Pathogen Reductions Associated with Traditional Processing of Landjäger

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ABSTRACT

To determine if traditional processing would reduce *Escherichia coli* O157:H7 (EC), *Listeria monocytogenes* (LM), and *Salmonella* spp. (S) in landjäger, beef and pork trim (95% lean) and pork fat was experimentally-inoculated to obtain a pathogen population of *ca.* 8 log₁₀CFU/g. The trim was spray-treated with 4.5 % lactic acid (30 min, 25°C), ground, mixed with seasonings, and a starter culture added. Sausages were stuffed, pressed, fermented (to pH 4.8), cold smoked, and dried to a water activity of 0.88. Sausages were vacuum packaged, stored (20 days, *ca.* 23°C), and evaluated for microbial populations, pH, and water activity. Lactic acid treatment of beef trim reduced EC, S, and LM 0.23, 0.42, and 0.22 log₁₀ CFU/g, respectively. Subsequent fermentation and drying reduced EC, LM, and S 3.94, 4.11, and 4.29 log₁₀ CFU/g respectively. Average log reductions of 7.83, 6.19, and 7.21 log₁₀ CFU/g were observed for EC, LM and S, respectively, for the duration of the study. This study demonstrates that traditional processing of landjäger may result in a *ca.* 5 log reduction of foodborne pathogens.

1. INTRODUCTION

Fermented, dry or semi-dry sausages are ready-to-eat (RTE), shelf-stable products that are produced by fermenting a seasoned (sugar, salt and various spices), raw-meat batter to a pH of ≤ 5.3 and a final moisture-to-protein (M:P) ratio of $\leq 3.1:1.0$ (American Meat Institute Foundation, 1997; USDA-FSIS, 2005). The fermentation can be carried out by microflora naturally present in the raw meat or by the direct addition of a commercial starter culture. Following fermentation, sausages may be smoked to enhance the development of desired flavor, color, and aroma. Finally, sausages are dried to remove up to 15% moisture for semi-dry sausages, or up to 50% moisture for dry sausages (American Meat Institute Foundation, 1997).

Traditionally, fermented dry and semi-dry sausages have been regarded as safe products, due to their low pH, low water activity (a_w), salt content, and the presence of competing microflora. Combined, these attributes are expected to limit survival and growth of pathogenic bacteria (Barbuti & Parolari, 2002; Ducic *et al.*, 2016; Ellajosyula *et al.*, 1998; Hwang *et al.*, 2009; Lindqvist & Lindblad, 2009). However, several cases of foodborne illnesses have been associated with these types of products. In 1994, *Escherichia coli* O157:H7 was responsible for an outbreak associated with dry-cured salami. More recently, Lebanon bologna was linked to an outbreak of *E. coli* O157:H7 (Hwang *et al.*, 2009). These outbreaks led the USDA-Food Safety and Inspection Service (USDA-FSIS) to reconsider the safety of these products and establish guidelines to ensure their safety (USDA-FSIS, 2001).

The USDA-FSIS guidelines provide five alternatives to thermal treatments for these types of RTE sausages, one of which is to scientifically validate an alternative process and required

lethality for pathogens of interest, by demonstrating a 5 log CFU/g reduction of *E. coli* O157:H7, if the sausage formulation contains beef products (USDA-FSIS, 2001). Other pathogens of concern in fermented, RTE sausages include *Salmonella* spp. and *Listeria monocytogenes*. Regulations for pathogen reductions and limits for allowable number of cells in the final product differ by type of pathogen, food product and regulating agency (FDA vs USDA). The USDA-FSIS has issued a “zero tolerance” policy for *Salmonella* spp. In addition, *L. monocytogenes* also has a zero tolerance in RTE foods, due in part to its high mortality rate (USDA-FSIS, 2014).

