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Pathogen Reductions during Traditional Fermentation and Drying of Pork Salamis

ABSTRACT

Traditional salami products are increasing in popularity in the United States. Meat processors strive to create high-quality niche products that are similar in quality to salamis of European origins, while also ensuring food safety. This experiment investigated the impact of casing type and an antimicrobial intervention on pathogen reductions in a non-heat treated pork salami. Cubed pork was experimentally inoculated with three strains each of *Escherichia coli* O157:H7 (EC), *Salmonella* spp. (S), and *Listeria monocytogenes* (LM) and sprayed with water (CTRL) or a 2.5% antimicrobial solution (TRT) prior to grinding. Dry ingredients and starter culture were mixed into the ground pork prior to its being stuffed into ~50 mm natural, collagen, and fibrous casings. Salamis were fermented (72 h), dried (21 days), vacuum-packaged, and stored at 20–22°C (28 days). No significant difference was observed between CTRL and TRT sausages, regardless of casing type, for pathogen populations during the sampling period. A 5 $\log_{10}$ CFU/g reduction was achieved for S and LM by the end of storage, but no combination of casing type and treatment achieved a 5 $\log_{10}$ CFU/g reduction of EC. This study validated the safety of fermented salamis manufactured without thermal processing and any additional lethality processes following fermentation and drying.

INTRODUCTION

Pathogenic bacteria are of concern in ready-to-eat (RTE) meat products because of the lack of further preparation (e.g., cooking) by the consumer, and these foodborne pathogens may cause illness if ingested. The United States Department of Agriculture, Food Safety and Inspection Service (USDA–FSIS) has issued guidelines and policies to help meat processors comply with government regulations to produce safe, RTE meat products. Regulations include implementing a valid Hazard Analysis Critical Control Point (HACCP) plan and utilizing Appendix A to address *Salmonella* spp. (S) and the "zero tolerance policy" to address *Escherichia coli* O157:H7 (EC) and *Listeria monocytogenes* (LM) in products containing beef and RTE meats, respectively *(21, 23)*. Processors may control for LM, especially in the post-processing environment, by using one of the three

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alternatives outlined in the FSIS Compliance Guideline *(25)*. To specifically address EC, USDA–FSIS has stated five practices that processors may utilize to ensure a safe product, one of which is to validate a 5 $\log_{10}$ lethality treatment *(22)*.

Multiple definitions of traditional foods are found in the literature. Bertozzi *(2)* defines a traditional food as "… representation of a group, it belongs to a defined space, and it is part of a culture that implies the cooperation of the individuals operating in that territory." Given this definition, Germany, Hungary, Italy, and several other European cultures are associated with the production of salami, with Italian salamis being the focus of this research project *(14)*.

Traditionally, Italian salamis are processed without a heat treatment and therefore are a raw, RTE product. Pork is used to produce Italian salamis because of the flavor and appearance pork provides to the finished product *(13)*. Processing consists of grinding or chopping the meat, adding a spice blend (including 2.5–3.0% salt and nitrite) and starter culture, stuffing the mixture into casings, fermenting, and drying at 15°C or less for up to six months to develop the quality attributes familiar to consumers *(13)*. The combination of salt content, reduced pH, presence of nitrite, and low water activity (a_w) work synergistically to create an appealing sensory profile as well as to inhibit microbial growth *(11, 20)*.

Despite being fermented and dried, non-heat treated sausages have led to several notable outbreaks *(3, 4)*. *Salmonella enterica* serotype Goldcoast in Thuringia, Germany was responsible for an outbreak of 44 cases from the consumption of a raw, fermented sausage *(4)*. In 2010, 69 salmonellosis cases were identified in France as being the result of the consumption of a dried pork sausage contaminated with *Salmonella enterica* serotype 4,12:i:- *(3)*.

One of the challenges facing meat processors of traditional salami in the United States is validating the safety of the product. Traditional products dating back to antiquity utilized the natural flora associated with the pork meat for fermentation, which may lead to an increased risk of spoilage and/or allow for pathogenic microorganisms to survive. Additionally, the pH of the product may not be controlled consistently, allowing for variability in product quality. Most salamis that are traditionally produced in modern times incorporate traditional methods in combination with modern technology. For example, starter cultures have been incorporated as a common ingredient primarily added for a more uniform and controlled acidification, which often results in a shorter fermentation period. Lactic acid bacteria used as starter cultures in fermented meats also have been selected because of their ability to produce bacteriocins and other antimicrobial compounds. Bacteriocins are known to inhibit the growth of Gram-positive spoilage bacteria and pathogens, such as LM *(15)*. Since many artisanal salamis have unique sensory attributes, it is important to select starter cultures that are able to replicate the traditional flavors and aromas across specific salamis while ensuring an appropriate acid production for safety.

