



Development of a Logistic Regression Plot for Predicting the Probability of Achieving a 7-Log Reduction of *Escherichia coli* O157:H7 During Beef Slow-cooking Processes

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Introduction

- 9 CFR § 318.17 requires that any cooked beef products must be processed to ensure ≥ 6.5 log reduction of *Salmonella*, as well as the reduction of other pathogens. Failure to achieve this lethality renders the product adulterated [1].
- As of 1994, *Escherichia coli* O157:H7 has adulterant status in beef [2], so cooking must ensure that survival of either pathogen is unlikely.
- Beef roasts (both whole and restructured) are commonly slow-cooked to impart desirable characteristics. However, slow cooking may enhance the heat resistance of organisms [3], in particular *E. coli* O157:H7.
- Government guidelines caution against slow cooking processes which maintain the temperature of product between 10°C and 54.4°C for more than 6 hours [4].
- Though USDA provides cooking guidelines, processors are also able to use other scientifically validated methods to demonstrate that their slow cooking processes meet the lethality performance standards [4].
- Many predictive tools have been created, but are based on broth studies and do not reflect growth or survival conditions in real product [5].
- The objective of this study was to develop a logistic regression plot to be used in creating a predictive tool for validating beef roast slow-cooking procedures.

Methods

Inoculum Preparation

- Five strains of *Escherichia coli* O157:H7 were grown anaerobically for 24 hrs at 35°C, re-suspended in Butterfield's Phosphate Diluent (BPD; Nelson-Jameson, Marshfield, WI, USA), and combined to make the inoculum.

Sample Preparation

- Small bags of 25 g lean (6-8% fat) ground beef were first inoculated with 1.0 mL manually dispersed cell suspension and then cooked according to one of four simulated cook schedules, based on a standard schedule (Figure 1).
- During 12 trials, samples were taken sequentially 9 times per trial (n=108). When sampling, each bag was held on ice for 30 sec to stop cooking.
- The bags were surface-sterilized with 70% EtOH, aseptically cut to remove the wire closure, and everted into a Whirl-pak filter bag (Nasco, Fort Atkinson, WI, USA) followed by dilution (99 mL BPD) and stomaching (30 sec at medium speed).

Cell Enumeration

- The stomached homogenate was diluted in BPD and spread-plated onto nutrient agar (NA; BD Difco, Sparks, MD, USA).
- After 60 min incubation at 35°C for injury-repair, the plates were overlaid with modified eosin methylene blue agar composed of lactose free EMB (BD BBL, Sparks, MD, USA) with added Sorbitol and NaCl. After a further 24 hrs of incubation at 35°C, *E. coli* O157:H7 colonies were counted.

Analysis

- As shown in Figure 2, the cumulative process lethality (F-value) for each sampling time was determined using a worksheet provided by the American Meat Institute [6] using a previously determined z-value (11.4°F*) and reference temperature of 130 F.
- Logistic Regression analysis of all log CFU vs. F-value data in R.2.5.0 statistical software (R Foundation for Statistical Computing, Vienna, AUT) was then used to plot the probability of a ≥ 7 -log₁₀ reduction of *E. coli* O157:H7 against the cumulative process F-value (Figure 3).

