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Modeling Time to Inactivation of *Listeria monocytogenes* in Response to High Pressure, Sodium Chloride, and Sodium Lactate

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ABSTRACT

A mathematical model was developed to predict time to inactivation (TTI) by high pressure processing of *Listeria monocytogenes* in a broth system (pH 6.3) as a function of pressure (450 to 700 MPa), inoculum level (2 to 6 log CFU/ml), sodium chloride (1 or 2%), and sodium lactate (0 or 2.5%) from a 4°C initial temperature. Ten *L. monocytogenes* isolates from various sources, including processed meats, were evaluated for pressure resistance. The five most resistant strains were used as a cocktail to determine TTI and for model validation. Complete inactivation of *L. monocytogenes* in all treatments was demonstrated with an enrichment method. The TTI increased with increasing inoculum level and decreasing pressure magnitude, from 1.5 min at 700 MPa and 2 log CFU/ml, to 15 min at 450 MPa and 6 log CFU/ml. Neither NaCl nor sodium lactate significantly influenced TTI. The model was validated with ready-to-eat, uncured, Australian retail poultry products, and with product specially made at a U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS)–inspected pilot plant in the United States. Data from the 210 individual product samples used for validation indicate that the model gives ''fail-safe'' predictions (58% with response as expected, 39% with no survivors where survivors expected, and only 3% with survivors where none were expected). This model can help manufacturers of refrigerated ready-to-eat meats establish effective processing criteria for the use of high pressure processing as a postlethality treatment for *L. monocytogenes* in accordance with FSIS regulations.

The ability of *Listeria monocytogenes* to grow at low temperatures, and under low-oxygen conditions *(11),* makes this microorganism a particular concern for producers of cooked, ready-to-eat (RTE) meat products *(14),* who spend great amounts of time and money to minimize the risk from recontamination during slicing and packaging operations. The incidence of *Listeria* contamination of RTE meat products has been shown to be highly variable *(6, 23, 24),* but there are encouraging indications that the incidence of *Listeria* contamination of U.S. RTE meats has fallen quite sharply since the early 1990s *(24).* It seems reasonable to assume that this is a direct result of efforts by the industry to improve sanitation practices, implement hazard analysis and critical control point (HACCP) procedures and adopt use of antilisterial ingredients such as lactates and diacetate.

In the United States, refrigerated, RTE cooked meat products are regulated by the U.S. Department of Agriculture (USDA). Under an interim final rule released on 6 June 2003 *(20),* the USDA Food Safety Inspection Service (FSIS) afforded RTE products produced with control programs to reduce or eliminate *L. monocytogenes,* or suppress or limit its growth, different regulatory treatment under three alternatives: (i) ''postlethality treatments'' *and* antimicrobial agents within a HACCP plan (Alternative 1), (ii) postlethality treatments *or* antimicrobial agents within a HACCP plan (Alternative 2); and (iii) sanitation procedures alone within a HACCP plan (Alternative 3).

Under Alternative 1 or Alternative 2, the FSIS would apply relatively less sampling to a product undergoing a postlethality treatment giving a ≥2-log reduction of *L. monocytogenes,* relatively more sampling to a product receiving a postlethality treatment giving between a 1- and 2-log reduction, and would consider <1-log reduction not eligible for those alternatives unless there is supporting documentation that the treatment provides an adequate safety margin *(21).*

Beyond the U.S. regulatory considerations, the required stringency of the postlethality treatment depends, in part, on the level of recontamination to be addressed. The International Commission on Microbiological Specifications for Foods (ICMSF), as an illustrative example, suggested a food safety objective that ''. . . the concentration of *L. monocytogenes* in frankfurters should not exceed 100 CFU/g at the time of consumption'' and illustrated how a process criterion that ''. . . the concentration of *L. monocytogenes*

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TABLE 1. Listeria monocytogenes *strains and their origins*

Culture collection source	Strain no.[a]	Original isolation source
CSIRO FNS	FRRW 2340	Salad with pasta, cheese, and ham or bacon
CSIRO FNS	FRRW 2341	Salad with pasta, cheese, and ham or bacon
CSIRO FNS	FRRW 2342	Ham
CSIRO FNS	FRRW 2343	Salad with pasta, cheese, and ham or bacon
CSIRO FNS	FRRW 2345	Ham
CSIRO FNS	FRRB 2472	ATCC strain Scott A (clinical specimen)
CSIRO FNS	FRRB 2542	Salami
CSIRO FNS	FRRB 2655	Chicken feathers
CSIRO FNS	FRRB 2657	Chicken skin
Ohio State Department of Agriculture	OSY 8578	

[a] FRRW, CSIRO Division of Food and Nutritional Sciences culture collection, Werribee, Victoria, Australia; FRRB, CSIRO Division of Food and Nutritional Sciences culture collection, North Ryde, New South Wales, Australia.

after cooking shall be ≤1 CFU/kg'' achieves that objective *(8)*. Assuming that this process criterion is met, and further assuming that recontamination increases the concentration as high as 10 CFU/g, the recontamination is equivalent to a 4-log increase in concentration. In these circumstances, ICMSF pointed out *(8)* that an in-pack postlethality treatment giving a 4-log reduction would still achieve the process criterion of ≤1 CFU/kg (and by extension, the Food Safety Objective of <100 CFU/g at the time of consumption).

High pressure processing (HPP) has been shown to successfully eliminate pathogenic and spoilage microorganisms in food *(7, 9, 15)*. Since HPP can be conducted on goods in their final packaging *(17)* and causes minimal changes in the nutritional and organoleptic properties of foods *(7, 17),* it could be ideally suited for use as a postlethality treatment for control of *L. monocytogenes* in RTE meat and poultry products. As part of a good overall HACCP program, HPP may also allow for the extension of refrigerated shelf life by inactivating spoilage microorganisms *(5, 7)*.

