

Effect of Water Activity on the Growth Kinetics of *Staphylococcus aureus* in Ground Bread Crumb

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ABSTRACT: The kinetics of *Staphylococcus aureus* (strains A, B, D) growth in bread crumb were determined as a function of water activity (A_w) from 0.836 to 0.909 at pH 5.2 to 5.5 and at 35 °C. Adding glycerol to the dough or equilibrating the bread over saturated salt solutions adjusted the A_w of the bread. Growth kinetics data, plotted as enumerated colony counts versus incubation time, were fitted using the logistic function to determine maximum growth rates. Similar maximum growth rates resulted, irrespective of the method used to adjust A_w . Extrapolation of growth rate- A_w results predicts the A_w corresponding to a zero growth rate.

Keywords: *Staphylococcus aureus*, growth kinetics, predictive microbiology, intermediate moisture (IM) food, water activity (A_w), pH

Introduction

THE RECENT SPATE OF PUBLICITY ILLUSTRATES the successful development of a barbecue chicken pocket sandwich satisfying stringent military storage requirements (ABC 2002; BBC 2002; CNN 2002; Cook 2002; Fabricant 2002; Graham-Rowe 2002; SBCCOM online 2003). Controlling food properties such as A_w mitigates moisture migration from the sauce to the bread and ensures microbiological stability, while achieving nutritious, high-quality products scoring high acceptance ratings by consumers. To satisfy consumers' need for variety, cheese and/or deli meat pocket sandwiches, in which the cheese and/or meat items are enrobed in bread, have been considered. We focus on the abilities of bread and cheese to act as food substrates that support *Staphylococcus aureus* growth by measuring the kinetics of growth over the A_w range of 0.836 to 0.909 at pH 5.2 to 6.0 to ensure the development of stable, safe products. Determinations of microbial growth in actual foods improves predictions of food safety that are based on near-optimal growth conditions achieved by desorption in laboratory medium (Gibson and others 1987; Walls and others 1996; McMeekin and others 1997; Baranyi and Roberts 2000; Stewart and others 2001; Castillejo-Rodríguez 2002). Cheese has been demonstrated to support *S. aureus* growth (Magrini and others 1983; Rajkowski and others 1994; Sutherland and others 1994).

Formulating and developing intermediate moisture foods to attain shelf stability with respect to microbiological growth and/or degradation reactions is generally referred to as *hurdle technology*, which encom-

passes a number of methods to impart food stability by controlling factors such as A_w , pH, atmospheric conditions, the levels and nature of various humectants, food additives, and preservatives, and the presence of competing microflora (Leistner and others 1981). Applying the concept of the "hurdle" approach to intermediate moisture (IM) foods meets consumer demand for more freshlike products while retaining the value of extended shelf life and avoiding excessive processing. Complex foods such as bilayered sandwiches and cream-filled cakes and pastries represent a special case for ensuring microbiological stability because of the potential for moisture transfer between the 2 phases during storage that may render a previously stable phase unstable (Tatini 1973; Rajkowski and others 1994). For example, dried salami slices stabilized at A_w below 0.85 did not support *S. aureus* growth; however, storage for 48 h of these meat slices in contact with cheese at $A_w = 0.91$ increased the A_w of these salami slices to 0.91 and they supported the growth of *S. aureus* (Rajkowski and others 1994).

The growth of *S. aureus* and its associated production of enterotoxin occur in an array of food systems exhibiting a wide range of environmental conditions (A_w , pH, temperatures, type and concentration of humectant, food matrix and laboratory medium, and under both aerobic and anaerobic conditions). *Staphylococcus aureus* thrives in terms of its abilities to grow and produce enterotoxin in the unique ecological niche (Lotter and Leistner 1978) characteristic of IM foods. Intermediate moisture foods are defined (Karel 1973) as displaying the following properties: A_w ranging from 0.7 to 0.9

and water content ranging from 20 to 50%. In general, *S. aureus* growth occurs in the pH range 4.5 to 9.3 (7.2 optimum) and a temperature range of 6.7 to 45.6 °C (37 °C optimum; Sutherland and others 1994). Scott (1953) found that the lowest A_w supporting *S. aureus* growth was 0.86 (at 30 °C) for dried milk and dried soup and 0.88 for dried mutton. He also found that A_w and not the water content was the factor that controlled growth and determined that growth was independent of the predominant solutes in the medium.

