RESEARCH REPORT
PORK SAFETY

Title: Efficacy of ultrasonic technology to reduce pathogens associated with fresh and ready-to-eat pork products - **NPB #07-195**

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Industry Summary:

To eliminate occurrences of pathogens in fresh or further processed pork products, coupled with the increased consumer demand for fresh, minimally processed foods, non-thermal technologies for pathogen reduction, such as ultrasonics, have been emerging. It was hypothesized that ultrasound could be used to decontaminate surfaces of raw and ready-to-eat pork products in order to reduce contamination and improve the microbiological safety of the food supply. The objectives of the study were to apply ultrasound technology to reduce microbial loads, enhance product quality, and extend shelf life of fresh and ready-to-eat pork products and to determine consumer acceptability of ultrasound-treated pork products and feasibility of using the technology in commercial pork processing operations. After a series of experiments with ultrasound demonstrated significant reductions in pathogens when suspended in buffers, experiments were performed with experimentally inoculated and vacuum packaged chops and ham slices. Vacuum packaged meats were used in subsequent experiments to simulate commercially processed products and to prevent the spread/aerosolization of pathogens during experiments. For the latter experiments, several different contact and non-contact ultrasound systems were identified, constructed, and/or evaluated. Despite observations of ultrasound activity and surface changes to the fresh pork surfaces, it does not appear that either contact or non-contact ultrasound under the conditions examined in this project can penetrate the 2 mil vacuum packaging materials and reduce pathogens (*L. monocytogenes* and *S.* Typhimurium) associated with the surface of the ham steaks or pork chops. Based on these findings, other non-thermal technologies (e.g. irradiation) may be better suited for controlling pathogens associated with packaged ready-to-eat and fresh pork products than ultrasound under the conditions described. Since we were not able to afford any reduction in the pathogens using ultrasound, and therefore could not demonstrate efficacy, no consumer sensory studies were conducted in this study.

Scientific Abstract:

The objectives of the study were to apply ultrasound technology to reduce microbial loads, enhance product quality, and extend shelf life of fresh and ready-to-eat pork products and to determine consumer acceptability of ultrasound-treated pork products and feasibility of using the technology in commercial pork processing operations.

After a series of experiments with contact ultrasound demonstrated significant reductions in pathogens when

suspended in buffers, experiments were performed with experimentally inoculated and vacuum packaged chops and ham slices. For these experiments, several different contact and non-contact ultrasound systems were identified, constructed, and/or evaluated. Despite observations of cavitation (bubble formation) and surface changes (whitening) to the fresh pork surfaces, it does not appear that either contact or non-contact ultrasound can penetrate the 2 mil vacuum packaging materials and reduce pathogens (*L. monocytogenes* and *S.* Typhimurium) associated with the surface of the ham steaks or pork chops. As such, other non-thermal technologies should be considered for reducing pathogens associated with fresh or further processed, vacuum packaged pork products.

Introduction

The food industry has an obligation to the consumer to deliver a product, which is wholesome, free of pathogenic bacteria and of high quality with a reasonable shelf life. The Centers for Disease Control estimates that foodborne illnesses account for 325,000 serious illnesses resulting in hospitalizations, 76 million cases of gastrointestinal illnesses, and 5,000 deaths each year (Mead et al., 1999). While many of these cases are undoubtedly the result of improper handling and cooking by either food processors, food service operations, or the consumer, attempts should be made to reduce the presence of food borne microorganisms in raw foods of animal origin and throughout production of further processed meats (i.e. ready-to-eat).

According to USDA estimates, between 6 and 33 million cases of foodborne illness due to *Campylobacter* spp. occur annually in the USA (Buzby et al., 1997ab). According to the USDA-Economic Research Service, *C. jejuni* and *C. coli* cause approximately 1,375,000-1,750,000 cases of foodborne illness and 100-511 deaths annually in the USA (Allos, 1998, Buzby et al., 1997 ab). The most frequently identified organism associated with diarrheal infections in humans is *C. jejuni*. The closely related species *C. coli* is less frequently a cause of human disease. The relative frequencies of isolation of these two species from humans with disease have varied in different reports, but *C. coli* appears to represent between approximately 3% and 10% (Griffith and Park, 1990). Other species such as *C. lari* and *C. fetus* appear to cause human disease less frequently than *C. jejuni* or *C. coli*. These figures underestimate the true incidence of campylobacteriosis because most cases are relatively mild and are never seen in a clinic or hospital setting. Despite the low mortality rate associated with *Campylobacter* spp., the disease has a significant economic impact, with an estimated annual cost of $1 billion in the U.S. alone (Skirrow and Blaser, 1992). Given these figures, novel and inexpensive measures to control the pathogen in fresh meat and poultry products are warranted.