Several studies have evaluated the survival of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. in various fermented sausages. Nissen & Holck (1998) evaluated the effect of storage temperature on pathogen survival and noted that after 5 months of refrigerated storage, both *E. coli* O157:H7 and *L. monocytogenes* could still be recovered, whereas *Salmonella* Kentucky was unable to be recovered. Furthermore, they concluded that storage at 20°C might be preferable for fermented sausages since none of the pathogens could be recovered after 5 months of storage at this temperature (Nissen & Holck, 1998). Ellajosyula *et al.* (1998) examined the effect of fermentation pH, heating temperature, and time on the reduction of *E. coli* O157:H7 and *S. Typhimurium*. They concluded that fermentation or heating alone was unable to obtain reductions of >2 log units, but the combination of both, fermenting to a final pH of 4.7 and gradual heating to 120°F in 10.5h, achieved reductions of >7 log units for both pathogens (Ellajosyula *et al.*, 1998). Ducic *et al.* (2016) demonstrated a 1.3 log CFU/g reduction for *E. coli* O157:H7 in beef sausage, a 1.4 log CFU/g reduction for *S. Typhimurium* in pork sausage, and 0.8 and 0.5 log CFU/g reductions for *L. monocytogenes* in pork and beef sausage, respectively (Ducic *et al.*, 2016). Similarly, Porto-Fett *et al.* (2008) demonstrated that fermentation and drying alone

reduced *E. coli* O157:H7 by 0.03 and 1.11 log₁₀ CFU/g in sausages fermented to pH 5.3 and 4.8, respectively, while *S. Typhimurium* and *L. monocytogenes* were reduced by 1.52 and 3.51 log₁₀ CFU/g and 0.07 and 0.74 log₁₀ CFU/g, respectively (Porto-Fett *et al.*, 2008).

Despite different experimental conditions (fermentation pH, starter cultures used or final a_w), studies have demonstrated that pathogens, although they can be reduced significantly by a combination of processes, may be present in the final product unless an adequate heat treatment is applied (Holck *et al.*, 2011; Hwang *et al.*, 2009; Lindqvist & Lindblad, 2009; Porto-Fett *et al.*, 2008).

Landjäger is a fermented, semi-dry sausage, originating from southern Germany. It is traditionally produced through fermentation at 24°C (75.2°F) and drying (18-14°C in 88 to 76% relative humidity) (Toldrá, Hui, Astiasarán, Sebranek, & Talon, 2014). Since landjäger is produced typically without a heat step, the safety and stability of the product is attributed to the low pH (≤ 5.0) and low a_w (≤0.90) to control pathogenic bacteria. For sausage processors who do not include a thermal treatment as part of their process, due to the potential unfavorable impact on product quality, alternative processes must be employed in order to ensure food safety. Therefore, processors must demonstrate pathogen reductions through validation studies that satisfy USDA-FSIS requirements for food safety (USDA-FSIS, 2001).

The objectives of the present research were to determine if a traditional landjäger process would result in a ca. 5 log₁₀ CFU/g reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. The information from this study may be of interest to researchers,

processors, and regulatory officials who are interested in determining the safety of landjäger and other similarly-produced, semi-dry fermented RTE sausages.