Additionally, other antimicrobials can be applied to raw meat surfaces or may be incorporated into the formulation, which serves as an extra hurdle to bacterial growth. The use of citric acid, lactic acid, acetic acid, or a combination of acids on fresh meat has been accepted as one such intervention to reduce pathogen populations *(26)*. A proprietary combination of lactic and citric acid is approved at the 2.5% level *(26)* and has been investigated for its efficacy in fresh meat to control the growth of *E. coli* O157:H7 and *Salmonella (12)*. However, very little information exists as to the use of such interventions in the production of fermented dry sausages.

Previous research in our lab has demonstrated that a pre-treatment of meat with lactic acid, followed by traditional processing of Landjäger with no thermal treatment, could bring about significant reductions of pathogens such as EC, LM, and S *(18)*. Therefore, the objective of this experiment was to validate a process used to make traditionally processed salami while incorporating modern technologies into the production process.

MATERIALS AND METHODS

Preparation of inoculum

Cultures of *E. coli* O157:H7 (EC; ATCC 43895, ATCC BAA-460, and PA-2), *Salmonella* spp. (S; *Salmonella enterica* serovar Typhimurium, ATCC 14028; *Salmonella enterica* serovar Montevideo, SMvo13; *Salmonella enterica* serovar Panama, ATCC 7378) and *L. monocytogenes* (LM; Scott A serotype 4b, H3396 serotype 4b, and FSL J1-129 serotype 4b) were obtained from the American Type Culture Collection (ATCC; Manassas, VA), Center for Disease Control and Prevention (CDC; Atlanta, GA), and the Microbiology Culture Collection at The Pennsylvania State University Food Science Department. Frozen cultures were transferred to fresh tryptic soy broth (TSB; Becton Dickinson and Company; BD, Sparks, MD) and incubated statically at 37°C under aerobic conditions for 24 h. The overnight cultures of EC, S, and LM were streaked onto Cefixime-tellurite Sorbitol MacConkey agar (CT-SMAC; BD), Xylose Lysine Deoxycholate agar (XLD; BD), and Modified Oxford agar (MOX; BD), respectively. Plates were incubated at 37°C for 24 h before culture confirmation tests were performed (EC: Remel, Lenexa, KS; S: Oxoid, Hants, United Kingdom; LM: Microgen Bioproducts, Camberley, UK). Individual colonies were used to inoculate 10 mL of fresh TSB and grown for 24 h at 37°C. The 24 h culture (10 mL) was added to bottles containing 240 mL TSB (total 250 mL per bacteria strain) and incubated for 24 h at 37°C to obtain cell concentrations of ~8 $\log_{10}$ CFU/mL (adapted from USDA Microbiological Laboratory Guidebook) *(24)*. The inoculation bath (8.38 $\log_{10}$ CFU/mL) was prepared by mixing the three strains of each bacterium together (2,250

mL total volume) in a sterile autoclave bin under a biological safety cabinet (Nuaire NU-425; Plymouth, MN).

TRIM INOCULATION AND PREPARATION OF SAUSAGES

Trim inoculation and sausage manufacturing were performed by personnel in the Penn State Food Safety Pilot Plant (BSL-2), with appropriate PPE being worn. Boneless pork shoulder butts (IMPS 406A; Indiana Kitchen, Delphi, IN) were received from a local distributor and stored frozen (-5°C) until use (< 1 month). The pork was thawed at 3.3°C prior to being cut approximately 2.54 cm × 2.54 cm cubes. Cubes were vacuum-packaged and stored (up to 48 h) at 2–4°C until ready for inoculation.

Pork cubes were placed in the inoculation bath and stirred every 5 min for a total of 30 min to allow for adequate pathogen attachment. The cubes were removed with a sterilized slotted spoon to allow excess liquid to drain off and then placed into a sterile tub. Following a 30-min chilling period (4°C), half of the pork cubes were spread into a single layer on trays and sprayed with tap water (23.9°C), using a hand-held tank sprayer (Model 67220; Hudson Mfg. Co, Chicago, IL), for 30 s on each side (CTRL) approximately 15–17 cm from the product surface. The remaining cubes were sprayed in the same manner as the CTRL group with a 2.5% Beefxide® solution (23.9°C; Birko, Henderson, CO) for 30 s on each side (TRT) to ensure even coverage. The CTRL and TRT groups were placed in separate sterilized plastic tubs and chilled (4°C) overnight before grinding.

After chilling (< 24 h), the meat was ground through a 6-mm plate (Model 177MG22; Avantco Equipment, Lancaster, PA), and the dry ingredients were added *(Table 1)*, followed by the starter culture (SafePro® B-LC-007, CHR Hanson, Milwaukee, WI). Three casing types were used: beef middles (natural casings; Globe Casings, Carlstadt, NJ), collagen (Devro, Columbia, SC), and fibrous (Globe Casings, Carlstadt, NJ). Casings for the CTRL sausage group were prepared by placing them in 3 L of warm tap water (41–45°C), and casings for the TRT sausage group were prepared in 3 L of a 2.5% Beefxide® solution (41–45°C). Casings were soaked in the corresponding liquid for a minimum of 10 min prior to use for stuffing. Sausages were stuffed (#50501-TSM 5lb stuffer; The Sausage Maker, Buffalo, NY) to an average weight of 204.4 ± 10.2 g and average diameter of 49.7 ± 1.2 mm. A total of 64 sausages per casing type per treatment (n = 384) were made for each replication. A reference sausage was used to determine product weight and diameter at each sample time for each casing type. Sausages were sprayed with distilled white vinegar (5% acetic acid; Giant Food Stores, LLC, Carlisle, PA) at days 11 and 25 to control mold growth on the casings.