Salmonella spp. also have been implicated in pork-borne illnesses for several decades. To cause illness in humans, *Salmonella* cells must be ingested. Common symptoms that are associated with *Salmonella* infection include nausea, vomiting, diarrhea, and severe abdominal pain (Jay, 2000 or Jay et al. 2005?). In some salmonellosis cases, severe dehydration can lead to death of infected individuals. Among more than 2,300 *Salmonella* serotypes, *S.* Typhimurium and *S.* Enteritidis are estimated to cause 43.5% of the human cases of salmonellosis . Of these two species, *S.* Typhimurium poses the most critical hazard to red meat safety because it is the serotype most frequently isolated from beef animals (15.7%) and market hogs (20.4%; Sarwari et al., 2001). This pathogen frequently has been isolated from the fecal matter and rumen contents of livestock (Gay et al., 1994).

It is widely accepted that the contamination of red meat carcasses with *Salmonella* occurs via fecal contamination and that the severity of contamination is largely due to the extent of infection in the live animal

and the handling of carcasses by slaughter employees (Humphrey, 1997). Despite efforts to remove *Salmonella* from meat surfaces during slaughter, carcasses may still contain small populations, which may be embedded in surface tissues and crevices or deposited on surfaces during fabrication and further processing. An extensive study of retail meats from Washington, D. C. grocery stores demonstrated that *Salmonella* still is present on 3.3% of fresh pork and 1.9% of fresh beef (Zhao et al., 2001).

Listeria monocytogenes has been recognized as a foodborne pathogen with major public health consequences. *L. monocytogenes* causes listeriosis, an illness that is potentially life threatening. *L. monocytogenes* gained prominence in the 1980's after several large outbreaks in the United States, Canada, and Europe established conclusively that consumption of contaminated foods is the primary means by which this microorganism is transmitted to humans (Ryser and Marth, 2000). Listeriosis may occur more frequently in people considered to be at higher risk for the infection (i.e., the elderly, young children, pregnant women, and those who have a preexisting illness or condition that impairs their immune system). However, foodborne illness can also occur in healthy people, following consumption of a very high number of organisms. It has been estimated that *L. monocytogenes* results in approximately 2500 illnesses and over 500 deaths annually in the U. S. Of the foods implicated in outbreaks or recalls, FSIS has identified the pathogen in numerous ready-to-eat (RTE) meat products, including jerky, sliced ham, and luncheon meats, cured sausage, smoked sausage, ham, cooked poultry, souse, chicken salad, and fajita strips. It is also been demonstrated that the organism can grow to dangerous levels on the product during long term, refrigerated storage (USDA-FSIS, 2007).

USDA issued regulatory guidelines that require meat and poultry processors to control *Listeria monocytogenes* in ready-to-eat products using one of three strategies (USDA-FSIS, 2007). These three strategies require that processors apply a post-lethality treatment to the product, alone (Alternative 2) or in combination with an antimicrobial agent or process (Alternative 1), or rely upon sanitation (Alternative 3) to suppress or limit growth of the pathogen following cooking and before packaging. When compared to Alternative 1, Alternatives 2 and 3 may increase costs associated with sanitation, testing, holding, recalls and outbreaks. Some processors have reported that extensive microbiological testing and holding of product under Alternative 3 is a significant financial burden. Development and validation of an economically feasible Alternative 1 strategy may provide pork processors with the necessary means to reduce *L. monocytogenes* contamination of their RTE meat products without the added costs of extensive microbiological testing (USDA-FSIS, 2007).

