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Simplified prediction of *Staphylococcus aureus* growth in a cooked meat product exposed to changing environmental temperatures in warm climates

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ABSTRACT

In this work, a simplified method is used to estimate the growth of *Staphylococcus aureus* in a pasteurized meat product left for several hours at environmental temperatures (diurnal time) in warm climates of different cities in Argentina. Hourly temperature data for a warm January (the hottest month of the year) day, and literature data on the kinetics of *S. aureus* growth inoculated in a pasteurized meat product were used for calculations. As shown by results, if a cooked meat product is left exposed to environmental temperature at diurnal time, predictions made when using a constant temperature value (i.e. average daily) may not be accurate. Growth estimations in contaminated food left under ambient conditions during diurnal time, should consider the changing environmental temperature for correct results.

Key words: *Staphylococcus aureus*, temperature, environment, predictive microbiology, cooked meat, generation time

RESUMEN

Predición simplificada del crecimiento de *Staphylococcus aureus* en productos cárnicos cocidos expuestos a temperaturas ambientes cambiantes en climas cálidos. En este trabajo se utiliza un método simplificado para predecir el crecimiento de *Staphylococcus aureus* en un producto cárnico pasteurizado dejado por varias horas a temperatura ambiente diurna en zonas de clima cálido. En la predicción, se utilizaron datos de la temperatura horaria para un día caluroso típico de enero (mes más caliente del año) en varias ciudades de la Argentina y datos de la literatura sobre tiempos de generación y tiempo *lag* de la bacteria inoculada en un producto cárnico pasteurizado. Los resultados indicaron que cuando el producto se deja a temperatura ambiente diurna durante varias horas, no se debe utilizar para la predicción un valor de temperatura promedio (ej.: temperatura media diaria), sino que hay que tener en cuenta la evolución de este parámetro a lo largo del período considerado.

Palabras clave: *Staphylococcus aureus*, temperatura, ambiente, microbiología predictiva, carne cocida, tiempo de generación

INTRODUCTION

Staphylococcus aureus is a bacterium with strains that are capable of producing a highly heat-stable toxin that causes illness in humans. Intoxication is caused by ingesting enterotoxins produced in food by *S. aureus*, usually because the food has been left at ambient temperature (13). Foods that require considerable handling during preparation and that are kept without refrigeration are usually involved in staphylococcal food poisoning; this bacterium is able to grow in a wide range of temperatures (7-48 °C) with an optimum at 35-37 °C, a range which may be frequent in warm climates. The toxin produced by *S. aureus* is very heat-stable and is not destroyed at normal cooking temperatures.

Many kinds of cooked foods are known to be displayed in restaurant windows at ambient temperatures for several hours. Cooked meats, ham, poultry, egg products, tuna, potato and macaroni salads are good environments for this bacterium to produce the toxin. Many foods are often prepared under unsanitary conditions and stored for long periods at ambient temperature before selling (for example, street foods), and the time lapse between food preparation and consumption is an important factor to consider in terms of hazard. For example, vendors cook the food in the morning and then store it at ambient temperatures for the rest of the day (3, 7).

It must be acknowledged that the term “ambient” temperature in relation to the risk assessment of bacterial growth is vaguely defined, since daily temperature is not constant and contaminated food products undergo a changing temperature environment. Thus, a constant temperature prediction may not be so useful. Risk assessment studies of food borne bacterial pathogens are

usually performed at constant temperature which mimics some average environmental temperatures. This procedure may not adequately reflect the actual temperature profile to which the bacterium is exposed at a given time period of the day.

It is the purpose of the present paper to estimate the extent of *S. aureus* growth which would occur when a cooked meat product is left for several hours at changing environmental temperatures during diurnal time under various warm climates. Temperature data corresponding to selected Argentinean cities were used for calculations.

MATERIALS AND METHODS

Data Analysis

Environmental (ambient) temperatures

Table 1 shows the geographical location of selected Argentine cities, namely Buenos Aires, Córdoba, Formosa, La Rioja, Posadas, Resistencia, San Juan, San Luis and Santiago del Estero, as well as their mean environmental (surface) temperatures for the month of January (the hottest month in summer) in the decade 1981-1990. Mean temperature values for January ranged around 24-27 °C for the different places. Data were obtained from the Argentinean Meteorological Service, and conventional temperature monitor stations were used to measure the environmental temperature.

