

## PREDICTIVE MODELING OF PATHOGEN GROWTH AND DEATH IN INTERMEDIATE MOISTURE (IM) RATIONS

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### ABSTRACT

A unique, "quasi-chemical" mathematical model was developed for the growth of *Staphylococcus aureus* in military shelf stable bread. This model accounts for the influences of  $A_w$ , pH, and temperature on the growth kinetics. The quasi-chemical mechanism postulates a series of four reaction steps with associated rate constants and the involvement of an antagonistic metabolite to depict growth dynamics that account for all four phases of the microbial lifecycle. The model is generalized to IM turkey meat and other microorganisms (*Listeria monocytogenes* and *Escherichia Coli* 0157:H7).

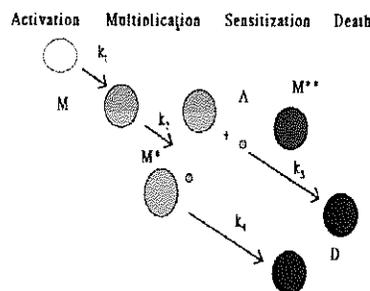
### 1. INTRODUCTION

"Hurdles" are adjustments in food formulations [e.g., water activity ( $A_w$ ), pH, natural antimicrobials] or processing or storage conditions that control bacterial growth while avoiding the excessive loss of vitamins and nutrients in foods attributable to conventional thermal processing ("canning"). Ration components stabilized in such ways are perceived as more fresh-like, retain more nutritive value, feature higher quality attributes, and are more fully consumed, characteristics that ensure adequate nutrition for the warrior to sustain full performance.

We propose a quasi-chemical model that predicts the growth and death of the key pathogens *S. aureus*, *L. monocytogenes*, and *E. Coli* in shelf stable military bread and turkey meat as functions of  $A_w$ , pH, and temperature. The model is based on a schematic chemical mechanism; the microbial processes are treated as a series of chemical steps with associated rate constants. The pivotal feature of this model is the concept of an antagonistic metabolite acting as an intercellular signaling molecule, an analog to those forming the basis for quorum sensing. The proposed role of this communication molecule enables modeling of the successive phases of the entire microbial lifecycle in an integrated and continuous fashion. This model provides a powerful tool in predicting the conditions to ensure microbiological safety in rations stabilized by "hurdles."

### 2. METHODOLOGY

The "reaction" steps of the quasi-chemical model (Figure 1) involve the conversion to actively metabolizing ( $k_1$ ); the multiplication of active cells ( $k_2$ ) with the formation of an antagonistic metabolite; a death step induced by interaction with the antagonist ( $k_3$ ); and a natural death step ( $k_4$ ). In this general form, the model simulates the sequential phases of the microbial lifecycle (lag, exponential, stationary, and death).

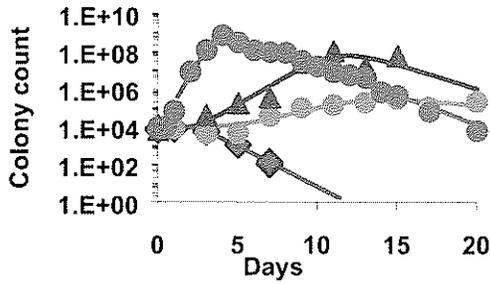


**Figure 1.** Mechanism of the quasi-chemical model (M = metabolizing cells, M\* = multiplying, A = antagonist, M\*\* = sensitized, and D = dead).

Shelf stable bread was prepared according to US military specifications. The  $A_w$  was varied by adding glycerol to the dough (desorptive) or by equilibrating bread over saturated  $BaCl_2$  or  $KCl$  salt solutions (adsorptive), and the pH was varied by the addition of glucono-delta-lactone (GDL). Final  $A_w$  and pH values were measured using an Aqualab CX-2 (Decagon, Inc.) and a Ross spear-tip electrode (Orion Research, Inc.), respectively. Turkey meat containing 6.3% glycerol and other additives was cooked, freeze-dried to adjust  $A_w$ , ground, and irradiated to remove competitive microflora. Samples were inoculated with cocktails of either *S. aureus* or *L. monocytogenes* or *E. Coli*, and the growth of microorganisms was determined by plate counting.