Ultrasound technology is the use of sound waves that generate energy at a frequency that disrupts biological structures, resulting in death of microbial cells. As the energy is transmitted through liquids, compression and expansion occur, resulting in bubbles or cavities (Figure 1; Scherba et al., 1991). When bubbles collapse, high pressures (up to 100 MPa), temperatures (up to 5000 K) and shear forces are briefly formed which result in disruption in the integrity of the cell wall. In addition, free radicals and hydrogen peroxide are formed that may act as a microbiocides (Scherba et al., 1991). These effects can be either beneficial or detrimental to the properties of the product being treated. High power ultrasound has been researched as a means of reducing the number of microorganisms. Ultrasonic energy can be used either as a sole means of destruction or coupled with other treatments, such as lower temperature or antimicrobials, to reduce microorganisms.

Previous research has demonstrated that ultrasound can affect the viability of microorganisms in suspension or foods. Ultrasound treatment has been successful in achieving 2.5 and 4-log reductions of *Salmonella* spp. respectively, in 0.1% peptone water (100 W, 160 kHz, 10 min, 5°C) and 0.78 $\log_{10}$ in chocolate (100 W, 160 kHz, 30 min, 42°C; Lee et al., 1989). Factors that would affect the microbiocidal ability of ultrasonic energy would include: amplitude of the waves, type of microorganisms, exposure time, composition of the suspending medium, volume and temperature of the food. Research by Hoover et al. (2002) also has demonstrated 98.12% (161 kHz, 30 sec) to 99.9% (93 kHz, 30 and 60 sec) destruction of dried *Bacillus thuringiensis kurstaki* (Bt)

spores, a taxonomic relative of *B. anthracis*, following treatment with pulsed non-contact ultrasound. Treatment times longer than 60 sec did not improve the efficacy. SEM micrographs suggested an alteration in the physical and physiological nature of the spores (Hoover et al., 2002).

An ultrasonic transducer has been developed for application in foods (Doores, unpublished data). This transducer can be submerged into food solutions/slurries or applied in close proximity to a food surface. Laboratory data has demonstrated a consistent 4 $\log_{10}$ CFU/ml (99.99%) decrease of log-phase cells of non-pathogenic *E. coli* suspended in phosphate buffer or milk (whole or skim) when subjected to a pulse wave of 24 KHz for up to 2.5 minutes (Doores, unpublished data). These preliminary findings suggest that fat concentration does not appear to interfere with the ability of ultrasound to reduce bacteria in milk or buffer suspensions.

While the prevalence of pathogens in fresh and ready-to-eat pork products is low, there is still a need to develop effective, novel, and inexpensive non-thermal methods that can be employed by processors to improve the safety and stability of their products without compromising consumer acceptability. Ultrasonic or ultrasound technology has the potential to be employed by pork processors to address these issues.

Objectives:

1. To apply ultrasound technology to reduce microbial loads, enhance product quality, and extend shelf life of fresh and ready-to-eat pork products.
2. To determine consumer acceptability of ultrasound-treated pork products and feasibility of using the technology in commercial pork processing operations.

Materials and Methods:

Initial experiments determined the temperature profile of the ultrasound technology using a contact ultrasound system (**Experiment #1**; Figure 1) and how the temperature could be controlled most efficiently and effectively. Since use of the transducer may elevate the temperature of the surrounding liquid, it was necessary to determine if it was possible to conduct experiments at refrigeration temperatures. An experimental set-up was devised that employed the use of a temperature-controlled reaction beaker attached to a water bath that allowed a liquid (glycerol and water) to flow around the beaker and allowed for cooling of liquid materials (Figure 1). The experiment was performed for three different temperatures (-5, -16, -18.5 ºC) and ultrasonic power levels (60, 80, 100%).

Figure 1

In another set of experiments (**Experiment # 2**), the overall effectiveness of contact ultrasound using the system described in Experiment 1 was performed against three pathogens (*Campylobacter jejuni* [CJ], Salmonella *Typhimurium* [ST], *Listeria monocytogenes* [LM]) suspended in a buffered peptone water solution. Cultures of approximately 7 $\log_{10}$ CFU/mL were washed in buffered peptone water and treated with the ultrasound (24

kHz) for 0, 2, 4, 6, 8, and 10 minutes at 5ºC. Samples were enumerated on non-selective media to determine remaining bacterial populations.

