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Effectiveness of a Laboratory-Scale Vertical Tower Static Chamber Steam Pasteurization Unit against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria innocua* on Prerigor Beef Tissue†

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ABSTRACT

A laboratory-scale vertical tower steam pasteurization unit was evaluated to determine the antimicrobial effectiveness of different exposure times (0, 3, 6, 12, and 15 s) and steam chamber temperatures (82.2, 87.8, 93.3, and 98.9°C) against pathogens (*Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria innocua*) inoculated onto prerigor beef tissue. Samples were collected and microbiologically analyzed immediately before and after steam treatment to quantify the effectiveness of each time-temperature combination. The 0-s exposure at all chamber temperatures (cold water spray only, no steam treatment) was the experimental control and provided ≤0.3 log CFU/cm^2 reductions. Chamber temperatures of 82.2 and 87.8°C were ineffective ($P > 0.05$) at all exposure times. At 93.3°C, significant reductions (>1.0 log CFU/cm^2) were observed at exposure times of ≥6 s, with 15 s providing approximately 1 log cycle greater reductions than 12 s of exposure. The 98.9°C treatment was consistently the most effective, with exposure times of ≥9 s resulting in >3.5 log CFU/cm^2 reductions for all pathogens.

Hazard analysis critical control point (HACCP) programs have been implemented in meat and poultry plants to prevent or minimize microbial, physical, and chemical hazards in edible products. HACCP is a ''systematic approach to the identification, evaluation, and control of food safety hazards'' *(9).* Antimicrobial intervention technologies for prerigor beef carcasses prior to chilling have become the cornerstone of most beef slaughter HACCP programs. Several carcass decontamination methods have been validated for use as critical control points for reducing bacterial populations on meat and poultry carcasses.

Steam pasteurization has been reported to be an effective method for decreasing bacterial populations on meat surfaces *(1, 6, 10–12).* An automated steam pasteurization system (SPS; Frigoscandia Food Processing Systems, Bellevue, Wash.) has been developed and commercially validated to reduce microbial populations on prerigor beef carcass sides *(10–12).* This commercial system is designed for medium and large beef slaughter operations and can ''pasteurize'' up to 400 carcasses (SPS 400) per h *(9, 10).* The three-phase process consists of surface water removal using vertical air blowers, surface pasteurization within a steam-flushed chamber for 6.5 to 10 s, and surface cooling with a cold water spray immediately prior to entering the chilling rooms. During the second phase, condensing steam uniformly blankets the carcass surface, rapidly elevating the carcass surface temperature to approximately 87.8°C *(11).*

The validated antimicrobial effectiveness of the large SPS unit and widespread installation by beef processors led to the conception of a steam pasteurization unit that could be used by smaller processing facilities as an automated intervention treatment for the entire carcass. A laboratory-scale steam treatment prototype was constructed to incorporate a static steam chamber instead of the steam-flushed chamber used in previous studies *(12).* The static chamber design provides a constant condensing steam environment for meat treatment instead of reflushing the chamber with steam for each treatment. The purpose of this study was to evaluate the antimicrobial effectiveness of a laboratory-scale vertical tower static chamber SPS against *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria innocua* attached to prerigor beef surfaces. Different exposure times and chamber temperature combinations were evaluated to guide further development of a small-scale commercial steam pasteurization unit.

MATERIALS AND METHODS

Steam pasteurization unit, vertical tower static chamber. A prototype steam pasteurization chamber was constructed using insulated stainless steel (Fig. 1). The rectangular steam chamber (119.4 by 53.3 by 53.3 cm) was enclosed on all sides except the bottom. The chamber was equipped with a metal cable block-and-tackle assembly to quickly suspend and hoist the meat samples in and out of the steam chamber. The steam compartment was mounted 142.2 cm above the laboratory floor using four square

