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The effect of lauric arginate on the thermal inactivation of starved *Listeria monocytogenes* in *sous-vide* cooked ground beef

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ABSTRACT

The aim of this study was to examine the efficacy of lauric arginate (LAE, 1000 ppm – 3000 ppm) as an assisting tool to reduce starved *Listeria monocytogenes* population in ground beef following *sous-vide* processing at different temperatures (55–62.5 °C). Ground beef mixed with LAE was vacuum sealed and a laboratory water bath was used for sous-vide cooking. Loglinear and Weibull models were fit to the survival microbial population and the D and Z-values were determined at 55–62.5 °C. Calculated D-values ranged from 33.62 to 3.22 min at temperature 55–62.5 °C. LAE at higher concentration is an effective antimicrobial to increase the inactivation of the pathogen in *sous-vide* cooking. With the addition of LAE, D-values at 55 and 62.5 °C determined by the Loglinear model decreased from 31.86 to 2.28 min (LAE 1000 ppm) and 16.71 to 0.56 min (LAE 3000 ppm), respectively; whereas the D-values at 55 to 62.5 °C determined by the Weibull model were 44.26 and 2.09 min (LAE 1000 ppm) and 22.71 and 1.60 min (LAE 3000 ppm), respectively. This study shows that *sous-vide* processing of ground beef supplemented with higher concentration of LAE effectively inactivates *L. monocytogenes* and thus, helps increase the microbiological safety and product quality.

1. Introduction

Listeria monocytogenes is a ubiquitous foodborne pathogen that remains a concern for food processors due to its psychrotrophic behavior. The pathogen is widely distributed in the environment (e.g., soil, water, silage, etc.) and can find its way into the food processing plant and cross-contaminates food contact surfaces and other processing equipment (Tiwari et al., 2015). *L. monocytogenes* grows and survives at a wide temperature range, from − 0.4 to 45 °C (ICMSF, 1996), and if ingested in contaminated foods, can cause listeriosis (food poisoning) outbreaks in humans (WHO/FAO, 2004). The impact of *Listeria* on food related diseases is widely reported. Todd and Notermans (2011) conducted a surveillance study to monitor the increase in outbreaks of listeriosis among developed countries over decades, with the incidence rate of human infections ranging from 0.3 to 1.3 per 100,000 population.

Thermal processing remains one of the most widely employed techniques for ensuring the food safety profile of a range of solid foods, including meat. One of the thermal processing techniques is *sous-vide* processing, i.e., food products packaged under vacuum are cooked under controlled conditions of temperature and time. This process of cooking is used to prevent losses of water and flavors, which helps to make pasteurized, ready-to-eat (RTE), refrigerated foods more desirable and to extend the shelf life of the products. However, adverse thermal impact on the quality of meat is often debated. As such, negative impacts on the quality of food can be reduced by manipulating the food formulation factors and thereby, rendering the pathogen more sensitive to the lethal effect of heat.

Lauric arginate (LAE; N-lauroyl-L-arginine ethyl ester monohydrochloride) is a cationic surfactant derived from lauric acid, L-arginine and ethanol (Ruckman, Rocabayera, Borzelleca, & Sandusky, 2004). This food grade antimicrobial agent/preservative has been approved by the United States-Food and Drug Administration (US-FDA, 2005) and the European Food Safety Authority (EFSA, 2007) as a 'generally recognized as safe' (GRAS) food additive, to use in foods such as RTE meat products (Ruckman, Rocabayera, Borzelleca, Sandusky, 2004). In the human body, it is rapidly metabolized to naturally occurring amino acids (dietary components), arginine and ornithine, and is thus known to be non-toxic (Hawkins, Rocabayera, Ruckman, Segret, & Shaw, 2009). It has a broad spectrum of antimicrobial activity and is

