of a Traditional Italian-Style Salami Manufacturing Process for Control of *Salmonella* and *Listeria monocytogenes*.” *Journal of Food Protection* 69 (4): 794–800. https://doi.org/10.4315/0362-028x-69.4.794.

Rahman, S. M. 2007. *Handbook of Food Preservation*. 2nd ed. Boca Raton: Crc.

Rivera, N., M.Bunning, and J. Martin. 2019. “Uncured-Labeled Meat Products Produced Using Plant-Derived Nitrates and Nitrites: Chemistry, Safety, and Regulatory Considerations.” *Journal of Agricultural and Food Chemistry* 67 (29): 8074–84. https://doi.org/10.1021/acs.jafc.9b01826.

Sebranek, J. G., A. L. Jackson-Davis, K. L. Myers, and N. A. Lavieri. 2012. “Beyond Celery and Starter Culture: Advances in Natural/Organic Curing Processes in the United States.” Meat Science 92 (3): 267–73. https://doi.org/10.1016/j.meatsci.2012.03.002.

Sebranek, J. G., and J. N. Bacus. 2007. “Cured Meat Products without Direct Addition of Nitrate or Nitrite: What Are the Issues?” *Meat Science* 77 (1): 136–47. https://doi.org/10.1016/j.meatsci.2007.03.025.

Sindelar, J. J., and A. L. Milkowski. 2011. “Sodium Nitrite in Processed Meat and Poultry Meats: A Review of Curing and Examining the Risk/Benefit of Its Use.” American Meat Science Association.

Sullivan, G. A., A. L. Jackson-Davis, S. E. Niebuhr, Y. Xi, K. D. Schrader, J. G. Sebranek, and J. S. Dickson. 2012. “Inhibition of *Listeria monocytogenes* Using Natural Antimicrobials

in No-Nitrate-Or-Nitrite-Added Ham." *Journal of Food Protection* 75 (6): 1071–76. https://doi.org/10.4315/0362-028x.jfp-11-511.

Sullivan, G. A., A. L. Jackson-Davis, K. D. Schrader, Y. Xi, C. Kulchaiyawat, J. G. Sebranek, and J. S. Dickson. 2012. "Survey of Naturally and Conventionally Cured Commercial Frankfurters, Ham, and Bacon for Physio-Chemical Characteristics That Affect Bacterial Growth." *Meat Science* 92 (4): 808–15. https://doi.org/10.1016/j.meatsci.2012.07.005.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 1995. "Processing Inspectors' Calculations Handbook." https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/7620.3.pdf.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2001. "Performance Standards for the Production of Processed Meat and Poultry Products. Proposed Rule. Federal Register." 2001. https://www.federalregister.gov/documents/2001/02/27/01-4420/performance-standards-for-the-production-of-processed-meat-and-poultry-products#:~:text=The%20proposed%20performance%20standards%20set,%2C%20plant%2Dspecific%20processing%20procedures..

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2021. Fratelli Beretta USA, Inc. Recalls Ready-to-Eat Uncured Antipasto Meat Products Due to Possible *Salmonella* Contamination | Food Safety and Inspection Service. November 18, 2021. https://www.fsis.usda.gov/recalls-alerts/fratelli-beretta-usa-inc.-recalls-ready-eat-uncured-antipasto-meat-products-due.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2022. *Salmonella* Outbreaks Linked to ItalianStyle Meats Outbreak Investigation After Action Review. Available at https://www.fsis.usda.gov/sites/default/files/media_file/2022-04/FSIS-After-Action-Review-2021-09_2022-01.pdf

Chapter 4
Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. During Fermentation, Drying, and Storage of Salami Cured with Various Sources of Nitrite

Abstract

Consumers' interest in 'clean label' foods has been associated with a decrease in purified nitrite application and an increase in meat products manufactured with naturally sourced curing agents. Natural curing agents are typically extracts of fruits or vegetables high in nitrate content. Concerns regarding pathogen inhibition in naturally cured meats have been raised due to nitrite's vast antimicrobial efficacy. Though research exists that addresses the safety of naturally cured meat, there is limited work that investigates this concern in naturally cured, fermented, and dried products. Therefore, research was conducted to determine the survival of *Escherichia coli* O157:H7 (EC), *Listeria monocytogenes* (LM), and *Salmonella* spp. (S) in raw, ready-to-eat salami manufactured with various sources of nitrite.

Three independent replications of salami were manufactured for the four treatments in this study: negative control, positive control (6.25% sodium nitrite), Swiss chard powder, and Prosur ® Natpre T-10 (dried fruit extract). The positive control and Swiss chard powder treatment groups were formulated to 156 ppm. All curing agents were formulated using manufacturers' guidelines for appropriate product utilization. To prepare salami, ground pork shoulder butts (IMPS 406) were inoculated with a three-strain culture of EC, LM and S to obtain ~7 $\log_{10}$ CFU/g. Inoculated pork was mixed with dry ingredients and starter culture, stuffed into ~55mm fibrous casings, fermented (pH < 5.0), and dried to a target water activity (a_w) of 0.88 in a commercial drying cabinet. After drying, salamis were vacuum packaged and stored at ambient temperatures (20 ± 0.003ºC). Salamis from each treatment were sampled everyday throughout fermentation, every week for seven weeks, and on day 118 for pathogen enumeration, pH, and a_w (n = 9; N = 431). Pathogen populations were analyzed using a mixed model procedure in SAS. This study demonstrated the significance of including a curing agent in combination with other

pathogen hurdles to achieve adequate control of EC, LM, and S in raw, RTE salami manufactured with various sources of nitrite.

Introduction

Salami is a Ready-to-Eat (RTE), fermented, cured, and dried meat product that typically consists of pork, beef, or a combination of both, mixed with curing agents, salt, sugars, spices, flavorings, and a starter culture (Toldrá, 2002; Maddock, 2015). After mixing meat and non-meat ingredients together, the meat batter is stuffed into either a natural or synthetic casings and then hung into an environmental chamber to ferment and dry. Traditional salamis are manufactured without a thermal lethality step. Though other meat products rely on cooking to inhibit pathogen growth and survival, the application of hurdle technology by combining low pH, redox potential, and water activity (a_w), high salt content, the addition of competitive microorganisms from a starter culture, and nitrite inclusion permit for the safe production of salami (Leistner & Gorris, 1995).

Nitrite is an essential, multifunctional ingredient in cured, processed meat products. Nitrite is responsible for many cured meat qualities such as color, flavor, and antioxidant activity, the ingredient is also well-documented as an effective inhibitor against pathogenic and spoilage organisms (Sindelar and Milkowski, 2011). Though best acknowledged for its inhibition of *Clostridium* spp. germination and toxin production, nitrite is also recognized for its control of *Escherichia coli* O157:H7 (EC), *Listeria monocytogenes* (LM), *Salmonella* spp. (S), *Staphylococcus aureus*, and *Bacillus cereus* (Milkowski et al., 2010). The effectiveness of nitrite against pathogenic organisms is increased when applied in combination with other intrinsic factors of the meat product, such as low pH and a_w, high salt concentrations, and other

antimicrobial agents (Tompkin, 2005). The mechanisms of pathogen control by nitrite are not well-understood, however, it is primarily thought that nitrite blocks essential compounds for pathogen growth and survival (e.g., oxygen, glucose, and other metabolic enxymes), penetrates cell membranes and therefore disturbing the ability of the cell to divide, and has bacteriostatic activity against pathogens (Yarbrough, et al., 1980; Buchanan et al., 1988; Duffy et al., 1994; Työppönen et al., 2002).

