RESEARCH NOTE…

Penetration of Surface-inoculated Bacteria as a Result of Electrically-generated Hydrodynamic Shock Wave Treatment of Boneless Skinless Chicken Breasts

T. A. Lorca [*1], J. R. Claus [†], J. D. Eifert [*], J. E. Marcy [*] , and S. S. Sumner [*]

[*] *Department of Food Science and Technology, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, VA 24060;and* [†] *Meat Science & Muscle Biology Laboratory, University of Wisconsin-Madison, 1805 Linden Dr. West, Madison, WI 53706.*

[1] To whom correspondence should be addressed: Tatiana A. Lorca, Department of Food Science & Technology, Virginia Tech, Blacksburg, VA, 24060; Phone: 540-231-9518 and Fax: 540-231-9293; e-mail: t_lorca@hotmail.com

Processing

Abbreviation Key: GFP = Green Fluorescent Protein; Rif = Rifampicin; HVADH = hydrodynamic shock wave treatment; µs = microsecond

ABSTRACT

The top surface of boneless skinless chicken breasts was inoculated with either Green Fluorescent Protein labeled *Escherichia coli (E. coli*-GFP) or rifampicin-resistant *E. coli (E. coli*-rif) and subjected to electrically-generated hydrodynamic shock wave treatment (HVADH). Cryostat sampling in concert with laser scanning confocal microscopy or plating onto antibiotic selective agar was used to determine if HVADH treatment resulted in the movement of the inoculated bacteria from the outer inoculated surface to the interior of intact boneless skinless chicken breasts. In HVADH-treated boneless skinless chicken breasts, marker bacteria were detected within the first 200 µm below the inoculated surface, 50-100 µm beyond the depth of untreated surface inoculated boneless skinless chicken breasts. The exact depth in which the marker bacteria were found was dependent on the cryostat sampling distance used. This suggests that HVADH treatments affect the movement of surface bacteria.

Keywords: Electrically-generated Hydrodynamic Shock Wave; Bacterial penetration; Chicken; Research note

INTRODUCTION

Consumer studies have shown that broiler breasts which are not subjected to a 4-7 hour postmortem mechanical restraint (aging) are unacceptably tough (Lyon and Lyon, 1990). Researchers have explored the use of many technologies for improving tenderness in early deboned chicken breasts, including marination, electrical stimulation, wing restraints, hydrostatic pressure, and hydrodynamic shock wave processing (HSW) (Meek et al, 2000; Sams *et al.*, 1989; Young and Lyon, 1997). In EHSW processing, an explosively-generated hydrodynamic shock wave (of microsecond duration) travels through a vacuum packaged raw muscle food submerged in water-filled treatment chamber (Long, 2000; Meek et al, 2000). 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 (Long, 2000). Investigators have demonstrated the ability of HSW treatment to improve tenderness in poultry and reduce broiler aging time. A 28.3 % reduction in Warner-Brazler shear (WBS) was found when early deboned broiler breasts (removed 45 min. post-mortem followed by 24 h storage at 4˚C) were subjected to HSW treatment (159 MPa) (Meek et al, 2000). Electrically-generated hydrodynamic shock wave processing (HVADH) is a novel application of existing HVAD processing to the tenderization of boneless skinless raw muscle foods (Long, 2000). Barbosa-Canovas *et al.,* (2000) suggest the nature of the foodstuff and the treatment parameters play a key role in whether the process yields bacterial reduction. Complex food matrices such as slurries with particulates or media with a high fat or high protein content may offer a protective effect on the biota within the food. Lorca *et al.* (2002a) found HVADH processing had no significant

reductive effect on the bacterial flora of ground beef or whole steaks, though the researchers did not investigate possible induced migration issues.

