Journal of Food Research; Vol. 6, No. 3; 2017
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

The Relative Efficacy of Thermal and Acidification Stresses on the Survival of *E. coli* O157:H7, *Salmonella,* and *Listeria Monocytogenes* in Ground Beef

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Received: March 14, 2017 Accepted: April 8, 2017 Online Published: April 25, 2017

doi:10.5539/jfr.v6n3p74 URL: https://doi.org/10.5539/jfr.v6n3p74

Abstract

To provide additional insights on pathogen survival, we evaluated the relative efficacy of acidification (pH 2.7), thermophilic treatment (55 °C), and low temperature pasteurization (68 °C) on the inactivation of *E. coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* in ground beef. A series of experiment was conducted under biosafety level 3 environments for assessing the impacts of heat and low pH on pathogen survival. Results showed that 5-log reductions of *E. coli* O157:H7 could take more than 2640 min at 55 °C, 134 min at 68 °C and 120 min under pH 2.7. Compared to *E. coli* O157:H7, the 5-log reduction of *Salmonella* was obtained in 4836, 126, 86 min at 55 °C, 68 °C, and pH 2.7, respectively. The 5-log reduction of *Listeria* was achieved in 4704, 200, and 115 min under 55 °C, 68 °C, and pH 2.7, respectively. The results of this study will provide additional insights for developing improved methods for controlling pathogens in ground beef.

Keywords: foodborne pathogens, thermal inactivation, acidification, ground beef, public health

1. Introduction

The U.S. Centers for Disease Control and Prevention (CDC) estimates that each year 48 million people in the U.S. are sickened by foodborne diseases, which results in 128,000 hospitalizations, and 3,000 deaths (National Center for Emerging and Zoonotic Infectious Diseases, 2016). More than 10-80 billion USD are involved annually in dealing with foodborne outbreaks in the U.S. (Bavaro, 2012). Each year approximately 73,000—97,000 and 37,000—169,000 illnesses are linked with Shiga toxin-producing and non-Shiga toxin-producing *E. coli*, respectively (CDC, 2011; Luchansky et al., 2014; Scallan et al., 2011). In addition to *E. coli*, *Listeria monocytogenes* causes more than 255 deaths annually. Further, *Salmonella* spp. alone is responsible for more than 1 million illnesses annually (Scallan et al., 2011). A foodborne diseases active surveillance network study conducted in 10 U.S. sites reported that *Salmonella* was the most common infectious pathogen between 1996 and 2010 (CDC, 2011).

Over the past 30 years, many illnesses and at least six outbreaks were related to Shiga toxin-producing *E. coli* O157:H7 in beef products (Beauchamp & Sofos, 2010; Luchansky et al., 2014). After the 1992-1993 multistate outbreaks of *E. coli* O157:H7, the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) enforced zero tolerance for *E. coli* O157:H7 in ground beef and trim, which was followed by mandated Hazard Analysis and Critical Control Point (HACCP) regulation (Wheeler, Kalchayanand, & Bosilevac, 2014).

In addition to various *E. coli* serogroups such as O157 and non-O157 (O26, O123, O111, O145, O45), which are already added to the list of adulterates in beef, a debate is ongoing on how to include *Salmonella* (Wheeler et al., 2014) in this group. *Listeria monocytogenes* is known for its ability to grow at even refrigeration temperature, which results in significant food safety issue (Pal, Labuza, & Diez-Gonzalez, 2008; Rhoades, Duffy, & Koutsoumanis, 2009). The rate of fatalities during *Listeria monocytogenes* is relatively higher in comparison with *E. coli* and *Salmonella.* For example, 78 (11.35%) of the 687 fatalities between 1996 and 2000 in the England and Wales were related to *Listeria monocytogenes;* while *Listeria* was the agent in only 221 (0.01%) of

1.7 million reported cases of foodborne illness (Adak, Meakins, Yip, Lopman, & O'Brien, 2005; Rhoades et al., 2009).

Although considerable regulations are in place for reducing the risk of pathogens in beef products in the U.S. and outside of the U.S., the prevalence of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* are not uncommon. For instance, Rhoades et al. (2009) reported the mean prevalence of *E. coli* O157 in 44% hides, 0.3% chilled carcasses, and 1.2% raw beef products. The prevalence of *Salmonella* was reported in 60% hides, 1.3% chilled carcasses, and 3.8% raw beef products. The mean prevalence of *Listeria monocytogenes* was reported in 12% hides, and 10% raw beef products (Rhoades et al., 2009). Cattle hides are considered to be the main source of carcass contamination during cattle processing (Arthur et al., 2007). The author reported that the prevalence of *E. coli* O157:H7 in hides varied from 50 to 94%. Another study reported the prevalence of *E. coli* O157:H7 and *Salmonella* on 46.9% and 89.9% of cattle hides, respectively (Brichta-Harhay et al., 2008). On average 33.3% of cattle hide were contaminated by both pathogens. The prevalence of *Listeria monocytogenes* was reported in 10.8% of hide samples (Wieczorek, Dmowska, & Osek, 2012).