Unfortunately, nitrite and the consumption of cured meat products have been repeatedly associated with an increased risk for some cancer types. Specifically, the American Institute for Cancer Research (AICR) states that there is convincing evidence for an increased risk for colorectal cancer and limited suggestive evidence for an increased risk for lung, pancreatic, stomach, esophageal, and nasopharyngeal cancers due to consuming processed meats manufactured with purified nitrite sources (AICR, 2021). Processed meat products have, therefore, been classified as carcinogenic to humans based on sufficient evidence found by the International Agency for Research on Cancer (IARC), an entity of the World Health Organization (WHO). Further, the IARC has stated that consuming a portion of cured processed meats as small as 50g increases the risk of colorectal cancer by 18% (WHO-IARC; 2015).

Due to health concerns associated with nitrite consumption, and despite the vast benefits nitrite inclusion has on cured meat products, consumer demands are driving the meat industry away from the application of purified nitrites and toward “clean label” alternatives (Sebranek et al., 2012). Though “clean label” does not have a strict definition, the term is broadly understood as the ingredient statement is short, easy to read, and perceived to be non-artificial or chemical sounding (Asioli et al., 2017). Generally, consumers have been found to prefer, and are willing to pay premiums for clean label products (Grant et al., 2021). Therefore, to meet consumer

demands, many meat processors have been observed to increase the application of natural nitrite sources in processed meat products (Bizzozero, 2017; Iqbal et al., 2021).

Natural alternatives to nitrite are typically from fruits or vegetables that are naturally high in nitrate content, like celery or swiss chard (Sebranek et al., 2012). Nitrate content in natural sources can, however, vary due to seasonal, geographical, growing practice, and genetic differences (Kalaycıoğlu and Erim, 2019). The United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) states in 9 CFR § 424.21 (c) that it is recommended that natural nitrite sources be formulated to the minimum nitrite content regulated for the product being manufactured and manufactured using the minimum times for fermentation, aging, and drying. However, processors may apply natural sources of nitrite to lower levels of ingoing nitrite content as these ingredients are not approved as curing agents, rather, they are considered antimicrobials and flavorings as stated in the USDA-FSIS Directive 7120.1, "Safe and Suitable Ingredients used in the Production of Meat and Poultry products".

Furthermore, lower concentrations of ingoing nitrite, ~ 40 ppm, have been shown to achieve desired cured meat quality attributes, whereas higher concentrations, ~120 ppm, are needed to prevent pathogen growth and toxin production in products labeled "keep refrigerated" (USDA-FSIS, 1995). Given the lack of regulations for minimum ingoing nitrite in products, like salami, that have been manufactured to ensure shelf stability through a combination of pH, moisture, and appropriate packaging controls (USDA-FSIS, 1995), processors may apply natural nitrite sources in reduced amounts as a method to reduce costs of processing (Sebranek and Bacus, 2007).

Salami and other cured, fermented, and dried meat products have been identified as the causative agent in multiple foodborne outbreaks. An industry changing foodborne outbreak in

1994 was linked to the consumption of EC contaminated dry-cured salami (CDC, 1995). This outbreak led to the USDA-FSIS proposing a rule for a 5 $\log_{10}$ reduction of Shiga Toxin-producing *Escherichia coli* (STEC) in dry and semi-dry products containing beef, and S in products containing meat of other species origins (USDA-FSIS, 2001; USDA-FSIS, 2023a). LM is the most frequently isolated pathogen in the USDA-FSIS fermented sausage monitoring program (USDA-FSIS, 2001). Over 69,000 lbs. of RTE charcuterie products that were deemed adulterated because of LM contamination in late January 2023 (USDA-FSIS, 2023b). Two outbreaks of S related to naturally cured, RTE, fermented, and dried meat products occurred in late 2021. The USDA-FSIS determined that antipasto Italian-style meat products manufactured with Swiss chard as the curing agent contaminated with S was the source of the outbreak that resulted in twelve hospitalizations from August to September 2021 (USDA-FSIS, 2021; CDC, 2021a). The investigation of this outbreak found that the processor did not have scientific validation of their process achieving a 5 $\log_{10}$ reduction of S. The second outbreak of S was linked to a salami stick manufactured with Swiss chard that resulted in 34 salmonellosis infections and seven hospitalizations across 10 states (CDC, 2021b). It was concluded that this outbreak was a result of the product manufacturer not having sufficient evidence to support the adequate control of S through their fermentation and drying steps (USDA-FSIS, 2022).

Concerns regarding pathogen inhibition in naturally cured processed meat products have been raised to due the variable nitrite content in natural nitrite sources, possibility of reduced ingoing nitrite formulations, and foodborne outbreaks related to naturally cured meat products (Rivera, Bunning, & Martin, 2019). The concern of natural nitrite sources has been well investigated in other meat products like deli-style turkey (Golden et al., 2014), ham (Sullivan et al., 2012), frankfurters (Jackson et al., 2011), and bacon (Gipe, 2012). There remains a gap,

however, in scientific literature that investigates the efficacy of natural nitrite sources on pathogen inhibition in RTE, cured, fermented, and dried meat products. Therefore, the objective of this research was to determine the fate of EC, LM, and S during fermentation, drying, and extended reduced oxygen, ambient storage of salami manufactured without a thermal lethality step.

Materials and Methods

Study Groups

This research consisted of four treatment groups of salami manufactured with or without commercially available curing agents. Treatment groups were no nitrites added (negative control; NC), purified nitrite (positive control; PC), Swiss chard (SC; Florida Food Products, LLC; Eustis, FL), and Natpre T-10 Cur SB (T-10; Prosur Inc.; Naperville, IL). All treatments were performed in three, independent replications and manufactured according to industry standards for raw, RTE salami.

Culture Selection and Inoculum Preparation

Three strains each of EC, LM and S were received from the American Type Culture Collection (ATCC; Manassa, VA), Centers for Disease Control and Prevention (CDC, Atlanta Georgia), and The Microbiology Culture Collection at the Pennsylvania State University (PSU) Food Science Department (University Park, PA). EC isolates EDL933 (ATCC 43895; ground beef outbreak) Sakai, and PA-2 (Hartzell, et al., 2011), LM serotypes Scott A, 1/2a isolate FSL R2-603 (deli meats outbreak) and 4b isolate H3396 (hot dog outbreak), and S serovars Typhimurium (ATCC 14028; chicken organs), Montevideo isolate SMvo13, and Derby (ATCC

7378; human isolate) were identified for use in this study.

Cultures of each organism were stored at -80ºC prior to use. A loopful of each frozen culture was aseptically transferred to 10 mL Tryptic Soy Broth (TSB; Becton, Dickinson, and Company; BD, Franklin Lakes, NJ) and incubated aerobically at 36ºC for 24 h. After incubation, overnight cultures were streaked onto Sorbitol MacConkey Agar supplemented with Cefixime-Tellurite (CT-SMAC; HiMedia Laboratories, LLC; HiMedia; Kelton, PA), Modified Oxford Agar (MOX; HiMedia), and Xylose Lysine Deoxycholate Agar (XLD; HiMedia) for EC, LM, and S isolation, respectively. CT-SMAC and XLD were incubated at 36ºC for 24 h and MOX was incubated at 28ºC for 48 h. Resulting isolated colonies of each pathogen strain were confirmed using protein agglutination tests (EC, S, & LM: Microgen® Bioproducts; Hardy Diagnostics; Santa Maria, CA).

