Staphylococcus aureus: Production of Extracellular Compounds and Behavior in Foods - A Review

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ABSTRACT

Growth of Staphylococcus aureus is accompanied by production of such extracellular compounds as hemolysins, nucleases, coagulase, lipase, and enterotoxins. Enterotoxins that can cause food poisoning are produced by about one-third of the coagulase-positive strains of S. aureus. The enterotoxins are a heterogeneous group of heat-stable, water-soluble, single-chain globular proteins having a molecular weight between 28,000 and 35,000 daltons. Production of enterotoxin by appropriate strains of S. aureus is affected by the nutritional quality and pH of the substrate, temperature, atmosphere, sodium chloride (and hence water activity), other chemicals, and competing microorganisms. Outbreaks of staphylococcal food poisoning are produced by about one-third of the coagulase-positive strains of S. aureus, but it can not be used as the sole determinant of pathogenicity (9,10). S. aureus possesses a species-specific antigenic determinant called polysaccharide A and thus can be differentiated from other staphylococcal species. Polysaccharide A is a component of the cell wall, consisting of a ribitol-type teichoic acid which is esterified with N-acetylglucosamine (72). Another characteristic of S. aureus is the ability to produce not only the extracellular enzyme, coagulase, but several other extracellular enzymes and toxins, many of which are toxic to humans and some animals.

The pathogenic capacity of a given strain of S. aureus is the combined effect of its ability to survive and multiply under a variety of conditions and to produce extracellular toxins. Among the extracellular toxins and enzymes produced by S. aureus are hemolysins, nucleases, coagulase, lipase, and staphylococcal enterotoxins. This review discusses these extracellular compounds, then considers growth and enterotoxin production by S. aureus in foods, and concludes with information on how growth and enterotoxin production are affected by food additives and by processing and storage of food.

EXTRACELLULAR COMPOUNDS OF S. AUREUS

Staphylococcal alpha-hemolysin

Staphylococcal bovine mastitis causes an enormous economic impact. S. aureus is one bacterium routinely associated with bovine mastitis, other microorganisms sometimes implicated include coliforms, Streptococcus agalactiae and Listeria monocytogenes. Whereas S. aureus is associated with mastitis approximately 20% of the time, research has shown that it is the staphylococcal alpha-hemolysin which may be leucocidal, enabling rapid multiplication and allowing for production of large amounts of toxin, including alpha-toxin (138).

Alpha-hemolysin, an extracellular toxin formed during the logarithmic phase of growth, has a molecular weight of...
10,000 to 50,000 daltons, depending on method of purification (31). The alpha-toxin possesses hemolytic, cytotoxic, dermonecrotic, and lethal activities. No other bacterial toxin is so versatile in its effects (31). A better understanding of this extracellular toxin is necessary for ultimate control of staphyloccal-associated mastitis.

**Staphyloccal nuclease**

Production of a heat-resistant nuclease appears to be uniquely associated with most coagulase-positive but not coagulase-negative strains of *S. aureus*. The enzyme is a phosphodiesterase with both endo- and exonucleolytic properties. It can cleave either DNA or RNA to produce 3’-phosphomonomonucleotides (72).

Staphyloccal nuclease is a compact globular protein consisting of a single polypeptide chain that can be isolated from, or near, the cell surface (103). Heating staphyloccal nuclease at 65°C causes structural disruption, but the changes are rapidly and completely reversible (72). Thermal stability of this enzyme, and its unique association with growth of *S. aureus*, have resulted in development of several methods to screen food products for presence of the nuclease. Its presence in food is used as an indirect measurement of growth of *S. aureus* in the product and thus indicates the likely presence of enterotoxin (56,59,79,112,124,136).

**Staphyloccal coagulase**

Only staphyloccal coagulase is considered to correlate with, if not be a determinant of, virulence. Seven antigenically different extracellular coagulases have been obtained from staphyloccoci, but the only pathogenic role suggested for the enzyme is the coating of the organisms with fibrin to inhibit phagocytosis (72). The enzyme exerts a clotting effect on plasma derived from several animal species, resulting in conversion of fibrinogen to fibrin, and a decrease in fibrinogen concentration in the circulating blood (19,130).

Coagulase does not function alone, but acts as a co-participant with other staphyloccal toxins and cell-contained factors in a complex pathogenic phenomenon (19,130). The combined action, in a susceptible or compromised host, enhances development of disease and can be directly related to virulence of *S. aureus* (30).

**Staphyloccal lipase**

Staphyloccal lipase acts on a variety of substrates, including plasma and the fats and oils that accumulate on the surface of the body. Lipase is an extracellular enzyme that is synthesized during the logarithmic growth phase, reaching maximum levels at the stationary phase of the culture (37,151). Production of large amounts of lipase accounts for increased virulence of many staphyloccal strains (31).

Lipolytic activity is especially common among coagulase-positive strains of *S. aureus*, regardless of the source from which the strain has been isolated. Coagulase-negative strains have been investigated for lipase production, with relatively few producing the enzyme (37).

Staphyloccal lipase acts on triglycerides, other esters, fats, and lipoproteins. Action of staphyloccal lipase on one of the lipoproteins of egg yolk, lipovitellenine, is used, in part, as the basis for identification of *S. aureus* on Baird-Parker Agar. Staphyloccal lipase also can hydrolyze lipoproteins present in blood serum (100,160).

Lipase activity occurs at pH values from 5.5 to 8.3, with the optimal pH for enzymatic activity being about 8.0. A second optimum also has been found at pH 5.5, but this has been disputed. The self-decomposition of lipid substrates at pH 5.5, rather than the presence of a second optimum for lipase activity has been established (31,126).

The optimum temperature for activity of staphyloccal lipase also has been disputed. Reported values for optimal activity range from 32 to 55°C. Tyski et al. (155) found that staphyloccal lipase could be stabilized by a substrate at a higher temperature. Such a phenomenon has been observed with lipase isolated from other bacteria (31).

The role of staphyloccal lipase in staphyloccal associated disease remains unclear. In human topical infections, the action of the lipase releases higher fatty acids from the skin of the host. These acids can be used by the bacterial cell for inclusion in the cell membrane or can be used to activate cellular metabolism (50). If this is true, intensification of intracellular metabolism may result in increased production and excretion of staphyloccal toxins, including staphyloccal enterotoxins.

**Staphyloccal enterotoxins**

In addition to extracellular coagulase, alpha-hemolysin and lipase, strains of *S. aureus* that are associated with food poisoning outbreaks produce extracellular enterotoxins, which are the protein toxins responsible for the symptoms.

Approximately one-third of the coagulase-positive strains of *S. aureus* produce enterotoxins that can cause food poisoning in humans (21). Foods commonly involved are improperly refrigerated custard or cream-filled bakery products, Ham, processed meat, ice cream, cheeses, hollandaise sauce, and chicken salad also are often implicated in outbreaks of staphyloccal food poisoning (22,92). It is noteworthy that foods containing the pre-formed enterotoxin are often normal in odor, appearance, and taste (21).

Staphyloccal food poisoning is characterized by severe cramping, abdominal pain, nausea, diarrhea, and vomiting that occur 2 to 6 h after ingestion of food in which *S. aureus* grew and produced enterotoxin (16,92). The short incubation time is indicative of a true food intoxication (16). Duration of acute symptoms is usually less than 24 h and the disease is rarely fatal, although fluid replacement may be necessary to compensate for the fluid lost through diarrhea and vomiting (31).

The enterotoxins are a heterogeneous group of water-soluble, single-chain globular proteins with a molecular weight between 28,000 and 35,000 daltons. The enterotoxins are strain-specific, although a strain of *S. aureus* can synthesize multiple toxin serotypes. Seven serologically distinct types [A, B, Cl, C2, D, E, and TST (toxic shock toxin)] of enterotoxin are recognized, with homogeneity existing between types A, B, and C2 (16). Enterotoxins A and D are most frequently associated with staphyloccal food poisoning (92), and enterotoxin B is most likely to be associated with
nosocomial infections (31). It also has been reported that staphylococcal enterotoxin A is a potent T-lymphocyte mitogen, causing induction of mitogen-type interferon synthesis in mouse spleen cells and human peripheral lymphocytes (74,80). All enterotoxins (A, B, Cl, C2, D, and E) except staphylococcal toxic shock toxin have been associated with foodborne disease (16,92). Toxic shock toxin is the cause of toxic shock syndrome in women and sometimes also in men (12).