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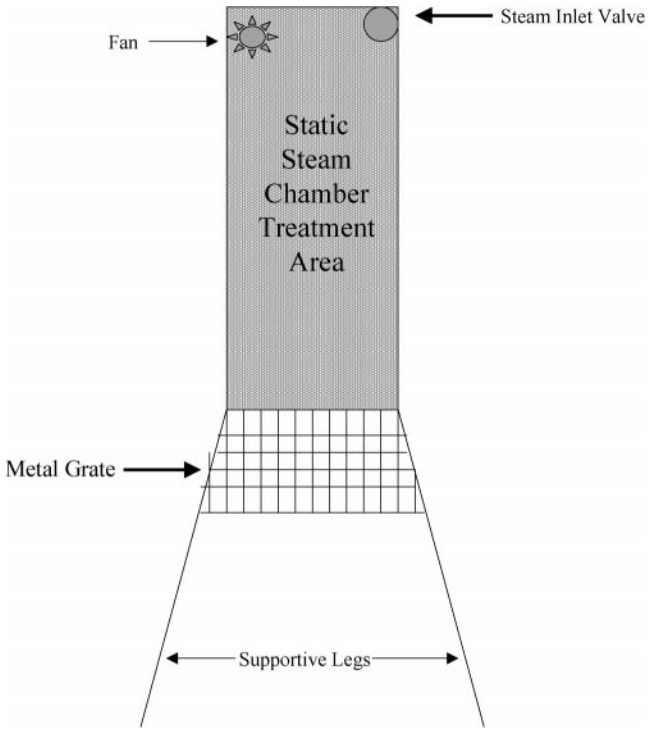

FIGURE 1. *Schematic diagram (side view) of the laboratory-scale vertical tower static chamber steam pasteurization system.*

tubular stainless steel legs. A protective metal grate was mounted at the bottom of the steam compartment to prevent laboratory personnel from reaching into the saturated steam atmosphere within the chamber. The compartment was filled with steam from a 1.9-cm-diameter inlet valve at the top of the chamber. A small fan was mounted inside the steam compartment and was used to infuse a small volume of air into the steam chamber to adjust chamber temperatures. The temperature inside of the static steam chamber was measured using a type K thermocouple (Omega Engineering, Inc., Stamford, Conn.) and recorded by a Hydrologger (Fluke, Everett, Wash.). The beef sample was hung on a bacon comb (a stainless steel multipronged meat hanger), the bacon comb was attached to the cable, and the meat was hoisted into the chamber for the designated exposure time.

Bacterial cultures. *Escherichia coli* O157:H7 (rifampicin-resistant strain developed by Dr. Gary Acuff, Texas A&M University, College Station, and obtained through Dr. James Dickson, Iowa State University, Ames), *Salmonella* Typhimurium (nalidixic acid–resistant strain, obtained from Dr. Stan Bailey, U.S. Department of Agriculture, Agricultural Research Service, Athens, Ga.), and *Listeria innocua* (streptomycin-resistant strain, obtained from the U.S. Meat Animal Research Center, Clay Center, Nebr.) were used for the inoculation studies. Cultures were maintained on Protect beads (Technical Service Consultants, Ltd., Heywood, Lancashire, UK) stored at −18°C.

Cultures were activated by aseptically adding one Protect bead to 9 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) and incubating at 35°C for 18 to 24 h. Growth of antibiotic-resistant *E. coli* O157:H7 was evaluated on tryptic soy agar (TSA; Difco, Becton Dickinson) containing 100 ppm rifampicin (Sigma, St. Louis, Mo.) (TSA-Rif). Antibiotic-resistant *Salmonella* Typhimurium was evaluated on MacConkey agar (Difco, Becton Dickinson) containing 200 ppm nalidixic acid (Sigma) (Mac-Na). Growth of antibiotic-resistant *L. innocua* was evaluated on TSA containing 500 ppm streptomycin (Sigma) (TSA-Str).

