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Modeling the Aerobic Growth and Decline of *Staphylococcus aureus* as Affected by pH and Potassium Sorbate Concentration

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ABSTRACT

The effects of pH (5.0, 5.2, 5.4, 5.6, and 5,8) and concentration of potassium sorbate (10.0 and 16.6 mM) at two water activity values (0.90 and 0.92) on the aerobic growth and decline of *Staphylococcus aureus* ATCC 6538P, 196-E, and FDA-C243 were studied using brain-heart infusion broth. The inoculum was approximately 4 to 5 log CFU/ml, and the incubation temperature was 30°C. Samples were periodically enumerated on tryptic soy agar. The Gompertz model was used to obtain microbial growth parameters, specific growth rate was obtained as a derived parameter, and the inhibition index was calculated. A linear model was fitted in cases of bacteriostatic or bactericidal action of the treatment. The ATCC 6538P strain showed the highest resistance in the range of tested conditions. Microbial behavior was modeled considering the main controlling factors, and a response surface methodology was used to determine the effects of undissociated acid concentration and pH. These results can be used to establish treatment conditions for microorganism growth or inhibition.

Staphylococcus aureus is recognized as a cause of food poisoning via a protein enterotoxin. Food poisoning occurs after food (initially contaminated with a toxigenic strain) is subjected to conditions permitting growth of the microorganism. Enterotoxins are generally produced in the late exponential or stationary phase of growth. The minimal number of microorganisms of *S. aureus* required to produce enough enterotoxin to cause food poisoning is believed to be about 10^7 CFU/g of food *(13)*. However, Bergdoll *(3)* reported that *S. aureus* must grow to approximately 10^5 CFU/g to produce toxin and cause illness. Foods that have been associated with staphylococcal intoxication include cooked meats (particularly hams), cream or custard-filled cakes, shellfish, salads (including potato, chicken, tuna, and ham *(2)*), chocolate milk *(8),* lasagna *(1),* and foods that have been prepared some time in advance of consumption and stored without adequate refrigeration after preparation *(18)*.

A key to controlling *S. aureus* and other foodborne pathogens is understanding the factors that influence their growth in foods and manipulating those factors to limit potential risks. A characteristic that distinguishes *S. aureus* from other pathogenic microorganisms is its tolerance to low water activity (a_w) and NaCl concentrations of up to 20%. Growth has been reported at a_w values as low as 0.83 *(23),* but those values are dependent on pH, temperature, humectant, and gaseous atmosphere *(15, 24, 25)*. Acidulants affect the growth of *S. aureus (6)*. This foodborne bacterial pathogen can tolerate a pH range of 4.2 to 9.3, and the range of 7.0 to 7.5 is optimal for growth *(2)*.

Sorbic acid has known antimicrobial properties in a wide range of products. The salts of sorbic acid, especially the potassium salt, are very important in applications because of their high solubility in water. Early in its use, sorbate was classified as relatively nontoxic. It was reported that sorbate can be metabolized by the organisms in a way similar to that of naturally occurring fatty acids. The World Health Organization has stipulated for sorbate an acceptable daily intake of 25 mg/kg of body weight *(21)*. Robach and Stateler *(17)* studied the combined effect of potassium sorbate with sodium chloride and different antioxidants on the growth of *S. aureus* in laboratory media at 37°C. Lahellec et al. *(12)* studied the effect of potassium sorbate on *S. aureus* 196-E in brain-heart infusion broth (BHI) at 37°C and demonstrated the influence of pH on the action of the preservative. Kreisman and Labuza *(11)* reported the effect of different factors on the growth of *S. aureus* in intermediate-moisture food (processed cheese with an a_w ranging from 0.81 to 0.94).

Baird-Parker and Kilsby *(2)* concluded that the logical approach to determining the probable behavior of pathogens in foods is the use of predictive models that estimate the microbial response to the primary factors affecting its growth and survival. Validated mathematical models have the potential to be invaluable tools for rapidly and objectively assessing the relative safety of food products.