The bulk muscle (excluding glandular tissue) in healthy meat animals is essentially sterile prior to slaughter (Niven, 1987). Contamination of outer surfaces occurs during processing, where blades, knives, or gloves may cross-contaminate the cut surfaces with gut or environmental flora while leaving the interior of the chicken breasts essentially sterile. Lorca *et al.* (2002b) reported minimal bacterial penetration occurred as a result of HSW processing of intact beef eye of round steaks. Like HSW, HVADH treatment destroys portions of muscle fibers via penetration of a large force from the outer surface into the muscle, therefore HVADH treatment may produce a non-intact boneless skinless chicken breast. Typical chicken breast cookery techniques which call for cooking to an internal temperature of 77˚C or until juices run clear are sufficient to inactivate pathogens of concern that may be on the surface of the breasts, such as salmonellae (Anon., 1995b). The concern with HVADH induced bacterial dislocation involves undercooking of treated breasts. If the treated breasts are undercooked (internal temperatures do not reach 77˚C), the internal temperatures achieved may not be sufficient to inactivate surface pathogens which may now be embedded and protected within the interior of the breast. This may create a microbiological safety hazard in boneless skinless chicken breasts tenderized by HVADH.

This study was performed in order to determine whether HVADH processing induced the bacterial dislocation of surface-inoculated bacteria deep into the breast tissue. If bacterial

migration occurred, undercooked HVADH-treated boneless skinless chicken breasts may harbor pathogens now protected by the bulk of the chicken breast from thermal inactivation.

MATERIALS AND METHODS

Preparation of marker microorganisms

Two strains of *Escherichia coli* were modified to express Green Fluorescent Protein (*E. coli*–GFP) or resistance to the antibiotic rifampicin (*E. coli*–Rif) as previously described (Lorca *et al.*, 2002b). The modified strains were used to create stock cultures which were stored at -80°C until use (Lorca *et al.*, 2002b). Fourteen hours prior to use, stock cultures were thawed on ice. *E. coli*-GFP cultures were placed into 25 mL LBAI medium containing 100 ug/mL ampicillin[1] + 0.25mM Isopropyl-Beta-D-thiogalactopyranoside[2] (IPTG) and grown aerobically at 37°C in a shaking incubator[3] for 4 hr to log phase (Anon., 1995a, Lorca *et al.*, 2002b). A frozen culture aliquot of *E. coli*-Rif was cultivated in 25 mL Trypticase Soy Broth (TSB)[4] with 100ug/mL rifampicin[5] and grown to log phase at 37°C in a shaking incubator (Lorca *et al.*, 2002b). Logarithmic growth of inocula was confirmed previously in the laboratory using growth curve experiments (Lorca *et al.*, 2002b). A 0.1 mL volume of *E. coli*-GFP in LBAI was spread-plated onto individual LB agar plates containing 100ug/mL ampicillin and 0.25mM IPTG (LBAI, [Anon., 1995a]) and the plates were incubated for 9 hr at 37°C to allow time for visible colonies to grow on the plates, forming lush bacterial lawns. A 0.1 mL volume of *E. coli*-Rif was spread-plated onto individual TSA agar plates containing 100ug/mL rifampicin (TSA-Rif, [Kaspar and

---

1 Sigma, St. Louis, MO
2 Pittsburgh, PA
3 4 L, Precision Scientific, Chicago, IL
4 Becton Dickinson, Cockeysville, MD
5 Sigma

Tamplin, 1993) and the plates were incubated for 9 hr at 37°C to allow time for visible colonies to grow on the plates, forming lush bacterial lawns.

Preparation of chicken samples

Fresh boneless skinless chicken breasts (approx 2.5 cm thick) purchased from a local grocery store were hand trimmed in the laboratory to form disks 6.4 cm in diameter and 2.54 cm in thickness, and then stored at 4°C for 24 hrs. Breast disks were randomly designated as "uninoculated control", "*E. coli*-GFP" or "*E. coli*-Rif" and treated accordingly. The top surface was inoculated by placing individual breast disks (fascia side down) onto a bacterial lawn of *E. coli*-GFP or *E. coli*-Rif in Petri dishes for 30 min. at room temperature. Inoculated breasts and uninoculated controls were individually vacuum-packaged[6] in reduced temperature shrink bags[7], with "top surface" and sample identity code (GFP, Rif, or uninoculated control) clearly marked on package. Packaged chicken breasts were dipped into 100°C water bath for 2-3 s, further vacuum packaged into individual bone-guard bags[8], dipped into 100°C water bath 3-5 s. The breast samples were transported in a Styrofoam™9 cooler on ice to the Canovanas, Puerto Rico Hydrodyne™ prototype[10] for HVADH treatment. Hot water dipping of packaged samples was performed in order to "shrink fit" the package to the chicken breast, reducing the void spaces between the chicken and the package which could contribute to failure of the packages during

6Ultravac model UV-250, Koch, Kansas City, MO
7 B-2651 Cryovac, Duncan, SC, 29334
8 B-2650, bone-guard, Cryovac, Duncan, SC, 29334
9 Dow Chemical Company, Midland, MI
10 Hydrodyne, Inc., Canovanas, PR

HVADH treatment. Double bagging of samples was performed to reduce the failure rate of the packages during HVADH treatment (Lorca *et al.*, 2002b).

