MODELING *E. COLI* O157:H7 SURVIVAL IN UNCOOKED, SEMI-DRY, FERMENTED SAUSAGE

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Abstract.

Inactivation of *E. coli* O157:H7 in uncooked fermented salami were used to develop models to describe survival of the pathogen. Two models were developed and included variables best describing *E. coli* O157:H7 inactivation. Model A included the variables a_w, pH, time and quadratic variables pH and time. Model B separated the processing stages into fermentation and drying. The fermentation included the variables pH and temperature × time (ttarea) and interaction between the two variables. The drying stage was modeled using the variables time and a_w and interaction between the two. The variables selected for inclusion in the models were significant at the $p<0.0001$ level. The models were validated using data from a trial not used to develop the model. The predicted values for the two models correlated well to the observed values (R^2 of 0.888 and 0.828 for Models A, B_{ferm}, and B_{drying}, respectively).

Background.

The two food associated outbreaks of hemorrhagic colitis resulting from *E. coli* 0157:H7 found in hamburgers sold at fast food restaurants in 1982 draw a lot of attention to this microorganism (*Riley et al., 1983*). *E. coli* O157:H7 has been noted for its acid adaptive and acid tolerant properties in a number of foods and under a variety of conditions (*Arnold and Kasper, 1995*). Fermented meat products have traditionally been considered relatively safe due to the intrinsic and extrinsic factors used in the processing system. However, outbreaks in 1994 and 1995 epidemiologically linked *E. coli* O157:H7 to the consumption of semi-dried fermented sausage in Washington State and *E. coli* O111:NM to the consumption of dry fermented sausage in Australia (*Anon., 1995*). In response to the Washington State outbreak, the USDA/FSIS required meat processors manufacturing fermented sausages to validate their processes according to one of the following options: 1) utilize a heat process (e.g. 145°F for 4 min), 2) include a validated 5-D inactivation treatment, 3) conduct a "hold and test" program for finished product, 4) propose other approaches to assure at least a 5-D inactivation, and 5) initiate a Hazard Analysis Critical Control Point (HACCP) system that includes raw batter testing and a 2-D inactivation.

Validation studies indicated that for a 5-D inactivation of *E. coli* O157:H7 to be achieved, a cooking step is required. Hinkens et al. (*1996*) observed a more than 5-D inactivation of *E. coli* O157:H7 for a pepperoni process when a heating step of 63°C, instantaneously, or 53°C for 60 min was included after fermentation.

Objectives.

There is limited information on the ability of *E. coli* O157:H7 to survive during manufacture of uncooked, semi-dried, fermented salami products. Data collected, in this experiment, regarding inactivation of *E. coli* O157:H7 in uncooked fermented salami, were used to develop models to describe survival of the organism.

Materials and Methods.

Bacterial Strains. A five-strain cocktail of *E. coli* O157:H7 was used to inoculate the meat batter. The strains used were 380-94 USDA (salami outbreak) and bovine strains *E. coli* 92005, *E. coli* 920081, *E. coli* 920026 and *E. coli* 920027. The methods used for bacterial preparation and inoculation are outlined by Nickelson et al. (*1996*).

Salami Processing. Processing was carried out according to conventional manufacturing procedures using ground meat, premixed spices, lactic acid culture and nitrite. Three separate batches were individually mixed for 30 s followed by *E. coli* O157:H7 cocktail and starter culture addition. Later, salt and the other ingredients were added, and mixed for 4 min to ensure uniform distribution. Products were dried for several weeks (until achieving 2 log reduction) at 80-85% RH.

Microbiological Analysis. Viable *E. coli* O157:H7 counts were determined in samples taken before inoculating the meat, prior to stuffing, after fermentation, at mid and final drying. Casings were removed aseptically and samples cut into multiple cross-sectional slices. Samples were serially diluted with 0.1% peptone water, surface plated (0.1 ml) in duplicate onto MSA plates and counted after incubating at 37°C for 18 h.

Chemical Analysis. Each salami stick was analyzed for moisture, fat, titratable acidity, water activity (a_w), protein, and salt according to established methods.

Statistical Analysis. Models were developed based on data from the different experimental series. Preliminary validation of the models was carried out using the data from one additional salami series representing three individual salami runs. The General Linear Model (GLM) in the Statistical Analysis System (SAS Institute Inc, Cary, NC) was used for model development and validation.

Results And Discussion.

Models were developed to describe the log reduction of *E. coli* O157:H7 in uncooked, semi-dry, fermented sausages. The models focused on different variables to determine which parameters would best describe the response of *E. coli* O157:H7 in these products. The first stage in model development was to hypothesize the form of the model. Quadratic and interaction response surface models were proposed.