Various investigators have concluded that the growth and inactivation of *S. aureus* are dependent not only on the pH of the environment but also on the identity and con-

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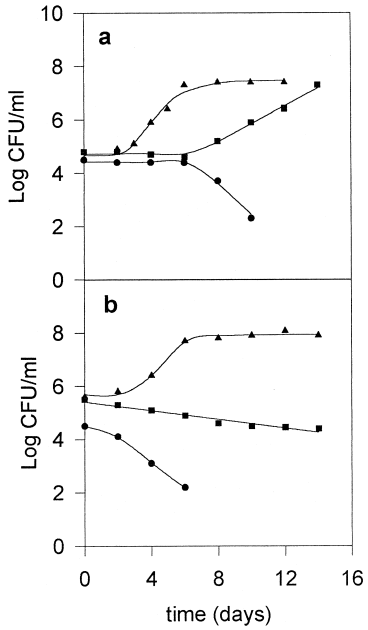

FIGURE 1. *Examples of Gompertz and linear model fittings for the aerobic growth and decline of* S. aureus *ATCC 6538P at a_w = 0.90: (a) 10.0 mM potassium sorbate; (b) 16.6 mM potassium sorbate. ●, pH = 5.0; ■, pH = 5.4; ▲, pH = 5.8. Symbols correspond to experimental data, and lines correspond to the models.*

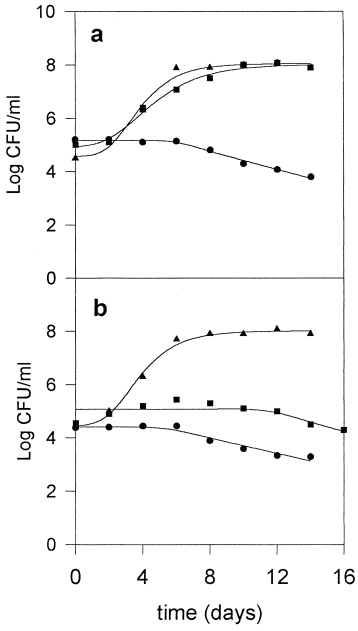

FIGURE 2. *Examples of Gompertz and linear model fittings for the aerobic growth and decline of* S. aureus *ATCC 6538P at a_w = 0.92: (a) 10.0 mM of potassium sorbate; (b) 16.6 mM potassium sorbate. ●, pH = 5.0; ■, pH = 5.4; ▲, pH = 5.8. Symbols correspond to experimental data, and lines correspond to the models.*

centration of the preservative used to control the growth. The simultaneous study of the quantitative effects of multiple physical and chemical factors influencing the growth of *S. aureus* is useful in developing predictive models. Sutherland et al. *(22)* analyzed the simultaneous effects of temperature, pH, and sodium chloride concentration on the growth of *S. aureus* using a predictive model. Response surface models for the effects of temperature, pH, and sodium chloride and sodium nitrite concentrations on the aerobic and anaerobic growth of *S. aureus* 196E were reported by Buchanan et al. *(4)*.

The objectives of the present study were (i) to determine the simultaneous effect of potassium sorbate (10.0 and 16.6 mM) and pH (5.0, 5.2, 5.4, 5.6, and 5.8) on the growth and decline kinetics of three strains of *S. aureus* in a laboratory culture medium with a_w values of 0.90 and 0.92, (ii) to obtain microbial growth and decline parameters by fitting adequate equations to microbial counts, (iii) to model microbial behavior with respect to the main controlling factors, and (iv) to establish a response surface model that could be used to determine treatment conditions that inhibit microorganism growth.

MATERIALS AND METHODS

Experimental procedure. Three strains of *S. aureus,* ATCC 6538P, 196-E, and FDA C243 (obtained from the culture collection of Instituto Nacional de Farmacología y Bromatología, Buenos Aires, Argentina) were cultured separately in 250-ml Erlenmeyer flasks containing 9 ml of BHI (Unipath CM 225, Oxoid, Basingstoke, UK) with a pH of 7 and a_w of 0.993 and subcultured for 3 consecutive days at 30°C for 24 h. The strains were incubated separately in a laboratory culture medium (BHI) at five pH levels (5.0, 5.2, 5.4, 5.6, and 5.8), two levels of a_w (0.90 and 0.92), and two concentrations of potassium sorbate (10.0 and 16.6 mM). Concentrations of 20.84 and 27.07% (wt/vol) NaCl were added to the BHI to achieve levels of a_w 0.92 and 0.90, respectively *(19)*. An electric hygrometer (PL 26 SF 00-421, Vaisala, Helsinski, Finland) with a measurement error of ±0.005 was used to determine a_w values; pH values of the broths were adjusted to different values by adding HCl and measured using a Metrohm E632 pH meter. Food-grade potassium sorbate (Anedra, San Fernando, Buenos Aires, Argentina) was supplemented to reach concentrations of 10.0 and 16.6 mM. Each culture system was dispensed in aliquots of 18 ml to 250-ml flasks and sterilized by autoclaving. The a_w of the culture media was measured after sterilization at 121°C for 15 min, and good agreement was found between calculated and measured a_w values. Each flask was inoculated with 2 ml of different strains diluted in BHI to about 4 to 5 log CFU/ml. Aerobic flasks were closed with hermetic seals. The flasks were incubated at 30 ± 0.5°C without agitation. Cultures at pH 7 without added potassium sorbate and at the two a_w levels served as control samples. At appropriate intervals, samples were removed aseptically to determine the number of microorganisms by preparing decimal dilutions in peptone water, plating 1 ml on tripticase/glucose/yeast extract agar, and incubating for 48 h at 30°C. A Ionomex colony counter was used to quantify the results. Each experiment was performed in triplicate. Results were expressed as log colony-forming units per milliliter.

