

Modeling the Growth/No Growth Interface of *Listeria monocytogenes* in Bologna

Yohan Yoon, Ifigenia Geornaras, Patricia A. Kendall¹, Keith E. Belk, John A. Scanga, Gary C. Smith, and John N. Sofos

SUMMARY

The objective of this study was to model the growth/no growth boundaries of *Listeria monocytogenes* in bologna sausage as a function of lactic acid concentration (0, 1, 2, 3, or 4%), dipping time (0, 1, 2, 3, or 4 min), and storage temperature (40, 45 or 50°F). *L. monocytogenes* was inoculated on both sides of bologna slices. Samples were dipped into lactic acid and were stored in vacuum packages for up to 60 days. Bacterial populations were enumerated on tryptic soy agar plus 0.6% yeast extract and PALCAM agar at time-zero, and the middle and end point of storage for each storage temperature. Significant ($P < 0.05$) increases in bacterial populations during storage were designated as “growth”, otherwise, they were designated as “no growth”. The growth response data were fitted with logistic regression to determine growth/no growth interfaces. Growth/no growth interface lines were generated for 10%, 50%, and 90% probability. The inhibitory concentration of lactic acid decreased as dipping time increased. At low storage temperatures (40 or 45°F), lower lactic acid concentration and shorter dipping times were needed to inhibit *L. monocytogenes* growth. In this study, the models developed may be useful in selecting appropriate lactic acid concentrations and dipping times in bologna processing to control of *L. monocytogenes*.

Key Words: *L. monocytogenes*, Lactic acid, Modeling, Bologna.

INTRODUCTION

Mathematical modeling has been advanced from experimental data on combinations of factors that affect growth of food-borne pathogenic microorganisms to predict their response in foods (Tienungoon et al., 2000). Ratkowsky and Ross (1995) introduced logistic regression to model the interface between growth and no growth. Recently, mathematical modeling of growth limits has been considered as a critical component of modern predictive microbiology (McMeekin et al., 2002). However, even though the majority of foods are solid, most models have been developed in broth media (Wilson et al., 2002). Hence, modeling the growth boundaries of foodborne pathogens should be evaluated in real foods for more realistic estimation of food safety risks. Since the Food Safety and Inspection Service of the United States Department of Agriculture (USDA/FSIS) enforces a ‘zero-tolerance’ policy to control *Listeria monocytogenes* on ready-to-eat meat and poultry products, studies to model the growth/no growth response of *L. monocytogenes* on bologna should be useful. Thus, this study modeled the growth/no growth boundaries of *L. monocytogenes* in bologna as a function of lactic acid concentration and dipping time, and storage temperature.

MATERIALS AND METHODS

Preparation of inoculum. The inoculum mixture consisted of 10 strains of *L. monocytogenes* (Scott A, NA-3, NA-19, 101M, 103M, 558, PVM1, PVM2, PVM3, and PVM4) and it was prepared as described by Bedie et al. (2001) and Samelis et al. (2001, 2002).

Preparation of samples and inoculation. The formulation and preparation of bologna (no antimicrobials included) used in the study was that described by Bedie et al. (2001) and Samelis et al. (2002). A 0.1 ml portion of the inoculum was spread

over on side of each slice of bologna (65 mm diameter, approximately 2 to 3 mm thickness) with a sterile bent glass rod, left at 40°F for 15 min for bacterial attachment, and then inoculated on the second side using the same procedure, as describe above.

Application of treatments.

Bologna slices were completely dipped into lactic acid (14 slices/300 ml of lactic acid solution; Purac, Montmelo, Spain) solutions (0, 1, 2, 3, 4%) for 0, 1, 2, 3, 4 min. Then, two samples were placed in a vacuum bag (Koch), vacuum packaged (Hollymatic Corp., Countryside, IL), and stored at 40, 45, and 50°F for up to 60 days, depending on storage temperature.

Microbiological analyses.

Bacterial populations were determined on tryptic soy agar (Difco, Bacton Dickonson, Sparks, MD) plus 0.6% yeast extract (Acumedia, Baltimore, MD) (TSAYE) and on PALCAM agar (Difco) at time-zero, the middle, and end point of storage. All plates were incubated at 86°F for 48 h (PALCAM) or at 77°F for 72 h (TSAYE).

Evaluation of growth/no growth.

