Staphylococcus aureus and Enterotoxin A in Cream and Butter

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Abstract

Cream was a satisfactory growth medium for *Staphylococcus aureus* at 37 C but supported only minimal growth at 23 C for 16 hr. After 24 hr at 37 C, > 1 μg enterotoxin A per 100 g cream was detected. When butter initially contained 10^5 to 10^6 staphylococci per gram, some growth occurred with 1% or less salt at 23 C. Growth was not evident in butter with 1.5% salt but staphylococci remained stable at 23 C for 14 days. However, this environment was detrimental to the organism during incubation at 10 C for 60 days. Whipping of butter had little effect on staphylococcal behavior over that in unwhipped butter stored at 23 C. At 10 C, the number of viable staphylococci in whipped butter remained nearly constant or increased slightly during 60 days of storage. In unwhipped butter, viable staphylococci markedly declined so that up to a 400-fold difference in numbers existed between the two products after 60 days. When cream inoculated with S. *aureus* was incubated and churned, enterotoxin A appeared in the butter. The ratio of toxin in buttermilk: butter was approximately 8 to 16:1.

Introduction

Dairy products, on occasion, become contaminated with *Staphylococcus aureus*, and thus can cause food intoxications (5, 6, 7, 8). Until recently, cream and butter, particularly the latter, were not considered as likely vehicles for staphylococcal enterotoxin. The Foodborne Surveillance Unit of the National Center for Disease Control (Atlanta, Georgia) reported no cases of staphylococcal food poisoning associated with butter until 1970 when 24 people became ill after consuming whipped butter (prepared by blending 4 oz milk with 6 lb butter) (9). Staphylococci were isolated from the butter but not the milk, and enterotoxin A was detected in the butter. Furthermore, a major company voluntarily recalled 75,000 lb of butter which contained up to 100 million staphylococci per gram and was suspected of causing illness in several consumers (10).

Cream used to manufacture butter usually contains 28 to 35% milk fat whereas butter, by federal law, must have a minimum of 80% fat. Hunziker (3) listed analyses from many butter samples: 77 to 84% fat (avg 80.85), 13 to 19% moisture (avg 15.88), 1 to 4% salt (avg 2.39), and 0.89% curd. Butter with 2.4% salt and 15.9% moisture has 15.1% brine (percent salt in available water) which is not conducive to growth of high-salt tolerant S. *aureus*.

This investigation was conducted to determine a) suitability of cream for staphylococcal growth and enterotoxin production, b) fate of S. *aureus* in stored butter, c) fate of S. *aureus* in salted butter, d) survival of staphylococci in whipped butter, and e) distribution of enterotoxin from cream between the aqueous and nonaqueous phases during buttermaking.

Materials and Methods

CULTURE

A culture of S. *aureus* strain 100 (commonly used for investigations since it produces only enterotoxin A) was obtained from K. F. Weiss (The Food Research Institute, University of Wisconsin). The organism was stocked on brain heart infusion (BHI) agar (Difco) slants and refrigerated. Before each experiment, the organism was transferred from a stock slant to BHI broth, incubated at 37 C for 24 hr, transferred to BHI broth a second time and used after 18 hr at 37 C. The 18-hr old cells were washed twice in cold (10 C) sodium phosphate buffer (pH 7.4), then an appropriate volume of the preparation or a dilution (in buffer) was used as inoculum.

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PREPARATION OF TEST MATERIALS

Pasteurized cream (36 to 40% fat) and butter [unsalted and regular (1.5% salt)] were obtained from the University of Wisconsin dairy plant.

Inoculation. Cream samples (300 g) were inoculated in a beaker. The cream was vigorously stirred with a glass rod, then distributed equally between two 8-oz prescription bottles.

Butter was divided into portions of proper weight and placed in beakers. The beakers were immersed in 45 C water until the butter was molten to facilitate uniform distribution of the inoculum. When required, salt was added at this stage (crystals were stirred into the butter). The butter then was inoculated and stirred vigorously with a glass rod. When butter was to be whipped, it was first cooled to 15 C; otherwise 11 g were distributed to paper cups (1 oz) before incubation. Use of these cups simplified bacteriological sampling and helped ensure uniformity. For toxin analyses 50 g of butter were distributed to 2-oz paper cups.