To prepare the inoculum, single, isolated colonies of each pathogen strain were independently inoculated, in duplicate, into 25 mL of TSB, resulting in 50 mL of overnight culture of each pathogen strain after a 24 h incubation at 36ºC. This step was done to achieve an approximate cell concentration of 8 $\log_{10}$ CFU/mL in each overnight culture (adapted from the USDA Microbiological Laboratory Guidebook, 2012). Overnight cultures were centrifuged at approximately 20ºC for 5 minutes at 11,000 x g (Avanti JLA-16.250; Beckman Coulter, Pasadena, CA). After centrifugation, the supernatant was poured off and disposed of, leaving only a Concentrated pellet of bacteria. Inoculum was prepared for inoculation by resuspending and combining remaining bacteria pellets with 2.5 mL Buffered Peptone Water (BPW; BD) within pathogen.

Salami Manufacturing and Inoculation

Pork shoulder butts (IMPS 406; sourced from local suppliers) were deboned, cubed to approximately 2.54 cm x 2.54 cm, and ground (GMG 180A; Hollymatic Corp.; Countryside, IL) to approximately 5 mm (400 Triumph 3/16" Holes #103421; Speco Inc.; Schiller Park, IL). Ground pork was vacuum sealed (50.8 cm x 71.2 cm bags of 3 mil thickness; Con Yeager Spice Company; Zelienople, PA) as 11.33 kg batches and was stored at ~4ºC for less than 24 h.

Ground pork was mixed (Hakka 15-Liter Capacity Tank Stainless Steel Manual Meat Mixer; Hakka Brothers; Hayward, CA) with EC, LM, and S inocula to obtain an inoculation concentration of ~7 $\log_{10}$ CFU/g of each pathogen in the meat batter before the addition of dry ingredients. Dry ingredients for each treatment were formulated on a meat block weight basis (table 4.1) PC was formulated to 156 ppm ingoing sodium nitrite according to regulated usage rates for comminuted and cured processed meat products (9CFR§424.21). SC was formulated based on the ingredient's standardized at 22,500 ppm sodium nitrite and manufacturers' recommendations for ingredient utilization to achieve 156 ppm ingoing nitrite (Personal Communication, February 8, 2022). T-10 was formulated to ~1% according to recommended usage rates provided from the ingredient manufacturer (Personal Communication, March 21, 2022). Ingoing salt amount in NC was adjusted to match the amount in PC. All treatments had a final salt content of 4.64 ± 0.12%. Starter culture (Safepro® B-LC 007 starter culture; CHR Hansen; Hoersholm, Denmark) combined with DI water was added to the meat batter after all dry ingredients were well-distributed.

Treatment Formulations

Ingredient	Negative Control	Positive Control	Swiss Chard	Prosur T-10
Pork Shoulder Butts (IMPS 406A)	96.29%	96.20%	95.84%	95.56%
Salt	2.54%	2.31%	2.31%	2.29%
Dextrose	0.58%	0.58%	0.58%	0.57%
White Pepper	0.25%	0.25%	0.25%	0.25%
Starter Culture	0.02%	0.05%	0.05%	0.05%
Granulated Garlic	0.21%	0.21%	0.21%	0.21%
Peppercorns	0.11%	0.11%	0.11%	0.11%
Curing Salt (6.25% NaNO2)		0.24%		
Swiss Chard			0.66%	
Prosur T-10				0.96%
Total	100%	100%	100%	100%

Table 4.1 Formulations for each treatment in percent of total batch.

Mixed meat batter was then hand stuffed (Model MF-15V; Walton's Incorporated; Wichita, KS) as individual sausages into 55-mm permeable, fibrous casings (Globe Casings; Carlstadt, NJ). Sausages from all treatments were stuffed to an average of 264.71 ± 19.79 g and average diameter of 52.09 ± 0.36 mm**.** Stuffed sausages were hung in a drying cabinet (AS50; Impianti Condizionamento Salumifici; Camposanto, Modena, Italy) for fermentation (72 h) to a target pH of 5.0 and drying to a target water activity (a_w) of 0.88 (see table 4.2 for drying cabinet program). Throughout drying, sausages were sprayed with distilled white vinegar as needed to prevent surface mold growth (5% acidity; Wegmans Food Markets, Inc.; Rochester, NY). Once meeting the target a_w (~21 d), salamis were removed from the drying cabinet, vacuum sealed (8" x 10" 3 Mil Nylon/Poly; Phoenix Scale & Food Equipment; Dallas, PA) and stored at ambient temperature (20 ± 0.003ºC).

Drying Cabinet Program

Phase	Min. Temp.	Max Temp.	Min. Humidity	Max. Humidity	Time (h)
Static Cooling	6	8	0	0	5
Hot Drip	24	26	0	0	36
Drying	24	26	55	65	12
Drying	22	24	60	70	12
Drying	20	22	65	75	12
Drying	18	20	65	75	12
Seasoning	16	18	65	73	24
Seasoning	14	16	66	73	24
Seasoning	11	13	67	72	0

Table 4.2 Drying cabinet program. Temperatures are in ºC. Humidity parameters are %relative humidity. Humidity programmed to '0' is at the same relative humidity as the environment. Phase times set to '0' run indefinitely until manually shut off.

Sampling Procedure

Salamis were randomly selected and evaluated in triplicate (n=9; N=432) on each sampling day (D): 0 (raw batter), 1, 2, 3, 7, 14, 21, 28, 35, 42, 49, and 118. Casing was removed aseptically and 20 g samples from the center of each sausage were combined then diluted with 240 g BPW, creating a 1:5 dilution, in a filtered stomacher bag (BagFilter P; Interscience Laboratories Inc.; St.-Normandy, France). The dilution was stomached at 230 rpm for 30 s (Stomacher® 400 Circulator; Seward Limited; West Sussex, UK). After stomaching, the stomachate was serially diluted in 9 mL BPW blanks to an appropriate dilution for the sampling day. Dilutions were spread plated, in duplicate, onto CT-SMAC, MOX, and XLD and incubated at 36ºC (CT-SMAC & XLD: 24 h; MOX: 48 h) for the enumeration of EC, LM, and S, respectively.

In addition to pathogen enumeration, pH (Testo 206-pH2 pH Meter; Testo, Inc.; Sparta, NJ) and a_w (AquaLab Water Activity Meter, Series 4TE; Decagon Devices, Inc.; Pullman, WA) were measured on the three salamis from each replication on every sampling day. pH was

measured from the core of the sausages. a_w was measured from a thin slice taken from the center of the sausages. Salt content was measured on D118. To measure salt content, a pulverized salami core from each treatment replication (10 g) was diluted with DI water (90 g) and boiled on a hot plate. The solution was filtered through a filter paper and a salt strip (Chloride QuanTab® Test Strips; Hach) was entered into the filtered liquid.