2. MATERIALS AND METHODS

2.1 Bacterial Strains

Three isolates of *E. coli* O157:H7, including ATCC 43895, Sakai, and PA-2; four strains of *Salmonella enterica subsp. enterica*, including serovars Typhimurium (ATCC 14028), Panama (ATCC 7378; human isolate), Derby (ATCC 6960; tank water and pork pies) and Montevideo; four isolates of *Listeria monocytogenes*, including Scott A, serotype 1/2a isolate FSL R2-603 (outbreak linked to deli meats in 2000), serotype 4b isolate H3396 (outbreak associated with hotdogs in 1998) and *L. monocytogenes* Pirie (ATCC 19115; human isolate) were obtained from the Food Microbiology Culture Collection (Department of Food Science, Pennsylvania State University). All cultures were stored at -80°C in tryptic soy broth (TSB) (Difco, Detroit, MI) supplemented with 20% glycerol (Sigma Aldrich, St. Louis, MO) to ensure viability. These frozen stock cultures were revived by transferring 0.1 ml of each frozen culture to 10 ml of fresh TSB and incubated aerobically at 37°C for 24h. A loopful of the overnight culture was streaked onto Tryptic Soy agar (TSA) plates and incubated at 37°C for 24 h to obtain isolated colonies. An overnight culture was prepared from isolated colonies of each pathogen that were transferred to 10 ml of fresh TSB and incubated at 37°C for 24 h. The inoculum for the experiments was prepared by transferring 1 ml of the of the previous overnight culture into 100 ml of fresh TSB and incubated overnight at 37°C for 24 h to obtain *ca.* 9 log₁₀ CFU/ml. Ten ml of the cultures were transferred to 500 ml of TSB for another 24 h at 37°C prior the experiment. To obtain a concentrated inoculum, a modified

procedure was used (Richard & Cutter, 2011). Equal volumes (500 ml) of each pathogen culture were centrifuged at 11,000 x g for 5 min in sterile centrifuge bottles, washed once with a volume of buffered peptone water (BPW), resuspended in fresh BPW at one tenth of the original TSB volume, and mixed together in the bottles prior to inoculation. Using this methodology, single-pathogen cocktail populations were found to be *ca.* 9.5, 8.7, and 9.1 log₁₀ CFU/ml for *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* respectively.

2.2 Inoculation and Processing of Trim

Trim (5 kg [11 lbs]; 4°C), containing pork boneless ham (1.8 kg [4 lbs]; 95% lean), beef knuckles (1.8 kg [4 lbs]), and pork back fat (1.6 kg [3 lbs]) was obtained from the Penn State Meat Laboratory (University Park, PA). Control (non-inoculated) and treatment trim were placed in autoclaved metal bins and inoculated either with sterile buffered peptone water (BPW; control) or the pathogen cocktail (250 ml). Trim pieces were turned and mixed in the bin to ensure even coverage. Inoculated trim was left undisturbed for 30 min to allow for pathogen attachment. Control and inoculated trim was then transferred and placed on sterile plastic trays and treated by spraying the inoculated surfaces with a solution of 4.5% lactic acid (25°C, pH 2.5; Birko, US) using a handheld tank sprayer (Leader Plus, Hudson, US) for 45 s. Trim was turned and sprayed again for 45 s to ensure adequate coverage, placed inside a cooler, and left to stand for another 30 minutes. After inoculation, the surface pH of the trim was measured using a surface pH probe (Model A57184, Beckman Coulter, US). The control and experimentally-inoculated trim were fine ground once through a 4.5 mm plate using a benchtop grinder (Universal Mincer, Jupiter, GE) and the resulting batter was seasoned with salt (NaCl; 2.93% wt/wt), red wine (1.63% wt/wt), dextrose (0.60% wt/wt), black pepper (0.32% wt/wt), granulated garlic (0.16% wt/wt), ground

caraway (0.16% wt/wt), ground coriander (0.09% wt/wt), curing salt (6.25% NaNO₂; 156 ppm), and sodium erythorbate (546 ppm). The mixture was hand massaged using sterile gloves until all ingredients were mixed uniformly into the batter. A commercial, freeze-dried starter culture (Safepro® B-LC-007; CHR Hansen, Denmark) containing *Pediococcus acidilactici*, *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Lactobacillus sakei*, and *Pediococcus pentosaceus* was revived following the manufacturer's recommendations. The revived starter culture was added to the meat batter, ca. 1.1 g per 5 kg (11 lbs) of meat to obtain ca. 7.2 log₁₀ CFU/g.