Experiment #3 was conducted to determine the effects of contact ultrasound on a series of ground pork slurries. Unfortunately, the protein, connective tissue and fat interfered with the transducer such that the transducer stopped working. At this point, it was determined that the ultrasound system could not be used with unpackaged pork. Given this issue, Experiment 3 employed the use of sachets of *Salmonella* Typhimurium packaged into two different types of vacuum packaging materials, based on thickness (2 and 3 mil). For these experiments, the pathogen was grown overnight in tryptic soy broth, washed in BPW, and transferred to sachets of vacuum bags and sealed via a heat sealer. These sachets were then fixed to the bottom of the reaction beaker and 75 mL of water was placed in the beaker to cover the sachet. Experiments were conducted with similar conditions conducted in Experiment 2. Sachets were subjected to ultrasound (24 mHz) for 0, 2, 4, 6, 8, and 10 minutes and removed. Then using a sterile needle, 1mL of the solution was withdrawn from the sachet, serially diluted and remaining populations were enumerated as described above.

Experiment #4. In the next series of experiments, a different transducer system (non-contact) was employed. Using a prototype system developed for de-icing of airplane wings, we could detect sufficient ultrasound activity on meat surfaces. The non-contact ultrasound system was constructed with a signal generator that utilized piezoelectric ultrasonic transducers (Bellefonte, PA), which were made from flat, aluminum crystals (3" diameter, 0.1" thick; Figure 8). For these experiments, fresh pork chops and ham steaks were cut to 5 cm x 5 cm x 0.5 (~25 cm^2). To the cut meat, ~6 $\log_{10}$ CFU/cm^2 *Salmonella* Typhimurium or *Listeria monocytogenes* were inoculated and vacuum packaged (Sealed Air, Duncan, SC; 3 mil thick). Packaged meat was surface-treated with couplant (ultrasound gel) to enhance ultrasonic activity and subjected to different resonance frequencies (RF) as described below.

For **Experiment #4,** ultrasound at a RF of ~35 (38-39 kHz, 0.2-0.3 amp, 150 ohms, 24-25 watts) was applied to the inoculated, vacuum packaged meat surfaces for up to 60 seconds (Figure 9).

Figure 8. Piezoelectric ultrasonic transducers (crystals).

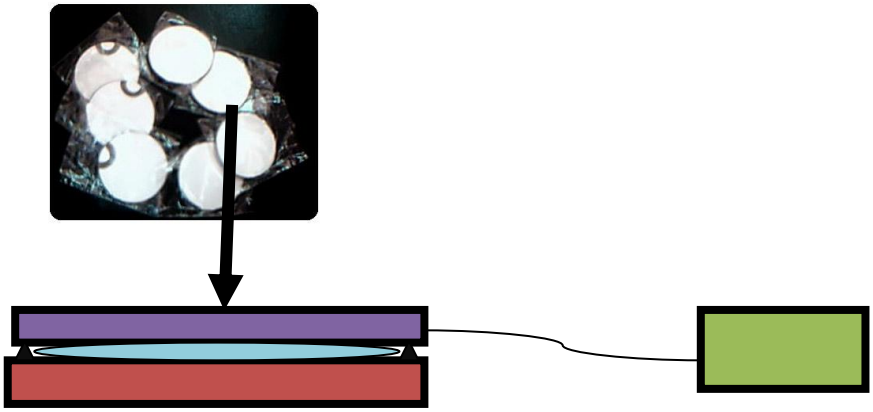


Figure 9. Set up for non-contact ultrasound.

Red=inoculated and vacuum packaged meat (pork chop/ham steak)
Blue=couplant (gel solution for transmitting ultrasonic waves)
Purple=crystal connected to signal generator
Black=separators
Green= signal generator

Experiments # 5 and #6: Using the same system as previously (Figure 9), non-contact ultrasound was conducted at a higher resonance frequency of 38 (~40-50 kHz, 0.5 amps, 150 ohms, 45-47 watts) and a longer time (up to 3 minutes).

In **Experiment #7,** a commercially available, ultrasonic jewelry cleaner was evaluated for reducing ~6 $\log_{10}$ CFU/cm^2 *Listeria monocytogenes* on ham steaks and ~6 $\log_{10}$ CFU/cm^2 *Salmonella* Typhimurium on fresh pork chops. For this experiment, experimentally inoculated (*S.* Typhimurium and *L. monocytogenes*), vacuum packaged ham steaks and pork chops were submerged in fresh, cold water, weighted and positioned above the transducers in the jewelry cleaner, and subjected to ultrasound for up to 3 minutes.