Statistical Analysis

Plate duplicates were averaged, and populations of EC, LM, and S were converted to $\log_{10}$ CFU/g prior to statistical analysis. Plates with no observed colony growth after incubation were assigned a concentration 0.01 $\log_{10}$ CFU/g less than their detection limit (0.40 $\log_{10}$ CFU/g) to incorporate them into the analysis. Enrichment procedures were not performed. Average pathogen populations were independently compared within treatment using a general linear model procedure with unique comparisons in a Statistical Analysis Software (SAS OnDemand Version 9.4; Sas Institute Inc.; Cary, NC). All results were analyzed using a mixed model procedure in SAS. The model included comparisons across treatments on a sampling day and a treatment group by sampling day interaction. pH and a_w were included in the model as fixed effects. Comparisons between pathogens were not made to maintain statistical power. A significance level of $P < 0.05$ was assigned to determined statistical significance in both analyses.

Results

pH and a_w

All salami treatments achieved the target pH of less than 5.0 after the first 24h of fermentation (average pH of all treatments = 4.79 ± 0.02). pH values increased gradually thereafter. The fixed effects of pH did not have a significant effect on pathogen populations in the salami throughout the duration of the study (EC: $p = 0.3562$; LM: $p = 0.4861$; S: $p = 0.6082$).

The average a_w remained above the lower a_w limit for EC and S growth ($a_w = 0.95$) until D3 in SC, and D7 in NC, PC, and T-10. The a_w of all treatment groups went below the lower limit for LM growth ($a_w = 0.92$) by D14. Salami were vacuum packaged upon meeting or going below the target a_w of 0.88, which was achieved by all salami treatment groups during week three of manufacturing (NC = 0.87; PC = 0.86; SC = 0.84; T-10 = 0.87). Final a_w on D118 for NC, PC, SC, and T-10 were 0.8332, 0.8398, 0.8251, and 0.8298, respectively. The fixed effects of a_w did not have a significant effect on pathogen populations throughout the duration of the study (EC: $p = 0.8364$; LM: $p = 0.8861$; S: $p = 0.9779$).

Bacteria

Curing agent treatment had a significant impact on all pathogen populations for the duration of the study ($p < 0.001$). Table 4.5 shows the EC populations and total reductions for each treatment group throughout the study. An increase in EC populations was observed for NC and T-10 on D3, the end of fermentation (0.08 $\log_{10}$ CFU/g and 0.24 $\log_{10}$ CFU/g, respectively). PC achieved the greatest reduction of EC populations at the end of the drying period (NC = 1.33; PC = 2.61; SC = 0.78; T-10 = 2.14). EC population reductions between D0 to D21, when salamis reached target a_w and were packaged, were: 1.33, 2.61, 0.78, and 2.14 $\log_{10}$ CFU/g for NC, PC,

SC, and T-10, respectively. EC populations on D21 in NC, 5.62 ± 0.11 $\log_{10}$ CFU/g, and SC, 5.44 ± 0.17 $\log_{10}$ CFU/g, were not significantly different ($p = 0.4628$). EC populations on D21 in PC and T-10 were 4.28 ± 0.25 $\log_{10}$ CFU/g and 4.37 ± 0.27 $\log_{10}$ CFU/g, respectively, and were not significantly different ($p = 0.7275$). Furthermore, EC in NC was significantly different from that in PC ($p < 0.0001$) and T-10 ($p = 0.002$) on D21. EC in SC on D21 was also significantly different from PC ($p = 0.001$) and T-10 ($p = 0.0031$). EC populations in NC on D49 were significantly different from all other treatment groups ($p > 0.05$). Total EC reductions between D0 and D118 for NC, PC, SC, and T-10 were 6.12, 6.50, 5.83, and 6.12 $\log_{10}$ CFU/g, respectively. All comparisons of EC populations between D0 and D118 were significant for each treatment ($p < 0.0001$). NC was the only treatment to have EC above the lower limit of detection on D118 (0.83 ± 0.28 $\log_{10}$ CFU/g).

***E. coli O157:H7* Results**

Phase	Sample Day	Negative Control	Positive Control	Swiss Chard	Prosur® T-10
Raw	0	$6.95 \pm 0.08^{A,a}$	$6.89 \pm 0.22^{AB,a}$	$6.22 \pm 0.20^{B,a}$	$6.51 \pm 0.11^{AB,a}$
Fermentation	1	$5.04 \pm 0.28^{A,b}$	$6.98 \pm 0.15^{B,a}$	$6.16 \pm 0.11^{C,a}$	$6.57 \pm 0.03^{BC,a}$
	2	$6.58 \pm 0.26^{A,a}$	$5.35 \pm 0.12^{B,b}$	$5.26 \pm 0.26^{B,b}$	$6.05 \pm 0.15^{A,b}$
	3	$7.07 \pm 0.09^{A,a}$	$6.80 \pm 0.07^{A,a}$	$4.63 \pm 0.19^{B,b}$	$6.75 \pm 0.04^{A,a}$
Drying	7	$6.11 \pm 0.12^{A,b}$	$6.58 \pm 0.11^{A,a}$	$5.94 \pm 0.04^{AB,a}$	$5.39 \pm 0.25^{B,b}$
	14	$6.57 \pm 0.03^{A,b}$	$5.07 \pm 0.16^{B,b}$	$4.11 \pm 0.39^{B,b}$	$5.73 \pm 0.22^{C,a}$
	21	$5.62 \pm 0.11^{A,c}$	$4.28 \pm 0.25^{B,c}$	$5.44 \pm 0.17^{A,a}$	$4.37 \pm 0.27^{B,b}$
Packaging	28	$4.87 \pm 0.49^{A,d}$	$3.01 \pm 0.09^{B,d}$	$3.60 \pm 0.10^{BC,b}$	$3.92 \pm 0.27^{C,b}$
	35	$3.22 \pm 0.28^{A,e}$	$3.01 \pm 0.45^{AB,d}$	$2.42 \pm 0.45^{B,c}$	$3.36 \pm 0.19^{A,c}$
	42	$2.52 \pm 0.22^{A,e}$	$2.60 \pm 0.36^{A,d}$	$2.19 \pm 0.26^{A,c}$	$2.19 \pm 0.19^{A,d}$
	49	$3.00 \pm 0.41^{A,e}$	$1.51 \pm 0.09^{B,e}$	$1.84 \pm 0.71^{B,c}$	$2.14 \pm 0.15^{B,d}$
	118	$0.83 \pm 0.28^{A,f}$	$0.39 \pm < 0.01^{A,f}$	$0.39 \pm < 0.01^{A,d}$	$0.39 \pm < 0.01^{A,e}$
	TR	**6.12**	**6.50**	**5.83**	**6.12**

Table 4.3 Average *E. coli* O157:H7 populations and reductions ($\log_{10}$ CFU/g ± Standard Error). Sampling days within a column that have a different lowercase letter than the previous day are significantly different ($p < 0.05$). Populations within a row that do not share an uppercase letter are significantly different ($p < 0.05$). Total reduction (TR) is the difference between populations ($\log_{10}$ CFU/g) on day 0 and day 118.

