Effects of explosively-generated hydrodynamic shock wave treatments on the microbial flora of beef steaks and ground beef, and *Listeria innocua*

T.A. Lorca [a*], J.R. Claus [b], J.D. Eifert [a], J.E. Marcy [a] , and S.S. Sumner [a]

[a] *Department of Food Science and Technology, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA 24060.*

[b] *Meat Science & Muscle Biology Laboratory, University of Wisconsin-Madison, 1805 Linden Dr. West, Madison, WI 53706.*

* Corresponding author. Mailing address: Department of Food Science and Technology, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA 24060. Phone: 540-231-9518. Fax: 540-231-9293. E-mail: t_lorca@hotmail.com

ABSTRACT

The effects of a non-thermal whole muscle food tenderization process, treatment by explosively-generated hydrodynamic shock waves (EHSW), on the microbial flora of whole steaks and ground beef, and broth cultures of *Listeria innocua* were studied. The nature, magnitude, and movement of the hydrodynamic shock wave (50-251 MPa) were controlled by varying the explosive (binary v. molecular) and size of charge used, and the distance between the explosive charge and the top surface of the sample. There was no significant difference ($P>0.05$) in the psychrotrophic, lactic, or coliform populations of EHSW-treated ground beef compared to untreated ground beef regardless of the nature, magnitude, or movement of the hydrodynamic shock wave. Submitting ground beef to two sequential EHSW treatments (both binary and molecular explosives) did not produce a significant difference ($P>0.05$) in the psychrotrophic, lactic, or coliform populations of EHSW-treated and untreated samples. EHSW treatment (215 MPa) did not extend ($P>0.05$) the shelf life of ground beef during a 5 d refrigerated storage study. No difference ($P>0.05$) was observed in levels of *L. innocua* in treated broth samples when compared to untreated control samples. EHSW treatments utilized in this study do not impact the ability of bacterial flora naturally present in the various samples to propagate and grow and thus under the conditions described in this study would not be effective in improving the keeping quality and increasing the bacterial safety of treated whole and ground beef samples.

Keywords: Hydrodynamic Shock Wave, beef, bacteria, Hydrodyne™, *Listeria innocua*

INTRODUCTION

Explosively-generated hydrodynamic shock wave (EHSW) treatment is a non-thermal tenderization technique that has been shown to increase instrumental and subjective tenderness in raw beef, lamb, pork, and poultry without affecting the functional properties of proteins (2,16,17,18,19,21). In EHSW processing, an explosively-generated hydrodynamic shock wave of μs duration travels through a vacuum packaged raw muscle food submerged in a water-filled treatment chamber (12,13). The wave front passes through substances with an acoustical match to water and dissipates energy into structures which are an acoustical mismatch to water, producing non-selective structural damage to the myofibrils in and near the Z-lines (10,25).

Because EHSW processing has been shown to tenderize whole muscle foods uniformly and throughout the entirety of the sample, the same shock waves that produce tenderization may produce shearing stress on bacteria and have a bactericidal effect. If this is the case, the technique may be explored as a non-thermal alternative to irradiation in the production of ground beef with a reduced bacterial load and perhaps be used to offer ground beef with a reduced level of potentially pathogenic bacteria (23,24). The bacteriological effects of the tenderization technique have been explored in a few studies with mixed results. Gamble et al (6) found EHSW treatment of 46 MPa ($P<0.05$) (but not treatment of 50-60 MPa [$P>0.05$]) to be effective at reducing the levels of the parasite *Trichinella spiralis* in infected pork loins, but that the treatment failed to affect their infectivity as evidenced by the ability of the recovered parasites to encyst into the muscles of mice following a 28 hr inoculation and incubation period. Berry et al. (2) reported that EHSW treatment of pork loins and beef reduced total plate counts by 14 and 12% after a 7 d storage period. Moeller et al. (17) found EHSW processing (69 MPa) had no significant effect ($P>0.05$) on the aerobic population (APC CFU/g, 25°C, 72 hr. incubation) nor

on the coliform population (MPN/g, 37°C, 48 hr. incubation) of pork loins. They concluded that submitting pork loins to EHSW processing did not affect their natural flora. Only two detailed published studies have shown any bactericidal effect of EHSW processing. Williams-Campbell and Solomon (23) found EHSW treatment (70MPa) of temperature abused (22 hrs at 23°C) beef and pork stew pieces produced a 1.7 log difference ($P<0.05$) among APC (48 hr at 30°C) when compared to untreated controls. The researchers also reported approximately a 3 log reduction ($P<0.05$) in treated (54 MPa, 62 MPa, and 70 MPa) temperature abused ground beef when compared to untreated controls. In a 2002 study, Williams-Campbell and Solomon (24) found that an EHSW treatment of 70 MPa produced initial 1 and 2 log reductions ($P<0.05$) in fresh ground beef and stew pieces as well as a 3 log reduction in treated ground beef stored at 5°C for 7 days. This treatment produced a 4.5 log difference ($P<0.05$) in the APC of treated fresh ground beef after 14 d of storage at 5°C when compared to untreated ground beef (4.5 log CFU/g vs. 9.0 log CFU/g).