Results and Discussion

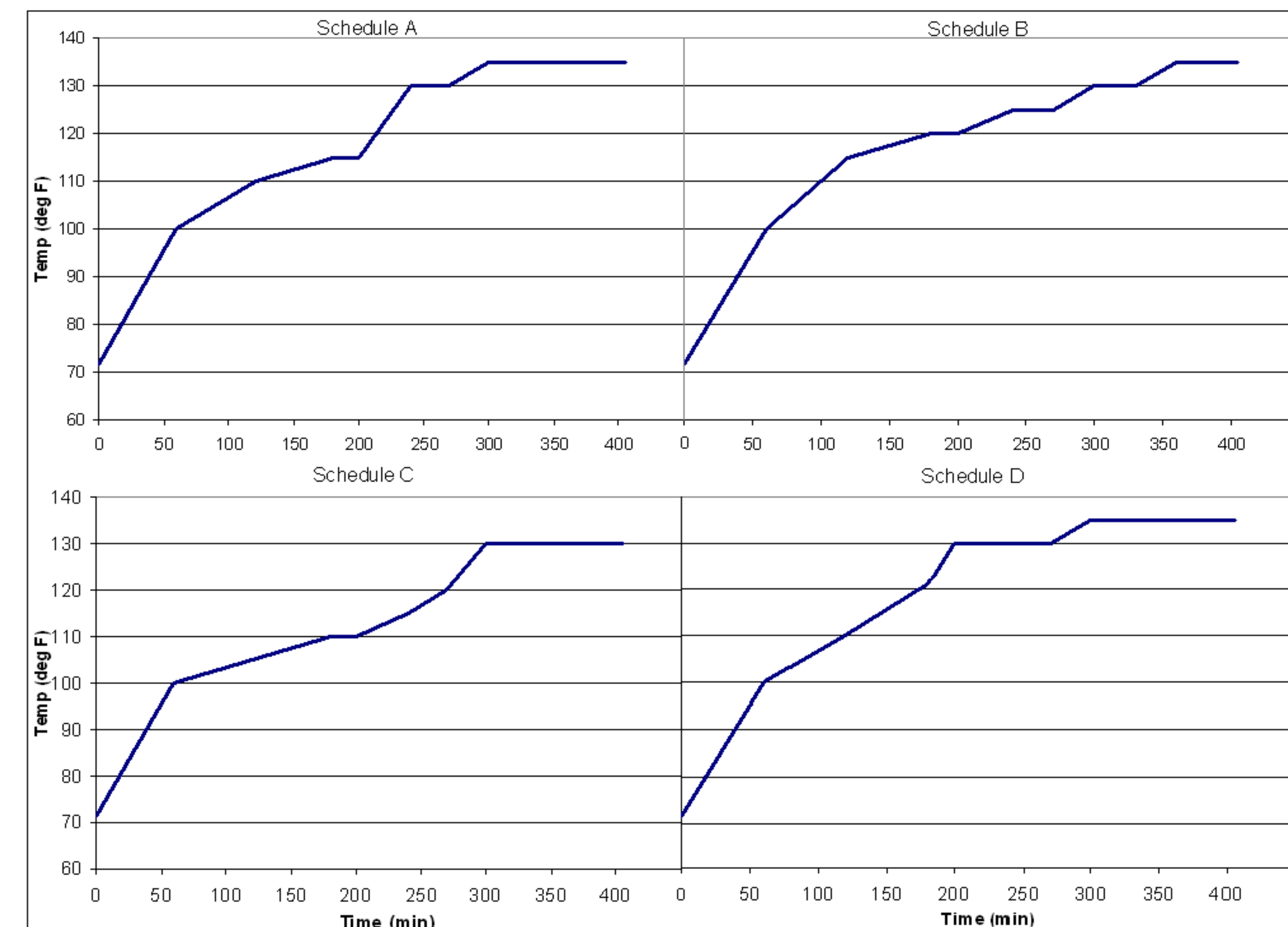


Figure 1: Modified 405-min cook schedules used for plot development. Schedule A: standard schedule for cooking beef roast interior to 135°F* - medium rare finish. Schedule B: standard schedule with slower come-up time (modified A). Schedule C: 130 F maximum internal temperature. Schedule D: sub-lethal hold (121°F) for 5 min before reaching 135°F. When the temperature was below 130°F for at least 3 hrs, *E. coli* O157:H7 grew, although the growth was not statistically significant.

*Note: Fahrenheit temperatures are the standard in the meat industry and are used in reference to industry practices.

PROCESS LETHALITY DETERMINATION

z = 11.4°F T ref = 130°F Product name: Ground Beef, Schedule A Date: Jan. 14, 2008

Time (min)	Core Temp (°F)	F-value (min)
0	62	0
20	62.4	0.00
40	69.8	0.04
60	88.7	0.09
80	109.7	0.30
100	109.6	0.62
120	110.7	0.96
140	114.6	1.79
160	114.6	2.89
180	115.2	3.59
200	130	14.08
220	129.6	33.24
240	129.6	51.86
260	129.7	70.58
280	134.6	99.08
300	134.5	150.56
320	134.5	199.89
340	134.5	249.18
360	134.5	298.51
380	134.5	348.75
405	134.5	406.14

Instructions:

1. Enter z and T reference values from challenge studies on your product, or reference values from the literature that properly represent your product.
2. Enter in-process times and temperatures in col. B and C (overwrite example data).
3. Total lethality will be the final computed cumulative F value.