Whipping. Butter held at 15 C was whipped by high-speed mixing for 5 min with a domestic-type electric mixer. Whipped butter was distributed to paper cups.

Incubation. Cream samples were stored at 23 and 37 C, whereas butter was held at 10 and 23 C (butter could not be maintained in a solid state at higher temperatures). When stored at 23 C, the molten butter was first solidified in a refrigerator for 20 min.

BUTTERMAKING

When cream was held at 37 C, the indigenous flora (predominantly bacilli) often grew rapidly and coagulated the product. Therefore, cream for buttermaking was sterilized by tyndalization, i.e. steamed for 1 hr on each of 3 succeeding days. A 2,500-ml volume of cream was sterilized, cooled, inoculated with *S. aureus* (to contain approximately 10^6 organisms per gram), and incubated at 37 C for 24 hr. It was then cooled to 7 C and held overnight. Two liters were added to a 4-liter mixing bowl equipped with a water jacket. Ice water was circulated through the jacket to maintain the cream at 13 C. Cream was mixed at low speed with a simple beater connected to a Hobart N-50 mixer. After the cream "broke" (oil-in-water emulsion inverted to water-in-oil), buttermilk was removed. A 500-ml volume of cool (13 C) tap water was added, the mixer operated for a short time, then 500 ml additional cool water were added and the mixer again operated briefly. The wash water was removed and the butter was "worked" briefly by hand.

BACTERIOLOGICAL DETERMINATIONS

Two replicates (11.0 g) were held at 45 C for 15 min (to soften the butter). Softened butter was then added to sterile buffered water (35 to 40 C), appropriate dilutions were made, and pour plates prepared according to recommendations of Standard Methods for the Examination of Dairy Products (13). Plates were poured with mannitol salt agar (Difco) and incubated at 37 C for 48 hr. Mannitol-fermenting colonies of proper size and appearance were recorded as *S. aureus* and occasionally were confirmed as staphylococci by microscopic examination.

ENTEROTOXIN ANALYSES

Two replicates of butter (50 g) and cream (100 g) were selected for toxin analyses and submitted to the laboratory of M. S. Bergdoll (The Food Research Institute, University of Wisconsin). All samples were extracted and tested with the microslide gel diffusion methods outlined by Zehren and Zehren (14). In addition, samples from the buttermaking experiment were analyzed by the reverse passive hemagglutination technique of Silverman et al. (11).

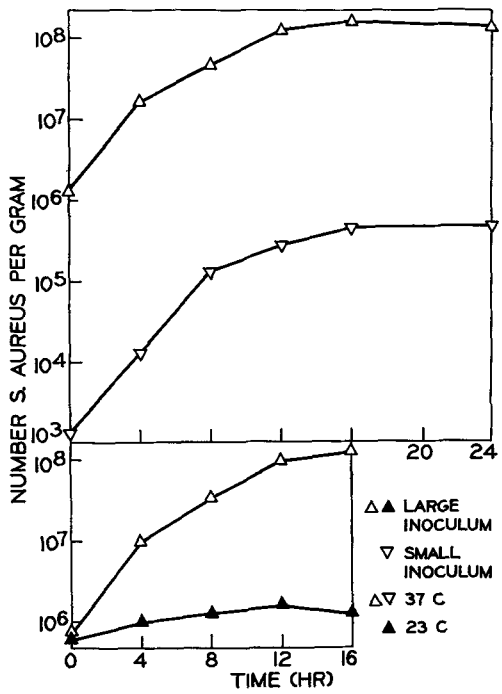

FIG. 1. Behavior of *Staphylococcus aureus* in cream held at 23 and 37 C for 16 to 24 hr.

Results

GROWTH OF *S. aureus* IN CREAM

Cream was a satisfactory growth medium for staphylococci at 37 C (Fig. 1). Numbers increased 10-fold within the first 4 hr, regardless of size of initial inoculum. When initial numbers were 10^6 per gram, they increased 100-fold within 16 hr, then entered a stationary phase. When the initial population was 10^3 per gram, numbers increased 350-fold within 16 hr, then were stationary. Cream inoculated to contain 10^6 staphylococci per gram and held at 23 C did not support appreciable growth during a 16-hr incubation.