HVADH treatment

Samples were treated in the HVADH unit (Fig. 1) of the Canovanas, Puerto Rico prototype (90% power [568 MPa]). The samples were removed from the treatment chamber, inspected for package integrity, frozen, and shipped via UPS for overnight delivery to the food microbiology laboratory at Virginia Tech for analysis. Unopened chicken breast samples were held at -20°C until sampling. All sampling was completed within 24 hrs of receipt to the laboratory. Thirty-two boneless skinless chicken breasts were treated per experiment (16 *E. coli*-Rif, 16*E. coli*-GFP), with each chicken breast receiving only one HVADH treatment (90% power, 11.8 kV). Those breasts not subjected to the Hydrodyne process were labeled inoculated controls and held on ice during HVADH treatment and then frozen with the HVADH treated breasts.

Sampling of frozen chicken breasts

Aseptic sampling of frozen chicken breasts followed previously published methods (Lorca *et al.*, 2002b). Frozen chicken breasts were aseptically removed from their packages under a laminar flow hood. The uninoculated side of each chicken breast was seared on an electric non-stick skillet preheated for 5 min to approx 200°C[11]. Searing for 2-3 s was done to achieve a cooked color appearance. Searing was performed to inactivate any bacteria that may

have been transferred from the "top" inoculated surface to the "bottom" surface during treatment. A nickel burnished steel cork borer[12] was flame-sterilized prior to use in removing core samples. The hot sterile cork borer was twisted and pushed from the seared uninoculated surface of the chicken breast to the inoculated surface in order to aseptically remove a core sample (ie., from the "clean" to "dirty" surface). The core sample was then pushed into the non-tapered end of a sterile 1000 ul pipette tip (approx 7.5 mm diameter) by aseptically and gently pushing the core through the borer with a sterile stick. Hot coring from the seared to the non-seared end ensured no bacteria were transmitted into the sample during the coring step. Five core samples were removed from each chicken breast and labeled. Cores were kept at -20°C until Cryostat sampling (approx 9 d).

Cryostat sampling of cores

Sampling of cores removed from frozen chicken breasts followed previously published methods (Lorca *et al.*, 2002b). The uninoculated end of each frozen core was mounted onto the center of a microtome die with fixing compound[13]. The inoculated end was exposed to be aseptically sliced and sampled at -20°C with a Cryostat[14]. Each core was sampled at 50 µm intervals and handled as previously reported (Lorca *et al.*, 2002b).

---

11 72020,West Bend,,27.9 cm
12 0.5 cm x 2.2 cm; d x l; Boekel, Fisher Scientific, Pittsburgh, PA
13 Tissuetek O.C. T. Compound, Miles Inc., Elkhart, IN)
14 Cryocut 1800, Reichert-Jung; Cambridge Instruments GMBH, Heidelberg, Germany; microtome blades Shandon Lipshaw High Profile Disposable blades No. 1001259, Pittsburgh, PA, autoclave sterilized

Laser scanning confocal microscopy examination

To determine the extent of Hydrodyne-assisted dislocation of bacterial cells into the HVADH-treated inoculated chicken breasts, *E. coli*-GFP HVADH-treated core slices, cores slices removed from *E.* coli-GFP non-HVADH treated controls, and uninoculated core slices were viewed by laser scanning confocal microscopy (LSCM)[15] as reported previously (Lorca *et al.*, 2002b). Slices from inoculated HVADH-treated chicken breasts in which GFP expressing bacteria were present beyond the maximum depth in the inoculated control chicken breasts were designated positive for Hydrodyne-assisted microbial penetration. Autofluorescence of the raw chicken during LSCM examination was reduced by utilizing a narrow band path filter[16], which allowed the photodetector to collect only the light emitted by GFP.