2.3 Sausage Preparation

The control and inoculated batter were hand-stuffed (The Sausage Maker, Buffalo, NY) into 32/35 mm hog casings (Quality Casings, Hebron, KY). Sausages, ca. 20 cm (8 in) long and weighing ca. 100 grams each, were linked in pairs and placed on a press (ca. 55 cm x 55 cm). The press was held together with 24" clutch lock bar clamps (Irwin Tools, Huntersville, NC) and then transferred to the smokehouse (Vortron model TR2-850, Beloit, WI), fermented at an average temperature of 23.3°C (74°F) and a relative humidity (RH) of 61% (U12 Stainless Steel Temp Logger; Onset HOBO Dataloggers, Bourne, MA) to a target pH of 4.8 (Testo pH2 model 206, Sparta, NJ). After fermentation (72 h), sausages were smoked (Hickory sawdust; The Frantz Co, Milwaukee, WI) for a period of 2 h at 30°C (87°F, RH 70%). After smoking, sausages were dried for 3 d at 21.6°C (average 71°F, RH 60%) to a target a_w of 0.88 (AquaLab 4 TE; Decagon, Pullman, WA). After drying, sausages were removed from the smokehouse, allowed to acclimate to ambient temperature (ca. 23°C), transferred to the laboratory, and vacuum packaged in 3-mil standard barrier vacuum bags (15.2 cm x 20.3 cm; OTR approx. 0.5-0.83 cc/100in²/24hr; Prime Source, Kansas City, MO) using an Ultravac UV-250 (Koch Equipment; Kansas City, MO, USA).

2.4 Microbial Analysis

At each sampling time (see Table 1), two sausages each, from the control and inoculated product, were aseptically removed for microbial analysis, pH, and a_w measurements. For microbial analysis, sausages were placed in sterile, filtered stomacher bags (BagFilter, InterScience; St. Norm, France), weighed, and diluted 1:10 in sterile buffered peptone water (BPW, Difco). Samples were stomached for 2 min at speed setting 3 (JumboMix® 3500 VP, InterScience). The resulting stomachate of each sausage was serially diluted in 9 ml of BPW and aliquots of 0.1 ml were spread plated in duplicate, onto selective agars to achieve a detection limit of *ca.* 0.5 log₁₀ CFU/g. When necessary, a 1 ml aliquot (0.5 ml in quadruplicate) was directly plated from the stomachate onto selective agars. Xylose lysine deoxycholate agar (XLD; Remel, Lenexa, KS), cefixime tellurite sorbitol MacConkey agar (CT-SMAC; Remel, Lenexa, KS) and modified Oxford agar with antibiotic supplement (MOX; Remel, Lenexa, KS) were used for the enumeration of *Salmonella* spp. (S), *E. coli* O157:H7 (EC), and *L. monocytogenes* (LM), respectively. Plates were incubated aerobically at 37°C for 24h (EC and S) or 48h (LM), after which typical looking colonies were enumerated manually. When no bacteria were detected during standard plating, a value of 1 CFU/g was used to allow for conversion to log₁₀ CFU/g. Enrichments were performed simultaneously during processing as follows. When counts on the plates were below the detection level (0.5 log₁₀ CFU/g), enrichments were used to determine presence of EC, S, and LM. Gram-negative (GN) broth (Difco, US) was used to enrich for EC, while lactose broth (LB) (Himedia, IN) and Rappaport-Vassiliadis (RV; Remel, Lenexa, KS) broth and University of Vermont Medium (UVM; Difco) and Fraser broth (Difco) were used as primary and secondary enrichments, for S and LM respectively. Enrichments were performed as previously described.

Briefly, 1 ml aliquots of stomachate were transferred to pre-enrichments (9 ml) broths (GN, LB, and UVM for EC, S, and LM, respectively), followed by selective enrichment in RV broth and Fraser broth for S and LM, respectively (Scheinberg, Valderrama, & Cutter, 2013). Enriched samples were incubated aerobically at 37°C, transferred into secondary enrichments the following day, and incubated as previously described. Enrichments were plated on selective agars and further confirmed by latex agglutination (Oxoid, UK). In each of the four independent replicates, two control (un-inoculated) and two of the inoculated sausages were sampled at each sampling interval. Bacterial numbers were expressed as log₁₀ CFU/g.

