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Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami ☆

Anna C.S. Porto-Fett [a,1], Jeffrey E. Call [a], Bradley E. Shoyer [a], Dolores E. Hill [b], Claudette Pshebniski [c], George J. Cocoma [d], John B. Luchansky [a,*]

[a] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Microbial Food Safety Research Unit, 600 East Mermaid Lane, Wyndmoor, PA, 19038, USA
[b] Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, 10300 Baltimore Avenue, Beltsville, MD, 20705, USA
[c] Santa Maria Foods ULC, Brampton, ON, L6T 5M4, CA
[d] Professional Resource Organization, 1405 Irvine Drive, Edmond, OK, 73003, USA

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ABSTRACT

We evaluated the effectiveness of fermentation, drying, and high pressure processing (HPP) to inactivate *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Trichinella spiralis* in Genoa salami produced with trichinae-infected pork. In addition, we evaluated the effectiveness of using HPP to inactivate *T. spiralis* larvae in pig masseter tissue. In part A, Genoa salami batter (about 2.3 log larvae/g) prepared with trichinae-infected pork was separately spiked with a five-strain cocktail of each microbial pathogen (about 7.0 log CFU/g) and subsequently fermented at 20 °C and about 90 to 95% RH for 6 h and then at 27 °C and about 90 to 95% RH for 26 h before being dried at 20 °C and about 65 to 75% RH for 40 h and then at 17 °C and about 65 to 75% RH to/for: A) 25 d (65 mm casing), B) a target a_w of 0.92 (65 mm casing), C) 35 d (105 mm casing), or D) a target a_w of 0.94 (105 mm casing). Inactivation of *L. monocytogenes, E. coli* O157:H7, and *Salmonella* spp. after fermentation and drying ranged from about 1.1 to 1.3, about 1.1 to 2.2, and about 4.2 to 4.8 log CFU/g, respectively. After drying, three replicate salami samples in each of two trials for each treatment were subjected to HPP. Pressurization at 600 MPa or at 483 MPa for 1 to 12 min reduced pathogen numbers by an additional 1.6 to ≥5.0 (*L. monocytogenes*), 4.7 to ≥5.8 (*E. coli* O157:H7), and 1.9 to 2.4 (*Salmonella*) log CFU/g. After storage for 28 d at 4 °C, *L. monocytogenes* levels decreased by up to an additional 3.0 log CFU/g, whereas an additional decrease of up to about 1.1 and 1.7 log CFU/g was observed for *E. coli* O157:H7 and *Salmonella*, respectively. In contrast, in each of three trials, *T. spiralis* was inactivated (about 2.3 log larvae/g) in Genoa salami by all treatments of fermentation and drying as confirmed by both microscopy and mouse bioassays. In part B, in each of two trials, a 10-g portion (2 replicates per treatment) of infected pig masseter muscle (about 3.4 log larvae/g) were pressurized at 483 and 600 MPa for 0.5 to 5 min. *T. spiralis* was inactivated in pig masseter by all treatments of HPP as confirmed by both microscopy and mouse bioassays. Thus, fermentation and drying and/or HPP of contaminated Genoa salami or pork are effective for inactivating *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* spp., and/or *T. spiralis* larvae. These data validate that HPP can be used as an alternate to curing for trichinae control and as a post-process intervention to meet performance standards and/or compliance guidelines for the three microbial pathogens evaluated herein.

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1. Introduction

Although a concerted effort by both the meat industry and regulatory agencies has appreciably reduced the incidence of *Listeria monocytogenes* and *Salmonella* spp. in fermented sausage, the USDA/FSIS reported the prevalence of these pathogens in fermented meats at about 1.4% (10 of 698 samples) and 3.3% (27 of 830 samples), respectively, in federally inspected plants between 1997 and 1999 (Levine et al., 2001). Likewise, from 1995 to 1999, 3445 regulatory samples of dry and semi-dry sausages tested negative for *Escherichia*

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* Corresponding author. Tel.: +1 215 233 6676; fax: +1 215 233 6581.
E-mail address: john.luchansky@ars.usda.gov (J.B. Luchansky).
[1] Present address: Oser Technologies, P.O. Box 62, Blacksville, WV, 26521, USA.

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coli O157:H7 (Levine et al., 2001). To further insure the safety of fermented meats, the USDA/FSIS established performance standards that require manufacturers of ready-to-eat (RTE) sausage to validate the lethality of their processes. For dry and semi-dry fermented meats, as detailed by Nickelson et al. (1996), the USDA/FSIS accepts the following five options for control of pathogens: i) utilize heat as detailed in 9 CFR 318.17, ii) use a validated 5D inactivation treatment, iii) conduct a hold-and-test program for finished product, iv) propose other strategies to achieve 5D inactivation, and iv) conduct raw batter-testing and deliver 2D inactivation (see also, Reed, 1995 and USDA-FSIS, 2003a). The USDA/FSIS performance standards also require processors of red meat and poultry products to achieve a 6.5-log (beef and pork) or a 7.0-log (chicken and turkey) reduction for *Salmonella*, as well as maintain a "zero tolerance" for *L. monocytogenes* in cooked and RTE meats (USDA-FSIS, 2001b; USDA-FSIS, 2003a). With further regard to *L. monocytogenes*, manufacturers of RTE red meat and poultry products are also required to include a post-process lethality step, or to include a post-process intervention to preclude outgrowth of this pathogen during refrigerated shelf life, or to deliver both lethality and inhibition to achieve the so called Alternative 1, 2, or 3 status (USDA-FSIS, 2001b; USDA-FSIS, 2003a).

Genoa salami, a dry sausage traditionally manufactured using raw pork, salt, and spices, relies on a decrease of pH and water activity (a_w) during fermentation and drying for both its quality and safety attributes. The USDA standard of identity for Genoa requires it to have a moisture-to-protein ratio (M:Pr) value of $\leq$2.3:1.0 (USDA-FSIS, 2005). To be categorized as shelf-stable, in association with other hurdles such as salt and curing agents, Genoa salami must attain: i) a pH of pH 5.2 and an a_w of <0.95, or ii) only a pH of <pH 5.0, or iii) only an a_w of <0.91 (http://www.fsis.usda.gov/PDF/FSRE_S-S_7Principles.pdf). Several investigators have evaluated the lethality of processes and/or have developed post-process interventions for microbial pathogens in fermented sausages (Calicioglu et al., 2001; Ihnot et al., 1998; Faith et al., 1998; Nightingale et al., 2006; Porto-Fett et al., 2008). Among the many biological, chemical, and physical interventions used to control and/or eliminate foodborne pathogens in fermented RTE meat products are the addition of bacteriocins and/or of food-grade antimicrobials to the formulation, and/or the application of high pressure processing (HPP), hydrodynamic pressure processing (HDP), heat, or E-beam irradiation to the finished product (Cabeza et al., 2009; Deumier and Collignan, 2003; Hinkens et al. 1996; Marcos et al., 2005). However, relatively little is known about the effectiveness of HPP when used in combination with fermentation and drying to inactivate targeted microbial pathogens such as *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. in Genoa salami. Thus, one objective of the present study was to evaluate modifications to a traditional/standard protocol for processing Genoa salami, alone and in combination with HPP, for efficacy towards the above mentioned microbial pathogens while enhancing product quality.

Trichinellosis remains one of the most significant zoonotic foodborne diseases worldwide (Dorny et al., 2009; Gottstein et al., 2009). It is commonly associated with the ingestion of raw or uncooked pork that becomes infected with the encysted larvae of *Trichinella spiralis* (Gamble, 1996; Mitreva and Jasmer, 2006). Modifications, including implementation of Good Agricultural Practices in swine production, have dramatically decreased the prevalence of trichinae in conventionally raised swine to virtually non-detectable levels in the United States over the past 60 years (Gamble et al., 1998; Gamble et al., 1999; Pyburn et al., 2005). Moreover, when present, levels of this parasite in pork are quite low, ranging from 0.003 to 1000 larvae/g (Doby, 1987; Gamble et al., 1999). Thus, pigs continue to harbor this parasite, albeit at reduced prevalence and levels than in the previous six decades and, therefore, may serve as a vector for its transmission to humans in the absence of Good Agricultural Practices, Good Manufacturing Practices, and/or Hazard Analysis Critical Control Point standards.

Although sporadic in occurrence, trichinellosis in humans still occurs in the U.S., usually from non-pork sources such as from feasting on improperly prepared game meats (Kennedy et al., 2009). However, the historical association of this parasite with swine is primarily responsible for Federal control measures that must be strictly followed during further processing of RTE pork products in the U.S. and in Canada (Gamble and Bush, 1999). More specifically, the control of *T. spiralis* in pork must be accomplished by mandatory carcass inspection of pigs at slaughter or by post-slaughter processing interventions, namely heating, freezing, and/or curing of products containing pork (USDA-FSIS, 2001a). In fact, prescribed conditions for the time and temperature of heating and/or freezing of pork are used as a trichinae intervention for all parts of the muscle tissue of pork or products containing pork tissue. Curing is also used as a trichinae intervention for products containing pork, including Genoa salami. However, the effectiveness of curing to eliminate *T. spiralis* larvae depends on a combination of various processing parameters and on the product formulation, specifically on the temperature and time of fermentation/drying and the salt level, respectively. Therefore, curing alone, as a post-slaughter intervention, is not recommended by the International Commission on Trichinellosis (ICT) as a stand alone method to inactivate *T. spiralis* larvae in products made with raw pork (Gamble et al., 2000). Additional/alternative processing technologies for trichinae control are considered on a case-by-case basis by USDA/FSIS using criteria described in more detail elsewhere (USDA-FSIS, 2001a).