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widely used in the food and food packaging industries to guard against the hazards associated with both Gram-positive and Gram-negative bacteria (Becerril, Manso, Nerin, & Gómez-Lus, 2013; Coronel-Leon et al., 2016), as well as molds. While alterations in the cytoplasmic membrane and external membrane were found in LAE treated Gram-negative *Salmonella* Typhimurium, alterations in cell membrane and cytoplasm were observed in Gram-positive *Staphylococcus aureus*. Nevertheless, disturbance in membrane potential and cell structure can lead to loss of cell viability, without causing lysis or disruption of treated cells (Rodriguez, Seguer, Rocabayera, & Manresa, 2004). Its effectiveness is well documented in the reduction of *L. monocytogenes* (Porto-Fett et al., 2010). Based on the mode of actions of different antimicrobials, the efficacy of LAE in combination with other preservatives has been reported to exhibit additive or synergistic effects against foodborne pathogens, such as *Salmonella* spp., *L. monocytogenes*, and *Escherichia coli* (Martin et al., 2009; Brandt et al., 2010; Stopforth, Visser, Zumbrink., van Dijk, & Bontenbal, 2010; Noll, Prichard, Khaykin, Sinko, & Chikindas, 2012; Soni, Desai, Oladunjoye, Skrobot, & Nannapaneni, 2012; Theinsathid, Visessanguan, Kruenate, Kingcha, & Keeratipibul, 2012). In a toxicological animal study with different levels of LAE, Ruckman et al. (2004) concluded that there was no sign of neurotoxicity even at high levels of LAE (50,000 ppm), helping to confirm the safety of LAE as a food additive.

Microbial starvation commonly exists in the food industry because usage of water and sanitizer in cleaning and sanitizing on food contact surfaces generally creates an inconducive environment for microorganisms. Exposure to nutrient shortages and chemical treatments may increase microorganisms' resistance to heating processes. For example, heat resistance of starved *L. monocytogenes* in flask of Trypticase Soy Broth with 0.6% yeast extract followed by treatment of heat shock was observed to increase (Lou & Yousef, 1997). The ability of pathogens to increase microbial resistance can result in reducing effectiveness of inactivation and preservation methods. Therefore, evaluating the efficiency of inactivation parameters on starved bacterial cells can help the food industry improve processing and ensure food safety and quality.

To our knowledge, there are no published studies on the thermal death time values of *L. monocytogenes* in *sous-vide* processed ground beef supplemented with LAE. Accordingly, the goal of this study was to determine the efficacy of LAE in rendering *L. monocytogenes* more sensitive to the lethal effect of heat under conditions that occur in the retail food industry. The thermal death time values of *L. monocytogenes* at 55, 57.5, 60 and 62.5 °C quantified in the current study can be used to validate the *sous-vide* cooking of beef to eliminate the pathogen and enhance the microbiological safety of the product during its intended shelf-life.

2. Material and methods

2.1. Bacterial cultures

Five *L. monocytogenes* strains that were isolated from human clinical cases (Scott A), a hot dog outbreak (H7762), steer/heifer (MF27137), ground chicken (MF38521), and fresh pork sausage (MF46869) were used in this study. Stock cultures were maintained at −80 °C in brain heart infusion broth (BHI; Labline-Thermo Scientific, Melrose Park, IL) with 20% v/v glycerol (Sigma-Aldrich, St. Louis, MO). The strains were thawed and individually grown by transferring 0.1 ml of culture to 10 ml BHI broth tubes and incubating for 24 h at 37 °C. A second transfer into fresh BHI tubes was made and BHI slants were streaked for each strain. Both slants and broth tubes were incubated for 24 h at 37 °C and then maintained at 4 °C. Biweekly culture transfers were made from the slants which were initiated monthly from the frozen stocks.

2.2. Preparation and starvation of inoculum

Overnight cultures (0.1 ml) were grown in BHI broth (50 ml) in 250 ml flasks at 37 °C for 18 h. The cultures were centrifuged twice at 4696 × g for 15 min at 4 °C with the pellet being washed once in 0.1% (w/v) peptone water (PW; Becton, Dickinson & Co., Sparks, MD) and finally suspended in 10 ml of Butterfield Phosphate Buffer (Neogen Corp., Lansing, MI; Juneja, Gonzales-Barron, Butler, Yadav, & Friedman, 2013). These new cultures were incubated at 37 °C for 24 h for starvation. The starved cultures were centrifuged and washed as described above and then suspended in 2 ml of 0.1% PW. These 2 ml cultures were combined in a sterile conical vial and vortexed for a minute to produce a five-strain cocktail of *L. monocytogenes* (7–8 $\log_{10}$ CFU/ml).

Table 1
Thermal inactivation kinetics in ground beef supplemented various concentrations of LAE[1] at different temperatures.