*Staphylococcus aureus* also has been implicated in pseudomembranous enterocolitis seen in patients after oral administration of broad-spectrum antibiotics that selectively permit overgrowth by drug-resistant enterotoxin-producing strains of staphylococci (31,72). Disease is manifested by abdominal cramps, severe diarrhea, dehydration, and electrolyte imbalance. Presence of drug-resistant enterotoxigenic strains of *S. aureus* isolated in pure culture from the feces of these patients, necrosis of the intestinal tract caused by secretion of the enterotoxins, and clinical manifestations distinguish this disease from staphylococcal food poisoning (72).

Much of the research on pathogenesis of *S. aureus* and mode of action of the enterotoxins has been done with primates. In the monkey, oral challenge with staphylococcal enterotoxins causes vomiting, diarrhea, and an acute inflammatory response in the gastric mucosa and small intestine (45). Intravenous injections with enterotoxins also elicit these effects, especially vomiting. Staphylococcal enterotoxins do not appear to act directly on intestinal cells, and thus are not considered to be classical enterotoxins, such as cholera toxin or *Escherichia coli* enterotoxins. The site of emetic action of the enterotoxin lies within the gastrointestinal tract (45). Action of the staphylococcal enterotoxins generates impulses that reach the subcortical vomiting center of the brain via the vagus nerve and sympathetic afferents (141). In effect, then, staphylococcal toxins can be regarded as neurotoxins based on their mode of action (138).

**Intracellular factors affecting enterotoxin production**

An extracellular protein is one that is completely dissociated from the cell and found free in the surrounding medium. Clarification of this definition is needed to include (a) compounds that are membrane-bound in young cells and released as the culture enters stationary phase, and (b) compounds that are solubilized by relatively mild procedures such as washing of the cells with water or concentrated salt solutions.

Research has found that compounds to be secreted by bacteria are not synthesized simultaneously as cytoplasmic and extracellular molecules; in other words, bacteria produce the compounds for the sole purpose of cell export (154). Synthesis and secretion of extracellular compounds are regulated within the bacterial cell and are related to the growth cycle of the cell (154). For example, staphylococcal enterotoxin A is produced during the log phase of the cell, whereas enterotoxin B is secreted during the late log and early stationary phases (11). Secondary metabolites can have an inhibitory effect on the microorganisms producing them. However, studies have shown that enterotoxin B-producing strains of *S. aureus* are insensitive to enterotoxin B throughout the growth cycle (66).

The question exists as to whether production of enterotoxin B is an adjustment of the bacterial cell to static growth conditions or if the enterotoxin is produced as a secondary metabolite. If cellular growth is the sole determinant of production of an extracellular compound, or is a key regulator, there would be reduced capacity for exoprotein formation by biosynthetic machinery during the log phase. Thus, while enterotoxin A is produced and secreted early during growth, enterotoxin B is not produced until the latter stages of growth, as determined by the genetic mechanism and not solely by growth conditions. In such instances, cells within the cultures that have recently ceased to multiply are considered to be responsible for formation of the secondary metabolite (31). Nonreplicating cells of *S. aureus* will synthesize enterotoxin B once they are committed to enterotoxin B production, if certain environmental and nutritional requirements exist (82). This indicates the presence of a toxin precursor pool in committed cells and different mechanisms for production of enterotoxins A and B (11,82).

Yield of most enterotoxins can vary with maxima of 500 µg/ml for type B-producing strains of *S. aureus* (76), 60 µg/ml for type C, 10 µg/ml for type A-producing strains (116,140). Selection of strains that produce greater quantities of enterotoxin (e.g. enterotoxin C rather than A- or B-producing strains) also can enhance the likelihood that staphylococcal food poisoning will occur. Many strains of *S. aureus* can produce two or more enterotoxins (97). If enterotoxins are synthesized simultaneously by staphylococcal strains, the additive effect of the enterotoxins will increase the potential for foodborne illness if the food is eaten (97).

**Extracellular factors affecting enterotoxin production**

**Nutritional factors.** Nutritional factors, in addition to affecting growth of *S. aureus*, will influence production of secondary metabolites. Iron, inorganic phosphate, and carbon dioxide or bicarbonate in the medium can modulate the yield of secondary metabolites (31). Increasing levels of magnesium, inorganic phosphate, or potassium in a chemically defined medium greatly enhances production of enterotoxin B (77). Magnesium also can enhance production of enterotoxin C, but has no effect on production of enterotoxin A by strains of *S. aureus* (93). No effect on production of enterotoxins A or C was noted from addition of iron, but iron did influence production of enterotoxin B (93).

Protein supplements increase growth and enterotoxin production by strains of *S. aureus*. Yeast-supplemented media enhanced growth as compared to those containing soy or fish protein concentrate. Production of enterotoxins A and D also was enhanced in yeast-supplemented media, but not in those with soy protein. Addition of yeast to whole milk enhanced growth of *S. aureus*, and production of enterotoxin was increased 100-fold (146).

Peters (105) attributed suppression of enterotoxin B synthesis by glucose to acid production that lowered the pH of the medium to a value at which enterotoxin was not produced. Morse et al. (94), however, reported repressed
enterotoxin B synthesis at glucose concentrations of 0.35% or greater in a controlled pH environment. Production of enterotoxin C was not affected by presence of 1 or 5% added sugar (sucrose, maltose, lactose, glucose, and glucose plus fructose) after 12 and 24 h of incubation, although 1% added sugar enabled the staphylococcal culture to significantly decrease the pH (163). Jarvis et al. (71) concluded that pH changes in media or food resulting from sugar metabolism were only partly responsible for the observed effect on production of enterotoxins A, B, and C by strains of S. aureus. In other words, products of sugar metabolism may act as catabolite repressors of enterotoxin production while allowing for good growth of the culture (105).

**pH value.** An environmental factor such as pH can alter the balance between the final products of differentiation and secondary metabolites. Both formation and rate of formation of staphylococcal enterotoxins are influenced by pH of the medium. At pH 5.0 and below, enterotoxin B-producing strains synthesize little enterotoxin, although there may be good growth of the bacterium (105). For enterotoxins A and C, pH 5.0 appears to be the minimal value permitting enterotoxin production (52, 150). The optimal pH for production of enterotoxins B and C is 6.8, and enterotoxin B is not produced at or above pH 9.0 (83). In phosphate buffered brain heart infusion (BHI) broth, enterotoxin B production occurs at pH values of 5.15 to 9.02. In non-buffered BHI broth, toxin production is restricted to pH 6.14 to 7.95 (123). Growth of *S. aureus* occurs over a wider pH range than does toxin production. Less time is required for production of detectable toxin within the range of pH 6.44 to 7.20 than at pH values above or below this range. Compared to enterotoxin B, production of enterotoxin A is more tolerant of pH. Enterotoxin A was produced at the same rate at all pH values down to the minimum value tested, pH 5.5 (76). Tatini et al. (145) reported that enterotoxin A was produced in sterile milk at pH 4.6 to 6.5, with both growth and enterotoxin concentration reduced at pH values below 5. Optimal conditions of incubation temperature and pH will greatly enhance staphylococcal growth and enterotoxin production. Maximum production of enterotoxins A and B was observed at pH 7.0 when strains were held at the optimal temperature for growth, 39.4°C (23, 104, 150).

