INFLUENCE OF FOOD ENVIRONMENTS ON GROWTH OF *STAPHYLOCOCCUS AUREUS* AND PRODUCTION OF VARIOUS ENTEROTOXINS[1]

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ABSTRACT

Though *Staphylococcus aureus* can grow in foods within a broad range of environmental conditions, production of enterotoxins occurs within a much narrower range. In situations that permit growth of S. *aureus*, oxygen tension and associative growth of other microorganisms affect enterotoxin production more adversely than other factors such as temperature, pH, and water activity. Minimal amounts of enterotoxins or none may be produced in raw, semiprocessed, or fermented foods when there is competitive growth of other microorganisms unless such growth is retarded by bacteriophages, antibiotics, organic acids, and processing conditions such as curing and heating.

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For *Staphylococus aureus* to grow and produce enterotoxins in a given food environment, nutritional requirements such as an organic source of nitrogen (amino acids), source of energy (carbohydrate or other), and sources of vitamins (thiamine and niacin), and minerals should be met. In addition, other environmental conditions must also be adequate, such as water activity, pH, absence of inhibitory substances, oxidation-reduction potential or oxygen tension, and temperature. Another factor which significantly affects growth of staphylococci in food products is the competitive growth of other microorganisms found in raw, semiprocessed and fermented foods. Food products undergo a series of changes as a result of their handling, processing, packaging, and storage, and these are unique for each product. Consequently, it is the interaction of environmental factors such as these which exist in foods at various phases of their handling, manufacture, and storage that result in permitting either unrestricted, restricted, or no growth and/or production of enterotoxins. The purpose of this paper is to review the significant interactions of environmental factors in foods that influence growth of S. *aureus* and production of enterotoxins.

S. *aureus* can grow and produce enterotoxins in a variety of substrates (foods or bacteriological media) under a wide range of environmental conditions. The ranges of these conditions for growth and for productin of enterotoxins are in Table 1. The ranges for production of enterotoxins are narrower than those required for growth of S. *aureus*.

Nutrients

The fact that a variety of food products have been involved in staphylococcal food poisoning outbreaks should affirm that there are very few foods in which S. *aureus* growth and production of enterotoxins are limited by unavailability of nutrients, with the exception of possibly some pastry and synthetic cream fillings. Early work of Cathcart et al. *(5)* with various pastry fillings and more recent work of Crisley et al. *(6)* with synthetic cream fillings point to the possible limiting influence of nutrients on growth of S. *aureus* in fillings prepared by blending ingredients with water instead of reconstituted dry milk. As shown by data in Table 2, cream fillings A and C made with water did not support growth of S. *aureus*, whereas these fillings supported extensive growth when prepared with reconstituted whole dry milk or prepared with water and placed in pie crust. Availability of nutrients in fillings made with milk or those added by the pie crust is a distinct possibility.

Water activity

Availability of moisture or water activity (a_w) influences growth of S. *aureus* in food products and the limiting a_w value varies with the nature of the product. The early work of Segalove and Dack *(27)* showed that S. *aureus* growth was inhibited when the moisture content of meat was 20% or lower. Scott *(26)*, in

TABLE 1. RANGES OF ENVIRONMENTAL CONDITIONS FOR GROWTH AND ENTEROTOXINS PRODUCTION BY *Staphylococcus aureus*

	For growth		For toxin production	
Condition	Optimum	Range	Optimum	Range
Water activity	>0.99	0.83->0.99	>0.99	0.86->0.99
pH	6-7	4-10	6-7	4-9.8
Temperature (°C)	37	7-47.8	40-45	10-46
% NaCl	0	0-20	0	0-10
Availability of oxygen	Aerobic	Aerobic-anaerobic	Aerobic	Aerobic-anaerobic

TABLE 2. GROWTH OF S. *aureus* (196E) IN SYNTHETIC CREAM FILLINGS MADE WITH WATER OR RECONSTITUTED WHOLE MILK AND IN PIE AT ROOM TEMPERATURE[a]