Definitions:

D-value: The time (in minutes) at an associated T ref required to kill 90% of the selected microorganism; a one log reduction.

z-value: The number of degrees F to change the D-value by a factor of ten.

F-value: The process lethality. The equivalent time of heating at a reference temperature.

Note: This model is a tool for calculating F-values. To ensure correct results, the proper z, T-ref, and D-values for each product and organism must be used.

Let Rate Om ih

where $\frac{F}{z}$

$$CPE_2 = F_1 + \frac{10^{-10}}{z(t_2 - t_1)}$$

Figure 2: The AMI Foundation lethality worksheet (above). Using the calculation (left) the worksheet determines the lethal rate per min [7], as well as the cumulative lethal rate (F-value), based on entered time (t) and temperature (T) data, a set z-value (11.4°F) and reference temperature (T_r: 130°F). Temperatures were monitored every min.

Logistic Regression Curve

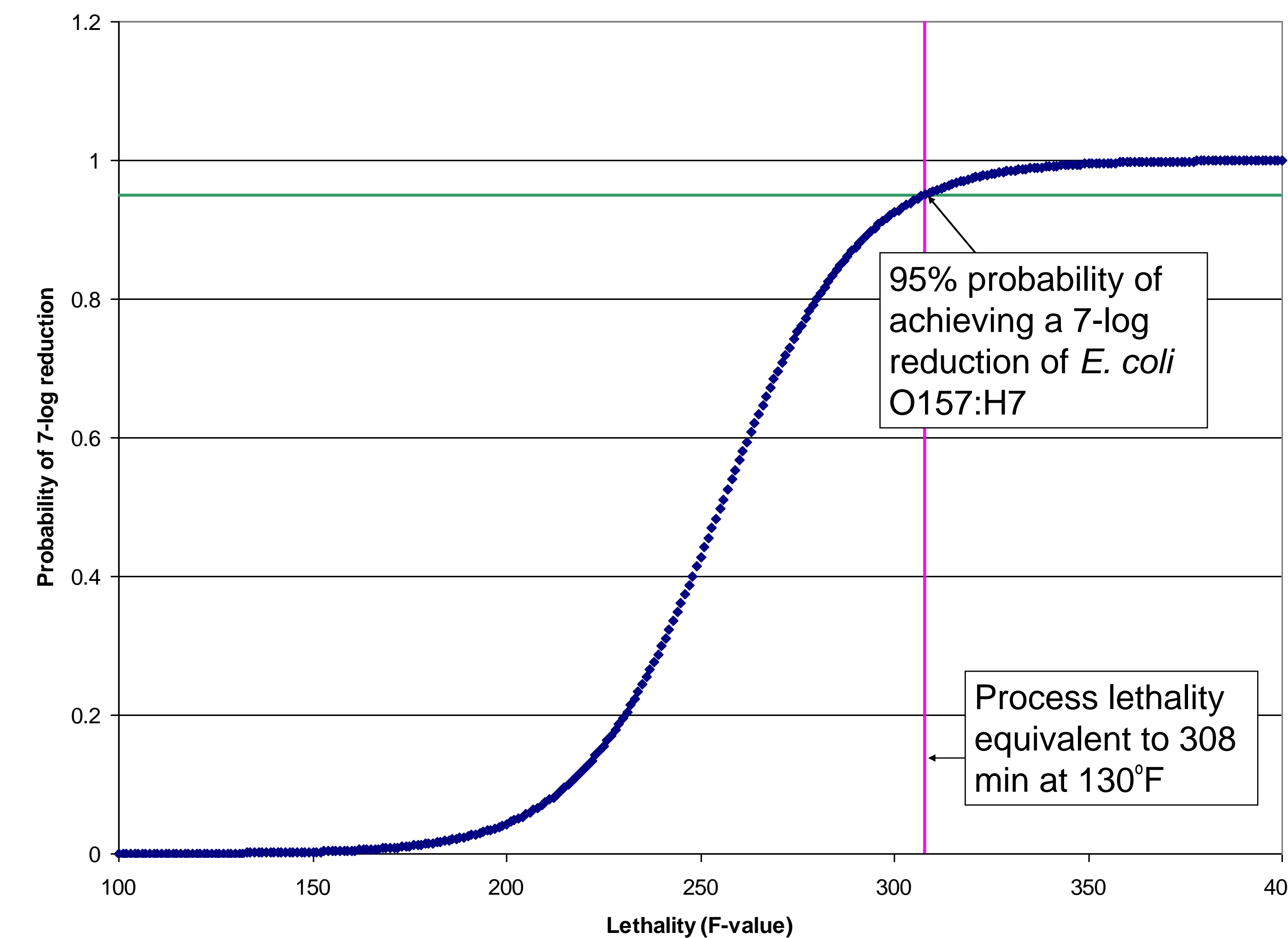


Figure 3: Logistic regression curve showing the probability that a given cumulative process lethality (F-value) during slow-cooking would result in a ≥ 7 -log reduction of *E. coli* O157:H7.

- The logistic regression curve was developed using a reference temperature of 130 F, and a z-value of 11.4 F - determined based on isothermal inactivation studies at 130°, 140°, and 150°F.
- Only samples exposed to F-values between 150 and 350 min were used to develop the curve. Below 150 min, there was little likelihood of achieving a ≥ 7 -log reduction, and above 350 min, a ≥ 7 -log lethality was achieved every time.

	Lethality <308 min.	Lethality ≥ 308 min.
<i>E. coli</i> O157:H7 kill < 7.0 log	ACCURATE (a) 85/108 (b) 4/12 (c) 30/36	FAIL-DANGEROUS (a) 0/108 (b) 0/12 (c) 0/36
<i>E. coli</i> O157:H7 kill ≥ 7.0 log	FAIL-SAFE (a) 8/108 (b) 2/12 (c) 1/36	ACCURATE (a) 15/108 (b) 6/12 (c) 5/36

Figure 4: Accuracy of a critical F-value of 308 min at predicting ≥ 7 -log reduction of *E. coli* O157:H7 in ground beef. Row (a) contains all sample results from the original 12 trials used in developing the logistic regression curve. Row (b) contains only the final sample (cooking was "done") for each of these 12 trials, and row (c) contains all samples from a subsequent set of 4 trials done to validate the logistic regression curve.