Temperature (°C)	D value Ll (min)	T4D (min) Ll[2]	D value Wb[3] (min)	T4D (min) Wb	z value (°C) Ll	z value (°C) Wb
Control						
55	33.62	134.48	43.74	174.96		
57.5	14.05	50.20	12.84	51.36		
60	5.88	23.53	7.76	31.04		
62.5	3.22	12.87	4.47	17.89	7.28	7.84
LAE 1000 ppm						
55	31.86	127.44	44.26	177.04		
57.5	12.46	49.84	15.22	60.88		
60	3.79	15.18	3.93	15.73		
62.5	2.28	9.12	2.09	8.36	6.33	5.48
LAE 3000 ppm						
55	16.71	66.84	22.71	90.84		
57.5	9.20	36.80	8.39	33.55		
60	1.16	4.66	1.17	4.66		
62.5	0.56	2.25	1.60	6.4	4.70	5.80

[1] LAE: lauric arginate.
[2] Ll: Loglinear.
[3] Wb: Weibull.

Table 2
Shape parameter values obtained from the Weibull distribution for treated and untreated ground beef with LAE at different temperatures.

Temperature (°C)	Shape parameter (β)		
	Control	LAE 1000 ppm	LAE 3000 ppm
55	1.27	1.33	1.42
57.5	0.93	1.18	0.94
60	1.26	1.04	1.23
62.5	1.95	0.89	0.28

β < 1: concave shaped curves; β > 1: convex shaped curves; β = 1: linear shaped; LAE: lauric arginate.

2.3. Sample preparation

Ground beef (73% lean) procured from a local market was divided into 50-g portions. The portions were mixed with 1000 or 3000 ppm LAE (Ethyl Lauroyl Arginate HCl; Mirenat-GA; Vedeqsa, Barcelona, Spain) using a KitchenAid mixer (model K5SSDWH, St. Joseph, MI) while the control samples were prepared without addition of lauric arginate. All samples were stored frozen for about 60 d until further use. Thawed 50-g sample bags were inoculated (0.1 ml) with the cocktail inoculum and thoroughly mixed both manually and with a Seward stomacher 400 (Seward, UK) for 2 min. Three-gram samples were aseptically transferred into filter stomacher bags (Nasco Whirl-