Cream filling[b]	S. *aureus* count/g at time (h) indicated								
	Filling made with						Filling (made with water)		
	Water			Whole milk			In the finished pie		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
A	1.2×10^3	3.3×10^2	NC[c]	1.2×10^3	4.9×10^7	1.0×10^9	6.1×10^2	1.0×10^7	4.9×10^7
C	1.1×10^3	4.6×10^2	6.3×10^2	1.2×10^3	5.9×10^5	1.7×10^8	9.6×10^2	6.6×10^5	6.7×10^7

[a]Data of Crisley et al. *(6)*.
[b]Filling A contained sodium benzoate and was of pH 8.5. Filling C contained sodium propionate and was of pH 5.85.
[c]NC = No detectable growth.

TABLE 3. INFLUENCE OF WATER ACTIVITY ON GROWTH AND ENTEROTOXIN PRODUCTION BY S. *aureus* 265-1 AT 35 C[a]

Beef[b]				Pork[b]			
S. *aureus*/g	Final pH	a_w[c]	Toxin A	S. *aureus*/g	Final pH	a_w[c]	Toxin A
1.6×10^8	4.7	0.96	–	2.4×10^8	5.0	0.97	+
3×10^9	5.3	0.94	+	2.7×10^9	5.3	0.94	+
1.2×10^9	5.5	0.88	+	1.7×10^9	5.4	0.90	+
1.0×10^9	5.5	0.86	–	1.1×10^9	5.5	0.86	+
4×10^4	–	0.82	–	3.1×10^7	5.8	0.83	–

[a]Data of Dr. Mike Hill, Armour & Co.
[b]Containing 3% dextrose and 100 ppm sodium nitrite.
[c]a_w was adjusted by adding NaCl.

TABLE 4. GROWTH OF S. *Aureus* IN CREAM FILLING AND IN DEVILS-FOOD CAKE AT ROOM TEMPERATURE (ca. 70F)[a]

Sucrose concentration		S. *aureus* count per g after incubation					
		Initial		5 days		12 days	
(Parts per part of water)	a_w	Bottle (x 10^5)	Cake (x 10^5)	Bottle (x 10^5)	Cake (x 10^5)	Bottle (x 10^2)	Cake (x 10^5)
2.7	–	2.9	2.9	3.0	2.0	1	17
2.4	0.80	3.2	3.2	3.1	2.0	1	6.8
2.1	0.795	1.8	1.8	6.3	3.2	1	4.6

[a]Data of Silliker and McHugh *(28)*

showing the influence of water activity on growth of S. *aureus*, demonstrated that the lower limit for foods or bacteriological media which permitted S. *aureus* growth was 0.86. He also demonstrated growth of S. *aureus* at a_w values of 0.86 or higher in three different foods, namely mutton, dried milk, and dried soup. The water content of these foods at 25 C was 23.3% for mutton, 15.7% for dried milk, and 62.5% for dried soup. Labuza et al. *(16)* while confirming these findings of Scott, also demonstrated that not only the a_w but also the mode of adjusting the a_w of the food system influences cell viability and growth. Pork slurry of an a_w as low as 0.84 (adjusted by adding glycerol) supported growth of S. *aureus*, whereas freeze-dried pork at an a_w of 0.90 did not support such growth. These authors found that the moisture content needed to yield the desired a_w was dependent on adsorption or desorption of water into the system. Data of Hill (*personal communication*) in Table 3 show the effect of water activity on growth of S. *aureus* and production of enterotoxin A in cured beef and pork. Growth of S. *aureus* did not occur in beef of 0.82 a_w and good growth occurred at 0.86 or higher a_w. On the other hand, pork supported somewhat slower but distinct growth of S. *aureus* at an a_w of 0.83. Enterotoxin A was detected at 0.88 and not 0.86 a_w in beef despite good growth, and enterotoxin was detected at 0.86 in pork. At 0.83 a_w in pork, the population may not have been high enough to yield a positive enterotoxin test. In beef with 0.97 a_w, there was a sharp decrease in pH probably because some acid-producing bacteria grew and this may explain the lack of toxin. Hill further noted that the limiting a_w (nutrient broth) was lower when vitamins (yeast extract) were added to the system, thus indicating the interactive influence of nutrients and a_w on growth and enterotoxin A production by S. *aureus*.