Hourly surface temperature records (24 measurements/day) for a single hot January day, (January 9th, 2006) in the selected locations were also obtained. The corresponding hourly temperature profiles in the selected cities are shown in Figure 1 (a, b, c).

For the purpose of the present work we considered that the cooked meat food was prepared (and contaminated) early in the morning (e.g. at 8:00 am) and then stored at environmental temperature during diurnal time before selling/consumption. As shown by the temperature profiles (Figure 1) at 8:00 am, temperature was about 25-30 °C in all locations; then slowly increased, remained several hours at 35-40 °C (close to the optimum temperature for *S. aureus* growth) and finally began to decline.

Growth kinetics of Staphylococcus aureus in a cooked meat product stored at constant temperatures.

The International Commission on Microbiological Specification for Food (ICMSF) (5) reported the generation time, (GT) (also called doubling time) and lag phase duration of *S. aureus* inoculated (3 log counts/g) in a pasteurized beef and kidney pie having pH 5.8 and water activity (a_w) 0.98, stored at various constant temperatures. During the lag phase, cells are adjusting to their new environment; during the exponential phase of growth, bacteria multiply by binary fission and the so-called stationary phase occurs when the bacterial level (*S. aureus,* in this case) reaches a high critical concentration (e.g. around 1 x 10^9). Figure 2 shows the correlation of these kinetic parameters (lag time and GT) with temperature in the range of interest of this work. It can be seen that the lag phase time (Figure 2a) rapidly decreased with increasing temperature approaching some sort of asymptotic value; GT (Figure 2b) also decreased with increasing temperature, both behaviours are within expectations (14).

In the present work, *S. aureus* growth estimations were made by using a simplified approach. It consists in using the well- known relationship between the number of bacteria at a given time (N_t), the original number of bacterial cells (No), GT, and time (t),

$$N_t = N_o \cdot 2^{t/GT} \qquad \text{equation 1}$$

where t is the time elapsed after completion of lag phase. For present predictions in cooked meat, N_o is taken as 3 log counts/g (inocula value) and the lag phase time is known (Figure 2a). The use of Equation 1 is valid only for predictions in the exponential growth phase of bacterial curve. In the present work, maximum predicted value of *S. aureus* counts in the cooked meat product is limited to, N_t = 1 x 10^6 cfu/g, which is within the exponential growth phase.

GT (4) were empirically correlated with temperature (in the range of 25-42 °C) by the following equation obtained from curve fitting of the data,

$$GT = -0.0185\,T^3 + 2.1314\,T^2 - 82.205\,T + 1083.5 \qquad \text{equation 2}$$

where T is temperature (°C).

Since we assumed the cooked food was prepared (and contaminated) at 8:00 am, the lag phase time was taken as 3 hours, which is the average of lag times at 25-30 °C (Figure 2a), since this is the environmental temperature range in all locations at that morning time. Subsequent growth of *S. aureus,*

Table 1. January mean environmental temperatures for decade 1980-1990 in selected Argentine cities

Place	Latitude (S)	Longitude (W)	Altitude (m)	January Mean Temperature(1) (°C)
Buenos Aires	34.58	58.48	25	25.1
Córdoba	31.19	64.13	474	23.5
Formosa	26.12	58.14	60	27.5
La Rioja	29.23	66.49	429	27.1
Posadas	27.22	55.58	133	26.9
Resistencia	27.45	59.05	52	27
San Juan	31.34	68.25	598	27
San Luis	33.16	66.21	713	24.4
Santiago del Estero	27.46	66.18	199	26.9

