Meat and Muscle Biology™

High-Pressure Processing Helps Meet the *Escherichia coli* O157:H7 and Shiga Toxin–Producing *E. coli* (STEC) Performance Standards for Beef Summer Sausage

Macc Rigdon[1], Harshavardhan Thippareddi[2], Chevise L. Thomas[1], Sanjay Kumar[2], Robert W. McKee[1], and Alexander M. Stelzleni[1]*

[1]Department of Animal and Dairy Sciences, University of Georgia, Athens, GA 30602, USA
[2]Department of Poultry Science, University of Georgia, Athens, GA 30602, USA
*Corresponding author. Email: astelz@uga.edu (Alexander M. Stelzleni)

Abstract: The United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) performance standards require that manufacturers of fermented beef sausages validate their processes to achieve a 5-log reduction of *Escherichia coli* O157:H7 and Shiga toxin–producing *E. coli* (STEC). Most processors rely on rapid fermentation to a low pH and a mild heat treatment to achieve the lethality performance standard. However, this process alters the sensorial characteristics of traditional fermented sausages. An alternative method to achieve lethality using high-pressure processing (HPP) during the manufacture of summer sausage with higher pH (5.0) and minimal heat treatment was evaluated. Sausages inoculated with circa 9.1 log CFU/g of *E. coli* O157:H7 and 8.9 log CFU/g of STEC were fermented to target pH values of 4.6 or 5.0. Subsequently, fermented sausages were heated to endpoint temperatures of 54.4°C, 48.9°C, or 43.3°C to the total process treatments of (1) Process A: pH 4.6 and 54.4°C, simulated cold air chilling, (2) Process B: pH 5.0 and 54.4°C, simulated cold air chilling, (3) Process C: pH 5.0 and 54.4°C, ice bath chilling, (4) Process D: pH 5.0 and 48.9°C, ice bath chilling, and (5) Process E: pH 5.0 and 43.3°C, ice bath chilling. After processing, the product was subjected to HPP (586 MPa; 4°C ± 2°C) for hold times of 1, 150, or 300 s and a nontreated (no HPP) control. All treatments subjected to HPP for 150 and 300 s reduced ($P \leq 0.05$) *E. coli* (O157:H7 and STEC) populations by >5.0 log CFU/g and >7.5 log CFU/g, respectively. The use of HPP allows for the production of more mild beef summer sausage (pH 5.0 and a mild thermal treatment of 43.3°C) while still achieving USDA-FSIS performance standards for lethality.

Key words: beef, safety, sausage, *Escherichia coli*, high-pressure, shelf-stable
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Introduction

Historically, dried and fermented meat products were regarded as safe for human consumption because of their use of multiple biological hurdles such as acidification, salt, competitive exclusion, nitrite/nitrate, drying, and sometimes even thermal treatment. However, after a foodborne outbreak of *Escherichia coli* O157:H7 in California and Washington that was associated with dried and fermented salami (CDC, 1995), the National Cattlemen's Beef Association assembled a team of industry representatives to address the control of *E. coli* O157:H7 and prevent future outbreaks associated with the organism (Nickelson et al., 1996). The representatives, known as the Blue Ribbon Task Force, recommended 5 options to reduce the risk of *E. coli* O157:H7 in fermented sausages. Option 4 was described specifically as any combined process that demonstrates at least a collective 5 log reduction of *E. coli* O157:H7 with precise documentation. Subsequently, the United States Department of Agriculture-Food Safety Inspection Service (USDA-FSIS)

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sampled over 3,400 samples of fermented and dried meat products for *E. coli* O157:H7 from March 1995 to December 1999 and reported that none of the samples tested positive for *E. coli* O157:H7 (Levine et al., 2001). Currently, there is limited literature evaluating the reduction of *E. coli* O157:H7 and Shiga toxin–producing *E. coli* (STEC) in non- to mild-thermally processed, mildly fermented sausages containing beef that successfully meet the guidelines set forth by the Blue Ribbon Task Force and USDA-FSIS without additional thermal hold times or post-thermal drying (Hinkens et al., 1996; Glass et al., 2012). Processors of products, using similar parameters (pH > 4.9 with a low degree of thermal processing or no thermal processing, and no additional drying step), currently operate under the in-plant validations described by Option 4 because of the lack of performance in the scientific literature, or by using Option 5, which allows the testing of raw materials, a validated 2 log reduction process, and additional supporting literature as part of their HACCP plan (Nickelson et al., 1996).

Calicioglu et al. (1997) demonstrated the effectiveness of mild fermentation (target pH 5.0) along with lower temperature cook (54°C) with thermal hold procedures (30 and 60 min) or more aggressive fermentation (pH 4.6) and low-temperature thermal processing (54°C) without additional thermal hold for summer sausage. Porto-Fett et al. (2010) reported more aggressive fermentation to a low ultimate pH (4.56 to 4.66) in conjunction with the use of elevated temperature high-pressure processing (HPP; 483 or 600 MPa; 23.3°C to 36.7°C) could be used to meet compliance guidelines for *E. coli* O157:H7 in Genoa salami. Although these methods were effective at creating a 5 log reduction, they fell short of meeting the growing consumer demand for specialty food products (Ilbery and Kneafsey, 1999; Guerrero et al., 2009), including sausages that are mildly fermented and low-temperature processed, which do not meet the recommended 5 log reduction outlined by Option 4.