Microbiological examination

To determine the extent of Hydrodyne-assisted penetration of viable bacterial cells, *E. coli*-Rif slices were pour plated onto TSA-Rif, incubated aerobically for 48 h, and examined for growth of visible colonies as previously reported (Lorca *et al.*, 2002b). Sampling depths of 100 µm were designated positive upon visual confirmation of growth on TSA-Rif.

---

15 Zeiss LSM 510, Thornwood, NY
16 FITC, 488 nm

RESULTS & DISCUSSION

As previously reported, no differences were observed in the growth characteristics of the parental strain *E. coli* and the two genetically modified strains, *E. coli*-Rif and *E. coli*-GFP (Lorca *et al.*, 2002b). Graphs generated from growth data over a 24 hour period did not significantly differ among the parental or modified strains (Lorca *et al.*, 2002b).

The HVADH unit was composed of an electrode housing, sample treatment chamber area, and power supply (Fig 1). The muscle food sample does not come into direct contact with the electrodes or the high voltage arc, but rather sits on top of a food grade rubber diaphragm situated within close proximity to the electrodes. An inflated rubber inner tube lightly pressed the muscle food onto the diaphragm, holding it in place during the discharge. The sample was surrounded on the top and bottom with a food grade rubber material (rubber tube on top and diaphragm on the bottom) with the ability to transmit the shock wave directly to the boneless skinless raw meat (Long, 2000). In effect, this unit subjects a meat or poultry sample to an instantaneous shock wave produced by an underwater electrical discharge through an electrode gap from a capacitor bank (Long, 2000). One 2 μs long discharge of the HVADH unit produces both a positive and a negative pressure shock wave and emission of photon radiation within the treatment area (Long, 2000). Pressures achieved during HVADH treatment have been consistently measured and extrapolated for 80% and 90% power settings for the unit, 505 MPa and 568 MPa respectively (Thomsen, 2000: personal communication). Martin (1960) stated that below the surface of water, the discharge of high voltage across an electrode gap is similar op the

detonation of explosives, creating high pressures in the surrounding aqueous medium, generating high pressure shock waves.

HVADH treatment induced limited dislocation of *E. coli*-GFP from the outer inoculated surface towards the interior of the intact chicken breasts (Table 1). Penetration occurred only to a maximum depth of 250 µm from the outer surface. HVADH treatment increased the depth at which *E. coli*-GFP was found by 200 µm beyond the depth seen in untreated inoculated control samples. In slices removed from HVADH-treated chicken breast samples, the marker bacteria appeared as plump rods displaying a bright green fluorescence, contrasted with the light gray background of the unstained chicken tissue. No bright green fluorescent plump rods were observed in slices removed from uninoculated chicken breast samples nor in slices representing a depth beyond 250 µm towards the interior of HVADH-treated chicken breasts inoculated with *E. coli*-GFP.

E. coli-Rif marker bacteria also exhibited minimal dislocation from the outer inoculated surface towards the interior of the intact chicken breast as a consequence of HVADH treatment (Table 2). Visual examination of bacterial growth on TSA-Rif plates revealed viable *E. coli*-Rif cells within the first two 100 µm segments removed from HVADH-treated inoculated chicken breasts, corresponding to a depth of 200 µm from the outer surface. Viable *E. coli*-Rif cells were detected within the first 100 µm segment of non-HVADH treated inoculated control chicken breasts, most likely reflecting surface inoculum (*E. coli*-Rif) and minimal dislocation into the tissue. Bacterial growth was not detected in plates reflecting a depth beyond 200µm from the

outer surface towards the interior of HVADH treated chicken breasts nor was it detected in plates reflecting a distance beyond 100 μm from the outer surface towards the interior of the non-HVADH treated inoculated control chicken breasts. Slices removed from the uninoculated control samples failed to produce visible growth on TSA-Rif plates, as expected.

The extent of which bacterial dislocation occurred in the non-HVADH treated controls is consistent with the observations of Prachaiyo and McLandsborough (2000), who reported that most bacteria in intact beef (beef top round roasts) were observed between 0 – 30μm from the surface of the meat. The distance range found by the researchers was encompassed within the first slices obtained from all chicken samples, with the first slice representing a distance of 50 μm for those inoculated with *E. coli*-GFP and 100 μm for those inoculated with *E. coli*-Rif. Lorca *et al.* (2002b) also noted a similar dislocation of surface-inoculated bacteria in non-HSW treated beef eye of round steaks.

