PS5.05 Meaningful modeling of microbial growth potential 263.00

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INTRODUCTION

Excellent models of the growth of pathogens over time exist, but are relatively limited in their usefulness to meat processors primarily because they need further validation in actual meat products. For example, the United States Department of Agriculture Agricultural Research Service (USDA-ARS) has developed their Pathogen Modelling Program (PMP) that includes various pathogen models (1). These models are useful to illustrate the influences of storage temperature, inclusion of various antimicrobial ingredients, and the like, but are limited with respect to the performance of the various antimicrobial ingredients to specific meat products. Of the six *Listeria monocytogenes* models in the PMP, only one is relevant to typical meat applications (ground ham stored anerobically) (1, 2). This paper offers some advice on methods of conducting such challenge studies to obtain commercially meaningful data on the growth potential of *Listeria monocytogenes*.

OBJECTIVES

There are three objectives: 1) explain useful experimental designs, 2) show a method of analyzing data using a time-to-growth technique, and 3) give a pertinent example using a published study assessing the usefulness of sodium benzoate as an antimicrobial agent in ready-to-eat (RTE) meats (3).

METHODOLOGY

Microbial challenge data in actual meat products can be difficult to assess caused by the variability observed in the data caused by a multitude of factors and the complexity of the meat products themselves. The data may also be limited in usefulness as well since many challenge studies target only one type of product and the results cannot be applied to other products. It is desirable from a research point of view to be able to create experimental designs that have as wide an applicability as possible and that are able to deal with the variability that can exist in microbial challenge data, especially those of potential public health significance.

A design of experiments approach using central composite designs offer a valuable means of dealing with the complexities of microbial challenge studies as long as certain considerations are well thought-out. These designs are efficient and offer a systematic investigation of the factors being tested as well as their interactions – the interactions perhaps being the most important part of the experiment. However, only a limited number of factors can be observed in any given experiment otherwise the scope of the experiment becomes unmanageable. The skill in developing models using these designs is to determine and control the important factors while minimizing and understanding the ones that cannot be controlled.

Several methods to assess the effectiveness of the factors being investigated exist; these can be broadly classified into two categories: kinetic models and time-to-growth models. Both can be quite useful. Where the regulatory authorities specify zero growth for pathogens, the time-to-growth offers simpler interpretation. In other cases, the kinetic models may be more useful.

The efficacy of sodium benzoate in combination with sodium diacetate, sodium chloride, and product moisture content was tested using a central composite statistical design. The design consisted of 16 factorial treatments augmented with eight axial (star) points and six center points for a total of 30 runs. The number of samples was divided into three blocks of 10 runs each. The order of the runs was randomized.

Time-to-growth (TTG) values were established by plotting the MOX counts for each product over the shelf life storage time. Growth was determined to occur once the average count (three samples per sampling period) exceeded one log greater then the inoculation level and was sustained over two sampling periods.

RESULTS AND DISCUSSION

The benzoate/diacetate example shows that sodium benzoate is an effective inhibitor of *Listeria monocytogenes* along with sodium diacetate, but its usefulness is also affected by the finished product moisture content (Fig. 2). This limitation is not of much consequence unless the amount of sodium benzoate is limited to a maximum of 0.1% -- the currently allow regulatory limit in the USA.

CONCLUSIONS

Such experimental designs as those illustrated here are quite useful, but must be checked for accuracy of product formulations and for the determination of time-to-growth (TTG). One must have clear in his mind the objectives of the experiment and then needs to be able to make educated choices on the proper factors, ingredients, storage conditions, and other factors that may influence the growth of the intended organism. Some of these can and should be controlled, but for some, control may be impossible.

REFERENCES

1 Anonymous,http://pmp.arserrc.gov/PMPHome.aspx. Accessed on May 10, 2009.

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3 Seman, D.L., Quickert, S.C., Borger, A.C., and Meyer, J.D. (2008). Inhibition of Listeria monocytogenes growth in cured ready-to-eat meat products by use of sodium benzoate and sodium diacetate. J. Food Protection, 71(7); 1386-1392.

4 Draper, N.R. 1988. Response surface designs, In Encyclopaedia of Statistical Sciences. S. Kotz and N.L. Johnson *Eds*. Wiley-Interscience, John Wiley and Sons, New York, 8:107-119.

Tables and Figures

Table 1. Desirable features of a response surface design

The response surface should:
1. Generate a satisfactory distribution of information about the behavior of the response variable throughout the region of interest
2. Ensure that the fitted value at x, $\hat{y}(x)$, be as close as possible to the true value at x, $\eta(x)$
3. Give good detectability of lack of fit
4. Allow transformations to be estimated
5. Allow experiments to be performed in blocks
6. Allow designs of increasing order to be built up sequentially
7. Provide an internal estimate of error
8. Be insensitive to wild observations and to violation of the usual normal theory assumptions
9. Require a minimum number of experimental points
10. Provide simple data patterns that allow ready visual appreciation
11. Ensure simplicity of calculation
12. Behave well when errors occur in the setting s of the predictor variables
13. Not require an impractically large number of predictor variable levels
14. Provide a check on the constancy of variance assumptions