In multi-ingredient products such as cakes and pies, the heterogeneous distribution of moisture could result in supporting S. *aureus* growth (in certain layers due to increasing a_w by migration of water) which otherwise could not occur. Data of Silliker and McHugh *(28)* show this aspect. Though neither the devil's food cake crumb nor the filling supported growth of S. *aureus*, filling when placed as layers in

cake supported growth of S. *aureus*, although S. *aureus* died in the filling placed in bottles (Table 4). Likewise, differences in compostion of various parts or areas that prevent migration of mositure (as in southern custard pie) can result in an extended lag in growth of S. *aureus* (23). Growth of S. *aureus* was retarded in the top surface due to high fat content of the top layer or to spraying with Myvacet, thus resulting in prevention of moisture migration to the top layer. The cut surface supported growth of S. *aureus* at an a_w of 0.925 and not at 0.90.

pH

Investigations on the effect of pH of certain food products show that growth of S. *aureus* could take place at values as low as 4.5 - 4.7. However, as observed by many investigators, the type of acidulant used for pH adjustment has a significant effect on the lower limit of pH which will permit growth of S. *aureus*. Growth and enterotoxin A production by S. *aureus* occurred at pH values of 5.0 or greater obtained with either lactic acid or HCl in reconstituted nonfat dry milk, whereas neither growth nor enterotoxin production took place at pH 4.5 when obtained with lactic acid (29). As has been pointed out by the studies of others (19), undissociated lactic acid at pH 4.6 or lower is destructive to S. *aureus*. Other organic acids such as citric, acetic, etc. have also been shown to inhibit growth of S. *aureus* (19), and the undissociated acid was responsible for this, as shown by Minor and Marth (19). Lechowich et al. (17) demonstrated that lowering pH of pork or ground commercial ham to 4.8 to 5.0 with lactic acid completely prevented anaerobic growth for 7 days at 30 C. Barber and Diebel (3) and Genigeorgis et al. (12) noted the interactive effect of pH and oxygen tension on growth and enterotoxin production by S. *aureus*. The latter authors found lack of enterotoxin B production at pH below 5.3 (adjusted with gluconodelta-lactone) in ham under anaerobic conditions. These authors (13) also found the lowest pH supporting enterotoxn C production in cured meats to be 4.7 under aerobic conditions, and that other factors such as presence of NaCl influenced enterotoxin C production; toxin C was produced at pH 4.7 with 3.2% and not with 7.85% brine concentration.. Scheusner and Harmon (25) observed lack of growth of S. *aureus* or production of enterotoxins in foods (doughnut and jelly pastry, cream fillings, salad dressings, ham salad, and creamed herring) at pH 4.8 or lower.

Availability of oxygen or oxygen tension

The early work of Davison and Dack pointed out that in canned corn, peas, oysters, and salmon, anaerobic conditions prevented or reduced the toxin production substantially (9). Vacuum packing (anaerobic atmosphere) was shown by Christiansen and Foster (7) to retard growth of S. *aureus* in chopped ham. Lechowich et al. (17) observed lack of S. *aureus* growth in the interior of ham (anaerobic conditions) during smoking of the product even though the temperature was in the growth range (70-118 F) for several hours. Growth, however, took place on the surface (aerobic conditions). However, Genigeorgis et al. (12) observed production of enterotoxin B in cured ham (finished product) under anaerobic conditions; toxin development was slower than under aerobic conditions. Growth of S. *aureus* and enterotoxin production ocurred in Canadian back bacon held in vacuum, partial vacuum, and under hydrogen or nitrogen at 37 C (32). On the other hand, Baird-Parker (2) was unable to detect enterotoxin B in UK-produced bacon held under vacuum at 25 C for 14 days. Also, he did not observe toxin production in cooked peeled prawns stored under $N_2 + CO_2$).