The objective of the following work was to investigate the effects of different variables encountered in EHSW processing on the microbial flora of whole steaks and ground beef, and *Listeria innocua.* Because researchers have shown that altering the magnitude of explosively-generated shock waves by creating different combinations of the magnitude of the explosive charge and the distance between the charge and the sample produced different tenderization effects in whole muscle foods (16,18), we altered both the magnitude and the nature of the shock waves. The magnitude of the shockwaves was altered by changing both the mass of the explosive charge used and the distance between the explosive charge and the top surface of the sample. The magnitude of the shockwaves created with the various combinations of mass and

distance were derived from pressure calculations for binary and molecular explosives derived for EHSW processing (4).

The effect of EHSW processing on less complex matrices was examined by using an aerated slurry composed of water and ground beef (slurry) and log phase cells of *L. innocua* suspended in Trypticase Soy Broth with Yeast Extract as samples. Adding air and decreasing the complexity of the samples by adding water may allow EHSW to produce a bactericidal affect. The energy produced by the explosive force may heat the air bubbles trapped in the slurry, adding heating stress to the shearing stress that is exerted on the bacterial membrane by the EHSW process.

The effects of multiple sequential treatments on the bactericidal properties of EHSW processing were also explored. It was believed that the first EHSW treatment may have the effect of injuring bacteria present in the sample and that subsequent EHSW treatments would then produce increased injury and cell death. If the package integrity held during multiple treatments and the multiple treatments proved successful, multiple EHSW treatments may be pursued as a method to reduce the bacterial levels in ground beef.

The ability of a 215 MPa EHSW treatment to extend the bacterial shelf-life of ground beef during a 5 d refrigerated study was also studied. The purpose of the refrigerated shelf life study was to gage both the initial and extended impacts that EHSW processing had on ground beef. If the levels of those members of the bacterial flora considered the most responsible for the bacteriological degradation of fresh ground beef during aerobic refrigerated retail storage did not differ between EHSW-treated and untreated ground beef samples at any point during the refrigerated storage study, then we could conclude that an EHSW treatment of 215 MPa had no effect on extending the bacterial shelf life of ground beef.

MATERIALS AND METHODS

Sample preparation. Fresh ground beef (15 kg) and whole raw eye of round steaks were purchased at a local grocery store. The ground beef was mixed in a sterile 4.5 stainless steel bowl for 5 min at setting 2 with a model K45SS stand mixer (KitchenAid, St. Joseph, MI) to homogenize the sample and then subdivided into 20 g and 60 g portions under a laminar flow hood. Twenty g portions were vacuum packaged into shrink bag (SB) packages using a model UV-250 Ultravac (Koch, Kansas City, MO) or vacuum packaged into Curlon (C) packages made of two 5.7 x 8.3 cm pieces of 8 mil Curlon ® Grade 9450-EE packaging film (O^2 barrier properties <0.2 $cm^3/100\ in^2$ per 24 hr. at 23°C and 0% RH [Curwood, Oshkosh, WI]). Fresh raw whole eye of round steaks were hand trimmed into 6.4 x 6.4 cm squares (1.3 cm thickness) and vacuum packaged into 9 x 9 cm C packages while 60 g portions were packaged into tube packages (TP) (Fig. 1). Because package survival was not consistent during the course of the studies, three package types were used for different tests in the studies, SB, TP, and C. Each SB package was made in the following manner: a 20 g portion was individually vacuum-packaged within reduced temperature shrink bags (Cryovac Division, W.R. Grace & Co.-Conn., Duncan, SC). Packaged samples were dipped into 100°C water bath for 2s, further vacuum packaged into individual bone-guard bags (Cryovac), and dipped into 100°C water bath for 3-5 s. Hot water dipping of packaged samples was performed in order to "shrink fit" the package to the sample, reducing the void spaces between the ground beef and the package which could contribute to failure of the packages during EHSW treatment. Double bagging of samples was performed to reduce the failure rate of the packages during EHSW treatment (Lorca et al., 2002). Each TP package was made in the following manner: A 25 cm clear colorless polyvinyl chloride (PVC)

