PROJECT FINAL REPORT
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Project: 02-226

Project Title: *Improving the Utilization of Microbial Pathogen Computer Models for Validating Thermal Processes in the Meat Industry*

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Organization: Michigan State University

Project Duration: March 2003 to February 2005 (extended to June 30, 2005)

Project Cost: $69,991

Executive Summary:

Federal regulations require the meat and poultry industry to validate the safety of ready-to-eat products. However, existing tools for calculating process lethality do not account for all of the relevant variables that might affect pathogen survival, and the acceptability of these tools for regulatory compliance has been recently questioned. Therefore, the overall goal of this project was to improve the tools used by the industry to ensure that thermal processes are meeting the USDA-FSIS lethality performance standards for ready-to-eat products. The specific objective was to improve the AMI Process Lethality Spreadsheet (AMI-PLS) by adding a user-friendly "front-end" that accounts for the effects of key product factors (e.g., species, fat content) on the thermal inactivation parameters for *Salmonella*.

From a variety of sources, 58 poultry data sets and 28 beef data sets were used to parameterize a complete secondary model for log-D, including all first-order, second-order, and two-term interaction terms, with temperature, fat content, and moisture content as independent variables. The R^2 and root mean squared error (RMSE) for the poultry model were 0.97 and 0.08518 log(min), respectively; the R^2 and RMSE for the beef model were 0.97 and 0.1365 log(min), respectively. These models were integrated directly into the AMI-PLS, and a user-friendly "input box" was added, so that the user inputs the product characteristics, and the AMI-PLS calculates and utilizes product-specific D-values in the lethality calculations. The modified AMI-PLS also generates confidence intervals (±95%) for direct prediction of lethality calculations (i.e., log reductions). The results demonstrate that the D-value for *Salmonella* in meat products could be successfully modeled in a general way, as a function of temperature, fat content, and moisture content for a given meat species. However, the confidence intervals (CI) for the D-value (and resulting lethality calculations) are still quite wide, because of the relatively large RMSE. Therefore, more data sets and/or advanced statistical simulations are needed to narrow the CI, to improve model accuracy, and to validate the model with independent data before the enhanced version of the AMI-PLS will be ready for distribution.

PROJECT OBJECTIVE:

To improve the AMI Process Lethality Spreadsheet (AMI-PLS) by adding a user-friendly "front end" that accounts for the effects of key product factors (e.g., species, fat content, and pH) on the microbial model parameters.

METHODS

Data

Data Source. Previous work (before 2004) was evaluated in the general area related to the project objective - product factors affecting thermal resistance of *Salmonella* in different kinds of meat (poultry, beef and pork). Most related studies were identified through ComBase (*1*), published studies (*3-9*), and in-house research data.

Data Selection. Data were selected only when they met the following requirements: 1) temperature of 50-70°C; 2) experimental methods stated clearly; 3) fat content and moisture content analyzed; 4) D-value reported or could be calculated from the data; and 5) good correlation (R^2>0.85) for logN vs. time in the data. After this screening, data from 18 different studies were selected for further analysis. A total of 58 and 28 D-values were included for poultry (turkey + chicken) and beef, respectively.

Model

Based on the temperature, fat content, and moisture content, the complete secondary model was selected by using back elimination (α =0.05). The independent variables were fat content, moisture content, and temperature. The dependent variable was logD. The model was:

$$LogD = a + b_{11}T + b_{12}F + b_{13}M + b_{21}TF + b_{22}TM + b_{23}FM + b_{31}T^2 + b_{32}F^2 + b_{33}M^2 + e$$

where D is the D value (min); T is temperature (°C); F is the fat content (%); and M is the moisture content (%).

AMI Spreadsheet Improvement

Currently, there are three key limitations to the AMI-PLS:

1. The *user* must provide the D and z values for *Salmonella* at a reference temperature (or, as is typical, use the example values, with unknown validity for a given product). Obviously, most users do not have the capacity to generate thermal inactivation parameters that are specific to their products.
2. The spreadsheet does not generate confidence intervals for the predicted lethality, thereby giving a false impression of absolute/deterministic accuracy for the resulting values.
3. The spreadsheet does not directly calculate log reductions for a given process, requiring the user to calculate log reductions from the F-values generated by the spreadsheet (which is not normal pre-existing knowledge for a typical user).