HPP is a technology currently being used in food production to improve the safety of raw fruit juices, deli meats, and other high water activity foods. During HPP, food products are typically subjected to 500 to 700 MPa of pressure by forced water displacement with the objective of eliminating pathogenic organisms. HPP inactivates potential pathogens through several mechanisms, including increased cell membrane permeability, changes in cell morphology, altered biochemical reactions, interference in genetic mechanisms, oxidative burst, and increasing pathogen sensitivity to reactive oxygen (Aertsen et al., 2005; Yaldagard et al., 2008; Sehrawat et al., 2021). Reactive oxygen species are produced by the organism in times of stress (Wuytack et al., 2003), such as during fermentation and drying in salt-cured products. It has also been shown that lower water activity may protect microbial cells during HPP. However, lower pH may increase microbial cell death and restrict sublethal repair (Linton et al., 1999a, 1999b; Yaldagard et al., 2008). Therefore, the objective of this study was to evaluate the fate of pathogenic strains of *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145 in mildly fermented, low-temperature thermally processed beef summer sausage using HPP. The hypothesis was HPP of mildly fermented, low-temperature thermally processed summer sausage can be used to meet the performance standards for *E. coli* O157:H7 and STEC outlined by Option 4 of the Blue Ribbon Task Force.

Materials and Methods

Culture preparation

For each of 3 replications, 5 rifampicin-resistant (100 μg/mL; Sigma-Aldrich, St. Louis, MO) strains of *E. coli* O157:H7 (USDA-FSIS 011–82, American Type Culture Collection [ATCC] 43888, ATCC 43889, ATCC 43890, and USDA-FSIS 45756) and 6 kanamycin-resistant (100 μg/mL; Sigma-Aldrich) serotypes of non-O157:H7 STEC (H30 [O26:H11], B395 [O111:H7], CDC 96–3285 [O45], CDC 90–3128 [O103:H2], CDC 97–3068 [O121], and CDC 83–75 [O145:HNM]) were used. All cultures were obtained from Dr. John Luchansky, USDA Agricultural Research Service, Wyndmoor, PA. Each isolate was maintained in glycerol at −80°C prior to use. Each isolate was then individually streaked on Sorbitol-MacConkey agar (Sigma-Aldrich) with its respective antibiotic at 37°C for 20 ± 2 h and maintained at 4°C until needed. Prior to incubation each serotype was confirmed by polymerase chain reaction and gel electrophoresis. One loopful of each isolate was transferred into individual 10 mL tubes of tryptic soy broth (TSB; Sigma-Aldrich) plus 2.5% glucose with 100 ppm of the respective antibiotic and incubated at 37°C for 24 ± 2 h. After the initial incubation, 1 mL of culture was aseptically transferred into individual 1 L TSB solutions with 2.5% glucose and its respective antibiotic. After incubation at 37°C for 18 h, inoculated strains were individually collected by centrifugation at 7,200 × *g* and 4°C for 10 min. After centrifugation, the supernatant was decanted, and cells were resuspended in 10 mL

of sterilized deionized (DI) water. Isolates were combined into 2 separate cocktails of O157 and non-O157 STEC cultures. Two DI water washes were used to remove the residual antibiotic from the cultures. After the final wash step, the pellet was resuspended in 70 mL of DI water prior to overnight storage (4°C ± 2°C) and inoculation.

Beef trimmings procurement and batter processing

Sausage production was performed following Rigdon et al. (2020) with modifications. For each replicate, 2 meat batters were produced at the University of Georgia Meat Science Technology Center and randomly assigned to target pH 4.6 or 5.0 after fermentation. Each batter consisted of 5 kg of beef trimmings blended to target 10% fat that were ground (Biro Model G58483, Cleveland, OH) through a 12.7 mm grind plate followed by a 4.76 mm grind plate (−1°C ± 1°C). After grinding, the meat was placed into a reverse action mixer (Model A-80, Koch, Kansas City, MO) and mixed with a seasoning block consisting of 2% salt (Morton Salt, Chicago, IL); 1.0% or 0.4% dextrose (for a targeted pH of 4.6 or 5.0, respectively); 0.25% sodium nitrite (156 ppm Prague powder); 0.13% black pepper, white pepper, and garlic powder; 0.06% ginger, coriander, and mustard; and 0.05% sodium erythorbate (539 ppm; all ingredients were provided by A.C. Legg, Calera, AL, unless otherwise noted). A commercially available frozen *Pediococcus acidilactici* starter culture (SAGA 200, Kerry, Rochester, MN) was used for natural fermentation by diluting 10 g of 12 log CFU/g starter culture in 236 mL of distilled water followed by mixing with the meat batter and seasoning for ~3 min. The prepared batter was then placed in plastic bags and transported (1.5 km) in coolers on ice to a biosafety level 2 laboratory for inoculation, stuffing, fermentation, and thermal processing.