Green Fluorescent Protein (GFP) is a fluorescent protein with absorption and emission peaks 395 nm and 509 nm, respectively. It was originally extracted from the jellyfish *Aequorea Victoria* (Chalfie *et al.*, 1994). Food microbiology researchers have used bacterial strains genetically modified to express GFP in concert with LSCM to study the interaction of *E. coli* with foodstuffs at the structural level (Prachaiyo and McLandsborough, 2000). In our studies, linking LSCM and GFP proved to be a powerful technique, allowing for visualization of individual cells among structures of the chicken tissue.

Tenderization techniques which produce physical disruption of tissues have been shown to translocate surface bacteria to the interior of the muscle where the bulk of the muscle may protect bacteria from thermal inactivation during traditional cookery techniques. Phebus *et al.* (1999) suggested blade-tenderized beef posed no additional safety hazard to consumers, observing that although the technique transferred 3-4% of the artificially inoculated surface bacteria into the interior of the muscle, cooking the steaks in an oven broiler to a target internal temperature of as low as 54°C (rare) produced a 5.0 log reduction (CFU/g) in treated steaks as compared to a 5.6 log reduction (CFU/g) in non-blade tenderized beef steaks. In 1999, Moeller *et al.* (1999) found that HSW processing had no significant effect on the natural flora of pork loins, though the effect of the process on pathogenic bacteria has not been published to date. Lorca *et al.* (2002b) found HSW processing induced migration of surface inoculated bacteria to the interior of HSW treated beef steaks, noting that penetration occurred only to a depth of 300 µm from the surface and would not be likely to pose a significant threat to consumers if the steaks were cooked to achieve an external cooked color change.

HVADH treatment caused limited penetration of both marker bacteria into the boneless skinless chicken breasts to a maximum depth of 200 µm (0.2 mm) in all chicken breast samples. United States Department of Agriculture (USDA) guidelines suggest raw poultry be cooked to an internal temperature of 77° C to inactivate salmonellae, pathogens of concern in poultry (Anon., 1995b). By following this guideline during proper cooking, consumers achieve thermal inactivation of salmonellae. Since the movement of the marker bacterium observed the treated boneless skinless chicken breasts was minimal, proper cooking of the product would be expected

to inactivate vegetative bacterial cells at this depth. Therefore, HVADH treated boneless skinless chicken breasts would not be expected to pose a bacterial hazard if the product was properly cooked. The extent of penetration would not appear to pose a safety hazard to consumers unless the treated chicken breasts were undercooked.

ACKNOWLEDGEMENTS

The authors would like to thank Hydrodyne Inc., Kellogg, Brown & Root, and Dynawave Inc. for their support of this research. Special thanks to Dr. Kristi DeCourcy of the Fralin Biotechnology Center at Virginia Tech and Elise Shumsky of Carl Zeiss Inc. for their assistance with the LSCM and to Jill Songer of the Histopathology Laboratory at the Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA for her assistance with the cryostat. In addition, thanks extend to Brian Smith for his assistance in the food microbiology laboratory at Virginia Tech and to Dr. Kali Kniel for her assistance with GFP.

REFERENCES

Anon. 1995a. *Escherichia coli*, plasmids, and bacteriophages. In Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., and Struhl, K. *Short Protocols in Molecular Biology* (3rd ed. pp.1.1-1.26). USA: John Wiley & Sons, Inc.

Anon. 1995b. Use a meat thermometer and take the guesswork out of cooking. USDA.Food Safety Inspection Service Publication. Consumer Education and Information Pamphlet.

Barbosa-Canovas, G., Zhang, Q., Pierson, M., and Schaffner, D. 2000 High voltage arc discharge. In Kinetics of microbial inactivation for alternative food processing technologies. J. Food Sci. Suppl.: 80-81.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W., and Prasher, D. 1994. Green Fluorescent Protein as a marker for gene expression. Science. 263:802-804.

Kaspar, C. and Tamplin, M. 1993. Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. Appl. Environ. Microbiol. 59(8):2425-2429.