Modeling of microbial growth. Mathematical models allowed us to analyze the effect of different factors on microbial growth parameters. One of the recommended models *(10, 26)* is the modified Gompertz equation:

$$\log N = \log N_0 + a \exp\{-\exp[-b\,(t - m)]\} \qquad (1)$$

where log N is the decimal logarithm of microbial counts, expressed in log colony-forming units per milliliter, at time t; log N_0 is the asymptotic log count as time decreases indefinitely (ap-

TABLE 1. *Specific growth and decline rates (*R*) for the three tested strains of* Staphylococcus aureus *corresponding to different conditions of a_w, total potassium sorbate concentration (C_t), pH, and undissociated sorbic acid concentration (uac)*

				R^a [log(CFU/ml)/days]		
a_w	C_t (mM)	pH	uac (mM)	ATCC 6538P	196-E	FDA C243
0.90	0.00	7.0	0.00	1.26	1.17	1.20
	10.00	5.8	0.82	0.71	0.65	0.70
	10.00	5.6	1.24	0.65	0.15	0.14
	16.60	5.8	1.36	0.47	0.25	0.29
	10.00	5.4	1.83	0.33	−0.10	−0.14
	16.60	5.6	2.05	0.08	−0.25	−0.23
	10.00	5.2	2.62	−0.53	−0.16	−0.25
	16.60	5.4	3.03	−0.12	−0.25	−0.28
	10.00	5.0	3.60	−0.57	−0.62	−0.65
	16.60	5.2	4.34	−0.56	−0.40	−0.52
	16.60	5.0	5.97	−0.60	−0.69	−0.67
0.92	0.00	7.0	0.00	1.42	1.44	1.35
	10.00	5.8	0.82	0.87	0.65	0.65
	10.00	5.6	1.24	0.78	0.57	0.57
	16.60	5.8	1.36	0.67	0.67	0.67
	10.00	5.4	1.83	0.49	0.33	0.32
	16.60	5.6	2.05	0.38	0.55	0.55
	10.00	5.2	2.62	−0.12	−0.09	−0.09
	16.60	5.4	3.03	−0.02	0.15	0.15
	10.00	5.0	3.60	−0.21	−0.22	−0.22
	16.60	5.2	4.34	−0.21	−0.13	−0.25
	16.60	5.0	5.97	−0.26	−0.32	−0.32

[a] Positive values of *R* correspond to growth, and negative values correspond to decline.

proximately equivalent to the log of the initial level of bacteria), expressed in log colony-forming units per milliliter; *a* is the log count increment as time increases indefinitely (i.e., number of log cycles of growth), expressed in log colony-forming units per milliliter; *m* is the time required to reach the maximum growth rate, expressed in days; and *b* is the relative growth rate determined as 1/days at time *m*.

From these parameters, the specific growth rate, $R = b \times a/e$, expressed as log colony-forming units per milliliter per day (with $e = 2.7182$), lag-phase duration, LPD $= m - (1/b)$, expressed in days, and maximum population density, MPD = log $N_0 + a$, expressed in log colony-forming units per milliliter, were derived.