(Cole-Palmer Instrument Co., Vernon Hills, IL] tube with 3.5 cm o.d., 2.5 cm id, 0.5 cm wall thickness was sealed on one end by heating the end of the tube to melting with a model AH 502 Master Flow Heat hot air gun (Master Appliance Corp., Racine, WI)and then crimping with a model 101028 stationary vice grip (Wilton, Schiller PK, IL) until cool to create a leak proof seal. PVC plugs were formed by baking a liquid PVC molding compound (Rutland Plastic Technologies, Pineville, NC) within a 2.6 cm i.d. cylindrical shaped steel mold for 60 min at 165°C until solid and allowed to cool. Each plug was cut to 2.5 cm in length and inserted into the sealed end of the TP package. The TP packages were hand washed under running warm soapy water, allowed to air dry, and rinsed with a 70% ethyl alcohol solution. The tubes were filled with the aid of a Fatosa model E 251 commercial sausage stuffer (Koch Supplies, Inc., Kansas City, MO) to reduce the amount of air introduced into the ground beef. The open end of the TP tube was capped with a PVC plug leaving approx 7.6 cm of excess tubing beyond the length of the PVC plug. The open end of the package was sealed as described above. For treatment in the Large Hemi Shell (LH) prototype, 15 SB packages were randomly selected and designated for EHSW treatment and 15 designated as untreated control samples. For treatment in the Small Tank (ST) prototype (Fig 2.), 11 C, 3 TP and 15 SB ground beef packages and 11 whole steak packages were randomly selected for EHSW treatment, leaving 3 TP, 15 SB, and 11 whole steak packages designated as untreated control samples. All samples with the exception of those used in the refrigerated storage study were held at 5°C for 48 hrs to bring the levels of psychrotrophs, lactic acid bacteria, and coliforms to approx 10^6, 10^5, and 10^3 CFU/g, respectively and transported on ice to the ST prototype or to the Large Hemi Shell ([LH] Fig. 3) prototype for EHSW treatment. Samples used for the 5 d refrigerated storage study were purchased within 2

hours of treatment within the ST prototype, packaged into TP packages as described above, and stored on ice until EHSW treatment.

Twenty-five mL log phase stock cultures of *Listeria innocua* were grown in Trypticase Soy Broth with Yeast Extract (TSBYE; Becton Dickinson, Sparks, MD) and stored as 1 mL stocks at -80°C following published methods (1,7). Four days prior to use, stock cultures were thawed on ice, placed into 20 ml TYSE medium and grown aerobically at 21°C in a shaking incubator (Precision Scientific, Chicago, IL) for 72 hr. One-milliliter aliquots were dispensed into sterile 1.5 ml polypropylene flat-top microcentrifuge tubes and centrifuged 3 min (21,000 x g) to pellet cells. The supernatant was aseptically removed, and the remaining pellet was washed one time with sterile TSYE broth, then recentrifuged to pellet. The pellet was then resuspended in 25 ml TSYE broth and grown aerobically in a shaking incubator for 24 h. at 21˚C to log phase. Logarithmic growth of inocula was confirmed previously in the laboratory using growth curve experiments on Oxford Agar (OA; Becton Dickinson, Sparks, MD). This ca. 10^6 CFU/ml stock culture was then poured into pre-assembled vinyl packages (VP) and allowed to overflow. VP Packages were formed in the following manner: Each VP package was composed of a 15.2 cm long clear colorless PVC tube (Cole-Palmer Instrument Co.) with an outer diameter of 2.5 cm and 1.9 cm id. One end was capped with a solid threaded PVC plug (McMaster-Carr, Atlanta, GA), and secured with a plastic cable tie (Garner Bender, Milwaukee, WI). The VP packages were hand washed under running warm soapy water, allowed to air dry, rinsed with a 70% ethyl alcohol solution, and filled when dry with the *L. innocua* broth culture. The open end of the VP tube was capped with a second threaded PVC plug leaving approx 5 cm of excess tubing beyond the length of the PVC plug. The second end was folded over and secured with a second cable tie. Five VP packages were placed side by side widthwise into a 20.3 cm x 30.5 cm 8 mil Curlon

bag (Curwood, Oshkosh, WI) and vacuum packaged, leaving a 10 cm x 20.3 cm pocket below the samples. A 2.54 cm slit was cut into the pocket of the Curlon bag, the vacuum broken, the pocket filled with water, and the slit sealed with 2-4 layers of adhesive duct tape. This package allowed precise placement of the samples directly in the center of the tank to provide equal exposure to the explosively generated shock waves. The slurry was formed in the following manner: Three hundred g of ground beef was added to 375 ml sterile distilled water, forming a blend of 1:1.25 (beef: water), and mixed again for 2 min at setting 2 with a stand mixer. Approx 20 ml of the slurry was poured into each of 20 VP packages as previously described and closed securely