Several studies have shown the ability of HPP to obtain substantial reduction of *L. monocytogenes (4, 9, 16)*. Most of these studies have used kinetic approaches, and various degrees of upward concavity (tailing) have been observed in the survivor curves. Although the concentration of viable *L. monocytogenes* has been reduced below the detection limit in a number of studies *(16, 18),* this is not the same as showing that all viable cells had been destroyed. For a meat processor considering the substantial investment required to install and operate HPP equipment, some assurance that HPP can eliminate *L. monocytogenes* from RTE meat products increases the value of the investment. According to the FSIS *(21),* Raghubeer and Ting demonstrated elimination of 4 log CFU/g *L. monocytogenes* from retail packaged sliced ham, turkey, chicken, and roast beef, but published details of that study are limited *(1)*.

The work presented here had two objectives. The first was to show, in model systems and in RTE meat, that HPP can eliminate substantial numbers of *L. monocytogenes* (as measured by an enrichment culture method). The second was to create a predictive model that describes the relationship between the effective HPP time, magnitude of pressure, initial microbial concentration, and concentrations of sodium chloride and sodium lactate on the inactivation of *L. monocytogenes*.

MATERIALS AND METHODS

Preparation of inoculum cultures. In preparation for all experiments, one loopful of cells of each isolate was transferred from glycerol stock (−80°C) into tryptic soy broth (TSB; 10 ml, pH 6.6, adjusted with 1 M HCl) and incubated for 18 h at 37°C. For initial screening of the pressure resistance, an aliquot (100 μl) of each available isolate was subcultured into prechilled TSB (50 ml, pH 6.6) at 15°C in a 250-ml bottle (SCHOTT, Elmsford, NY) and incubated for 72 h at 15°C and 40 strokes a min in a shaking water bath (model U07030, Lauda, Lauda-Königshofen, Germany), after which time the cells were in the early stationary phase of growth (approximately 9 log CFU/ml). For time to inactivation (TTI) studies, initial overnight growth from the glycerol stock culture was in each of five replicate tubes of TSB per isolate (10 ml, pH 6.3, adjusted with 1 M HCl). Aliquots (100 μl) of these overnight cultures were used to inoculate fresh tubes of TSB (9.9 ml) that were incubated with shaking as described above, resulting in early-stationary-phase cells (approximately 9.3 log CFU/ml).

Screening of *L. monocytogenes* isolates for their relative resistance to pressure. Ten *L. monocytogenes* isolates originally from a variety of sources, including processed meats, were evaluated for their relative resistance to HPP (Table 1). Each isolate was initially identified as *L. monocytogenes* by using colony morphology on tryptone soya agar (Oxoid, Ltd., Basingstoke, UK), Gram stain, catalase production, and a positive CAMP test. Final confirmation was obtained with *Listeria* API strips (bioMérieux, Marcy l'Etoile, France).

Stationary-phase cultures of each isolate prepared as above were individually diluted in fresh prechilled TSB (pH 6.6 to facilitate comparison with data from Hayman et al. *(7)*) to obtain a final concentration of 6 log CFU/ml, and were stored on ice. Approximately 5 ml of the 6-log CFU/ml diluted cultures were each dispensed separately into five sterile sample tubes (transfer pipettes 273-205, Samco Scientific Corp., San Fernando, CA) and the tubes heat sealed. The samples were then placed into bags, covered with freshly made 0.2% (vol/vol) Proxitane (a blend of acetic acid, peracetic acid, and hydrogen peroxide; Solvay Intertox, Sydney, Australia) to protect the high pressure unit from contamination in the event that a sample tube were to leak, and the bags were heat sealed, excluding as much air as possible. These

bags were heat sealed into a second bag, filled with an ice-and-water slurry, again excluding as much air as possible. Samples were held on ice for not more than 30 min prior to pressure treatment and were treated at 600 MPa for 10, 20, 30, 40, and 60 s by using a 2-liter high-pressure unit (Avure Technologies, Kent, WA). The time to reach 600 MPa (come-up time) was approximately 10 s, and pressure release after the indicated hold times was <2 s. The unit does not have temperature control and was run under ambient conditions (initial temperature of approximately 20°C). However, the temperature of the samples prior to pressure treatment and immediately after removal from the unit after processing was approximately 1°C. All samples were held on ice for not more than 2 h prior to plating.

Pressure-treated samples were removed from the bags, and the tubes were surface disinfected with 1% (vol/vol) sodium hypochlorite. A small hole was cut into the top of the tube with sterile scissors. The sample was transferred to a sterile 1.5-ml Eppendorf tube (Interpath, Caringbah, Australia) by using a 1-ml sterile syringe. Serial dilutions were made with sterile 0.1% (wt/vol) peptone water (Amyl Media, Dandenong, Australia), and samples were plated onto replicate tryptone soya yeast extract agar (pH 7.4, 30 g of TSB, 6 g of yeast extract [Amyl Media], and 14 g of agar [Leiner Davis Gelatin, Sydney, Australia] per 1,000 ml of distilled water). The plates were incubated at 37°C for 72 h before enumeration. Experiments were performed in duplicate for each isolate, on separate days.

Preparing inocula for TTI studies. The five isolates most resistant to pressure, FRRB 2542, FRRB 2655, FRRW 2343, FRRB 2472, and OSY8578, were used as a cocktail to generate TTI data.

Cocktails were prepared by adding 1 ml of each isolate to the same 5 ml of TSB, giving 10 ml of a cocktail containing approximately 9 log CFU/ml. Serial dilutions were made in TSB (pH 6.3) to concentrations of approximately 8, 6, and 4 log CFU/ml. These cocktails were transferred (100 μl) into experimental media (9.9 ml, TSB, pH 6.3 to match the pH of RTE meats, supplemented with NaCl and/or sodium lactate) to achieve final cell concentrations of approximately 6, 4, and 2 log CFU/ml. Carnitine hydrochloride (Sigma-Aldrich Pty., Ltd., Castle Hill, New South Wales, Australia) was added to experimental media, prior to autoclaving, to a final concentration of 1 mM to simulate the level found naturally in meat products, because carnitine has been shown to function as both an osmoprotectant and a cryoprotectant for *L. monocytogenes (2).* Approximately 1.5 ml of each cocktail was transferred into 1-ml Nalgene Cryovials (In Vitro Technologies Pty., Ltd., Noble Park, Victoria, Australia), filling them to leave minimal headspace. The vials were stored on ice until pressure treated (30 to 60 min).