(1) calculated from mean daily temperature measurements of all days of January

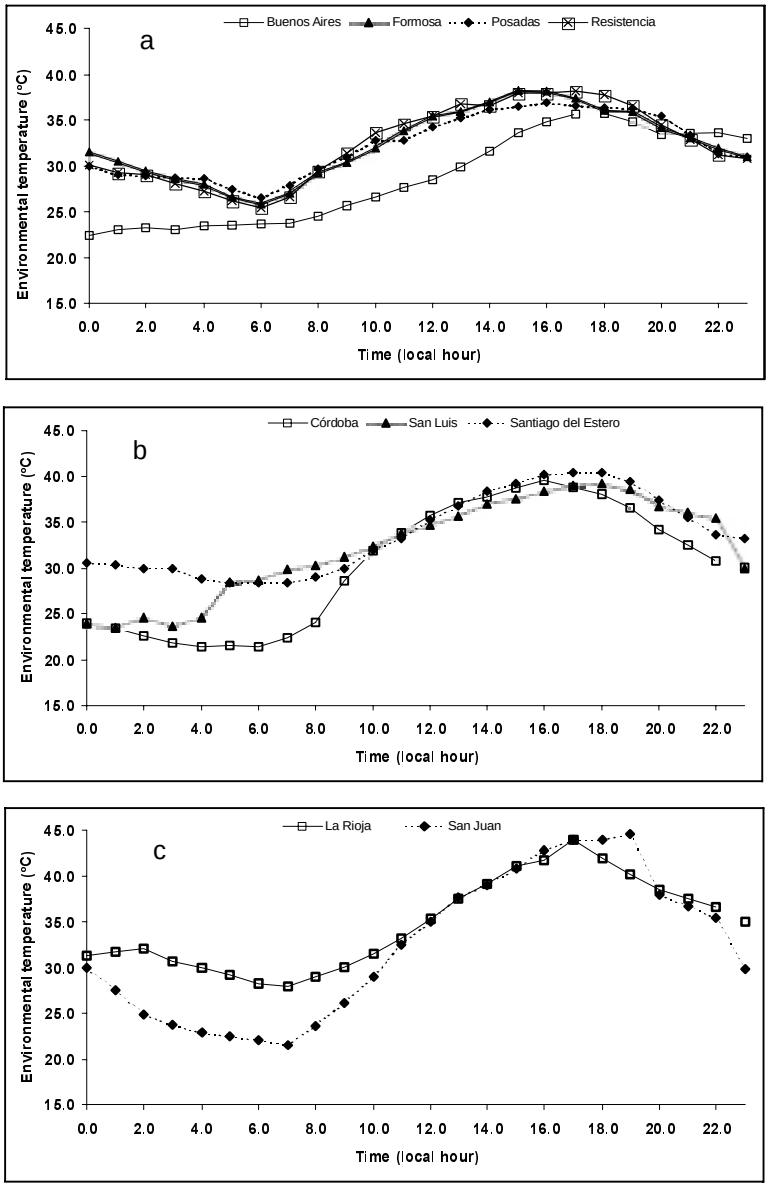

Figure 1. Hourly environmental temperatures for January 9th, 2006 in selected Argentine cities:
(a) Buenos Aires, Formosa, Posadas and Resistencia
(b) Córdoba, San Luis and Santiago del Estero
(c) La Rioja and San Juan

exposed at the changing environmental temperatures in selected cities was calculated (equation 1) by accumulated sum of various one- hour intervals as the time interval considered. For each interval, GT was evaluated (equation 2) at the middle point temperature of any one hour interval. Calculations ended by the time the staphylococcal population (N_t) reached 1 x 10^6 CFU/g, which is a count number likely to be associated with enterotoxin production. The number of staphylococci required to cause illness cannot be predicted with certainty because many variables may affect the amount of enterotoxin produced in a contaminated food. Stewart et al. (12) cited that according to a 1992 guideline given by the U.S. Food and Drug Administration, the effective dose of enterotoxin may be achieved when the population of *S. aureus* reaches a level of > 10^5 CFU/g. Niskanen and Nurmi (9) reported that contaminations of *S. aureus* over 2 x10^6 CFU/g were associated with enterotoxin production in dry sausage, and Walls and Scott (13) determined that the toxin was not produced in a cooked meat product (1.2% NaCl, pH 5.6 incubated at 35 °C) until counts increased to a level greater than 1.2 x 10^6 CFU/g.

