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The Efficacy of Ultrasound on The Inactivation of Shiga Toxin-Producing Cells of *Escherichia Coli* in Raw Beef Trim

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Abstract

Shiga toxin-producing Escherichia coli (STEC) has been recognized as a human pathogen since 1982. However, the non-O157 STEC strains have been implicated in many human illnesses and seem to cause disease as severe as the potent E. coli O157: H7. Hence, the objective of this study was to evaluate the effectiveness of organic acids such as lactic and citric acid combine containing surfactants such as sodium lauryl sulfate in combination with ultrasound treatment for the inactivation of E. coli O26:H11, O45:H2, O103:H2, O111: H-, O121:H19, O145: NM and O104:H4 on beef trim when compared to E. coli O157:H7. The beef trim was inoculated (ca. 8.0 log CFU/g) with a seven-strain cocktail of rifampicin-resistant STEC strains (STEC-7: O111: H, O45: H2, O103: H2, O104: H4, O121: H19, O145: NM and O26: H11) and O157: H7. Inoculated meat was portioned at 25 grams and stored at 4ºC for up to 8-18 h. Beef trim samples were subjected to the ultrasound treatment with operating frequency of 20 kHz at room temperature and resident times of 0, 10, and 15 min. Treated and untreated samples were stomached and plated on Sorbitol MacConkey agar with rifampicin (100 µg/ml) followed by incubation at 37ºC for 24 h, the treatments were conducted in three replications. The effect of the antimicrobial treatments of beef trim showed neither the O157: H7 and non-O157 STEC cocktail were significantly ($p > 0.05$) inactivated in the beef trim to an acceptable level. The effect of ultrasonic treatment and the combination of ultrasound and antimicrobial treatments did not show any significant ($p > 0.05$) reduction of the microbial populations to an appreciable level in beef trim. The impact of the ultrasound and antimicrobial was very low (< 1 log CFU/g) on the inactivation of microbes in beef trim.

Keywords: *STEC, beef trim, ultrasound, antimicrobial, cocktail.*

Introduction

In the United States, human consumption of food products contaminated with *Escherichia coli* O157:H7 and/or non-O157:H7 Shiga toxin-producing *E. coli* (STEC) results in many illnesses, hospitalizations, and deaths each year [1]. Although serotype O157H7 strains of STEC have been recognized as a human pathogen since 1982, the non-O157 STEC (O26, O103, O45, O111, O121, and O145) have more recently been implicated in human illnesses and are capable of causing disease as severe as that caused by *E. coli* O157:H7. The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) considers serotype O157:H7 and the above mentioned six non-O157 *E. coli* serogroups as adulterants in non-intact beef products, such as ground beef [2,3]. According to Gould et al. (2013) [4], outbreaks involving STEC have been linked to not-ready-to-eat (NRTE) and non-intact beef products and a variety of other food commodities.

The applications of thermal and non-thermal interventions have been previously evaluated regarding efficacy in reducing or controlling the risk of STEC populations in various foods, including beef products. Since no intervention system is 100% effective, a multiple-hurdle systems approach has gained popularity in the food industry [5]. Novel non-thermal technologies such as

ultrasound (US), ultraviolet (UV) light and high-pressure processing (HPP) technologies are regarded as alternatives to traditional thermal processing. These technologies inactivate microorganisms (up to 5-6 log reduction) at near-ambient temperatures without significant degradation of the food components and while preserving the sensory and nutritional quality of foods [6].