Determining time to inactivation. Initially, 36 treatments were tested in a full-factorial design (Table 2), consisting of pressure (600, 650, and 700 MPa), inoculum level (6, 4, and 2 log CFU/ml), NaCl concentration (1 and 2% [wt/vol]), and sodium lactate concentration (0 and 2.5% [wt/vol]). For each treatment, multiple sets of five replicate tubes were prepared as described above. Sets of five inoculated tubes were pressure treated for various hold times, starting with a short hold time expected to leave surviving *L. monocytogenes,* and a long hold time expected to leave no survivors. After pressure treatment, the contents of the tubes were tested for viability (with a modification of the FSIS method as described in the ''Viability testing'' section, below) and the number of tubes at each time with recoverable *Listeria* was recorded. Additional times were tested, working from the longest time with survivors and back from the shortest time with no survivors, until the interval was small relative to the total treatment time (generally <30 s). The TTI was defined as the time for the first negative result. In all, 341 separate sets of 5 tubes were pressure treated (for a total of 1,705 individual tubes observed). Pressure hold time ranged from a minimum of 30 s for a 2-log CFU/g inoculum treated at 700 MPa with survivors observed, to a maximum of 40 min for a 6-log CFU/g inoculum and no survivors observed. The actual number of different pressure hold times per treatment varied, depending on how readily the endpoint criteria were attained. The smallest number of runs, each of five tubes, needed to determine TTI for a single treatment was 3 and the largest was 16.

HPP was carried out in five individual pressure chambers in the multivessel high pressure apparatus U111 (Unipress Equipment, High Pressure Research Centre, Warsaw, Poland), with the bath precooled to 4°C. The five replicate samples were pressure treated simultaneously for the appropriate pressure-time combination, with a come-up time of 70 to 80 s, and a decompression time of approximately 30 to 45 s. The samples were removed from the pressure vessel and stored at 4°C for 72 h to facilitate recovery of potentially pressure-injured cells prior to viability testing. The postpressurization hold time was based on our own (unpublished) experience with recovery of *L. monocytogenes* from pressure-treated foods.

After statistical analysis of the results from this design, an additional 14 treatments were identified with SAS PROC OPTEX (SAS Institute Inc., Cary, NC) to extend the experimental pressure range. Experimental details were as above, except that at lower pressures the times to inactivation were generally longer, and an interval of up to 2 min between the shortest time with no survivors and the longest time with survivors was considered acceptable.

Viability testing. Testing for the presence of viable cells was carried out with the FSIS method *(19),* modified to end after the examination of modified Oxford plates. (Identification on modified Oxford was considered sufficient, because pure cultures of *L. monocytogenes* were used.) For all pressure treatments, data points were recorded as the number of positive (for *L. monocytogenes*) samples out of five replicates. Different times at pressure were tested for each treatment so that a positive result (e.g., 3 of 5) and a negative (0 of 5) were obtained for each treatment. The TTI was defined as the time for the first negative result.

Statistical analysis and diagnostics. Survival analysis in Minitab Statistical Software, Release 14 for Windows (Minitab, Inc., State College, PA) was used to develop a predictive model for TTI of *L. monocytogenes* as a function of the independent variables of inoculum level, pressure level, NaCl, and sodium lactate. Values were normalized prior to model development by using the formula [(level − mean of design levels)/standard deviation of design levels], e.g., [(log inoculum level − 4)/2], [(pressure − 600)/108.0123], [(NaCl − 1.5)/0.707], [(sodium lactate − 1.25)/1.768].

The first step was a comparison of different error distributions available within Minitab. The Weibull and lognormal distributions both gave good descriptions of the data, with similar log likelihood. The log likelihood from the lognormal model was slightly larger than that from the Weibull model, so the lognormal distribution was used for additional model development.

The model development process was iterative. The analysis began with a model that was all-inclusive. This model included all four main effects (normalized as described above), inoculum level (inoc), pressure level (mpa), NaCl (nacl), and sodium lactate (nalac); two quadratic effects ($inoc^2$ and mpa^2); and the six two-

TABLE 2. *Experimental matrix used in modeling studies*

Treatment	MPa	Initial log CFU/ml[a]	NaCl (%, wt/vol)	Na lactate (%, wt/vol)
1	600	2	1.0	0.0
2	600	2	1.0	2.5
3	600	2	2.0	0.0
4	600	2	2.0	2.5
5	600	4	1.0	0.0
6	600	4	1.0	2.5
7	600	4	2.0	0.0
8	600	4	2.0	2.5
9	600	6	1.0	0.0
10	600	6	1.0	2.5
11	600	6	2.0	0.0
12	600	6	2.0	2.5
13	650	2	1.0	0.0
14	650	2	1.0	2.5
15	650	2	2.0	0.0
16	650	2	2.0	2.5
17	650	4	1.0	0.0
18	650	4	1.0	2.5
19	650	4	2.0	0.0
20	650	4	2.0	2.5
21	650	6	1.0	0.0
22	650	6	1.0	2.5
23	650	6	2.0	0.0
24	650	6	2.0	2.5
25	700	2	1.0	0.0
26	700	2	1.0	2.5
27	700	2	2.0	0.0
28	700	2	2.0	2.5
29	700	4	1.0	0.0
30	700	4	1.0	2.5
31	700	4	2.0	0.0
32	700	4	2.0	2.5
33	700	6	1.0	0.0
34	700	6	1.0	2.5
35	700	6	2.0	0.0
36	700	6	2.0	2.5
Extended design treatment				
37	450	2	1.0	0.0
38	450	2	1.0	0.0
39	450	2	1.0	2.5
40	450	2	2.0	0.0
41	450	2	2.0	2.5
42	450	2	2.0	2.5
43	450	4	1.0	2.5
44	450	6	1.0	0.0
45	450	6	1.0	0.0
46	450	6	1.0	2.5
47	450	6	2.0	0.0
48	450	6	2.0	0.0
49	450	6	2.0	2.5
50	450	6	2.0	2.5

[a] Sample size pressure treated was approximately 1.5 ml.

factor interactions (inoc × mpa, inoc × nacl, inoc × nalac, mpa × nacl, mpa × nalac, and nacl × nalac). Quadratic terms for nacl and nalac were not included because it is not possible to assess curvature of a factor with only two levels. After each analysis, the least significant factor was dropped, and the data were reanalyzed. This process was repeated until all the remaining effects were significant ($P < 0.05$).