One loop of rehydrated culture was added to 100 ml of TSB and incubated at 35°C for 18 h. Two 100-ml bottles of TSB were inoculated for each bacterial culture. All cultures were centrifuged at 15,300 × *g* at 4°C for 10 min. The supernatant was discarded, and each pellet was rehydrated with 100 ml of 0.1% peptone diluent. Two rehydrated culture pellets of each pathogen were transferred to a handheld pump-spray bottle (inoculum bottle) to achieve a total volume of 600 ml and approximately 10^8 CFU/ml of each pathogen. A 1-ml aliquot of the inoculum mixture was serially diluted using 0.1% peptone diluent and was spiral plated onto TSA-Rif, Mac-Na, and TSA-Str plates to enumerate *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. innocua*, respectively. A new inoculum mixture was prepared for each replication immediately prior to use. Mean *E. coli* O157:H7 concentrations in the inoculum solution were 9.1 log CFU/ml when the first meat sample was inoculated and 9.0 log CFU/ml after the last meat sample was inoculated. Mean *Salmonella* Typhimurium and *L. innocua* concentrations were 8.8 and 9.5 log CFU/ml, respectively, at the start and 8.8 and 9.4 log CFU/ml, respectively, at the end of sample inoculation.

Preparation of meat samples. Four fed (fattened) beef steers were purchased from local producers, and animals were slaughtered on three successive days in the Kansas State University meat science abattoir using slaughter procedures approved by the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service. The first day, two steers were slaughtered; the second animal was slaughtered approximately 4 h after the first (animal = replicate). One steer was then slaughtered on each of the two following days (carcass side = replicate). A 300-cm^2 stainless steel template was used as a guide to remove fascia samples (approximately 2 cm thick) with a scalpel after hide removal, evisceration, carcass splitting, and washing. The cutaneus trunci samples were placed into a plastic bag inside an insulated container for holding and transportation to the microbiology laboratory. At the laboratory, the plastic bags containing the samples were placed in a 35°C incubator to maintain postslaughter temperature. Meat samples were utilized within 4 h of slaughter, thus meeting the definition of prerigor given by Faustman *(7).* All experiments were replicated six times.

Inoculation of meat surfaces. Meat samples were randomly removed from the incubator at approximately 10-min intervals. Each sample was placed onto a clean sheet of freezer paper (poly coated), and a stainless steel bacon comb was inserted through one edge of the sample to suspend the meat during inoculation and subsequent steam pasteurization treatments. The meat sample was suspended in the center of a sealed plexiglass inoculation chamber (62 by 42 by 60.5 cm). The nozzle of the inoculum bottle was placed into a 1.5-cm-diameter hole in the center of the door of the inoculation chamber. The fascia side of the sample was mist inoculated with four sprays from the inoculum bottle positioned 31.5 cm away from the suspended meat sample. The meat sample was then placed (inoculated side up) onto a clean sheet of freezer paper for 5 min at room temperature to allow for pathogen attachment. Significant attachment of bacteria has been reported to occur on both lean and adipose tissue after 1 min *(2, 3).*

Steam pasteurization treatment. Four chamber temperatures (82.2, 87.8, 93.3, and 98.9°C) and six exposure times (0, 3, 6, 9, 12, and 15 s) were tested. After the sample was suspended on the hook, it was quickly hoisted into the steam chamber with the pull cable for the specified treatment time. Exposure time was defined as the time at which the entire beef sample had completely entered the boundary steam layer at the bottom of the steam compartment

TABLE 1. *Mean reductions of inoculated* E. coli *O157:H7 on samples of freshly slaughtered beef after treatment in a laboratory-scale static chamber steam pasteurization unit*

Treatment temperature (°C)	Mean initial count (log CFU/cm^2)	Count reduction (log CFU/cm^2) after[a]:					
		0 s	3 s	6 s	9 s	12 s	15 s
82.2	6.1	0.0 A	0.2 AB	0.3 AB	0.2 AB	0.2 AB	0.3 AB
87.8	6.0	0.3 AB	0.0 A	0.1 A	0.3 AB	0.6 ABC	0.6 AB
93.3	6.0	0.0 A	0.3 AB	1.0 BC	1.8 DE	1.6 CD	2.6 EFG
98.9	6.0	0.0 A	2.1 DEF	2.8 FG	4.1 HI	3.2 GH	4.7 I

[a] Means with a letter in common are not significantly different ($P > 0.05$). The SE of the least squares mean = 0.33, $n = 144$.

until the time it completely exited this layer. At the end of the treatment time, the meat sample was quickly lowered and immediately cooled with 15 sprays from a pump-spray bottle filled with cold water (approximately 4.4°C) that was held in an ice chest. The sample was then placed on a clean sheet of freezer paper.