LM populations in the treatments employed throughout the study are shown in Table 4.4. PC and SC achieved the greatest reduction of LM on D3, the last day of the fermentation phase (PC = 1.98 $\log_{10}$ CFU/g; SC = 1.67 $\log_{10}$ CFU/g). NC, however, had increased from 7.12 ± 0.06 to 7.18 ± 0.09 $\log_{10}$ CFU/g from D0 to D3. LM subjected to T-10 had a 0.39 $\log_{10}$ CFU/g decrease from D0 to D3. Similar trends in LM reductions were observed on D21. LM reductions from D0 to D21, when salami achieved the target a_w, were 1.06, 2.35, 2.57, and 1.19 $\log_{10}$ CFU/g for NC, PC, SC, and T-10, respectively. Furthermore, PC differed significantly from NC ($p < 0.0001$), SC ($p = 0.0054$), and T-10 ($p < 0.0001$) on D21. SC was also significantly different from NC ($p < 0.0001$) and T-10 ($p < 0.0001$). NC was not significantly different from T-10 on D21 ($p = 0.39630$). LM populations in NC on D118 were significantly different from PC, SC, and T-10 ($p < 0.0001$). A significant difference in LM populations was not seen between days in NC until comparing between D21 and D28 ($p < 0.0001$). Populations of LM subjected to PC, SC, and T-10 did not differ significantly on D118 ($p > 0.05$), but NC differed significantly from PC, SC, and T-10 ($p < 0.0001$). All reductions from D0 to D118 for all treatments were significant ($p < 0.0001$). Total LM reductions for NC, PC, SC, and T-10 were 4.55, 6.89, 6.4, and 6.94 $\log_{10}$ CFU/g, respectively. NC did not achieve a 5 $\log_{10}$ reduction of LM throughout the entirety of the study, including extended ambient storage. Furthermore, LM populations in T-10 and NC, unlike PC and SC, did not go below the lower limit of detection.

***L. monocytogenes* Results**

Phase	Sample Day	Negative Control	Positive Control	Swiss Chard	Prosur® T-10
Raw	0	$7.12 \pm 0.06^{AB,a}$	$7.28 \pm 0.04^{A,a}$	$6.79 \pm 0.13^{B,a}$	$7.48 \pm 0.13^{A,a}$
Fermentation	1	$7.07 \pm 0.11^{A,a}$	$5.97 \pm 0.17^{B,b}$	$5.61 \pm 0.04^{B,b}$	$6.85 \pm 0.07^{A,b}$
	2	$6.96 \pm 0.16^{A,a}$	$5.60 \pm 0.17^{B,b}$	$4.85 \pm 0.13^{B,c}$	$6.83 \pm 0.07^{A,b}$
	3	$7.18 \pm 0.09^{A,a}$	$5.30 \pm 0.04^{B,b}$	$5.12 \pm 0.14^{B,b}$	$7.09 \pm 0.03^{A,b}$
Drying	7	$6.83 \pm 0.02^{A,a}$	$5.26 \pm 0.15^{B,b}$	$4.95 \pm 0.26^{B,b}$	$6.87 \pm 0.13^{A,b}$
	14	$6.58 \pm 0.1^{A,a}$	$5.08 \pm 0.29^{B,b}$	$4.62 \pm 0.11^{C,b}$	$6.47 \pm 0.09^{A,c}$
	21	$6.54 \pm 0.04^{A,a}$	$5.11 \pm 0.07^{B,b}$	$4.49 \pm 0.12^{C,b}$	$6.75 \pm 0.12^{A,b}$
Packaging	28	$5.11 \pm 0.38^{A,b}$	$3.93 \pm 0.06^{B,c}$	$3.78 \pm 0.14^{B,c}$	$6.22 \pm 0.07^{C,c}$
	35	$5.68 \pm 0.12^{A,c}$	$4.17 \pm 0.23^{B,c}$	$3.18 \pm 0.09^{C,d}$	$5.82 \pm 0.09^{A,d}$
	42	$5.49 \pm 0.17^{A,b}$	$3.90 \pm 0.23^{B,c}$	$2.99 \pm 0.12^{C,d}$	$5.30 \pm 0.19^{A,e}$
	49	$5.00 \pm 0.23^{A,d}$	$2.97 \pm 0.24^{B,d}$	$3.83 \pm 0.30^{C,b}$	$4.43 \pm 0.13^{D,f}$
	118	$2.63 \pm 0.15^{A,e}$	$0.39 \pm < 0.01^{B,e}$	$0.39 \pm < 0.01^{B,c}$	$0.54 \pm 0.10^{B,g}$
	TR	**4.55**	**6.89**	**6.4**	**6.94**

Table 4.4 Average *L. monocytogenes* populations and reductions (log_{10} CFU/g ± Standard Error). Sampling days within a column that have a different lowercase letter than the previous day are significantly different ($p < 0.05$). Populations within a row that do not share an uppercase letter are significantly different ($p < 0.05$). Total reduction (TR) is the difference between populations (log_{10} CFU/g) on day 0 and day 118.

No initial inoculation populations of S differed significantly across treatments ($p > 0.05$; Table 4.5). At the end of fermentation (D3), NC was the only treatment to be observed to have an increase (0.29 log_{10} CFU/g) in S. NC was significantly different from PC ($p = 0.0001$) and SC ($p < 0.0001$) on D3. At the end of drying (D21), NC, PC, SC, and T-10 achieved S reductions of 0.58, 2.17, 2.3, and 0.73 log_{10} CFU/g respectively. S populations in NC, 5.73 ± 0.18 log_{10} CFU/g, and T-10, 5.58 ± 0.11 log_{10} CFU/g, did not differ significantly on D21 ($p = 0.5528$). S populations in PC and SC on D21 were 4.63 ± 0.07 and 4.56 ± 0.21 log_{10} CFU/g, respectively, and were not significantly different ($p = 0.7069$). S populations in NC were significantly different from those in PC and SC on D21 ($p < 0.0001$). T-10 was also significantly different

from PC and SC on D21 ($p < 0.0001$). On D49, NC was the only treatment statistically different from any other treatments ($p < 0.0001$). All treatments were below the lower limit of detection of S and were not significantly different on D118 ($p > 0.05$). All total reductions from D0 to D118 were significantly different ($p < 0.0001$). Total reductions of S from D0 to D118 were 6.4, 6.59, 6.74, and 6.39 $\log_{10}$ CFU/g for NC, PC, SC, and T-10, respectively.

***Salmonella* Spp. Results**

Phase	Sample Day	Negative Control	Positive Control	Swiss Chard	Prosur® T-10
Raw	0	$6.79 \pm 0.06^{A,a}$	$6.98 \pm 0.19^{A,a}$	$7.13 \pm 0.06^{A,a}$	$6.77 \pm 0.05^{A,a}$
Fermentation	1	$7.22 \pm 0.12^{A,a}$	$6.83 \pm 0.19^{AB,a}$	$6.67 \pm 0.19^{B,a}$	$6.71 \pm 0.15^{B,a}$
	2	$7.06 \pm 0.15^{A,a}$	$6.38 \pm 0.14^{BC,a}$	$5.97 \pm 0.16^{C,b}$	$6.61 \pm 0.16^{AB,a}$
	3	$7.00 \pm 0.15^{A,a}$	$6.08 \pm 0.18^{BC,a}$	$5.72 \pm 0.16^{C,b}$	$6.53 \pm 0.19^{AB,a}$
Drying	7	$6.38 \pm 0.11^{AC,b}$	$6.10 \pm 0.11^{C,a}$	$5.41 \pm 0.11^{B,b}$	$6.53 \pm 0.19^{A,a}$
	14	$6.23 \pm 0.26^{A,b}$	$5.55 \pm 0.03^{B,b}$	$5.37 \pm 0.07^{B,b}$	$5.59 \pm 0.12^{B,b}$
	21	$5.73 \pm 0.18^{A,c}$	$4.63 \pm 0.07^{B,c}$	$4.56 \pm 0.21^{B,c}$	$5.58 \pm 0.11^{A,b}$
Packaging	28	$5.41 \pm 0.19^{A,c}$	$3.70 \pm 0.13^{B,d}$	$3.55 \pm 0.13^{B,d}$	$4.84 \pm 0.13^{C,c}$
	35	$4.34 \pm 0.24^{A,d}$	$2.11 \pm 0.31^{B,e}$	$2.13 \pm 0.14^{B,e}$	$3.65 \pm 0.09^{C,d}$
	42	$3.19 \pm 0.22^{A,f}$	$2.56 \pm 0.20^{B,e}$	$2.27 \pm 0.16^{B,e}$	$2.40 \pm 0.26^{B,d}$
	49	$3.27 \pm 0.21^{A,e}$	$1.27 \pm 0.16^{B,f}$	$1.28 \pm 0.36^{B,f}$	$1.61 \pm 0.21^{B,d}$
	118	$0.39 \pm < 0.01^{A,g}$	$0.39 \pm < 0.01^{A,g}$	$0.39 \pm < 0.01^{A,g}$	$0.39 \pm < 0.01^{A,d}$
	TR	**6.4**	**6.59**	**6.74**	**6.38**