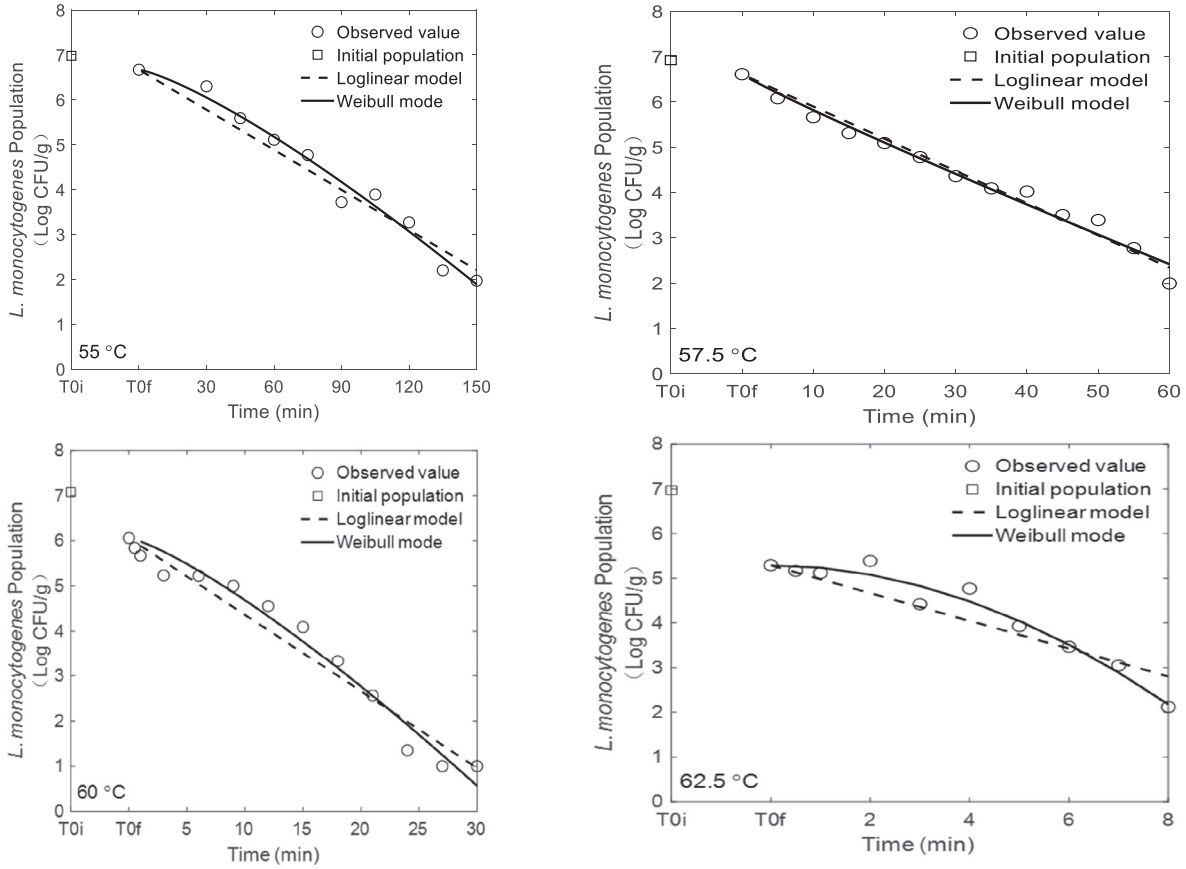

Fig. 1. Survival curves of *L. monocytogenes* in ground beef samples cooked under *sous-vide* of control samples at 55, 57.5, 60 and 62.5 °C. Loglinear model (dotted line) and Weibull (solid line) model.

Pak, Fort Atkinson, WI), pressed into a thin layer and sealed using a vacuum sealer (model A300/16, Multivac Inc., Kansas City, MO).

2.4. Sous-vide cooking and thermal treatment

To simulate the cooking of rare roast beef as performed in the food industry, 3-g meat sample bags were placed in a wire rack and fully immersed in a temperature-controlled water bath (Thermo NESLAB RTE-17, Neslab Instruments, Inc., Newington, NH). The temperatures of the water and the meat samples were monitored using a type K thermocouple (Omega Engineering Inc., Stamford, CT) and recorded using a temperature data logger (SPI50, Dickson, Addison, IL). For the meat samples, the thermocouple was inserted in the center of an uninoculated meat bag. The temperature of the water bath was programmed to increase linearly from 23 °C to four specific target temperatures (55, 57.5, 60 and 62.5 °C) in 1 h. Meat sample bags were pulled at predetermined times once the target temperature was reached. The bags were subsequently immersed in an ice/water slurry and analyzed within 30 min. The total heating times ranged from 60 to 150 min at 55 °C, 48 to 60 min at 57.5 °C, 1.5 to 30 min at 60 °C, and 1.5 to 8 min at 62.5 °C and sampling frequency ranged from every 10–30 min 55 °C to 0.25 to 1 min at 62.5 °C.

2.5. Microbiological analysis and enumeration

Each heat-treated sample bag was opened aseptically, combined with 3 ml of 0.1% PW and vigorously mixed for 2 min using a stomacher (Bag Mixer 100 Mini Mix, Interscience, St. Nom, France). After 10-fold serial dilutions, 100 µl were spread onto Tryptic Soy Agar (TSA; Becton, Dickinson & Co., Sparks, MD) plates. The plates were left for 2 h and overlaid afterwards with Difco Oxford Medium Base with Difco Modified Oxford Supplement (MOX; Becton, Dickinson & Co., Sparks, MD). Colonies were enumerated [colony forming units (CFU)/g] after 48 h of incubation at 30 °C. All experiments were independently performed twice.

2.6. Microbial inactivation models

Two inactivation models (Log-linear and Weibull) were fitted to the changes in microbial population by using the MATLAB (version 2018b, The MathWorks, Inc. Natick, MA). Log-linear is a simple first-order inactivation model that shows exponential inactivation by using the following equation:

$$y = y_0 - \frac{t}{D} \quad (1)$$

where y represents observed population of *L. monocytogenes* (Log CFU/g) and y_0 represents initial population of *L. monocytogenes* (Log CFU/g); t represents time and D, decimal reduction "D-value", represents the inactivation time (min) at a given temperature.