**Temperature.** Temperature also plays a major role in expression or suppression of secondary metabolites. Staphylococci are more resistant to adverse environmental conditions than are most non-spore-forming bacteria. Dietrich et al. (40) monitored growth of and enterotoxin B production by *S. aureus*. Maximum growth of *S. aureus* at 25°C did not correspond to the maximum amount of enterotoxin detected. Dietrich et al. (40) observed decreased growth of *S. aureus* and a corresponding increase in amount of enterotoxin detected as the temperature was increased from 25°C. The optimal temperature for staphylococcal enterotoxin B-production was 37°C. A further increase of temperature to 40°C resulted in decreased growth of *S. aureus* and a decrease in detectable amount of enterotoxin. The higher temperatures had a greater effect on production of enterotoxin than on growth of *S. aureus* (40). Scheusner et al. (123) observed a temperature maximum near 45°C for production of enterotoxins A, C, and D, with a corresponding minimum temperature of 19°C. Enterotoxin B production was observed over the range of 13 to 39°C. Vandenbosch et al. (157) found that the optimum temperature for production of enterotoxins B and C was 40°C, with a range of 20 to 45°C for their production in a liquid medium. Tatini (143) observed optimal growth for strains of *S. aureus* at 37°C, with enterotoxins produced over the range of 10 to 46°C. Maximum levels of enterotoxin were detected when *S. aureus* was grown at 40 to 45°C (143). Scheusner et al. (123) also noted that variation among strains of staphylococci influenced toxin production above and below 26°C. The ranges of concentration of specific nutrients and of environmental conditions are narrower for expression of secondary metabolites than for growth of the producing bacterium. Accordingly, despite bacterial growth, toxin formation may not occur.

**Atmospheric conditions.** Although strains of *S. aureus* are facultatively anaerobic, the amount of enterotoxin produced and the rate of enterotoxin production under anaerobic conditions are considerably less than when oxygen is present (11). Aerated cultures may produce as much as 10 times more enterotoxin B than do cultures held under the same conditions but incubated in an atmosphere of 95% parts nitrogen and 5 parts carbon dioxide (23). Levels of enterotoxin B produced by cultures at 12 and 24 h increased two- to threefold with aeration (163). Excessive aeration, or oxygen levels, also can decrease amounts of enterotoxin produced without affecting growth rate of the culture (11). Carpenter and Silverman (23) found 10% to be the optimal dissolved oxygen concentration for enterotoxin production.

**Sodium chloride.** One of the important characteristics influencing growth and enterotoxin production by strains of *S. aureus* is their ability to grow and survive in relatively high salt concentrations. A concentration of 5% NaCl enhanced growth of *S. aureus* compared to a salt-free milk control (23). Salt concentrations of 7.5 and 10% slightly retarded growth (23). A salt concentration of 10% or greater was inhibitory to production of enterotoxin B, and to a lesser extent inhibited production of enterotoxin A (104). At 15% salt, growth was repressed during the first 24 h, but strains of *S. aureus* grew at a slow rate after an initial lag (63). In an artificial medium containing up to 5% sodium chloride, growth of *S. aureus* and production of enterotoxin B were not affected (83). Smaller amounts of enterotoxin were produced in the presence of 7 to 9% salt, and none in the presence of 12 to 14% NaCl, although *S. aureus* grew (83). In the presence of 10% NaCl, enterotoxin B was produced when the culture was held at 37°C but not at 20 to 30°C (52). The effects of salt concentration and pH combined to inhibit enterotoxin production. Production of enterotoxin B was inhibited to a greater degree by these combined effects than was production of enterotoxin A (104). Temperature and pH had a greater effect than salt concentration on enterotoxin production (83).

**Other chemicals.** Minor and Marth (88) studied loss of viability by *S. aureus* in acidified media. Inactivation oc-
curred in the presence of all acids tested, with a mixture of lactic and hydrochloric acid being the most effective. Temperature of incubation, inoculum size, and presence of salts of the acids tested all influenced survival of *S. aureus* (88). Chemicals present in foods or added as preservatives, as well as those chemicals associated with sanitary practices in the food industry, also are inhibitory to growth and toxin production by strains of *S. aureus*. Hydrogen peroxide produced by lactobacilli, or added to a food product, can inhibit growth of *S. aureus* (36). Iodine at 1000 mg/L was effective in reducing numbers of *S. aureus* (127). Growth can be inhibited by diacetyl at concentrations of 0.075% or above (57). In addition, branched and straight chain amines have antimicrobial activity against strains of *S. aureus* (35).

**Water activity.** In addition to tolerating relatively high salt concentrations, *S. aureus* can tolerate the presence of other chemicals which result in reduced water activity within a food product. *S. aureus* is comparatively more able than other bacteria associated with foods to tolerate low water activity values (92). The minimal water activity for growth of *S. aureus* is 0.860 (134). This value is influenced by the oxidation-reduction (O/R) potential of the food, temperature, pH, and chemical preservatives, and thus may vary (29,134).

**Competing microorganisms.** In addition to nutritional requirements, pH, temperature, atmosphere, salt concentration, water activity, presence of other microorganisms can enhance or inhibit growth of staphylococci and thus can influence toxin production (42,55,73,110,152,153). Foods containing *S. aureus* often are contaminated with other bacteria that predominate in the microflora. Bacterial interactions may result, not in inhibition of growth and toxin production by *S. aureus*, but in enhancement of growth and in production of greater levels of enterotoxin by modifying environmental conditions.

Duitschaever and Irvine (42) studied the effect of *Penicillium* sp. on growth and production of enterotoxin by *S. aureus*. A large lot of Cheddar cheese contaminated with extreme surface growth of *Penicillium* sp. yielded 630 staphylococci/g, of which 30% were coagulase-positive strains. While all sections of the cheese yielded staphylococci, only subsurface sections yielded coagulase-positive strains. All coagulase-positive strains produced at least small amounts of enterotoxin D. The pH gradient resulting from mold growth favored staphylococcal growth, and thus enterotoxin production by the contaminating strains of *S. aureus* (42).

Studies have shown that certain microorganisms have a marked negative effect on growth of *S. aureus* and on enterotoxin production. Noleto and Bergdoll (96) studied production of staphylococcal enterotoxin in the presence of nonenterotoxigenic strains of *S. aureus*. Such strains inhibited growth of toxin-producing strains in milk, but not to a degree sufficient to prevent enterotoxin production. Interactions in broth between a strain of *Pediococcus cerevisiae* and toxin-producing strains of *S. aureus* resulted in growth of the staphylococci, with a corresponding 20-fold decrease in levels of enterotoxins A, B, and C produced by the respective strains (58). No enterotoxin D was produced by staphylococcal strains known to synthesize that toxin (58). McCoy and Farber (84) studied the interaction of selected strains of bacteria on growth and toxin production by *S. aureus*. A strain of *Serratia marcescens* and two of *Escherichia coli* prevented enterotoxin A production by an enterotoxogenic strain of *S. aureus* in cooked beef slurries stored at 35°C, but did not affect growth of the bacterium. When slurries of cooked beef and ham were held at 25°C, all microorganisms tested, with the exception of *Bacillus cereus*, reduced the amount of enterotoxin produced, or prevented enterotoxin production entirely (84). In ham slurries held at 25 or 35°C, production of enterotoxin A by *S. aureus* was stimulated by *B. cereus* (84).

An investigation on the interaction between an enterotoxin B-producing strain of *S. aureus* and *Pseudomonas aeruginosa* found a marked decrease in toxin production, reduced growth by the staphylococcal strain, and loss of salt tolerance by *S. aureus* (32). Reduced salt tolerance was related to cellular membrane damage, possibly associated with production of staphyloytic enzymes by *P. aeruginosa* (32). Haines and Harmon (58) studied the specific interaction between *Streptococcus lactis* and *S. aureus* in a broth culture. They noted growth of *S. aureus*, but no enterotoxin production. Inhibition of growth and enterotoxin production by streptococci was enhanced at temperatures below 30°C, and by controlling the size of the staphylococcal inoculum. Lactic acid bacteria can initially stimulate growth of strains of *S. aureus*, with such stimulation being greatest at 30 or 37°C (75).

Spillman et al. (135) studied the antimicrobial activity of commercial yogurts, as well as of cultures of lactobacilli, against test organisms, including *S. aureus*. They found the inhibitory effect on growth of the test organisms, including *S. aureus*, was not the result of antimicrobial activity within the yogurt or from the lactic cultures, but was the result of lactic acid (135). Research results of Chordash and Potter (30) disputed these findings. When staphylococcal enterotoxin A was added to inoculated media containing species of *Lactobacillus*, *Streptococcus*, or *Leucconostoc*, the amount of enterotoxin detected decreased with growth of the microorganisms. When enterotoxin A was added to media acidified to pH 3 to 6, but containing no added lactic acid cultures, pH had no effect on levels of enterotoxin A recovered. Chordash and Potter (30) attributed the effect on level of enterotoxin to factors other than the presence of lactic acid.