High pressure processing (HPP) has received considerable attention as a post-processing intervention to eliminate pathogens in RTE products, especially products that might be altered by other interventions, notably by thermal treatment. Although numerous studies have been conducted to determine the effect of HPP on microbial pathogens directly in foods, including *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* (Hayman et al., 2004; Jofré et al., 2008; Marcos et al., 2005; Morales et al., 2006, Murano et al., 1999; Simpson and Gilmour, 1997), few have been conducted on foodborne parasites such as *T. spiralis* (Ohnishi et al., 1992; Ohnishi et al., 1994; Gamble et al., 1998; Lindsay et al., 2005). In the present study, we evaluated the effect of fermentation/drying on viability of *T. spiralis* in Genoa salami. We also evaluated HPP for efficacy towards *T. spiralis* larvae in trichinae-infected pig masseter muscle as an alternate to curing for trichinae control. We also validated the integrated lethality of a post-process HPP treatment in combination with a traditional protocol for production of Genoa salami towards *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* to confirm that existing performance standards and/or compliance guidelines were being met.

2. Material and methods

2.1. Microbial strains and preparation of inocula

The multi-strain cocktails (Table 1) of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. were confirmed, cultured, combined, and/or maintained as described previously (Porto et al., 2002; Porto-Fett et al., 2008). The nematode, *T. spiralis* (Table 1), was maintained by successive passage in female Sprague–Dawley rats (Covance, Denver, PA). Every 60 d the infected rats were killed using CO_2 gas, and viable larvae were collected by artificial digestion from the muscle tissue and inoculated into rats by oral lavage as described below (Gamble, 1996). The commercial starter cultures (Kerry Bio-Science, Rochester, MN) used in the production of the Genoa salami, that being Bactoferm HPS (*Pediococcus acidilactici*), Saga 75 (*P. pentosaceous*), and Saga 444 (*Kocuria varians*), were maintained and prepared according to the instructions provided by the manufacturer.

Table 1
Strains of *L. monocytogenes, E. coli* O157:H7, *Salmonella*, and *T. spiralis* used in this study.

Microbial strains	Strain #	Other designation	Source	Type[a]	Ref.
L. monocytogenes	MFS2		Environmental isolate from a pork processing plant	1/2a	Porto et al. (2002)
	MFS102	H7776	Frankfurter isolate, 1998 BilMar outbreak	4b	CDC (1998)
	MFS104	ScottA	Clinical isolate, 1983 Massachusetts pasteurized milk outbreak	4b	Linnan et al. (1988)
	MFS105	LM-101 M	Beef and pork sausage isolate	4b	Glass and Doyle (1989)
	MFS110	F6854	Turkey frankfurter isolate	1/2a	Schwartz et al. (1988)
E. coli	FSIS OB070361		Ground beef isolate	O157:H7	USDA-FSIS (2007)
	JBL2139	C7927	Clinical isolate, 1991 Massachusetts apple cider outbreak	O157:H7	Besser et al. (1993)
	JBL2347	SLH21788	Clinical isolate, 1994 Wisconsin daycare outbreak	O157:H7	Proctor (1996)
	JBL1411	EC505B	Beef isolate	O157:H7	Calicioglu et al. (2001)
		380-94	Salami outbreak	O157:H7	Anonymous (1995)
S. typhimurium	MFS-248		Hog carcass isolate		Wonderling et al. (2003)
S. typhimurium	MFS-330		Hog carcass isolate		Wonderling et al. (2003)
S. copenhagen	8457		Pork isolate		Juneja et al. (2001)
S. typhimurium	FSIS OB060362		Clinical isolate associated with pork sausage		USDA-FSIS Outbreaks and Special Projects Laboratory, Athens, GA
S. typhimurium	JBL3270	H3380	Clinical isolate	DT104	Briggs and Fratamico (1999)
T. spiralis	T1		Pig isolate		Gamble (1996)

[a] Serotypes for *L. monocytogenes* and *E. coli* strains; Phage types for *Salmonella* strains.

2.2. Recovery of T. spiralis by artificial digestion

As described by Gamble (1996), a 10-g sample of pork, pig masseter tissue, or Genoa salami and/or an entire rat or mouse was transferred to a blender cup (Model MB-101; Homeland Housewares, Pacoima, CA) previously sanitized with a quaternary ammonium-based germicide, multipurpose detergent (Misty® Biodet ND32; Amrep, Marietta, GA) and containing 50 mL of artificial digestion fluid (about pH 2.0), that being 1% pepsin (wt/vol; Fisher Scientific, Rochester, NY) and 1% hydrochloric acid (vol/vol; Mallinckrodt Chemicals, Phillipsburg, NJ). After blending for eight-second intervals a total of three successive times, an additional 250 mL of digestion fluid and a magnetic stir bar were added to each sample, and the contents were vigorously mixed for up to 1 h in an incubator while maintained at 47 °C. Next, samples were filtered through a stainless-steel #45 size sieve (355 μm; U.S. Standard Sieve, Thomas Scientific, Swedesboro, NJ) to remove undigested debris and then allowed to settle for about 1 h at room temperature. The sediment was washed with sterile distilled water and allowed to resettle until the supernatant was clear. Samples were then fed to mice or examined under a stereo microscope as detailed below.

2.3. Inoculation of pigs with Trichinella larvae and harvesting of infected pork meat

For each of three trials, to establish infection, two, three-month-old, castrated male pigs (Yorkshire X Duroc crossbreed) were fed only one gelatin capsule (#13; Torpac Inc., Fairfield, NJ) using a 2 3/8" metal balling gun (Model #4000; Ideal Instruments/Neogen Inc., Lexington, KY) containing about 10,000 *T. spiralis* muscle larvae suspended in tap water. Infective larvae used for the inoculum were recovered from the above mentioned Sprague–Dawley rats by artificial digestion. Inoculated pigs were housed in separate pens and were quarantined from non-infected pigs in accordance with the Animal Welfare Act, Guide for the Care and Use of Laboratory Animals (http://www.nap.edu) and with the approval of the USDA/ARS Beltsville Area Institutional Animal Care and Use Committee (BAA-CUC) under protocol #08-044. The pigs were fed about 2 lbs of a 16% protein-based feed (Purina Growena; Land O'Lakes Purina Feed, LLC, Glenview, IL) per day, with water available *ad libitum*. After about 35 d, pigs were humanely euthanized by chemical anaesthesia in accordance with methods provided in the Guide for the Care and Use of Laboratory Animals. The triceps, picnics, hams, neck, rump, and loins were hand trimmed of excess fat and connective tissue with an alcohol-sterilized sterile butchers' knife to yield a total of about 50 to 70 lbs of meat from each animal. The resulting large chunks/portions of infected meat were cut into smaller pieces and transported on ice to the USDA/ARS Eastern Regional Research Center (ERRC; Wyndmoor, PA) where it was placed into sterile nylon-polyethylene bags (Prime Source Packaging Ltd., Houston, TX), vacuum-sealed to 950 mbar (Ultravac-500; Bunzl/Koch, Kansas City, MO), and then stored at 4 °C for up to 7 d. Whole masseter muscle was separately harvested from each pig and transported on ice to the ERRC where it was also vacuum-sealed and then stored at 4 °C for up to 7 d. Larval burdens in harvested pork and masseter samples were determined as described below within 3 d following slaughter/harvest. Storage at 4 °C has little effect, if any, on viability of trichinae larvae. More specifically, Kotula et al. (1990) reported 100% survival of *T. spiralis* after storage for 135 d at −3.9 °C and Hill et al. (2009) reported survival for 106 h at −6.6 °C in pork meat within bags from each the air was expelled just prior to heat sealing.

2.4. Inoculation of batter and processing of Genoa

As described in more detail below, salami was prepared with the assistance of, and in collaboration with, a commercial processor using their standard make protocol and with adherence to the guidelines in 9 CFR 318.10 (USDA-FSIS, 2001a) for "curing" of sausage as delineated under method 6. In brief, approximately 200 lbs (about 90 kg) of raw salami batter was prepared in each of six batches. In three of these six batches, salami was prepared using trichinae-infected pork [35 lbs or 13.6 kg (17.6%)], but without the masseter tissue, obtained as described above, along with boneless, skinless pork picnics [103.6 lbs or 49.4 kg (52.3% of total weight)] and pork trim [59.4 lbs or 27 kg (30% of total weight)] that were purchased fresh from a local wholesaler. Regardless, for the three batches of salami that did not contain *T. spiralis* larvae, pork picnics were substituted for the infected pork. This blend of pork meat was mixed using a sanitized mixer/grinder (Model 4346; Hobart Corporation, Troy, OH) to achieve a ratio of about 80% lean meat to 20% fat. The blend was subsequently coarse ground through a grinding plate with 3/8" holes (Model 4346; Hobart), and then stored overnight at 4 °C, for convenience, in large (100 lbs capacity), covered, high-density polyethylene containers (Bunzl/Koch). The mixer/grinder was sanitized with a quaternary ammonium-based germicide, multipurpose detergent (Misty® Biodet ND32), rinsed thoroughly for 3 min with hot water (87.8 °C), and then allowed to air dry at room temperature for up to 1 h. The coarse ground pork was re-loaded into the sanitized mixer/grinder, and the following dry ingredients and spices were added: high-grade sodium chloride (2.9%; The Canadian Salt Company Ldt., Pointe Claire, QC, Canada), dextrose (1%; Tate & Lyle of Nealanders International Inc., Mississauga, ON, Canada), white pepper (0.08%; Wiberg Canada