Ultrasound is a form of energy generated by sound waves with frequencies greater than the upper limit of the human hearing range (> 20 kHz) [7]. These low and high-energy ultrasonic systems are classified by their power (W), sound energy density (W/m^3) and sound intensity (W/m^2). Ultrasound energy is used in food processing, and it is classified as a "green" technology due to its limited or no negative effect on the environment, allowing the technology to be both energy efficient and sustainable [8]. The US technology is unique in that it has the ability to eliminate microorganisms and enzymes without destroying nutrients in foods, unlike thermal processing [9]. Microbial inactivation by US results from intracellular cavitation which causes the thinning of cell membranes and heating and production of free radicals, hence microbial inhibition [10,11]. The wave compression and rarefaction cycles of US energy generate a negative pressure, and cavitation bubbles are primarily formed causing the breaking of cell walls in decrease in the cell permeability. Many parameters, such as frequency and amplitude of ultrasound waves and the temperature and viscosity of the liquid medium, influence the degree of cavitation [12]. During the collapse of the cavitation bubbles, hydroxyl radicals are produced which recombine to form hydrogen peroxide; the latter or and molecular hydrogen, which have an antimicrobial effect due to the different mechanisms such as microstreaming that causes the thinning of the cell membrane and hence cause damage to the DNA [13].

Chemical treatments using organic acids such as lactic and citric acid represent another non-thermal process. Hence, these treatments are used to control microbial growth on high fat products (i.e. whole fatty carcasses). Since water is hydrophobic and a poor wetting agent, food grade surfactants can be used to increase the wettability and enhance the exposure of pathogens to the antimicrobial treatment [14]. Sodium lauryl sulfate (SLS) is a generally regarded as safe (GRAS) food additive in the concentration range of 10 to 5,000 ppm in animal fats, vegetable oils, fruit juices and beverages, gelatin, marshmallows and egg whites [15]. Dychdala (1983) [16] concluded that SLS causes membrane damage and protein denaturation of microbial cells when the pH of the solution is below 4.0. Tamblyn and Conner (1997) [17] also demonstrated that combining 125 ppm of SLS in 0.5% LA reduced the initial counts of *Salmonella Typhimurium* attached to broiler skins by 1.3 log CFU.

Although studies have been published on the effect of organic acid-surfactants on the inactivation of *E. coli* [18], the multiple-hurdle effect of organic acid, surfactant, and ultrasound has not been evaluated on different serotypes of STEC. Therefore, the objective of this work was to evaluate the effectiveness of organic acids in combination with surfactants and ultrasound treatment to inactivate STEC on beef trim.

Materials and Methods

Preparation of bacterial strains

The following eight rifampin (100 µg/mL; Sigma Chemical Company, St. Louis, MO) resistant-strains of Shiga toxin-producing *Escherichia coli* (STEC-8) were prepared and maintained as described by Porto-Fett et al. (2016): H30 (serotype O145:NM), and USDA-FSIS 011-82 (serotype O157:H7). The inoculum was prepared by taking an isolated colony of each STEC strain and transferring it to a separate test tube containing 10 mL of Brain Heart Infusion (BHI; Becton, Dickinson Company, Sparks, MD) broth that was subsequently incubated for ca. 20 ± 2h at 37°C. Next, the contents of each tube (10 ml) of the freshly-grown eight strains of STEC were combined (80 mL total). The single strain of *Escherichia coli* O157:H7 was prepared as described above by Porto-Fett et al. (2016) [19].

Inoculation of beef trim samples

Beef trim (93:7 percent lean: fat) was obtained from a local wholesale store in Huntsville, Alabama and screen for STEC that was not inoculated. Twenty-five grams of beef trim was used for each treatment tested (three replications per treatment). Samples were inoculated with both a non-O157H7 STEC cocktail and a single strain of O157:H7 for one min and transferred into a sterile filter stomacher bag. To allow for bacterial attachment, samples were stored at 4°C for 18 h.

Application of organic acids in combination with sodium lauryl sulfate

The inoculated beef trim samples were treated with either 2.4% lactic/citric acid (LA/CA Purac CL 21/80) (v/v), 0.5% sodium lauryl sulfate (SLS), or 2.4% LA/CA + 0.5% SLS blend (v/v). Using sterile tongs, samples were immersed in the appropriate treatment for either 10 or 15 min and then transferred to a sterile filtered stomacher bag for microbiological analysis. Controls consisted of inoculated samples that were dipped in water.