To control the pathogens in ground beef, methods including acidification, radio frequency, and thermal treatment are reported. The inactivation results of foodborne pathogens in ground beef by the energy of radio frequency indicated that the cooking of meatballs with the combination of radio frequency and convection cooking reduced the levels of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* to the non-detectable levels in 5.5 minutes (Schlisselberg et al., 2013). It has also been reported that the efficacy of treatments with regards to pathogen inactivation changes with products and strains (Vasan, Geier, Ingham, & Ingham, 2014). The authors found significant differences in decimal reduction of *E. coli* among various strains at 55 °C. The time for *Salmonella* decimal reduction was found to be significantly less than O157 and non-O157 Shiga toxigenic *E. coli* (Vasan et al., 2014).

A study focused on understanding of the fate of *Listeria monocytogenes* and *Salmonella* during preparation of ground beef jerky showed that the population of both the organisms was reduced by 4-3 orders of magnitudes, respectively, in 480 minutes of drying at 60 °C. The acid treatments for reducing *E. coli* O157:H7and *Listeria monocytogenes* levels in beef trim were found to be effective (Conner, Kotrola, Mikel, & Tamblyn, 1997). The authors reported that the use of 2 and 4% acid spray in beef trim stored at 4 °C reduced *Listeria monocytogenes* (initial concentrations 3 $\log_{10}$ CFU/g) by 0.36 $\log_{10}$ CFU/g and 0.44 $\log_{10}$ CFU/g, respectively. The reduction in *E. coli* O157:H7 was lower than *Listeria monocytogenes* under similar conditions (Conner et al., 1997).

Another study by Juneja (Juneja & Eblen, 1999) reported that lowering the pH increases the heat sensitivity of *Listeria monocytogenes* in beef slurry. Despite these numerous studies, which were executed in last decades, additional understanding is still needed to improve the inactivation of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ground beef, particularly, when ground beef is mixed continuously during cooking, and moisture content is high. Therefore, the goal of this study was to enhance the understanding of how acid and heat treatments influences *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inactivation in ground beef under high moisture and continuous mixing conditions. The specific objectives were to: 1) study the relative efficacy of thermophilic temperature (55 °C) on the inactivation of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*; 2) assess the inactivation of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* under low temperature pasteurization (68 °C) condition; and 3) determine the survival pattern of these pathogens under low pH (≈ 2.7) condition. Subsequently, models were developed to understand the relationships between time-temperature-pathogen inactivation and to predict the required time for pathogen reduction by 5-order of magnitude.

2. Materials and Methods

2.1 Beef Feedstock Preparation and Experiment Setup

The beef (chuck boneless eye steak) used for this experiment was purchased from the local grocery store (Walmart, Dixon, CA). The characteristics of beef (total nitrogen, protein, crude fat, and ash content) were analyzed (shown in Table 1). These parameters were analyzed at the University of California (UC) Davis Analytical Lab using the standard protocol (UC Davis Analytical Laboratory, 2015). Prior to starting the experiment, the beef was stored at -20 °C in the Extension Lab of School of Veterinary Medicine, University of California- Davis. To prepare the feedstock for the experiment, the beef was thawed with water, and subsequently, 200 g of beef was weighted and mixed with 800 ml of deionized (DI) water in the blending machine (Ninja Ultima Blender, Model BL800, 1500 Watt/ 2.5 peak HP motor). The experiment was conducted inside a biosafety cabinet (level III) to minimize the pathogen risks to the personnel involved in the experiment as well as possible ambient contamination. Two 1 L glass beakers were used as reactors for the experiment.

These two reactors were designated as two replicates (R1 and R2) for each set of experiment (i.e., thermophilic, low temperature pasteurization, and acidification). The glass beakers were placed in the water bath under exactly similar conditions (Figure 1). To control the temperature of the beef slurry in the two reactors used for digestion, the temperature of the water bath was regulated. The stirring of the beef slurry was performed using two overhead mixers (CAFRAMO, Model BDC250U1) in all the three stages. During the thermophilic and pasteurization processes, stirring speed was 50 RPM, and during the acidification process, the stirring speed was 200 RPM to create the uniformity in the temperature and digestion conditions of beef slurry emulsion. Stirring also created an aerated environment within the reactors.

Table 1. Properties of beef used for the experiment

Content	Mean ± Standard Deviation (%)
N (Total)	13.8 ± 0.1
Protein	86.0 ± 0.4
Crude Fat	14.3 ± 0.3
Ash	3.5 ± 0.0

NOTE: Protein results are based on the factor for alfalfa.