A total of 75 combinations (temperature × lactic acid × dipping time) in two replicates (two samples/replicate) for each combination were studied. Microbiological data were converted into log₁₀ CFU/cm² before being analyzed. To determine growth/no growth response, surviving bacterial populations were analyzed using the mixed model procedure of SAS[®] (SAS institute version 8.2, Cary, NC). All least squares means were compared by pairwise t-test. Significant ($P < 0.05$) increases in bacterial populations during storage were designated as “growth (1)”, otherwise as, “no growth (0)”.

Model development for growth/no growth interface. The growth response data were fitted to logistic regression model using SAS[®] to determine the growth/no growth interfaces. The following equation was fitted to datasets of bologna.

$$\text{Logit}(P) = a_0 + a_1 \cdot T + a_2 \cdot LA + a_3 \cdot DT + a_4 \cdot T \cdot LA + a_5 \cdot T \cdot DT + a_6 \cdot LA \cdot DT + a_7 \cdot T^2 + a_8 \cdot LA^2 + a_9 \cdot DT^2$$

¹ Department of Food Science and Human Nutrition, Colorado State University, Fort Collins. 80526-1571

where, *Logit* (*P*) is an abbreviation of $\ln [P/(1-P)]$, *P* is the probability of growth (in the range of 0 to 1), *a_i* are coefficients to be estimated, *T* is storage temperature, *LA* is lactic acid concentration, and *DT* is dipping time. The automatic variable selection option with a stepwise selection method was used to determine the parameters.

RESULTS AND DISCUSSION

In general, total bacterial populations (TSAYE) were higher than bacterial populations recovered with PALCAM due to possible growth of natural flora (data not shown). Bacterial populations recovered with PALCAM were used for model development. The coefficients of parameters were obtained by fitting growth response data with the logistic regression model. However, there were no storage temperature effects observed for growth response. The observed growth response data were identical for bologna at low storage temperatures (40 and 45°F) (Figure 1). Linear predicted growth/no growth interfaces were generated for 10, 50, and 90% probability of *L. monocytogenes* growth in bologna (Figure 1). The inhibitory concentrations of lactic acid solutions were reduced as dipping time increased (Figure 1). For observed growth responses, storage at low temperatures allowed inhibition at shorter dipping times compared to storage at 50°F (Figure 1). Since the model used in this study was developed in a solid food and good performances were observed (data not shown), the new procedure, developed with the results from real foods in this study may be practical in generating growth/no growth models.

IMPLICATIONS

The study provides quantitative data on the antimicrobial effect of lactic acid, and the model developed may be useful in selecting appropriate lactic acid concentrations and dipping times needed for adequate control of *L.*

monocytogenes. In addition, the novel procedure developed to product growth/no growth by modeling may be useful in predictive microbiology.

ACKNOWLEDGEMENTS: This work was supported by the National Integrated Food Safety Initiative of the United States Department of Agriculture Cooperative State Research, Education and Extension Service (agreements 2004-51110-02160 and 2005-51110-03278) and by the Colorado State University Agricultural Experiment Station.

LITERATURE CITED

- Bedie, G. K., J. Samelis, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith. 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* postprocessing contamination on frankfurters stored at 4°C in vacuum packages. *J. Food Prot.* 64:1949-1955.
- McMeekin, T. A., J. Olley, K. Presser, D. A. Ratkowsky, and T. Ross. 2002. Predictive microbiology: toward the interface and beyond. *Int. J. Food Microbiol.* 73:395-407.
- Ratkowsky, D. A., and T. Ross. 1995. Modelling the bacterial growth/no-growth interface. *Lett. Appl. Microbiol.* 20:29-33.
- Samelis, J., G. K. Bedie, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4°C in vacuum packages *J. Food Prot.* 65:299-307.
- Samelis, J., J. N. Sofos, M. L. Kain, J. A. Scanga, K. E. Belk, and G. C. Smith. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing to sliced pork bologna stored at 4°C in vacuum packages. *J. Food Prot.* 64:1722-1729.
- Tienungoon, S., D. A. Ratkowsky, T. A. McMeekin, and T. Ross. 2000. Growth limits of *Listeria*

monocytogenes as a function of temperature, pH, NaCl, and lactic acid. *Appl. Environ. Microbiol.* 66: 4979-4987.

Wilson, P. D. G., T. F. Brocklehurst, S. Arino, D. Thuault, M. Jakobsen, M. Lange, J. Farkas, J. W. T. Wimpenny, and J. F. Van Impe. 2002. Modelling microbial growth in structured foods: towards a unified approach. *Int. J. Food Microbiol.* 73:275-289.