Experiment #8: Using the contact ultrasound system from Experiment #1, experimentally inoculated, pre-cut, vacuum packaged ham steaks and chops were subjected to ultrasound by vertically hanging the vacuum-packaged meat in a beaker of fresh, cold water where maximum cavitation (bubble bombardment) would occur (Figure 10). The horn was submerged approximately 5 cm below the water surface and 10 cm from the hanging vacuum packaged meat. Ultrasonic power at 100% was employed for up to 6 minutes at ~30C for Experiment #8 with fresh water changes between each sample.

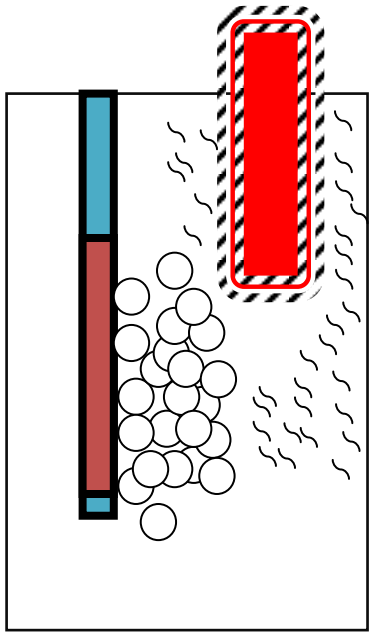


Figure 10. Set up for contact-ultrasound with meat; Red=ultrasound horn that generates energy→water movement (squiggles)→cavitation/bubbles (white circles)→ bubbles bombard surface of vacuum packaging bag; Turquoise=vacuum packaging bag (2 mil); Rose=inoculated meat (ham/chop)

With little to no success in reducing pathogens associated with vacuum-packaged pork using the other ultrasound systems, a custom-made, high-intensity, ultrasonic cavitation, resonant cavity was designed, fabricated (Figure 11), and evaluated.

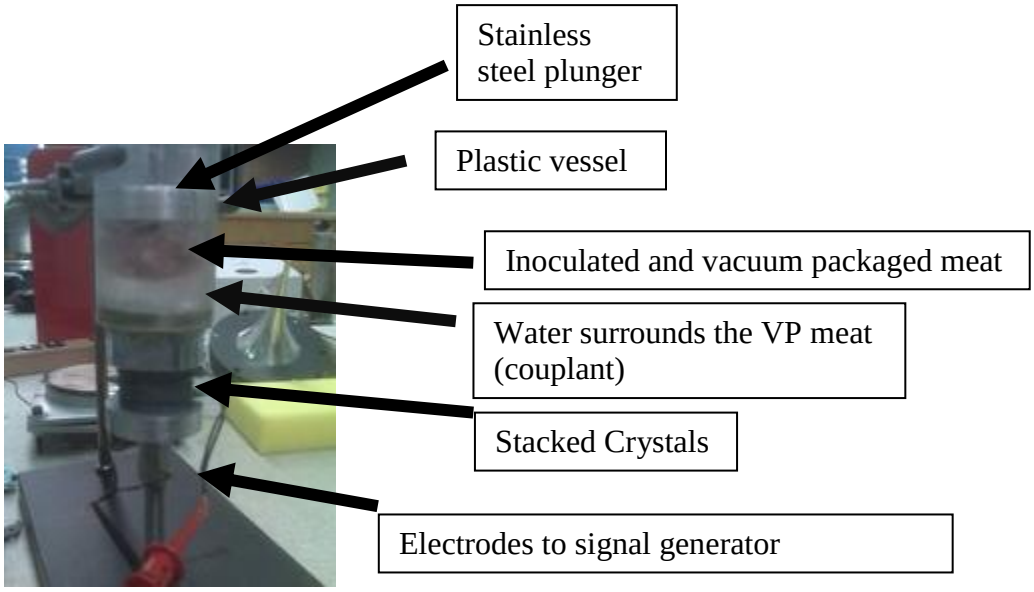


Figure 11. Custom-made ultrasound transducer for Experiment #9.

For **Experiment #9**, non-contact ultrasound was employed using the system described in Figure 11 for up to 3 min at 32 or 50 kHz (100% power) with experimentally inoculated, vacuum packaged (2 mil; Sealed Air, Duncan, SC) meat. In the low frequency (32 kHz) treatments, there were more visible bubbles than in the higher frequency (50 kHz) treatments. The few bubbles in the higher frequency ultrasound were larger. Most of the meat was between 1 cm and 2.5 cm from the bottom surface of the vessel and it was difficult to keep this distance consistent with every sample during treatments. After ultrasonic treatments, sample temperatures were elevated, so fresh cold water was added to every sample.