Our modification of the AMI-PLS includes: 1) development of a user-friendly front-end (programmed via Visual Basic) for input of product attributes; 2) integration of the new generalized model for the D-value; and 3) direct calculation of log reduction, and the confidence interval for those calculations. The front-end asks the user for the product species, product type, and product composition (fat, moisture, and pH). After the user enters this information and clicks the "submit" button, the parameters for the logD model are calculated (in the background) and are imported directly into the spreadsheet. According to the data input, the spreadsheet then reports the reference D-value at 62.8°C (±95% confidence interval), and the accumulated log reduction at each processing time (±95% confidence interval).

RESULTS & DISCUSSION

Data Analysis. *Salmonella* Senftenberg is known to be significantly more thermally resistant than other *Salmonella* serovars, but it is seldom the source of outbreaks related to meat and poultry. Inclusion of *S.* Senftenberg in thermal inactivation studies significantly skews the results, when compared to other studies that do not include this serovar. Therefore, data sets that included *Salmonella* Senftenberg were excluded from this study, in order to start with original data that represented serovars and cocktails with similar inherent heat resistance. Also, because the research methods were different from one laboratory to the other, and the range of D-values and z-values were large, some outlier data (where predicted minus actual logD was greater than 0.3 log[min]) were excluded, due to the need for more data to verify/corroborate those values. The data used for the model are listed in Table 1 (poultry, 5 different studies from 4 researchers, 58 data sets) and Table 2 (beef, 3 different studies from 3 researchers, 28 data sets). The ranges of temperature, fat content, and moisture content for poultry were 55-65°C, 1-14.2%, and 67-75% respectively; the ranges of temperature, fat content, and moisture for beef were 55-65°C, 4.8-24%, and 57-72.4% respectively.

Model Selection. The final model for poultry was:

$$\log D = -92.589 + 0.750F + 1.633M + 1.240T - 0.0035F^2 - 0.0101M^2 - 0.01T^2 - 0.0099FM + 0.0024MT$$

The R^2 was 0.97, and observed vs. predicted Log D is shown in Figure 1. The root mean squared error (RMSE) was 0.08518 log(min).

The final model for beef was:

$$\log D = -237.59 + 2.307F + 3.309M + 4.086T - 0.0048F^2 - 0.0375FT - 0.0569MT$$

The R^2 was 0.97, and observed vs. predicted Log D is shown in Figure 2. The RMSE was 0.1365 log(min).

AMI Spreadsheet Improvement. The user-friendly front-end (Figure 3) includes the product species selection, product type selection, and product parameters input. After inputting this information, clicking the submit button leads the user to the spreadsheet (Figure 4). The data currently included in the model encompass only beef and poultry, with no variation in product structure (e.g., whole vs. ground samples), and no meaningful variation in pH. Therefore, other species and product structures are currently non-active choices in the input box, and the pH input is merely returned in the output worksheet.

The other current limitation with the model+spreadsheet is that the confidence intervals (±95%) for the predicted log reductions are still relatively wide. For example, when we input a particular time-temperature data set into the spreadsheet, the predicted log reduction was 7.9;

however, the 95% confidence interval was 5.3 to 11.7 log reductions. It is important to recognize two points surrounding this issue. First, the current version of the AMI-PLS generates a single value for process lethality, which fails to inform the user of the inherent uncertainty in any microbiological data and associated model predictions; therefore, it is important to include this type of information in future versions of the tool. Secondly, any model aimed at achieving universal validity will inherently have a relative wide confidence band; however, our goal is to have the most accurate model possible, with the best estimates of the confidence intervals that we can generate. Overall, however, we believe that the confidence bands resulting from the models reported in this study are still too wide, and our future work will aim at improving this result.