Table 2 shows the drying cabinet (Model AS50/A; I.C.S. of Vanni Sprocatti & C. snc; Camposanto (Modena), Italy) parameters for temperature, relative humidity (RH) and time for each phase during the process. Sausages were fermented to a pH of 4.9 ± 0.1 and dried to an a_w of < 0.88, with 38–40% weight loss. Following ripening, salamis were vacuum-packaged (Ultravac UV-250, UltraSource, Kansas City, MO) in 3 mil poly bags (101.6 mm × 254.0 mm; OTR average 60 cc/m^2/24 h; UltraSource, Kansas City, MO) and stored at 20–22°C for up to 28 days. Two independent replications of this experiment were performed.

Microbial analysis

Samples were collected every 24 h for the first 96 h, after which samples were collected weekly. Three salamis (n = 3) each for CTRL and TRT sausage groups and per casing type were collected per sample time to make a composite sample for each casing type. Samples were individually prepared by creating a 1:5 dilution in Buffered Peptone Water (BPW; Hardy Diagnostics; HD; Santa Maria, CA) into a filtered stomacher bag (Interscience, St.-Normandy,

TABLE 1. Formulation for the production of pork salamis

Ingredient*	Grams
Salt	457.2
Dextrose	133.4
Potassium nitrate (KNO_3)	16.4
Sodium erythorbate	10.4
Curing salt (6.25% $NaNO_2$)	47.6

*Ingredients were added to 42 lb of ~80/20 (% lean % fat) pork shoulder butt.

TABLE 2. Drying cabinet settings during the manufacture of dry pork salamis

Phase	Temperature °C	RH %*	Time
Static Cooling	6–8	0	5 h
Hot Drip	24–26	0	36 h
Drying	24–26	55–65	12 h
Drying	22–24	60–70	12 h
Drying	20–22	65–75	12 h
Drying	18–20	68–78	24 h
Drying	16–18	72–80	24 h
Drying	14–16	75–82	24 h
Seasoning	12–14	75–80	24 h
Seasoning**	12–14	77–85	21 d

*RH% shows the range (min–max) of values controlled for in each phase by the cabinet microprocessor. An input of "0" indicates that humidity was not controlled by the cabinet and utilized the ambient humidity present from the product.

**During this phase, the drying cabinet had a run–pause cycle of 30-min run time and 45-min pause time for the fan.

France). Samples were homogenized for 1 min at 230 RPM (Stomacher® 400 Circulator; Seward Limited; West Sussex, UK) and 10 ml of each stomachate was saved to create the composite sample (30 ml) for each casing type within treatments. The composite sample was vortexed and then serially diluted, using 9 ml of BPW.

Aliquots (0.1ml) were plated in duplicate on CT-SMAC, XLD, and MOX to determine survival of EC, S, and LM, respectively. Tryptic soy agar (TSA; HD) was also plated for total bacteria counts. CT-SMAC, XLD, and TSA plates were incubated at 37°C for 24 h, and MOX plates for 48 h at 37°C, in accordance with the USDA Microbiological Laboratory Guidebook *(24)*.

Samples were enriched and plated simultaneously for the various sampling times when colonies were below the detection limit (0.40 CFU/g). For EC enrichment, 1 ml of the stomachate was transferred to 9 ml of Gram Negative broth, Hajna (BD) and incubated for 24 h at 37°C. The enrichment was streak-plated onto CT-SMAC, incubated for 24 h at 37°C and examined for typical colonies. For S enrichment, 1 ml of the stomachate was transferred to 9 ml of lactose broth (HiMedia, Mumbai, India) and grown for 24 h at 37°C, followed by transfer of 1 ml to 9 ml of Rappaport-Vassiliadis R10 broth (HD). After 24 h of incubation at 37°C, the Rappaport-Vassiliadis R10 broth was streak-plated onto XLD, incubated at 37°C for 24 h and examined for colonies. For LM enrichment, 1 ml of the stomachate was transferred to 9 ml of University of Vermont (UVM) Modified Listeria Enrichment broth (HD) and grown for 24 h at 37°C, followed by transfer of 1 ml to 9 ml of Fraser broth (Oxoid; Basingstoke, UK). The Fraser broth enrichment was grown for 24 h at 37°C, streaked onto MOX agar, incubated at 37°C and examined for the presence or absence of colonies after 48 h of incubation. Colonies were verified by use of confirmation tests (EC: Remel; S: Oxoid; LM: Microgen Bioproducts).