Gilliland and Speck (54) related inhibition of growth of *S. aureus* by lactic acid cultures to presence of both known and unknown inhibitory compounds, as well as acid production by the streptococci. Richardson and Divatia (118) noted that an active culture of lactic streptococci inhibited growth and toxin production by strains of staphylococci producing enterotoxins A, B, C, and D in sterile milk held at 32°C for 24 h. Further investigation revealed that an unknown chemical compound with characteristics of amino acids was possibly involved in inhibition of growth and toxin production. Research indicated that inhibition was independent of acid production (118).

Results from research designed to study interactions...
between enterotoxin producing strains of *S. aureus* and other microorganisms are far from conclusive. Variability in pH, temperature, and microbial strains makes it difficult to generalize about the effect of microbial interactions on growth of *S. aureus* and enterotoxin production in foods. Relative numbers of microorganisms present, and populations of each strain, will play a role in controlling growth and in ultimately determining the microbial load and microbial composition of the food product. Prevention of staphylococcal food poisoning, then, must be rooted in strict sanitary conditions of manufacture and temperature control of food products that could provide a medium for staphylococcal growth and enterotoxin production.

**GROWTH AND ENTEROTOXIN PRODUCTION BY S. AUREUS IN FOODS**

Staphylococcal food poisoning can be associated with a variety of foods. Foods often implicated include red meats (especially processed meats), poultry (especially chicken salad), hollandaise sauce, and dairy products (especially dried milk and cheeses). Ham and ham products are the vehicles associated with as many as 30% of the outbreaks of staphylococcal food poisoning (22,133). Other foods commonly implicated are improperly refrigerated custard or cream filled bakery products (92).

Most food poisoning outbreaks in which *S. aureus* is the causative agent result from the combined effects of contaminating the food through improper (unsanitary) handling, coupled with holding of the food at the wrong temperature, including inadequate cooling (21). The ability of strains of *S. aureus* to grow and produce enterotoxin over a wide range of environmental conditions, heat resistance of the toxins, and mishandling of food account for the continued importance of *S. aureus* as a major cause of food poisoning in the United States.

Many persons harbor staphylococci on their skin as a component of their indigenous microflora, and asymptotically in their nasal cavity and throat. The most probable source of contamination of foods by *S. aureus* is from people. Reali (114) examined nasal swabs of healthy individuals for enterotoxigenic strains of *S. aureus*. Of the enterotoxigenic strains identified, 76% produced either enterotoxin A or B, or both. Reali (114) concluded that contamination of foods by toxin-producing strains of *S. aureus* can be related to food handlers carrying *S. aureus* asymptomatically. Thus raw ingredients, equipment, or finished products can become contaminated from this source (114,161).

Payne and Wood (103) sampled a variety of foods for presence of *S. aureus*. Of 200 strains isolated, 125 were toxin producers, with enterotoxin A-producing strains being the most prevalent (59 strains) and enterotoxin B-producing strains being the least prevalent (4 strains). Payne and Wood (103) noted that 26, or 21%, of the toxigenic strains could produce multiple toxin serotypes. Wieneke (161) also investigated the prevalence of staphylococcal strains in raw and cooked food products. Enterotoxin D-producing strains predominated (47.7%), with staphylococcal strains in raw and cooked foods also likely to produce enterotoxins C (35.1%) and A (26.1%) (161). Upon direct examination of 113 staphylococcal strains associated with food poisoning cases, 88 produced enterotoxin A, 48 produced enterotoxin D, and 46 produced multiple toxin serotypes, predominantly toxin serotypes A and D (161).

Strains of *S. aureus* associated with staphylococcal infections exhibit a different pattern of enterotoxin production than do staphylococci associated with food poisoning. Enterotoxin A-producing strains were most often isolated from hospital patients, with strains also often producing serotypes B and C (161). Of those strains of *S. aureus* isolated from hospital patients, 27.9% produced multiple toxins (161).

Correlation between staphylococcal food poisoning, staphylococcal infections, and presence of staphylococcal enterotoxins appears to be as much the result of the strains of *S. aureus* that produced the enterotoxin as the medium that allowed for staphylococcal growth. Strains of *S. aureus* isolated from processed poultry, poultry farms, and processing facilities produced almost exclusively enterotoxin D, with some strains producing enterotoxin A, or both enterotoxins A and D (62). Enterotoxigenic strains of *S. aureus* isolated from both healthy and infected hospital patients, produced enterotoxin A and B, or both, but predominantly enterotoxin B (114).

To further understand the relationship between toxin-producing strains of *S. aureus* and their presence in foods of hospital patients, Reali (114) examined cultures isolated from foods but not associated with food poisoning outbreaks. The data indicate 47% of the strains were enterotoxigenic and produced predominantly enterotoxin B. Data from this and other studies indicate that although a relationship appears to exist between the type of enterotoxin-producing strains and their isolation from a food or hospital patient, no generalization is possible (62,103,114,161).

**Staphylococci and enterotoxins in foods other than dairy products**

Minor and Marth (91) reviewed the literature on staphylococci and staphylococcal enterotoxins in meat, bakery products, and other non-dairy foods. Their extensive review covered information on a number of foods associated with staphylococcal food poisoning.

The ability of *S. aureus* to grow and produce enterotoxin over a range of environmental conditions accounts, in part, for the variety of foods implicated in staphylococcal food poisoning. Contamination of foods from environmental sources or by infected food handlers also accounts for the presence of staphylococci in properly processed foods. In addition, the ability of staphylococci to grow and produce toxin at temperatures often associated with improper holding of foods (25 to 35°C) also plays a major role.

A voluntary recall by a firm in Denver, Colorado occurred in 1970 after fresh frozen country-style wide egg noodles were found to contain staphylococci (5). In mid-July 1978, 25 people became ill after consuming sterilized vanilla custard (15). The custard contained greater than 100 million
staphylococci/ml, and enterotoxin A was detected in a sample of the product. Preparing conditions were adequate, but post-processing contamination occurred, and *S. aureus* grew when the product was stored without refrigeration (15). A catered event in August 1984 for 300 people resulted in 27 people contracting staphylococcal foodborne disease. The vehicle of transmission was Virginia ham (6). The length of time the ham was held at an elevated temperature contributed to the outbreak of food poisoning.

Staphylococcal food poisoning outbreaks that involve an infected food handler may result in several foods becoming vehicles for foodborne illness. In June 1985, an outbreak of staphylococcal food poisoning was traced to contaminated ham, deviled eggs, and potato salad. Mayonnaise used to prepare the potato salad and whipped topping for strawberry shortcake contained small numbers of staphylococci (7). The food had been prepared by a food handler who contaminated the products, and then the food was improperly refrigerated (7).

A number of foods available to the consumer are possible vehicles for staphylococcal food poisoning. Foods with the appropriate pH, water activity, and ingredients (e.g., nutrients) held at temperatures conducive to growth of *S. aureus*, require that special attention be given to sanitary conditions during manufacture, handling, and storage.

**Bakery products.** Local grocery stores and bakeries are capitalizing on the consumer’s desire for fresh baked goods. Numerous baked goods are available, including vegetable- and cheese-filled rolls and croissants, pastries and croissants with sweetened fillings, and cream-filled rolls. Many of these products are bought as snack items. It is hard for individual bakers to judge demand for these products; thus, they may be held for several days before they are bought and consumed.

A number of bakery products were sampled for microbial count, including staphylococcal count, and moisture (120). Moisture content varied from 6.4 to 16.9%, with aerobic plate counts ranging from 120 to 61,000 colony forming units (CFU)/g. Staphylococcal counts averaged over 13,000 CFU/120 g and accounted for 15% of aerobic bacteria isolated (120). All cultures of *S. aureus* isolated produced enterotoxins, with A, B, and E detected, and enterotoxin B predominant (124). Sankaran and Leela (120) concluded that strains of *S. aureus* isolated from bakery products could produce enterotoxin if the foods were subjected to improper holding temperatures for sufficient time (120).