The Weibull model is an inactivation model that demonstrates the decline in microbial numbers as a cumulative distribution of heat lethality. The Weibull model includes two model parameters, shape β and scale D. The scale parameter accounts for various shaped survival curves such as concave ($\beta < 1$), convex ($\beta > 1$) or linear ($\beta = 1$) shaped curves and decimal reduction (D-value) time (min) taken to achieve a 1 log CFU reduction in microbial population:

$$y = y_0 - [t/D]^{\beta} \quad (2)$$

The Z-values were determined as the negative inverse slope of the log D-values versus temperature:

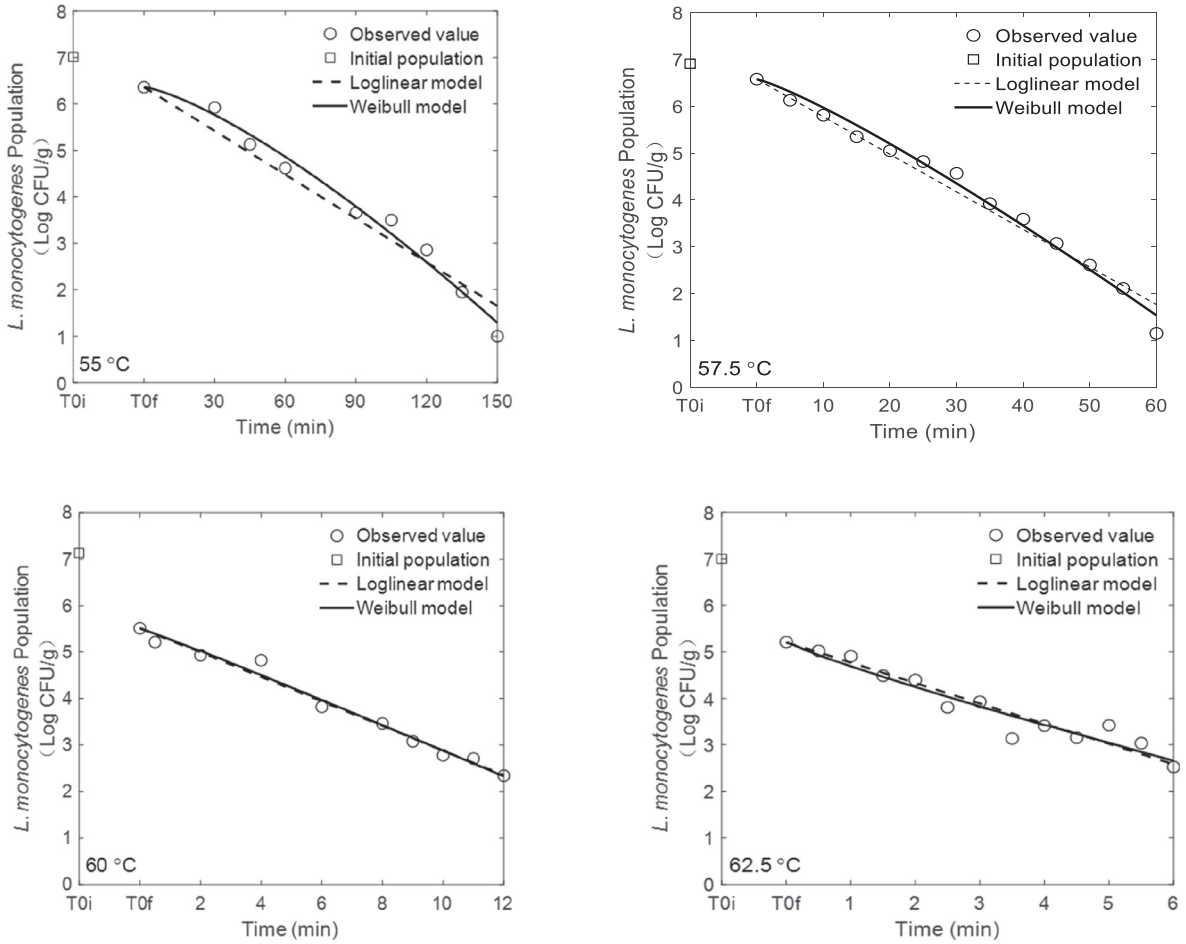

Fig. 2. Survival curves of *L. monocytogenes* in ground beef samples cooked under *sous-vide* of 1000 ppm LAE treated samples at 55, 57.5, 60 to 62.5 °C. Loglinear model (dotted line) and Weibull (solid line) model.

$$\log(D) = \log(D_0) - \frac{t}{Z} \quad (3)$$

2.7. Model evaluation & statistical analysis

All statistical analyses were performed using MATLAB. The performances of inactivation models were compared using root mean square root (RMSE), coefficient of determination (R^2) and sum of squared error (SSE). All values were calculated with 95% confidence intervals.