Validation of the model in food: Australia. Three sliced RTE Australian retail poultry products, with varying NaCl concentrations, were used in challenge studies to validate the model. When pureed 1:1 (wt/vol) with water, these products spanned the range of NaCl levels used to generate the data for model development. The three products tested were chicken breast A (847 mg of sodium per 100 g), chicken breast B (1,890 mg of sodium per 100 g), and turkey breast (737 mg of sodium per 100 g). None of the products contained sodium lactate. Experiments were conducted with each product in both sliced and pureed format. For each product, the puree was split into two aliquots, one of which was supplemented with sodium lactate to a final concentration of 2.5% (wt/wt). Prior to pressure treatment, purees were inoculated to 2 or 4 log CFU/g with a *L. monocytogenes* cocktail, prepared as described previously. The sliced products were inoculated with approximately 4 log CFU per pack of *L. monocytogenes* cocktail to confirm that a minimum of a 4-log reduction of *L. monocytogenes* was achievable in product. The same five-isolate cocktail as used for the model development was used for validation experiments.

To prepare meat purees, a blender (Waring Laboratory, Torrington, CT) was surface sterilized by soaking in 25% bleach (4 g/liter of available chlorine) for 15 to 30 min before use and in between the preparation of each meat product. Approximately 40 g of sliced meat product was blended with 40 ml of sterile diluent (water or 3.9% sodium lactate solution, as required) until a smooth puree was achieved. Puree was transferred (2 ml) into sterile tubes, and was inoculated with the cocktail of *L. monocytogenes* (0.5 ml, the cocktails prepared as described previously), to achieve final concentrations of 2 or 4 log CFU/ml. The relatively large inoculation volume was to aid in even distribution of the inoculum throughout the sample.

Purees (1.5 ml) were transferred into 1-ml Nalgene cryovials for HPP and stored on ice until pressure treated (60 to 90 min). Purees were pressure treated at 600 MPa, 4°C (initial temperature) in the U111 Multivessel Apparatus for times longer and shorter than that of TTI predicted by the model. Samples were then incubated for 72 h at 4°C to facilitate recovery of potentially pressure injured cells prior to viability testing. Survival of cells after processing was determined with the viability testing procedure described previously.

Slices were individually placed in pouches (Cryovac Australia, Melbourne, Australia) and the weight (approximately 40 g) recorded. Five replicate samples were prepared for each treatment. Inoculum (10 μl of a 6-log CFU/ml cell suspension per g of meat) was massaged into the slice, as much air as possible was removed, and the pouch was heat sealed. Slices were then packed in a secondary bag containing 0.2% (vol/vol) Proxitane sanitizer to protect the high pressure unit from contamination in the event that a sample bag were to leak. The secondary bag was then heat sealed in a tertiary bag filled with ice-water slurry and immediately transferred to the high pressure unit.

Slices were processed in the 2-liter (Avure Technologies) high pressure unit at 600 MPa and ambient temperature. Five slices of each product were treated for 210 s, and the remaining five slices per product for 300 s. Samples were then incubated for 72 h at 4°C prior to testing for viability. To test for the presence of viable *L. monocytogenes,* 2 ml of sterile water per g of product was added aseptically to the slices, the bag resealed, and processed in a stomacher for 2 min. Detection of viable cells in the sample was then tested by using the method described previously.

Validation of the model in food: United States. Additional challenge studies were conducted with uncured RTE poultry products produced in an FSIS-inspected pilot plant facility in the United States. These products were formulated with varying levels of NaCl and sodium lactate, and were more representative of RTE poultry products available in U.S. retail markets.

Five formulas of two products, sliced turkey and diced chicken, were tested. On each test date, packages were aseptically opened and 25-g portions placed in vacuum pouches (Koch Supplies, Kansas City, MO). Each sample was inoculated with 250 μl of inoculum (10 μl per g of meat, the *L. monocytogenes* cocktail), prepared as described previously, to achieve initial levels of either 2 or 4 log CFU per package. Inoculated meat samples were massaged for 30 s and vacuum packaged with a Multivac A300 packaging machine (MultiVac, Wolfertschwenden, Germany). Samples were triple bagged with a sanitizer layer as described previously, and stored at 4°C for not more than 30 min prior to pressure treatment.

Samples were processed with a Quintus 35 L-600 Food Press (Avure Technologies) at ambient temperature (approximately 20°C) according to the parameters outlined in the experimental matrix. Treated samples were stored at 4°C for 72 h prior to analysis to facilitate recovery of potentially pressure injured cells. Samples were analyzed for the presence of viable *L. monocytogenes* cells according to the modified FSIS procedure described above in the ''Viability testing'' section.