Wyatt and Guy (164) studied the presence of enterotoxigenic strains of *S. aureus* in retail pumpkin pie. One of four pies purchased from a retail outlet in Oregon and held at 25°C for 72 h contained more than 2400 staphylococci/g. The internal temperature during baking of pumpkin pie should exceed 108°C for 1 min, which would be sufficient to kill all viable staphylococci. Thus contamination after cooking probably was responsible for the level of *S. aureus* detected (164). When retail pumpkin pies were inoculated with *S. aureus* (100/g) and held at 4, 25, and 35°C, growth occurred at 25 and 35°C, but not at 4°C. Numbers of staphylococci exceeded 1 million/g within 24 h at 25 and 35°C, with greater than 100 million/g detected in pies held at 25 and 35°C for 48 h (94). Added potassium sorbate (0.25%) was not effective against *S. aureus*. All pies inoculated with *S. aureus* and held at 25 or 35°C for 48 h contained detectable enterotoxin A (164).

**Dried meat products.** Other foods available at retail outlets and handled by employees and/or consumers can harbor sufficient levels of *S. aureus* to be potential health hazards. Adesiyun (2) analyzed samples of dried beef and dried fish for *S. aureus*. The samples contained 0.9 million and 4.6 million CFU/g, respectively. Storage for 28 d at room temperature resulted in a 100-fold decrease in staphylococcal count (2). No enterotoxin analysis was done during this study. However, presence of large numbers of coagulase-positive staphylococci is cause for concern and could indicate presence of levels of enterotoxin sufficient to cause food poisoning.

A study with beef jerky revealed that numbers of *S. aureus* present in meat used in jerky manufacture decreased during the heat-drying process (64). Further reductions in staphylococcal count were observed with refrigerated storage of the product. Need for using wholesome meats in production of microbiologically safe beef jerky was emphasized (64). Although dry and semi-dry sausage products usually are not considered to be potential vehicles for staphylococcal food poisoning, Genoa and Italian dry salami have been implicated in some outbreaks of illness (25-27).

**Vegetables and legume products.** Vegetables, including green beans and corn (18) and mushrooms (81), have been studied as possible vehicles for staphylococcal food poisoning. Foster et al. (49) found that addition of textured soy protein to regular ground beef had no effect on total microbial load of the product, including number of *S. aureus*. Craven et al. (34) showed that soy protein supported growth and enterotoxin production by *S. aureus* S-6, with both enterotoxin A and B detected in most products. Although beef and pork sausage were excellent media for growth of *S. aureus*, addition of 20% soy products neither inhibited nor stimulated growth of the pathogen (34). Presence of soy products did affect enterotoxin production, however (34). Beef with textured soy protein or soy protein concentrate had markedly less enterotoxin B than did the control. Pork sausage with soy protein isolate had appreciably more enterotoxin B than did the control or sausage with either soy protein concentrate or textured soy protein (34). Use of soy products as meat extenders in ground meats and in sausage-type products, or for their functional properties, seems to be of less interest today than several years ago. Soy products, when added to some other foods, can enhance staphylococcal enterotoxin production. Thus soy products should only be used in foods processed under sanitary conditions and held at appropriate storage temperatures.

**Pork products.** Ham and ham products often are associated with outbreaks of staphylococcal food poisoning (22,133). Steele and Stiles (137) studied the food poisoning potential of vacuum-packaged sliced ham. Microbial competition affected the ability of pathogenic bacteria to grow in the...
product. Steele and Stiles (137) found that holding the product at elevated temperatures during storage was necessary for S. aureus to develop to levels associated with the potential for food poisoning. Similar results were obtained with bologna and chopped ham (137).

Another processed pork product, precooked canned bacon, harbored S. aureus in the range of 100 to 140,000/g (109). Loss of viability by S. aureus before testing the product was possible; thus, preformed staphylococcal enterotoxin could have been present at levels associated with staphylococcal food poisoning. Wide variations in water activity (0.86-0.96), and the moisture/salt ratio (8.73-21.44), were partly responsible for survival of S. aureus and contributed to development of potentially hazardous populations in the product (109).

Poultry products. Poultry products also have been involved in staphylococcal food poisoning outbreaks. S. aureus has been directly implicated in a food poisoning outbreak involving barbecued chicken (108). Studies have determined that processing procedures greatly influence contamination of poultry carcasses with S. aureus, and specific phage types indigenous to processing facilities play a significant role in contamination of carcasses (98,128,129).

Although poultry carcasses often are heavily contaminated with competing bacteria, presence of staphylococci contributes to the potential for recontamination after cooking. Yang et al. (165) studied growth of strains of S. aureus isolated from a turkey processing facility and inoculated them into ground turkey muscle contaminated with spoilage bacteria. Growth did not occur at 7 or 10°C. Cooking the meat before inoculation reduced the population of spoilage bacteria and allowed growth of strains of S. aureus at 15 and 20°C (165).

Hard-boiled eggs were involved in two food poisoning outbreaks in Wisconsin in the 1970s (60). Enterotoxigenic strains of S. aureus were implicated, and enterotoxin B was detected. Handling of the eggs by a person harboring enterotoxigenic staphylococci, or water contaminated with staphylococci and used to cool the eggs, were probable sources of contamination (60). Control of the temperature at which cooked eggs are stored, and adherence to sanitary practices when handling such eggs will help control staphylococcal growth and enterotoxin production in eggs.

Staphylococci and enterotoxins in dairy products

Many dairy products are satisfactory media for growth of pathogenic bacteria, including enterotoxigenic strains of S. aureus. Large outbreaks of foodborne illness in 1985 and associated with other pathogenic bacteria in dairy products commanded national attention. An outbreak of listeriosis in California caused by contamination of Mexican-style soft cheese with Listeria monocytogenes and the outbreak of salmonellosis in Illinois and surrounding states resulting from contamination of 2% low-fat milk with Salmonella typhimurium re-emphasized the need for control of pathogenic bacteria in dairy products.

In addition to their extensive review of the literature on non-dairy products, Minor and Marth (90) reviewed information on the presence of S. aureus and enterotoxin production in dairy foods. Their review included a discussion of food poisoning outbreaks and factors affecting staphylococcal growth and enterotoxin production. More recent information is summarized in the following paragraphs.

Fluid milks. Fluid milk is less often associated with large staphylococcal food poisoning outbreaks than are dairy products as dried milk and cheeses. Presence of staphylococci in milk and dairy products can be related to contamination of milk from the bovine udder, human sources, environmental sources, or by pasteurization contamination from improperly cleaned and sanitized equipment. Ahmed et al. (3) investigated the presence of enterotoxigenic strains of S. aureus in milk and dairy products. Of 75 staphylococcal cultures, 27 strains (36%) were enterotoxigenic, producing enterotoxins A, B, or E, or combinations of these enterotoxins. dos Santos et al. (122) surveyed raw and pasteurized milk for staphylococci. S. aureus was present in 46.9% of the raw milk samples. Of pasteurized milk samples, 6% contained S. aureus, with numbers averaging over 18,000 CFU/ml (122).

Tatini et al. (145) found that microbial competition greatly influenced growth of S. aureus in raw whole milk. Growth and production of enterotoxin were observed in heat-treated milk. Donnelly et al. (41) noted that raw or pasteurized low-count milk was a better medium for staphylococcal growth and enterotoxin production than was high-count raw milk. Although staphylococci are destroyed by pasteurization, the ability of S. aureus to grow and produce enterotoxin in milk is of concern when coupled with improper pasteurization of raw milk containing a large population of staphylococci and/or heat-stable enterotoxin and the possibility of post-processing contamination.

Walker and Harmon (158) studied growth of enterotoxigenic S. aureus in whole and skim milk and broth at 5 to 37°C. Growth did not occur at 5°C in any medium. At 10 and 22°C, the medium had little effect on duration of the logarithmic growth phase. When the temperature was increased to 30 and 37°C, no difference in generation time was seen for growth in skim and whole milk but staphylococcal strains did have significantly shorter generation times at these temperatures in broth than in milk (158). Staphylococcal strains were more persistent at higher temperatures in broth and skim milk than in whole milk. A decrease in population in whole milk was related to presence of free fatty acids, specifically capric, caprylic, and lauric, released by action of staphylococcal lipases on the milk fat (158). Fatty acids released by lipases are toxic to S. aureus (156).