Inoculation and processing

Prior to inoculation, 1 chub from the target pH 4.6 batter and 4 chubs from each target pH 5.0 batter were stuffed (~0.45 kg; Mighty Bite, LEM Products, West Chester, OH) into 5.08 cm fibrous mahogany casings (ViskoTeepak, Kenosha, WI) and tied closed. The preinoculated chubs were randomly assigned to one of the total process treatments (described as follows) for post-thermal processing and chilling proximate analysis. The remaining batter was spread out in plastic trays lined with 4 layers of wax-coated butcher paper. Inoculum cocktails were dropped onto the respective flattened meat batter using a pipette and hand mixed wearing double latex gloves and following aseptic procedures. Sausage batters were inoculated with O157:H7 and non-O157 STEC inoculums at the same time. Pretests showed no competitive exclusion and no difference in inoculum performance when used combined or individually. Following the pipetting of both O157:H7 and non-O157:H7 STEC cocktails, approximately 50 g of each batter was collected and placed in a sterile stomacher bag for microbial analysis to determine the inoculation levels for O157:H7 and non-O157:H7 STEC. The remaining batters were individually placed into the stuffer, and one chub for each target pH was stuffed (~0.45 kg) for postfermentation microbial analysis, followed by one chub for each total process treatment (one chub for target pH 4.6; 4 chubs for target pH 5.0). All chubs were tied shut, hung on a smoke cart, and placed in an Alkar smokehouse (Model 8770–4–12000, Alkar, Lodi, WI). Sausages were allowed to ferment until endpoint pH was reached with dry bulb at 43.3°C and wet bulb at 40.5°C (95% relative humidity [RH]). The remainder of the cooking schedule and parameters followed the procedures outlined by Rigdon et al. (2020). Briefly, dry bulb temperatures were increased to 62.8°C and wet bulb at 40.5°C (RH 85%) for 30 min followed by a dry bulb temperature of 73.9°C and wet bulb at 71.5°C (RH 90%) for the remainder of the cooking cycle. The chubs assigned to each total process treatment were removed from the smokehouse, double wrapped in poly bags to prevent cross contamination, and placed in an ice water bath for rapid chilling after they reached their endpoint cooking temperatures of 43.3°C, 48.9°C, and 54.4°C. Additional chubs (pH 4.6 and 5.0) cooked to 54.4°C were removed from the smokehouse and placed into a cooler with ice (not touching the ice) to simulate cold air chilling in a cooler. These parameters were arranged into 5 total process treatment groups: pH 4.6 heated to 54.4°C with simulated cold air chilling (Process A), pH 5.0 heated to 54.4°C with simulated cold air chilling (Process B), pH 5.0 heated to 54.4°C with rapid ice bath chilling (Process C), pH 5.0 heated to 48.9°C with rapid ice bath chilling (Process D), and pH 5.0 heated to 43.3°C with rapid ice bath chilling (Process E; Table 1). The overall treatment design was such that each treatment was evaluated as a total process combining target pH through fermentation, degree of thermal processing, and chilling procedure.

High-pressure processing

After chilling, the day after processing, each inoculated sausage chub was aseptically cut so that 6 cm was

Table 1. Treatment combinations for summer sausage processing

Treatment	Target pH	Temperature[1], °C	Chilling[2]	High-Pressure Processing[3], s			
Process A	4.6	54.4	Cold air	0	1	150	300
Process B	5.0	54.4	Cold air	0	1	150	300
Process C	5.0	54.4	Ice bath	0	1	150	300
Process D	5.0	48.9	Ice bath	0	1	150	300
Process E	5.0	43.3	Ice bath	0	1	150	300

[1]Endpoint internal temperature at which samples were removed from smokehouse and placed in chilling.

[2]Cold air = summer sausage chubs were suspended in cooler with ice, ensuring they were not touching the ice, to simulate cold air (cooler) chilling; ice bath = summer sausage chubs were double bagged in poly bags to prevent cross contamination and placed in an ice water bath to stop the cooking process and simulate rapid chilling.

[3]Samples were subjected to high-pressure processing at 586 MPa, 4°C.

removed from each end and then 5 slices were hand cut from the middle portion of the chub to approximately 3-mm thickness and individually vacuum packaged (B-620 series; 30 to 50 cm^3 $O_2/m^2/24$ h/ 101,325 Pa/23°C; Cryovac, Sealed Air Corporation, Charlotte, NC) for HPP. One slice from each treatment was assigned to 1 of 4 HPP times: 0, 1, 150, or 300 s, with 1 slice being sampled immediately for post-cook and chill enumeration. Vacuum-packaged samples were then bagged together by HPP time, vacuum-sealed, and placed in a third bag, which was also sealed. All 4 sample bags were then shipped (0°C ± 1°C) overnight to the University of Nebraska–Lincoln to be high-pressure processed at 4°C ± 2°C and 586 MPa for their designated time and then returned overnight for enumeration. As explained by Rigdon et al. (2020), the 0 s samples were packaged and shipped with the other samples, remaining in cold storage while the other samples were exposed to HPP. The total time from processing to post-HPP sampling was 5 d. The timepoint of 1 s was selected because this is the minimum time for the pressure chamber to achieve 586 MPa and release, demonstrating the effects of pressure alone. One-hundred fifty seconds was selected as the lower end of the common time utilized for meat products subjected to HPP (150 to 180 s), whereas 300 s, a doubling of time under pressure, represented an extended time. Pressures up to 600 MPa are commonly used in the industry to help ensure a level of safety and have been shown to be effective against pathogenic *E. coli* (Gill and Ramaswamy, 2008; Omer et al., 2010; Simonin et al., 2012; Hygreeva and Pandey, 2016) in various meat products.