2.5 Statistical Analysis

Bacterial populations (averaged duplicates) were converted to log₁₀ CFU/g to allow for analysis and transformation of data in order to meet the assumptions of the statistical test. Log reductions were calculated by subtracting final log counts (B) from initial log counts (A): Log reduction (LR) = log₁₀ (A) – log₁₀ (B). Resulting data were analyzed using the SAS statistical program version 9.4 (SAS Institute, Cary, NC). The mixed procedure was used to analyze the effect of pH, a_w, pathogen type, replicate number, and time on log counts. The Kenward-Roger method was used to determine degrees of freedom as it adjusts for bias, improves the F statistic and is especially useful with small sample sizes (Schaalje, McBride, & Fellingham, 2002). Fixed variables were pathogen type, replicate number, and the interactions between pathogen and time, and pathogen and replicate number. Time was included as a repeated effect with pathogen type by replicate number as the subject. Least squares means and standard errors were determined for all fixed effects. The regression procedure was used to analyze the effect of pH and a_w on pathogen log reductions. Significance was declared at $p < 0.05$.

3. RESULTS

The inoculation level in the trim was *ca.* 8.50, 7.81, and 7.85 log₁₀ CFU/g of product for EC, S, and LM respectively. Initial pH of the trim was on average, 6.10 and 6.08 for control and treatment trim, respectively. Surface pH of trim after lactic acid treatment was 5.52 and 5.44 for control and treatment trim, respectively. Reductions obtained by lactic acid treatment of the trim after inoculation with the pathogen cocktail and prior to grinding were not statistically significant. Log reductions observed for 4.5% lactic acid spray treatment of pathogens were on average, 0.23, 0.42, and 0.22 log₁₀ CFU/g for EC, S and LM, respectively.

Using the methodologies described above, landjäger processing resulted in a consistent reduction of internal pH to values of ≤ 5.0 after fermentation. Fermentation was stopped once sausages reached target pH, with the average internal pH ranging from 4.9 to 5.0, for control and treatment sausages, respectively. After fermentation, sausages were dried to an average a_w of ≤ 0.88 , after which sausages were vacuum packaged. As expected, there were no significant differences ($p > 0.05$) in pH or a_w between control sausages and treatment sausages throughout the process, indicating that the addition of a pathogen cocktail did not affect the growth and performance of the starter culture (Figures 1 and 2).

Data on the behavior of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* during the processing of landjäger sausage is presented in Table 1. In general, reductions for all three pathogens were similar, as demonstrated in Figure 3. Interestingly, there were some notable differences as to when reductions became statistically significant ($p < 0.05$) for each pathogen. Reductions for EC were significant after the process of fermentation was concluded; however,

for S, significant reductions occurred up to 24 h of fermentation, while reductions for LM only became significant after smoking had occurred (Table 1).

The process of fermentation alone resulted in reductions of 2.92, 1.57, and 3.64 log₁₀ CFU/g for EC, LM, and S, respectively; whereas, drying provided additional reductions of 1.02, 2.54, and 0.65 log₁₀ CFU/g, respectively. Subsequent log reductions for EC after fermentation, were not significant ($p > 0.05$) until day 12 of vacuum packaged storage at 23°C. Reductions were significant from smoking at 96 h to day 16 (552 h) of vacuum packaged storage at 23°C. By day 20 of storage, EC was no longer recoverable (negative by enrichment). In contrast, S was not reduced significantly after fermentation. By day 12 of vacuum packaged storage, S was no longer recoverable (confirmed negative by enrichment). LM reductions after smoking (96 h) were no longer significant ($p > 0.05$) and it continued to be recoverable until the last day of vacuum packaged storage.

The regression model showed that both pH and a_w contributed to log reductions for EC, whereas S and LM were mostly affected by a_w . These results are in contrast with those reported elsewhere. Researchers found that a_w was more significant than pH in reducing EC, while pH was more significant than a_w in reducing LM (Hwang *et al.*, 2009). It is somewhat unexpected that a_w impacted LM survivability more than pH, given that reductions during storage were not considered statistically significant.