Corporation, Oakville, ON, Canada), sodium ascorbate (0.05%; H & A Industrial Inc., Toronto, ON, Canada), garlic powder (0.02%; Wiberg), sodium nitrate (0.015%; Wiberg), and sodium nitrite (0.005%; Wiberg). The blended batter was then mixed for about 1 min before about 31 g of Bactoferm HPS, about 10.4 g of Saga 75, and about 8.5 g of Saga 444 collectively diluted with a total of 15 mL of sterile water (final concentration of about 6.0 log CFU/g of batter) and 900 mL of either the *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. pathogen cocktails (final concentration of about 7.0 log CFU/g of batter) were added to the batter. Mixing was continued for an additional 2 min, and then the inoculated salami batter was fine ground through a 3/16" grinding plate (Model 4346; Hobart) and stuffed into either about 65 mm (about 2.5 in.) diameter × about 160 mm (about 6.3 in.) length or about 105 mm (about 4.0 in.) diameter × about 200 mm (about 8.0 in.) length cellulose-based casings (Naturin R2; Weinham, Germany) using either a stainless steel table-top manual piston stuffer (24 lbs capacity; Model FD-9051200; F. Dick, Esslingen, Germany) or a floor-type, hydraulic-driven piston stuffer (50 lbs capacity; Model SC-50, Koch Equipment, Kansas City, MO). After stuffing, the salami chubs were hand tied with twine, transferred to a temperature- and humidity-controlled walk-in incubator (EJS Systems Inc., Changrin Falls, OH) with an air flow of 1.0 to 1.5 m/s, and hung vertically on racks so that the individual chubs were not touching. The relative humidity (RH) and air temperature were controlled and measured using the Dynamist 2000 System (EJS) and Partlow MRC5000 chart recorder (EJS). The salami was held/fermented at 20 °C and about 90 to 95% RH for 6 h, and then at 27 °C and about 90 to 95% RH for 26 h. Next, the chubs were dried at 20 °C and about 65 to 75% RH for 40 h, and then at 17 °C and about 65 to 75% RH for/to: A) 25 d (65 mm casing), B) a target a_w of 0.92 (65 mm casing), C) 35 d (105 mm casing), or D) a target a_w of 0.94 (105 mm casing). Treatments A and C are the standard/traditional make protocols practiced by our cooperating processor for small and large casing sizes, respectively. The end point for drying used for treatments A and C were determined as prescribed in 9 CFR 318.10 for salami prepared with added salt of less than 2.9 lbs per hundredweight and a casing diameter at stuff of either 65 mm or 105 mm. The drying times for treatments B and D were selected based on a target a_w value of 0.92 or 0.94, respectively, to yield the best quality product in terms of taste and texture, in combination with high pressure processing (HPP) for trichinae control. Following fermentation and drying, the chubs were treated by HPP and subsequently stored at 4 °C as described below.

2.5. Pressurization of Genoa salami and pig masseter muscle

i) HPP of Genoa: After fermentation and drying, but prior to refrigerated storage, large (105 mm casing) and small (65 mm casing) diameter chubs or portions therefrom were separately transferred to nylon-polyethylene bags (Prime Source Packaging) and vacuum-sealed to 950 mbar. Each chub was then transferred to a second nylon-polyethylene bag and vacuum-sealed a second time to 950 mbar. Salami chubs were then shipped overnight on ice to either Gridpath Inc. (Stoney Creek, ON, Canada), Virginia Polytechnic Institute and State University (VPI; Blacksburg, VA), or the National Center for Food Safety and Technology (NCFST; Summit Argo, IL) for treatment by HPP. The VPI and NCFST are equipped with a 35-liter capacity high pressure processing unit (Model Quintus Food Press 35L-600; Avure Technologies, Kent, WA). This unit can generate up to 600 MPa (87,000 psi) at temperatures between 40° and 95 °F. Gridpath is equipped with a commercial 55-liter capacity high pressure processing unit (Model 6000/55; NC Hyperbaric, Burgos, Spain). Salami chubs were pressurized at 600 MPa (87,000 psi) for 0, 1, 2, 3, 5, or 7 min or at 483 MPa (70,000 psi) for 0, 5, 7, 10, or 12 min. For salami treated with HPP, the average come-up-times (CUT) were 131.4 s (± 16.3 s) for 87,000 psi and 114.7 s (± 19.0 s) for 70,000 psi. The average initial temperatures of the water in the pressure vessel were 66.9 °F (± 4.04 °F) for 87,000 psi and 66.7 °F (± 3.84 °F) for 70,000 psi. For each pressure level, the above mentioned values for CUT and initial temperature are the average of 96 measurements. Each measurement was a separate run in the HPP unit, that being pressurization of three chubs for a given pressure level and exposure time for each pathogen in each of two trials. The pressure release times were instantaneous. The temperature range attained after pressurizing to 87,000 psi was 83° to 98 °F and was 74° to 91 °F after pressurizing to 70,000 psi. The pressure-treated salami was placed on ice and shipped via overnight courier to ERRC for microbiological analyses. Relative to the levels of pressure applied, 600 MPa for 3 min was tested because it has been approved as a technology for treating meats and is being used by the industry to process RTE whole muscle and sliced meats to enhance quality, extend shelf-life, and inactivate pathogens, particularly *L. monocytogenes* (Avure). The lower pressure level, 483 MPa, was selected to evaluate if it would be lethal for the target pathogens, but less disruptive to the product. *ii) HPP of masseter muscle*: In each of two trials, two 10-g portions of tissue were aseptically harvested from random areas of masseter muscle (about 3.4 log larvae/g) that were previously removed from trichinae-infected pigs as described above. The excised masseter tissue was separately vacuum packaged and then shipped overnight on ice to VPI for HPP or was pressure treated at ERRC (2-liter capacity; Model 2L, Avure). The Model 2L unit can generate up to 690 MPa (100,000 psi) at temperatures ranging from 50° to 194 °F. The samples of masseter tissue were subjected to 600 MPa (87,000 psi) for 0.5, 1, 1.5, or 3 min, or to 483 MPa (70,000 psi) for 0, 1, 2, 3, or 5 min. For masseter muscle treated with HPP, the average CUT were 122.5 s (± 1 s) for 87,000 psi and 101.3 s (± 3.8 s) for 70,000 psi. The average initial temperatures of the water in the pressure vessel were 69.0 °F (± 0 °F) for 87,000 psi and 77.3 °F (± 2.6 °F) for 70,000 psi. The above mentioned values for CUT and initial temperature are the average of 96 measurements. Each measurement was a separate run in the HPP unit, that being pressurization of for a given pressure level and exposure time for a single trial. The pressure release times were instantaneous. The temperature range attained after pressurizing to 87,000 psi was 81° to 86 °F and was 85° to 88 °F after pressurizing to 70,000 psi. The Avure 2L does not record the water temperature inside the vessel during come up and pressurization. However, because the pressure applied was the same as for the units used at VPI and GridPath we assume that the same increase in temperature was achieved on the Avure 2L unit. In addition, for each exposure time we programmed the Avure 2L unit to achieve the target pressurization level within a 5-min period. After treatment, the samples were shipped via overnight courier on ice to ERRC and/or processed within 24 h to screen for viable larvae as described below.

2.6. Storage of Genoa salami

Following HPP treatment, chubs previously inoculated with *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. were removed from the bags, placed on Styrofoam trays (1012S, Genpak, Glens Falls, NY), and sliced/sectioned with the aid of an ethanol-sterilized knife. Each slice/section (about 120 g for 65 mm casing or about 430 g for 105 mm casing) was separately placed into nylon-polyethylene bags (Prime Source Packaging) and vacuum sealed. Samples were incubated at 4 °C for 0, 7, 14, and 28 d. It was not possible to test salami for viable larvae of *T. spiralis* during refrigerated storage, since prior treatment by fermentation and drying were lethal for *T. spiralis.*

2.7. Recovery of L. monocytogenes, E. coli O157:H7, Salmonella spp., and indigenous flora from Genoa salami

In each of two trials, for each diameter of salami, fermentation/drying condition, HPP treatment, and storage time, a total of three

salami samples were analyzed for the presence and/or levels of either *L. monocytogenes, E. coli* O157:H7, or *Salmonella* spp. Single 25 g portions from each sample were transferred into sterile filtered stomacher bags (K & R Technologies, Frederick, MD) containing 25 ml of 0.1% sterile peptone water and stomached for 2 min (Stomacher 400; Seward, Cincinnati, OH). The resulting slurry, with or without prior dilution in 0.1% sterile peptone water, was surface-plated (100, 250, or 500 μL) onto Modified Oxford agar (MOX; Difco, Becton, Dickinson and Company, Franklin Lakes, NJ) for enumeration of *L. monocytogenes*, onto MacConkey sorbitol (SMAC; Difco) for enumeration of *E. coli* O157:H7, or onto Rappaport-Vassiliadis agar (RV; Difco) for enumeration of *Salmonella* spp. Typical colonies of each pathogen were counted after aerobic incubation of these plates at 37 °C for either 48 h (MOX) or 24 h (SMAC and RV). When *Salmonella* spp., *E. coli* O157:H7, or *L. monocytogenes* numbers decreased to below detection (≤0.3, ≤0.3, and ≤1.0 log CFU/g, respectively) by direct plating, the presence or absence of each pathogen was determined by selective enrichment essentially as described (Hinkens et al., 1996; Ihnot et al., 1998; Cook, 1999). Likewise, at selected intervals after stuffing and after drying, total aerobic plate counts (TPC) were enumerated by spread plating the resulting slurry, with or without prior dilution in 0.1% sterile peptone water, onto BHI agar plates and incubating for 72 h at 30 °C. For enumeration of total lactic acid bacteria (LAB), the slurry was spread-plated, with or without prior dilution in sterile 0.1% peptone water, onto Mann, Rogosa and Sharp (MRS; Difco) agar plates and incubated anaerobically (10.1% carbon dioxide, 4.38% hydrogen and balance nitrogen; Bactron IV Anaerobic/Environmental Chamber, Sheldon Manufacturing Inc., Cornelius, OR) for 48 h at 37 °C.