Model A. The equation for Model A was as follows:

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \alpha_{22} x_2^2 + \alpha_{33} x_3^2 + \gamma_0$$

where:

y = *E. coli* O157:H7 log reduction in uncooked fermented salami
x_1 = a_w of uncooked fermented salami
x_2 = pH of uncooked fermented salami
x_3 = time of processing at specific stages of uncooked fermented salami
α_0 = estimate for the y intercept
$\alpha_1 x_1$ = estimate for the linear effect of independent variable a_w
$\alpha_2 x_2$ = estimate for the linear effect of independent variable pH
$\alpha_3 x_3$ = estimate for the linear effect of independent variable time
$\alpha_{22} x_2^2$ = estimate for the quadratic curvature effect of independent variable pH
$\alpha_{33} x_3^2$ = estimate for the quadratic curvature effect of independent variable time
γ_0 = error term

The model explained 89% ($R^2 = 0.888$) of the sample variation of *E. coli* O157:H7 log reduction with the remainder explained by random error. A t-test was conducted to identify the important α parameters to include in the proposed equation. The results showed pH and time as the most important variables for predicting *E. coli* log reduction ($p < 0.0001$). However, all other variables were significant ($p < 0.01$) allowing acceptance of the alternative hypothesis that the estimated α parameters were nonzero. The data also provided information to explain the relationship of the α parameters and *E. coli* O157:H7 reduction. The pH and a_w variables were negatively correlated to *E. coli* log reduction. Therefore, as pH or a_w decreases, the reduction in *E. coli* O157:H7 counts will increase. Furthermore, the time variable showed a positive correlation indicating that as time increases *E. coli* O157:H7 log reduction increases.

Model B. Two separate equations were hypothesized for Model B to describe the response of *E. coli* O157:H7 in uncooked, fermented salami. Overall, process was separated into two stages, fermentation and drying. This allowed for inclusion of an important variable, time and

temperature (ttarea), that described the fermentation stage as total-area based on fermentation time multiplied by fermentation temperature.

The ttarea variable and the interaction between ttarea and pH variable were significant ($p < 0.0001$). The significant interactive variables were those that described the relationship between *E. coli* O157:H7 log reduction and variables pH and ttarea. The slope of *E. coli* O157:H7 log reduction and one of the interactive variables (pH) depended on the value of the other interactive value (ttarea) and *vice versa*. The utility of the model was highly significant ($p < 0.0001$) for predicting *E. coli* O157:H7 log reduction suggesting that at least one parameter is nonzero.

The model explained 83% of the sample variation of *E. coli* O157:H7 log reduction with the remainder explained by random error. A test on the individual parameters showed that both ttarea and ttarea and pH interaction were significant ($p < 0.0001$) and important variables for predicting *E. coli* O157:H7 log reduction; permitting acceptance of the alternative hypothesis that the α parameters are nonzero.

Validation of the fermentation equation with separate experimental data showed a good agreement between the observed and predicted reduction in *E. coli* counts. Evaluation of the scatter diagram indicated strong agreement ($R^2 = 0.965$) between the predicted and observed *E. coli* O157:H7 log count reductions for Model B fermentation.

Conclusions.

The objective of this project was to use data from commercial fermented sausage manufacturing processes to model the reduction of *E. coli* O157:H7 populations in uncooked fermented salami. It was estimated that fewer than 50 organisms may have been present in the dry fermented salami which caused infection in the Washington State outbreak, so it is important to be able to predict the efficacy of production practices. The processes examined differed in time, temperature and product formulation. Fermentation alone produced a mean log reduction in *E. coli* O157:H7 count ranging from 0.30 to 1.33 whereas the mean log reduction in *E. coli* O157:H7 count obtained during the drying stage ranged from 1.37 to 2.70. Other researchers have obtained reductions in *E. coli* O157:H7 between log 0.41 to 1.39 during the fermentation stage and between 0.43 to 1.36 during the drying stage of fermented sausage processing under a variety of time/temperature regimens (*Ellajosyula et al., 1998*).

The models were developed and validated to describe the response of *E. coli* O157:H7 in uncooked, fermented salami. The observed *E. coli* O157:H7 log reductions fitted reasonably well with the predicted values. Model A was developed to allow for the best fit to the data for predicting log reduction of *E. coli* O157:H7 in uncooked, fermented salami.

Pertinent Literature.

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Model B hypothesized for the fermentation stage was:

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_{12} x_1 x_2 + \gamma_0$$

where:

- y = *E. coli* O157:H7 log reduction during the fermentation stage
- x_1 = total area of fermentation stage (time × temp)
- x_2 = pH of uncooked during fermentation stage
- α_0 = estimate for the y-intercept
- α_1 = estimate for the linear effect of ttarea.
- α_2 = estimate for the linear effect of pH
- α_{12} = estimate for the interactive effect of ttarea and pH
- γ_0 = error term

The equation for the fermentation stage for predicting *E. coli* O157:H7 log reduction was:

$$y = 0.764 + 0.0049x_1 - 0.123x_2 - 0.0009x_1x_2 + \gamma_0$$

where:

- y = *E. coli* O157:H7 log reduction during the fermentation stage
- x_1 = total area of fermentation stage (time × temp)
- x_2 = pH of uncooked during fermentation stage
- $x_1 x_2$ = estimate for the interactive effect of ttarea and pH
- γ_0 = error term

The response surface diagram generated from the model shows that the ability of *E. coli* O157:H7 to survive in the product decreases as the pH decreases and as the fermentation time/temperature function increases (Fig. 1).

All variables tested (i.e. a_w, time and the interaction between a_w and time) were significant ($p < 0.0001$). An F test for model utility was also significant ($p < 0.0001$) suggesting it is a useful model for predicting *E. coli* O157:H7 survival in fermented sausage (Table 1).