There is ample evidence for much lower limiting values of A_w for *S. aureus* growth, depending on the interactive effects of critical growth factors and the nature of the food substrate. One report showed that the limiting A_w for growth of *S. aureus* was 0.83 to 0.84 at 35 °C in Brain Heart Infusion (BHI) medium (pH 7.2), pork extract (pH 6.0), and beef extract (pH 6.1; Chirife and others 1982). Labuza and others (1972) observed the slow growth of *S. aureus* in pork at $A_w = 0.84$ (25 °C and pH 7.25). The data of Hill (as cited by Tatini 1973) showed that the minimum A_w for growth of *S. aureus* in pork was 0.83 (35 °C and pH 5.5) and 0.86 in beef (35 °C and pH 5.8). Lee and others (1981) measured the growth of *S. aureus* in precooked bacon at $A_w = 0.84$ (25 °C), which was also capable of supporting production of enterotoxin A.

In comparison, the A_w parameters for anaerobic growth of *S. aureus* are not as well established, but the minimal A_w for growth is slightly higher than for aerobic conditions. Scott (1953) determined that *S. aureus* grew in nutrient broth with A_w of 0.92 and not with A_w of 0.88 (30 °C). He also pre-

dicted, based on this work, that no growth would occur at $A_w = 0.90$. Lee and others (1981) reported that the minimal A_w for growth of *S. aureus* at 37 °C in precooked bacon was 0.90.

As indicated earlier, pH also influences the growth of *S. aureus* in IM foods. Boylan and others (1976) established that *S. aureus* growth in hennican (a ground IM product) was slower at pH 5.2 than pH 5.6 ($A_w = 0.91$ and 22 °C). Hennican contains a mixture of peanuts, chicken, milk, raisins, and honey. Hansemann and others (1980) determined that *S. aureus* growth in IM meat was inhibited with A_w at or below 0.84 (37 °C and pH 4 to 6) or with pH at or below 4.5 (37 °C and $A_w = 0.80$ to 0.95). Interestingly, synergism between these 2 physical parameters was found: an additional narrow "zone" of no-growth occurred in the A_w range of 0.84 to 0.87 while the pH was between 4.5 and 4.7 (37 °C).

The recent report of 300 cases of *S. aureus* food poisoning (ANZFA 2002) follows other major outbreaks and recalls of food due to staphylococcal contamination, such as dried milk in Japan (DJ/AFPE 2000), wet bean curd in the U.S. (Safety Alerts 2000), and pasta products in Canada (CFIA 2001). These incidents exemplify the gravity of the adverse impact of *S. aureus* enterotoxin on human health and its potential for economic loss and negative publicity on industry (AFPE 2000). Information gathered by the Centers for Disease Control (CDC) from 1973 to 1987 shows that *S. aureus* accounted for 13% of all foodborne disease outbreaks, and 14% of the cases due to bacterial pathogens (Bean and Griffin 1990); and from 1993 to 1997 the CDC figures were correspondingly 1.5% and 1.6% (Olsen and others 2000), although these figures may underestimate the numbers involved because cases often are unreported.

Consequently, we investigated the kinetics of *S. aureus* growth in shelf-stable IM bread, systematically measuring and analyzing the growth kinetics over the entire range of A_w and pH conditions that support growth of this microorganism. These results establish a comprehensive database characterizing the growth kinetics of *S. aureus* in an actual food system. Determining the sensitivity of kinetics parameters to A_w can provide guidelines for ensuring that IM, cereal-based, multicomponent food products are safe, stable, and acceptable.