RESULTS

Screening of *L. monocytogenes* isolates for their relative resistance to pressure. The 10 *L. monocytogenes* isolates pressure treated at 600 MPa (1°C) varied widely in their sensitivity to pressure (Fig. 1), with more than a 5-log difference in the reduction at a 60-s pressure hold time between the most and the least sensitive strains. Isolates 2542 and 2655 were the most resistant, with only a 1.1-log reduction achieved, followed in relative resistance by isolate 2343 (1.4-log reduction), isolate 2472 (2.2-log reduction), and isolate OSY8578 (2.5-log reduction). Isolates 2657 and 2341 were the most sensitive, with >6- and >5-log reductions in cell numbers, respectively. Similar pressure resistance screening studies were conducted by Hayman et al. *(7),* but at an initial temperature of approximately 20°C (600 MPa, 60 s, pH 6.6), and they observed an overall larger reduction in numbers after equal time at 600 MPa than we did. However, the relative resistance of the different strains was similar in both studies. Four of our five most resistant strains were in the top five of Hayman et al. and they did not test OSY 8578, our fifth most resistant strain. The four least resistant strains were the same in both studies. The five isolates most resistant to pressure (2542, 2655, 2343, 2472, and OSY8578) were used for all subsequent experiments.

Time to inactivation. Table 3 summarizes the observed TTI for each experimental treatment. In the initial factorial design, the most noticeable effect was an increase in TTI with increasing inoculum level. The NaCl and sodium lactate levels had no noticeable effect on TTI, and even pressure did not seem to consistently affect the TTI over the range of 600 to 700 MPa. This pressure range was chosen based on previous work at the Commonwealth Scientific and Industrial Research Organisation, Division of Food and Nutritional Sciences (North Ryde, New South Wales, Australia), which indicated that complete inactivation of *L. monocytogenes* could not be achieved at ≤550 MPa in a milk system in less than 20 min, and 700 MPa was the upper pressure limit of the equipment. It was possible to model the data to this point, but the resulting model was rather unsatisfying, since it indicated a maximum TTI at 650 MPa, with the TTI falling sharply as pressure both increased and decreased. Therefore, it was decided to expand the experimental design to include treatments at 450 MPa (Table 2). In the additional treatments at 450 MPa, the TTI was noticeably longer than at the higher pressures, which was logical. Figure 2 illustrates the relationship between TTI and pressure. In all, over 1,700 individual data points were used to estimate TTI across all treatments.

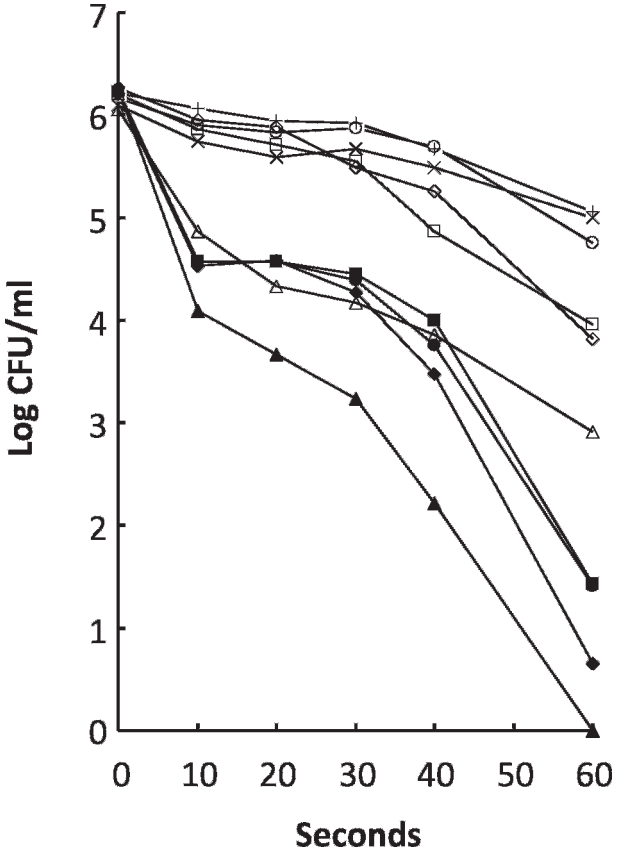

FIGURE 1. Listeria monocytogenes *pressure treated in tryptic soy broth (pH 6.6) at 600 MPa, 1°C (initial sample temperature). Experiments were conducted in replicate. The limit of detection was 10 CFU/ml. Strains: +, FRRB2542; ×, FRRB2655; ○, FRRW2343; □, FRRB2472; ◇, OSY8578; △, FRRW2345; ●, FRRW2340; ■, FRRW2342; ♦, FRRW2341; ▲, FRRB2657.*

All treatments were able to achieve complete inactivation of *L. monocytogenes,* with no viable cells recovered from an enrichment method after pressure treatment. In a proportion of the cases, it was hard to discern clearly the TTI because of sporadic positive samples that were observed at times after the first appearance of five of five tubes with no recoverable *L. monocytogenes*. Though these were believed to represent false-positive results, it did not seem reasonable to ignore the presence of these results. In these cases, the TTI was recorded as the shortest time when

TABLE 3. *Observed time to complete inactivation of* Listeria monocytogenes, *pressure treated at 4°C (initial temperature) in tryptic soy broth supplemented with sodium chloride and sodium lactate*

Design	Inoculum level (log CFU/ml)	Pressure (MPa)	Time to inactivation (min) in:			
			1% (wt/vol) NaCl		2% (wt/vol) NaCl	
			0% (wt/vol) lactate	2.5% (wt/vol) lactate	0% (wt/vol) lactate	2.5% (wt/vol) lactate
Original	2	600	2.0	1.5	2.0	2.0
	2	650	2.5	2.5	2.0	2.0
	2	700	1.5	1.5	1.5	1.5
	4	600	4.5	2.5	3.0	4.0
	4	650	3.0[Ca]	3.0[C]	3.5	4.0
	4	700	3.0[C]	2.0[C]	3.0[C]	1.5[C]
	6	600	7.5	7.0[C]	6.5	3.5[C]
	6	650	9.5	8.5	12.0	4.0[C]
	6	700	8.0	5.0[C]	8.0[C]	5.0[C]
Extended	2	450	7.5	4.0[C]	5.0[C]	7.5
	2	450	6.0	NT[b]	NT	5.0
	4	450	NT	9.5	NT	NT
	6	450	13.0[C]	13.0[C]	14.0	16.0
	6	450	14.0	NT	15.0	12.0[C]

[a] The letter C indicates a censored observation recorded at the first time all five tubes were negative. Censoring indicates that one or more tubes showed positive at a later time point.
[b] NT, not tested.

all five tubes were negative, but the time was also shown as a ''censored'' observation, reflecting the uncertainty about what happened after that point.