RESULTS

Predictive microbiology involves the use of mathematical models to predict the growth, survival and inactivation responses of microorganisms to different environmental conditions. Sigmoidal functions have been used to accurately model the typical microbial growth curve from the

lag to the stationary phase and provide the mathematical basis for estimating parameters, such as the maximum growth rate, (1, 4, 10, 15). Models are normally developed under static conditions (growth rates and lag times are measured at a series of set temperatures, water activity values, and pH levels) and the results are combined to describe the effects of each factor or a combination of factors on population development. Subsequently, models must be validated in foods under conditions that mimic situations encountered in normal practice.

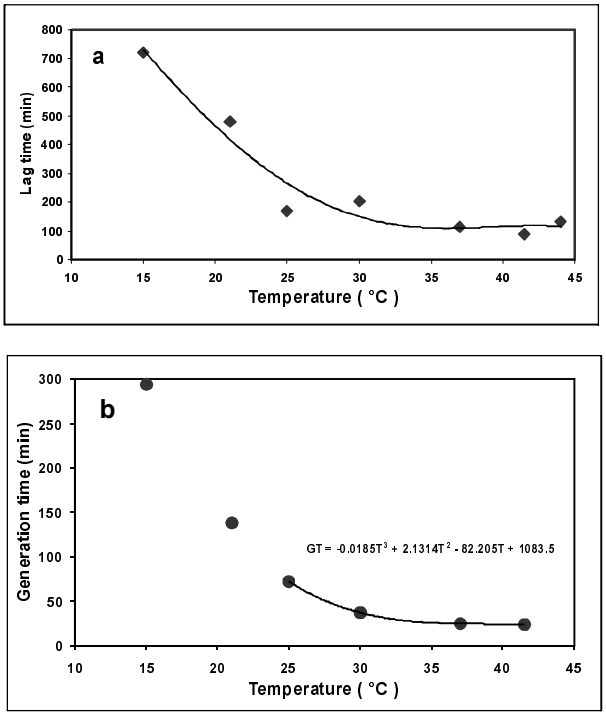

Figure 2. Correlation of kinetic parameters for growth of *Staphylococcus aureus* in cooked beef and kidney pie (ICMSF, 1996)
(a) Lag time versus temperature
(b) Generation time versus temperature

A great concern for predictive microbiology is estimating the growth under changing temperature conditions (4). Shaw (11) and later other authors (1, 2, 8) reported on the effect of fluctuating temperatures on microbial growth. Depending on the magnitude of the temperature deviation, the organism may change its growth rate to a characteristic rate of the new temperature, or it may stop growing if a lag phase is introduced. Langeveld and Cuperus (6) found that bacteria within the exponential phase immediately respond to a change in temperature. Zwietering *et al.* (14) also indicated that shifts during the exponential phase in a moderate temperature range, result in immediate exponential growth at the growth rate associated with the new temperature. However, shifts to or from low temperatures resulted in an adaptation period.

Present predictions considered that *S. aureus* cells contaminating the cooked meat continuously adapt to a new growth rate characteristic of the new temperature, when exposed to changing environmental temperature at selected diurnal times (Figure 1). This assumption is based on two important facts: 1) the changing environmental temperature occurs in a range which is close to the optimum for *S. aureus* growth, and b) the average rate of diurnal temperature increase in the growth period is low, being somewhere between 0.7-1.2 °C/hour in Resistencia, Posadas, Formosa, San Luis and Córdoba and 1.4- 2.0 °C for Buenos Aires, Santiago del Estero, La Rioja and San Juan (Figure 1) . These factors mean that

Table 2. Time for a 3-fold log increase (from inocula of 10^3 CFU/g) of *S. aureus* count in cooked beef and kidney pie: calculations using different environmental temperature approaches.