Materials and Methods

Bread preparation

Shelf-stable bread was prepared precisely according to Military Specification

MIL-B-44360A, 11 March 1993, with dough conditioner "Control S" (ADM Arkady, Olathe, Kans., U.S.A.) added. The bread formulation consisted of the following ingredients (on a weight percent basis): bread flour, 50.09; water, 28.73; shortening, 8.60; glycerol, 6.29; yeast (Red Star Active Dry Yeast, Universal Foods Corp., Milwaukee, Wis., U.S.A.), 2.23; salt, 1.28; sucrose ester (sucrose ester stearate having a hydrophilic-lipophilic balance [HLB] number of 16, Mitsubishi-Kagaku Foods Corp., Tokyo, Japan), 0.99; "Control S", 0.63; gum arabic (Gum Technology, Tucson, Ariz., U.S.A.), 0.50; calcium sulfate (ADM Arkady), 0.25; xanthan gum (Kelco, Chicago, Ill., U.S.A.), 0.25; encapsulated potassium sorbate (Balchem Corp., Slate Hill, N.Y., U.S.A.), 0.13; cream flavor (Haarmann & Reimer, Teterboro, N.J., U.S.A.), 0.04. The dough conditioner imparts (USANRDEC 1998) both crumb softening and dough strengthening characteristics, thereby allowing for better machinability of the dough and an improved final product with regard to increased water absorption, improved gas retention, increased resistance to collapse at higher shear rates, and improved tolerances to mixing. "Control S" contains the following compounds: diacetyl tartaric acid esters of monoglycerides and diglycerides, fungal alpha amylase, ascorbic acid, cysteine hydrochloride, and azidocarbonimide.

All dough samples were worked and baked identically to minimize any differences between bread samples. The bread was made by mixing the dry ingredients for 10 min in a mixer (Hobart, Troy, Ohio, U.S.A.) fitted with a dough hook at speed 1. The shortening was added and mixed, and then the water/glycerol mixture was added. The dough was mixed at speed 2 for about 15 min until dough development was complete and allowed to rest at room temperature for 15 min. Batches of approximately 6 kg were produced. A Fortuna Automat (Admatic, Eatontown, N.J., U.S.A.) was used to divide the dough into 60- to 65-g pieces. Each piece was rolled flat and placed in a pan (24 wells per pan) for proofing. The dough was proofed at 85 °F (29.4 °C) and 85% relative humidity for 60 min in a proof-er (Hobart). After proofing, lids were placed on the trays to control the height of the bread loaf and baked at 350 °F (176 °C) for 14 min in a convection oven (G.S. Blodgett Corp., Burlington, Vt., U.S.A.). After baking, the loaves were removed from the pans and cooled on metal racks to an internal temperature of 176 to 230 °F (80 to 100 °C). The loaves were sealed in military trilaminar pouches with an oxygen scavenger (Fresh

Pax®, Multisorb Technologies, Inc., Buffalo, N.Y., U.S.A.) without vacuum. The bread was stored at 39.2 °F (4 °C) until use. Bread prepared with this formulation remains shelf-stable and freshlike for 3 y of storage without refrigeration in compliance with military specifications.

Two cheddar-type process cheese products with relatively low A_w were purchased (Gamay Flavors, New Berlin, Wis., U.S.A.). The 2 cheese products had similar moisture contents of about 40% and corresponding A_w and pH values of either 0.870 and 6.04 or 0.851 and 5.94. Formulation data for the cheese were not available from the producer.

Water activity adjustment of bread

The A_w of the bread was adjusted to specific levels by desorptive and adsorptive techniques. The first method for adjusting the A_w of the bread was to vary the amount of glycerol added to the dough mixture (with compensatory adjustments in the total amount of flour added) prior to baking. Glycerol levels of 0 to 12% produced A_w values in the bread of 0.909 to 0.793. At 12% added glycerol, the dough became more difficult to mix. The second method for adjusting the A_w of the bread entailed equilibrating 55 g of ground bread crumb (containing 6.3% glycerol in accordance with military specifications) spread in a sterile aluminum foil pan over saturated salt solutions of either BaCl_2 ($A_w = 0.902$) or KCl ($A_w = 0.849$; Fisher, Fair Lawn, N.J., U.S.A.) in a 10-L evacuated dessicator at 25 °C. Appropriate proportions of each equilibrated crumb type were mixed then allowed to re-equilibrate for 30 d to attain bread samples with intermediate water activities.

pH adjustment of bread

The pH of the bread was adjusted by the dissolution of 0.05, 0.1, 0.2, or 0.3% nonencapsulated glucono-delta-lactone (GDL, Glucona America, Inc., Janesville, Wis., U.S.A.) into the water phase of the dough before mixing with the dry components. The effects of GDL on the final pH of the bread are discussed below.