Model development and diagnostics. The final model was

$$\ln \mathrm{TTI} = 1.3806 + (0.6942 \times \mathrm{inoc}) - (0.2416 \times \mathrm{mpa}) + (0.1942 \times \mathrm{inoc} \times \mathrm{mpa}) + 0.2934 \times \mathrm{mpa}^2)$$

Where ln TTI is the natural logarithm of time to inactivation in minutes.

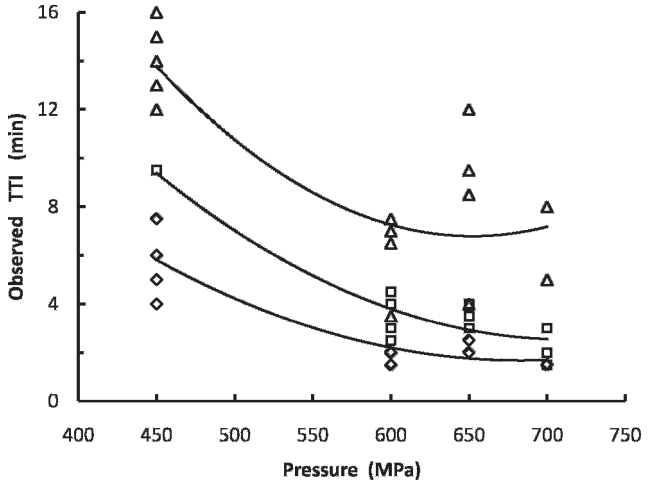

FIGURE 2. *Observed time to inactivation (TTI) at different pressures in tryptic soy broth (pH 6.3) inoculated with 2.0 (◇), 4.0 (□), or 6.0 (Δ) log CFU/ml and with an initial temperature of 4°C. Data from all tested levels of NaCl (1 and 2% [wt/vol]) and sodium lactate (0 and 2.5% [wt/vol]) are included but not separately identified.*

Details of the regression output are shown in Table 4. The overall log likelihood was −41.043, and 17 of the 50 observations were censored. For diagnostic purposes, the plot of predicted TTI versus observed TTI ($R^2 = 0.8624$) (Fig. 3) and a normal probability plot of residuals (not shown) were examined and no unusual patterns or anomalies were detected. Since NaCl and sodium lactate concentrations did not have statistically significant effects on TTI, the model contains no terms for these variables. Hence, TTI can be illustrated with reference simply to pressure and initial load of *L. monocytogenes*. The central prediction of TTI is shown in Figure 4 as a contour plot spanning the experimental range.

Validation in foods: Australia. Examples of validation results for Australian pureed product are shown in Table 5. By using the model, the predicted TTI for samples pressure treated at 600 MPa and with an initial cell concentration of 2 log CFU/ml was 119 s. In purees of Australian retail RTE meat products treated for 50 s, all of the samples retained recoverable *L. monocytogenes* after pressure treatment, as predicted. Two purees showed total inactivation of *L. monocytogenes* after 110 s, and one more showed total inactivation after 140 s of pressure treatment. No *L. monocytogenes* was recovered from any sample tested after being held for 180 s at pressure. Overall, the agreement between predicted and observed TTI was good. In a total of 12 trials (five replicate tubes per trial), 9 trials resulted in survivors or no survivors exactly as predicted, 2 trials resulted in no survivors where survivors were predicted, and only 1 trial yielded recoverable *L. monocytogenes* where none were predicted.

The predicted TTI for pureed samples pressure treated at 600 MPa and with an initial cell concentration of 4 log

TABLE 4. *Regression results from survival analysis in Minitab by using a lognormal error distribution*[a]

Predictor	Coefficient	SE	*z*	*P* value	Normal 95% CI	
					Lower	Upper
Intercept	1.38063	0.048591	28.41	0.000	1.28539	1.47587
inoc	0.694163	0.035919	19.33	0.000	0.623762	0.764563
mpa	−0.24165	0.056086	−4.31	0.000	−0.35157	−0.13172
inoc × mpa	0.1942	0.038515	5.04	0.000	0.118711	0.269688
mpa^2	0.293434	0.059051	4.97	0.000	0.177697	0.409171
Scale	0.180033	0.021325			0.142733	0.227079

[a] Of 50 total observations, 17 were ''right censored'' at the time when total inactivation was first observed. Overall log likelihood = −41.043.

CFU/ml was 239 s. Complete inactivation of *L. monocytogenes* at 600 MPa was observed after 210 s in five of the six purees examined, and after 360 s in the sixth (Table 5). It should be noted that the NaCl concentration of 3% in the sixth puree was outside the design range for the model, where predictions would be expected to be less reliable (even though, across the experimental design range, salt had no significant effect). From the total of 12 trials (five replicate tubes per trial), the results were as predicted in 6 trials, no survivors were seen where survivors were predicted in 5 trials, and survivors were seen where none were predicted in only 1 trial (Table 5).

The predicted TTI for 4 log CFU *L. monocytogenes* per pack of sliced Australian poultry product pressure treated at 600 MPa was 239 s. In two products, complete inactivation of *L. monocytogenes* was observed after 210 s of pressure treatment, slightly shorter than the predicted 239 s (Table 6). In one product, viable *L. monocytogenes* were recovered after 210 s of pressure but not after 300 s. From a total of six trials (five replicate packs per trial), four trials gave results exactly as predicted, and two trials showed no survivors where survivors were expected (Table 6).

Validation in foods: United States. Examples of validation results for U.S. sliced product are shown in Table 7. The predicted TTI for *L. monocytogenes* in sliced turkey samples at an initial 2 log CFU per pack was 229 s at 500 MPa and 104 s at 650 MPa. Complete inactivation of *L. monocytogenes* was observed after 195 s at 500 MPa and 90 s at 650 MPa for all turkey formulations tested. From a total of 20 samples tested, 10 samples resulted in no survivors as predicted, and 10 gave no survivors where survivors were expected.