BEHAVIOR OF *S. aureus* IN BUTTER

Survival in regular butter. Regular butter (1.5% salt) stored at 10 C was detrimental to staphylococci (Fig. 2). When a large inoculum (10^6/g) was used, cell numbers decreased 35-fold in 14 days and decreased an additional 40-fold between the 30th and 60th day of storage. Numbers of staphylococci in butter inoculated with 10^3/g, declined 100-fold, leaving a residual population which persisted throughout the remainder of the experiment. In contrast, initial numbers of staphylococci did not change appreciably in regular butter at 23 C for 14 days, regardless of inoculum used (Fig. 3).

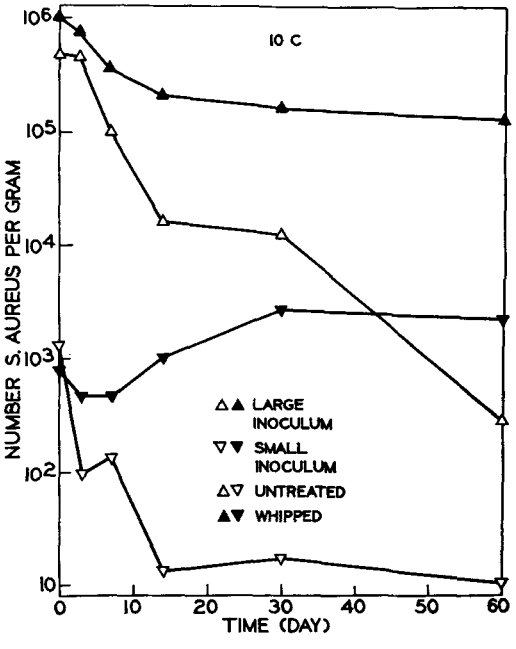

FIG. 2. Survival of *Staphylococcus aureus* in untreated and whipped butter stored at 10 C for 60 days.

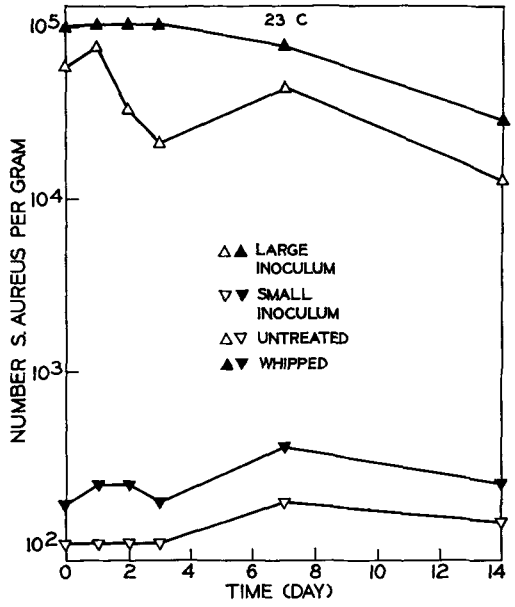

FIG. 3. Survival of *Staphylococcus aureus* in untreated and whipped butter stored at 23 C for 14 days.

Effect of whipping on survival in regular butter. Substantial differences in survival of *S. aureus* were observed between untreated and whipped regular butter (1.5% salt) at 10 C

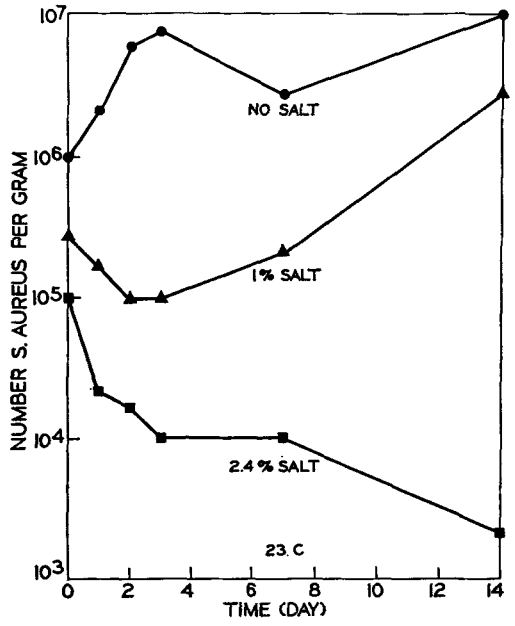

FIG. 4. Growth inhibition of *Staphylococcus aureus* in butter by salt during storage at 23 C.