Physical and microbiological measurements

Five replicates were measured for each bread crumb preparation to determine the total percent moisture, A_w , and pH of each sample. For moisture determinations, precisely weighed, 3- to 4-g samples of bread crumb were dried at 70 °C for 18 to 24 h in a vacuum oven, then reweighed to calculate the total percent moisture in the sample. To measure A_w , ground bread crumb was placed in the sample cup of an Aqualab CX-

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Table 1—Effect of glycerol on shelf-stable bread

% Glycerol	% Moisture	A_w	pH ^a
0.0	27.54	0.909	5.22
3.0	27.27	0.891	5.47
4.0	26.08	0.880	5.48
5.0	26.30	0.871	5.41
6.3	26.90	0.866	5.54
7.0	26.43	0.859	5.48
9.0	25.72	0.839	5.45
12.0	25.88	0.793	5.53

^aDetermined by AOAC 2000 method.

2 (Decagon, Inc., Pullman, Wash., U.S.A.) with a temperature-controlled chamber and tested at 25 °C. Following the AOAC (2000) procedure, the pH was determined by mixing 10 g of ground bread crumb in 100 mL of distilled and deionized water (boiled and cooled). After equilibrating for 15 min and decanting the supernatant, the pH was measured using a combination tip electrode (Orion, Inc., Beverly, Mass., U.S.A.) connected to a digital pH meter (model 720A, Orion, Inc., Beverly, Mass., U.S.A.). Direct pH measurements of the solid bread crumb were made using a ROSS® combination spear-tip pH electrode for foods (Orion, Inc.) connected to the pH meter (model 720A, Orion, Inc.), and the averages of readings taken at 3 different locations in the sample were recorded.

Microbiological analyses of the uninoculated bread samples showed that the samples contained < 10 CFU (colony-forming units)/g with regard to aerobic plate counts, and yeast and mold. The samples were also found to be negative for *S. aureus*, *Escherichia coli*, and anaerobic counts.

Organisms, culture maintenance, and inoculum preparation

The cocktail of *S. aureus* used in this study comprised 3 strains: *S. aureus* A-100, an enterotoxin A (SEA)-producing strain (E. M. Powers, Natick Soldier Center, Natick, Mass., U.S.A.); *S. aureus* ATCC 14458, an enterotoxin B (SEB)-producing strain (American Type Culture Collection, Manassas, Va., U.S.A.); and *S. aureus* 993, an enterotoxin D (SED)-producing strain (Toxin Technology, Sarasota, Fla., U.S.A.). The cultures were maintained on slants of tryptic soy agar (DIFCO, Sparks, Md., U.S.A.) supplemented with 0.5% yeast extract (DIFCO) and transferred monthly. The media used in the inoculum preparation were trypticase soy broth (TSB, Becton-Dickson, Cockeysville, Md., U.S.A.) and Plate Count Agar (PCA, DIFCO, Detroit, Mich., U.S.A.). Three separate inocula were prepared by incubating the inocu-

lated medium (culture from slant suspended in TSB and 1 drop inoculated per 10 mL TSB in screw-cap tube) at 35 °C for about 18 h, then repeating the process for another 18 h. Next, 0.3 mL of each TSB inocula were spread-plated on PCA and incubated at 35 °C for 18 h. The cells were harvested from the PCA by scraping the surface of the agar with a sterile glass "hockey stick" and rinsing the plate with Butterfield's phosphate buffer to produce a 10-mL "stock" culture for each organism. The stock culture was diluted to give a Klett₅₄ colorimetry reading (Klett-Summerson Photoelectric Colorimeter, A. H. Thomas Co., Philadelphia, Pa., U.S.A.) of about 125 to 135 Klett units (approximately 10⁹ cells/mL). The cultures were mixed in equal proportions and diluted with Butterfield's phosphate so that an inoculum of 0.01 mL added to the ground bread crumb produced a concentration of approximately 10⁴ CFU/g.