EHSW treatment. Samples designated for EHSW treatment and untreated control samples were transported either to the Buena Vista facility (Dynawave, Inc., Buena Vista, VA) for treatment in the LH prototype (Fig 2.) or to the Virginia Tech facility for treatment in the ST prototype (Fig 3.) and treated as follows. Samples treated in the LH prototype (Fig. 2) were subjected to EHSW treatments as shown in Table 1. The binary explosive used was composed of liquid nitro methane and solid ammonium nitrate (Donovan Commercial Industries, Nortonville, KY) and not explosive until mixed on site. The molecular explosive, Pentaerythritol tetranitrate (PETN) (Donovan Commercial Industries), was purchased as a preformed shaped charge. Packaged samples were secured with plastic cable ties into a hemi shell shaped basket, and placed into the tank. Each of the binary explosive charges used in the treatments was placed into a plastic zip-seal bag hand-molded around an electrically detonated blasting cap connected to wire leads. The wire leads were in turn connected to an electronic detonation device located outside of the tank, the zip bag closed, secured with duct tape, and suspended at the specified

distance above the top surface of the samples with a wire. The lid of the tank was secured to the treatment chamber and the tank flooded with cold water to remove all void spaces within the tank. The explosive was detonated, the samples removed from the tank after treatment, inspected for package integrity, and placed on ice with the untreated control samples for transport to the food microbiology laboratory at Virginia Tech for microbiological analysis within 4 hours of treatment. This was repeated for all of the EHSW treatments within the LH prototype.

Samples treated in the Small Steel prototype (Virginia Tech, Blacksburg, VA) were treated as shown in Table 1. The unit (ST; Fig. 2) was previously described (23). The tank was flooded with water, the samples were lowered into the tank and allowed to sink to the bottom. For treatments requiring the molecular explosive be set at a distance of 10 cm from the sample, the TP packaged samples were suspended individually with fishing line within the center of a stainless steel insert with a wall thickness of 2.5 cm, an outer diameter of 20.3 cm, and a height of 48 cm. The molecular sheet explosive was folded around the blasting cap and taped to the interior wall of the insert at a set distance of 10 cm from the sample. The steel insert was used in order to decrease the distance between the sample and the explosive, increasing the magnitude of the resultant hydrodynamic shock wave which strikes the sample. After the samples were placed in the tank, the tank was flooded to remove void spaces and the hemi shell lid secured. Charges were detonated as described above. Each treated sample (with the exception of those subjected to 116 and 170 MPa treatments which received two sequential treatments) received only one EHSW treatment. The samples were removed from the tank after treatment, inspected for package integrity, and placed on ice for transport to the food microbiology laboratory at Virginia

Tech for analysis. Only samples which retained their package integrity were used for microbiological analysis. Untreated control samples were held on ice during EHSW treatment. Treated samples were placed on ice with control samples until microbiological analysis was performed at the food microbiology laboratory within 3 hr of EHSW treatment.

Microbiological sampling and examination. SB samples were opened aseptically in a laminar flow hood, dispensed into sterile strainer stomacher bags along with 180 ml of 0.1 % peptone diluent, blended for 2 min. with a model 4000 Stomacher Lab Blender (Tekmar, Cincinnati, OH), and then serially diluted in 0.1 % sterile peptone. Serial dilutions were pour plated in duplicate onto Tryptic Soy Agar ([TSA] Becton Dickinson, Sparks, MD), DeMan, Rogosa, Sharpe Agar ([MRS] Becton Dickinson), and Violet Red Bile Agar ([VRB] Becton Dickinson) and incubated aerobically to enumerate psychrotrophs ([PPC] 72 hr. @ 21˚C), lactobacilli ([LAB] 72hr. @ 21˚C), and coliforms ([CC] 48 hr. @ 35˚C) respectively. Colonies were enumerated following published methods (15).

