Developing Validation Models for E. Coli *0157 Inactivation in Dry Fermented Sausages*

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Abstract

After the 1994 *E. coli* 0157:H7 outbreak associated with the consumption of semi-dried, fermented sausage in Washington, the USDA-FSIS has required processors to either validate their processes or include a step that will assure a 5 log reduction of the pathogen. The presentation will illustrate the work done in our laboratory to validate sausage fermentation (i.e., inoculated studies) for different commercial processes, and the initial development of three models to validate the process when the USDA-FSIS two log *E. coli* reduction option is chosen. The models include variables such as water activity, pH, fermentation and drying time, as well as the interactions among these variables. The predictive abilities of the models were confirmed by linear regression, comparing values for *E. coli* survival derived from the models with experimental values obtained from data that were not used to construct the models. Response surface diagrams were also produced to demonstrate the effects of the different variables.

Introduction

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 demonstrate 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. However, various processors are reluctant to apply a heat treatment because it may modify the sensory properties of the product, or they do not have the necessary equipment. These processors are choosing the fifth option listed above, which requires HACCP implementation and demonstrating a 2-D *E. coli* 0157:H7 inactivation.

There is limited information on the general ability of *E. coli* O157:H7 to survive during manufacture of uncooked, semi-dried, fermented salami products. Ellajosyula et al. (1998) proposed a model that includes fermentation pH (5.2 or 4.7), final heating temperature (43, 46 or 49°C) and time (3 to 20 hrs, depending on temperature). Riordan et al. (1998) have also proposed a model that indicates salt concentration (2.5 to 4.8%), final pH (4.4 to 5.6) and nitrite concentration (100 - 400 ppm). In our study, data collected from various *E. coli* O157:H7 validation studies (2D inactivation option) of uncooked, commercial fermented salami were used to develop models to describe survival of the organism.

Methods

The trials were conducted according to the USDA-FSIS guideline/requirements for validating fermented sausage processes (Nickelson et al., 1996). Accordingly, a five-strain cocktail of *E. coli* O157:H7 was used to inoculate the meat batters. 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 products were processed according to conventional manufacturing procedures using ground meat, premixed spices, lactic acid culture and nitrite. For each trial, three separate batches were individually mixed for 30 seconds followed by *E. coli* O157:H7 cocktail and starter culture addition. Later, salt and the other ingredients were added, and mixed for 4 minutes to ensure uniform distribution. Products were dried for several weeks (until achieving 2 log reduction) at 80-85% RH. Viable *E. coli* O157:H7 counts were

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determined in samples taken before inoculating the meat, prior to stuffing, after fermentation, at mid and final drying (as required by the USDA-FSIS procedure). 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. In addition, salami sticks were analyzed for moisture, fat, titratable acidity, water activity (a_w), protein, and salt according to established methods. 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 trials. The General Linear Model (GLM) in the Statistical Analysis System (SAS Institute Inc, Cary, NC) was used for model development and validation.

Discussion

Three main 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 (Pond et al., 2001). The first stage in model development was to hypothesize the form of the model. Because of the assumption made and because of their ease of use, quadratic and interaction response surface models were proposed.

The equation for Model A was:

$$y = ßo + ß_1x_1 + ß_2x_2 + ß_3x_3 + ß_{22}x_2^2 + ß_{33}x_3^2 + \varepsilon$$

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
$ßo$	=	estimate for the y intercept
$ß_1x_1$	=	estimate for the linear effect of independent variable a_w
$ß_2x_2$	=	estimate for the linear effect of independent variable pH
$ß_3x_3$	=	estimate for the linear effect of independent variable time
$ß_{22}x_2^2$	=	estimate for the quadratic curvature effect of independent variable pH
$ß_{33}x_3^2$	=	estimate for the quadratic curvature effect of independent variable time
ε	=	error term

The model explained 89% ($R^2 = 0.88$) 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 ß 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 ß parameters were nonzero. The data also provided information to explain the relationship of the ß 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.