The Gompertz equation was applied to every culture in which microbial growth was detected. The equation was fitted to growth data using the nonlinear regression modulus of SYSTAT software (Systat, Inc., Evanston, Ill.). The selected algorithm calculated the set of parameters with the lowest residual sum of squares and a 95% confidence interval for *S. aureus* growth. When the preservatives showed a bactericidal effect, a linear model was applied:

$$\log N = \log N_0 + R(t - \mathrm{LPD}) \qquad (2)$$

In this case, R is the decline rate, expressed in log colony-forming units per milliliter per day, and adopted negative values. In the cases of bacteriostatic effect, the slope of equation 2 was close to zero.

TABLE 2. *Maximum population density MPD (obtained as a derived parameter of the Gompertz model) for three tested strains of* Staphylococcus aureus *corresponding to different conditions of a_w, total potassium sorbate concentration (C_t), pH, and undissociated sorbic acid concentration (uac)*

				MPD [log(CFU/ml]		
a_w	C_t (mM)	pH	uac (mM)	ATCC 6538P	196-E	FDA C243
0.90	0.00	7.0	0.00	9.2	8.5	9.2
	10.00	5.4	1.83	7.5	4.8[a]	5.1[a]
	10.00	5.6	1.24	7.4	7.5	6.2[a]
	10.00	5.8	0.82	7.5	7.4	7.3
	16.60	5.8	1.36	7.9	6.9	6.9
0.92	0.00	7.0	0.00	9.2	9.1	9.2
	10.00	5.4	1.83	8.0	6.8	6.8
	10.00	5.6	1.24	8.0	7.2	7.2
	10.00	5.8	0.82	8.1	7.7	7.4
	16.60	5.6	2.05	7.8	7.3	7.3
	16.60	5.8	1.36	8.0	7.7	7.6

[a] When a bacteriostatic or bactericidal effect was observed, the inoculum level was reported.

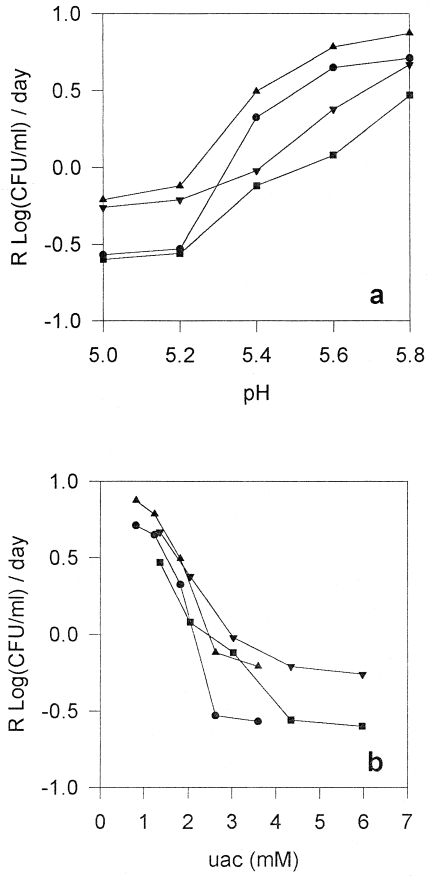

FIGURE 3. *Effect of (a) pH and (b) uac on specific growth rate of* S. aureus *ATCC 6538P.* ●, *10.0 mM potassium sorbate, a_w = 0.90;* ■, *16.6 mM potassium sorbate, a_w = 0.90;* ▲, *10.0 mM potassium sorbate, a_w = 0.92;* ▼ *16.6 mM potassium sorbate, a_w = 0.92.*

TABLE 3. *Coefficients of equations 5 and 6 that give specific microbial growth rate (*R*) and inhibition index (II) dependence on control factors and statistical parameters of the regressions*[a]

a_w		Coefficient	ATCC 6538P	196-E	FDA C243
0.90	*R*	K_1	0.114 (0.019)[b]	0.053 (0.011)	0.069 (0.014)
		K_2	−1.754 (0.228)	−1.267 (0.135)	−1.483 (0.169)
			r^2 = 0.881	r^2 = 0.930	r^2 = 0.924
			RSS = 2.251[c]	RSS = 1.409	RSS = 1.742
			F = 29.793	F = 253.202	F = 42.290
	II	K_3	−0.104 (0.019)	−0.068 (0.019)	−0.069 (0.021)
		K_4	1.513 (0.237)	1.576 (0.236)	1.582 (0.260)
			r^2 = 0.837	r^2 = 0.867	r^2 = 0.875
			RSS = 1.686	RSS = 2.131	RSS = 2.054
			F = 20.484	F = 26.114	F = 21.057
0.92	*R*	K_1	0.142 (0.013)	0.131 (0.013)	0.122 (0.015)
		K_2	−1.467 (0.157)	−1.312 (0.158)	−1.262 (0.180)
			r^2 = 0.939	r^2 = 0.928	r^2 = 0.897
			RSS = 2.244	RSS = 1.889	RSS = 1.661
			F = 61.967	F = 51.424	F = 34.839
	II	K_3	−0.145 (0.017)	−0.123 (0.014)	−0.129 (0.015)
		K_4	1.318 (0.210)	1.343 (0.178)	1.404 (0.188)
			r^2 = 0.901	r^2 = 0.902	r^2 = 0.901
			RSS = 2.347	RSS = 1.715	RSS = 1.882
			F = 36.265	F = 36.999	F = 36.505