CONCLUSIONS

According to the data analysis and modeling, the D-value for thermal inactivation of *Salmonella* in meat and poultry products could be successfully modeled in a general way, as a function of temperature, fat content, and moisture content. However, further research is needed to validate the model with independent results. Additionally, the confidence intervals (CI) for the D-value (and resulting lethality calculations) are still quite wide, because of the relatively large RMSE. Therefore, more data sets and/or advanced statistical simulations are needed to narrow the CI, to improve model accuracy, and to validate the model with independent data before the enhanced version of the AMI-PLS will be ready for distribution.

IMPACT

This grant has funded two years of a Ph.D. student in food science, with a focus on microbial safety of meat and poultry products. This project forms the core of the student's dissertation, for which the final year is being supported by leveraging funding from other sources. The student has authored and presented a paper on this project at ICoMST 2005 in Baltimore, Maryland, and will be refining and submitting that work shortly to a peer-reviewed journal for publication.[2] The generalized model and user-interface generated in this project indicate that it should be feasible, within the next year or two, to deploy an updated version of the AMI-PLS with enhanced functionality that improves the ability of processors to generate accurate and reliable thermal process lethality calculations.

CURRENT AND FUTURE PLANS

We submitted a grant proposal (Dec 2004) to the USDA National Integrated Food Safety Initiative, to continue this project – aimed at the refinements and validations necessary to make the enhanced version of the AMI-PLS ready for distribution. That proposal was unfunded. We are refining the proposal and plan to resubmit it in Fall 2005.

The Ph.D. student on this project is currently incorporating data from several recent projects at MSU, in which thermal inactivation data for *Salmonella* in whole-muscle products (beef, pork, and turkey) have been generated and compared to equivalent ground products. These data are being used to add additional functionality to the user-interface, so that the program will generate thermal inactivation parameters that are structure-specific (i.e., ground or whole-muscle). Lastly, inoculated, pilot-scale challenge studies are being planned to generate independent data for validation of the generalized models included in the modified AMI-PLS.

REFERENCES

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Table 1. List of product parameters and D-values taken from literature for thermal inactivation of *Salmonella* in poultry.

Log D	Fat	Moisture	Temp	D value	Log D	Fat	Moisture	Temp	D value
0.875061	1	72	58	7.5	0.897627	1.1	72.3	55	7.90
0.658965	1	72	60	4.56	1.026125	1.1	72.3	55	10.62
0.184691	1	72	62.5	1.53	1.118595	1.1	76.3	55	13.14
-0.22915	1	72	65	0.59	0.895975	13.02	64.5	55	7.87
0.887054	7	72	58	7.71	0.913814	13.02	66.5	55	8.20
0.693727	7	72	60	4.94	1.039811	13.02	66.5	55	10.96
0.267172	7	72	62.5	1.85	0.994757	13.02	68.5	55	9.88
-0.25964	7	72	65	0.55	0.975891	1.1	72.3	55	9.46
0.839478	10	67	58	6.91	1.168203	1.1	71.5	55	14.73
0.710117	10	67	60	5.13	1.151982	1.1	71.9	55	14.19
0.161368	10	67	62.5	1.45	1.067443	1.1	71.4	55	11.68
-0.24413	10	67	65	0.57	0.993877	1.1	72	55	9.86
0.869818	12	68	58	7.41	1.079543	1.1	72.5	55	12.01
0.7348	12	68	60	5.43	1.016616	1.1	72.4	55	10.39
0.25042	12	68	62.5	1.78	1.089552	1.1	71.9	55	12.29
-0.22915	12	68	65	0.59	1.034227	13.02	68.5	55	10.82
0.870404	8.85	70.2	58	7.42	-0.29243	6.3	72	65	0.51
0.683047	8.85	70.2	60	4.82	0.931458	9	68	58	8.54
0.178977	8.85	70.2	62.5	1.51	0.732394	9	68	60	5.4
-0.09691	8.85	70.2	65	0.8	0.064458	9	68	62.5	1.16
0.665581	14.2	73	57	4.63	-0.27572	9	68	65	0.53
0.130334	14.2	73	60	1.35	0.956168	12	69	58	9.04
0.868056	2	75	58	7.38	0.740363	12	69	60	5.5
0.683947	2	75	60	4.83	0.113943	12	69	62.5	1.3
0.056905	2	75	62.5	1.14	-0.30103	12	69	65	0.5
-0.38722	2	75	65	0.41	0.850033	8.45	71.75	58	7.08
0.865104	6.3	72	58	7.33	0.716003	8.45	71.75	60	5.2
0.670246	6.3	72	60	4.68	0.133539	8.45	71.75	62.5	1.36
0.064458	6.3	72	62.5	1.16	-0.22915	8.45	71.75	65	0.59

