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A PREDICTIVE MODEL: *LISTERIA* IN SOUTH AFRICAN PRODUCED HAM

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Background

As a result of several outbreaks worldwide of foodborne infection, *Listeria monocytogenes* is now of considerable concern to the food industry (3). It is evident from surveys done in Pretoria (7), that processed products in South Africa are contaminated with *Listeria*, with the highest incidence in ham (14 % of all ham samples obtained were contaminated with this organism).

Modelling microbial growth responses is recognised as a cost-effective alternative to traditional challenge testing of products to assess their microbiological safety. By constructing a model which predicts the growth of *Listeria* a food product, i.e. ham, food processors will be able to design procedures to safeguard ham against this pathogen and predict the possibility of growth in the event of a breakdown in specified production schedules.

Objective

The object of this study was to construct a model which predicts the growth and survival of *Listeria monocytogenes* in South African produced ham.

Methods

To manufacture ham, lean pork was tumbled with 27 % curing solution. The solution contained different concentrations of NaCl (0 %, 1.5 %, 3 %) and $NaNO_2$ (0 %, 0.01 %, 0.02 %) combinations. After processing, 10 g of each sample was inoculated with *Listeria monocytogenes*, stomachered and incubated at different incubation temperatures (0 °C, 7 °C, 18 °C). Each combination was sampled at least 40 times over time for *L. monocytogenes* counts, in order to generate sufficient points (15 - 20) for an adequately fitted growth curve.

The mentioned procedures were repeated (3 times) and regression analysis and Gompertz function fitting were used to construct a predictive model. At each combination of temperature, NaCl and $NaNO_2$, the bacterial count was modelled as a function of time using the Gompertz growth curve given by $L_{(t)} = A + C \exp\{-\exp[-B(t-M)]\}$ where $L_{(t)}$ is the Log_{10} count at time t. This allows the growth curve to be summarised in four parameters B, M, C, and A where A is the asymptotic log-count as t decreases indefinitely, C is the asymptotic amount of growth that occurs as t increases indefinitely, and B is the relative growth rate at M, where M is the time at which the growth rate is a maximum (4). The statistical programming language Genstat 5 (2) was used to fit Gompertz curves and for modelling of data.

The function chosen to model the parameters was a polynomial of the form $\text{Ln } y = a + b_1T + b_2S + b_3N + b_4TS + b_5TN + b_6SN + b_7T^2 + b_8S^2 + b_9N^2$. In this expression y is the response variable (the parameter to be modelled), T, S and N represent temperature (C), NaCl (% w/v) and $NaNO_2$ (% w/v). The coefficients a, b1-9 were determined by least square sums. This quadratic response surface model was chosen for simplicity (4).

Results and discussion

In our investigation, temperature (as expected), had the greatest influence on the inoculated organism. The average lags percentage variance accounted for 95.7) at 18 C were 9.7 hours, at 7 °C 58.8 hours and at 0 °C, 824.9 hours. The average Generation Times (GT) (percentage variance accounted for 98.2) were 2.1 (18 °C), 15.3 (7 °C) and 121.2 (0 °C). Studies in chicken broth and pasteurized milk confirmed the ability of this pathogen to multiply at low temperatures (-0.1 to -0.4 °C) (8). Growth of the organism in ham at 0 °C was very slow; while the *Listeria* cells were still in the exponential phase the structure of the incubated hams changed to a mushy mass. The change could be the result of the ham being broken down enzymatically.

Shahamat and coworkers (6) examined effects of various concentrations of sodium nitrite and sodium chloride on growth of *L. monocytogenes* in Tryptose Soy Broth at different temperatures and pH values. When incubated at 37, 22 and 4 °C in broth at pH 7.4, it grew at nitrite concentrations as high as 25 000, 30 000 and 10 000 ppm, respectively. Inhibitory effects of nitrite were enhanced at pH 6.5, particularly at lower incubation temperatures, with complete inhibition caused by 1 500 ppm nitrite at 4 °C. Inhibitory effects of nitrite were most pronounced at 4 °C when the chemical was combined with sodium chloride.

The nitrite concentrations being used in foods are lower than the concentrations used in above mentioned experiments. The lower concentrations used in this study (0, 100 and 200 ppm) had an effect on the growth of the pathogen in ham, although not total inhibition. In the ham produced, 0.02 % (200 ppm) nitrite had a greater inhibition effect (longer generation time) (2.3 h at 18 °C) than 0.01 % (100 ppm) nitrite (1.8 h at 18 °C), and 0.01 % (100 ppm) nitrite had a greater effect than 0 % (1.6 h at 18 °C). The same effect could be seen in ham produced with 1.5 and 3 % salt and incubated at the different temperatures (Figs. 1,2,3).