[a] df = 8; $P \leq 0.001$ in all cases.
[b] Standard deviation of coefficients is given in parentheses.
[c] RSS, sum of squares of the regression.

Inhibition index. To analyze the inhibitory action of reduced a_w and sorbic acid concentration, an inhibition index (II) was defined as follows:

$$\text{II} = 1 - [\log(N/N_0)_{\text{treated}}/\log(N/N_0)_{\text{control}})] \quad (3)$$

where N is the number of microorganisms at time t and N_0 is the initial level of microorganisms.

The values of $\log(N/N_0)_{\text{treated}}$ and $\log(N/N_0)_{\text{control}}$ were evaluated at 10 days of storage time. It must be noted that if II = 1, then the microorganisms in the treated samples remain in the lag phase ($N = N_0$). If II surpasses 1, then bactericidal action takes place, and $\log(N/N_0)_{\text{treated}}$ has negative values. II = 0 indicates microbial growth similar to that of control samples. Moreover, II values between 0 and 1 reflect definite microbial growth at a lower rate than that of control samples because of the preservative action.

Statistical analysis. Response surface analysis was applied to study interactions between factors affecting growth (pH and sorbic acid concentration). The stepwise procedure was used to analyze the simultaneous dependence of specific microbial growth, decline rate, and II on pH and sorbic acid concentration at each a_w level. The stepwise selection method inserts variables until the regression equation is satisfactory. This method is recommended as one the best for variable selection *(5)*. The statistical treatment of data was performed using the SYSTAT statistical package, which provides coefficients and corresponding standard deviations.

RESULTS AND DISCUSSION

Gompertz and linear equations were fitted to microbial counts of the three strains of *S. aureus,* which were collected at different pH values, potassium sorbate concentrations, and two levels of a_w. Experimental data obtained by our research team were reported previously *(9)*. The Gompertz model allowed prediction of the entire sigmoidal curve. Linear regressions were fitted in cases of bacteriostatic or bactericidal action of the preservative. In all cases, good agreement between experimental data and predicted values was obtained. Examples of sigmoidal curves and linear fittings are shown in Figures 1 and 2 for *S. aureus* ATCC 6538P at different a_w values, pH levels, and potassium sorbate concentrations.

Considering that the antimicrobial action of weak acids is generally attributed to the undissociated fraction, its effect on the growth parameters of *S. aureus* strains was analyzed. Sorbic acid is a monoprotic weak acid with a dissociation constant $K_a = 1.78\ 10^{-5}$ mol/L ($pK_a = 4.75$). The undissociated concentration of a weak acid (uac) depends on pH and was calculated as follows:

$$\text{uac} = C_t[H^+]/(K_a + [H^+]) \quad (4)$$

where C_t is the total acid concentration and K_a is the dissociation constant of sorbic acid.

Tested conditions of total acid concentration, pH of the broths, and undissociated sorbic acid concentration (uac, in mmol/L) are shown in Table 1. Growth and decline rates (R) for the three *S. aureus* strains, derived from the Gompertz equation or from the slopes of linear fittings, are also shown. Strain ATCC 6538P had the greatest resistance to the treatments, with higher specific growth rate values than the other tested strains.