In addition to microbial analyses, pH (Testo 206-pH2; Testo, Inc.; Sparta, NJ) and a_w (AquaLab Water Activity Meter, Series 4TE; Decagon Devices, Inc.; Pullman, WA) were measured at each sampling time for each casing type within treatments. Reference sausages were weighed (Taylor TE22FT; Taylor USA, Oak Brook, IL) and product diameter (DialMax 41104; Wiha Tools USA; Monticello, MN) recorded at each sampling time.

Statistical analysis

This experiment utilized a completely randomized design. Prior to any statistical analyses, bacterial populations were first converted to $\log_{10}$ CFU/g. For plates with zero populations, a CFU count of 0.01 less than the detection limit (0.39 $\log_{10}$ CFU/g) was assigned to incorporate into the analyses. Results were analyzed by a mixed model procedure using a Statistical Analysis Software (Version 9.4, SAS Institute Inc., and Cary, NC), and unique comparisons of the mean pathogen populations were performed using the GLM procedure in SAS. The model included differences between the CTRL and TRT groups, differences within a casing type by day for CTRL and TRT, differences between

the casing types at each sample period for CTRL and TRT, and a casing type by time interaction for CTRL and TRT. A significance level of $P < 0.05$ was used to determine significant differences.

RESULTS

There was no significant difference between CTRL and TRT casing types for populations of EC ($P = 0.1645$), S ($P = 0.3746$), or LM ($P = 0.1762$) by the end of the sampling period *(Tables 3–5)*. The pH did not differ between the CTRL and TRT sausages (data not presented); however, the CTRL sausages were observed to have a pH 0.1–0.3 higher than the TRT sausages during fermentation, drying, and especially after vacuum packaging (data not presented). A_w did not differ between CTRL and TRT sausages; CTRL sausages had an average final a_w of 0.87, regardless of the casing type, while the TRT sausages, regardless of casing type, had an average final a_w of 0.85 (data not presented).

Casing type did not have an effect on remaining EC populations in the CTRL ($P = 0.5315$) or TRT sausages ($P = 0.9193$). *Table 3* demonstrates the remaining EC populations for both CTRL and TRT sausages. Following treatment sprays, EC decreased by 3.18 $\log_{10}$ CFU/g on the trim used for the CTRL sausages and decreased by 2.4 $\log_{10}$ CFU/g on the trim used for the TRT sausages. An increase in EC populations was seen with both CTRL and TRT sausages between 24 h (grinding) and 48 h (24 h of fermentation).

Within the CTRL sausage group, EC populations decreased by 1.49, 3.19, and 1.49 $\log_{10}$ CFU/g in the natural, collagen, and fibrous casings, respectively, following fermentation and drying *(Table 3)*. EC populations for the TRT sausages decreased by 2.15, 2.16, and 2.18 $\log_{10}$ CFU/g in the natural, collagen, and fibrous casings, respectively, during fermenta-

TABLE 3. Average *E. coli* O157:H7 populations ($\log_{10}$ CFU/g + SE*) in sausages made with natural, collagen, and fibrous casings following treatments with water or Beefxide, fermentation, drying, and vacuum packaged storage

	Natural		Collagen		Fibrous	
Sample time	**CTRL**	**TRT**	**CTRL**	**TRT**	**CTRL**	**TRT**
0 h	$6.88 + 0.64^{a}$	$6.48 + 0.76^{ac}$	$6.88 + 0.64^{ac}$	$6.48 + 0.76^{ac}$	$6.88 + 0.64^{ac}$	$6.48 + 0.76^{acd}$
24 h	$3.70 + 1.31^{b}$	$4.08 + 0.90^{bd}$	$3.70 + 1.31^{b}$	$4.08 + 0.90^{bd}$	$3.70 + 1.31^{b}$	$4.08 + 0.90^{be}$
Reduction^	**3.18**	**2.40**	**3.18**	**2.40**	**3.18**	**2.40**
48 h	$6.84 + 0.04^{a}$	$6.79 + 0.03^{ac}$	$6.85 + 0.04^{ac}$	$6.68 + 0.01^{ae}$	$6.81 + 0.15^{ac}$	$6.75 + 0.06^{ac}$
96 h	$6.57 + 0.17^{a}$	$6.30 + 0.31^{af}$	$6.56 + 0.32^{ac}$	$6.35 + 0.16^{ae}$	$6.30 + 0.35^{ac}$	$6.43 + 0.04^{ac}$
Reduction	**+2.87**	**+2.22**	**+2.86**	**+2.27**	**+2.60**	**+2.35**
11 days	$6.40 + 0.31^{a}$	$6.38 + 0.01^{af}$	$6.35 + 0.29^{ac}$	$6.29 + 0.07^{ab}$	$6.38 + 0.25^{ac}$	$6.25 + 0.14^{a}$
18 days	$6.19 + 0.33^{ac}$	$6.14 + 0.15^{ab}$	$6.02 + 0.28^{a}$	$5.92 + 0.04^{abe}$	$6.30 + 0.32^{a}$	$5.89 + 0.12^{ab}$
25 days	$5.78 + 0.32^{acd}$	$5.49 + 0.22^{ab}$	$5.62 + 0.23^{a}$	$5.47 + 0.13^{ab}$	$5.52 + 0.61^{a}$	$5.29 + 0.10^{ab}$
32 days	$5.39 + 0.47^{acd}$	$4.33 + 0.33^{abe}$	$3.69 + 0.28^{c}$	$4.32 + 0.08^{abe}$	$5.39 + 0.29^{a}$	$4.30 + 0.30^{abe}$
Reduction	**1.18**	**1.97**	**2.87**	**2.03**	**0.91**	**2.13**
39 days	$4.39 + 0.11^{ad}$	$3.51 + 0.36^{abe}$	$3.63 + 0.18^{bc}$	$3.51 + 0.62^{cde}$	$4.24 + 0.29^{ac}$	$3.04 + 0.30^{ce}$
45 days	$3.77 + 0.01^{abd}$	$3.60 + 0.30^{cdef}$	$3.39 + 0.19^{bc}$	$3.34 + 0.40^{cde}$	$3.63 + 0.13^{bc}$	$3.83 + 0.51^{bde}$
53 days	$3.56 + 0.34^{abd}$	$2.22 + 0.92^{cde}$	$2.79 + 0.15^{bc}$	$2.37 + 1.07^{cd}$	$3.12 + 0.05^{cb}$	$3.21 + 0.55^{bcf}$
60 days	$3.13 + 1.16^{b}$	$2.56 + 0.96^{cde}$	$2.91 + 0.46^{bc}$	$2.46 + 1.46^{cd}$	$3.35 + 0.19^{cb}$	$2.57 + 1.39^{def}$
Reduction	**2.26**	**1.77**	**0.78**	**1.86**	**2.04**	**1.73**
TR*	3.75	3.92	3.97	4.02	3.53	3.91