Inoculation of bread crumb and cheese samples

To begin the growth kinetics experiments, bread pouches were opened aseptically, crusts were removed from the bread loaves, and the crumb from 15 to 20 loaves (approximately 30 g/loaf) was ground and mixed in a blender (Waring, Torrington, Conn., U.S.A.). Aliquots (10 g) were dispensed into sterile 250-mL Seward Stomacher bags (Seward, Ltd., London, England, U.K.). This process was duplicated for each type of bread sample. Similarly, 10-g aliquots of cheese samples were dispensed into individual sterile 250-mL Seward Stomacher bags (Seward, Ltd.).

Each individual sample, whether bread crumb or cheese, was inoculated with cells in the Stomacher bag and mixed with a sterile applicator. The Stomacher bags containing inoculated samples were folded, placed inside individual oxygen-impermeable trilaminar pouches (Cadillac Products, Inc., Paris, Ill., U.S.A.), and then sealed under 28 psi of vacuum (Röschermatic Vacuum Packaging Machine, Reiser & Co., Canton, Mass., U.S.A.). All of the samples prepared for growth kinetics experiments (about 30 per test condition) were inoculated and sealed on the same day and stored overnight at 4 °C. Time zero was considered the following day, and all samples were incubated at 35 °C to promote proliferation of the *S. aureus* inoculum.

Microbiological examination of test samples

Duplicate sample pouches were withdrawn at regular intervals for assay by microbiological enumeration. The vacuum-

sealed pouches were opened aseptically and 90 mL of cold (4 °C) Butterfield's phosphate was added directly to the sample in the Stomacher bag. In the case of the cheese products, the Butterfield's phosphate was warmed to 45 °C before use (AOAC 2000). The contents were mixed for 2 min in a stomacher (LabBlender 80, Seward Medical, London, England, U.K.). Appropriate dilutions were made in Butterfield's phosphate and 0.1 mL of diluted sample was plated in duplicate by spread-plate technique on Baird-Parker agar (DIFCO, Sparks, Md., U.S.A.) supplemented with egg yolk tellurite (DIFCO). The petri plates were incubated for 48 h at 35 °C before colonies were counted using a New Brunswick colony counter (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.).

Growth curves and the logistic function expression

Growth curves of *S. aureus* in bread crumb, plotted as colony counts versus time, conformed to the 4-parameter logistic function expression and in all cases gave excellent fits ($R^2 = 0.939$ to 0.996) using the curve-fitting software SigmaPlot 2000 (Jan-del Scientific, San Rafael, Calif., U.S.A.):

$$\log N_t = \frac{(\log N_f/N_i)}{(1 + \exp [b(t_m - t)])} + \log N_i$$

The sigmoidally shaped logistic function is commonly used for expressing microbial growth dynamics: N_t is the microbial load at time t ; N_i is the initial microbial load; N_f is the final microbial load; t_m is the time at which the growth rate is maximal; and b , the maximum growth rate, is the slope of the line tangent to the curve at t_m . Fitting the data with other exponential negative linear functions available in SigmaPlot 2000 such as the 4-parameter Gompertz equation yielded nearly identical fits and values for growth parameters. Zwietering and others (1990) and Gibson and others (1987) found comparable curve-fitting results using the logistic equation and the Gompertz equation for *S. aureus* growth and for *C. botulinum* growth in pork slurries, respectively.

Results and Discussion

TABLE 1 DEMONSTRATES THE EFFECT OF added glycerol on the moisture content, A_w , and pH of shelf-stable bread.

In general, as the level of glycerol added to the dough increased, the A_w of the baked bread crumb decreased, but the total moisture content (average = 26.5%) and the pH (average = 5.5 by AOAC 2000) remained nearly constant. For bread containing 6.3% added glycerol and equilibrated over satu-

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rated salt solutions, the A_w was adjusted to values of 0.836, 0.842, 0.866, and 0.890.