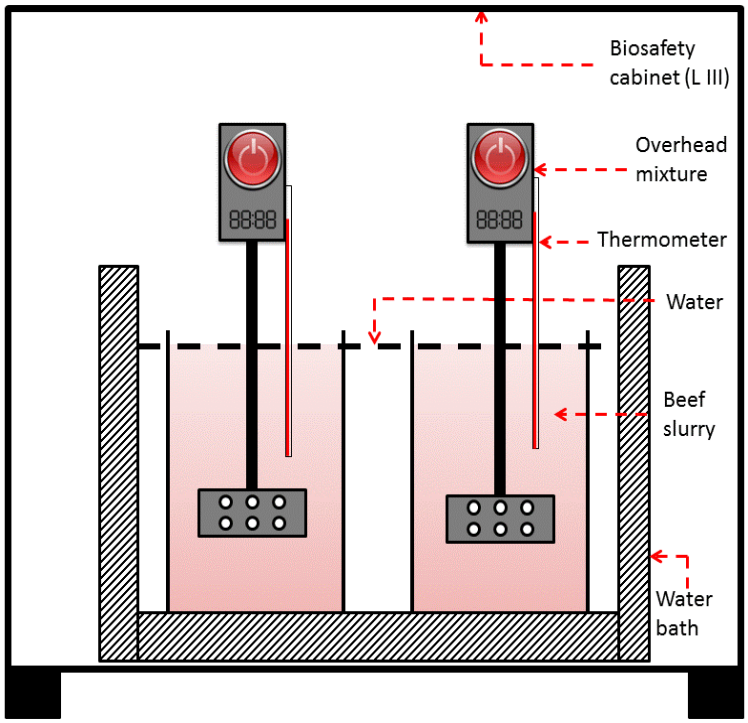

Figure 1. The experimental setup of water bath, beakers and overhead mixer inside the biosafety cabinet

Using the experiment setup shown in Figure 1, a series of experiments were conducted to test the inactivation of *E. coli* O157, *Salmonella Typhimurium LT2*, and *Listeria monocytogenes* under multiple incubation conditions. To test the impacts of heat stress, the incubations were performed at 55 °C (thermophilic) and 68 °C (low temperature pasteurization). For testing the impacts of acidification, the feedstock pH was lowered to 2.7 using phosphoric acid. The incubation at 55 °C was performed for 4 days (5640 min ≈ 94 h). The incubation under low temperature pasteurization condition (68 °C) was conducted for 210 min (3.5 h). The acidification experiment was executed for 2 h.

2.2 Pathogen Inoculation and Testing

To test pathogen inactivation, the beef slurry was inoculated with known concentrations of human pathogens (*E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium LT2, and *L. monocytogenes*) at room temperature. To prepare inoculum of these pathogens, the fresh pathogen cultures of *E. coli* O157:H7 (ATCC #35150), *S. typhimurium* LT2 (ATCC #700720), and *L. monocytogenes* (ATCC # BAA- 679D-5) were prepared in the lab prior to starting the experiment. Difco LB Broth Miller (Luria-Bertani) growth media was used for growing *E.*

coli O157:H7 and *S. typhimurium* LT2. BBL Brain Heart Infusion was used for growing *L. monocytogenes*. The overnight cell growths of these pathogens in respective media were used to form the pellets (by centrifugation) of each pathogen. A 4 ml volume of pure culture of each pathogen was pelletized using micro-centrifuge (Thermo Scientific, Sorvall Legend X1R, Langenselbold, Germany) at 8000 RPM for 10 min. Subsequently, the cell pellets were dissolved into the beef slurry, and mixed 10 min at room temperature.

The pathogen-inoculated beef slurry was digested under thermophilic and pasteurization temperature to understand the pathogen inactivation in ground beef. The acidification experiment was performed at room temperature (22 ^{0}C), while other two experiments at desired temperature (i.e., 55 and 68 °C) conditions. Samples were collected from the experimental digesters over the period of experiments, and pathogens levels were analyzed within 24 h of sample collection. Pathogens were enumerated using selective agar media following the FDA BAM method (Biswas, Pandey, & Farver, 2016; Pandey et al., 2016; USFDA, 2016).

For pathogen enumeration, the digested beef slurry samples were streaked (i.e., plated) on MacConkey II Agar (Becton, Dickinson and Company, Sparks, MD, USA), Difco XLD Agar (Becton, Dickinson and Company, Sparks, MD, USA), and PALCAM Agar (with selective supplement) (HiMedia Laboratory Pvt Ltd, Mumbai, India) plates for testing the concentrations of *E. coli* O157:H7, *S. typhimurium* LT2, and *L. monocytogenes*, respectively. Total number of samples collected in thermophilic, pasteurization, and acidification were 26, 20, and 18, respectively. All the samples were tested in duplicates. During the acidification study, food grade phosphoric acid was used to reduce the pH of beef slurry. The total of 5 ml phosphoric acid (H_3PO_4) was used for reducing the pH from 5.9 to 2.7 in each reactor. A pH meter (Omega Engineering, INC., Stamford, CT, USA) equipped with a pH and temperature probe was used to measure the pH and temperature during the experiment.

2.3 Data Analysis

Data analyses were performed in GraphPad Prism 6 and Microsoft Excel 2010 software. Descriptive statistics (average, standard deviation, minimum, maximum) were calculated in Excel. The function "analyze" of GraphPad Prism 6 was used to compare the slopes of linear curves of inactivation data of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. The linear regression and 95% confidence bands were used to compare the data. The linear regression equations of pathogen inactivation obtained from GraphPad Prism 6 (2016) were used to predict the time required for 5-log reduction of each pathogen.