*SE is standard error; TR is total $\log_{10}$ reduction.

^{abcdef}Different letters in the same column denote significant differences ($P < 0.05$) within each treatment type. No significant difference was observed between casing types.

^Reductions are the differences between the last day of a phase and the last day of the previous phase.

tion and drying. Following vacuum packaged storage, CTRL sausages had an additional reduction of 2.26, 0.78, and 2.04 $\log_{10}$ CFU/g for natural, collagen, and fibrous casings, respectively for EC. Following vacuum packaged storage, EC for TRT sausages exhibited a 1.77, 1.86, and 1.73 $\log_{10}$ CFU/g reduction for natural, collagen, and fibrous casings, respectively *(Table 3)*.

Casing type had no effect on remaining S populations in the CTRL ($P = 0.6581$) or TRT groups ($P = 0.9734$). *Table 4* shows S populations for both CTRL and TRT sausages. The treatment sprays provided a 0.38 $\log_{10}$ and 0.34 $\log_{10}$ CFU/g reduction on pork trim used for the CTRL and TRT sausages, respectively. S populations decreased between 0.35 and 0.51 $\log_{10}$ CFU/g during the first 24 h of fermentation for all sausages.

S populations decreased by 2.21 $\log_{10}$ CFU/g in the natural casing, 2.46 $\log_{10}$ CFU/g in the collagen casing, and 2.62 $\log_{10}$ CFU/g in the fibrous casing for the CTRL sausages after the completion of fermentation and drying. TRT sausages exhibited S reductions of 2.76 $\log_{10}$ CFU/g in the natural casing, 2.77 $\log_{10}$ in the collagen casing, and 2.89 $\log_{10}$ CFU/g in the fibrous casings during fermentation and drying. Following vacuum packaged storage, S populations in the CTRL sausages had decreased 2.90, 2.84, and 2.00 $\log_{10}$ CFU/g in the natural, collagen, and fibrous casings, respectively. S populations in the TRT sausages decreased 3.46, 3.44, and 3.02 $\log_{10}$ CFU/g in the natural, collagen, and fibrous casings, respectively.

Casing type did not have an effect on LM populations in the TRT sausages ($P = 0.1610$), but did have an effect on LM

TABLE 4. Average *Salmonella* spp. populations ($\log_{10}$ CFU/g + SE*) in sausages made with natural, collagen, and fibrous casings following treatments with water or Beefxide, fermentation, drying, and vacuum packaged storage