Table 2. List of product parameters and D-values taken from literature for thermal inactivation of *Salmonella* in beef.

Log D	Fat	Moisture	Temp	D value
0.507856	7	71	58	3.22
0.390935	12	65	58	2.46
0.396199	18	62	58	2.49
0.206826	24	57	58	1.61
0.956649	4.8	72.4	55	9.05
0.354108	4.8	72.4	58	2.26
-0.24413	4.8	72.4	61	0.57
-0.82391	4.8	72.4	64	0.15
1.023252	4.8	72.4	55	10.55
0.332438	4.8	72.4	58	2.15
-0.38722	4.8	72.4	61	0.41
-1.1549	4.8	72.4	64	0.07
1.01157	4.8	72.4	55	10.27
0.313867	4.8	72.4	58	2.06
-0.36653	4.8	72.4	61	0.43
-0.85387	4.8	72.4	64	0.14
1.342028	19.1	63.4	55	21.98
0.419956	19.1	63.4	58	2.63
-0.18709	19.1	63.4	61	0.65
-0.79588	19.1	63.4	64	0.16
1.270912	19.1	63.4	55	18.66
0.5302	19.1	63.4	58	3.39
-0.24413	19.1	63.4	61	0.57
-0.69897	19.1	63.4	63	0.2
0.937016	12.45	65.5	58	8.65
0.738781	12.45	65.5	60	5.48
0.176091	12.45	65.5	62.5	1.5
-0.17393	12.45	65.5	65	0.67

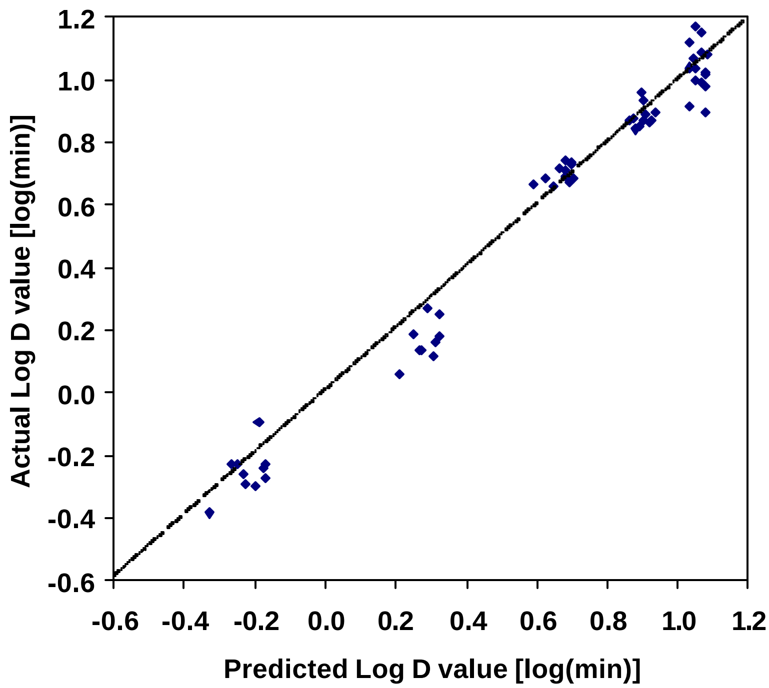

Figure 1. Observed vs. predicted log D from generalized model for thermal inactivation of *Salmonella* in poultry products.