3. Results and Discussion

3.1 Change in Temperature and pH During Experiment

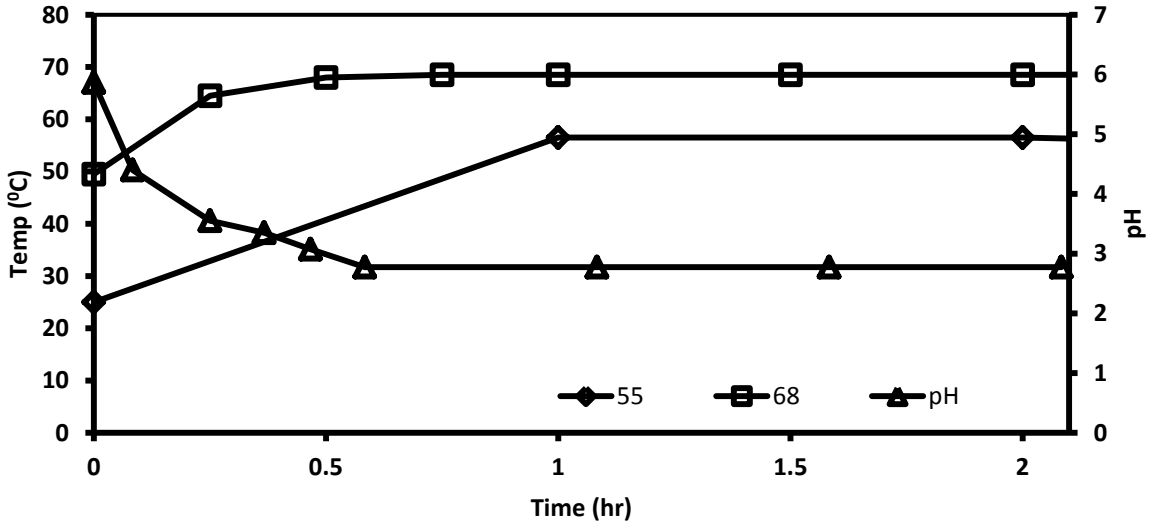

Figure 2. Change in temperature and pH at thermophilic, pasteurization and acidification experiments

Figure 2 shows the temperature profile during the thermophilic and pasteurization processes and change in pH of the acidification experiment in a single graph. For thermophilic process (55 ℃), it took 1 h to raise the slurry temperature to 55 ℃ from room temperature (25 ℃). During this process water bath temperature was maintained at 55 ℃. During pasteurization (68 ℃) experiment, the temperature of the beef slurry was increased

to 50 ℃ within short period after placing the beakers in water bath (maintained at 68 °C), the temperature increased from 50 ℃ to 68 ℃ in 30 min (Figure 2). During acidification process, the pH drop was relatively sudden after phosphoric acid was added to slurry.

3.2 Observations of Pathogens Reduction at Thermophilic Stage (55 ℃)

The changes in *E. coli* O157, *Salmonella*, and *Listeria monocytogenes* concentrations over time at 55 ℃ are shown in Figure 3. The total duration for the inactivation procedure was set 94 h. During the first 6 h of experiment, samples were collected hourly and then once daily for the next 3 days. The initial concentration of *E. coli* O157 was 9.6 $\log_{10}$ CFU/ ml. Subsequently, there was a sharp decline (4.2-log reduction) during the first 6 h of experiment. There was only 3-log reduction in next 50 h, which indicates that the majority of pathogens were reduced within first 6 h of experiment. There was no *E. coli* O157 positive sample in the beef slurry at the end of experiment.

The initial concentration of *Salmonella* in beef slurry was 10 $\log_{10}$ CFU/ ml. There was a 3.2-log reduction in the first 6 h. A total of 5.6-log reduction was achieved at the end of experiment. Among the pathogens, *Salmonella* survival was longer compared to the *E. coli* at 55 ℃. The initial concentration of *Listeria* was 9.3 $\log_{10}$ CFU/ml. There was a 3-log reduction in concentration of *Listeria* in beef slurry over the first 6 h of experiment and total 5.4 log reduction at the end of experiment.