Steam chamber temperatures were monitored as previously described and adjusted by using a small fan placed inside the top of the vertical tower chamber. Temperatures could be reduced by increasing the fan speed (incorporating more air into the saturated steam).

Sample collection and microbiological analyses. After inoculation and before steam treatment, two cores (ca. 2 mm thick) were excised from the sample using flame-sterilized forceps, scalpels, and stainless steel coring devices (representing 18.16 cm^2 total exterior surface area sampled). The cores were placed into a sterile filter stomacher bag and homogenized with 50 ml of 0.1% peptone diluent for 2 min using a stomacher. Serial dilutions were made using 0.1% peptone diluent, and the appropriate dilutions were spiral plated onto the culture media. All plates were plated in duplicate and incubated at 35°C for 24 h.

After each steam pasteurization treatment, two cores were excised from the sample and homogenized with diluent as described above. Three plates of each medium were used to spread plate a total of 1 ml of sample (0.33 ml per plate) directly from the initial diluted sample. Serial dilutions were made using 0.1% peptone diluent, and the appropriate dilutions were spiral plated onto TSA-Rif, Mac-Na, and TSA-Str plates. All plates were plated in duplicate and incubated at 35°C for 24 h.

Statistical analyses. Pathogen counts were calculated, averaged, and converted to log CFU/cm^2. The experimental design was a split plot, with a 4 (temperature: 82.2, 87.8, 93.3, and 98.9°C) × 6 (exposure time: 0, 3, 6, 9, 12, and 15 s) factorial design. The subplot treatment was sampling time (before and after steam treatment). The data were analyzed using the MIXED linear models procedure in SAS (Statistical Analysis Institute, Cary, N.C.). Means were separated using the differences of the least square means. Differences were considered significant at $P \leq 0.05$. The results from these analyses allowed for the determination of significant differences in bacterial counts before and after treatment for each pathogen type treated at different temperatures and for different exposure times.

The general linear model was used to analyze bacterial reductions from each treatment temperature and exposure time for all pathogens. Means were separated using the least significant difference procedure. Differences were considered significant at $P \leq 0.05$. The results from these analyses were used to determine the optimum treatment temperature and exposure time for the pathogens tested.

RESULTS AND DISCUSSION

Statistical analysis of the pathogen populations before and after steam treatment indicated an interaction ($P \leq 0.05$) between treatment temperature and exposure time. As the temperature and exposure time increased, pathogen populations after treatment decreased. Before steam treatment, mean inoculation concentrations of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. innocua* on beef tissue surfaces were 6.0, 5.6, and 6.2 log CFU/cm^2, respectively. A cold-water spray with no steam exposure served as the control treatment and resulted in pathogen reductions of ≤0.3 log CFU/cm^2 (Tables 1 through 3). All treatment combinations using 82.2 and 87.8°C chamber temperatures and 93.3°C for 3 s were ineffective, producing log reductions similar to those of the control treatment ($P > 0.05$).

Treatment combinations of 93.3°C for ≥6 s and 98.9°C for ≥3 s reduced *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. innocua* populations after treatment by 1.0 to 4.8 log CFU/cm^2 ($P \leq 0.05$). Although the results were significantly different from the controls, exposure of samples at 93.3°C for 6 s resulted in marginal (1.0 to 1.3 log CFU/cm^2) reductions. Moderate reductions (1.6 to 1.8

TABLE 2. *Mean reductions of inoculated* Salmonella *Typhimurium on samples of freshly slaughtered beef after treatment in a laboratory-scale static chamber steam pasteurization unit*

Treatment temperature (°C)	Mean initial count (log CFU/cm^2)	Count reduction (log CFU/cm^2) after[a]:					
		0 s	3 s	6 s	9 s	12 s	15 s
82.2	5.7	0.3 ABC	0.1 AB	0.3 AB	0.2 AB	−0.2 A	0.2 AB
87.8	5.7	0.2 AB	0.1 AB	0.2 AB	0.0 A	0.2 AB	0.7 BCD
93.3	5.6	0.0 A	0.0 A	1.3 BCD	1.6 DE	1.7 DE	2.7 EF
98.9	5.6	−0.4 A	1.4 CD	2.9 F	3.6 F	3.5 F	4.8 G

[a] Means with a letter in common are not significantly different ($P > 0.05$). The SE of the least squares mean = 0.42, $n = 144$.