Table 4.5 Average *Salmonella* spp. populations and reductions ($\log_{10}$ CFU/g ± Standard Error). Sampling days within a column that have a different lowercase letter than the previous day are significantly different ($p < 0.05$). Populations within a row that do not share an uppercase letter are significantly different ($p < 0.05$). Total reduction (TR) is the difference between populations ($\log_{10}$ CFU/g) on day 0 and day 118.

Discussion

The USDA-FSIS (2001) recommends a 5 $\log_{10}$ reduction of S in dry and semi-dry meat products and at least a 5 $\log_{10}$ reduction of STEC in dry and semi-dry meat products containing beef. Though a 5 $\log_{10}$ reduction of LM is preferable for a greater margin of safety in fermented

meat products, at least a 3 $\log_{10}$ reduction of LM is recommended to be achieved during a lethality treatment of RTE shelf-stable products. In this study, pathogen reductions on D21 are critical as this is when salami achieved the target a_w of $\leq$ 0.88, were vacuum packaged, and would be distributed to consumers on an industry scale. Unfortunately, no treatment group achieved a 5 $\log_{10}$ CFU/g reduction of any pathogen by D21 in this study. However, as described in "option #5" of the Blue Ribbon Task Force (1996), processors may use the results from this study as scientific validation of a 2 $\log_{10}$ reduction of LM and S in their Hazard Analysis Critical Control Point (HACCP) plan for manufacturing raw, RTE pork salami cured with purified nitrite or Swiss chard that includes an analytical method for raw batter testing. Processors are recommended to sample 15, 25g samples per lot of product. This research may be used to support the application of "option #5" in the Blue Ribbon Task Force because PC and SC achieved a > 2 $\log_{10}$ CFU/g reduction of LM and S at the time of packaging. NC and T-10 achieved LM reductions of 1.06 and 1.19 $\log_{10}$ CFU/g, respectively, and S reductions of 0.58 and 0.73 $\log_{10}$ CFU/g, respectively by D21. Therefore, this study does not serve as scientific validation for a 2 $\log_{10}$ reduction of LM or S in raw, RTE salami manufactured without a curing agent or with T-10. Though PC and T-10 achieved > 2 $\log_{10}$ CFU/g reduction of EC on D21, processors should not use this study as validation for products containing beef as more research is needed to support the manufacturing procedures in this study for fermented and dried products containing beef.

The successful management of processing controls in this research was exhibited by the fixed effects of pH and a_w not having a significant impact on any pathogen population differences between treatments throughout the duration of this study. Further, successful processing controls also were displayed by differences in pathogen populations between

treatment groups only being significantly impacted by the treatment itself ($p < .0001$). All salami treatments were formulated to, and manufactured with the same, ingoing, amount of B-LC 007 starter culture, which is a mixed culture of microorganisms. An organism included in the starter culture is *Pediococcus acidilactici*, which produces the bacteriocin, pediocin, known to have strong antagonistic properties against LM (Nielson et al., 1990). Despite the equivalence of bacteriocin inclusion and processing control parameters, LM populations in NC were significantly different from PC and SC throughout the entirety of the study, apart from initial inoculation populations. Furthermore, LM populations in T-10 were significantly different from PC and SC throughout the study until D118 ($p < 0.05$) and were not significantly different from NC until D28. Prosur® states that there are no nitrates or nitrites in Natpre T-10 (Prosur®, N.D.). Therefore, the observed differences between LM populations in salami manufactured with and without nitrite sources may be due to the inhibition sodium nitrite has on LM (Buchanan et al., 1989; Duffy et al., 1994; Ngutter & Donnelly, 2003).

The current study exhibited similar reductions of EC, LM, and S reductions in cured, non-thermal lethality treated pork salami (McKinney et al., 2019) and in LM and S reductions in duck salami (Watson et al., 2021) when formulated to 156 ppm ingoing sodium nitrite. The study presented here is the first of its kind to exhibit the longevity of combining curing agents and other pathogen hurdles to control EC, LM, and S during extended ambient storage of raw, RTE salami. All treatments in this study achieved and exceeded a 5 $\log_{10}$ total reduction of EC and S by day 118. NC was the only treatment that did not achieve a 5 $\log_{10}$ reduction of LM (4.55 $\log_{10}$ CFU/g total LM reduction). Therefore, this finding demonstrates the importance of including a curing agent for the control of LM during extended, reduced oxygen storage of salami at ambient temperatures.

Other research has also investigated naturally cured, RTE meat products. Golden et al., (2014) surface inoculated fully cooked, deli-style turkey breasts with LM. The turkey breasts were prepared with various concentrations of purified nitrite or cultured celery powder. The researchers found that the concentration of nitrite, rather than the source, was the most critical factor in inhibiting LM. This knowledge was applied when formulating treatments for this research as PC and SC were both intentionally formulated to 156 ppm ingoing nitrite. Another study found that LM was better controlled than EC or S in pork bellies injected with brines prepared with natural or purified sources of nitrite (Gipe, 2012). Similarly, when comparing the total reductions of EC, LM, and S in the research detailed here, though not statistically compared, it can be inferred that nitrite sources serve as a bigger hurdle to LM than EC or S in raw, RTE salami.

It is important to acknowledge that this research represents a worst-case-scenario for RTE, fermented, and dried meat products manufactured using various curing agents without a thermal lethality step. A processor may use this experiment as scientific validation for manufacturing raw, RTE salami with purified nitrite or Swiss chard. Additionally, it is recommended to follow good manufacturing procedures during salami processing using the methods described in this research. More research is recommended to investigate the safety of naturally cured salami when applying other antimicrobial treatments or ingredients, using other types of raw meat materials, and using different style casings or product diameters. Furthermore, analytical measurements of residual nitrite in the product throughout the duration of a challenge study similar to the one discussed here would add valuable knowledge by providing the ability to correlate nitrite levels to pathogen survival.

References

American Institute for Cancer Research (AICR). 2021. "Processed Meat (Sausages, Ham, Bacon, Hot Dogs, Salami)." American Institute for Cancer Research. March 31, 2021. https://www.aicr.org/cancer-prevention/food-facts/processed-meat/.

Asioli, D., J. Aschemann-Witzel, V. Caputo, R. Vecchio, A. Annunziata, T. Næs, and P. Varela. 2017. "Making Sense of the 'Clean Label' Trends: A Review of Consumer Food Choice Behavior and Discussion of Industry Implications." *Food Research International* 99 (1): 58–71. https://doi.org/10.1016/j.foodres.2017.07.022.