TP samples were aseptically sampled as follows: Each tube was flame sterilized and aseptically sampled to remove 50 g ground beef from the interior of the package and placed into a sterile stomacher bag. The contents of the stomacher bag were hand mixed through the bag for 2 min to distribute the bacteria evenly throughout the meat. Five 10 g samples were removed, blended with 90 ml sterile 0.1 % peptone in a stomacher strainer bag, and serially diluted. Serial dilutions were pour plated in duplicate to enumerate PPC, LAB, and CC as described above. Storage study samples packaged in TP packages were aseptically opened as described above. Approx 50 g of ground beef removed from the package, placed into a sterile stomacher bag and hand mixed through the bag to evenly distribute the microorganisms throughout the 50 g sample.

Five 10 g samples were aseptically removed, placed onto ethanol-sterilized individual foam trays (large trays trimmed to 6.4 cm ^{2}), wrapped with 1 layer of Saran (0.5 mil, O_2 transmission rate of 2.4 $cm^3/100\ in^2$ x d x atm @ 23°C, 10% RH [Dow Chemical Corp. Midland, MI]), and stored aerobically at 4°C for the duration of the 5 d study. Each 10 g package was referred to as a "mini pack" (MP). The above procedure was followed for all 10 EHSW-treated and 10 non-treated samples. PPC, LAB, and CC were enumerated on day 0,1,3, and 5 as described above. Aerated slurry samples were opened aseptically in a laminar flow hood and serially diluted to enumerate PPC, LAB, and CC as described above.

Each whole steak sample was aseptically opened under a laminar flow hood, placed onto the sterile side of a stomacher bag (bag was opened on 3 sides and unfolded to reveal the sterile interior surface of the bag) and sliced into two halves (3.2 cm x 3.2 cm x 1.3 cm thickness) with a flame-sterilized non-serrated knife. One of the halves was examined for the presence of microorganisms throughout the entirety of the steak by blending in a sterile glass jar of a model 91-203 blender (Waring Product Div., New Hartford, CT) for 2 min with the appropriate volume of 0.1 % peptone to create an initial 1/10 dilution. Serial dilutions were pour plated in duplicate to enumerate PPC, LAB, and CC as described above. The surface bacterial flora was sampled from the second half of the steak by swabbing a 9 cm^2 area with a sterile calcium alginate-tipped swab moistened in a sterile 9 ml 0.1% peptone blank. After swabbing the area 2 times in each direction, the tip of the swab was broken off into the blank. Each swab sample was serially diluted, plated in duplicate, and examined for PPC, LAB, CC populations as discussed above and reported as microbial parameter/cm^2.

Microbiological examination of *L. innocua* broth culture samples occurred immediately upon arrival at the Food Microbiology Laboratory, within four hours of EHSW treatment. *L.*

innocua broth samples were opened aseptically in a laminar flow hood, poured into sterile screw cap tubes, and vortexed. One ml aliquots were serially diluted in 0.1 % peptone, pour plated onto Oxford Agar, and incubated aerobically for 72 hrs. at 21˚C. *L. innocua* was enumerated following published methods (7).

Statistical design and analysis. The experimental design was a randomized complete block with all EHSW treatments and their respective untreated controls. Data was analyzed using JMP (SAS Institute Inc., Cary, N.C.) for Analysis of Variance (ANOVA) to determine if the means of the bacterial levels (log CFU/ g beef) between EHSW treated and non-treated control samples were significantly different. Values for microbial levels are represented as geometric means.

RESULTS

No difference ($P > 0.05$) was observed between EHSW-treated ground beef and untreated controls for the psychrotrophic population (PPC), lactic acid bacterial population (LAB), nor the coliform population between samples subjected to EHSW processing compared to untreated control samples regardless of the magnitude of the pressure front achieved during processing or the type of explosive used. Both treated and untreated ground beef samples contained levels of 10^6, 10^5, and 10^3CFU/g for PPC, LAB, and CC respectively. In addition, no difference ($P>0.05$) was found amongst any of the different EHSW treatments. Regardless of the magnitude of pressure front created within the treatment chamber (50, 77, 78, 87, 97, 116, 170, 201, 215, 251, 350-541 MPa), bacterial levels of both EHSW-treated and untreated control samples were found to be 10^6, 10^5, and 10^3CFU/g for PPC, LAB, and CC respectively. The elevated bacterial levels seen in all ground beef samples can be attributed to the 48 hr 5°C aging period the samples were subjected to prior to EHSW treatment. No difference ($P > 0.05$) was observed between ground beef subjected to EHWS processing (87 MPa) and untreated ground beef for the psychrotrophic population (PPC), lactic acid bacterial population (LAB), nor the coliform population regardless of the size of the water column above the explosive charge (15 and 7 cm). Again all bacterial levels were found to be 10^6, 10^5, and 10^3CFU/g for PPC, LAB, and CC respectively. Similar results were obtained for whole steak samples. No difference ($P>0.05$) was observed in the surface bacterial populations (PPC, LAB, or CC) recovered from whole eye of round steaks subjected to EHW-processing (87 MPa) when compared to untreated control samples. Analysis of the bacterial levels recovered from entire EHSW-whole steaks (4.0 Log CFU/cm^2 ± 0.2 S.D. PPC, 3.1 Log CFU/cm^2 ± 0.3 S.D. LAB, 2.6 Log CFU/cm^2 ± 0.5 S.D. CC) were not different ($P>0.05$) from those recovered from untreated whole steaks (3.8 Log CFU/cm^2 ± 0.3 S.D. PPC,