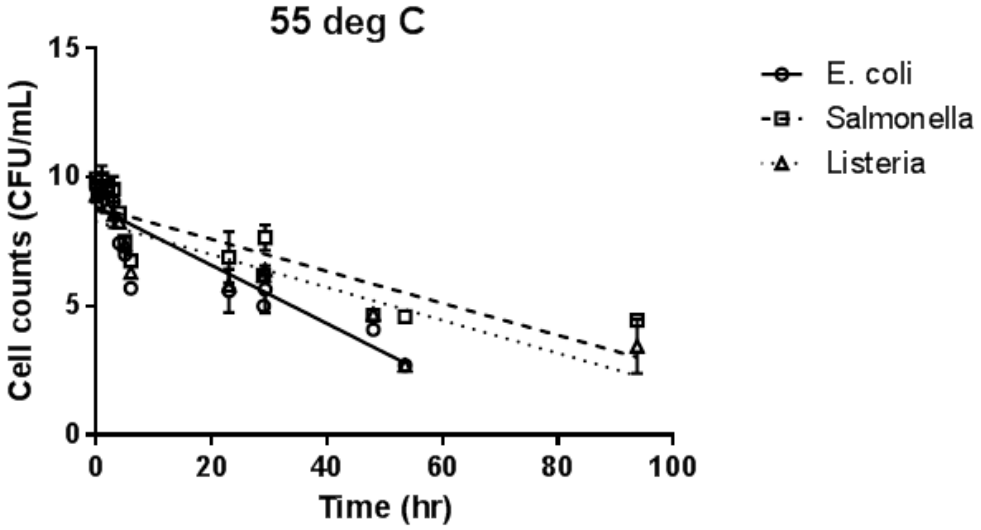

Figure 3. Change in concentrations of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* over time at 55 ℃

3.3 Observations of Pathogens Reduction in Pasteurization Temperature (68 ℃)

The change in *E.coli* O157, *Salmonella*, and *Listeria monocytogenes* concentrations at 68 ℃ is shown in Figure 4. At 68 ℃, the samples were collected at 15 min interval during the first hour of experiment and at 30 min interval beyond 60 min of digestions to the end of experiment. The reduction of *E.coli* O157 was gradual and went to undetectable level by the end of experiment in both the reactors. The starting concentration of *E. coli* O157 in beef slurry was 9.8 $\log_{10}$ CFU/ ml. There was a 5.4-log reduction during the first 90 min of experiment. The total duration of experiment was 3.5 h. The concentration of *E.coli* O157 was reduced to undetectable level after 3 h.

The initial concentration of *Salmonella* was 9.7 $\log_{10}$ CFU/ml. There was a 6.5-log reduction of *Salmonella* concentration at 68 ℃ during the first 90 min of experiment. *Salmonella* levels reached to undetected level at the end of experiment. The initial concentration of *Listeria* was 9.4 $\log_{10}$ CFU/ml. There was a 4.4 log reduction in *Listeria* concentration over the period of 90 min. At the end of the experiment, *Listeria* concentrations were undetectable in digested samples.

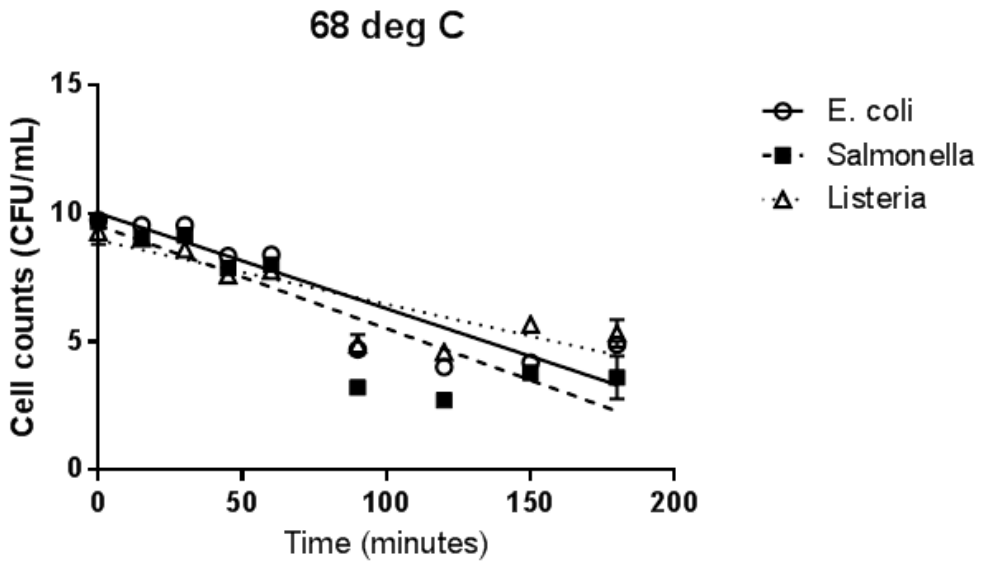

Figure 4. Change in concentrations of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* over time at 68 ℃

3.4 Observations of Pathogens Reduction in Acidification

The change in *E. coli* O157, *Salmonella*, and *Listeria monocytogenes* concentrations over the time is shown in Figure 5. During the acidification phase, we gradually added 1 ml of phosphoric acid (H_3PO_4) to observe the reduction of pH. The initial pH of beef slurry was 5.87 (Figure 2). The initial concentration of *E. coli* O157 was 9.4 $\log_{10}$ CFU/ ml. The results showed that by gradually adding 5 ml of phosphoric acid, the pH dropped to 2.77 in 30 min. A 5.7 log reduction of *E. coli* O157 was observed compared with the initial concentration. The initial concentration of *Salmonella* was 9.6 $\log_{10}$ CFU/ ml. *Salmonella* concentration was reduced by 6.9-log in 35 min after adding 5 ml of phosphoric acid. The initial concentration of *Listeria* was 8.6 $\log_{10}$ CFU/ ml, and 3.4-log reduction was observed after adding the 5 ml of phosphoric acid, which resulted in a drop of slurry pH to 3.1. During acidification, *Listeria* concentration was reduced by 4.9-log by the end of experiment.