The data of Barber and Diebel (3) show the influence of oxygen tension of sausage mix on production of enterotoxin A. With no oxygen, enterotoxin was not detected despite attainment of 1×10^8 S. *aureus*/g. Enterotoxin A was detected at 5% or higher oxygen tension and above 5% the amount of toxin produced was greater; toxin was detectable at 1.4×10^7 S. *aureus* at 10% oxygen tension and 1.5×10^8 S. *aureus* at the 5% level. Genigeorgis and Pureha (14) observed lack of enterotoxin C production in cured meats under anaerobic conditions. We observed (*unpublished data*) production of enterotoxins A, B, C, and D in sliced straight bologna placed under a partial vacuum.

Temperature

Temperature has a direct influence on production of enterotoxins in that much smaller amounts are produced with a decrease of temperature within the range of 10 C to 40-45 C. Genigeorgis et al. (12) observed enterotoxin B production in ham at 10 C. We found small amounts of enterotoxins A, B, C, and D to be produced in cooked ground beef, ham, and bologna at 10 C (*unpublished data*). Fung (11) observed production of enterotoxin B at 40 C in fish protein concentrate and when it was added to beef broth, wheat flour, and corn flour, Pivnick et al. (21) observed toxin A production by S. *aureus* inoculated into cut pieces of barbecued chicken when incubated at 35 C. On the other hand, whole barbecued chickens inoculated with 9 stains of S. *aureus* did not support production of detectable enterotoxin A after 24 h at 40, 42.5, and 45 C probably because of anaerobic conditions. Scheusner and Harmon (25) observed production of enterotoxins A, B, C, and D in vanilla pudding at 19, 26, 37, and 45 C. Toxins B, C, and D were detected at much lower populations at 45 C

than at 19, 26, or 37 C. Temperature also affects growth of S. *aureus* by altering the microbial activities of other microorganisms found in raw, unheated or semiprocessed, and fermented foods.

Associative growth of other microorganisms

The studies of Peterson et al. *(20)*, Troller and Frazier *(33)*, Post et al. *(22)*, Angelotti et al. *(1)*, Casman et al. *(4)*, Keosyan and Bennett *(15)*, and Rieter et al. *(24)* show that staphylococci grow poorly in mixed culture. Data of McCoy and Faber *(18)* show that certain microorganisms inhibit or stimulate enterotoxin production. Inhibition of growth by S. *aureus* could be caused by competition for nutrients or production of antibiotics, H_2O_2, or organic acids. Donnelly et al. *(10)* showed that in high count raw milk S. *aureus* did not grow well at an inoculum level of 10^4/ml and with an inoculum level of 10^6/ml S. *aureus* was able to grow and produce enterotoxin A only at 35 C. However, after pasteurization of the milk, growth and toxin production by added S. *aureus* occurred at all temperatures of 25 C or higher. In our studies *(29)* we found that in mixed population growth, even in very low bacteria count (40/ml) raw milk, S. *aureus* can be inhibited and that a higher population of S. *aureus* than in a pure culture is needed to yield a positive enterotoxin test. Growth of S. *aureus* to about 3×10^6/ml gave a positive toxin test in pure culture, whereas $7\text{-}8 \times 10^5$ S. *aureus*/ml in mixed culture growth (commercial cheese milk) did not yield a positive test for enterotoxin. Also Keosyan and Bennett *(15)*, Casman et al. *(4)*, Hill (*personal communication*), and Scheusner and Harmon *(25)* did not find detectable enterotoxin A in a competitive environment produced in custard and ground beef or pot pies.