Values of MPD for each studied strain at different uac and pH values for the two levels of a_w (0.90 and 0.92) are shown in Table 2. Values of MPD for a_w of 0.90 and 0.92

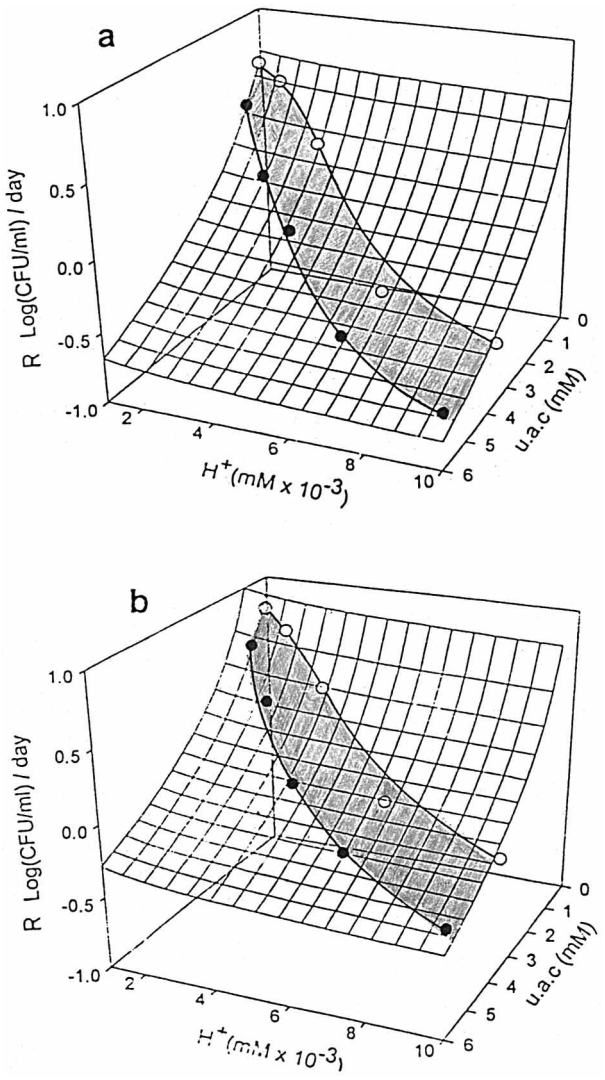

FIGURE 4. *Surface plots showing the dependence of R on pH and uac for* S. aureus *ATCC 6538P at* $a_w = 0.90$ *(a) and* $a_w = 0.92$ *(b). Potassium sorbate concentration: ○, 10.0 mM, ●, 16.6 mM. The shaded zone indicates the region where the model is valid.*

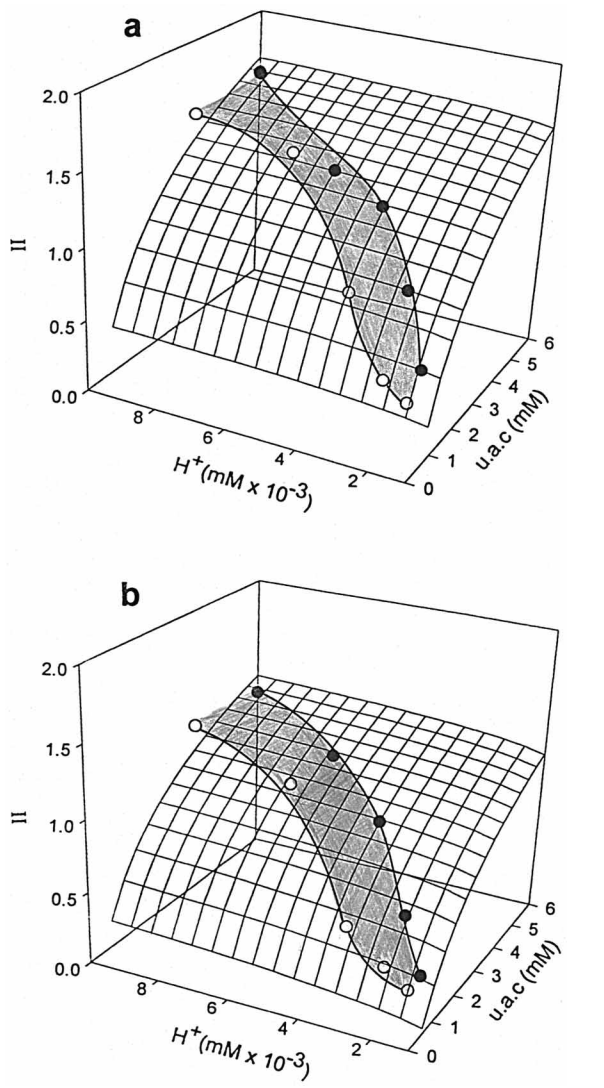

FIGURE 5. *Surface plots showing the dependence of Inhibition Index (II) on pH and uac for* S. aureus *ATCC 6538P at* $a_w = 0.90$ *(a) and* $a_w = 0.92$ *(b). Potassium sorbate concentration: ○, 10.0 mM, ●, 16.6 mM. The shaded zone indicates the region where the model is valid.*