Results obtained in this study indicate that traditional processing of landjäger sausage, coupled with vacuum packaged storage at ambient temperature can result in a safe product. Moreover, initial trim treatment with lactic acid might contribute to the observed pathogen

reductions. Overall, total log reductions by the end of the storage period were over 5 log₁₀ CFU/g for each pathogen and only LM could be recovered in very low amounts or was only positive in landjäger samples following enrichment.

4. DISCUSSION

In this study, lactic acid treatment (4.5%) of trim resulted in lower reductions of the pathogens, when compared to those reported elsewhere (Wolf *et al.*, 2012; Fouladkhah *et al.*, 2012; Harris, Miller, Loneragan, & Brashears, 2006). For example, Wolf *et al.* (2012) demonstrated a 0.5 to 1 log₁₀ CFU/g reduction in pathogens experimentally inoculated onto trim. These findings are not unexpected, given that Wolf *et al.* (2012) treated trim by submersion in a 4.4% lactic acid solution, which may have provided better coverage. Similar reductions were observed by Fouladkhah *et al.* (2012) in which counts of *E. coli* O157:H7 were reduced by 0.5 and 1 log₁₀ CFU/g following immersion of experimentally-inoculated beef in a 5% lactic acid solution at 25°C and 55°C, respectively. Ellebracht *et al.* (1999) inoculated beef trimmings with EC and S, treated the trim with hot water (95°C), alone or in combination with a 2% lactic acid solution, and ground the treated beef. Results demonstrated that hot water treatment alone resulted in 0.9 and 0.7 log₁₀ CFU/g of EC and S, respectively, while treatments with hot water and 2% lactic acid resulted in reductions of 1.5 and 1.8 log₁₀ CFU/g, respectively (Ellebracht, Castillo, Lucia, Miller, & Acuff, 1999). More recently, Blagojevic *et al.* (2015) treated beef trimming intended for dry fermented sausage production by submersion in a 4% hot lactic acid solution. These treatments resulted in reductions of 3.9 and 3.6 log₁₀ CFU/g for EC and S, respectively, in the final product; however reductions for LM were insignificant (Blagojevic *et al.*, 2015) In this study, a room temperature solution of lactic acid was used. It has been suggested that application of lactic acid

to beef trim at warmer temperatures might improve pathogen reductions on trim. Therefore, the lower reductions obtained by the lactic acid treatment in the present study could in part, be due to its lower temperature upon application.

Although pathogen reductions with the lactic acid treatment were lower than expected, small reductions from several hurdle techniques, could contribute to the overall log reduction goal. It must be noted that an artificially high inoculum level was purposely used in this study in order to maintain high enough counts to be able to observe the effect of sausage processing on the survival of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*. This level of contamination is not expected to reflect a real processing scenario.

Previous research exploring the efficacy of fermentation and drying to reduce pathogen loads during sausage production to the levels required by USDA-FSIS standards has demonstrated that this process alone is unlikely to achieve such reductions (Hwang *et al.*, 2009; Porto-Fett *et al.*, 2008). Results have been variable across studies and may be influenced by sausage fermentation pH, final water activity, initial pathogen loads, storage temperature and overall experimental design (i.e. surface inoculation versus batter inoculation). To date, no research has demonstrated a 5 log reduction for *E. coli* O157:H7 in semi-dry sausages. Porto-Fett *et al.* (2008) studied the viability of multi-strain mixtures of *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 inoculated into soudjuk-style fermented, semi-dry sausages. The sausages fermented to a final pH of 4.8, exhibited reductions of 0.74, 3.51, and 1.11 log₁₀ CFU/g for *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7, respectively. These reductions are consistent with what others have reported previously. Ducic *et al.* (2016) reported reductions of 0.8 and 0.5 log₁₀ CFU/g reductions for *L. monocytogenes* in pork and beef sausages, respectively. In addition, the

308 authors reported reductions of 1.4 log₁₀ CFU/g in pork sausages and 1.3 log₁₀ CFU/g in beef
309 sausages for *Salmonella* spp. and *E. coli* O157:H7, respectively (Ducic *et al.*, 2016).