2.8. Recovery of *T. spiralis* from Genoa salami and pig masseter

For Genoa salami, in each of three trials, for each diameter of salami, fermentation/drying treatment, and HPP treatment, a total of two 10-g salami samples were analyzed for the presence of viable *T. spiralis* larvae via microscopy and/or bioassay. Likewise, for pig masseter muscle, in each of two trials, a total of two, 10-g masseter tissue samples were analyzed to determine the effect of HPP on the viability of *T. spiralis* larvae. After digestion of each test sample as described above, a 0.1- to 1-ml portion was examined via microscopy (Model MZFLIII; Leica Microsystems, Wetzlar, Germany) at 30× to 50× magnification to enumerate and determine the presence of viable/non-viable larvae by visual inspection. Alternatively, digested samples were orally gavaged into mice for determining infectivity (see below). *i) Infectivity assay in mice*. All samples were tested for the presence of infective larvae by orally gavaging 100 μL of the digested samples into female Swiss-Webster mice (Taconic, Albany, NY) using a 1-mL syringe (Becton-Dickinson) and a 20-gauge, 1.5" length oral gavage needle (Cadence Science, Lake Success, NY) essentially as described by Hill et al. (2007). Approximately 30 d post-infection, mice were euthanized by CO_2 asphyxiation and then skinned and eviscerated in accordance with the Guide for Animal Care and Use of Laboratory Animals. Eviscerated and skinned mice were transferred to sterile nylon-polyethylene bags and placed at 4 °C for up to 3 d. Each mouse was separately digested in total as described above to subsequently examine microscopically for the presence of larvae in the muscle tissue.

2.9. Physical–chemical analyses of Genoa salami

For each trial and for each treatment, the a_w of one chub/portion per treatment was measured at each sampling interval by placing a nominal amount (about 3 g) of salami into a sampling cup and analyzing it using an electronic water activity meter (Aqualab Model Series 3; Decagon Devices, Pullman, WA) according to the manufacturer's instructions. For pH measurements, a 25-g portion of a chub was transferred to a filtered stomacher bag containing 25 ml of 0.1% peptone water and then macerated for 2 min. For each trial and for each pathogen, the pH of one chub/portion per treatment was measured after fermentation using a model 6000P pH/temperature electrode and a model 5500 pH meter (Daigger, Vernon Hills, IL). The proximate composition of Genoa salami was determined in one of the six trials using a 100-g composite sample comprised of the three salami portions taken directly after fermentation/drying ($N=1$ trial, $n=3$ salami portions per trial). Proximate composition analyses were performed according to methods approved and described by the Association of Official Analytical Chemists (McNeal, 1990) as conducted by a commercial testing laboratory.

2.10. Statistical analyses

Data were analyzed using version 9.1.3 of the SAS statistical package (SAS Institute Inc., Cary, NC). When the three pathogens tested were inoculated into Genoa salami batter, analysis of variance (ANOVA) was performed to evaluate the effects of fermentation, drying, and HPP on pathogen reduction over time. When microbial numbers decreased to below detection (≤0.3, ≤0.3, and ≤1.0 log CFU/g, respectively), a value of zero was used for determination of the arithmetic mean. The individual means and standard deviations of pathogen viability in Genoa salami for each treatment were determined from the average of three samples at each sampling interval for each of the two trials. Differences in the proximate composition among treatments A, B, C, and D were also evaluated using ANOVA. Mean separations were performed using the Bonferroni LSD method.

3. Results

3.1. Levels of indigenous flora

Direct plating and/or enrichment of the six batches of skinless pork picnics and pork trim before inoculation revealed the absence of any indigenous *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. by both direct plating (≤1.0 log CFU/g) and by enrichment (data not shown). The average initial levels of TPC and LAB in batter after stuffing were 7.75 ± 0.11 log CFU/g and 7.56 ± 0.12 log CFU/g, respectively. The average levels of TPC in Genoa salami after drying for treatments A, B, C, and D were 9.07 ± 0.23, 8.32 ± 0.70, 8.85 ± 0.08, and 8.49 ± 0.70 log CFU/g, respectively, whereas the average levels of LAB in Genoa salami after drying for treatments A, B, C, and D were 8.72 ± 0.62, 8.67 ± 0.36, 8.56 ± 0.77, and 9.11 ± 0.14 log CFU/g, respectively. Essentially there were no appreciable differences in the initial and in the final levels of TPC and LAB among treatments A, B, C, and D. Also, there were no appreciable differences in the levels of TPC and LAB for a given treatment.

3.2. Proximate composition of Genoa salami

Results from proximate composition analyses (Table 2) did not show significant ($P>0.05$) differences in the levels of carbohydrates, fat, nitrite, or acidity among treatments A, B, C, and D. Regarding the levels of ash, moisture, protein, M:Pr ratio, salt, a_w, and pH, proximate composition analyses revealed only subtle differences ($P<0.05$) for a given chemical parameter among treatments A, B, C, and D. Our data confirmed that, with the exception of treatment D, treatments A, B, and C were in compliance with the USDA/FSIS guidelines for the M:Pr ≤ 2.3:1.0 to be designated as Genoa salami. However, all treatments achieved the combined pH (pH<5.2) and a_w (a_w 0.95) parameters/values to be designated by USDA/FSIS as shelf-stable (http://www.fsis.usda.gov/PDF/FSRE_SS_7Principles.pdf). The average initial pH of the salami batter before stuffing was pH 6.04 ± 0.02 (Table 3). No statistical differences ($P>0.05$) in pH values were observed among treatments after fermentation and drying. After fermentation and

Table 2
Proximate composition of raw batter and Genoa salami.

Analyses	Batter[1]	Genoa salami treatments[2]			
		A	B	C	D
Ash (g/100 g)	3.74 ± 0.07[d]	6.07 ± 0.08[a]	4.85 ± 0.26[b,c]	5.22 ± 0.03[b]	4.68 ± 0.01[c]
Carbohydrates (g/100 g)	1.05 ± 0.45[a]	0.10 ± 0.0[a]	0.71 ± 0.67[a]	0.32 ± 0.31[a]	0.93 ± 0.08[a]
Fat (g/100 g)	19.48 ± 2.73[a]	27.90 ± 0.52[a]	26.73 ± 4.25[a]	24.14 ± 1.69[a]	21.56 ± 0.98[a]
Moisture (g/100 g)	59.30 ± 2.20[a]	37.81 ± 0.50[c]	44.58 ± 6.35[b,c]	46.89 ± 1.26[b,c]	53.03 ± 1.22[a,b]
Protein (g/100 g)	16.43 ± 0.72[d]	28.43 ± 0.30[a]	23.64 ± 1.81[b]	23.51 ± 0.01[b]	19.82 ± 0.30[c]
M:Pr	3.61:1 ± 0.04[a]	1.33:1 ± 0.03[d]	1.57:1 ± 0.20[d]	1.99:1 ± 0.04[c]	2.68:1 ± 0.11[b]
Salt (g/100 g)	3.01 ± 0.06[d]	4.88 ± 0.04[a]	3.94 ± 0.13[c]	4.23 ± 0.01[b]	3.75 ± 0.00[c]
Nitrite	1.33 ± 0.52[a]	1.0 ± 0.00[a]	1.0 ± 0.00a	1.0 ± 0.00[a]	1.5 ± 0.70[a]
Acidity (%)	0.66 ± 0.01[b]	2.31 ± 0.04[a]	2.13 ± 0.30[a]	2.01 ± 0.10[a]	1.84 ± 0.03[a]
Water activity	0.961 ± 0.007[a]	0.884 ± 0.009[c]	0.926 ± 0.011[c]	0.920 ± 0.008[c]	0.940 ± 0.005[a,b]
pH	5.80 ± 0.08[a]	4.56 ± 0.14[b]	4.65 ± 0.21[b]	4.64 ± 0.09[b]	4.66 ± 0.09[b]

[1] Proximate composition analyses conducted in Genoa salami batter were determined in each of the six trials performed using a 100-g composite sample comprised of the two batter portions taken directly before fermentation/drying ($N = 6$ trial, $n = 2$ batter portions per trial).
[2] Proximate composition of Genoa salami was determined in one of the six trials using a 100-g composite sample comprised of the three salami portions taken directly after fermentation/drying ($N = 1$ trial, $n = 3$ salami portions per trial).

drying, the pH of the salami decreased to pH 4.56 ± 0.14, 4.65 ± 0.21, 4.64 ± 0.04, and 4.66 ± 0.09 for treatments A, B, C, and D, respectively. The average initial a_w of the salami batter before stuffing was a_w 0.961 ± 0.007 (Table 4); however, after fermentation and drying, the average a_w for treatments A, B, and C were significantly ($P<0.05$) lower than for treatment D. The average a_w of the resulting salami generated by treatments A, B, C, and D was a_w 0.884 ± 0.009, 0.926 ± 0.011, 0.920 ± 0.008, and 0.940 ± 0.005, respectively. For treatments C and D, the target a_w values of 0.92 and 0.94 were achieved after 14 ± 3 d and 22 ± 2 d of drying, respectively. After HPP and subsequent storage at 4 °C for 28 d, the a_w decreased to about a_w 0.875, 0.919, 0.913, and 0.930 for treatments A, B, C, and D, respectively.

3.3. Viability of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in Genoa salami after fermentation and drying

For Genoa salami stuffed into either a 65-mm or a 105-mm diameter casing there were no significant differences ($P>0.05$) in the comparative viability of *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* after fermentation (Tables 5, 6, and 7, respectively). After drying, for a given pathogen there were no significant ($P<0.05$) differences in viability between treatments A and B and between treatments C and D. For comparisons among the three pathogens, after drying, there were significant ($P<0.05$) differences in viability of *L. monocytogenes* and *E. coli* O157:H7 compared to viability of *Salmonella* spp. for a given treatment. Results showed that *Salmonella* spp. decreased to a greater extent after drying for all treatments in Genoa salami than either *L. monocytogenes* or *E. coli* O157:H7 for both casing sizes tested. Total reductions after fermentation and drying of Genoa salami ranged from about 1.1 to 1.3, about 1.1 to 2.2, and about 4.2 to 4.8 log CFU/g for *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp., respectively.

