Effects of electrically-generated hydrodynamic shock waves on the microbial flora of ground beef

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HVADH on ground beef microflora…

Abstract

The affects of electrically-generated hydrodynamic shock wave treatment (High Voltage Arc Discharge Hydrodyne [HVADH]), on the psychrotrophic, coliform, and *Escherichia coli* populations of ground beef were examined. No difference (P>0.05) was observed among stated populations between HVADH treated (505 MPa, 568 MPa) and untreated samples. No difference (P>0.05) was found between the two HVADH treatments for any microbiological parameter. Results suggest HVADH does not affect the microflora of ground beef and may not affect pathogens commonly associated this product such as *E. coli*. The technique is not a suitable non-thermal treatment to reduce the bacterial load of ground beef nor as a method to reduce levels of *E. coli* 0157:H7.

Keywords: High Voltage Arc Discharge, beef, bacteria, Hydrodyne

Introduction

Investigators have demonstrated the ability of explosively-generated hydrodynamic shock wave (EHSW) treatment treatments to improve tenderness in raw poultry, beef, lamb, and pork (Meek et al., 2000; Moeller et al., 1999; Solomon, 1998). This technology is being investigated a non-thermal tenderization alternative to such treatments as electrical stimulation, chemical enzyme treatments, or mechanical severance. 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). 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 (Kolsky, 1963; Long, 2000). While the tenderization effects have been studied extensively, the bacterial implications of EHSW processing are not yet clear. Because EHSW processing has been shown to tenderize whole muscle foods uniformly and throughout the entirety of the sample (Zuckerman and Solomon, 1998), the same shock waves that produce the tenderization may produce shearing stress on bacteria and have a bactericidal effect. If that 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 offer ground beef with a reduced level of potentially pathogenic bacteria (Williams-Campbell and Solomon, 2001; 2002). Researchers have shown the technique (46 MPa) effectively reduces ($P<0.05$) levels of the parasite *Trichinella spiralis* in infected pork loins, although the treatment had no affect on the infectivity of the parasites (Gamble et al., 1998). Moeller et al. (1999) found EHSW processing (69 MPa) had no effect ($P>0.05$) on either the aerobic (APC CFU/g, 25°C, 72 hr. incubation) or the coliform (MPN/g, 37°C, 48 hr. incubation) populations of pork loins, concluding EHSW processing did not affect the natural flora of pork loins. Lorca et al. (2002) found the treatment did not affect the bacterial flora of whole and ground beef samples utilizing pressures between 50 MPa – 251 MPa generated by either binary or molecular explosives. Neither the results of Lorca et al. (2002), nor those of Moeller et al. (1999) are consistent with those reported by Williams-Campbell and Solomon (2001; 2002), the only researchers to report that EHSW treatment produced a bactericidal effect. The researchers 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 noted approximately a 3 log reduction ($P<0.05$) in treated (with distance and explosive mass combinations producing pressures of 54 MPa, 62 MPa, and 70 MPa) temperature abused ground beef when compared to untreated controls. 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 latest evolution of EHSW processing (High Voltage Arc Discharge Hydrodyne [HVADH]) utilizes an electric discharge or series of discharges to create hydrodynamic shock waves to tenderize whole boneless skinless foods. Martin (1960) stated that below the surface of water, the discharge of high voltage across an electrode gap is similar to the detonation of explosives, generating high pressure shock waves by creating high pressures in the surrounding aqueous medium. As with EHSW processing, possible bacteriological effects of the HVADH system may be attributed to the nature and magnitude of the pressure wave produced.

Studies performed in the 1960’s with High Voltage Arc Discharge (HVAD) treatment chambers in which the foodstuff was placed into direct contact with the electrodes may add insight to bacteriological effects which may be observed in the electrically-generated hydrodynamic shock wave treatment (HVADH). HVAD processing achieved pasteurization of fluids by applying rapid discharge voltages through an electrode gap below the surface of an aqueous medium (Gilliland and Speck, 1967a,b). Researchers found that that pressure waves generated during HVAD treatment (40-250 MPa) had no bactericidal effect on their own, that microbial reductions observed in treated bacterial suspensions were attributed to the combination of pressure and the generation of chemical compounds within the medium during treatment, termed electrohydraulic shock (Edebo and Selin, 1968).