3. Results

3.1. Microbial inacitvation

The efficacy of adding 1000 and 3000 ppm LAE to ground beef on thermal inactivation of *L. monocytogenes* following *sous-vide* beef processing at various temperatures was studied. The results were shown in Table 1. As expected, the D-values obtained from the Loglinear and Weibull model both decreased when temperature increased for the control and LAE-treated samples. For the Weibull model, the D-value of control samples decreased from 43.74 to 12.84 min at 55 to 57.5 °C, and further reduced to 7.76 min at 60 °C and 4.47 min at 62.5 °C. The addition of 1000 ppm of LAE added into samples slightly decreased the D-value of *L. monocytogenes* at all temperatures, except that at 55 °C and 57.5 °C in the Weibull model. The Weibull model showed the D-values of 44.26 min (55 °C) and 15.22 min (57.5 °C) in samples supplemented with 1000 ppm LAE, which were slightly higher than the D-value in control samples.

Compared to the control samples, D-values further decreased when the level of LAE in samples increased to 3000 ppm, 22.71 min at 55 °C in the Weibull model compared to 43.74 min at 55 °C that was estimated for the control samples. The decreasing trend of the D-value of *L. monocytogenes* with addition of LAE was noticed in the log-linear model as well. The D-value was calculated as 12.87 min at 62.5 °C of control sample while a reduced D-value of 9.12 min was calculated for samples supplemented with 1000 ppm LAE at the same temperature. Furthermore, the D-value decreased to 2.25 min at 62.5 °C when the level of LAE increased to 3000 ppm. LAE treatment showed an antimicrobial ability which reflected on the z-value as well. For the Loglinear model, the z-value of control was estimated at 7.28 °C while it decreased to 6.33 °C when 1000 ppm LAE was added. With 3000 ppm LAE treatment, the z-value further reduced to 4.70 °C. Similarly, for the Weibull model, the z-values were estimated to be 7.84 °C, 5.48 °C, and 5.80 °C for the control samples, 1000 ppm and 3000 ppm LAE, respectively.

Table 2 shows the shape parameter "β" of Weibull model fitted to *L. monocytogenes* population during *sous-vide* processing at 55, 57.5, 60 and 62 °C. The survival curves were mostly convex ($\beta > 1$) shaped for the control samples (Fig. 1, Fig. 2, Fig. 3), with the exception of 57.5 °C. For the samples treated with 1000 ppm LAE, the survival curves were convex for temparature from 55 °C to 60 °C, but for temperature 62.5 °C curve was concave. The survival curves for 3000 ppm LAE exhibited a different trend. For temperature 55 °C and 60 °C, the survival curves were convex while the opposite for temperature 57.5 °C and 62.5 °C. The β values were in the 0.28–1.95 range, and very close to 1 in several cases, which indicates that there was not very strong concave or convex