3.4. Viability of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in Genoa salami after HPP and storage

Storage of Genoa salami without prior HPP treatment had comparatively little effect on *L. monocytogenes* (Table 5) and *E. coli* O157:H7 (Table 6), whereas levels of *Salmonella* spp. (Table 7) decreased by as much as about 1.5 log CFU/g at 4 °C within 28 d. In contrast, HPP had a significant effect ($P<0.05$) on the extent of inactivation of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in Genoa salami compared to salami that were not treated with high pressure (Tables 5, 6, and 7, respectively). Regardless of exposure time for HPP, when salami was pressurized at 600 MPa there were significant ($P<0.05$) differences in viability of *L. monocytogenes* between treatments A and B or between treatments C and D. However, regardless of the duration of the application of high pressure, when salami was pressurized at 483 MPa after fermentation/drying, no statistical ($P>0.05$) differences were observed in viability of *L. monocytogenes* between treatments A and B or between treatments C and D. Regardless of the amount of pressure or the time of exposure, there were significant ($P<0.05$) differences in viability of *L. monocytogenes* between treatments A and B or between treatments C and D after 28 d of refrigerated storage. Likewise, regardless of pressure level, or time of exposure, or length of storage, there were no statistical differences ($P>0.05$) in the extent of inactivation of *Salmonella* spp. or *E. coli* O157:H7 in Genoa salami between treatments A and B or between treatments C and D. For control samples of salami that were not treated via HPP, regardless of the conditions for drying, there were significant ($P<0.05$) differences in viability of *L. monocytogenes* and *E. coli* O157:H7 compared to viability of *Salmonella* spp. when Genoa salami were subsequently stored 4 °C for up 28 d; more cells of *L. monocytogenes* or *E. coli* O157:H7 survived fermentation and drying than did cells of *Salmonella* spp.

Following pressurization at 600 MPa for 1 to 5 min or at 483 MPa for 5 to 12 min, numbers of *L. monocytogenes* were reduced by an additional 1.6 to ≥5.0 log CFU/g compared to their levels after fermentation and drying. However, regardless of the pressure or time of exposure, during 28 d of storage at 4 °C, *L. monocytogenes* levels decreased by up to an additional 3.0 log CFU/g in treatments A, B, and D, whereas levels of the pathogen increased by 0.4 to 2.2 log CFU/g in treatment C. When salami was not treated via HPP, numbers of *L. monocytogenes* were reduced by up to an additional 0.4 log CFU/g during storage at 4 °C for up to 28 d. With regard to *E. coli* O157:H7 and *Salmonella* spp., regardless of the amount of pressure or the time of exposure, it was only possible to recover these pathogens via enrichment. Nonetheless, following pressurization and storage, *E. coli* O157:H7 numbers were reduced by an additional ≥4.7 to ≥5.8 log CFU/g, whereas numbers of *Salmonella* spp. were reduced by an additional ≥1.9 to ≥2.4 log CFU/g compared to levels present after fermentation and drying. When salami was not treated with pressure, numbers of *E. coli* O157:H7 and *Salmonella* spp. were reduced by up to an additional about 1.1 and 1.7 log CFU/g, respectively. Our results established that it was possible to achieve a total reduction of about 3.6 to 6.1 log CFU/g of *L. monocytogenes*, about ≥6.2 log CFU/g of *E. coli* O157:H7, and about ≥6.6 log CFU/g of *Salmonella* spp. by fermentation and drying in combination with HPP. Collectively, these results established the effectiveness of HPP as a post-process treatment to inactivate *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in Genoa salami.

3.5. Viability of *T. spiralis* in Genoa salami after fermentation, drying, and HPP

Regardless of the processing conditions, fermentation and drying alone were sufficient for reducing the initial load (about 2.3 log

Table 3
Average of pH values following processing, pressurization, and storage of Genoa salami.

Processing step:		Casing diameter										Casing diameter									
		65 mm										105 mm									
Batter[a]		5.80±0.08										5.80±0.08									
Fermentation[a]		4.58±0.12										4.58±0.12									
Drying to target[b] Time or a_w		A) 25 days 4.56±0.14					B) a_w 0.92 4.65±0.21					C) 35 days 4.64±0.04					D) a_w 0.94 4.66±0.09				
HPP at 600 MPa for (min)[c]:		0	1	2	3	5	0	1	2	3	5	0	1	2	3	5	0	1	2	3	5
		4.63 (0.10)	4.59 (0.18)	4.71 (0.01)	4.72 (0.03)	4.73 (0.03)	4.76 (0.14)	4.87 (0.04)	4.77 (0.11)	4.87 (0.01)	4.86 (0.03)	4.64 (0.04)	4.57 (0.14)	4.63 (0.06)	4.67 (0.04)	4.69 (0.06)	4.66 (0.13)	4.74 (0.12)	4.68 (0.10)	4.61 (0.14)	4.73 (0.11)
Storage at 4 °C for:	7 days	4.65 (0.22)	4.79 (0.16)	4.83 (0.03)	4.79 (0.12)	4.72 (0.26)	4.62 (0.18)	4.71 (0.11)	4.79 (0.08)	4.66 (0.18)	4.82 (0.08)	4.63 (0.21)	4.70 (0.15)	4.80 (0.13)	4.67 (0.22)	4.82 (0.13)	4.55 (0.13)	4.62 (0.15)	4.64 (0.11)	4.68 (0.04)	4.56 (0.20)
	14 days	4.71 (0.23)	4.92 (0.06)	4.89 (0.01)	4.78 (0.19)	4.78 (0.23)	4.64 (0.15)	4.82 (0.05)	4.77 (0.04)	4.70 (0.13)	4.60 (0.21)	4.65 (0.18)	4.76 (0.11)	4.79 (0.01)	4.70 (0.16)	4.61 (0.16)	4.60 (0.14)	4.69 (0.04)	4.76 (0.07)	4.64 (0.13)	4.59 (0.15)
	28 days	4.73 (0.25)	4.95 (0.04)	4.96 (0.04)	4.69 (0.19)	4.93 (0.06)	4.67 (0.18)	4.84 (0.02)	4.84 (0.01)	4.68 (0.17)	4.85 (0.03)	4.69 (0.23)	4.78 (0.11)	4.86 (0.16)	4.68 (0.19)	4.85 (0.13)	4.62 (0.13)	4.79 (0.15)	4.71 (0.12)	4.63 (0.15)	4.68 (0.10)
HPP at 483 MPa for (min)[c]:		0	5	7	10	12	0	5	7	10	12	0	5	7	10	12	0	5	7	10	12
		4.63 (0.10)	4.65 (0.23)	4.89 (0.01)	4.86 (0.06)	4.79 (0.11)	4.76 (0.14)	4.70 (0.01)	4.71 (0.01)	4.73 (0.02)	4.68 (0.11)	4.64 (0.04)	4.65 (0.13)	4.72 (0.14)	4.71 (0.15)	4.71 (0.07)	4.66 (0.13)	4.51 (0.14)	4.62 (0.06)	4.61 (0.06)	4.62 (0.04)
Storage at 4 °C for:	7 days	4.65 (0.22)	4.77 (0.17)	4.90 (0.05)	4.81 (0.03)	4.69 (0.18)	4.62 (0.18)	4.72 (0.10)	4.85 (0.16)	4.77 (0.04)	4.66 (0.16)	4.63 (0.21)	4.71 (0.14)	4.77 (0.09)	4.80 (0.14)	4.69 (0.17)	4.55 (0.13)	4.63 (0.01)	4.66 (0.06)	4.62 (0.11)	4.61 (0.12)
	14 days	4.71 (0.23)	4.74 (0.19)	4.78 (0.31)	4.67 (0.21)	4.89 (0.03)	4.64 (0.15)	4.75 (0.01)	4.76 (0.0)	4.70 (0.12)	4.79 (0.03)	4.65 (0.18)	4.68 (0.11)	4.75 (0.01)	4.62 (0.14)	4.75 (0.11)	4.60 (0.14)	4.59 (0.07)	4.67 (0.09)	4.60 (0.12)	4.68 (0.04)
	28 days	4.73 (0.25)	4.85 (0.06)	4.92 (0.02)	4.71 (0.17)	4.84 (0.16)	4.67 (0.18)	4.84 (0.00)	4.83 (0.01)	4.71 (0.24)	4.81 (0.12)	4.69 (0.23)	4.75 (0.08)	4.84 (0.23)	4.68 (0.20)	4.75 (0.14)	4.62 (0.13)	4.67 (0.11)	4.71 (0.10)	4.63 (0.15)	4.67 (0.08)

[a] Mean of six trials (±standard deviation) ($N=6$ trials, $n=3$ samples per trial).
[b] Mean of six trials (±standard deviation) ($N=6$ trials, $n=1$ sample per trial).
[c] Mean of two to four trials (±standard deviation) ($N=2$ to 4 trials, $n=1$ sample per trial).

Table 4
Average of a_w values following processing, pressurization, and storage of Genoa salami.