	Natural		Collagen		Fibrous	
Sample time	**CTRL**	**TRT**	**CTRL**	**TRT**	**CTRL**	**TRT**
0 h	7.48 + 0.22^{a}	7.28 + 0.31^{a}	7.48 + 0.22^{a}	7.28 + 0.31^{a}	7.48 + 0.22^{a}	7.28 + 0.31^{a}
24 h	7.10 + 0.0^{a}	6.94 + 0.14^{a}	7.10 + 0.01^{a}	6.94 + 0.14^{a}	7.10 + 0.01^{a}	6.94 + 0.14^{ab}
Reduction^	**0.38**	**0.34**	**0.38**	**0.34**	**0.38**	**0.34**
48 h	6.60 + 0.36^{a}	6.47 + 0.19^{abd}	6.75 + 0.35^{a}	6.53 + 0.22^{ab}	6.69 + 0.28^{a}	6.43 + 0.25^{ac}
96 h	6.16 + 0.33^{a}	5.92 + 0.20^{ac}	6.14 + 0.26^{a}	5.91 + 0.25^{ac}	5.95 + 0.11^{a}	5.85 + 0.31^{ac}
Reduction	**0.94**	**1.02**	**0.96**	**1.03**	**1.15**	**1.09**
11 days	5.79 + 0.16^{a}	5.16 + 0.24^{ac}	5.79 + 0.08^{a}	5.12 + 0.41^{a}	5.86 + 0.09^{a}	5.42 + 0.24^{ac}
18 days	5.58 + 0.29^{a}	5.11 + 0.80^{a}	5.48 + 0.19^{a}	4.58 + 1.19^{a}	5.21 + 0.21^{a}	4.94 + 1.34^{a}
25 days	5.03 + 0.68^{a}	4.35 + 1.80^{a}	4.85 + 0.26^{a}	4.07 + 1.67^{a}	5.04 + 0.06^{a}	4.20 + 1.31^{a}
32 days	4.39 + 0.78^{a}	3.84 + 1.94^{ab}	4.29 + 1.04^{a}	3.83 + 1.81^{a}	4.45 + 0.14^{a}	3.71 + 1.99^{a}
Reduction	**1.77**	**2.08**	**1.85**	**2.08**	**1.50**	**2.14**
39 days	2.29 + 0.01^{b}	0.78 + 0.39^{cd}	2.48 + 0.31^{b}	0.39 + 0.01^{d}	2.15 + 0.75^{b}	0.39 + 0.00^{d}
46 days	1.97 + 0.72^{b}	0.78 + 0.39^{bc}	2.11 + 0.81^{b}	0.39 + 0.01^{cd}	2.31 + 0.50^{b}	1.02 + 0.63^{d}
53 days	2.07 + 1.67^{b}	0.39 + 0.00^{c}	1.65 + 1.26^{b}	0.69 + 0.31^{bcd}	1.90 + 1.03^{b}	1.15 + 0.76^{bcd}
60 days	1.49 + 1.10^{b}	0.38 + 0.01^{c}	1.45 + 1.07^{b}	0.39 + 0.01^{cd}	2.45 + 0.24^{b}	0.69 + 0.31^{cd}
Reduction	**2.90**	**3.46**	**2.84**	**3.44**	**2.00**	**3.02**
TR*	5.99	6.90	6.03	6.89	5.03	6.59

*SE is standard error; TR is total $\log_{10}$ reduction.

^{abcdef}Different letters in the same column denote significant differences ($P < 0.05$) within each treatment type. No significant difference was observed between casing types.

^Reductions are the differences between the last day of a phase and the last day of the previous phase.

TABLE 5. Average *L. monocytogenes* populations (log_{10} CFU/g + SE*) in sausages made with natural, collagen, and fibrous casings following treatments with water or Beefxide, fermentation, drying, and vacuum packaged storage

	Natural		Collagen		Fibrous	
Sample time	**CTRL**	**TRT**	**CTRL**	**TRT**	**CTRL**	**TRT**
0 h	$7.55 + 0.09^{a}$	$7.37 + 0.12^{a}$	$7.55 + 0.09^{a}$	$7.37 + 0.12^{a}$	$7.55 + 0.09^{a}$	$7.37 + 0.12^{a}$
24 h	$7.43 + 0.01^{a}$	$7.18 + 0.19^{ab}$	$7.43 + 0.01^{a}$	$7.18 + 0.19^{a}$	$7.43 + 0.01^{a}$	$7.18 + 0.19^{ab}$
Reduction^	**0.12**	**0.19**	**0.12**	**0.19**	**0.12**	**0.19**
48 h	$6.87 + 0.16^{ab}$	$6.71 + 0.02^{ac}$	$6.84 + 0.12^{ab}$	$6.87 + 0.08^{a}$	$6.94 + 0.28^{ab}$	$6.71 + 0.04^{abc}$
96 h	$5.75 + 0.42^{bc}$	$5.93 + 0.17^{ac}$	$5.94 + 0.26^{bc}$	$5.39 + 0.06^{ab}$	$5.82 + 0.34^{bcd}$	$5.13 + 0.08^{cd}$
Reduction	**1.68**	**1.25**	**1.49**	**1.79**	**1.61**	**2.05**
11 days	$5.52 + 0.33^{bcd}$	$5.41 + 0.08^{cde}$	$5.52 + 0.26^{bc}$	$5.72 + 0.12^{a}$	$5.52 + 0.10^{cde}$	$5.60 + 0.03^{bd}$
18 days	$5.00 + 0.31^{ab}$	$5.31 + 0.07^{bcd}$	$5.27 + 0.23^{ab}$	$5.39 + 0.06^{ab}$	$5.07 + 0.08^{ac}$	$5.14 + 0.08^{cd}$
25 days	$3.94 + 0.12^{c}$	$4.55 + 0.02^{df}$	$4.18 + 0.05^{c}$	$4.46 + 0.23^{c}$	$4.96 + 0.55^{ac}$	$4.50 + 0.13^{d}$
32 days	$3.94 + 0.15^{ce}$	$4.38 + 0.12^{ef}$	$4.26 + 0.10^{c}$	$4.68 + 0.24^{bc}$	$4.18 + 0.06^{d}$	$2.81 + 0.55^{e}$
Reduction	**1.81**	**1.55**	**1.68**	**0.71**	**1.64**	**2.32**
39 days	$2.77 + 1.07^{de}$	$1.18 + 0.78^{g}$	$2.29 + 0.01^{d}$	$1.65 + 0.47^{d}$	$3.41 + 0.90^{de}$	$1.29 + 0.29^{f}$
45 days	$1.93 + 0.58^{fg}$	$2.10 + 0.14^{h}$	$2.16 + 0.53^{d}$	$2.33 + 0.27^{d}$	$3.03 + 1.11^{ef}$	$2.19 + 0.15^{eg}$
53 days	$1.35 + 0.35^{f}$	$2.02 + 0.13^{h}$	$1.48 + 0.24^{d}$	$1.96 + 0.09^{d}$	$1.87 + 1.47^{fg}$	$1.81 + 0.51^{fg}$
60 days	$0.70 + 0.30^{g}$	$0.79 + 0.09^{g}$	$0.54 + 0.16^{d}$	$0.69 + 0.31^{e}$	$0.79 + 0.39^{g}$	$0.89 + 0.51^{h}$
Reduction	**3.24**	**3.59**	**3.72**	**3.99**	**3.39**	**1.92**
TR*	6.85	6.58	7.01	6.68	6.76	6.48