Figure 1. Schmetic of a 2^3 Central composite design

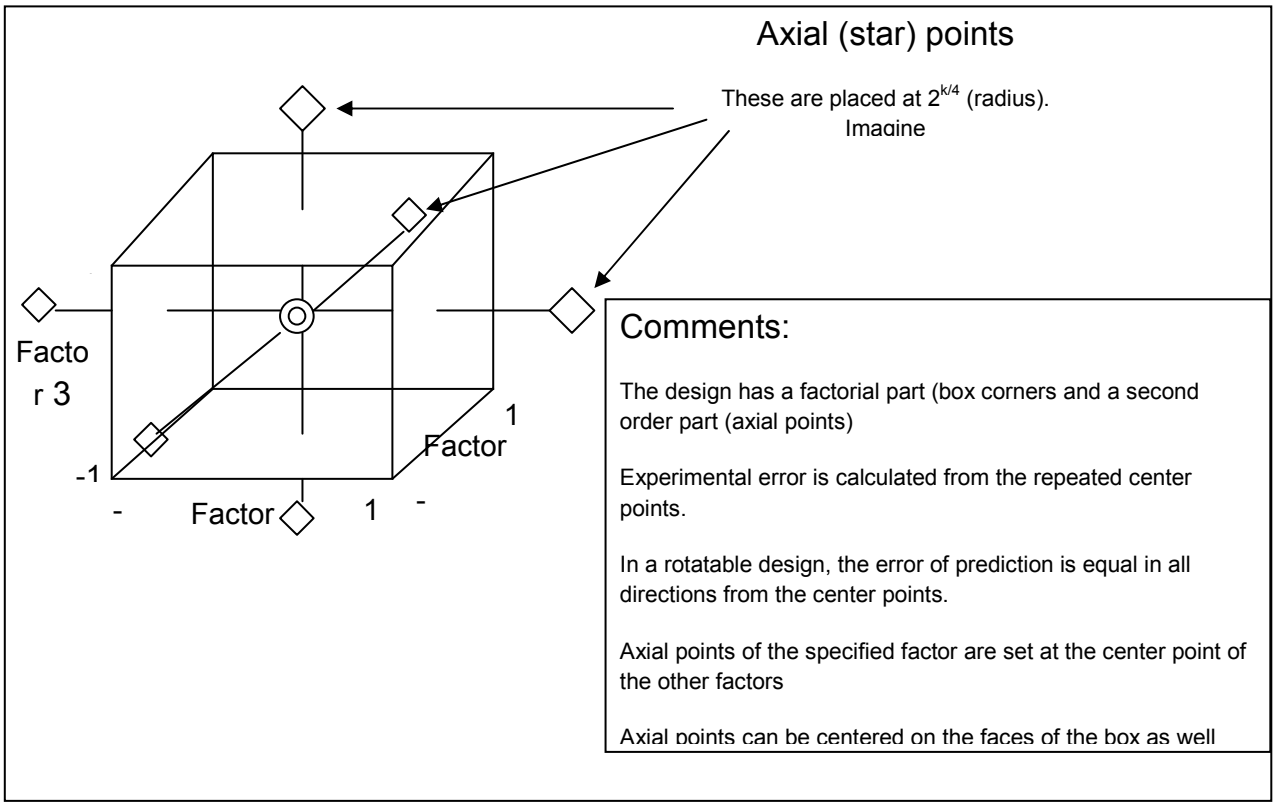


Table 2. Regression with Life Data to Determine Factor effects on Time-to-Growth

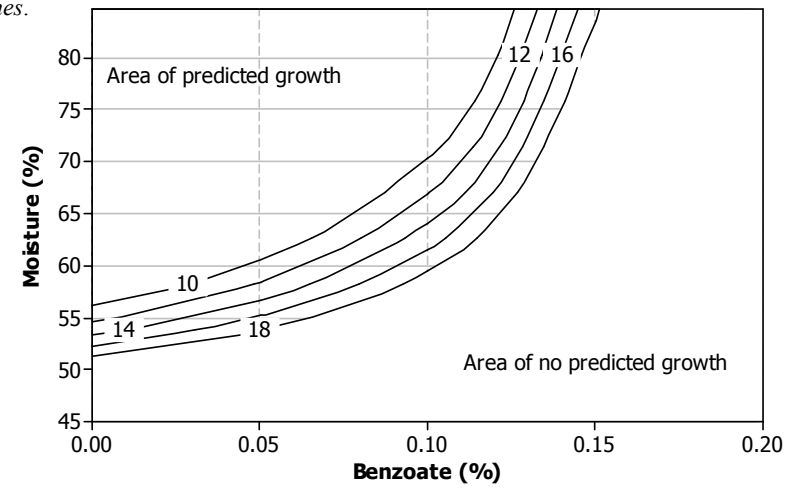
General form:

Prediction = constant + coefficient(predictor) +. . . coefficient(predictor) + scale(error term)

$Y = \beta 0 + \beta 1x1 + \ldots \beta\rho x\rho + \sigma\varepsilon$

- Y = log failure time
- Predictors (x1, x2, . . . xρ); these can be continuous or categorical; can include interactions, e.g., β1(x1*x2), or β2(x2)
- Scale (σ); the scale parameter. For Weibull, scale = 1.0/shape
- Error term (ε); the random error term
- Minitab offers eight lifetime distributions: Weibull (default), smallest extreme value, exponential, normal, lognormal, logistic, and loglogistic
- Usually choose the one that minimizes the log-likelihood and exhibits the best fit of points on the probability plot. Usually the Weibull and lognormal work well.

Figure 2. Contour plot of the nfluence of product moisture and sodium benzoate on the time-to-growth of *Listeria monocytogenes*.



Salt content set at 2% and Diacetate content set at 0.1%