Long, J. 2000. Treatment of meat by capacitor discharge. United States Patent #6,120,818.

Lorca, T., Claus, J., Eifert, J., Marcy, J., and Sumner, S. 2002a. Effects of electrically-generated hydrodynamic shock waves on the microbial flora of ground beef. J. Food Sci. *In Review.*

Lorca, T., Pierson, M., Claus, J., Eifert, J., Marcy, J., and Sumner, S. 2002b. Penetration of surface-inoculated bacteria as a result of hydrodynamic shock wave treatment of beef steaks. J. Food Prot. 65:616-620.

Lyon, B. and Lyon, C. 1990. Texture profile of broiler pectoralis major as influenced by postmortem de-boning time and heat method. Poultry Sci. 69: 329-340.

Martin, E.A. 1960. Experimental investigation of a high-energy density, high pressure arc plasma. J. App. Phys. 31:255-267.

Meek, K., Claus, J., Duncan, S., Marriott, N., Solomon, M., Kathman, S., and Marini, M. 2000. Quality and sensory characteristics of selected post-rigor, early deboned broiler breast meat tenderized using hydrodynamic shock waves. Poultry Sci. 79:126-136

Moeller, S., Wulf, D., Meeker, D., Ndife, M., Sundararajan, N., and Solomon, M. 1999. Impact of the hydrodyne process on tenderness, microbial load, and sensory characteristics of pork longissimus muscle. J. Anim. Sci. 77:2119-2123.

Niven, C. 1987. Microbiology and parasitology of meat; part 1 - microbiology. In J.F. Price, & B.S. Schweigert, *The Science of Meat and Meat Products* (3rd ed., pp217-263). Connecticut: Food & Nutrition Press, Inc.

Phebus, R., Marsden, J., Thippareddi, H., Sporing, S., and Kastner, C. 1999. Blade tenderization and food safety. Recip. Meat Conf. Proc. 52:71-72.

Prachaiyo, P. and McLandsborough, L. 2000. Microscopic method to visualize *Escherichia coli* interaction with beef muscle. J. Food Prot. 63:427-433.

Sams, A., Janky, D., and Woodward, S. 1989. Tenderness and R-value changes in early harvested broiler breast tissue following post-mortem electrical stimulation. Poultry Sci. 68: 1232-1235.

Solomon, M. 1998. The Hydrodyne process for tenderizing meat. 51st Annu. Recip. Meat Conf. Proc. 51:171-176.

Solomon, M., Long, J., and Eastridge, J. 1997. The Hydrodyne: a new process to improve beef tenderness. J. Anim. Sci. 75:1534-1537.

Young, L. and Lyon, C. 1997. Effect of calcium marination on biochemical and textural properties of pre-rigor chicken breast meat. Poultry Sci. 76:197-201.

Table 1. High voltage arc discharge(HVADH) induced penetration of *E. coli*-GFP cells in boneless skinless chicken breasts.

Chicken breast Sample	Percentage of samples found positive within each depth from the surface to the interior depth of the chicken breast (µm) [a]						
	0 - 50	50 - 100	100 - 150	150 - 200	200 - 250	250 - 300	300 - 350 [c]
HVADH-treated (n=80)	100	100	100	89	71 [b]	0 [b]	0 [b]
Control (n=40)	90[b]	0 [b]	0 [b]	0 [b]	0 [b]	0 [b]	0 [b]

[a] Depth measured in µm from the surface to the interior of the chicken breast

[b] No *E.* coli-GFP was detected in any slices beyond this depth

Table 2. High voltage arc discharge (HVADH) induced penetration of *E. coli*-Rif cells in intact boneless skinless chicken breasts.

Chicken breast Sample	Percentage of samples found positive within each depth from the surface to the interior depth of the chicken breast (µm) [a]			
	0 - 100	100 - 200	200 - 300	300 – 400
HVADH-treated (n=80)	100	90 [b]	0 [b]	0 [b]
Control (n=40)	100 [b]	0 [b]	0 [b]	0 [b]

[a] Depth measured in µm from the surface to the interior of the chicken breast

[b]No *E.* coli-GFP was detected in any slices beyond this depth

Figure 1. Diagram of electrically-generated hydrodynamic shock wave processing prototype (HAVDH) used for tendering boneless skinless whole muscle foods