The predicted TTI for 4 log CFU *L. monocytogenes* per pack of sliced poultry product was 384 s at 500 MPa and 227 s at 650 MPa. Complete inactivation was observed after 345 s at 500 MPa and 180 s at 650 MPa (Table 7) for all turkey formulations and for chicken formulated with 1 or 2% NaCl and 2.5% lactate. As predicted, viable *Listeria* were recovered after 315 s at 500 MPa in chicken formulated with varying NaCl levels and 1.25% or no lactate. *Listeria* was also recovered from samples pressure treated for 150 s at 650 MPa, as predicted, in chicken formulated with 1.55% NaCl and 1.25% lactate, and 2% NaCl with no lactate (Table 7). For 40 samples tested, 22 resulted in survivors or no survivors as predicted, 15 gave no survivors where survivors were predicted, and only 3 samples resulted in survivors where none were expected.

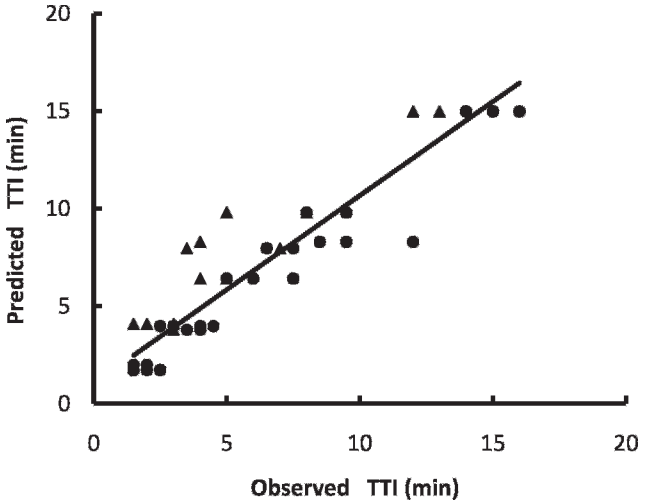

FIGURE 3. *Plot of predicted time to inactivation versus observed time to inactivation for pressure-treated* Listeria monocytogenes. ●, *Uncensored observations;* ▲, *censored observations.* $R^2 = 0.8624$.

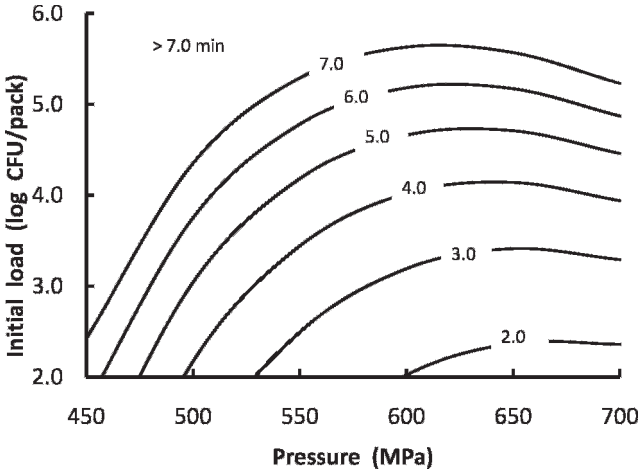

FIGURE 4. *Illustration of predicted time to inactivation (TTI) in minutes for different initial loads of* Listeria monocytogenes *subjected to different pressure levels. Contours are shown at 1-min intervals between 2 min (bottom right) and 7 min (top left).*

TABLE 5. *Examples of viability scores for pureed Australian product*[a]

Product	NaCl (%, wt/wt)	Na lactate (%, wt/wt)	Predicted TTI (s)	No. of tubes positive for growth after treatment for[b]: 210 s	300 s	360 s
Chicken	1.3	0	239	0/5[c]	0/5[d]	
	1.3	2.5	239	0/5[c]	0/5[d]	
	3.0	0	239		3/5[e]	0/5[d]
	3.0	2.5	239	0/5[c]	0/5[d]	
Turkey	1.2	0	239	0/5[c]	0/5[d]	
	1.2	2.5	239	0/5[c]	0/5[d]	

[a] Samples inoculated with 4 log CFU/ml *Listeria monocytogenes* cocktail and pressure treated at 600 MPa, 4°C (initial temperature).
[b] Via enrichment: limit of detection greater than 1 CFU in a 25-g sample (FSIS).
[c] No survivors where survivors predicted.
[d] Survivors as predicted.
[e] Survivors where no survivors were predicted.

When looking across all the validation tests, we see that from 210 individual samples tested, 122 (58.1%) gave survivors (29 [13.8%]) or no survivors (93 [44.3%]) exactly as predicted, 81 (38.6%) gave no survivors where survivors were predicted, and in only 7 (3.3%) were unexpected survivors seen (Fig. 5). This strongly suggests that the model is practically useful across the range of conditions tested and is likely to be somewhat conservative.

One note of caution is warranted. The model contains a quadratic pressure term and implies that the shortest TTI is seen at 680 MPa for a 2-log inoculum, falling to 644 MPa for a 4-log inoculum and 609 MPa for a 6-log inoculum. While it is counterintuitive that the minimum TTI is seen below the maximum pressure in the experimental design range, it is of little practical consequence at a commercially realistic pressure as shown by the validation results at 600 MPa. However, extrapolation beyond the design range, never to be undertaken without the utmost caution, is strongly counterindicated in this case. Predictions made above 700 MPa would be expected to be safe, but giving misleadingly long process times.