Results

Experiment #1. The results of the experiment indicated that by cooling as described, ultrasound experiments performed in the beaker at -16ºC and 100% power level did not elevate temperatures of liquids >25ºC after 60 minutes (Experiment 1; Figure 2).

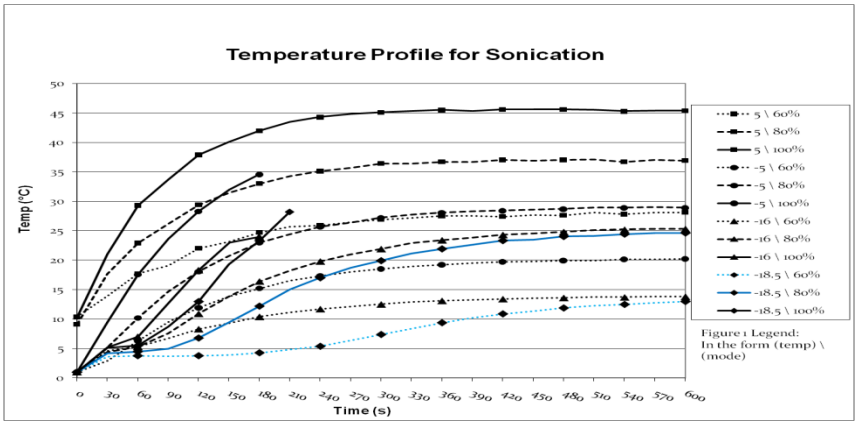
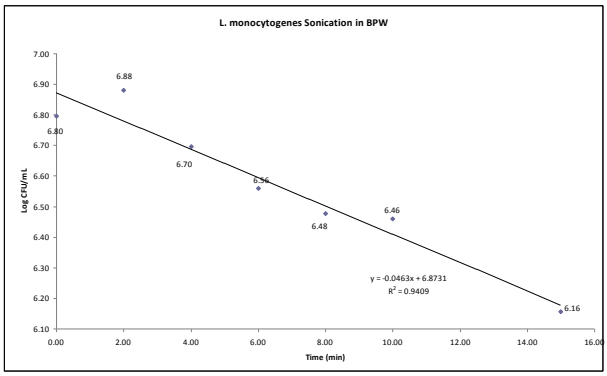
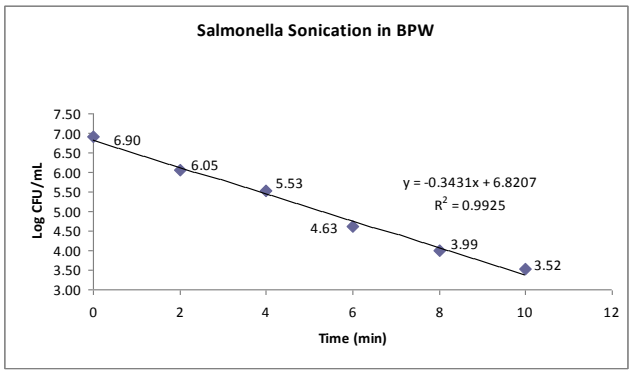


Figure 2: **Experiment #1.** Temperature profiles of distilled water following sonication under various chilling temperatures (-5, -16, -18.5 ºC) and ultrasonic power levels (60, 80, 100%).

Experiment #2. According to Figures 3, 4, 5, populations of pathogens were reduced significantly using the ultrasound system. D-values, under the described conditions, were calculated for each of the pathogens. D-

values are the time it takes to reduce the pathogen 90% (1 log). For these experiments, D-values were found to be 21.6 minutes for LM, 2.91 minutes for ST, and 2.43 minutes for CJ.





Figures 3 and 4: **Experiment #2.** Remaining populations of *Listeria monocytogenes* and *Salmonella* spp. following sonication in buffered peptone water (BPW). n=3.