3.0 Log CFU/cm^2 ± 0.3 S.D. LAB, 2.4 Log CFU/cm^2 ± 0.4 S.D. CC CFU/g). Multiple EHSW treatments performed at 116, 170, or 215 MPa did not affect (P>0.05) the bacterial flora (PPC, LAB, CC) of ground beef samples when compared to untreated control samples. During the 5 d storage study, no difference (P>0.05) in the psychrotrophic, lactic or coliform levels was observed between fresh ground beef submitted to EHSW processing (215 MPa) and fresh ground beef not submitted to EHSW processing after the initial testing date (date 0). At each testing day during the five day refrigerated storage study, no difference (P>0.05) was observed between those fresh ground beef samples submitted to EHSW processing (215 MPa) and those samples not submitted to EHSW processing.

An EHSW treatment of an estimated pressure of 350-541 MPa on the viability of a log culture of *L. innocua* did not affect (P>0.05) the levels of the marker organism when compared to untreated control samples. The same treatment did not produce a difference (P>0.05) in the psychrotrophic, lactic, or coliform populations of an aerated ground beef and water slurry. No difference (P>0.05) was observed between ground beef treated by EHSW (350-541 MPa) when compared to untreated ground beef.

DISCUSSION

The nature of the shock wave was altered by using two types of explosives to generate the EHSW, one binary explosive composed of liquid nitro methane and solid ammonium nitrite and one molecular explosive composed of PETN. Because of their composition, these explosives generate different products of chemical reaction, have different burn rates, and thus have different shattering effects (brisance) (13). Liquid nitro methane has a burn rate of 6,705 m/s (3,13). Neither the nitro methane nor the ammonium nitrate are explosive until mixed on site and detonated with an electric blasting cap connected to an electronic detonation bridge (12). Detonation of this binary explosive releases 25-30% of the energy of chemical reaction as a slow-moving shock wave and the remainder as a high energy gas bubble composed of CO_2, CO, NO_2, and H_2 (Kafakis, 2002: personal communication). PETN is a pre-manufactured commercially-available single molecule chemical explosive with a burn rate of 8, 260 m/s (3, Karfakis, 2002: personal communication; 13). Although detonation does produce a small high energy gas bubble, the shock wave preceding the gas release carries 55% or more of the energy of chemical reaction and causes most of the structural damage to the sample before the lower energy gas bubble reaches the sample. The shock wave produced is therefore mostly dependent on the rate of chemical reaction after detonation and should therefore produce a higher shock energy than the binary explosive discussed previously (Kafakis, 2002: personal communication). Thus the resultant shock waves the two explosives produce are different and were expected to exert different effects on the PPC, LAB, and CC of treated samples. It was believed that using the two different explosives to generate hydrodynamic shock waves would affect the bacterial flora differently. The researchers believed that a pressure front with more energy and a greater

magnitude would have a larger bactericidal effect than a smaller pressure front with less energy. This was not observed.

The movement of the HSW was controlled by changing the height of the water column above the explosive charge (15cm v. 7 cm). It was believed that altering the height of the water column above the explosive charge would increase the shattering effect of the shock waves and perhaps produce a bactericidal effect on the microbial flora.

The results indicate that EHSW processing does not affect the bacterial populations which are responsible for bacterial degradation during aerobic refrigerated storage (psychrotrophic, lactic, or coliform populations) in either fresh or ground beef aged for 48 hrs (5°C) prior to EHSW treatment. EHSW treatment did not affect the levels of surface bacteria in fresh whole eye of round steaks, nor did it exhibit any bacterial shelf life extension in treated fresh ground beef. The choice of explosive (whether a binary or molecular explosive) did not have an effect on the bacterial levels of ground beef, as neither molecular nor binary explosive treatments produced a difference in the bacterial levels of ground beef. It is possible that the parameters examined in these studies produced some level of sub-lethal injury to a portion of the bacterial cells on the surface of the ground beef sample, but that enough of the cells within the center of the package were not affected by the EHSW treatment. Under ideal conditions, as are found within an incubator set to the optimum growth temperatures of each type of microorganism examined, the doubling time for a population can be as little as 20-30 min (8). It is possible that enough cells were unaffected by the EHSW treatments that they outgrew the injured cells. The researchers attempted to confirm this during the present study with the multiple discharge testing at 116 and 170 MPa. No difference was observed between treated and non-treated samples after two subsequent EHSW treatments. Perhaps additional subsequent