Microbial sampling

Fifteen grams from the inoculated batter, postfermentation sample, and HPP samples were individually placed in sterile filter stomacher bags (VWR, Radnor, PA) with 25 mL of 0.1% peptone water (Difco, BD, Franklin Lakes, NJ) and stomached (Seward, West Sussex, UK) for 90 s at 230 rpm. After stomaching, 20 mL was removed from the stomacher bag and placed in sterile dilution tubes, with 10 mL aliquoted to each of 2 tubes: 1 tube for rifampicin-resistant O157 strains and 1 tube for kanamycin-resistant non-O157 strains. Serial dilutions (1:10) were performed using 0.1% peptone water containing 100 ppm of rifampicin or kanamycin in the final concentration for the respective organisms. Samples were plated in duplicate on 3M Petrifilm aerobic plate count (APC) film and *E. coli*/Coliform Count Plate (EC) film (3M, St. Paul, MN) with 1 mL aliquots and incubated at 37°C for 48 h. Raw counts were log transformed for statistical analysis and reporting.

Proximate analysis

To calculate the moisture to protein ratio (M:P), moisture was determined from the noninoculated samples subjected to the same fermentation, thermal processing, and HPP as the inoculated samples using a forced air oven (Fisher Scientific, Pittsburgh, PA). Aluminum pans were placed in a forced air oven at 100°C for 48 h and then placed in a desiccator until used. Two grams from each sample was measured in duplicate and placed into one of the aluminum pans. The pans were then placed in the forced air oven at 100°C for 18 h (AOAC, 2000). After oven drying, the samples were moved to a desiccator and allowed to equilibrate and cool for 10 min. Percent moisture was calculated as % moisture = ([wet sample weight − dry sample weight]/wet sample weight) × 100.

Crude protein was determined using a nitrogen auto-analyzer (Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI) for the quantification of *N* content (0.1 ± 0.05 g) and multiplied by 6.25 to be expressed as percent crude protein.

Water activity was read on each total process by HPP hold time treatment combination using an AQUALAB water activity meter (AQUALAB 4TE, METER, Pullman, WA) upon return from HPP. Sausage pH was taken immediately after the fermentation step by directly probing the sausage for pH measurement and again after the product cooled using a 1:10 dilution method in DI water using a Hanna Instruments edge pH meter with a general-purpose

glass body bulb probe (Hanna Instruments, Smithfield, RI) (Koniecko, 1979).

Titratable acidity was analyzed as directed by the starter culture manufacture. After chilling, 30 g of the summer sausage was added to 180 mL DI water (60°C) and homogenized for 60 s. The homogenate was allowed to stand at room temperature for 10 min and then transferred to a 400 mL beaker through Whatman grade 1 filter paper (Cytiva, Marlborough, MA). The beaker was placed on a stir plate and slowly stirred while a bulb probe attached to a pH meter (Hanna Instruments) was suspended in the beaker. The solution was titrated with 0.1 N sodium hydroxide 1 mL at a time to pH 8.3. Titratable acidity was calculated and reported as percent lactic acid.

Statistical analysis

A randomized block design was used to evaluate the populations of *E. coli* O157:H7 and non-O157 STECs during the processing of summer sausage following 5 total process applications and 4 HPP time exposures for each of the 5 processes to determine their effects on the reduction of *E. coli*. Microbial sampling was performed on the uninoculated meat batter, on the inoculated meat batter, on the fermented sausage, after chilling prior to shipment, and after HPP. Microbial counts were transformed and reported as log CFU per gram of sample. Data were analyzed using analysis of variance (ANOVA) by PROC GLM of SAS v. 9.4 (SAS Institute, Cary, NC) as one-way ANOVAs to compare means within total process, including HPP times, and to compare means within sampling timepoint across total processes. For uninoculated and inoculated sampling, the batter sample was the observational and experimental unit. The postfermentation sampling observational and experimental unit was the randomly assigned chub from each batter. For postchill and post-HPP sampling, the randomly assigned chub from each batter was considered the whole plot, and packaged slices taken from the chub were the subplot and observational and experimental units. Means were separated using Tukey's studentized range test, and means were considered different at $\alpha < 0.05$.

Results

Sausage proximate analysis

After processing, neither water activity nor moisture to protein ratio were different ($P = 0.06$ and $P = 0.38$, respectively; Table 2). Sausage pH immediately after the fermentation step as measured by direct probing was 4.6 for high dextrose (1.0%; Process A) and 5.0 for low dextrose (0.4%; Processes B to E) sausages. However, during the initial cooking step, the pH continued to decline, yielding a difference in ultimate pH that was slightly lower than what was targeted for all thermal endpoints ($P < 0.01$; Table 2). Although total process combinations were originally described as 4.6 (Process A) and 5.0 (Processes B to E), it should be noted that the ultimate pH of Process A was 4.5, whereas Processes B to E were 4.8. In a similar study conducted by Calicioglu et al. (1997), summer sausage products were produced to target pH of 4.6 and 5.0; however, in their study, the ultimate pH was 4.5 and 4.9, respectively. It is common during the fermentation of products to surpass the target pH because of continued fermentation during the temperature gradient in the initial portion of the cooking step. In the current study, the lactic acid bacterial culture was not inactivated until it reached a temperature of approximately 54.4°C. Titratable acidity was also calculated and, as expected, followed a similar trend to pH in which Process A sausages (target pH 4.6) had greater ($P < 0.05$) titratable acidity than Process B to E sausages (target pH 5.0), which were all similar ($P > 0.05$). All process