Typical growth kinetics curves for *S. aureus* in ground bread crumb containing various amounts of added glycerol are shown in Figure 1. Fitting the data with the 4-parameter logistic function generated the smooth sigmoidal curves. This curve fitting provides the best value for the maximum growth rate, b , which is a condition-dependent variable defined by $\Delta [\log (\text{CFU}/\text{mL})] / \Delta$ days and whose units are reciprocal days. With 12.0% glycerol added to the bread, only death kinetics were observed.

The fitted values for t_m , b , and N_f from analysis of the experimental data determined for bread prepared by the desorptive and adsorptive techniques are compiled in Table 2 and arranged in the order of decreasing A_w . The time (days) elapsed to reach the point of maximum growth (t_m , column 3) increases as A_w decreases. Though not shown, a plot of t_m against A_w follows approximately a power law or hyperbolic expression. In any case, it is reasonable to expect that lowering the A_w will increase t_m to the point of achieving total inhibition. Correspondingly, the maximum growth rate decreases with decreasing A_w (as A_w decreases from 0.909 to 0.836, b decreases from 1.80 to 0.14 per day). The final microbial load, N_f , also decreased with decreasing A_w (column 5). These results demonstrate that the growth kinetics of *S. aureus* in bread depend strongly on A_w and that the growth kinetics are empirically fit by the logistic function. These results agree with the conclusion of Troller and Stinson (1975) that limiting the amount of available water suppresses growth rates and maximum final counts.

The effect of A_w on $\Delta [\log (\text{CFU}/\text{mL})] / \Delta$ days or maximum growth rate, b , is displayed in Figure 2. The results demonstrated a linear relationship between the maximum growth rate and A_w ($R^2 = 0.946$), irrespective of whether the A_w was achieved

Table 2—Logistic function fitted parameters for *Staphylococcus aureus* growth in ground bread

A_w	% Glycerol	t_m (d)	b (d ⁻¹)	$\log (N_f)$
0.909	0.0	1.67	1.80	8.45
0.891	3.0	1.97	1.50	8.27
0.890 ^a	6.3	2.10	1.58	6.97
0.871	5.0	2.77	1.00	7.98
0.866	6.3	6.77	0.68	7.74
0.866 ^a	6.3	1.50	0.71	6.16
0.859	7.0	6.98	0.52	6.69
0.842 ^a	6.3	4.00	0.46	5.55
0.839	9.0	7.05	0.35	5.29
0.836 ^a	6.3	7.00	0.14	4.51

^aDenotes A_w achieved through equilibration method.

by varying the amount of glycerol added to the dough mixture or by equilibrating ground bread crumb containing 6.3% glycerol over various saturated salt solutions. Consequently, the most important factor controlling growth is the A_w and the glycerol concentration exerts its influence on growth by its effects on A_w . Based on linear regression analysis of the data in Figure 2, the straight line crosses the x-intercept, at the point that the maximum growth rate is zero (that is, A_w equals approximately 0.83).

Figure 3 illustrates a hypothetical curve drawn to represent a more encompassing view of the response of growth rate to A_w . This larger sigmoidal curve is not strictly linear through the straight-line portion of the data (Figure 2), but it approximates the response of the maximum growth rate outside of the experimentally determined domain (Figure 3). At A_w of 0.83 and below, the curve projects leveling off. The growth of *S. aureus* is not supported in this A_w regime and there is no further decrease in maximum growth rate. In the upper region of the curve, above A_w of 0.91, this hypothetical curve projects that the increases in maximum growth rate will become less pronounced as A_w increases, eventually leveling off.

Table 3—Effects of glucono-delta-lactone (GDL) on the physical properties of bread containing 6.3% glycerol

% GDL	pH ^a	pH ^b	A_w	% Moisture
0.0	5.34	5.38	0.865	27.24
0.05	5.17	5.36	0.867	27.16
0.1	5.10	5.31	0.859	26.22
0.2	5.07	5.19	0.854	25.26
0.3	4.95	4.97	0.864	25.57

^{a, b}Determined by AOAC 2000 and pH electrode, respectively.

Glucono-delta-lactone is an acidulant used to decrease the pH of bread. Table 3 lists the effects of added GDL on the physical properties of the shelf-stable bread.