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. 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. 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).

There is no information available on the effects that hydrodynamic shock waves generated by electrical discharges have on the bacterial flora of whole raw muscle foods and raw ground beef. The following study was conducted in order to observe the affects of HVADH processing on the microbial flora of ground beef. Specifically, the researchers investigated the affect on the segments of the bacterial biota which would most likely contribute to the aerobic refrigerated bacterial degradation of ground beef, the psychrotrophic and coliform populations. The affects of the technology on the foodborne pathogen *Escherichia coli* was also investigated in order to observe whether the treatment might be effective as an alternative non-thermal treatment to irradiation in the production of ground beef with reduced levels of pathogenic *E. coli*.

Materials and Methods

Sample preparation

Fresh ground chuck beef (7.9 kg) was 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. Sixty g portions were packaged into tube packages (TP) with the aid of a Fatosa model E 251 commercial sausage stuffer (Koch Supplies, Inc., Kansas City, MO) as previously described (Lorca et al., 2002). Twenty five TP packages were randomly selected and designated for treatment at 80% power, 25 were randomly selected for treatment at 90 % power, and 25 were randomly selected to serve as non-treated control samples. All samples were held on ice until HVADH treatment within 48 hours of packaging.

HVADH treatment.

Samples were treated in the capacitor discharge unit of the Canovanas, Puerto Rico prototype (Hydrodyne Inc., Canovanas, PR) at 80 or 90% power (505 and 569 MPa respectively [Thomsen, 2000: personal communication]). The samples were removed from the treatment chamber, inspected for package integrity, and placed on ice until microbiological testing at the facility.

Microbiological sampling.

TP samples were aseptically sampled as described previously (Lorca et al., 2002), blended for 2 min with a model 4000 Stomacher Lab Blender (Tekmar, Cincinnati, OH), and serially diluted with 0.1 % peptone. Serial dilutions were plated in duplicate onto 3M Petrifilm: aerobic plates (3M Health Care Inc., St. Paul, MN), coliform plates (3M Health Care Inc.), and *Escherichia coli* plates (3M Health Care Inc.), and placed on ice in a small flexible insulated cooler for transport to the Food Microbiology Laboratory at Virginia Tech for incubation and analysis. Petrifilm plates were carried by hand during transport and not submitted to airport agricultural X-rays. Upon arrival at Virginia Tech within 24 hours of testing, the plates were incubated aerobically to enumerate psychrotrophs ([PPC] 72 hr. at 21˚C), coliforms ([CC] 48 hr. at 35˚C), and *Escherichia coli* ([EC] 48 hr. at 37˚C) respectively. Colonies were enumerated following published methods (Maturin and Peeler, 1998).

Statistical design and analysis.

The experiment was set up as a randomized complete block design, with 3 treatments (HVADH treatment at 505 MPa, 568 MPa, and no treatment [control]). 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 and Discussion