- Using $F \geq 308$ min as a guide for obtaining sufficient lethality in slow-cooking of ground beef provided accurate or fail-safe predictions for all samples and processes. In no cases did reaching this F-value fail to achieve ≥ 7 -log reduction in *E. coli* O157:H7, i.e. 0% fail-dangerous (red numbers).
- Only about 7% of samples exhibited fail-safe results (≥ 7 -log reduction obtained at an $F < 308$ min, pink numbers).

Conclusions

- Achieving an F-value of ≥ 308 min resulted in adequate destruction of *E. coli* O157:H7 in ground beef (n=108).
- FSIS guidelines specify only 121 min at 130°F for a 6.5-log reduction in *Salmonella* [4], but the guidelines don't account for come-up time or potential differences between *E. coli* O157:H7 and *Salmonella*. Thus our results are not directly comparable to the USDA guideline.
- We observed that a slow cook process could enhance heat resistance of *E. coli* O157:H7 or allow growth at low temperatures during come-up periods. The logistic regression curve accounts for these possibilities.
- Use of a critical F-value of ≥ 308 min in predicting process lethality resulted in no fail-dangerous predictions and only about 8% fail-safe predictions.
- The logistic regression curve method can be used to create a predictive tool for determining the safety of slow-cooked beef roasts. However, varying z-values and reference temperatures will alter the target F-value, and should be properly selected prior to safety analysis.

References

- [1] 9 C.F.R. § 318.17 (2008).
- [2] USDA. 2004. FSIS Directive 10,010.1 Revision 1.
- [3] Mackey, B., Derrick, C. 1986. *J. Appl. Bacteriol.* 61, 389-394.
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- [5] Tamplin, M. 2002. *J. Food Protect.* 65, 1535-1540.
- [6] American Meat Institute Foundation, Washington, DC, USA <http://www.amif.org/ht/d/sp/i/26870/pid/26870>
- [7] Scott, J., Weddig, L. Principles of Integrated Time-Temperature Processing. 1998 Meat Industry Research Conference, Philadelphia, PA.

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