Table 2. Least-squares means of proximate analysis of summer sausage products

	Cooking Treatment[1]						
Trait	Process A	Process B	Process C	Process D	Process E	SD	*P* Value
pH	4.5^b	4.8^a	4.8^a	4.8^a	4.8^a	0.14	<0.01
Titratable acidity[2], %	1.66^a	1.22^b	1.18^b	1.22^b	1.19^b	0.09	<0.01
Water activity	0.96	0.97	0.97	0.97	0.97	0.003	0.06
Moisture:protein	2.7	2.8	2.9	2.9	3.0	0.19	0.38

[1]Cooking treatment endpoint parameters are as follows: A = pH 4.5, 54.4°C, simulated cold air chilling; B = pH 4.8, 54.4°C, simulated cold air chilling; C = pH 4.8, 54.4°C, rapid ice bath chilling; D = pH 4.8, 48.9°C, rapid ice bath chilling; E = pH 4.8, 43.3°C, rapid ice bath chilling. SD = standard deviation, $n = 3$ for each total process.

[2]Expressed as percent lactic acid.

[a–c]Means within an attribute with differing superscript differ; $\alpha < 0.05$.

treatments met the USDA's standards for a shelf-stable sausage with moisture to protein ratio ≤ 3.1:1 and pH ≤ 5.0.

Microbial sampling of Escherichia coli O157:H7

The raw meat blocks were sampled prior to inoculation, and no rifampicin-resistant organisms were detected by direct plating. Although samples were plated on both APC and EC film, all treatment, timepoint, and treatment by timepoint samples plated on EC film had either numerically or statistically lower counts than those plated on APC film. The use of EC film, as a selective media, may not have given sublethally injured *E. coli* O157:H7 cells the time or ability to recover. Therefore, the APC film provided a more conservative estimate for reductions than EC film, and APC counts were subsequently reported. The subsequent inoculation yielded 9.2 log CFU/g of *E. coli* O157:H7 in the Process A batter (Table 3). After fermentation to pH 4.6, populations of *E. coli* O157:H7 decreased 1.6 log CFU/g ($P = 0.84$). Although these reductions were associated with the postfermentation pH values, it is important to note that the total fermentation was unable to be recorded as the pH continued to drop after the fermentation step and into the cooking steps. Process A finished with an ultimate pH of 4.5 and thermal endpoint of 54.4°C, resulting in a total reduction of 6.4 log CFU/g ($P < 0.01$), exceeding the 5 log reduction required by USDA-FSIS. Further reductions could not be realized with HPP for hold times of 0 or 1 s ($P > 0.60$); however, HPP exposure for 150 and 300 s provided additional reductions of 0.7 and 1.8 log CFU/g ($P > 0.11$) *E. coli* O157:H7 for total reductions of 7.1 and 8.2 log CFU/g, respectively.

Process B to E batters were inoculated with 9.1 log CFU/g of *E. coli* O157:H7 (Table 3). They were then fermented to a target pH of 5.0, which decreased *E. coli* O157:H7 populations by 0.6 log CFU/g ($P = 0.57$). Through subsequent thermal treatment and chilling, Process B finished with 4.8 pH and 54.4°C thermal endpoint, using simulated cold air chilling, reducing *E. coli* O157:H7 populations by 5.1 log CFU/g ($P < 0.01$), and meeting the required 5 log reduction after thermal processing and chilling. Additional control against *E. coli* O157:H7 could be ensured with the use of HPP for 1, 150, or 300 s ($P < 0.01$). These HPP hold times further increase the reductions of target organisms by 1.5, 1.7, and 2.8 log CFU/g, respectively. Consequently, the total reductions of *E. coli* O157:H7 for Process B with pressurization at 586 MPa for 1, 150, and 300 s were 6.6, 6.8, and 7.9 log CFU/g, respectively. *E. coli* O157:H7 counts from Process C were not different ($P \geq 0.37$) from those noted in Process A or B after thermal processing or subsequent HPP exposure ($P \geq 0.85$). However, within Process C, HPP exposure for 150 and 300 s resulted in lower *E. coli* O157:H7 counts ($P > 0.05$) compared with samples not exposed to HPP. Although statistical differences were not found between Processes A, B, and C at the various timepoints, Process C did require at least minimal exposure to HPP (1 s) to meet the 5 log reduction threshold, whereas Processes A and B met a 5 log reduction after thermal processing and chilling. These data indicate that how sausages are chilled may impact overall reductions of *E. coli* O157:H7 when summer sausage products are cooked to an internal temperature of 54.4°C. Although the chilling rate in and of itself

Table 3. Least-squares means (± standard deviation) of *Escherichia coli* O157:H7 populations (CFU/g) in all beef summer sausage