For the second model (Model B), two separate equations were hypothesized to describe the response of *E. coli* O157:H7 in uncooked, fermented salami. Overall, the process was separated into two stages, fermentation and drying. This allowed for inclusion of an important variable, time and temperature (ttarea), that describes the fermentation stage as total-area based on fermentation time multiplied by fermentation temperature.

The equation for the fermentation stage was:

$$y = ßo + ß_1x_1 + ß_2x_2 + ß_{12}x_1x_2 + \varepsilon$$

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
$ßo$	=	estimate for the y-intercept
$ß_1$	=	estimate for the linear effect of ttarea.
$ß_2$	=	estimate for the linear effect of pH
$ß_{12}$	=	estimate for the interactive effect of ttarea and pH
ε	=	error term

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

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

The response surface diagram generated from the model showed 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.

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 ß 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 equation.

The third model (Model C) was proposed to include a variable that described the time at which the samples were exposed to a pH $\leq$ 5.3 (pHt). The equation was:

$$y = ßo + ß_1x_1 + ß_2x_2 + ß_{12}x_1x_2 + \varepsilon$$

where:

- y = *E. coli* O157:H7 log reduction during the fermentation stage
- x_1 = a_w of the uncooked fermented salami
- x_2 = time at pH $\leq$ 5.3
- $ßo$ = estimate for the y-intercept
- $ß_1$ = estimate for the linear effect of a_w
- $ß_2$ = estimate for the linear effect of pHt (time at pH $\leq$ 5.3)
- $ß_{12}$ = estimate for the interactive effect between a_w and pHt
- ε = error term

The actual equation for describing the *E. coli* O157:H7 log reduction was:

$$y = 10.6 - 10.5x_1 - 0.148x_2 + 0.0208x_1x_2 + \varepsilon$$

Conclusions

The intrinsic factors of a food product are very important in determining the product's shelf life and safety. The final average a_w of the salami products ranged from 0.796 to 0.923. A reduced a_w impacts bacteria cells by increasing the lag phase and decreasing the growth rate, resulting in a reduction in bacterial population. The pH of food products also influences the survival of microorganisms in foods. Bacteria normally have an intracellular pH of around 7.0. The pKa of an acid and the pH of a food will determine the amount of dissociated and undissociated acid that will occur in a product. Foods that have a high percentage of undissociated acid will lead to the acid being able to permeate the cell wall and become dissociated due to the higher intracellular pH of the bacteria (Barbut, 2001). This results in an increased expenditure of energy to remove intracellular H^+ ions, which often results in death of the cell. In addition, these factors may work in combination (the so-called "hurdle effect"), thereby increasing the destruction of bacteria in foods. The intrinsic factors that occur during the processing of fermented meat products are vital for achieving product stability and safety. The objective of the study 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 might 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 and 1.39 during the fermentation stage and between 0.43 and 1.36 during the drying stage of fermented sausage processed under a variety of time/temperature regimens (Ellajosyula et al., 1998).

In the present study, the observed *E. coli* O157:H7 log reductions fitted reasonably well with the predicted values. As food processing and distribution systems become more complex, innovative approaches will be needed to provide effective means for addressing, assessing and monitoring food safety issues. A tool presented in this paper was the modeling of a real food system and the validation of the model as a means of assessing manufacturing procedures. Modeling food-manufacturing processes may serve as an important and useful tool in assessing food safety risks. However, modeling a dynamic biological system presents many challenges for describing how microorganisms respond to their environment. To improve the predictability of these models, further research and data collection on other important variables should be assessed for possible inclusion into these models (e.g. casing diameter; preliminary results will be discussed during the presentation). This may provide greater confidence in predicting the reduction of *E. coli* O157:H7 in uncooked fermented sausages and lead to industrial implementation. It is important to note that predictive modeling should not be the sole means for monitoring and assessing microbial growth. However, in conjunction with other food safety tools such as hazard analysis critical control point (HACCP) and risk assessment, predictive modeling provides a sound and logical approach for addressing food safety hazards.

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