Location	Time for a 3-fold log increase (from 10^3 CFU/g) following a 3 hour lag time (hour)			
	Using hourly temperatures in specified diurnal period, January 9th, 2006	Using mean temperature of January 9th, 2006		Using mean January temperature for decade 1980-1990
Buenos Aires	5.4	7.2	(28.5 °C)(1)	11.7
Posadas	4.0	5.9	(32.1 °C)	9.0
San Luis	4.0	6.0	(32.0 °C)	12.9
Formosa	3.9	5.8	(32.4 °C)	8.3
Resistencia	3.9	5.8	(32.4 °C)	8.9
Córdoba	3.9	7.2	(30.2 °C)	14.7
Sgo. del Estero	3.8	5.2	(33.8 °C)	9.0
San Juan	3.8	5.9	(32.2 °C)	8.9
La Rioja	3.7	5.0	(34.8 °C)	8.8

(1) mean daily temperature for January 9th, 2006.

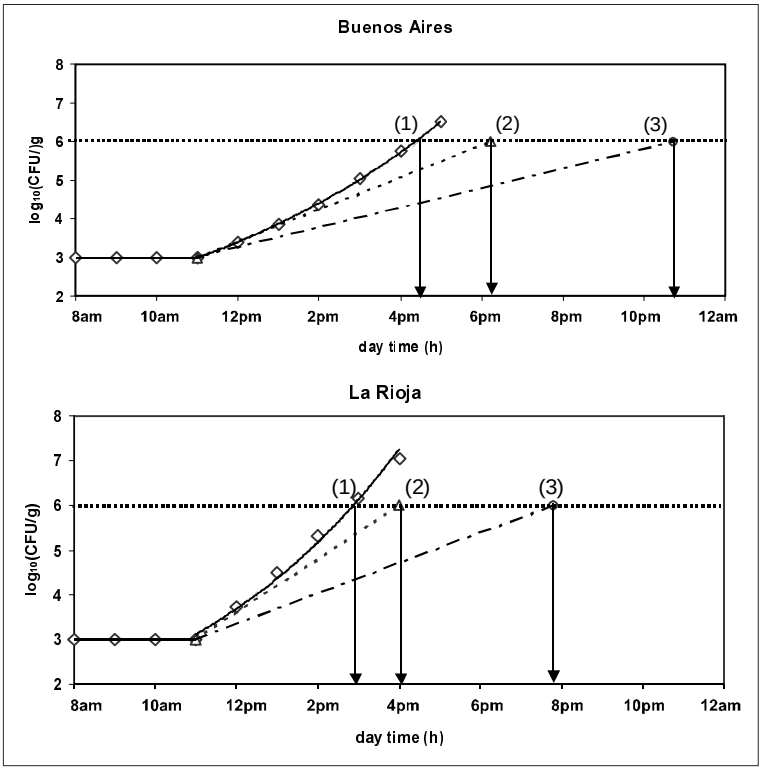

Figure 3. Comparison of *S. aureus* growth in cooked meat product predicted using different environmental temperatures approaches. (1) specified hourly temperatures for January 9 th, 2006; (2) mean temperature for January 9th, 2006, (3) mean January temperature over decade 1981-1990.

a "heat shock" (which may affect bacterial cells) never occurred. Furthermore, environmental temperature changes (to or from) are far away from the minimal value for growth, (7.0-8.0 °C).

Table 2 shows calculated times for a 3-fold log increase (from inocula of 10^3 CFU/g) of *S. aureus* count in cooked beef and kidney pie using different environmental temperature approaches: a) hourly temperatures at a specified diurnal period on January 9th, 2006; b) mean temperature for January 9th, 2006 and c) mean January temperature for the decade 1981-1990. Time for a 3-fold log counts goes from as low as 3.7 hours for La Rioja to 5.4 hours for Buenos Aires. Growth times predicted using daily mean environmental temperature are higher than those calculated using the actual (changing) environmental temperature; and this is due to the cooling effect of nights. Also, using the mean January temperature in the decade 1981-1990 lead to even higher predicted times to reach 3- fold log counts (total count 1 x 10^6 CFU/g). Figure 3 illustrates this behaviour for Buenos Aires and La Rioja.

It may be concluded that mean environmental temperatures (i.e. mean daily values) may not adequately model *S. aureus* response when a contaminated food is left for several hours at "ambient" conditions during diurnal time (time abuse-temperature). The present results are also useful to compare growth estimates which would occur in different warm climates.

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