			High-Pressure Hold Time			
Inoculation[1]	Postferment[1] (pH)	Postchill[2]	Control	1 s	150 s	300 s
$9.2 \pm 0.06^{a,z}$	$7.6 \pm 0.93^{a,z}$ (4.6)	(54.4°C T) $2.9^{*} \pm 2.32^{b,x}$	$2.8 \pm 1.72^{b,z}$	$2.9 \pm 1.28^{b,z}$	$2.1 \pm 1.67^{b,z}$	$1.2 \pm 1.59^{b,z}$
$9.1 \pm 0.29^{a,z}$	$8.5 \pm 0.04^{a,z}$ (5.0)	(54.4°C T) $4.0^{*} \pm 1.11^{b,x}$	$3.8 \pm 0.90^{b,z}$	$2.6 \pm 2.07^{b,z}$	$2.4 \pm 1.85^{b,z}$	$1.4 \pm 1.97^{b,z}$
9.1 ± 0.29^{a}	8.5 ± 0.04^{ab} (5.0)	(54.4°C RC) $5.0 \pm 0.93^{bc,xy}$	$4.8 \pm 0.89^{bc,z}$	$2.8^{*} \pm 2.31^{c,z}$	$2.5 \pm 1.93^{c,z}$	$1.4 \pm 2.00^{c,z}$
9.1 ± 0.29^{a}	8.5 ± 0.04^{ab} (5.0)	(48.9°C RC) $7.9 \pm 0.76^{ab,yz}$	$7.7 \pm 0.90^{ab,y}$	$5.4 \pm 1.80^{bc,z}$	$2.5^{*} \pm 1.94^{cd,z}$	$1.5 \pm 2.02^{d,z}$
9.1 ± 0.29^{a}	8.5 ± 0.04^{ab} (5.0)	(43.3°C RC) $8.5 \pm 0.04^{ab,z}$	$8.5 \pm 0.05^{ab,y}$	$6.7 \pm 1.06^{b,z}$	$3.0^{*} \pm 0.58^{c,z}$	$1.3 \pm 1.73^{c,z}$

[1]Statistical analysis was conducted with the proper degrees of freedom. Inoculation and postferment means were carried down for within total process comparisons. Total processes are in order from A to E; $n = 3$ for each total process by sampling timepoint.

[2]Chilling method denoted by T = simulated cold air chilling or RC = rapid ice bath chilling.

[*]First point in each total process to achieve a 5-log CFU/g reduction.

[a–d]Means with different superscripts within a total process differ; $\alpha < 0.05$.

[x–z]Means with different superscripts within a sampling time differ; $\alpha < 0.05$.

was not analyzed between Processes B and C (similar pH and endpoint temperature), the ice bath chilling procedure of Process C would be expected to hasten the rate of chilling compared with cold air (Process B) because of the heat transfer rate difference between ice water and air.

The pH of sausage produced in Process D was 4.8 after thermal processing to 48.9°C, yielding a process reduction of 1.2 log CFU/g prior to HPP ($P > 0.27$). Pressurization of Process D to 586 MPa for 150 and 300 s showed a total decrease of 6.6 and 7.8 log CFU/g in populations of *E. coli* O157:H7, respectively ($P < 0.01$). Holding samples for 1 s at 586 MPa did improve reductions ($P < 0.05$) of O157:H7 serotypes to 3.7 log CFU/g compared with inoculation levels but did not meet USDA-FSIS guidelines for a total 5 log reduction.

Sausages cooked to 43.3°C and fermented to pH 4.8 (Process E) resulted in 0.6 log CFU/g reductions for the total process prior to HPP. *E. coli* O157:H7 populations from inoculation, postfermentation, postchilling, and after shipment were not different ($P = 0.54$). After 1 s of HPP, populations had a total process reduction of 2.4 log CFU/g ($P < 0.03$) compared with inoculation levels. Longer HPP hold times of 150 and 300 s further reduced populations of *E. coli* O157:H7 in total Process E by 6.1 and 8.0 log CFU/g, respectively ($P < 0.01$).

Microbial sampling of non-O157:H7 Escherichia coli

Similar to the results of *E. coli* O157:H7, STEC plated on EC film had either statistically or numerically lower recovery than samples plated on APC film. Therefore, for the reasons previously mentioned, only results from APC film plating were reported. Initial inoculation of STEC yielded 8.9 log CFU/g for total Process A and 9.0 log CFU/g for Processes B to E ($P = 0.57$; Table 4). After fermentation, Process A fermented to pH 4.6 and achieved a 1.5 log CFU/g reduction ($P = 0.18$), whereas Processes B to E fermented to pH 5.0 and reduced 0.6 log CFU/g of STECs ($P = 0.56$).

As previously mentioned, total Process A fermented to a final pH of 4.5 and cooked to 54.4°C, resulting in a total STEC reduction of 6.2 log CFU/g after simulated cold air chilling ($P < 0.01$). After shipment and return of samples (0 s HPP), Process A STEC reductions totaled 7.4 log CFU/g ($P < 0.01$). After HPP hold times of 1, 150, and 300 s, reductions of at least 7.8 log CFU/g were achieved, with 150 and 300 s being below detection limits (<0.6 log CFU/g; $P < 0.01$).