Application of ultrasound in combination with organic acids

Twenty-five gram samples were submerged in a sterile beaker that contained one of the following treatments: 2.4% lactic/citric acid (LA/CA) (v/v), 0.5% sodium lauryl sulfate (SLS), or 2.4% LA/CA + 0.5% SLS combined (v/v). The LA/CA blend was comprised of 21% LA and 80% CA. Following this, samples were treated with 20-kHz ultrasound (Misonix Sonicator 3000 Ultrasonix Cell Disruptor with Temperature Control, Vernon, Hills, IL). The system was operated in a continuous sonication mode at an operating frequency of 20 kHz and resident times of 10 or 15 min and power level of 5 (36 watts) or 10 (75 watts). Treatments that did not contain LA/CA were sonicated in deionized water.

Microbiological analysis

Twenty-five grams of beef trim, both inoculated (experimental) and non-inoculated (control) samples, were separately tested (three replications per treatment). After treatment, cells of STEC were recovered by adding each sample into a sterile filter bag with 60 mL of sterile 0.1% peptone water (Difco, Becton, Dickinson Co., Sparks, MD) and macerating for 2 min at 230 rpm in a stomacher (Stomacher 400, Seward, Cincinnati, OH). After stomaching, appropriate serial dilutions of the filtrate were prepared using 0.1% peptone water and 0.1 mL was surface plated in duplicate on Sorbitol-MacConkey (SMAC; Difco) agar plates plus rifampicin (100 µg/mL). Plates were incubated for 24 h at 37°C and surviving cells were enumerated. When testing negative for the pathogen by direct plating (≤ 0.40 log CFU/g), samples were enriched as described previously [20].

Statistical analysis

The data was analyzed using the ANOVA (analysis of variance) procedure SAS software (Version 9.3, SAS Institute Inc., Cary, NC). Duncan's Group Mean Comparison Test was used for the mean comparison of the treatments that were found to be significant. All experiments were replicated three times, and statistical tests were performed at 5% level of significance.

Results and Discussion

Effect of ultrasound and antimicrobials on survival of STEC on beef trim

The effect of organic acid/surfactant and ultrasound treatment to inactivate STEC were evaluated on beef trim inoculated with the non-O157 cocktail or single strain of O157:H7. The antimicrobial treatments for beef trim showed that neither *E. coli* O157:H7 or the non-O157 STEC cocktail were significantly ($p > 0.05$) inactivated with the organic acid/surfactant dip treatments or the ultrasound treatments. Although not significant, the variation of the ultrasonic power level showed 0.39- 0.41 log reduction. However, the periods of resident time during the US treatment produced a 0.22-0.59 log reduction, which yielded a significant effect ($p < 0.05$).

Harris et al. (2006) [21] reported that the treatment of beef trim with antimicrobial interventions using 2% and 4% acetic and lactic acids, reduced levels of *E. coli* O157:H7 and *Salmonella* Typhimurium on beef trim by 1.5 to 2.0 logs. In the same study, the authors reported that higher concentrations of organic acids did not have any added benefits over lower concentrations. The 2.4% concentration mixture of LA/CA with the STEC-7 cocktail resulted in a higher rate (0.03/min) of reduction while the SLS at 0.5% concentration and SLS/LA/CA combined resulted in a lower reduction rate at 0.02 and 0.19/min compared with the *E. coli* O157:H7 strain shown in Figure 1. Figures 2 and 3 shows the effect of ultrasonic holding times and power level on the inactivation of microbes in beef trim. *Escherichia coli* O157:H7 exhibited a higher rate of reduction when held for 15 min, whereas the non-O157 cocktail resulted in a high reduction rate at 10 min.