Cream and butter. Ikram and Luudeecke (70) studied staphylococcal growth in a variety of fluid dairy products with various amounts of milk fat. S. aureus strain 100 grew better in skim and whole milk than in half-and-half or whipping cream. Enterotoxin production also was influenced by the growth medium. Skim and whole milk were better media for enterotoxin production than were half-and-half or cream (70). Minor and Marth (89) studied growth of S. aureus in cream at 37°C. Cream supported production of
enterotoxin A, with greater than 1 µg/100 g produced in cream after 24 h at 37°C. When butter was evaluated as a medium for growth and enterotoxin production by S. aureus, staphylococci survived in untreated and whipped butter at 23°C but not at 10°C. Enterotoxin was not detected in butter (1.5% salt) or whipped butter inoculated and stored at 23°C for 14 d (89).

One outbreak, and two likely outbreaks of staphylococcal food poisoning have been associated with butter. The first well-documented case of staphylococcal food poisoning associated with butter was reported in 1970 (24). Whipped butter, made by whipping butter together with milk, was implicated. S. aureus and enterotoxin A were present in the butter used (24). Also in 1970, a major food processor voluntarily recalled more than 75,000 lb. of butter in a multi-state area because of excessive bacterial contamination and the presence of S. aureus (119). Illness was reported by consumers after consumption of the product (119). In 1977, presumed staphylococcal food poisoning was associated with whipped butter (28). Again, a multisite recall of the product resulted after 100 people became ill. Up to 10 million S. aureus/g were isolated from lots of the whipped butter (28). Minor and Marth (89) observed that presence of salt, number of staphylococci, and whipping could influence the fate of S. aureus in butter. Presence of S. aureus in such high numbers in butter products, and involvement of these products in staphylococcal food poisoning, are considered unusual.

Nonfat dry milk. Nonfat dry milk and foods containing nonfat dry milk have been implicated in a number of staphylococcal food poisoning outbreaks. McDivitt et al. (85) stated that a low bacterial count for nonfat dry milk could be misleading as an indication of the safety of the product. Thompson et al. (148) found that the number of surviving bacteria increased as the temperature used for spray-drying increased. The effect of drying microorganisms also varied, with bacteria that were more resistant to drying surviving spray-drying and persisting in storage. S. aureus in relatively resistant to drying and to heat (72).

Frozen dairy desserts. Presence of S. aureus in frozen dairy desserts also has been established (14,48,142). Batish and Chander (14) tested samples of kulfí, kulfa, and ice cream for presence of enterotoxigenic staphylococci. Each of 50 samples tested contained S. aureus. Sixty nine of the 190 isolates characterized as S. aureus produced enterotoxin. Enterotoxin A was produced by 48 isolates, 19 produced enterotoxin B, and enterotoxins C1 and E were each produced by 1 isolate (14). Tamminga et al. (142) detected S. aureus sporadically in 100 ice cream samples. They observed substances or properties inhibitory to microbial growth in 86 samples. Tamminga et al. (142) found no correlation between a positive test for presence of S. aureus and microbial quality, although presence of S. aureus would indicate poor sanitary practices and improper storage temperatures since staphylococcal counts commonly decrease during frozen storage (142).

Cultured dairy products. Ghoniem (53) studied survival of S. aureus in yogurt. Temperature and pH were monitored, and staphylococcal counts were made. During storage at -1°C, the pH gradually increased from 5.0 to 7.0 by the 29th d of storage. During storage at 4°C, the pH fell from 5.0 to 4.5 by the 12th d of storage and fluctuated only slightly from this value. At 30-32°C, the initial pH of 4.0 was maintained during the first 12 d of storage, then the pH gradually increased to 5.5 by the 19th d of storage. Viable staphylococci were present in yogurt for 50 d at -1°C, for 29 d at 4°C, and for 20 d at 30-32°C (53).

These results are in sharp contrast to the work reported by Minor and Marth (86,87). For sour cream, buttermilk, and yogurt inoculated with staphylococci, pH of the products stored at 7 and 23°C varied less than 0.2 unit from initial values of 4.1-4.4, depending on the product and brand. Numbers of S. aureus were greatly reduced in all products held at 7 and 23°C after 7 d of storage, although staphylococci survived longer in sour cream than in buttermilk or yogurt. Minor and Marth (87) found no effect of storage temperature on survival of S. aureus in buttermilk or yogurt, but survival was enhanced slightly in sour cream stored at 7°C rather than 23°C.

Cheese. Numerous studies have been completed to determine the fate of S. aureus in cheese and cheese products. Fitz and Owens (47) analyzed 70 samples of Cheddar cheese for presence of S. aureus. For samples that tested positive, staphylococcal counts ranged from 200 to 25,000/g. All strains isolated were determined to be of bovine origin, with no human strains detected. Naguib et al. (95) observed that greater than 65% of Ras cheese samples were contaminated coagulase-positive S. aureus. Studies on the effect of cheesemaking on presence of staphylococci in Ras cheese indicated that ripening milk up to the time rennet was added did not affect number of staphylococci, but the remaining processing steps, including pressing, substantially decreased the number of staphylococci detected. Numbers of S. aureus were maintained for up to 10 d of ripening, followed by a gradual and progressive decrease in numbers. After 38 d of storage no staphylococci were detected in the cheese (95).

Tod et al. (149) evaluated blocks of Swiss-type cheese implicated in staphylococcal food poisoning outbreaks for presence of S. aureus and enterotoxin. Staphylococcal counts varied from less than 25 up to 100 million/g, with an average among the blocks of 10,000 to 1 million S. aureus/g (149). Enterotoxins A and B were detected. Of 186 samples tested for enterotoxin B, 72.6% contained detectable amounts of the enterotoxin. Enterotoxin A was detected in 52.5% of 122 samples tested for the enterotoxin. The pH values of contaminated cheese did not differ significantly from normal values for Swiss-type cheese (149).

Van Schouwenburg-van Foeken et al. (125) manufactured Gouda cheese of normal acid/pH. Enterotoxin was detected after 24 h in cheese samples containing greater than 10-100 million enterotoxigenic S. aureus/g. Amounts of enterotoxin varied, with 1 µg of enterotoxin A, 2 µg of enterotoxin B, and 0.5 µg of enterotoxin C detected/100 g of cheese (125). Tatini et al. (147) could not detect enterotoxin A in blue cheese with staphylococcal counts of 25-50 million/g and made with normal starter activity, or in blue
cheese with 20 million \( S. aureus \) /g and manufactured so starter activity was reduced. Enterotoxin A could not be detected in mozzarella cheese with 20 million \( S. aureus \) /g, but was detected in brick cheese with over 50 million \( S. aureus \) /g, and in Swiss cheese with 7-13 million \( S. aureus \) /g (147). Tatini et al. (144) detected enterotoxin A more frequently in Colby than Cheddar cheese when both types of cheese were made with normal starter activity. Growth of \( S. aureus \) to at least 15 million staphylococci/g in Colby and at least 28 million/g in Cheddar cheese resulted in production of detectable levels of enterotoxin A. Tatini et al. (144) also reported the ability of enterotoxin A to persist for over 3 years in Cheddar cheese made with a normal or inhibited starter culture.

Abo-Elnaga and Kandler (1) studied the fate of coagulase-positive staphylococci during the manufacture of Camembert cheese. They (1) suggested that presence of enterotoxins, and the potential for Camembert cheese to cause staphylococal food poisoning, were related to growth of \( S. aureus \) during the first 12 h of manufacture which could occur if the milk was contaminated with large numbers of staphylococci. The fate of \( S. aureus \) during the making of Brazilian Minas cheese varied with processing procedures used, with use and viability of starter, with use of raw or pasteurized milk, and with level of staphylococcal inoculum (121). Strain variation and ripening time also had a significant effect on the fate of \( S. aureus \) in commercially manufactured Minas cheese (121). Staphylococci multiplied during manufacture of the cheese (121). During ripening, staphylococcal counts decreased as pH decreased. After 3 d, the number of staphylococci/g had decreased by over 90%. When the surface of cheese was contaminated with \( S. aureus \) after being held in the brine tank, staphylococci did not multiply.