Total Process B reached a final pH of 4.8 and was cooked to 54.4°C with a total process reduction of 4.9 log CFU/g after chilling ($P < 0.01$), falling just short of meeting the USDA-FSIS–required 5-log reduction. However, holding Process B summer sausage for additional time (without HPP exposure) resulted in a 5 log CFU/g reduction ($P < 0.01$). HPP sausages from Process B for 1 s reduced 7.8 log CFU/g of non-O157 STEC ($P < 0.01$) compared with inoculation level. Continuing to hold the pressure at 586 MPa for 150 or 300 s reduced populations of non-O157 STEC by at least 8.2 log CFU/g ($P < 0.01$) compared with inoculation level. Fermentation to a final pH of 4.8 and cooking to 54.4°C with rapid ice bath chilling (Process C) did not create differences ($P < 0.05$) from total Process B within sampling timepoint ($P > 0.80$). However, Process C did require HPP for 1 s to achieve

Table 4. Least-squares means (± standard deviation) of non-O157 Shiga toxin–producing *Escherichia coli* populations (CFU/g) in all beef summer sausage

			High-Pressure Hold Time			
Inoculation[1]	Postferment[1]	Postchill[2]	Control	1 s	150 s	300 s
8.9 ± 0.16^{a,z}	7.4 ± 0.86^{a,z} (4.6)	(54.4°C T) 2.7* ± 2.18^{b,z}	1.5 ± 2.08^{b,z}	<0.6^{b,z}	<0.6^{b,z}	1.07 ± 1.35^{b,z}
9.0 ± 0.20^{a,z}	8.4 ± 0.24^{a,z} (5.0)	(54.4°C T) 4.1 ± 0.82^{b,z}	4.0* ± 0.87^{b,yz}	1.4 ± 1.80^{c,z}	<0.6^{c,z}	0.8 ± 0.98^{c,z}
9.0 ± 0.20^{a}	8.4 ± 0.24^{a} (5.0)	(54.4°C RC) 5.0 ± 1.03^{b,yz}	4.9 ± 0.95^{b,xy}	2.7* ± 2.14^{bc,yz}	<0.6^{c,z}	<0.6^{c,z}
9.0 ± 0.20^{a}	8.4 ± 0.24^{a} (5.0)	(48.9°C RC) 7.8 ± 0.86^{a,y}	7.7 ± 0.59^{a,wx}	5.8 ± 1.54^{a,xy}	1.9* ± 2.73^{b,z}	<0.6^{b,z}
9.0 ± 0.20^{a}	8.4 ± 0.24^{a} (5.0)	(43.3°C RC) 8.3 ± 0.32^{a,y}	8.5 ± 0.04^{a,w}	7.4 ± 0.86^{a,x}	3.4* ± 1.04^{b,z}	1.13 ± 1.41^{c,z}

[1]Statistical analysis was conducted with the proper degrees of freedom. Inoculation and postferment means were carried down for within total process comparisons. Total processes are in order from A to E; $n = 3$ for each total process by sampling timepoint.

[2]Chilling method denoted by T = simulated cold air chilling or RC = rapid ice bath chilling.

[*]First point in total process to achieve 5 log CFU/g reduction.

[a–c]Means with different superscripts within a total process differ; $\alpha < 0.05$.

[w–z]Means with different superscripts within a sampling time differ; $\alpha < 0.05$.

a 5-log reduction (6.4 log CFU/g; $P < 0.01$), whereas holding for 150 and 300 s reduced the populations below detectable levels, reducing by greater than 8.3 log CFU/g of the target organism ($P < 0.01$).

Total Process D was fermented to a final pH of 4.8 and thermally processed to 48.9°C, reducing non-O157 STEC populations by 1.2 log CFU/g after ice bath chilling ($P = 0.28$). For Process D, additional reductions were not achieved after shipping (0 s HPP; $P = 0.96$). HPP sausages from Process D for only 1 s had a total process STEC reduction of 3.2 log CFU/g ($P = 0.08$). It was not until Process D was subjected to at least 150 s HPP at 586 MPa that a reduction greater than 5 log was achieved (7.1 log CFU/g; $P < 0.01$). Continuing to hold the sausage at 586 MPa for 300 s reduced STEC populations by greater than 8.3 log CFU/g, below detectable levels ($P < 0.01$).

Process E STEC had similar counts to Process D after chilling, shipping (0 s HPP), 1 s HPP, 150 s HPP, and 300 s HPP ($P > 0.60$). Similar to Process D, Process E did not achieve a 5-log reduction until after it was subjected to 150 s of HPP (5.6 log CFU/g; $P < 0.01$). Holding Process E summer sausage at 586 MPa for 300 s created an additional reduction of 2.3 log CFU/g ($P = 0.03$) for a total reduction of 7.9 log CFU/g ($P < 0.01$).

Discussion

Prior work has shown that fermentation to pH < 5.0 plus heating to 62.8°C (145°F) or postfermentation (pH < 4.8) heating to an internal temperature of 53.3°C (128°F) for 1 h resulted in a 5-log reduction of *E. coli* O157:H7 for a beef and pork pepperoni product (Hinkens et al., 1996). Furthermore, reductions were maintained for both processing scenarios throughout drying for up to 18 d to a moisture:protein representative of a dry pepperoni product. In following work including *E. coli* O157:H7 and non-O157 STEC (Glass et al., 2012), it was reported that when a similar pepperoni product was fermented (pH ≤ 4.6), thermally processed, and held at 53.3°C for 1 h, it required an additional 7 to 14 d of drying at 12.8°C to achieve a 5-log reduction for *E. coli* O157:H7 and non-O157:H7, respectively. Comparatively, in the present study, when summer sausages were fermented to pH 4.5 or 4.8 and thermally processed to 54.4°C (with no extended hold time) and exposed to simulated cold air chilling, they were able to achieve a 5-log reduction in *E. coli* O157:H7; however, non-O157 STEC required additional hold time in cold storage to achieve a 5-log reduction when the summer sausages were fermented to the greater pH of 4.8.