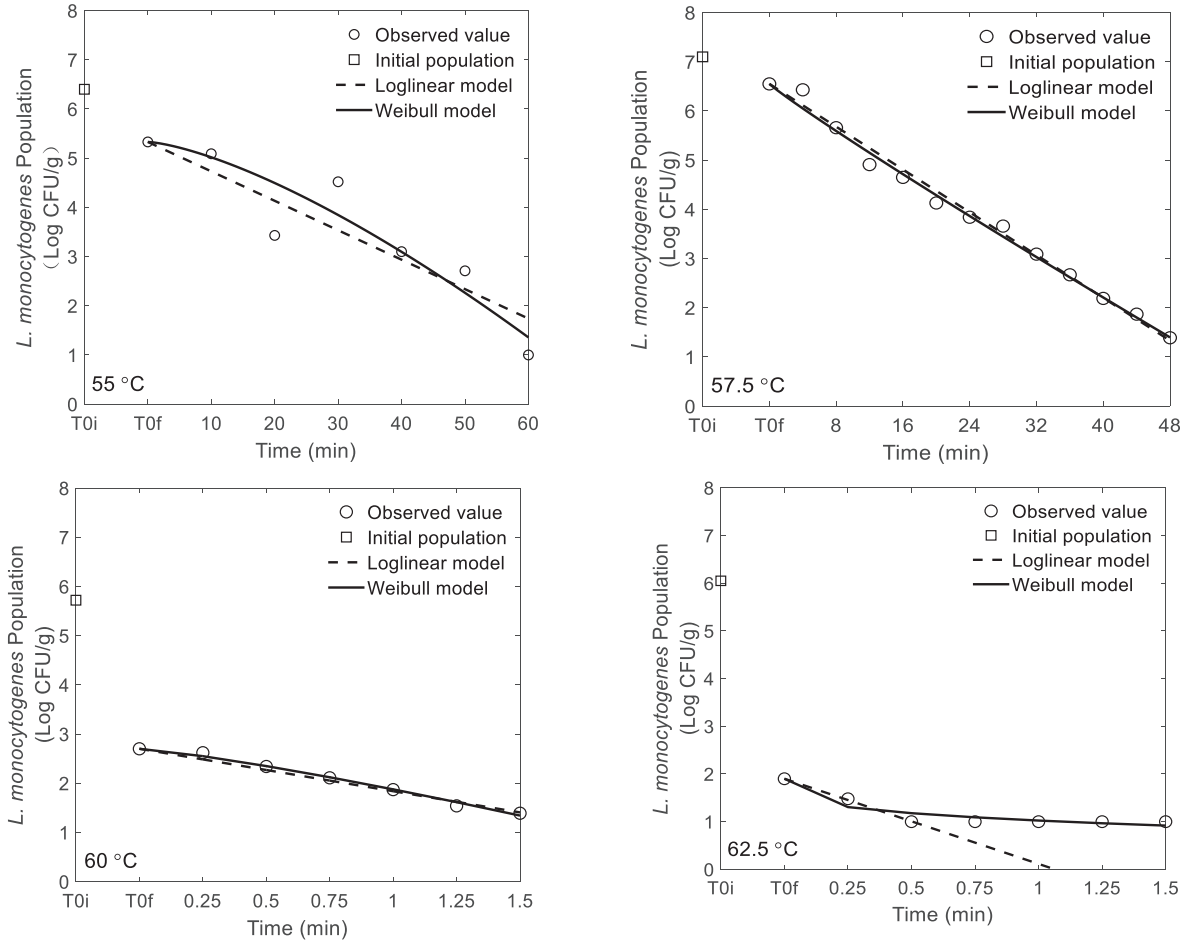

Fig. 3. Survival curves of *L. monocytogenes* in ground beef samples cooked under *sous-vide* of 3000 ppm LAE treated samples at 55, 57.5, 60 to 62.5 °C. Loglinear model (dotted line) and Weibull (solid line) model.

Table 3
Comparison of the goodness of fit of the Loglinear and Weibull models for the survival curves of *L. monocytogenes* in ground beef with added LAE at 55 to 62.5 °C.

	Log Linear			Weibull		
	Control	LAE 1000 ppm	LAE 3000 ppm	Control	LAE 1000 ppm	LAE 3000 ppm
Temperature (55 °C)						
RMSE	0.33	0.36	0.62	0.24	0.23	0.62
R^2	0.96	0.96	0.83	0.98	0.98	0.86
SSE	0.98	1.04	2.31	0.47	0.38	1.92
Temperature (57.5 °C)						
RMSE	0.20	0.19	0.17	0.20	0.19	0.16
R^2	0.98	0.99	0.99	0.98	0.99	0.99
SSE	0.48	0.38	0.35	0.44	0.38	0.29
Temperature (60 °C)						
RMSE	0.38	0.16	0.08	0.33	0.16	0.05
R^2	0.96	0.98	0.98	0.97	0.98	0.99
SSE	1.77	0.19	0.03	1.19	0.19	0.01
Temperature (62.5 °C)						
RMSE	0.42	0.23	0.02	0.23	0.23	0.13
R^2	0.86	0.93	0.99	0.96	0.93	0.90
SSE	1.58	0.59	0.02	0.41	0.59	0.08

RMSE: root mean square root; SSE: sum of squared error

curve. These β values for the Weibull model also suggest that a Loglinear model may generally define the inactivation pattern of *L. monocytogenes* in our studies very well.

Table 3 presents the goodness of fit statistics for all the fitted survival curves. Overall, the Loglinear and Weibull models showed a good fit with low RMSE (0.13 to 0.42) for all treatment except samples supplemented with 3000 ppm LAE at 55 °C (0.62). A similar trend was observed for R^2 values for both Loglinear and Weibull model, respectively. R^2 values were ranged from 0.90 to 0.99 with the exception that the R^2 values of samples added with 3000 ppm LAE at 55 °C were 0.83 for the Loglinear model and 0.86 for the Weibull model, respectively. In addition, control samples at 62.5 °C showed a R^2 value as 0.86. In general, the SSE of Loglinear and Weibull survival curves showed relatively higher values, ranging from 0.02 to 2.31, compared to RMSE. The samples with addition of 1000 ppm and 3000 ppm LAE were identified with the SSE of 1.04 and 2.31, respectively, for the Loglinear model while control sample illustrated the SSE as 1.77 for the Loglinear model. Similar trend was found in the Weibull model that the SSE of sample supplemented with 3000 ppm LAE at 55 °C was 1.92 and control sample at 60 °C was 1.19.