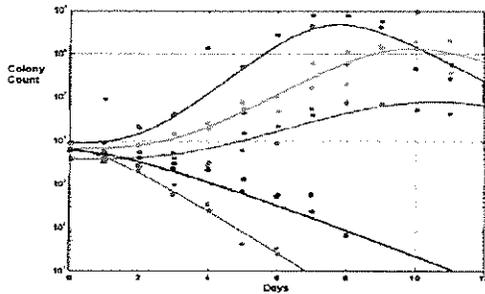
### 3. RESULTS

The quasi-chemical model is remarkably robust in fitting the observed *S. aureus* growth dynamics in all conditions. The growth of *S. aureus* in bread diminished as the  $A_w$  decreased from 0.91 to 0.79 (Figure 2). As the  $A_w$  value decreased, the lag time



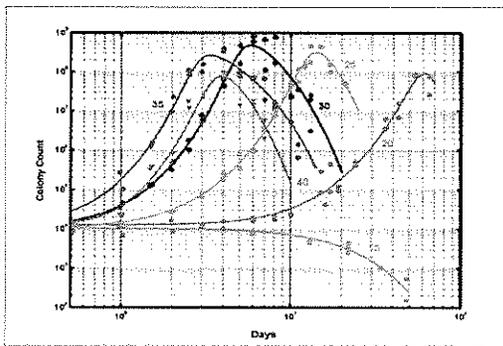
**Figure 2.** Experimental data and fitted curves for *S. aureus* growth/death for descending values of  $A_w$  over the range 0.91 to 0.79.

increase and growth rate decreased. At  $A_w = 0.84$ , only death kinetics were observed. Similarly, as the pH decreased from 5.4 to 4.5 (GDL concentrations of 0.0 to 0.3%) at constant  $A_w = 0.87$  there was also a corresponding decrease in the growth of *S. aureus* (Figure 3). Eventually, at pH 4.7 and 4.5, only death kinetics were observed.



**Figure 3.** Experimental data and fitted curves for *S. aureus* growth/death for pH 5.4, 5.2, 5.0, 4.8, and 4.5.

Varying the temperature dramatically influences the growth kinetics (Figure 4). At 15 °C, death kinetics are observed. As the incubation temperature increases, the lag time decreases and growth rate

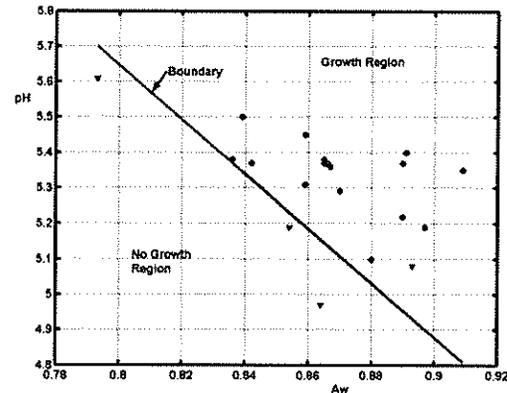


**Figure 4.** Experimental data and fitted curves for *S. aureus* growth for varied temperature (see text).

increases, reaching a maximum at  $T = 35$  °C. Above 35 °C, the growth kinetics begin decreasing. This observation is consistent with the optimal growth temperature of *S. aureus* being at 37 °C. Over the range 20 – 35 °C, the maximum number of cells was the same.

Similar behaviors were observed with turkey meat as the substrate, although the precise responses of the microorganisms differed from each other according to their unique characteristics. For example, the minimal  $A_w$  for growth in turkey was 0.86, 0.90, and 0.92 for *S. aureus*, *L. monocytogenes*, and *E. coli*, respectively. More importantly, the minimal  $A_w$  for *S. aureus* growth in turkey meat ( $A_w = 0.86$ ) was close to but higher than that observed in bread ( $A_w = 0.84$ ). Differences between the simple bread matrix and the more complex turkey meat matrix might account for this difference.

Interrelating the fitted rate constants to  $A_w$  and pH establishes the combinations of  $A_w$  and pH that either support or inhibit growth (Figure 5). Such boundary line data are useful in product development and in writing Hazard Analysis and Critical Control Points (HACCP) plans.



**Figure 5.** Calculated growth/no-growth boundary for *S. aureus* in bread.

#### 4. CONCLUSIONS

The development of rations is geared towards meeting the nutritional needs of a rapidly deployable, mobile, and versatile warfighter. Rations stabilized by “hurdles” generally have higher consumer acceptance and are more fully consumed by the warfighter. The quasi-chemical model can be used to predict guidelines for ensuring safety when formulating novel rations and ration items that are not processed to the point of sterility.