Hinkens et al. (1996) and Glass et al. (2012) targeted a dry fermented product (moisture:protein ≤ 1.6), whereas the current study focused on a semidry (moisture:protein ≤ 3.1) fermented product, which can be met under various processing conditions without relying on the additional space and labor required with extended thermal hold times or extended drying periods. Additionally, Hinkens et al. (1996) stated that in some cases a greater thermal processing temperature can lead to undesirable color and texture or might not be feasible for some processors because of other circumstances. There are numerous reasons a fermented product may not be processed to greater temperatures, be it color and texture preference, associated cost, or process deviation. In the current work, it was shown that moisture:protein meeting the required guidelines of ≤3.1 could be met in all processing scenarios, but at the lower processing temperatures and greater pH, the additional intervention of HPP was required to meet the 5-log reduction requirement for both *E. coli* O157:H7 and non-O157 STEC, giving processors in this product category additional options.

Benito et al. (1999) suggested that pressure-resistant strains of *E. coli* O157:H7 can be isolated from clinical patients with foodborne illness and that those strains were also heat resistant, giving rise for concern that the use of HPP in conjunction with low-temperature cooking may not be effective. Additionally, several studies have shown that the growth phase of cells during HPP can affect the baroprotective properties of microorganisms (Cheftel, 1995; Benito et al., 1999; Baccuss-Taylor, 2015). These baroprotective properties can be due, in part, to an increase in heat shock proteins over exponential phase cells (Aertsen et al., 2005); this is important because of the extended time of natural fermentation. Aertsen et al. (2005) stated that this phenomenon can be important in the production of high pressure–processed foods in conjunction with mild heat treatment. Although HPP exposure led to further reductions of *E. coli* O157:H7, especially for Processes C, D, and E, and all processes had >5 log CFU/g reductions after 150 s HPP exposure, all processes had *E. coli* O157:H7 within the countable range after 300 s at 586 MPa. Further research is warranted to determine if fermentation and low-temperature cooking induce baroprotective properties in summer sausage products.

Omer et al. (2015) reported that the use of HPP at 600 MPa for 10 min on sausages fermented to pH 4.7 and dried to a water activity of 0.85 obtained an approximate 2 log CFU/g reduction attributed to the

fermentation and drying alone, whereas pressurization attained an additional 3 log CFU/g reduction of *E. coli* O103. These researchers showed a 5 log CFU/g total process reduction from a drier product than that of the current study. Porto-Fett et al. (2010) also reported reductions of *E. coli* O157:H7 as high as 6 log CFU/g attributed to pressurization alone for Genoa salami with pH values ranging from 4.6 to 4.86 and water activity ranging from 0.88 to 0.94 units. Although the use of HPP on some low-moisture products may not be effective, the pressurization of semidry and dry sausage products in the presence of small amounts of acid, from fermentation, may increase the effectiveness of HPP for these moisture-reduced foods, helping to attain log reductions required by USDA-FSIS.

Similar findings to the current study were noted when sausages were fermented to a target pH of 4.6 and 5.0 and then cooked to 54.4°C, showing that the product continued to ferment during the cooking stages to a final pH of 4.5 and 4.9, respectively (Calicioglu et al., 1997). Calicioglu et al. (1997) reported reductions of *E. coli* O157:H7 populations for the parameters similar to those in the current study, achieving fermentation reductions of 1.3 and 0.3 log CFU/g for pH declines to 4.5 and 4.9, respectively. Reductions reported by Calicioglu et al. (1997) for sausages fermented to pH 4.9 and then cooked to 54.4°C with subsequent chilling were less than those in the current study; however, the current study demonstrated reductions at or below the required 5-log reduction for all thermal treatments fermented to pH 4.8. The use of HPP at 586 MPa for as little as 150 s on sausages fermented to a pH as high as 4.8 and thermally processed as low as 43.3°C exceeded the USDA-FSIS requirement for the 5-log reduction of *E. coli* O157:H7 and achieved a 6-log reduction or greater. Sausages produced using any of the total processes described in this study with the use of 300 s of HPP at 586 MPa ensured further safety, surpassing a 6-log reduction. These parameters can be used in a complete process following Option 4 of the Blue Ribbon Task Force (Nickelson et al., 1996) to achieve a validated process of production under USDA-FSIS inspection.

Collectively, the findings presented in the current study provide a method for processors of low-temperature thermally processed and mildly fermented summer sausage to ensure microbiological safety against pathogenic *E. coli*. Overall, *E. coli* O157:H7 and non-O157 STEC populations/reductions were similar with regard to meeting a 5-log reduction within a total process, with the exception of Process B. Although Process B met a 5-log reduction for *E. coli* O157:H7 after thermal processing and chilling, the STEC took additional time and did not meet a 5-log reduction until after the samples were shipped and returned with the HPP samples. These results indicate that thermal processes as low as 43.3°C and fermentation endpoints as high as pH 4.8, in conjunction with HPP at 586 MPa for 150 s or greater, can meet a 5-log reduction as suggested by USDA-FSIS, giving semidry fermented sausage producers alternatives if using Option 4 of the Blue Ribbon Task Force.

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