4. Discussion

The results showed that 1000 ppm and 3000 ppm LAE aided in reducing *L. monocytogenes* population densities during *sous-vide* processing of ground beef at different temperatures (55, 57.5, 60 and 62.5 °C). The inactivation models (Loglinear and Weibull) fiited to the survival curves showed the models fit the data well. A decreasing trend of D-values was observed with the aid of LAE treatment (1000 ppm and 3000 ppm) at all temperature, except that at 55 and 57.5 °C the samples supplement with 1000 ppm LAE showed slightly higher D-values in the Weibull model. In the Loglinear model, the D-values of samples treated

with 1000 ppm LAE at 55 and 57.5 °C were slightly lower comparing to the D-values of control sample. The results indicated that lower concentration of LAE (1000 ppm) may not have strong antimicrobial ability under lower temperatures (55 and 57.5 °C). A significant decrease of D-values between samples with 3000 ppm LAE treatment and control samples was observed in both Loglinear and Weibull model at all temperatures, which illustrated that *L. monocytogenes* was more sensitive to higher concentration of LAE even under relatively low temperatures (55 and 57.5 °C). In another study, frankfurters treated with 5,000 ppm of LAE resulted in about 2-log reduction of *L. monocytogenes* within 48 h (Taormina & Dorsa, 2009), which supported the bactericidal effect of LAE on *L. monocytogenes*.

Loglinear model and Weibull model were used to fit the survial curves for temperature from 55 °C to 62.5 °C with control samples and LAE treated samples. Overall, both models fit the data well except control samples at 62.5 °C where the Weibull model illustrated a better fit compared to the Loglinear model by having a greater R^2 value.

The D-values of starved *L. monocytogenes* cells in control samples were reduced from 33.6 min at 55 °C to 5.88 min at 60 °C. Similar results showed that the D-values of starved *L. monocytogenes* decreased from 24.61 min at 56.3 °C to 2.83 min at 60 °C during heat treatment for bologna (Grosulescu, Juneja, & Ravishankar, 2011). The D-value of non-starved *L. monocytogenes* was obtained for 3.2 min at 55 °C and 0.15 min 60 °C in ground beef follow by *sous-vide* cooking (Bolton et al., 2000). A significant increase of the D-values was observed between non-starved and starved *L. monocytogenes* cells, which indicated that starved *L. monocytogenes* cells have higher heat resistance.

Knowledge of the thermal inactivation kinetics of inoculating pathogens in thermally processed meat products is necessary to design adequate thermal processes and to ensure the elimination of pathogens.

The current study suggests that the addition LAE in ground beef followed by *sous-vide* processing effectively controlled *L. monocytogenes* survial and the fitted inactivation models provides an understanding of thermal resistance behavior. Higher concentration of LAE increased the sensitivity of the *L. monocytogenes* to heat, and thus, minimizing any adverse effect which is essential for product quality. Decimal reduction time values obtained in the present study cannot be placed into context with previous studies due to the unavailability of published research on heat inactivation of *L. monocytogenes* in beef supplemented with LAE. LAE is proven to have anti-microbial activity in several studies. For example, in a study provided by Porto-Fett et al. (2010), when frankfurters were treated with 22 or 44 ppm LAE, *L. monocytogenes* levels were reduced by 2.0 log CFU/package within 2 h.

5. Conclusions

Supplementing beef with 1000 ppm and 3000 ppm LAE followed by *sous-vide* processing at different temperatures showed a greater reduction in *L. monocytogenes* population when compared to a control. The Loglinear inactivation model as well as the Weibull inactivation model was fitted to survival curves and showed an overall good model fit for all the samples. The D-values significantly decreased among all samples (untreated and treated samples) with increasing temperatures. This study shows the *L. monocytogenes* cells which have been starved may have higher heat resistance. Further study is needed to explore the heat resistance of starved *L. monocytogenes* cells. Higher concentrations of LAE in beef increased the sensitivity of the *L. monocytogenes* to *sous-vide* treatment, thereby extending the shelf-life and improving the product quality, which is desirable for food processors, regulators and consumers.

Declaration of Competing Interest

The authors have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109280.

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