No difference ($P>0.05$) was observed in the psychrotrophic population between HVADH-treated samples and untreated controls (7.3 Log CFU/g ± 0.1 S.D.) for either treatment at 505 MPa (7.2 Log CFU/g ± 0.1) or 568 MPa (7.4 Log CFU/g ± 0.2). HVADH-treatment of 505 MPa did not yield a difference ($P>0.05$) in PPC levels when compared to samples treated at 568 MPa. No difference ($P>0.05$) was observed in the CC population between HVADH-treated samples and untreated controls (4.3 Log CFU/g ± 0.1 S.D.) for either treatment at 505 MPa (4.1 Log CFU/g ± 0.1) or 568 MPa (4.2 Log CFU/g ± 0.2). HVADH-treatment of 505 MPa did not yield a difference ($P>0.05$) in CC levels when compared to samples treated at 568 MPa. No difference ($P>0.05$) in the *E. coli* population was observed between HVADH-treated (505 MPa = 1.6 Log CFU/g ± 0.3; 568 MPa = 1.8 Log CFU/g ± 0.3) and untreated ground beef samples (1.7 Log CFU/g ± 0.1). Neither HVADH treatment produced a difference ($P>0.05$) in either the psychrotrophic or coliform count when compared to untreated controls. This was consistent with the work of Moeller et al. (1999) who found that hydrodynamic shock wave processing had no significant effect on the natural flora of pork loins. The researchers did not observe a difference ($P>0.05$) in the aerobic population (APC CFU/g, 25°C, 72 hr. aerobic incubation) nor in the coliform population (MPN/g, 37°C, 48 hr. aerobic incubation). The results are consistent with the work of Lorca et al. (2002) who found that explosively-generated EHSW treatments ranging from 50 MPa-215 MPa had no effect on psychrotrophs or coliforms. Neither result, nor the results of the present study, is consistent with the findings of Williams-Campbell and Solomon

(2001; 2002). Our studies suggest that HVADH processing as carried forth in the current prototype may not be adequate to alter the bacterial flora of ground beef.

In a study evaluating the effects of electrically-generated shock waves, Edebo *and* Selin (1968) 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. Gilliland and Speck (1967a,b) noted adding 0.05% bovine serum albumin to an aqueous suspension of *E. coli* (approx. 10^8 CFU/ml) reduced the bactericidal activity of HVAD treatments. Though the HVAD unit used by the researchers required direct contact between the electrodes and the food during treatment, perhaps the ground beef used in the current study produced the same reduced bactericidal effect.

Future research should explore whether multiple sequential HVADH treatments produce a bactericidal effect. This was attempted in the current study, but multiple discharges proved too destructive on the TP package. Upon the second HVADH treatment, the package integrity of the samples was lost and we therefore could not ensure bacterial contamination did not occur during treatment. Samples with a thickness smaller than the TP packages used in the present study could be tested, first ensuring that the thinner package holds its structural integrity during HVADH processing. A greater surface area would be exposed to the hydrodynamic shock wave and to the UV radiation emitted during the process, perhaps yielding bactericidal effects. The effects of HVADH treatment should be observed at the cellular level as well, to observe whether the process produces injury to the cellular membrane.

Acknowledgements

The authors would like to thank Hydrodyne Inc., Kellogg, Brown & Root, and Dynawave Inc. for their support of this research. Special thanks to Brian Smith for his assistance in the food microbiology laboratory at Virginia Tech.

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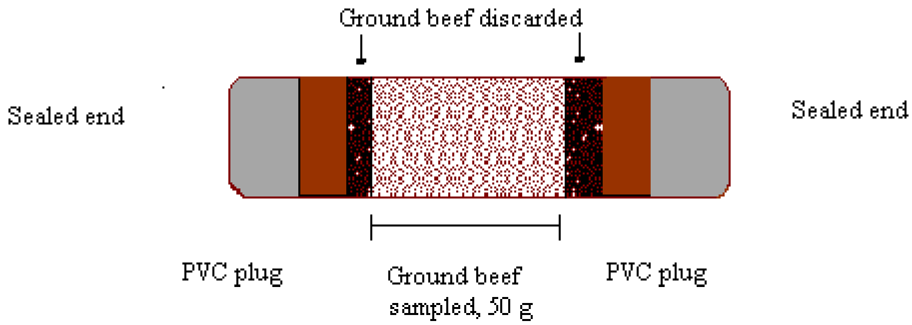
Figure 1. Schematic representation of the TP package used in HVADH processing

Figure 2. Diagram of electrically-generated hydrodynamic shock wave processing prototype (HAVDH) used for tendering boneless skinless whole muscle foods. (*Refer to Figure 3, Chapter 2 [Review of the Literature], p 20*).

Fig. 1.

Top


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