Results obtained for pH by the AOAC 2000 method, in which the sample is diluted 10-fold with water, were consistently lower than the direct measurements of pH using the ROSS[®] combination spear-tip electrode. Presumably, the results using the ROSS[®] electrode more accurately represent the pH within the bread product because measurements are not obscured by incomplete aqueous extraction, which only includes substances soluble in water. The average total moisture content and average A_w remained constant with increasing GDL concentrations at about 26.29% and 0.862, respectively.

Figure 4 illustrates some representative growth kinetics in shelf-stable bread with added GDL. Increasing the percent GDL in the dough formulation to decrease the pH of the bread protracted the observed lag time, decreased the maximum growth rate (from 0.831/d to 0.460/d at 0.0 and 0.1% added GDL, respectively), and decreased the attainable final colony count. At levels of added GDL equal to or above 0.2%, only death kinetics with no demonstrable lag time were observed (data not shown).

Stewart and others (2001) developed an empirical expression for constructing "growth/no-growth" contour lines for the

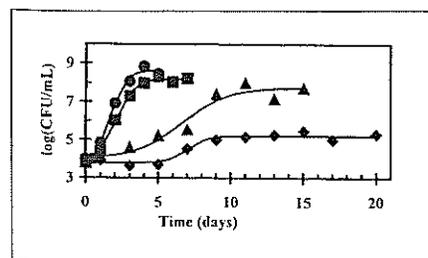


Figure 1—*Staphylococcus aureus* growth kinetics as a function of glycerol content (● = 0.0% added glycerol, $A_w = 0.909$; ■ = 3.0%, 0.891; ▲ = 6.3%, 0.866; and ◆ = 9.0%, 0.839)

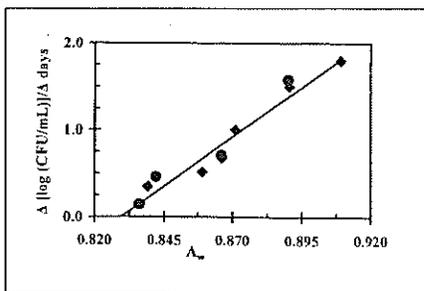


Figure 2—Effect of A_w on maximum growth rate of *Staphylococcus aureus* in shelf-stable bread (◆ = desorptive A_w adjustment using glycerol; ● = the adsorptive technique)

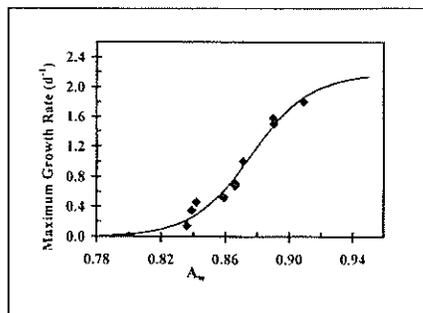


Figure 3—Hypothetical curve expanding view of maximum growth rate (b) compared with A_w

growth of a cocktail of *S. aureus* cells using BHI broth containing potassium sorbate or calcium propionate, glycerol as the humectant, and HCl. In conditions of 500 ppm potassium sorbate, A_w in the range of 0.84 to 0.86, and pH in the range 4.5 to 7.0, the expression predicts that neither growth nor toxin production will take place within 180 d of incubation at 37 °C. In contradistinction, we observed the growth of *S. aureus* in shelf-stable bread in nearly identical experimental conditions (650 ppm potassium sorbate, $A_w = 0.84$, pH = 5.45, and temperature = 35 °C). Our kinetics data in bread demonstrate significant *S. aureus* growth of approximately 1 log-cycle (Figure 1) after about 9 d and t_m is approximately 0.25 d. The information gathered in food products provides a better index of microbial growth than that gathered in broth.

The kinetics behavior of *S. aureus* was examined in experiments using the 2 cheddar-types of process cheese as potential sandwich fillers. As mentioned earlier, the relatively low A_w of these cheese-types, presumably, would reduce moisture transfer from cheese to bread or cheese to meat and reduce the potential for the growth of *S. aureus* in the latter 2 foods.