Processing step:		Casing diameter										Casing diameter									
		65 mm										105 mm									
Batter[a]		0.961 (0.007)										0.961 (0.007)									
Fermentation[b]		0.959 (0.003)										0.959 (0.002)									
Drying to target[b] Time or a_w		A) 25 days 0.884 (0.009)					B) a_w 0.92 0.926 (0.011)					C) 35 days 0.920 (0.008)					D) a_w 0.94 0.940 (0.005)				
HPP at 600 MPa for (min)[b]:		0	1	2	3	5	0	1	2	3	5	0	1	2	3	5	0	1	2	3	5
		0.884 (0.009)	0.885 (0.007)	0.883 (0.010)	0.887 (0.008)	0.881 (0.009)	0.926 (0.011)	0.927 (0.010)	0.926 (0.011)	0.926 (0.008)	0.929 (0.009)	0.920 (0.008)	0.917 (0.009)	0.921 (0.009)	0.923 (0.006)	0.921 (0.006)	0.940 (0.005)	0.941 (0.006)	0.940 (0.005)	0.942 (0.003)	0.942 (0.004)
Storage at 4 °C for:	7 days	0.879 (0.010)	0.879 (0.011)	0.883 (0.009)	0.887 (0.009)	0.887 (0.010)	0.924 (0.011)	0.926 (0.008)	0.926 (0.008)	0.927 (0.009)	0.924 (0.010)	0.913 (0.009)	0.916 (0.006)	0.916 (0.008)	0.914 (0.008)	0.915 (0.008)	0.936 (0.007)	0.935 (0.007)	0.934 (0.006)	0.936 (0.007)	0.935 (0.006)
	14 days	0.876 (0.014)	0.876 (0.008)	0.874 (0.016)	0.878 (0.011)	0.879 (0.010)	0.922 (0.009)	0.922 (0.013)	0.922 (0.013)	0.923 (0.011)	0.923 (0.010)	0.915 (0.010)	0.911 (0.010)	0.912 (0.014)	0.916 (0.011)	0.912 (0.010)	0.934 (0.008)	0.932 (0.008)	0.934 (0.008)	0.932 (0.008)	0.934 (0.008)
	28 days	0.877 (0.010)	0.870 (0.019)	0.872 (0.014)	0.876 (0.010)	0.872 (0.013)	0.922 (0.009)	0.919 (0.010)	0.919 (0.010)	0.918 (0.010)	0.917 (0.013)	0.913 (0.006)	0.911 (0.007)	0.915 (0.005)	0.916 (0.005)	0.906 (0.006)	0.930 (0.007)	0.929 (0.009)	0.929 (0.007)	0.930 (0.007)	0.931 (0.009)
HPP at 483 MPa for (min)[b]:		0	5	7	10	12	0	5	7	10	12	0	5	7	10	12	0	5	7	10	12
		0.884 (0.009)	0.885) (0.011)	0.886 (0.011)	0.886 (0.011)	0.885 (0.011)	0.926 (0.011)	0.927 (0.011)	0.926 (0.011)	0.928 (0.011)	0.927 (0.011)	0.920 (0.008)	0.921 (0.003)	0.924 (0.007)	0.923 (0.006)	0.921 (0.005)	0.940 (0.005)	0.936 (0.004)	0.942 (0.002)	0.942 (0.003)	0.941 (0.005)
Storage at 4 °C for:	7 days	0.879 (0.010)	0.876 (0.011)	0.880 (0.007)	0.879 (0.015)	0.881 (0.008)	0.924 (0.011)	0.926 (0.011)	0.926 (0.007)	0.923 (0.015)	0.928 (0.008)	0.913 (0.009)	0.912 (0.010)	0.917 (0.006)	0.911 (0.008)	0.914 (0.008)	0.936 (0.007)	0.937 (0.007)	0.936 (0.005)	0.936 (0.006)	0.936 (0.011)
	14 days	0.876 (0.014)	0.873 (0.009)	0.875 (0.009)	0.878 (0.008)	0.879 (0.012)	0.922 (0.009)	0.925 (0.009)	0.921 (0.009)	0.925 (0.008)	0.923 (0.012)	0.915 (0.010)	0.912 (0.011)	0.912 (0.012)	0.912 (0.008)	0.913 (0.007)	0.934 (0.008)	0.934 (0.007)	0.935 (0.007)	0.935 (0.007)	0.935 (0.009)
	28 days	0.877 (0.010)	0.880 (0.008)	0.877 (0.008)	0.877 (0.008)	0.875 (0.012)	0.922 (0.009)	0.920 (0.008)	0.921 (0.008)	0.919 (0.008)	0.921 (0.012)	0.913 (0.006)	0.912 (0.005)	0.907 (0.007)	0.912 (0.007)	0.912 (0.004)	0.930 (0.007)	0.928 (0.007)	0.933 (0.006)	0.930 (0.006)	0.931 (0.006)

[a] Mean of two trials (±standard deviation) ($N=2$ trials, $n=2$ samples per trial).
[b] Mean of six trials (±standard deviation) ($N=6$ trials, $n=3$ samples per trial).

Table 5
Recovery of *L. monocytogenes* (log CFU/g) following processing, pressurization, and storage of Genoa salami.

Processing step:		Casing diameter										Casing diameter									
		65 mm										105 mm									
Batter		7.14[a] (0.17)										7.14 (0.17)									
Fermentation		6.44 (0.41)										6.41 (0.36)									
Drying to target time or a_w		A) 25 days 5.84 (0.22)					B) a_w 0.92 6.08 (0.12)					C) 35 days 6.02 (0.09)					D) a_w 0.94 6.07 (0.12)				
HPP at 600 MPa for (min):		0	1	2	3	5	0	1	2	3	5	0	1	2	3	5	0	1	2	3	5
		5.84 (0.22)	4.22 (0.43)	4.14 (0.40)	3.29 (1.65)	3.46 (0.57)	6.08 (0.12)	3.81 (0.54)	3.23 (0.61)	2.82 (0.92)	2.14 (1.08)	6.02 (0.09)	1.80 (1.56)	1.90 (1.25)	≤1.0 (0.0) [3/6][b]	≤1.0 (0.0) [3/6]	6.07 (0.12)	2.91 (1.16)	2.10 (1.14)	1.86 (1.36)	1.53 (1.38) [2/2]
Storage at 4 °C for:	7 days	5.59 (0.24)	3.72 (0.19)	3.23 (0.40)	3.18 (0.42)	2.60 (0.59)	6.14 (0.18)	2.36 (1.20)	2.79 (0.94)	2.36 (0.88)	2.36 (0.88)	5.73 (0.24)	2.50 (0.79)	1.77 (0.81)	1.60 (1.10)	1.46 (0.79)	6.10 (0.24)	2.83 (1.03)	1.70 (1.34)	1.89 (0.72)	≤1.0 (0.0) [4/4]
	14 days	5.75 (0.28)	3.57 (0.46)	3.20 (0.71)	2.23 (0.65)	2.40 (1.03)	6.09 (0.26)	2.68 (0.26)	1.28 (1.03) [2/4]	1.34 (0.68) [1/1]	1.56 (0.20)	5.92 (0.14)	2.56 (0.63)	1.62 (1.14) [1/1]	1.49 (1.26) [1/1]	≤1.0 (0.45) [4/4]	6.0 (0.17)	1.71 (1.06) [1/1]	≤1.0 (1.18) [4/4]	≤1.0 (0.0) [5/5]	≤1.0 (0.0) [4/4]
	28 days	5.47 (0.27)	3.09 (0.71)	3.08 (0.54)	3.00 (0.61)	2.59 (0.60)	5.81 (0.26)	1.71 (0.58)	≤1.0 (0.0) [5/5]	≤1.0 (0.0) [4/4]	≤1.0 (0.0) [5/5]	5.59 (0.43)	3.02 (0.32)	2.34 (0.93)	2.40 (1.09)	2.26 (1.36) [1/1]	5.84 (0.30)	1.83 (1.32)	1.19 (1.06) [2/2]	≤1.0 (0.0) [4/4]	≤1.0 (0.0) [6/6]
HPP at 483 MPa for (min):		0	5	7	10	12	0	5	7	10	12	0	5	7	10	12	0	5	7	10	12
		5.84 (0.22)	4.02 (0.22)	3.15 (1.48)	4.29 (0.20)	3.64 (0.73)	6.08 (0.12)	3.96 (0.52)	3.07 (0.47)	3.35 (0.16)	3.09 (0.41)	6.02 (0.09)	2.34 (1.67) [1/1]	2.01 (1.53) [1/1]	≤1.0 (0.0) [3/6]	1.15 (1.18) [1/4]	6.07 (0.12)	2.92 (0.85)	2.29 (1.25)	2.18 (1.30) [1/1]	1.86 (1.34)
Storage at 4 °C for:	7 days	5.59 (0.24)	3.48 (0.58)	3.48 (0.36)	3.31 (0.33)	3.30 (0.58)	6.14 (0.18)	3.30 (0.39)	2.75 (0.71)	2.77 (0.41)	2.16 (0.39)	5.73 (0.24)	2.59 (0.36)	2.28 (1.0)	2.21 (0.74)	1.71 (1.02) [1/1]	6.10 (0.24)	1.96 (1.75) [2/2]	1.36 (0.86) [1/1]	≤1.0 (0.0) [3/3]	≤1.0 (0.0) [5/5]
	14 days	5.75 (0.28)	3.63 (0.53)	3.41 (0.70)	2.98 (1.13)	3.33 (0.74)	6.09 (0.26)	2.84 (0.94)	2.60 (0.13)	1.42 (0.82) [1/1]	1.50 (0.79)	5.92 (0.14)	2.06 (1.20) [1/1]	1.73 (0.82)	1.33 (0.85) [1/1]	1.37 (1.06) [2/2]	6.0 (0.17)	≤1.0 (0.83) [2/2]	≤1.0 (0.0) [2/5]	≤1.0 (0.0) [5/5]	≤1.0 (0.0) [6/6]
	28 days	5.47 (0.27)	3.10 (0.46)	3.10 (0.25)	3.52 (0.33)	3.08 (0.60)	5.81 (0.26)	1.02 (0.68)	≤1.0 (0.0) [2/2]	≤1.0 (0.0) [2/2]	≤1.0 (0.0) [3/6]	5.59 (0.43)	2.56 (0.40)	2.55 (0.86)	2.83 (0.55)	2.52 (0.54)	5.84 (0.30)	1.31 (1.02) [1/3]	≤1.0 (0.0) [3/3]	≤1.0 (0.0) [3/3]	≤1.0 (0.0) [4/4]

[a] Log CFU/g average of 6 samples (±standard deviation) ($N = 2$ trials, $n = 3$ replicates; detection limit by direct plating = ≤1.0 log CFU/g).
[b] Values in brackets are numbers of samples negative by enrichment from among the total number of samples that were enriched.