Bizzozero, J. . 2017. "75% of Consumers Will Pay Extra for Clean Label Ingredients." Food & Beverage Insider. March 15, 2017. https://www.foodbeverageinsider.com/market-trends-analysis/75-consumers-will-pay-extra-clean-label-ingredients.

Buchanan, R. L., H. G. Stahl, and R. C. Whiting. 1989. " Effects and Interactions of Temperature, PH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the Growth of *Listeria monocytogenes*." *Journal of Food Protection* 52 (12): 1–7. https://doi.org/10.4315/0362-028X-52.12.844.

Centers for Disease Control and Prevention (CDC). 1995. "*Escherichia coli* O157:H7 Outbreak Linked to Commercially Distributed Dry-Cured Salami -- Washington and California, 1994." www.cdc.gov. March 10, 1995. https://www.cdc.gov/mmwr/preview/mmwrhtml/00036467.htm.

Centers for Disease Control and Prevention (CDC). 2021a. "CDC: *Salmonella* Outbreaks Linked to Italian-Style Meats." Centers for Disease Control and Prevention. August 31, 2021. https://www.cdc.gov/*Salmonella*/italian-style-meat-08-21/index.html.

Centers for Disease Control and Prevention (CDC). 2021b. "CDC: *Salmonella* Outbreak Linked to Salami Sticks." Centers for Disease Control and Prevention. October 23, 2021. https://www.cdc.gov/*Salmonella*/i45-10-21/index.html.

Duffy, L.L., P.B. Vanderline, and F.H. Grau. 1994. "Growth of *Listeria monocytogenes* on Vacuum-Packed Cooked Meats: Effects of PH, Aw, Nitrite and Ascorbate." *International Journal of Food Microbiology* 23 (3-4): 377–90.

Gipe, A. . 2012. "Investigation of Quality Attributes and Inhibition of Foodborne Pathogens in 'No-Nitrate or Nitrite-Added' Bacon." PhD Dissertation , The Pennsylvania State University.

Golden, M. C., L. M. McDonnell, V. Sheehan, J. J. Sindelar, and K. A. Glass. 2014. "Inhibition of *Listeria monocytogenes* in Deli-Style Turkey Breast Formulated with Cultured Celery

Powder and/or Cultured Sugar-Vinegar Blend during Storage at 4°C." Journal of Food Protection 77 (10): 1787–93. https://doi.org/10.4315/0362-028X.JFP-14-059.

Grant, K. R., R. K. Gallardo, and J. J. McCluskey. 2021. "Consumer Preferences for Foods with Clean Labels and New Food Technologies." *Agribusiness* 37 (4). https://doi.org/10.1002/agr.21705.

Hartzell, A., C.Chen, C. Lewis, K. Liu, S. Reynolds, and E.G. Dudley. 2011. "*Escherichia coli*O157:H7 of Genotype Lineage-Specific Polymorphism Assay 211111 and Clade 8 Are Common Clinical Isolates within Pennsylvania." *Foodborne Pathogens and Disease* 8 (7): 763–68. https://doi.org/10.1089/fpd.2010.0762.

Iqbal, J., D. Yu, M. Zubair, M. I. Rasheed, H. Muhammad U. Khizar, and M. Imran. 2021. "Health Consciousness, Food Safety Concern, and Consumer Purchase Intentions toward Organic Food: The Role of Consumer Involvement and Ecological Motives." *SAGE Open* 11 (2): 215824402110157. https://doi.org/10.1177/21582440211015727.

Jackson, A. L., C. Kulchaiyawat, G. A. Sullivan, J. G. Sebranek, and J. S. Dickson. 2011. "Use of Natural Ingredients to Control Growth of *Clostridium Perfringens* in Naturally Cured Frankfurters and Hams." *Journal of Food Protection* 74 (3): 417–24. https://doi.org/10.4315/0362-028x.jfp-10-365.

Kalaycıoğlu, Z., and F. B. Erim. 2019. "Nitrate and Nitrites in Foods: Worldwide Regional Distribution in View of Their Risks and Benefits." *Journal of Agricultural and Food Chemistry* 67 (26): 7205–22. https://doi.org/10.1021/acs.jafc.9b01194.

Leistner, L., and L. G.M. Gorris. 1995. "Food Preservation by Hurdle Technology." *Trends in Food Science & Technology* 6 (2): 41–46. https://doi.org/10.1016/s0924-2244(00)88941-4.

Maddock, R. . 2015. "US Products- Dry Sausage." In *Handbook of Fermented Meat and Poultry*, 295–98. Chichester, West Sussex, Uk ; Hoboken, Nj: Wiley, Blackwell.

McKinney, S., C. Cutter, and J. Campbell. 2017. "Pathogen Reductions during Traditional Fermentation and Drying of Pork Salamis." *Meat and Muscle Biology* 1 (3): 129–29. https://doi.org/10.22175/rmc2017.122.

Milkowski, A., H. K. Garg, J. R. Coughlin, and N. S. Bryan. 2010. "Nutritional Epidemiology in the Context of Nitric Oxide Biology: A Risk–Benefit Evaluation for Dietary Nitrite and Nitrate." *Nitric Oxide* 22 (2): 110–19. https://doi.org/10.1016/j.niox.2009.08.004.

Ngutter, C., and C. Donnelly. 2003. "Nitrite-Induced Injury of *Listeria monocytogenes* and the Effect of Selective versus Nonselective Recovery Procedures on Its Isolation from Frankfurters." *Journal of Food Protection* 66 (12): 2252–57. https://doi.org/10.4315/0362-028x-66.12.2252.

Nielsen, J. W., J. S. Dickson, and J. D. Crouse. 1990. "Use of a Bacteriocin Produced by Pediococcus Acidilactici to Inhibit *Listeria monocytogenes* Associated with Fresh Meat." *Applied and Environmental Microbiology* 56 (7): 2142–45. https://doi.org/10.1128/aem.56.7.2142-2145.1990.

Prosur ®. n.d. "Natpre T-10: No Hidden Nitrates or Nitrites." Prosurla.com. https://prosurla.com/en/our-natural-solutions/natpre-t-10-clean-label/.

Rivera, N., M. Bunning, and J. Martin. 2019. "Uncured-Labeled Meat Products Produced Using Plant-Derived Nitrates and Nitrites: Chemistry, Safety, and Regulatory Considerations." *Journal of Agricultural and Food Chemistry* 67 (29): 8074–84. https://doi.org/10.1021/acs.jafc.9b01826.

Sebranek, J. G., A. L. Jackson-Davis, K. L. Myers, and N. A. Lavieri. 2012. "Beyond Celery and Starter Culture: Advances in Natural/Organic Curing Processes in the United States." Meat Science 92 (3): 267–73. https://doi.org/10.1016/j.meatsci.2012.03.002.

Sebranek, J. G., and J. N. Bacus. 2007. "Cured Meat Products without Direct Addition of Nitrate or Nitrite: What Are the Issues?" *Meat Science* 77 (1): 136–47. https://doi.org/10.1016/j.meatsci.2007.03.025.

Sindelar, J. J., and A. L. Milkowski. 2011. "Sodium Nitrite in Processed Meat and Poultry Meats: A Review of Curing and Examining the Risk/Benefit of Its Use." American Meat Science Association.