at pH 7.0 without potassium sorbate varied between 8.5 and 9.2 log(CFU/ml) for the three strains. When a bacteriostatic or bactericidal effect was observed, the inoculum level was reported. Magrini et al. *(14)* analyzed the effect of water activity on the growth of different strains of *S. aureus* in cheese and showed that at 30°C, strain ATCC 6538P grew well at a_w values of 0.993, 0.970, and 0.950; little effect of reduced a_w values on MPD was observed, and values of MPD close to 8 log(CFU/ml) were reported. Buchanan et al. *(4)* studied the effects and interactions of temperature (T = 12 to 42°C), initial pH (P = 4.5 to 8.4), NaCl (S = 0.5 to 16.5% wt/vol), and sodium nitrite (N = 0 to 200 mg/l) on the aerobic and anaerobic growth of *S. aureus* 196-E using BHI broth. The quadratic response surface model reported by Buchanan et al. *(4)* was applied in our work for aerobic conditions with $N = 0$ (deleting the effect of sodium nitrite) to compare results of maximum population densities in systems without potassium sorbate. The equation $\ln(\text{MPD}) = 1.4074 + 0.00765 \times T + 0.1588 \times P + 0.0330 \times S + 0.00241 \times TP - 0.0000980\ TS - 0.00355 \times PS - 0.000413 \times T^2 - 0.0129 \times P^2 - 0.00122 \times S^2$ was fit with the following data: T = 30°C, pH = 7, and S = 15%, yielding an MPD of 7.8 log(CFU/ml). In the present work for *S. aureus* 196-E, growing at a_w = 0.92 (20.84% wt/vol NaCl), P = 7, and T = 30°C, an MPD of 9.1 log(CFU/ml) was obtained, which is close to the value estimated with the equation and reported by Buchanan et al. *(4)*.

Buchanan et al. *(4)* also reported a quadratic response surface model for the b parameter of Gompertz. The proposed equation with $N = 0$ was $\ln(b) = -10.8812 + 0.2551 \times T + 1.0648 \times P - 0.2653 \times S - 0.00133 \times TP + 0.00516 \times TS - 0.00723 \times PS - 0.00273 \times T^2 - 0.0563 \times P^2 + 0.00308 \times S^2$, which led to R = 1.77 days^{-1} when T = 30°C, P = 7, S = 15%, and a = 4.0 log(CFU/ml). In comparison to this result, a value of R = 1.44 day^{-1} was obtained in the present work for *S. aureus*

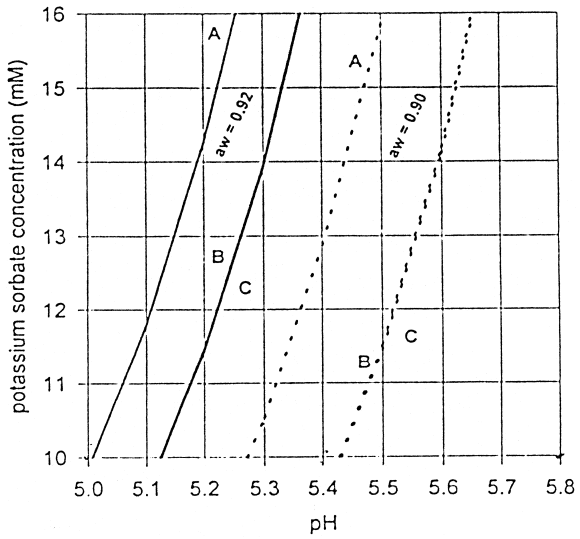

FIGURE 6. *Curves corresponding to II = 1 for the three tested strains of* S. aureus*: A (ATCC 6538P), B (196E), C (FDA C243). Full lines correspond to a_w = 0.92 and dotted lines to a_w = 0.90.*

196-E at a_w = 0.92 (20.84% wt/vol NaCl), P = 7, and T = 30°C.