treatments would have had a bactericidal effect. The packages did not retain structural integrity after two treatments, making it impossible to eliminate the effects of environmental contamination.

The results observed in this work were consistent with the work of Moeller et al. (17) who found that EHSW treatment of raw boneless pork loins did not affect the aerobic populations of treated samples. Neither the present work nor that of Moeller et al. (17) is consistent with the work of Williams-Campbell and Solomon (23,24). The researchers found that a single 69 MPa treatment reduced the bacterial content of beef and pork stew pieces as well as ground beef (23). They found this EHSW treatment of temperature abused (22 hrs at 23°C) beef and pork stew pieces produced a significant 1.7 log difference among APC (48 hr at 30°C) when compared to untreated controls. The researchers also noted a 3 log reduction in treated temperature abused ground beef when compared to untreated controls. An initial 1 and 2 log reduction in fresh ground beef and stew pieces as well as a 3 log reduction in treated ground beef stored at 5°C for 7 days was noted. The published are based on studies using small sample sizes (n=3, 5, or 10 for both EHSW and untreated controls), non-standard microbiological plating methods (48hr at 30°C, The Association of Official Analytical Chemists [AOAC], Food and Drug Administration [FDA] and the United States Department of Agriculture [USDA] list 35°C for 48 hr. as the incubation protocol for APC), and a lack of speculation on the biological importance of their findings. When dealing with microorganisms capable of doubling in number every 20 min under ideal conditions, one must question the importance of reducing a microbiological population by 1 log when the population has the ability to recover by a minimum of 1 log within 60 mins. (8). Williams-Campbell and Solomon (24) found the process (100g binary explosive, 30.5 cm from the sample) produced a 4.5 log difference in the aerobic

plate count (48 hr at 30°C) of treated fresh ground beef after 14 d of storage at 5°C when compared to untreated ground beef (4.5 log CFU/g vs. 9.0 log CFU/g). All ground beef samples were stored in a single layer of Cling Wrap (Glad; Danbury, CT; 100% polyethylene, oxygen permeability not specified but expected between 550-600 cc/24 hr) to simulate retail storage. Although the results of Williams-Campbell and Solomon (24) conflict with the data presented by Moeller et al. (17), one must keep in mind that the researchers appear to be sampling the mesophilic (30°C for 48 hr.) portion of the ground beef biota as opposed to the psychrotrophic biota sampled in that by Moeller et al. As with the 2001 studies, Williams-Campbell and Solomon (24) used a small sample size for their microbiological examinations (n=5).

Prior to the publication of research studies, Solomon et al. (21,22) suggested EHSW treatment may inactivate pathogens naturally present in whole muscle foods, making treated steaks a more bacteriologicaly safe product than untreated steaks. In a 1997 report, Berry et al. (2) reported finding a bacterial reduction in both pork and beef. This cannot be confirmed by their report since the researchers did not report detailed materials and methods in which they disclosed sample treatment before and after EHSW treatment. The 14% difference in microbiological levels the researchers reported may have been the result of a number of factors, including temperature abusing the control samples during the EHSW treatments (2). The results of Williams-Campbell and Solomon (23,24) provide the only supporting evidence.

Although EHSW tenderization was not designed as a treatment for ground beef, retail ground beef was chosen as the test sample for the majority of the present studies reported in this manuscript. Its uniformity and ease for sub-sampling would have allowed the researchers to observe any changes in the bacterial flora with ease. Since researchers have reported EHSW treatments produced uniform tenderization along the entirety of the whole muscle food (22), it

was important to determine whether the technique also affected the bacterial flora on both the surface and throughout the entirety of the sample. The effect on the surface bacteria of whole steaks was observed through surface swabs and the effect on the bacteria throughout the entirety of the sample was observed by sampling ground beef.