Reiter et al. (117) observed that staphylococci multiplied more rapidly in cheese when the starter was inhibited by phage than when the starter remained active. In Cheddar cheese made with slow starter activity, little decrease in numbers of staphylococci was seen, even after 18 months, in contrast to the rapid decrease seen in cheese made with normal starter activity. Reiter et al. (117) attributed the decrease in numbers of viable staphylococci to acid production by the streptococcus starter (pH effect) as well as to the competitive effect of an unknown thermolabile inhibitor in milk. Tatini et al. (144) also noted that starter failure influenced the ability of \( S. aureus \) to grow and produce enterotoxin. In Colby and Cheddar cheeses, starter failure resulted in extensive growth of \( S. aureus \) and production of detectable levels of enterotoxin A when staphylococcal counts reached 3-5 million/g (144).

Ibrahim et al. (69) observed inhibited growth and enterotoxin production by \( S. aureus \) in Cheddar cheese made with induced starter failure. Not salting the curd at the end of cheddaring, pressing the curd at low ambient temperatures, and minimizing pressing time inhibited growth of \( S. aureus \) (69). Storage of salted and unsalted cheese at 4°C resulted in decreased counts of \( S. aureus \), with no effect on enterotoxin concentration. At 11°C, salted Cheddar cheese was a favorable medium for growth and enterotoxin production by \( S. aureus \), whereas staphylococcal counts decreased in unsalted cheese at 11°C (69).

When Cheddar cheese was made with a starter having variable activity, Ibrahim et al. (68) observed a significant effect of starter on the fate of \( S. aureus \). Salting during processing enhanced the staphylococcal count and enterotoxin production. During storage, staphylococcal counts and levels of enterotoxin increased in salted cheese held at 11°C (68). At 4°C, staphylococcal counts decreased in salted cheese. In unsalted cheese, no change in enterotoxin concentration during storage was seen at 4 or 11°C, but a sharp decrease in levels of \( S. aureus \) occurred.

Koenig and Marth (78) studied behavior of \( S. aureus \) in Cheddar cheese made with sodium chloride or a mixture of sodium and potassium chloride. Lowest staphylococcal counts were found in unsalted Cheddar cheese, and highest counts appeared in cheese salted at a concentration of 2.4% sodium chloride. A mixture of sodium and potassium chloride (1:1 molar basis) in Cheddar cheese resulted in lower counts of \( S. aureus \) than in Cheddar cheese salted with sodium chloride alone (78). Presence of the potassium ion was assumed to be partly responsible for increased bacteriostatic activity of the mixture over that of sodium chloride alone. Levels of staphylococcal enterotoxins were affected by salt concentration and incubation temperature, but not by type of salt used. When Cheddar cheese was manufactured with 1.0% starter and salted with sodium chloride or a mixture of sodium and potassium chloride, mean log counts of \( S. aureus \) were 0.90 ± 0.15 and 0.92 ± 0.36, respectively, lower than log counts of staphylococci in cheese made with 0.5% starter (78). Percent starter used did not significantly affect average enterotoxin level detected in Cheddar cheese.

FOOD ADDITIVES AND PROCESSING AND STORAGE OF FOOD AFFECT STAPHYLOCOCCI AND ENTEROTOXIN PRODUCTION

Staphylococci are injured when subjected to sublethal stresses caused by heat, cold, irradiation, or chemicals. Injury is manifested by loss of membrane integrity, and cellular components are found in the environment around the cell (65,67,131). Smith et al. (131) reported that leakage of cellular components from stressed cells did not always indicate injury, and leakage could occur during heating even though few injured cells were found. Injured cells have only minimal metabolic activity, and enterotoxins are not synthesized (20). Zayaitz and Ledford (166) observed that acid-injury of staphylococcal cells did not result in damaged cellular membranes, or in leakage of 260 or 280 nm-absorbing material. However, activities of staphylococcal coagulase and thermonuclease were reduced in acid-injured cells. Acid-injury was determined to be sublethal, and recovery did occur with subsequent growth of \( S. aureus \).

If injury is not lethal and the stress is eliminated, bacteria can undergo repair and growth can be re-initiated (65,162). Witter (162) noted that Baird-Parker Agar was useful in detecting stressed staphylococci, and this medium
did not present a secondary stress to injured cells. Iandolo and Ordal (69) found the heat-injured cells of *S. aureus* could undergo repair when transferred to media containing amino acids, glucose, phosphate, and magnesium. Stiles and Witter (139) found that heat-injured, salt-sensitive staphylococci recovered their salt tolerance without growth in the presence of 5% glucose or galactose. They found no other carbohydrates or metabolites that had a similar effect on recovery. Stiles and Witter (139) also reported that cell growth, cell wall synthesis, protein synthesis, divalent cation-dependent enzyme systems were not involved in the recovery phenomenon.

**Thermal treatment**

Firstenberg-Eden et al. (46) studied the effect of thermal treatment of milk on death and injury of *S. aureus*. Cultures were injured as soon as heating began, and higher temperatures resulted in greater initial thermal shock. At 50 to 60°C, injury was more rapid than death; at temperatures greater than 60°C, death occurred. The effect of temperature on injury or in causing death coincided at 70°C (46). Increased resistance of staphylococci to thermal destruction at 60°C was noted in skim milk containing 26-57% sucrose and in pork macerate containing 8.4-8.5% NaCl (132).

Studies have determined the effect of heat treatment on survival of *S. aureus* (17, 159, 167). Individual strains of *S. aureus* were subjected to subpasteurization temperatures of 64, 65, and 67°C in milk for 21 s, with survival rates of 46, 20, and 3%, respectively (167). Bhatt and Bennett (17) heated a mixture of 171 strains of *S. aureus* in milk. They found 1.5% survived after 30 min at 62°C and 0.38% survived after 15 s at 72°C, with no survivors after 45 min at 62°C or 35 s at 72°C. Minimum pasteurization temperatures and times for milk are 63°C for 30 min or 72°C for 15 s.

Walker and Harmon (159), working with selected strains of *S. aureus*, found that survival rates were greater in skim milk and in Cheddar cheese whey than in whole milk or phosphate buffer. At 60°C, 12 min were required to kill 99,999% of a population of *S. aureus* in whole milk, and about 21 min were required for equivalent destruction in skim milk and whey (159).

Microbial load also influences the rate of thermal destruction. During thermal treatment, logarithmic destruction occurs, from an initial count of 10 million *S. aureus* per ml until 1000 to 100 cells or less remain per ml (61). Destruction of the remaining staphylococcal cells occurs gradually. Harmon (61) pointed out that the age of bacterial cells influences their resistance to thermal destruction. A threefold increase in time required for thermal destruction was noted for a culture of *S. aureus* as the age of the culture increased from 12 to 60 h or more (61).

Thermal resistance will influence the effects of heat treatment on *S. aureus*. Such resistance can be imparted by the medium and/or solutes, or by age of the culture, and can vary with staphylococcal strain. Walker and Harmon (159) found that staphylococci had greater heat resistance in skim milk and Cheddar cheese whey than in phosphate buffer or whole milk, with least resistance in whole milk and most resistance in Cheddar cheese whey. Thermal resistance varied among strains of *S. aureus*, but was consistent for each strain (159). Heat resistance was also influenced by age of the culture. Cultures grown for 60 or 228 h were several times more resistant than the same cultures grown for only 12 h. In phosphate buffer at 55°C, a 12-h-old culture had a D-value of 0.95 min; a 228-h-old culture had a D-value of 3.0 min (159). Zottola et al. (167) also found that microbial load influenced thermal resistance, with a large microbial load enhancing heat resistance compared to that when reduced populations were present. El-Banna and Hurst (43) found that *S. aureus* grown in a rich peptone medium containing 1 M NaCl at 46°C was more heat resistant than were cells grown at 37°C. When the D-values at 60°C were determined for these cultures in milk, green beans, peas, and beef slurry, values for cells grown at 46°C were about 4 times greater than those of cells grown at 37°C. In media treated with acetic or lactic acid, cells grown at 46°C also survived longer than did cells grown at 37°C (43).