TABLE 3. *Mean reductions of inoculated* L. innocua *on surfaces of freshly slaughtered beef after treatment in a laboratory-scale static chamber steam pasteurization unit*

Treatment temperature (°C)	Mean initial count (log CFU/cm^2)	Count reduction (log CFU/cm^2) after[a]:					
		0 s	3 s	6 s	9 s	12 s	15 s
82.2	6.3	0.1 A	0.2 A	0.2 AB	0.2 AB	0.1 A	0.2 AB
87.8	6.3	0.0 A	0.1 A	0.1 A	0.2 A	0.5 AB	0.5 AB
93.3	6.2	0.1 A	0.3 AB	1.1 BC	1.7 CD	1.6 CD	2.6 EF
98.9	6.2	0.1 A	2.4 DE	2.9 EF	4.0 GH	3.3 FG	4.6 H

[a] Means with a letter in common are not significantly different ($P > 0.05$). The SE of the least squares mean = 0.32, $n = 144$.

CFU/cm^2) were observed at 93.3°C for 9- and 12-s exposures. A marked increase in treatment lethality occurred as exposure time at 93.3°C increased from 12 to 15 s, with the 15 s of exposure providing approximately 2.6 log CFU/cm^2 reductions in all pathogen populations. Similar reductions occurred at 98.9°C at 6 s of exposure. The 98.9°C steam chamber temperature with exposure times >6 s were the most effective for generating large reductions in pathogen populations (≥3.2 log CFU/cm^2). As expected, the greatest reductions (≥4.6 log cycles) were achieved at this temperature using a 15-s exposure.

Reductions of 4.0 log CFU/cm^2 and 2.2 log CFU/cm^2 of inoculated *L. innocua* after steam treatment have been reported by Cygnarowicz-Provost et al. *(4)* (115 to 136°C for 30 to 40 s) and Morgan et al. *(8)* (127°C for <1 s), respectively. Other authors have reported a decrease in naturally occurring levels of *Salmonella* on various meat products after treatment with steam. Davidson et al. *(5)* reported a 50% decrease in the incidence of *Salmonella* on whole and cut-up chicken pieces treated with a continuous flow of steam for 20 s. *Salmonella* contamination was reduced by steam treatment to undetectable levels on freshly slaughtered beef (treatment of 90.5 to 94.0°C for 6 or 8 s) *(11)* and on freshly slaughtered pork (steam continuously sprayed on the carcass surface for 30 s) *(1)*.

Data from the current study indicate that steam treatment combinations of 93.3°C for 6 to 15 s and 98.9°C for 3 to 15 s provided >1 log CFU/cm^2 reductions of inoculated bacterial pathogens. A 98.9°C chamber temperature for ≥9 s of carcass exposure time is recommended for future commercial unit testing based upon these microbiological results. These operational parameters provided ≥3.2 log CFU/cm^2 reductions for all three pathogens. The results from this study apply to small pieces of meat from freshly slaughtered cattle. Further studies are needed using commercial-scale prototype units to validate these findings and to determine quality (color) effects on beef carcass sides from freshly slaughtered cattle. Since the beginning of commercial SPS installations in 1996, a large volume of observational data has been generated by companies who use the technology. Similar time and temperature combinations have routinely resulted in acceptable carcass color by the end of ordinary chilling times used by the industry.

The data from this laboratory experiment indicate the importance of applying steam so as to consistently maintain a target chamber temperature in SPS units. Slight variations in chamber temperature or exposure time will have marked effects on the level of microbial reduction achieved.

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