Table 6
Recovery of *E. coli* O157:H7 (log CFU/g) following processing, pressurization, and storage of Genoa salami.

Processing step:		Casing diameter										Casing diameter									
		65 mm										105 mm									
Batter		7.20[a] ± 0.15										7.20 ± 0.15									
Fermentation		6.46 ± 0.14										6.60 ± 0.14									
Drying to target time or a_w		A) 25 days 5.03 ± 0.35					B) a_w 0.92 6.03 ± 0.27					C) 35 days 5.59 ± 0.81					D) a_w 0.94 6.10 ± 0.28				
HPP at 600 MPa for (min):		0	1	2	3	5	0	1	2	3	5	0	1	2	3	5	0	1	2	3	5
		5.03 (0.35)	≤0.30 (0.0) [1/4][b]	≤0.30 (0.0) [2/5]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	6.03 (0.27)	≤0.30 (0.0) [3/4]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	5.59 (0.81)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	6.10 (0.28)	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]
Storage at 4 °C for:	7 days	4.77 (0.08)	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	6.17 (0.21)	≤0.30 (0.0) [4/4]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	5.24 (0.02)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	6.11 (0.27)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
	14 days	4.49 (0.29)	≤0.30 (0.0) [3/4]	≤0.30 (0.0) [3/4]	≤0.30 (0.0) [3/5]	≤0.30 (0.0) [5/6]	5.98 (0.34)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	4.82 (0.34)	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	5.95 (0.44)	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]
	28 days	4.20 (0.38)	≤0.30 (0.0) [1/4]	≤0.30 (0.0) [0/5]	≤0.30 (0.0) [2/6]	≤0.30 (0.0) [3/6]	5.92 (0.16)	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [4/6]	4.47 (0.72)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	6.0 (0.24)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
HPP at 483 MPa for (min):		0	5	7	10	12	0	5	7	10	12	0	5	7	10	12	0	5	7	10	12
		5.03 (0.35)	≤0.30 (0.0) [0/2]	≤0.30 (0.0) [1/3]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [5/6]	6.03 (0.27)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	5.59 (0.81)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	6.10 (0.28)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]
Storage at 4 °C for:	7 days	4.77 (0.08)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	6.17 (0.21)	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	5.24 (0.02)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	6.11 (0.27)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
	14 days	4.49 (0.29)	≤0.30 (0.0) [3/5]	≤0.30 (0.0) [3/3]	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [4/6]	5.98 (0.34)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	4.82 (0.34)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	5.95 (0.44)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]
	28 days	4.20 (0.38)	≤0.30 (0.0) [3/3]	≤0.30 (0.0) [3/5]	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [5/6]	5.92 (0.16)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	4.47 (0.72)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	6.06 (0.24)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]

[a] Log CFU/g average of 6 samples (± standard deviation) ($N = 2$ trials, $n = 3$ replicates; detection limit by direct plating = ≤0.3 log CFU/g).
[b] Values in brackets are numbers of samples negative by enrichment from among the total number of samples that were enriched.

Table 7
Recovery of *Salmonella* (log CFU/g) following processing, pressurization, and storage.

Processing step:		Casing diameter										Casing diameter									
		65 mm										105 mm									
Batter		6.95[a] (0.13)										6.95 (0.13)									
Fermentation		5.61 (0.13)										5.91(0.13)									
Drying to target time or a_w		A) 25 days 2.50 (0.23)					B) a_w 0.92 2.21 (0.52)					C) 35 days 2.18 (0.65)					D) a_w 0.94 2.74 (0.77)				
HPP at 600 MPa for (min):		0	1	2	3	5	0	1	2	3	5	0	1	2	3	5	0	1	2	3	5
		2.50 (0.23)	≤0.30 (0.0) [5/5][b]	≤0.30 (0.0) [4/5]	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [6/6]	2.21 (0.52)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [0/4]	≤0.30 (0.0) [4/5]	2.18 (0.65)	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.74 (0.77)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
Storage at 4 °C for:	7 days	1.70 (0.58)	≤0.30 (0.0) [4/5]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [5/6]	1.66 (0.90)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.76 (0.40)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.55 (0.85)	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
	14 days	1.88 (0.31)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	2.64 (0.75)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.09 (1.1)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.87 (1.22)	≤0.30 (0.0) [4/4]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
	28 days	1.09 (0.53)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.45 (0.50)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.07 (0.31)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	1.08 (0.76)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
HPP at 483 MPa for (min):		0	5	7	10	12	0	5	7	10	12	0	5	7	10	12	0	5	7	10	12
		2.50 (0.23)	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [4/6]	≤0.30 (0.0) [5/6]	2.21 (0.52)	≤0.30 (0.0) [4/4]	≤0.30 (0.0) [3/4]	≤0.30 (0.0) [4/4]	≤0.30 (0.0) [5/5]	2.18 (0.65)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.74 (0.77)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
Storage at 4 °C for:	7 days	1.70 (0.58)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.66 (0.90)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	2.76 (0.40)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [4/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.55 (0.85)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
	14 days	1.88 (0.31)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.64 (0.75)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.09 (1.1)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.87 (1.22)	≤0.30 (0.0) [4/5]	≤0.30 (0.0) [5/5]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]
	28 days	1.09 (0.53)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	2.45 (0.50)	≤0.30 (0.0) [5/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.07 (0.31)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	1.08 (0.76)	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]	≤0.30 (0.0) [6/6]

[a] Log CFU/g average of 6 samples (±standard deviation) ($N = 2$ trials, $n = 3$ replicates; detection limit by direct plating = ≤0.3 log CFU/g).
[b] Values in brackets are numbers of samples negative by enrichment from among the total number of samples that were enriched.

larvae/g of batter) of *T. spiralis* to below detection (Fig. 1). The loss of viability and/or infectivity of the larvae within Genoa salami was confirmed by both microscopy and mouse bioassays. Likewise, regardless of the time or level of pressure treatment, HPP was sufficient for reducing the initial load (about 3.4 log larvae/g) of *T. spiralis* larvae in pig masseter muscle to below detection. The loss of viability and/or infectivity of the larvae in masseter following HPP was also confirmed by both microscopy and mouse bioassays.

4. Discussion

Genoa salami has been prepared using traditional processes and has been enjoyed and consumed worldwide largely without incident for centuries. Since Genoa is typically (primarily) made from pork, processors are required to practice heating, freezing (sometimes referred to as certification), and/or curing to control *T. spiralis* in pork. Among these, certification, the process of storing pork for prescribed times at relatively cold temperatures, is perhaps the most practiced, but probably not the most cost effective approach. Likewise, heating is not an option for some meat products, including Genoa salami, due to likely untoward effects on quality. Therefore, curing, the process of holding and drying salami for prescribed times and temperatures based on casing size and on salt content is a viable, but not necessarily preferred or effective, approach for trichinae control. Given that the existing make procedures also impact both the safety and quality of Genoa, one objective of the present study was to evaluate the equivalency of HPP as an alternative to freezing or curing for ensuring that pork and pork products are trichinae-free. A second objective was to assess the lethality contributed by fermentation/drying and a post-process treatment via HPP on viability of *L. monocytogenes*, *Salmonella* spp., and/or *E. coli* O157:H7. For these reasons, it was essential that we obtained pork that was harvested from swine that were experimentally inoculated with *Trichinella* larvae and assemble a multi-disciplinary team to address these objectives, so that the results could be directly used by the industry, so that the process would pass muster with regulators, and so that the finished product would satisfy consumers. As described herein, these objectives were met in full.

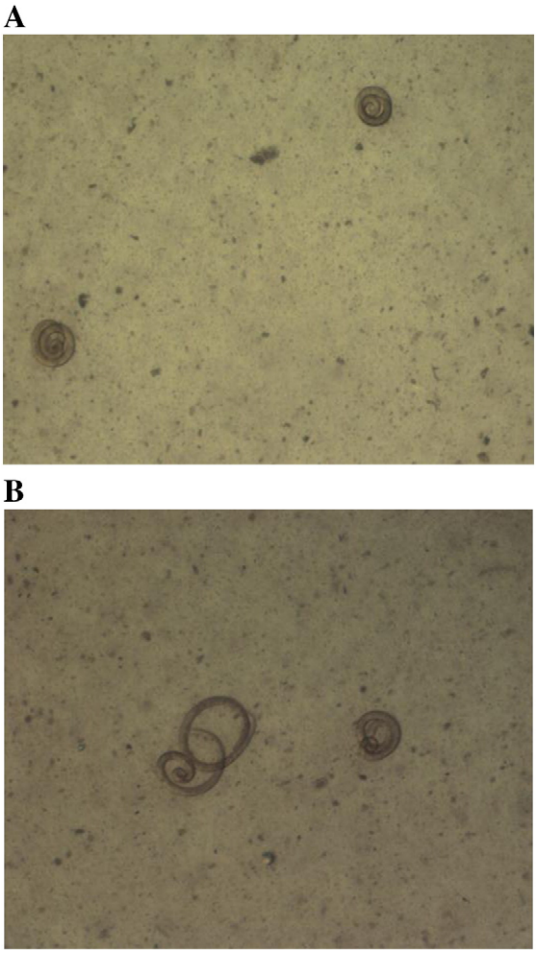

Fig. 1. Panel A. Live larvae (coiled) of *T. spiralis* recovered before fermentation/drying of from Genoa salami. Panel B. Dead larvae (uncoiled) *T. spiralis* recovered after fermentation/drying of Genoa salami.