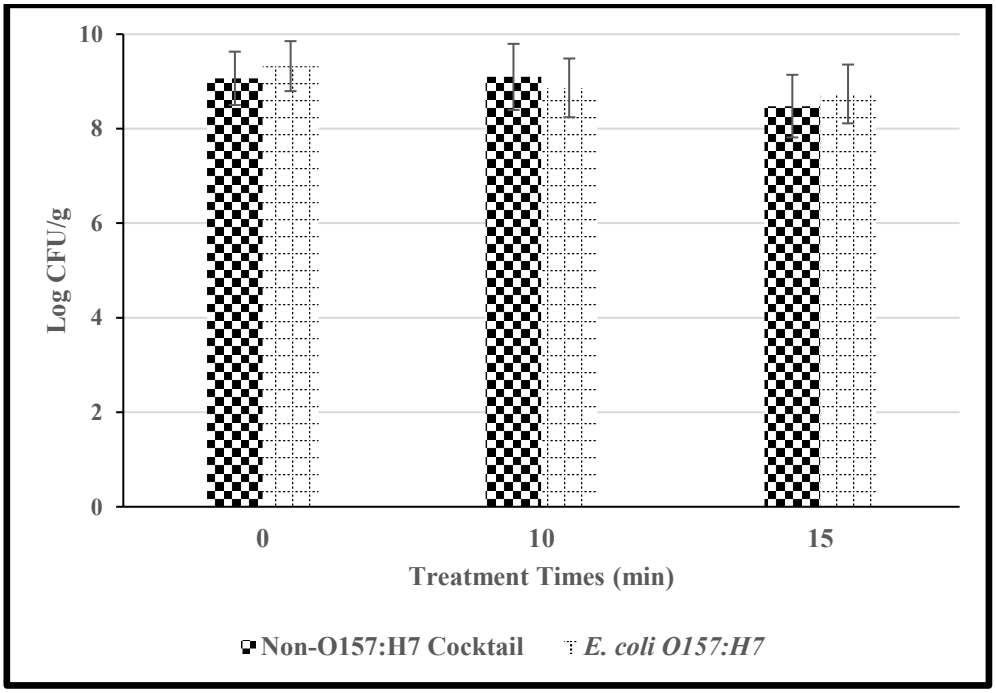

Figure 1: Survival of non-O157:H7 cocktail and *E. coil* O157:H7 on beef trim. The following treatments were applied: 2.4% lactic/citric acid (LA/CA), 0.5% sodium lauryl sulfate (SLS), 2.4% LA/CA+0.5% SLS, and inoculated untreated control.