310 The results observed in the present study demonstrated higher reductions after
311 fermentation and drying for all three pathogens than those previously reported, although they
312 did not achieve a 5 log reduction. It has been previously noted that maturation
313 (fermentation/drying) time, especially for sausages that do not undergo a heat treatment, is
314 instrumental in reducing pathogenic bacteria, and that shorter maturation times could result in
315 insufficient pathogen reductions (Lindqvist & Lindblad, 2009).

316 The reductions observed in this study are probably the result of a combination of various
317 hurdle techniques applied throughout processing. First, the use a commercial starter culture
318 ensured a controlled and consistent fermentation, which resulted in a rapid drop in pH to <5.0.
319 Secondly, the commercial starter culture used in this study also served as a bio-protectant, due
320 its ability to produce pediocin (a type of bacteriocin) which has known anti-listerial properties.
321 Researchers have shown that *L. monocytogenes* can be inhibited by competing lactic acid
322 bacteria that are added as a starter culture, in part due to the production of organic acids and/or
323 bacteriocins by the organisms (Nieto-Lozano *et al.*, 2010; Raimondi, Popovic, Amaretti, Di Gioia,
324 & Rossi, 2014).

325 The use of a starter culture for fermentation is recommended over a natural inoculation,
326 since it provides a consistent and controlled fermentation. In contrast, Ducic *et al.* (2016) used
327 natural microflora present in the meat for the purpose of fermentation. The use of natural
328 microflora is discouraged as it can lead to inconsistent fermentations and negative sensory

attributes of the final product. In addition, throughout the process and storage time, lactic acid bacteria continued to survive in high amounts (*ca.* 6 log; data not shown), which could have outcompeted injured pathogenic bacteria that had survived the process. The reduction of available water to the desired target ($a_w \leq 0.88$) is essential to prevent the growth of bacteria during long term storage. In this study, it appears that storage temperature also contributed to overall reductions. Several studies have noted that higher storage temperatures are preferred over refrigerated temperatures for reducing pathogens (Lindqvist & Lindblad, 2009; Nissen & Holck, 1998; Porto-Fett *et al.*, 2008).

Reductions for *Salmonella* spp. after fermentation and drying in this study, were higher than reductions observed for *E. coli* O157:H7 and *L. monocytogenes*. Ellajosyula *et al.* (1998) found that *Salmonella* spp. were more susceptible to fermentation pH and high temperature treatments. The authors also noted that *S. Typhimurium* was as susceptible as *E. coli* O157:H7 in fermented and heat-treated Lebanon bologna (Ellajosyula *et al.*, 1998). *L. monocytogenes* may survive better than *E. coli* O157 and *S. Typhimurium* in fermented and dried sausages, which is not unusual, given that the pathogen is a Gram-positive bacterium (Ducic *et al.*, 2016; Ellajosyula *et al.*, 1998; Hwang *et al.*, 2009; Lindqvist & Lindblad, 2009; Nissen & Holck, 1998).

In this study, landjäger processing, including treatment of trim with a 4.5% lactic acid solution and subsequent fermentation, smoking, drying, and vacuum packaged storage at 23°C were evaluated for reducing pathogen populations. EC and S were reduced to undetectable levels by the end of vacuum packaged storage (day 20), while LM continued to be recovered, albeit in very low amounts or only by enrichment. In conclusion, the proposed landjäger sausage process could help achieve higher reductions of EC, LM, and S when combined with strict process controls