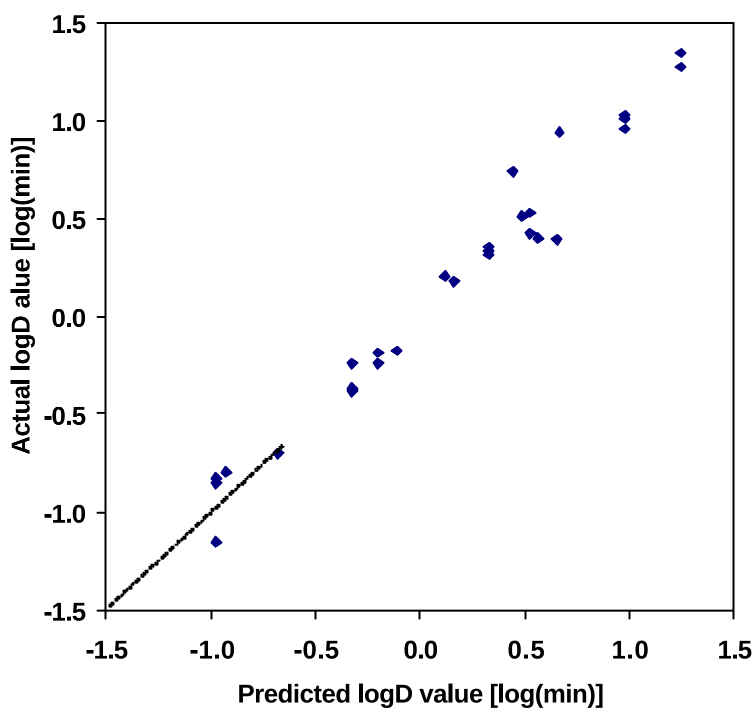

Figure 2. Observed vs. predicted log D from generalized model for thermal inactivation of *Salmonella* in beef products.

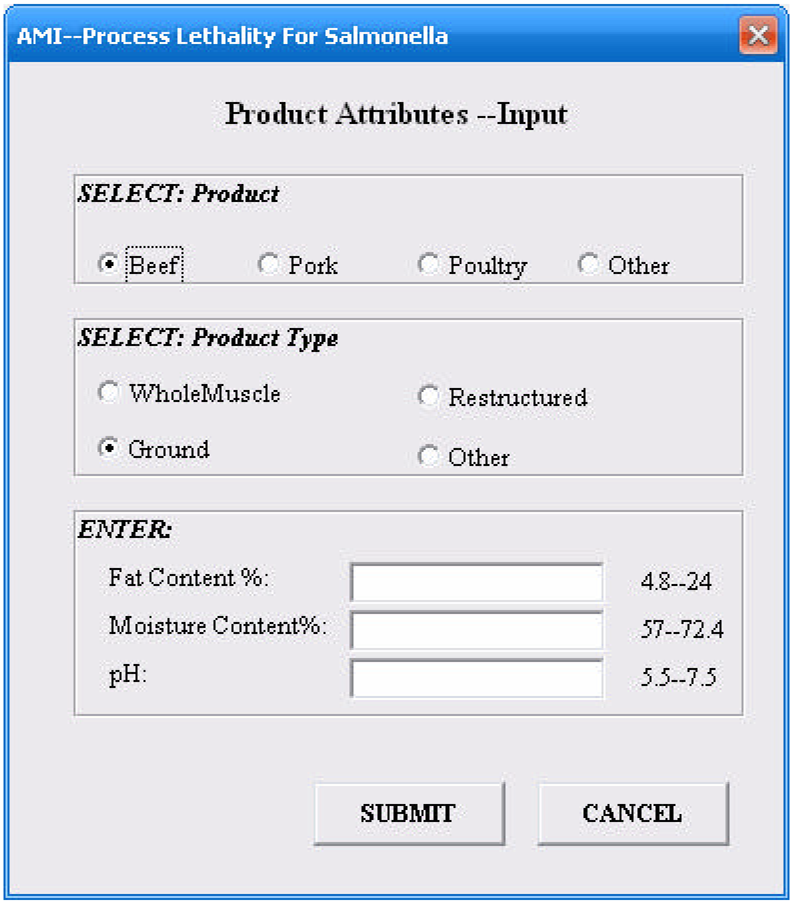

Figure 3. The user-interface for input to the enhanced process lethality spreadsheet.