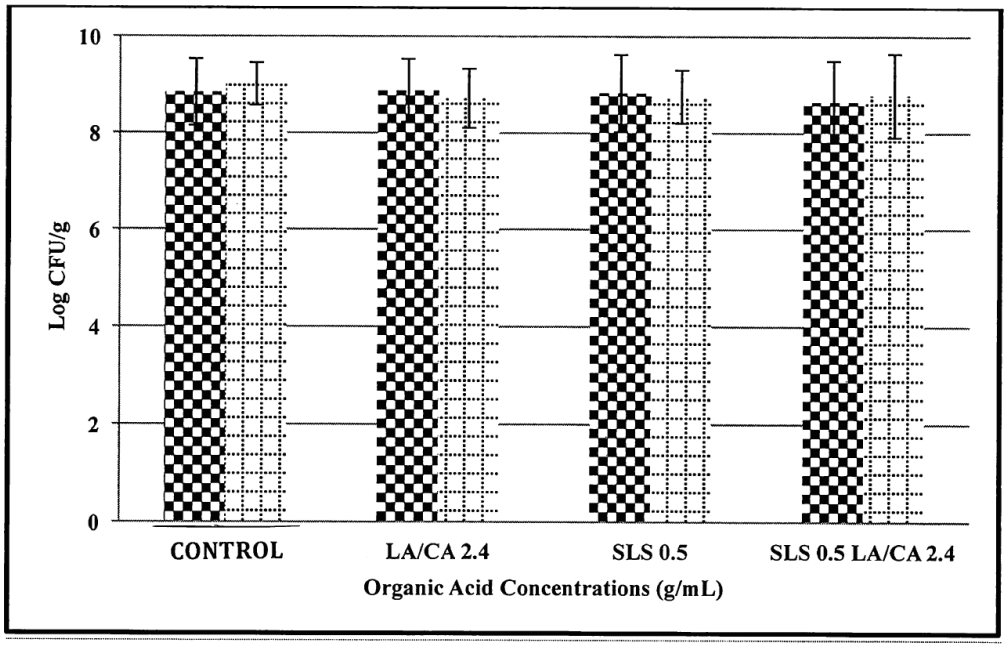

Figure 2: The effect of ultrasonic treatment on inactivation of non-O157:H7 cocktail and *E. coli* O157:H7 on beef trim.

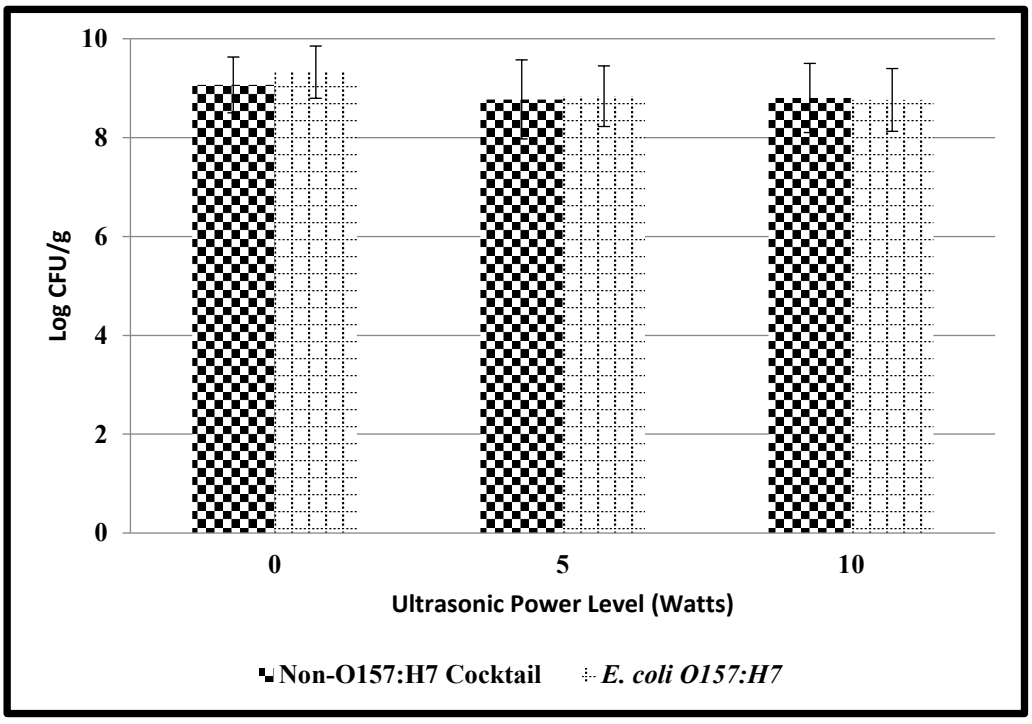

Figure 3: The effect of ultrasonic power level on inactivation of non-O157:H7 cocktail and *E. coli* O157:H7 on beef trim.

The antimicrobial treatments tested herein for beef trim showed neither the O157:H7 and non-O157 STEC cocktail were significantly ($p > 0.05$) inactivated in the beef trim. The impact of the ultrasound and antimicrobial treatment was very low (< 1 log CFU/g) on the inactivation of microbes in beef trim.

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