Featuring A_w levels of 0.870 and 0.851 and pH values of 6.04 and 5.94, respectively, the cheese products were expected to support *S. aureus* growth, based on our findings with bread. In both cases, not only was no growth detectable, but as Figure 5 shows, only death kinetics were observed. From the resulting exponential death curves, rate constants for destruction were calculated as $-0.28/d$ and $-0.31/d$ for the cheese-types with $A_w = 0.870$ and 0.851, respectively. As noted in the product information sheet, these cheeses contain sodium lactate and sorbic acid, although their precise quantitative levels were not made available. Sorbic

acid (Eklund 1983) and sodium lactate (de Wit and Rombouts 1990) have putative antimicrobial effects on *S. aureus* growth, and possibly their individual or combined concentrations in the cheeses were sufficient to induce the observed death kinetics of *S. aureus*. The presence of unknown levels of inhibitory substances preclude characterizing the growth kinetics of *S. aureus* in these food substrates as functions of A_w and pH and comparing the results to those obtained with bread. In any case, it appears that a cheese product with these general properties would be a suitable filling for a pocket sandwich with or without added IM meats and probably would not foster the growth of *S. aureus*.

Conclusions

THE GROWTH OF *S. AUREUS* IN BREAD depends strongly on A_w , and the kinetics behavior conforms to the sigmoidal shape of the 4-parameter logistic function. The maximum growth rate depends strongly on the measured A_w , irrespective of whether the desorptive (addition of glycerol) or adsorptive (equilibration over salt solutions) method was used to adjust A_w . In bread, A_w at or below 0.83 does not support the growth of *S. aureus*. This latter conclusion is similar to the results for aerobic *S. aureus* growth in pork (Hill, as cited by Tatini 1973) and in precooked bacon (Lee and others 1981). Lowering the pH of shelf-stable bread at A_w approximately 0.862 also decreases the growth rate of *S. aureus*, inhibiting growth altogether at pH approximately 5.2 (0.2% GDL). In contrast, growth was actively inhibited and death kinetics predominated in process cheddar cheese, despite A_w values of 0.85 or 0.87 and a pH of 5.9 to 6.0. The presence of antimicrobial compounds may have influenced these results.

Recognizing the limitations of empirical/semi-empirical models for predicting the kinetics of bacterial growth and decay, we have developed a phenomenologically

based model (Taub and others 2000; Feeherry and others 2001) that is in preparation for publication that predicts growth/no-growth boundaries based on variations in environmental conditions of A_w and pH. Future investigations will attempt to discern the influence of other variables such as incubation temperature and residual oxygen levels on the growth kinetics of *S. aureus* in IM foods, and details on the mathematical formulation and its validation will be published separately.

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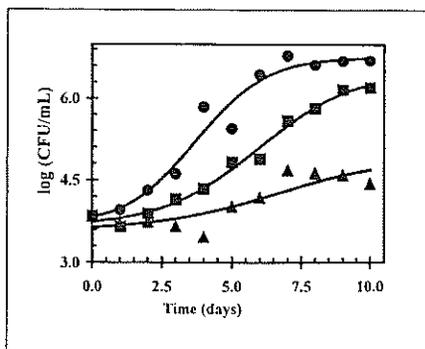


Figure 4—Kinetics growth curves of *Staphylococcus aureus* in ground bread crumb as functions of added glucono-delta-lactone (GDL) (● = 0.0% GDL, pH 5.38; ■ = 0.05%, pH 5.36; and ▲ = 0.1%, pH 5.31).

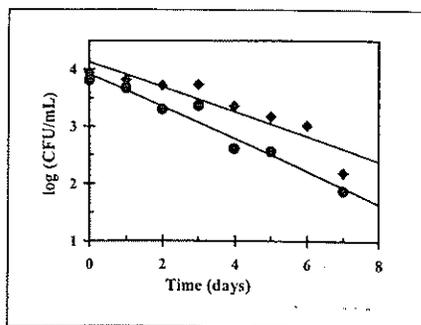


Figure 5—Death kinetics for *Staphylococcus aureus* in cheese with low A_w (◆ = 0.870; ● = 0.851).

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