With respect to the microbial pathogens tested in the present study, our results established total reductions of about 1.1 to 1.3, about 1.1 to 2.2, and about 4.2 to 4.8 log CFU/g of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella*, respectively, following fermentation and drying of Genoa salami. These results are in general agreement with other reports wherein cells of *L. monocytogenes* were more resistant than cells of *E. coli* O157:H7 and *S. typhimurium* to fermentation and drying of fermented sausages (Nightingale et al., 2006; Nissen and Holck, 1998; Porto-Fett et al., 2008). In general, for all treatments, an additional reduction of about 1.6 to ≥5.0, ≥4.7 to ≥5.8, and ≥1.9 to ≥2.4 log CFU/g in *L. monocytogenes*, *E. coli* O157:H7, and *S. typhimurium* numbers, respectively, was achieved after Genoa salami was pressurized at 600 MPa for up to 5 min or at 483 MPa for up to 12 min and then stored refrigerated for up 28 d. Previous studies reported that Gram-positive bacteria are generally more pressure resistant than Gram-negative bacteria, presumably due to intrinsic differences in their morphology, cell membrane, and cell wall, as well as to differences in how they respond to biochemical and genetic stresses (Mackey et al., 1994; Simpson and Gilmour, 1997; Ritz et al., 2000; Tholozan et al., 2000). However, in this study, differences in pressure resistance between *L. monocytogenes* and *Salmonella* could not be established since comparatively too few cells of the latter were available following fermentation and drying to squarely quantify the subsequent lethality of HPP towards these two pathogens. A direct comparison was feasible between *L. monocytogenes* and *E. coli* O157:H7, because both of these pathogens survived at similar levels after drying. In this regard, our data are in complete agreement with data published by others in that *L. monocytogenes* (Table 5) was more resistant to pressurization than *E. coli* O157:H7 (Table 6). That being said, our findings showed that *L. monocytogenes* was inactivated to a greater extent after pressurization in treatments B, C, and D when compared to treatment A. These results may be attributed to the lower a_w value for chubs inoculated with *L. monocytogenes* that were processed by treatment A (about a_w value of 0.88) when compared with a_w values of chubs processed by the other treatments (about a_w values of ≥0.92). In general, lower a_w values have a baro-protective effect for pathogens when they are subjected to high pressure treatments (Rubio et al., 2007).

Our findings validate that processing Genoa salami under the conditions tested herein does not provide a favorable environment for survival of *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella*. Our findings also validate that HPP is an effective post-processing intervention for reducing relatively low levels of *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* in Genoa salami. Collectively, the conditions used in this study for fermentation, drying, HPP, and storage of Genoa salami are sufficient to meet the current performance standards of a 5.0-log reduction for *E. coli* O157:H7 and a 6.5-log reduction for *Salmonella* (Nickelson et al., 1996; Reed, 1995, United States Department of Agriculture/Food Safety and Inspection Service, USDA-FSIS, 2001b). It also satisfies compliance guidelines for *L. monocytogenes* that requires processors of RTE meat products to deliver a post-process lethality treatment, in this case HPP, and/or allow for no more than a 1.5- to 2.0-log increase over shelf life (United States Department of Agriculture/Food Safety and Inspection Service, USDA-FSIS, 2001b; Anonymous, 2003). For treatments A, B, and D we observed both lethality and inhibition. For treatment C we observed

lethality, but levels of listeriae increased by >2.0 log CFU/g during refrigerated storage. Thus, relative to *L. monocytogenes*, these data make it possible for a processor to self affirm Alternative 1 status by following the make protocol described and validated herein for treatments A, B, and D, and to self affirm Alternative 2 status for treatment C. These data should also be applicable to related products without the need to conduct a bona fide process validation for each pathogen and each process and/or at each establishment. More specifically, it is neither possible nor practicable for a given plant to obtain trichinae-infected pork and/or to obtain pork that may be naturally contaminated with *L. monocytogenes*, Shiga toxin-producing *E. coli*, and/or *Salmonella* spp. Likewise, it would not be prudent or allowable to intentionally contaminate product with these pathogens in a plant that processes food for human consumption. Furthermore, there is a paucity of information on acceptable surrogates for the above mentioned pathogens and considerable ongoing debate as to the selection and comparative utility of using surrogates rather than pathogens for challenge studies. As such, it would be more beneficial from a public health standpoint to conduct and subsequently publish a scientifically-sound validation study, such as the one detailed herein, that was performed by an accredited/competent facility and to then subsequently verify that the validated processing conditions and product type/formulation were reduced to routine practice in the processing plant. It is probable that such conditions would be equally effective for like processes and for processes that are less stringent from the perspective of pathogen viability. It is also probable that the results validated herein would be directly applicable to like products and to products with formulations that are less favorable to the survival/growth of target pathogens. The precedent for this strategy can be found in the NCBA Blue Ribbon Task Force (see Nickelson et al., 1996; Reed, 1995).

With respect to the nematode tested, as an alternative to curing and/or certification, one objective of the present study was to evaluate the effect of HPP as a post-process intervention following fermentation/drying to inactivate *T. spiralis* larvae in both infected pig muscle and in Genoa salami. Regardless of the processing treatment, fermentation and drying of Genoa salami was sufficient to inactivate *T. spiralis* larvae by ≥2.3 log larvae/g. These results are in general agreement with other studies reporting that curing and fermentation/drying reduced levels of *T. spiralis* larvae in Genoa salami. As an example, Childers et al. (1982) reported that when Genoa salami (stuffed into a 98-mm diameter casing) was formulated with 1.67 or 3.3% of sodium chloride, fermented at 35 °C or 46.1 °C, and then dried at 7.8 °C for up 35 d, no viable larvae were recovered by artificial digestion after 10 and 5 d of drying time, respectively. Similar results were reported by Smith et al. (1989) who evaluated the effect of salt level on the viability of *T. spiralis* larvae in Genoa salami (stuffed into a 80-mm diameter) that were dried for up to 90 d at 7 °C and 80% RH. They reported loss of viability and/or infectivity of larvae from Genoa salami formulated with 2.0% ($a_w = 0.955$), 2.75% ($a_w = 0.950$), or 3.3% ($a_w = 0.930$) salt after 13 d of drying.

In the present study, pressures of either 483 or 600 MPa for 1.0 or 0.5 min, respectively, inactivated *T. spiralis* larvae in masseter muscle (about 3.4 log larvae/g) harvested from infected pigs. Thus, if fermentation and drying were not effective, then HPP would effectively inactivate the about 2.3 log larvae/g of *T. spiralis* larvae that were present in the Genoa salami prepared in this study. Our findings are also in general agreement with those of Ohnishi et al. (1992) who demonstrated that if muscle tissue from mice infected by oral gavage with 200 larvae was subsequently treated with ≥200 MPa (29,000 psi), then the *T. spiralis* larvae were inactivated. In a related study, Ohnishi et al. (1994) demonstrated that pressures ≥200 MPa for 10 min induced histological and morphological changes in *T. spiralis* that may result in the loss of viability and/or infectivity of the larvae. In contrast, Gamble et al. (1998) reported that hydrodynamic treatments of 55 to 60 MPa when applied to infected pork tenderloin were not sufficient to completely inactivate *T. spiralis* larvae. Collectively, these data confirm that pressures of ≥200 MPa are required to insure for inactivation of trichinae in muscle tissue/meat.

For the Genoa salami evaluated in the present study, the standard/traditional make protocol was established based on specified holding and drying times and temperatures determined by its salt content and casing size as prescribed for trichinae control (United States Department of Agriculture/Food Safety and Inspection Service, 2001a). On occasion, consumer preferences may prompt processors to alter certain parameters, such as the salinity, acidity, and/or moisture content, to generate a product that is more or less tart, salty, and/or firm. However, in practice, this is not so easily done, because as mentioned above, the basic parameters for fermentation and drying are essentially fixed based on salt content and casing diameter. As such, to comply with existing policies, it would be necessary to include some other "intervention" for trichinae control before key processing parameters could be modified. The technology evaluated in the present study as an alternate method for trichinae control was HPP. However, since holding and drying alone were effective at reducing the population of viable larvae from an initial level of about 2.3 larvae/g to below detection as determined using a mouse bioassay, it was not possible to quantify the efficacy of HPP towards *T. spiralis* directly in Genoa. Nonetheless, it was possible to quantify the effect of HPP on trichinae larvae in pig masseter muscle, a raw, whole-muscle piece of tissue harvested directly from trichinae-infected pigs. As an aside, meat from the same pigs from which masseter tissue was harvested was also used to prepare the Genoa salami in this study. Our data validated that a 3.2-log reduction of trichinae larvae was achieved in as little as 1.0 min using 483 MPa and in as little as 0.5 min using 600 MPa. Based on these data, HPP would be an effective alternative to heating, freezing, or curing for control of trichinae in Genoa-type salami and related whole-muscle products such as prosciutto, capocollo, and Parma ham. Moreover, when applied after fermentation and drying, HPP was also quite effective at reducing levels of *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella*. These data are significant in the context of HPP being used as a post-process intervention to provide lethality towards *L. monocytogenes* and for limiting its subsequent outgrowth during shelf life. These data also validate that treatments A, B, C, and D along with HPP satisfy the requisite performance standards for red meat and poultry products for *E. coli* O157:H7 (>6.2 log CFU/g reduction) and *Salmonella* (≥6.6 log CFU/g reduction) (Nickelson et al., 1996; Reed, 1995; United States Department of Agriculture/Food Safety and Inspection Service, USDA-FSIS, 2001b).

Collectively, our findings confirmed that Genoa salami prepared using the make protocols described in this study generates appreciable reductions in levels of *L. monocytogenes*, *E. coli* O157:H7, *Salmonella*, and *T. spiralis*, and meets all existing regulatory requirements for product wholesomeness. Regarding the potential impact and practical application of the findings of this study, our validation of HPP as an alternative technology for trichinae control would allow processors to adjust the prescribed times and temperatures for holding and/or drying to produce a product that was more desirable to some consumers from a taste/texture perspective and still be in compliance with requisite regulatory policies. As an added benefit, HPP would also make it possible for a processor to satiate the performance standards for *E. coli* O157:H7 and *Salmonella* as well as the current guidelines for *L. monocytogenes*.

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