The effect of salt on the growth of *L. monocytogenes* was different at different incubation temperatures, but the same pattern remained throughout the use of different nitrite concentrations (longest generation time in ham produced with 0.02 % nitrite and shortest without etc.). In ham incubated at 18

°C, the organism had the longest generation time (2.0 h) when 3 % salt was included and the generation time of the organism in ham produced with 1.5 % salt was longer (1.8 h) than that of ham produced without salt (1.6 h). At the lower incubation temperatures (0, 7 °C), salt appeared to have a different effect on the organism. The lower the salt concentration was, the shorter was the generation time (Figs. 1,2,3). These results are in accordance with the results obtained by Cole and coworkers (1) on the survival of the organism - low concentrations of salt provided a slight protective effect against inactivation of *L. monocytogenes* - except that these results were obtained at low pH values, lower than the pH of the ham produced. Mckellar (5) also found that growth was stimulated by 40 % with 2 % NaCl. The physiological basis of the protective and stimulating effect of salt at low temperatures is unknown.

Conclusions

Temperature has the greatest effect on the growth of *Listeria monocytogenes*. Ham should be processed, transported and stored (until consumption) at *ca* 0 °C. Nitrite is also of great consequence to the inhibition of the organism in ham. NaCl could be important to inhibit growth of the organism in ham at higher temperatures, but at lower temperatures the opposite is true. According to the constructed model, ham processed with 0.02 % nitrite, without salt, and incubated at 0 °C, will be the best safeguard against *L. monocytogenes*.

One important application of this model (and others) will be in providing a quantitative dimension to the establishment and implementation of HACCP programs. The developed model is not a substitute for good professional expertise in microbiology including laboratory support. The model provides a means of acquiring an estimate of the likely behaviour of *L. monocytogenes* in S.A. produced ham. To provide an example of how the model could assist a HACCP team in the processing of ham, consider a hypothetical product with a sodium chloride content of 1.5 % and a sodium nitrite content of 0.02 % is to be held at 18 °C for 12 hours because of a breakdown in the processing line. According to the model, if *Listeria* was present it would have been in exponential growth and there is a possible *Listeria* risk. The HACCP team should therefore have the product tested for the organism. Was the same product held at 18 °C for 6 hours, the organism would be in lag phase and one would not have to test for the organism.

Models developed in other countries (developed from results obtained from media) should be compared to this model to ascertain whether it is necessary to repeat the experiments with other pathogens and/or products or not.

Literature

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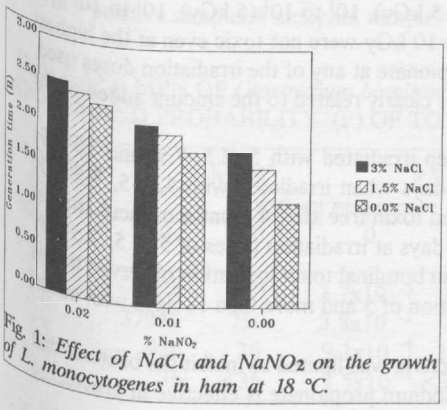

Fig. 1: *Effect of NaCl and $NaNO_2$ on the growth of L. monocytogenes in ham at 18 °C.*

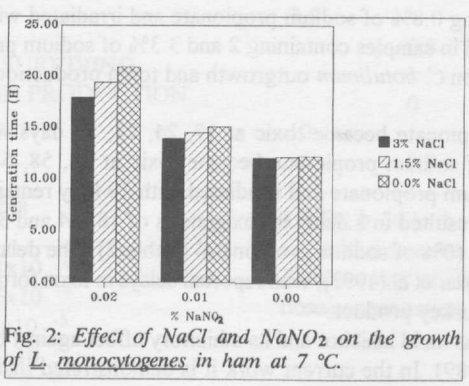

Fig. 2: *Effect of NaCl and $NaNO_2$ on the growth of L. monocytogenes in ham at 7 °C.*

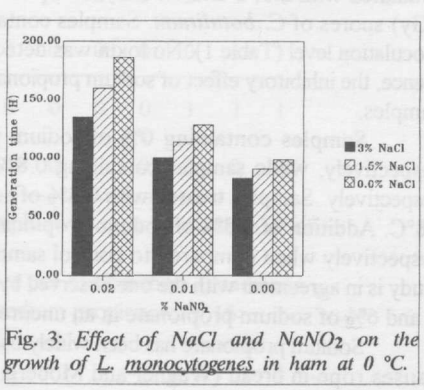

Fig. 3: *Effect of NaCl and $NaNO_2$ on the growth of L. monocytogenes in ham at 0 °C.*