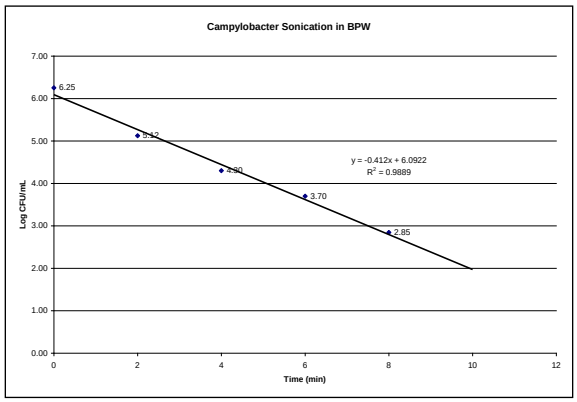
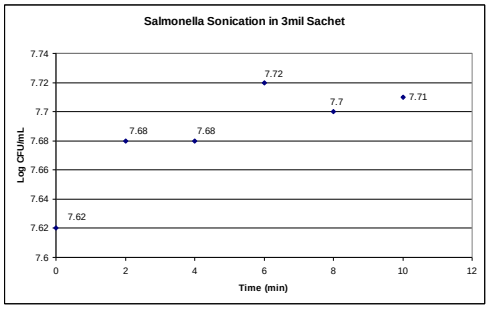
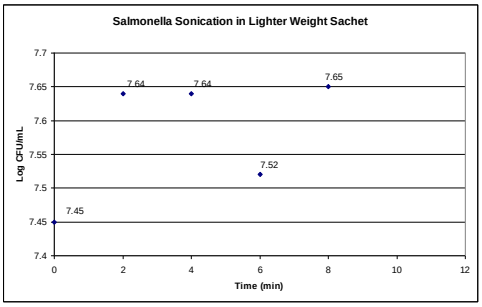


Figure 5: **Experiment # 2.** Remaining populations of *Campylobacter jejuni* following sonication in buffered peptone water (BPW). n=3.

Experiment #3. Figures 6 and 7 demonstrated that the pathogens were not reduced following ultrasound in the sachets. This experiment clearly demonstrates that ultrasound, under the conditions described, will not penetrate 3 mil vacuum packaging materials.





Figures 6 and 7: **Experiment 3.** Remaining populations of Salmonella *Typhimurium* suspended in buffered peptone water (BPW) and sonicated in a 3 and 2 mil sachet. n=3.

Experiment #4. Using a non-contact ultrasound system under the conditions described, no reductions in the pathogens were observed.

Experiments #5 and #6. No reductions in the pathogens were observed using the non-contact ultrasound system.

Experiment #7. For this experiment, visible bubbles were not observed but strong vibrations associated with ultrasound were detected. While no reductions in the pathogens were observed on treated samples, a slight discoloration/whitening of the fresh pork chop meat surface was observed on samples following treatment.

Experiment #8. In these experiments, large bubbles were observed moving and bombarding the surface of the vacuum packaged meat, especially at 4, 5, and 6 minutes. While no reductions in the pathogens were observed for any of the experimentally inoculated meat samples, a slight discoloration/whitening of the fresh pork chop meat surface was observed following treatments at 5 and 6 minutes.

Experiment #9. There was a visible increase in pores in the ham steaks following the 2-minute treatments at the lower frequency. The pork chops also exhibited more white spots after treatments. During processing for microbial analyses, it was noted that pork chops processed very easily (broke apart) indicating some type of tenderization may have occurred during ultrasound treatment. While there was a slight reduction (<0.5 $\log_{10}$ CFU/cm^2) in *S.* Typhimurium on fresh pork surfaces as compared to controls, it was not considered significant. No reduction was observed for *L. monocytogenes* following similar treatments.

Discussion

A series of experiments were conducted using contact and non-contact ultrasound systems. Despite observations of intense cavitation and surface changes to the fresh pork surfaces with a custom-made, high-intensity, ultrasonic cavitation, resonant cavity , we hypothesize that the ultrasound cannot penetrate the 2 mil vacuum packaging materials and reduce pathogens (*L. monocytogenes* and *S.* Typhimurium) associated with the surface of the ham steaks or pork chops. Given these findings, other interventions (i.e., irradiation) appear to be better suited for controlling pathogens associated with packaged ready-to-eat and fresh pork products than ultrasound under the conditions described. Unfortunately, we were not able to afford any reduction in the pathogens using ultrasound or determine if it could improve shelf life of pork products. Since we could not demonstrate efficacy, no consumer sensory studies were conducted in this study.

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