*SE is standard error; TR is total log_{10} reduction.

^{abcdefgh}Different letters in the same column denote significant differences ($P < 0.05$) within each treatment type. No significant difference was observed between casing types.

^Reductions are the differences between the last day of a phase and the last day of the previous phase.

populations in the CTRL sausages ($P = 0.0192$). *Table 5* shows LM populations for both CTRL and TRT sausages. The treatment sprays provided a 0.12 log_{10} and 0.19 log_{10} CFU/g reduction on pork trim used for the CTRL and TRT sausages, respectively. LM populations decreased 0.31 to 0.59 log_{10} CFU/g within the first 24 h of fermentation.

LM populations in the CTRL sausages decreased 3.61, 3.29, and 3.37 log_{10} CFU/g in the natural, collagen, and fibrous casings, respectively, during fermentation and drying. LM populations in the TRT sausages decreased 2.99, 2.69, and 4.56 log_{10} CFU/g in the natural, collagen, and fibrous, respectively. An additional 3.24 to 3.72 log_{10} reduction was observed following vacuum packaging for the CTRL sausages, while an additional 1.92 to 3.99 log_{10} reduction was achieved for the TRT sausages.

Specific differences in LM populations between casings were observed at day 25 and day 39 in the CTRL sausages. At day 25, pathogen populations in fibrous casings were significantly different when compared with collagen ($P = 0.0036$) and natural casings ($P = 0.0081$). At day 39 of vacuum packaged storage, pathogen populations in fibrous casings were significantly different compared with LM in collagen casings ($P = 0.0053$). For TRT sausages, at day 32 LM populations in fibrous casings were significantly different compared with collagen ($P < 0.0001$) and natural casings ($P = 0.0004$). However, there were no significant differences in pathogen populations, regardless of casing type, by the last day of vacuum packaged storage.

DISCUSSION

This experiment is one of the first to demonstrate the safety of fermented and dried salami that does not include a thermal processing step *(18)*. Despite the lack of a heat treatment, the procedures and ingredients used in the current experiment were able to achieve a 5 $\log_{10}$ reduction of S and LM. This result is due to the combined effects of hurdles presented to the pathogens, such as by-products of lactic acid bacteria, reduced pH, decreased a_w, and environmental conditions during storage.

Antimicrobial solutions have also been shown to be effective against microorganisms *(10, 19)*. Laury et al. *(12)* examined the efficacy of Beefxide® on beef trim and achieved a 1.4 $\log_{10}$ CFU/100 cm^2 reduction in EC and 1.1 $\log_{10}$ CFU/100 cm^2 reduction for S immediately after spray application. Bacterial populations measured in the first 24 h (data not shown) of the current study exhibited small reductions of EC and S on pork trim immediately after application of a 2.5% Beefxide® solution. The differences observed between the studies may be due to the sampling method. Laury et al. *(12)* used microbial swabs covering 100 cm^2, while this experiment used a thin portion of the top muscle layer for sampling.