for pH and a_w . It is important to note that some EC outbreaks associated with fermented sausages, such as the 2011 Lebanon bologna outbreak, have occurred due either to improper processing techniques or the use of supporting scientific research that was not reflective of the plant's processing conditions (Sartz *et al.*, 2008; USDA-FSIS, 2013). Therefore, when producing fermented sausages that do not have a cooking step as part of processing, it is imperative that other pathogen control measures, such as a low pH or low a_w are being achieved consistently. In addition, it is important to begin with good quality raw materials that have low microbial loads. This research could help processors establish and/or validate parameters (pH and a_w) for the manufacture of semi-dry fermented sausages using very similar conditions. More research is needed, regarding the potential of pre-treating trim with antimicrobial agents and its effects on the subsequent reductions of pathogens during fermented sausage processing. Additionally, research is needed to determine the effects of sub-lethal hurdle interventions throughout processing on the survival of injured cells, which could remain viable in the product during storage.

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Table 1. Populations ($\log_{10}$ CFU/g) and log reductions of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. through landjäger processing.

	<i>E. coli</i> O157:H7	<i>L. monocytogenes</i>	<i>Salmonella</i> spp.
4.5% Lactic Acid (LA) Spray Treatment			
Prior LA (Initial)	8.50 ± 0.37^A	7.85 ± 0.34^A	7.81 ± 0.54^A
Post LA	8.27 ± 0.40^A	7.63 ± 0.32^A	7.39 ± 0.45^A
Log Reduction	0.23 ± 0.29	0.22 ± 0.04	0.42 ± 0.37
Fermentation through drying and storage			
Prior Fermentation (0h)	7.40 ± 0.10^{Aa}	6.70 ± 0.12^{Aa}	6.31 ± 0.51^{Aa}
After Fermentation (72h)	4.48 ± 0.59^{Ab}	5.13 ± 0.38^{Aa}	2.67 ± 0.84^{Ab}
After Smoking (96h)	4.55 ± 0.27^{Ab}	3.62 ± 0.41^{Ab}	1.79 ± 0.96^{Ab}
After Drying (168h/d0)	3.46 ± 0.40^{Ab}	2.59 ± 0.88^{Ab}	2.02 ± 1.00^{Ab}
Log reduction*	$5.04 \log_{10} \text{ CFU/g}$	$5.26 \log_{10} \text{ CFU/g}$	$5.79 \log_{10} \text{ CFU/g}$
Final Storage (648h/d20)	0.67 ± 0.23^{Ac}	1.66 ± 0.11^{Ac}	0.60 ± 0.23^{Ab}
Log Reduction	6.73 ± 0.13	5.04 ± 0.18	5.71 ± 0.28
Total Reduction**	$7.83 \log_{10} \text{ CFU/g}$	$6.19 \log_{10} \text{ CFU/g}$	$7.21 \log_{10} \text{ CFU/g}$

Note: $\pm$ denotes the standard errors of the mean, log reductions calculated by subtracting final log counts from initial log counts. Log reductions with different upper case letter superscripts in the same row and different lowercase letters in the same column are significantly different ($p < 0.05$). *Log reduction calculated as Prior LA(Initial) log count – After drying log count.

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Figure Legends

Figure 1. pH Reduction During Processing of Landjäger.

Figure 2. a_w Reduction During Processing of Landjäger.

Figure 3. Populations of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. during Processing of Landjäger.

FIGURE 1

Note: Control = non-inoculated sausages; TMT = sausages inoculated with pathogen cocktail. Control sausages serve as a point of comparison for a normal fermentation, indicating that the addition of high amounts of pathogens did not adversely affect the process.

FIGURE 2

Note: Control = non-inoculated sausages; TMT = sausages inoculated with pathogen cocktail. Control sausages serve as a point of comparison for a normal process, indicating that the addition of high amounts of pathogens did not adversely affect the process.

532 FIGURE 3

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534 Note: Behavior and reduction trend of EC, LM and S cocktail in inoculated sausages throughout the sausage production and
 535 storage time (beginning of fermentation to last day of storage).