The results of the studies performed in this work show that EHSW processing does not affect ($P>0.05$) psychrotrophic, lactic, or coliform populations in treated ground beef or an aerated beef and water slurry. The same was observed in treated broth cultures of *L. innocua* ($P>0.05$). Although *L. monocytogenes* would not be expected to pose a bacteriological health hazard in ground beef, its non-pathogenic surrogate, *L. innocua*, was chosen as the marker organism in the study. It is a non-pathogenic microorganism which proliferates at psychrotrophic temperatures and it is an organism commonly used by food microbiology researchers. Its presence in the LH prototype would thus not pose a bacteriological hazard had package integrity been compromised during EHSW treatments.

In a study evaluating the effects of electrically-generated shock waves, Edebo and Selin (5) suggested that although hydrodynamic shock wave treatment would be expected to produce both a shearing stress and a squeezing effect on bacterial cells, a pressure of 500 MPa with a duration of 10 μs applied to an individual bacterial cell was distributed evenly along the length of the cell. The pressure difference created along the length of the cell would be expected to only reach 1/1000 of the total pressure applied, negligible compared to the estimated internal pressure of each bacterial cell (approx 40 MPa). Thus the lack of bactericidal activity observed in the present study is understandable. The pressure fronts achieved in this study would be expected to create a pressure difference of less than 0.001 MPa along the length of an individual bacterial cell.

The results show the treatments tested did not have a bactericidal effect, suggesting the parameters used in the technology thus far are not sufficient to reduce the number of bacteria present in a muscle food during treatment nor would they be sufficient to reduce the presence of pathogenic bacteria in a sample during treatment. Future research should focus on the effects of the technology on bacterial cells at the cellular level in order to determine whether ESHW produces any damage to the bacterial membrane or to ATPase systems as observed in hydrostatic treatment systems (20). Although the magnitudes of pressure (and exposure time to high pressure) in the present EHSW technology are much smaller than those observed in high pressure hydrostatic treatments, researchers have suggested that the dynamic movement of the pressure fronts in EHSW processing may exhibit similar bactericidal effects (23). The results of the present study suggest otherwise.

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Table 1. Explosively-generated hydrodynamic shock wave treatments used in the present study.

Magnitude Pressure Front (MPa)	Mass Explosive Charge (g)	Distance (cm) [a]	Sample Type	Sample No.
Binary explosive				
50	75	46	Ground beef	10
77	100	28	Ground beef	5
78	450	46	Ground beef	10
87 [b]	100	28	Ground beef	11
			Whole steaks	11
87 [c]	100	28	Ground beef	11
			Whole steaks	11
97	100	23	Ground beef	10
116	75	18	Ground beef	5
170	75	13	Ground beef	5
201	60	10	Ground beef	5
Molecular explosive				
215	40	4	Ground beef	30
251	60	10	Ground beef	5
350-	150	11	*Listeria innocua*	20
541			Ground beef	20
			Slurry	20

[a] distance between explosive charge and top surface of the packaged sample

[b] 15 cm water column above the explosive, 4 °C water in treatment chamber

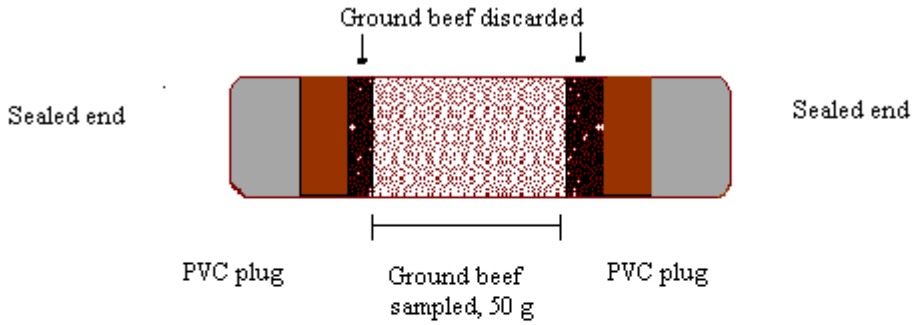
[c] 7 cm water column above the explosive, 4 °C water in treatment chamber

Fig. 1. Schematic representation of the TP package used in the current study.

Fig. 2. Diagram of Large Stainless Steel Hemi Shell prototype for explosively-generated hydrodynamic shock wave treatment of raw meats (EHSW). (*Refer to Figure 2, Chapter 2, p 18*).

Fig. 3. Diagram of Small Stainless Steel Tank prototype for explosively-generated hydrodynamic shock wave treatment of raw meats (EHSW). (*Refer to Figure 2, Chapter 2, p 18*).

Fig. 1 Top (↑)


Ground beef discarded
Sealed end
Sealed end
PVC plug
Ground beef sampled, 50 g
PVC plug