3.5 Statistical Analysis and Prediction of Desired Time for 5-Log Reduction in Concentration

The concentrations of the three pathogens at each sampling event are reported as average concentrations of two samples obtained from the two reactors. The summary of the quantitative results of regression analysis is provided in Table 2. The results showed that *E. coli* O157:O7 will take relatively shorter time to inactivate at 55 ℃ compared to *Salmonella* and *Listeria monocytogenes*. The trend line slope was higher for *E. coli* O157:H7 (-0.11) compared to *Salmonella* (-0.06) and *Listeria* (-0.06). The regression analysis showed better r^2 value for *E. coli* O157:H7 (0.81) compared with *Salmonella* (0.71) and *Listeria* (0.75). There was a significant ($P<0.05$) difference in slopes between *E. coli* O157:H7 versus *Salmonella*, and *E. coli* O157:H7 versus *Listeria* (i.e., 95% confidence intervals did not overlap). However, there are no significant differences between *Salmonella* versus *Listeria monocytogenes* (confidence interval did overlap).

The summary of the best fit lines at the inactivation temperature of 68 ℃ are also provided in Table 2. The regression lines show that *Salmonella* takes relatively shorter time to inactivate at 68 ℃ compared with *E. coli* O157: H7 and *Listeria monocytogenes*. All the three pathogens seem to show similar inactivation patterns. The slope of the trend line was higher for *Salmonella* (-0.040) and *E. coli* O157:H7 *(-0.037)* compared with *Listeria (-0.025)*. The regression lines showed r^2 value of 0.84, 0.79, and 0.91 for *E. coli* O157:H7, *Salmonella*, and *Listeria*, respectively. There was no significant difference between slopes of *E. coli* O157:H7, *Salmonella*, and *Listeria*.

The results of regression analysis of acidification are provided in Table 2. The regression shows that *E. coli* O157:H7 takes relatively shorter time for inactivation during acidification compared with *Salmonella* and *Listeria monocytogenes*. The slope of the regression line was higher for *E. coli* O157:H7 (-0.089) compared to *Salmonella* (-0.058) and *Listeria* (-0.043). The regression fit lines showed r^2 values of *E. coli* O157:H7 (0.86), *Salmonella* (0.77), and *Listeria* (0.81). There was a significant (*P<0.05*) difference in slopes between *E. coli* and *Listeria*. There are no significant (*P<0.05*) differences between *Salmonella* and *Listeria*. Also there was no significant (*P<0.05*) differences between *E.coli* and *Salmonella* inactivation (confidence interval did overlap).

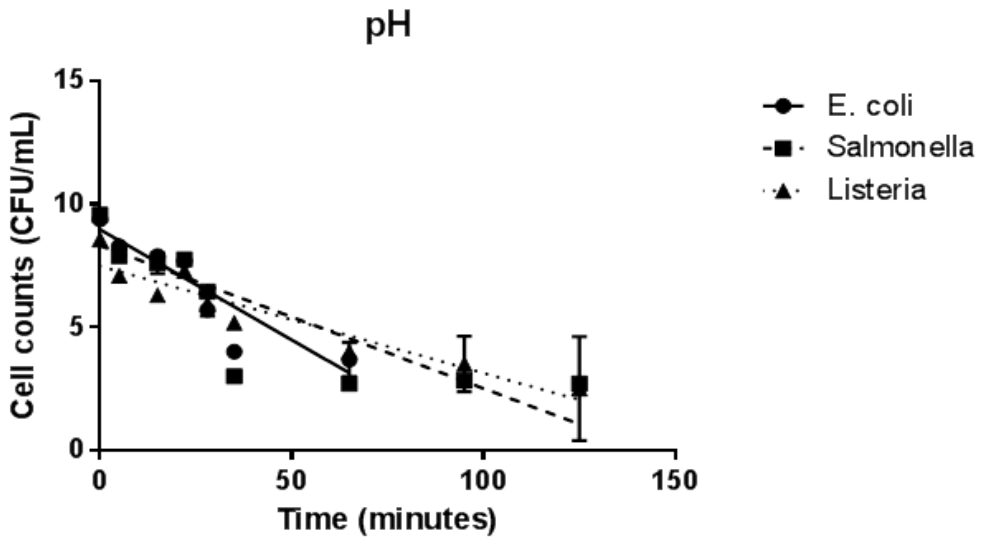

Figure 5. Change in concentrations of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* over time during acidification phase

Table 2. Statistical summary of the inactivation lines of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* at 55 ℃, 68 ℃, and acidification