Thermal treatment also has a noticeable effect on staphylococcal enterotoxins. Staphylococcal enterotoxins are proteins, which during heat treatment, undergo denaturation that can lead to a partial or total loss of activity. Renaturation of reactivation of enterotoxins can occur, with recovery of serological activity. Reichert and Fung (115) found that enterotoxin B lost 60-70% of its activity rapidly during the first few minutes of heating at 80 and 100°C. The remaining 30-40% of activity was lost much less rapidly. During storage or during continued heating or reheating to a higher temperature, enterotoxin B could recover some lost activity. Reactivation of heated enterotoxin B was observed even when small initial quantities of enterotoxin were present, and greater reactivation was noted when the heating time was less than 5 min at any temperature (115).

**Irradiation**

In addition to heat treatment, irradiation has been proposed as an effective means of controlling microbial growth in foods. Ultraviolet (UV) irradiation effectively kills bacteria and yeasts (13). Barluzzi et al. (13) reported that UV irradiation destroyed 99.9% of viable staphylococci in water, and 98.4% of viable cells in milk. The effect of irradiation on enterotoxins has also been studied. Read and Bradshaw (113) observed that a 50-kGy dose of gamma irradiation was required to inactivate 97.7% of a concentration of 31 μg of enterotoxin B/ml of Veronal buffer. In milk, a 200-kGy dose of irradiation inactivated 98% of 30 μg of enterotoxin B/ml. The dose of irradiation required to inactivate 90% of the active enterotoxin was 27 kGy in Veronal buffer, and 97 kGy in milk (113).

**Freezing**

Demchick et al. (39) found that repeated freeze-thaw cycling did not necessarily produce a significant degree of sublethal injury in *S. aureus*. Destruction of staphylococci did occur when low pH values were combined with freeze-thaw stress. Fast freezing followed by fast thawing, at pH values above 4.5, ensured survival of *S. aureus* (39). At pH
values of most frozen foods, repeated freeze-thaw stress would probably not lead to a substantial decrease in the number of contaminating staphylococci. Fung and Vandenbosch (51) showed that S. aureus cells injured by freeze-drying synthesized enterotoxin when the cells were rehydrated and allowed to repair. El-Banna and Hurst (43) found that length of survival in nonfat dry milk after freeze-drying was greatest for staphylococcal cells grown at 46 rather than at 37°C. Cells with the highest D-value survived longest after freeze-drying.

**Solute**

Hurst (65) showed that addition of NaCl, KCl, glucose, or sucrose to media permitted growth and enterotoxin production by S. aureus at temperatures at least 2°C higher than in unsupplemented growth media. Smith et al. (132) observed that increased thermal resistance in the presence of solutes was caused more by the chemical nature of the solute than by the effect of the solute on water activity. They (132) also found that solutes could reduce leakage of cellular components from cells that had undergone sublethal injury. Addition of 5% sodium chloride to a suspension of 50 g of ground beef and 150 ml of distilled water prevented leakage of UV-absorbing material from cells and reduced leakage of magnesium ions (132). Allwood and Russell (4) showed that addition of 34% sucrose prevented leakage of 260-nm-absorbing material from cells during heating of staphylococci. The biochemical basis for the interaction between solute concentration and temperature in preventing cellular injury has not yet been determined.

**Food additives**

Palumbo and Smith (101) incorporated food additives into ground beef agar and studied their effect on repair of cellular injury in S. aureus. Nitrite, ascorbate, lactic acid, and water activity-lowering substances (glycerol, NaCl, KCl, and sucrose) added to ground beef agar permitted repair of sublethally injured S. aureus. Repair occurred most rapidly at 35°C. Palumbo and Smith (105) also found that repair of cellular injury could occur in meat-foods with various food additives present, at temperatures from 20 to 45°C.

Presence of food additives can affect growth and enterotoxin production by S. aureus. Ayaz et al. (8) observed inhibition of growth of S. aureus in the presence of butylated hydroxyanisole (BHA) and/or butylated hydroxytoluene (BHT). Increasing levels of BHT and BHA from zero to 150 ppm and 200 ppm, respectively, resulted in increasing the degree of inhibition (8,102). Complete inhibition of S. aureus occurred in brain heart infusion (BHI) broth with 1.12 mM of BHA or 0.70 mM of BHT as well as in BHI broth with a combination of 0.25 μM of both BHA and BHT (8). Pierson et al. (107) found 200 ppm of BHA and 500 ppm of propylparaben to be bactericidal to S. aureus. No viable cells were detected after 6 h upon addition of 1500 ppm of each compound. Ayaz et al. (8) studied the effect of preservatives on enterotoxin production. They observed that presence of 0.84 μM or more of BHA or 0.47 μM or more of BHT inhibited growth of S. aureus so enterotoxin was not produced (8).

In a model system with water activity and pH values adjusted to resemble process cheese, presence of food additives affected staphylococcal growth to various degrees (102). Potassium sorbate at 0.25% had no appreciable effect on growth, whereas BHA (100 ppm) and BHT (200 ppm) inhibited growth to some extent. Neither BHA nor BHT prevented growth when S. aureus was inoculated into cheese spread (102). Potassium sorbate in combination with BHA, BHT, and propylgallate (PG) exerted greater bactericidal and bacteriostatic effects on S. aureus at pH 5 than at pH 7 (102). Strain variation also played a major role in determining which combinations of potassium sorbate and antioxidants were bacteriostatic and bactericidal.

Degre and Sylvestre (38) investigated the mode of action of BHA against S. aureus strain Wood 46. The antioxidant was absorbed and was determined to be bactericidal to growing staphylococcal cells, but had no effect on non-growing suspensions at 50 μg of BHA/ml. Concentrations over 100 μg/ml were lethal to both growing and non-growing cultures, and were related to leakage of nucleotides from within the cells (38).

Pierson et al. (106) observed that 0.13 and 0.26% potassium sorbate, with no nitrite, were most effective in suppressing staphylococcal growth in bacon through 14 d of storage at 27°C. When stored at 13°C, bacon containing nitrite and potassium sorbate contained fewer viable staphylococci (less than 1000/g) after 7 d than did bacon containing potassium sorbate alone (1900/g). A study by Elliott et al. (44) found that antimicrobial activity was enhanced with a synergistic relationship between potassium sorbate and modified atmosphere. Antimicrobial activity was optimal at pH 5.5 for a system consisting of 1.5% sorbate/100% carbon dioxide (44).

**Cellular repair**

Collins-Thompson et al. (33) demonstrated that when an enterotoxigenic strain of S. aureus was heat-injured and then transferred to artificial media, the cells underwent repair and subsequently grew and produced enterotoxin B. A 5- to 6-h lag phase occurred before growth, and a delay in enterotoxin production also was noted when compared to uninjured cells. The lag phase could correspond to the time necessary for cellular repair. Once growth was re-initiated, enterotoxin production was similar to that observed for uninjured cells (33).

Results of Collins-Thompson et al. (33), Reichert and Fung (115), and Fung and Vandenbosch (51) suggest that S. aureus cells injured by food processing procedures could potentially repair themselves and initiate toxin production in foods. Similarly, Reichert and Fung (115) found that reactivation of denatured enterotoxins can occur under suitable storage conditions, which would lead to recovery of activity of the proteins. If a food is underprocessed, with sublethal rather than lethal injury to S. aureus, and is stored at an elevated temperature which allows growth and enterotoxin production by the pathogen or reactivation of enterotoxin, if present, then the likelihood exists that an outbreak of food poisoning will occur.
CONCLUSION

One million enterotoxigenic staphylococci/ml or g may be necessary to cause staphylococcal food poisoning from consuming relatively fresh foods that supported growth of the pathogen (92). In processed foods that have undergone thermal treatment, absence of viable staphylococci may not indicate a safe food product because preformed enterotoxin is heat resistant (115). Studies have shown that presence of less than 1 ug of enterotoxin A/20 g of cheese can produce symptoms of food poisoning in human volunteers, although symptoms of food poisoning in human volunteers, although

The importance of control of growth of enterotoxigenic strains of S. aureus cannot be overemphasized. In addition, presence of staphylococcal enterotoxins in foods that have contained large populations of viable cells of S. aureus must be considered. Improved quality and safety of foods will result from a better understanding of the conditions necessary to control growth and survival of pathogenic bacteria, including S. aureus, as well as control of the production of toxic compounds by the bacteria and development of methods to determine the presence of the toxic compounds in foods.

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