DISCUSSION

A postpackaging lethality process equivalent to a 4-log reduction of *L. monocytogenes* would meet ICMSF illustrative guidelines *(8)* for protecting public health and is consistent with published risk assessment studies *(22)*. Several authors have shown that HPP can give substantial reductions in viable *L. monocytogenes (3, 10, 12, 13, 18),* though considerable curvature or tailing was observed in survival curves determined in food systems *(3, 18)*. The tailing phenomenon introduces considerable uncertainty into the interpretation of lethality curves, including the question of whether complete inactivation of *L. monocytogenes* can be achieved with high pressure. The approach taken here was to process to complete inactivation from different initial levels of *L. monocytogenes*. The results clearly demonstrate that high pressure treatment can achieve complete inactivation of *L. monocytogenes* in broth media from starting levels as high as 6 log CFU/ml (1.5 ml treated) and in uncured RTE cooked meats or cooked meat purees from starting levels up to 4 log CFU per pack (the highest level tested). There is no reason to believe that higher levels could not be inactivated by high pressure applied for longer periods.

The model developed in this study indicates that pressure treatment at 600 MPa (the current maximum realistic commercial pressure), with an initial product and process temperature of 4°C, will eliminate up to 4 log CFU per pack of *L. monocytogenes* in less than 4 min (and less than 5 min, even when predicting at the upper 95% confidence interval of the model).

The FSIS has listed HPP as a potential postlethality treatment that may be used to reduce or eliminate *L. monocytogenes* in RTE meat and poultry products, if included in the HACCP plan and the effectiveness is

TABLE 6. *Viability scores for sliced Australian product*[a]

Product	NaCl (%, wt/wt)	Predicted TTI (s)	No. of tubes positive for growth after treatment for[b]: 210 s	300 s
Chicken	2.1	239	0/5[c]	0/5[d]
Shaved chicken breast	4.8	239	5/5[d]	0/5[d]
Turkey	1.9	239	0/5[c]	0/5[d]

[a] Product was inoculated with 4 log CFU per pack of *Listeria monocytogenes* cocktail and pressure treated at 600 MPa. Inoculated product in a sealed bag was overwrapped with another bag containing ice-water slurry. The whole was processed in a vessel at ambient temperature.
[b] Via enrichment: limit of detection greater than 1 CFU in a 25-g sample (FSIS).
[c] No survivors where survivors predicted.
[d] Survivors as predicted.

TABLE 7. *Examples of viability scores for U.S. poultry products*[a]

Product	NaCl (%, wt/wt)	Na lactate (%, wt/wt)	Treatment time (s)	Result (for growth)[b]	
				Predicted	Observed
Turkey	1.0	2.5	180	Positive	Negative
	1.0	2.5	270	Negative	Negative
	1.0	0	180	Positive	Negative
	1.0	0	270	Negative	Negative
	1.5	1.25	180	Positive	Negative
	1.5	1.25	270	Negative	Negative
	2.0	2.5	180	Positive	Negative
	2.0	2.5	270	Negative	Negative
	2.0	0	180	Positive	Negative
	2.0	0	270	Negative	Negative
Chicken	1.0	2.5	150	Positive	Negative
	1.0	2.5	270	Negative	Negative
	1.0	0	150	Positive	Negative
	1.0	0	270	Negative	Negative
	1.5	1.25	150	Positive	Positive
	1.5	1.25	270	Negative	Negative
	2.0	2.5	150	Positive	Negative
	2.0	2.5	270	Negative	Negative
	2.0	0	150	Positive	Positive
	2.0	0	270	Negative	Negative

[a] Samples inoculated with 4 log CFU per pack *Listeria monocytogenes* cocktail and pressure treated at 650 MPa, 4°C initial product temperature, pressure vessel at ambient temperature. Predicted time for complete inactivation is 227 s.
[b] Via enrichment: limit of detection greater than 1 CFU/g in a 25-g sample (FSIS).

validated *(21)*. The FSIS considers a 2-log or greater reduction of *L. monocytogenes* to be a ''higher-level'' treatment, qualifying for relatively less FSIS sampling. The model predicts elimination of 2 log CFU per pack in 2 min, and elimination of 3 log CFU per pack in 3 min, representing a more stringent standard than a 2-log reduction. These predictions are consistent with process times used commercially in certain U.S. meat processing operations and give confidence in the efficacy of those operations.

Comparing the screening results of Hayman et al. *(7)* with our own (Fig. 1) leads us to believe that faster inactivation would be achieved with a starting temperature closer to 20°C. However, the difference may not be important in meat. Chen et al. *(4)* showed no significant difference between lethality of HPP (400, 500, and 600 MPa) applied at 4 or at 21°C against *L. monocytogenes* cocktails inoculated into ground chicken that had been previously sterilized by autoclaving. Either way, the ability to achieve rapid and complete inactivation of *L. monocytogenes* at the lower temperature makes the process more compatible with the operation of a commercial meat processor. We anticipate that the model presented here, along with the supporting validation data, will make a significant contribution to establishing appropriate processing criteria and for the effective use of HPP as a postlethality treatment for *L. monocytogenes*.

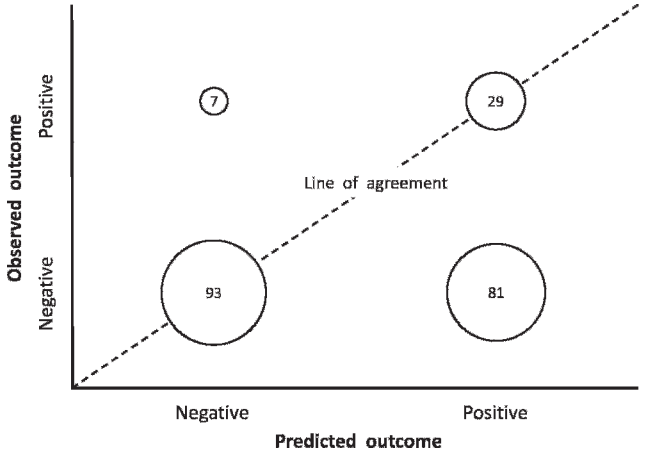

FIGURE 5. *Bubble plot summarizing observed versus predicted* Listeria monocytogenes *growth responses from all 210 validation samples.*

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