Best-fit values	*Ecoli* O157:H7	*Salmonella*	*Listeria monocytogenes*
55 ℃			
Equation	Y = -0.1132*X + 8.853	Y = -0.06202*X + 8.835	Y = -0.06379*X + 8.279
Slope (95% Confidence Intervals)	-0.1403 to -0.08613	-0.07874 to -0.04530	-0.08029 to -0.04730
R square	0.8110	0.7096	0.7451
P value	< 0.0001	< 0.0001	< 0.0001
68 ℃			
Equation	Y = -0.03723*X + 10.01	Y = -0.04026*X + 9.535	Y = -0.02493*X + 8.954
Slope (95% Confidence Intervals)	-0.04740 to -0.02705	-0.05174 to -0.02879	-0.03281 to -0.01705
R square	0.8411	0.7885	0.7518
P value	< 0.0001	< 0.0001	< 0.0001
Acidification			
Equation	Y = -0.08977*X + 8.974	Y = -0.05811*X + 8.321	Y = -0.04348*X + 7.482
Slope (95% Confidence Intervals)	-0.1137 to -0.06584	-0.07700 to -0.03922	-0.05460 to -0.03236
R square	0.8611	0.7726	0.8111
P value	< 0.0001	< 0.0001	< 0.0001

The statistic and log linear regression equations of pathogen inactivation in thermophilic, pasteurization, and acidification processes are shown in Table 2. The model based on log linear regressions were used to determine the required time for 5-log reduction of *E. coli*, *Salmonella*, and *Listeria* during thermophilic, pasteurization, and acidification processes. Figure 6 shows estimated inactivation time for 5-log reduction of each pathogen. At 55 ℃, the desired inactivation time for 5-log reductions of *E. coli* O157 was found to be 44.2 h compared to 80.6 h for *Salmonella* and 78.4 h for *Listeria monocytogenes.* Previous studies found quicker pathogen destruction time compared to the current study. Doyle and Schoeni (1984) observed the log reduction time of 39.8 min for *E.coli* O157:H7 in ground beef at 54.5 ℃ (pH of 5.7), and 0.16 min of log reduction time for *E.coli* O157:H7 at 64.3 ℃ at pH 5.7 with 0.25 NaCl solutions. Line et al. (1991) detected that the log reduction time varied between 4-7.4 min at 57 ℃ in lean and fatty beef and 0.2-0.5 min at 63 ℃ while the initial concentration of *E.coli* O157:H7 was 6-7 $\log_{10}$ CFU/ g in the inoculated ground lean or fatty beef. In a similar study, Vijay K Juneja, Snyder, and Marmer (1997) inoculated beef with *E.coli* O157:H7 and found log reduction time of 21.5 min at 55 ℃ and approximately 0.4 min at 65 ℃. To investigate the thermal inactivation of *E.coli* O157 in ground beef, OrtaRamirez et al. (1997) found log reduction time of 46.1, 6.4, .04, and 0.1 min at 53, 58, 63, and 68 ℃. Guo, Piyasena, Mittal, Si, and Gong (2006) used non-pathogenic *E.coli* K12 to evaluate the influence of cooking on the inactivation of *E.coli* in ground beef and showed that no *E.coli*/ *coliform* (initial concentration

$<10^3$ CFU/ g) or *E.coli* K12 (10^7 CFU/ g) was detected after 4.5 min of radiofrequency cooking or 152 min of water bath cooking at 72 ℃. In this study at 68 ℃, the estimated 5-log reductions time for *E. coli* O157 and *Salmonella* was determined to be 134.3 and 124.2 min, respectively. While studying *Salmonella* inactivation, OrtaRamirez et al. (1997) found the log reduction time of 53, 15.2, 2.1, and 0.2 min in ground beef at 53, 58, 63, and 68 ℃, respectively. V. K. Juneja, Eblen, and Ransom (2001) found 9 min of log reduction time at 58 ℃ and around 1 min of log reduction time at 65 ℃ for beef (12.5% fat), which was with inoculated with *Salmonella spp*. Craven and Blankenship (1983) observed the log reduction time of 0.9 min for *Salmonella spp* in Beef at 60 ℃ at 5.9 pH with 0.23 NaCl solution. Murphy, Duncan, Johnson, Davis, and Smith (2002) studied the thermal inactivation of *Salmonella* serotypes and *Listeria innocua* in different meat products and found that the log reduction time was 9.09 min in beef patties at 55 ℃ and 0.97 min at 65 ℃ while the initial concentration of Salmonella was 7-8 $\log_{10}$ CFU/g.