In fermented foods such as cheese and sausage, growth of S. *aureus* takes place to a certain extent depending on the type of lactic culture and its activity. As was shown by Reiter et al. *(24)* and ourselves *(30)*, S. *aureus* is inhibited by lactic culture growth and phage lysis of the culture leads to extensive growth of S. *aureus* even with a minimal S. *aureus* contamination of cheese milk. Data of Daly et al. *(8)* also show that lactic cultures will inhibit S. *aureus* growth in fermented sausage. Our data *(30)* with cheese show that a very high inoculum and attainment of numbers $> 2\text{-}3 \times 10^7$/g is needed to yield a positive test for enterotoxin, whereas phage lysis and a consequent unrestricted growth of S. *aureus* to only $3\text{-}5 \times 10^6$/g yielded a positive test for enterotoxin in Cheddar or Colby cheeses. Slow starter activity (partial lysis of lactics by phage) could permit toxin production with only a limited extent of S. *aureus* growth. On the other hand, even complete and early starter failure and growth of S. *aureus* to 5×10^7/g did not result in detectable enterotoxin A in Blue Cheese *(31)*.

SUMMARY

Whether or not S. *aureus* can grow and/or produce detectable amounts of enterotoxins in a given food product depends on the nature of the food product (composition, raw, semiprocessed, or fermented) and the interactive influence of the existent environmental conditions during various phases of handling, processing, packaging, and storage. Despite attainment of high S. *aureus* populations, enterotoxins production is inhibited by the independent and interactive effects of temperature, pH, oxygen tension, water activity, and competitive growth of other microorganisms.

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THEODORE E. MINOR RECEIVES RESEARCH AWARD

The Richard M. Hoyt Memorial Award is presented annually by the American Dairy Science Association to a graduate student in recognition of research efforts which apply directly to solving problems facing the dairy industry. The award was initiated in 1971.

This year's award winner, Dr. Theodore E. Minor, was born in Otsego, Michigan, in 1940. He received the B.A. degree in biology from Eastern Michigan University in 1965 and the M.S. degree in biology from Central Michigan University in 1968. While pursuing graduate study for the M.S. degree, he also held a full-time position as applications technologist at the Dow Chemical Company in Midland, Michigan.

Minor enrolled at the University of Wisconsin in 1968 and recently received his Ph.D. degree. His graduate program encompassed a major in bacteriology and a minor in food science. Research by the recipient of the award was concerned with the behavior of *Staphylococcus aureus* in acidic environments occurring in such cultured dairy foods as cheese, buttermilk, sour cream, and yogurt. Data he gathered established the pH values needed before growth of S. *aureus* in milk is inhibited or before the organism is inactivated. Minor demonstrated that S. *aureus* survives only briefly in such cultured foods as sour cream, buttermilk, and yogurt. Other data obtained by the recipient of the award indicate that S. *aureus* can grow and produce enterotoxin in cream and that butter made from such cream can be toxic, although highest concentrations of enterotoxin appear in the buttermilk. He recognized that the fate of S. *aureus* in a food is not determined by a single factor but by a multiplicity of conditions. Hence, his experiments are among the first to consider the impact of several food-related conditions on survival of S. *aureus*. Papers with the recipient as the senior author have appeared in the *Journal of Milk and Food Technology, Journal of Dairy Science, Applied Microbiology,* and the *Indian Journal of Nutrition and Dietetics.*

Dairy foods may be abundant and excellent in body, texture, appearance, and nutritive quality, but they are totally unacceptable if they cause illness in the consumer. Staphylococcal food poisoning is one illness that has been attributed to certain dairy products. Research results obtained by Minor should aid the dairy industry to improve further the safety of its products. The recipient conducted his research under the guidance of Dr. Elmer H. Marth, Professor of Food Science and Bacteriology at the University of Wisconsin in Madison.