Ellebracht et al. *(8)* demonstrated that a 2% lactic acid spray on beef trimmings proved most effective for reducing EC and S. Typhimurium, compared with a hot water wash. Lactic acid may be used at concentrations up to 5% in sprays and dips, and may provide a higher level of efficacy to reduce pathogenic microorganisms on pork trim *(26)*; however, it may impart quality defects, such as the development of off-flavors or aromas and color deterioration of the raw meat. These defects were not observed (color) or measured (flavor) in the current study.

Following the spray treatment, the CTRL sausages had higher EC and S reductions than the TRT, but had lower reductions of LM. This observation is opposite of what is expected, since Beefxide® targets Gram-negative microorganisms. The TRT sausages exhibited the largest decrease in pathogens for most casing types following fermentation and drying, and had fewer pathogens present throughout vacuum packaged storage. This finding demonstrates that application of an antimicrobial early in the process has a long-term effect when followed by fermentation, drying, and vacuum packaging.

No difference was observed between CTRL and TRT samples taken 24 h following the treatment sprays. One explanation for this result could be that the cells used to inoculate the pork trim were washed off the surface. Bacteria attach in two phases: a loose attachment phase, in which bacteria can still be removed, and a more permanent attachment, in which bacterial removal is more difficult. Berry and Cutter *(1)* inoculated beef with non-acid adapted EC and sprayed it with water and organic acids in an attempt to decontaminate the beef tissue. An approximately 1.5 to 2.0 $\log_{10}$ reduction was achieved after 24 h for tissue treated with tap water.

Alternatively, in an *in vitro* experiment using a pork product, EC appeared to have developed a protective effect in products sprayed with an organic acid and stored at 4°C, resulting in more bacteria surviving, compared with pork not sprayed with an organic acid *(6)*. Cheng and Kaspar *(5)* found bacterial acid tolerance is prolonged at colder temperatures, which may explain why more bacteria were able to survive following the Beefxide® spray and storage at 4°C.

Previous research has demonstrated the ability to achieve a 5 $\log_{10}$ reduction in Landjäger following an antimicrobial spray, fermentation, cold smoking, and drying period *(18)*. Although the process used to manufacture Landjäger also was effective at reducing pathogens prior to packaging, a 5 $\log_{10}$ CFU/g reduction was not achieved in the current experiment for any of the three pathogens investigated, until the product was subjected to vacuum packaged storage *(18)*.

Holding vacuum packaged products may be problematic for processors, who want to ship their product as quickly as possible to maximize shelf-life and quality. Holding a product to ensure that pathogenic bacteria loads are at an acceptable level also increases the likelihood that an employee would ship the product too soon, which could lead to a potential recall.

Given these problems, additional lethality treatments may be necessary to achieve a 5 $\log_{10}$ reduction sooner than can be achieved with the one week of vacuum-packaged storage. Ducic et al. *(7)* used pasteurization of dry sausages following a fermentation and drying period of 15 days, but found that pasteurizing at high temperatures had a negative impact on sausage quality. Porto-Fett et al. *(17)* demonstrated that a high pressure processing (HPP) treatment (600 MPa for 5 min) achieved a 5 $\log_{10}$ CFU/g reduction of S and LM in Genoa salami. Although HPP achieved a 5 $\log_{10}$ reduction, the research did not address how HPP may affect product quality.

This experiment used ingredients that would be common among processors and presented a worse-case scenario by using minimal ingredients to produce a shelf-stable product. Additional non-meat ingredients added to the formulation, such as garlic and other seasonings, may assist in further destruction of pathogens. The addition of more ingredients could bind water molecules and decrease a_w, making it more difficult for pathogens to grow and survive. Certain ingredients have also demonstrated antimicrobial properties. Linares et al. *(16)* studied the antimicrobial effects, *in vitro*, of garlic and red wine in chouriço (chorizo) and found that both garlic powder and juice were able to inhibit LM and *S.* Garlic powder and garlic juice worked synergistically with red wine to increase the antimicrobial effect in the meat batter. Essential oils of several herbs and spices have also been found to decrease LM, S, and *S. aureus* populations in dry sausages *(9)*.

Although this process should be accepted by the USDA–FSIS as validation for HACCP systems during the production of pork salami, caution should be taken for salamis produced in beef middle casings because of the inability of the current study to achieve a 5 $\log_{10}$ reduction of EC. Instead, processors could consider implementing a raw product testing program on incoming beef products to ensure that there is < 2 $\log_{10}$ CFU/g of EC before starting the salami process.

This experiment provides valid scientific support for HACCP systems when meat processors follow the critical parameters (antimicrobial spray of raw trim, fermentation to pH ≤ 5.0 and drying to $a_w \leq 0.88$) used in the manufacture of dry, fermented salamis that do not undergo a heat treatment. It is advised that salami manufacturing facilities utilize good manufacturing practices and purchase raw ingredients from reputable suppliers to minimize the potential for pathogenic contamination of raw materials. Although there was no significant difference between the CTRL and TRT sausages, it is also recommended that processors spray trim with an antimicrobial prior to grinding to achieve additional reductions of pathogens. Additional validation support is necessary if processors want to utilize beef middle casings or beef as a component of the formulation to produce this type of product.

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