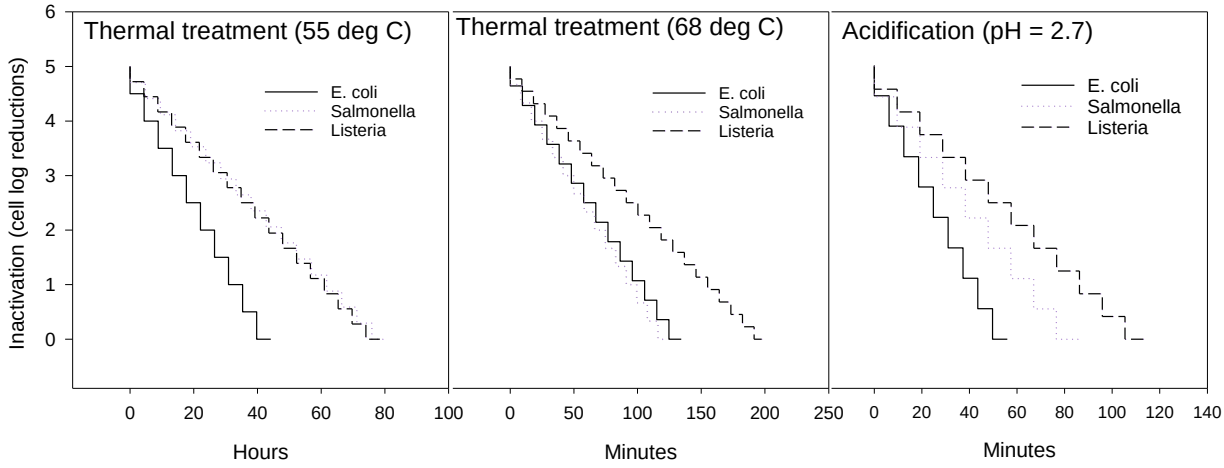

Figure 6. Estimated inactivation time for 5-log reduction of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* under heat treatment and low pH conditions

From projected calculation, the 5 log reduction of *Listeria* at 68 ℃ will occur in 200 min. Hansen and Knochel (1996) observed the log reduction time of 27-29 min at 56 ℃ and about 2 min at 64 ℃ when evaluating the thermal inactivation of *Listeria monocytogenes* in minced beef. In the previous study by Murphy et al. (2002), where the initial concentration of *Listeria* was 7-8 $\log_{10}$ CFU/g, the log reduction time was 19.52 min at 55 ℃ and 0.76 min at 65 ℃. Vijay K Juneja (2003) found the log reduction time of 0.75 min at 65 ℃ in inoculated ground beef with *Listeria monocytogenes* (initial concentrations of 8-9 $\log_{10}$ CFU/ml). In inoculated ground beef with known concentration (9 $\log_{10}$ CFU/ml) of *Listeria monocytogenes*, Huang (2009) observed that it took about 2 min to achieve 5-log reduction at 63 ℃ and about 30 min at 57 ℃. Above studies showed considerable differences in required inactivation time among various pathogens in heat treatment.

Previous studies by de W Blackburn, Curtis, Humpheson, Billon, and McClure (1997) hypothesized that increase in acidity or alkalinity can increase the rate of inactivation of pathogens such as *Salmonella enteritidis* and *E.coli* O157:H7. During acidification phase of this study, however, the inactivation of each pathogen was relatively greater in lower incubation time. The estimated 5-log reduction times for *E. coli* O157, *Salmonella* and *Listeria monocytogenes* were found to be 55.9, 86.0, and 115 min, respectively. Vijay K Juneja and Eblen (1999) determined the effects of temperature, pH, salt, and sodium phosphate on the inactivation of *Listeria monocytogenes* in beef slurry. Authors found that at 55 ℃ while the log reduction time was 5.35 min at pH 4, it was 21.64 min at pH 8 (i.e., reduced time under low pH). Authors also showed that at 65 ℃ the log reduction time was 0.26 min at pH 4 and 0.24 min pH 8.

4. Conclusions

In this experiment, we studied the relative efficacy of thermal and acidification treatments on the inactivation of *E.coli* O157, *Salmonella*, and *Listeria monocytogenes* in ground beef. The relative inactivation of pathogens was studied for 94 h at 55 °C, 3.5 h at 68 °C and 2 h for pH 2.77. Observations were used to develop regression equations, which predict the time required for 5-log reduction of *E. coli* O157:H7, *Salmonella*, and *Listeria* at 55 °C, 68 °C and pH 2.7. Results showed that time required for 5-log reduction changes with temperature and species. For *Salmonella*, 5-log reductions at 55 °C, 68 °C, and pH 2.77 were obtained at 80.6 h, 2.1 h, and 1.43 h,

respectively. Similar reductions in *Listeria* were achieved at 78.4 h, 3.33 h, and 1.92 h, respectively. The concentration of *E. coli* was reduced by 5-log in 44, 2.2, and 2 h at 55 °C, 68 °C and pH 2.7, respectively. We anticipate that the results of this study will be useful for improving the existing models capable of predicting the various pathogen inactivations under different treatment conditions such as acidification, pasteurization, and thermophilic environment.

Acknowledgments

The authors thank the Division of Agriculture and Natural Resources, Veterinary Medicine School Extension, and University of California Cooperative Extension (UCCE) for supporting this work. Authors also appreciate Dr. Philip Kass, Professor in the Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis for his feedback in the use of statistical model.

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