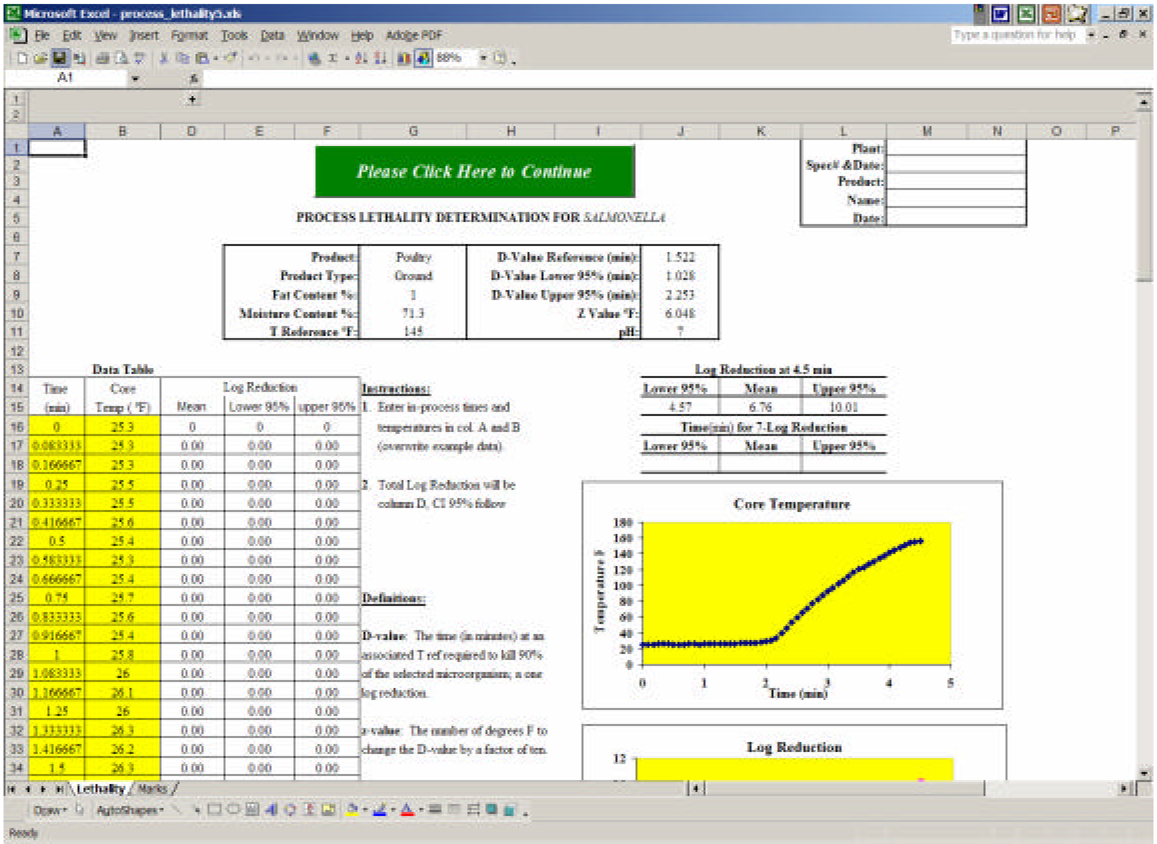

Figure 4. The spreadsheet showing product attributes, reference D-value with 95% confidence interval, and resulting log reductions with confidence limits.