Sutherland et al. *(22)* studied the growth of *S. aureus* as affected by NaCl concentration, pH, and storage temperature in a laboratory medium. In that work, results reported by different authors were summarized. For example, Magrini et al. *(14)* reported a generation time (GT) of 6.7 to 8.5 h for *S. aureus* at 30°C, pH 6, and a NaCl concentration of 8.0% (wt/vol) in BHI/buffer. GT values were converted to R values using the following relationship: R = log2/GT. The corresponding range of R values, 1.078 to 0.89 log(CFU/ml)/days, was close to that obtained in our work (Table 1). Similarly, Scott *(20)* reported GT = 5.56 h (R = 1.30 log(CFU/ml)/days) for *S. aureus* growing in a mineral salt mix used to adjust a_w with an equivalent NaCl concentration of 14.9% (wt/vol).

Figure 3a and 3b shows the effect of pH and undissociated sorbic acid concentration (uac) on specific growth and decline rate (R) of *S. aureus* ATCC 6539P at two levels of a_w and total potassium sorbate concentration. Similar curves were obtained for the other strains. The effect of preservative concentration was more marked at a_w = 0.92 than at a_w = 0.90.

Analysis of the results showed that R values at each a_w level depend on pH and uac. Thus, at each a_w condition, uac alone did not account for all the observed effects. It was assumed that the antimicrobial action of the preservative is not only a function of the undissociated acid concentration but also of the dissociated one *(7, 16)*. Two independent variables were fixed; we selected log(uac) and pH. Each pair of conditions determined the corresponding concentrations of undissociated and dissociated acid.

The simultaneous dependence of the specific microbial growth or decline rates (R) on the selected variables at each a_w level was analyzed. Different models were fitted, and those with the highest correlation coefficients and minor errors in the estimated parameters were selected. The following equations were obtained by stepwise analysis with SYSTAT software:

$$R = K_1\ \mathrm{pH} + K_2\ \log(\mathrm{uac}) \quad (5)$$

and

$$\mathrm{II} = 1 + K_3\ \mathrm{pH} + K_4\ \log(\mathrm{uac}) \quad (6)$$

Coefficients (K_1, K_2, K_3, and K_4), their standard deviations, and statistical parameters are shown in Table 3; the effect of pH on II was more marked at a_w = 0.92 than at 0.90. Examples of surface plots corresponding to equations 5 and 6, obtained by fitting R and II values of the *S. aureus* ATCC 6538P strain are shown in Figures 4a, 4b, 5a, and 5b. The shaded zones indicate the region where the model is valid.

Equation 6 was applied assuming II = 1 to obtain the set of operating conditions (range of potassium sorbate concentration and pH values) that leads to a bacteriostatic effect of the treatment, maintaining the microorganism in lag phase. Combining equations 4 and 6, the following expression was obtained:

$$C_t = 10^{(-K_3/K_4)\mathrm{pH} - \log[H^+/(K_a + H^+)]} \quad (7)$$

Curves of Figure 6 correspond to the plot of total sorbate concentration (C_t) versus pH with the condition II = 1 for the three tested strains at two levels of a_w (0.90 and 0.92). The curves of Figure 6 delimit zones that indicate the operating conditions that inhibit growth of *S. aureus* at 10 days of storage. This is related to the definition of II (equation 3), which considers not only R values but also LPD and evaluates microbial counts after 10 days of storage. The zone at the left of the curve corresponds to values of II $>$ 1 (bactericidal effect), and the zone at the right of the curve corresponds to II $<$ 1 (reduced microbial growth rate). *S. aureus* strains 196-E and FDA C243 had similar resistance to the applied inhibitory treatments, and their curves are superimposed. For example, at pH = 5.3 and a_w = 0.92, the bacteriostatic effect was achieved using 14 mM potassium sorbate for strains 196-E and FDA C243; higher concentrations were necessary for ATCC 6538P, which had the highest resistance to the applied treatments (Fig. 6). When 16 mM potassium sorbate was applied to the more resistant strain, II values greater than 1 were obtained at pH values $<$5.3 and 5.5 for a_w values of 0.92 and 0.90, respectively. When a concentration of 10 mM was applied, lower pH levels were required (5.0 and 5.3 for a_w values of 0.92 and 0.90, respectively) to achieve an inhibitory treatment. Thus, this plot is useful for technological purposes and allows treatment conditions for control of microbial growth to be established.

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