Sullivan, G. A., A. L. Jackson-Davis, S. E. Niebuhr, Y. Xi, K. D. Schrader, J. G. Sebranek, and J. S. Dickson. 2012. "Inhibition of *Listeria monocytogenes* Using Natural Antimicrobials in No-Nitrate-Or-Nitrite-Added Ham." *Journal of Food Protection* 75 (6): 1071–76. https://doi.org/10.4315/0362-028x.jfp-11-511.

Toldrá, F. . 2002. *Dry-Cured Meat Products*. Connecticut, USA: Food & Nutrition Press, Inc.

Tompkin, R. B. . 2005. "Antimicrobials in Good." In *Antimicrobials in Food*, edited by P. Michael Davidson, John N. Sofos, and A.L. Branen. Taylor & Francis.

Työppönen, S., E. Petäjä, and T. Mattila-Sandholm. 2002. "Bioprotectives and Probiotics for Dry Sausages." *International Journal of Food Microbiology* 83 (3): 233–44. https://www.sciencedirect.com/science/article/pii/S0168160502003793?via%3Dihub.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2012. Microbiological laboratory guidebook. Available at http://www.fsis. usda.gov/science/microbiological_lab_ guidebook/.

U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS). 2023b. "Daniele International LLC Recalls Ready-To-Eat Sausage Products due to Possible Listeria Contamination | Food Safety and Inspection Service." Www.fsis.usda.gov. April 28, 2023. https://www.fsis.usda.gov/recalls-alerts/daniele-international-llc-recalls-ready-eat-sausage-products-due-possible-listeria.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 1995. "Processing Inspectors' Calculations Handbook." https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/7620.3.pdf.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2001. "Performance Standards for the Production of Processed Meat and Poultry Products. Proposed Rule. Federal Register." 2001.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2021a. Fratelli Beretta USA, Inc. Recalls Ready-to-Eat Uncured Antipasto Meat Products Due to Possible *Salmonella* Contamination | Food Safety and Inspection Service. November 18, 2021. https://www.fsis.usda.gov/recalls-alerts/fratelli-beretta-usa-inc.-recalls-ready-eat-uncured-antipasto-meat-products-due.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2022. *Salmonella* Outbreaks Linked to ItalianStyle Meats Outbreak Investigation After Action Review. Available at https://www.fsis.usda.gov/sites/default/files/media_file/2022-04/FSIS-After-Action-Review-2021-09_2022-01.pdf

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2023a. "FSIS Ready-To-Eat Fermented, Salt-Cured, and Dried Products Guideline." May 5, 2023.

U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). 2023b. "Daniele International LLC Recalls Ready-To-Eat Sausage Products due to Possible *Listeria* Contamination | Food Safety and Inspection Service." Www.fsis.usda.gov. April

28, 2023. https://www.fsis.usda.gov/recalls-alerts/daniele-international-llc-recalls-ready-eat-sausage-products-due-possible-listeria.

Watson, S. C., C. N. Cutter, and J. A. Campbell. 2021. "Fate of *Salmonella* Spp., *Listeria monocytogenes*, and *Campylobacter* Spp. During Fermentation and Drying of Duck Salami." *Food Protection Trends* 41 (4): 423. https://doi.org/10.4315/1541-9576-41.4.423.

World Health Organization, International Agency for Research on Cancer (WHO-IARC). 2015. "IARC Monographs Evaluate Consumption of Red Meat and Processed Meat." IARC. https://www.iarc.who.int/wp-content/uploads/2018/07/pr240_E.pdf.

Yarbrough, J. M., J. B. Rake, and R. G. Eagon. 1980. "Bacterial Inhibitory Effects of Nitrite: Inhibition of Active Transport, but Not of Group Translocation, and of Intracellular Enzymes." *Applied and Environmental Microbiology* 39 (4): 831–34. https://doi.org/10.1128/aem.39.4.831-834.1980.

Chapter 5
Conclusions and Future Directions

Conclusions

The research presented displays the antimicrobial efficacy of including a nitrite source in combination with other hurdles to inhibit the growth of pathogens throughout fermentation, drying, and extended, reduced oxygen storage in salami manufactured without a thermal lethality treatment. All treatment and control groups exceeded a 5 $\log_{10}$ reduction of *Escherichia coli* O157:H7 and *Salmonella* spp. by D118, demonstrating the effectiveness of hurdles during extended ambient storage regardless of nitrite source or inclusion. Results from this research also show the importance of the inclusion of curing agents in salami for controlling the survival of *Listeria monocytogenes* during extended storage periods. Salami manufactured without a nitrite source achieved a 4.55 $\log_{10}$ CFU/g reduction of LM while those manufactured with purified nitrite achieved 6.89 $\log_{10}$ CFU/g reduction on D118.

It is critical to acknowledge the reductions of all pathogens when salami reached the target water activity (a_w) of ≤ 0.88 and were packaged as this is when product would be distributed to consumers. No salami, regardless of nitrite inclusion, achieved a 5 $\log_{10}$ reduction of any pathogen upon achieving the target a_w (day 21 of sampling). Reductions of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. were observed across all treatments. Salami manufactured with purified nitrite controlled all pathogens most consistently and achieved the highest reductions of pathogens, apart from salami manufactured with Swiss chard, which achieved a 2.57 $\log_{10}$ reduction of *L. monocytogenes*, whereas the salami manufactured with purified nitrite achieved a 2.35 $\log_{10}$ reduction. Though none of the salami achieved a 5 $\log_{10}$ reduction of the three pathogens, processors may follow "Option #5" of the Blue Ribbon Task Force, wherein it states that if a process achieves a 2 $\log_{10}$ reduction of the hazard of concern a processor may have an analytical testing scheme for testing the raw batter from every batch of

fermented products for the presence of pathogens. For products not containing beef, processors may display a 2 $\log_{10}$ reduction of either *L. monocytogenes* or *Salmonella* spp. The research presented may serve as scientific support for a process manufacturing fermented and dried salami with purified nitrite or Swiss chard, with the condition that both curing agents must be formulated to 156 ppm nitrite and have 2.31% ingoing salt. Moreover, the results of the current research may not be used as scientific support for processors choosing to not include a curing agent or to manufacture products with Prosur ® T-10.

The research presented is the first of its kind to determine the survival of pathogens in salami manufactured with various sources of nitrite, without a thermal lethality step, and during extended ambient storage. Therefore, this study may serve well as the foundation of future research and other scientific innovations.

Future Directions

Future work is recommended to expand on the knowledge gained from the results discussed in this thesis. First, it is recommended to determine the antimicrobial efficacy of the curing agents applied at various utilization rates. Furthermore, there may be a correlation between clean label products and lowered salt content. Therefore, the survival of pathogens in naturally cured salami manufactured with low ingoing salt content should be explored. Additionally, diameter size impacts the drying time of salami. It has been shown that smaller diameter, faster drying products can achieve less pathogen reductions than larger diameter products with the same formulation and a_w. It would be advantageous to investigate pathogen survival in products of varying diameter size when manufactured with various curing agents. The study discussed here investigated the survival of pathogens pork salami only. The ability of

natural curing agents to control pathogens of concern should be explored in other fermented, semi-dried, dried, comminuted, or whole muscle products of various species origins. Lastly, residual nitrite content was not evaluated on the salami investigated in this study. Investigating residual nitrite throughout the duration of a similarly designed study to the one discussed here would allow a correlation to be made between nitrite levels to pathogen populations in the product.