(Fig. 2). After 60 days, survivors were 400-fold greater in whipped than in untreated butter regardless of inoculum used. On the other hand, survival did not differ measurably between regular and whipped butter at 23 C for 14 days (Fig. 3).

Inhibition of growth by salt. Figure 4 shows the behavior of *S. aureus* in butters of different salt contents, inoculated with 10^5 to 10^6 cells per gram and stored at 23 C. Numbers increased 10-fold in salt-free butter during 14 days but declined slightly in butter with 1% salt during the first 2 days, then gradually increased 50-fold between the 3rd and 14th day of storage. A 125-fold decrease in cells occurred gradually during 14 days in butter with 2.4% salt.

PRODUCTION OF ENTEROTOXIN A IN CREAM AND BUTTER

When pasteurized cream was inoculated with 10^6 *S. aureus* per gram and stored at 37 C for 24 hr, $>$ 1 μg enterotoxin A per 100 g cream was detected with microslide gel diffusion. When regular (1.5% salt) and regular whipped butters were similarly inoculated and stored at 23 C for 14 days, no toxin was detected on the microslides.

STAPHYLOCOCCI AND ENTEROTOXIN IN BUTTERMAKING

Table 1 lists the number of *S. aureus* and amounts of enterotoxin A detected during buttermaking. Sterilized cream with an initial 10^6 staphylococci per gram contained 6×10^7 organisms per gram after 24 hr at 37 C. Substantial enterotoxin A was detected in the cream. Buttermilk contained slightly more staphylococci than cream (perhaps by breaking up of clumps during churning). Toxin in buttermilk was approximately at the same concentration as in cream [which was expected because the toxin is water-soluble (6)]. Although the data indicate that buttermilk contained more enterotoxin than cream, the difference is probably not significant because of the variability encountered with serological tests such as reverse passive hemagglutination. Wash water contained 10-fold fewer organisms than buttermilk and less enterotoxin A. Staphylococci in butter were 100-fold fewer than in the buttermilk. Detectable toxin was recovered from butter and the approximate ratio of toxin in buttermilk to butter was 8 to 16:1.

TABLE 1. *Staphylococcus aureus* and enterotoxin A detected in samples collected during buttermaking.

Sample	*S. aureus* (no./g)	Enterotoxin A (μg per 100 g) Microslide method		RPHA method[a]
Cream	6×10^7	(+)	$\geq$1	2
Buttermilk	2×10^8	(+)	$>$1	4
Wash water	2×10^7	(+)	$\leq$1	0.5
Butter	7×10^6	(+)	$<$0.5	0.25-0.5

[a] Reverse-passive hemagglutination.

PH OF CREAM AND BUTTER DURING STORAGE

When pasteurized cream was inoculated to contain 10^6 *S. aureus* per gram and incubated at 37 C for 24 hr, the pH decreased from 6.5 to 5.1 to 5.3. The pH of butter, on the other hand, was stable regardless of incubation temperature or time (change in pH was $\leq$ 0.3 unit).

Discussion

BEHAVIOR OF STAPHYLOCOCCI IN CREAM

Results show that cream used to manufacture butter can support growth of *S. aureus* and production of enterotoxin A, if the cream is stored at elevated temperatures or held for extended periods at lower temperatures.

When initial staphylococci in cream were $<10^6$/g, growth was limited before numbers approached the maxima often encountered in other foods and artificial media. The extent of growth in cream may be restricted by metabolic activity of the staphylococcus, e.g. lipolysis. The lipase of *S. aureus* was reported by Vadehra and Harmon (12) to be very active on milk fat. Approximately 56% of the fat was hydrolyzed by coagulase-positive strains of *S. aureus* during 8 days at 30 C and .05% of one fatty acid thus released completely inhibited growth of staphylococci.

The pH of cream decreased and this may have been inhibitory to staphylococci. In our laboratory (4) we showed that staphylococcal growth in milk during 12 hr at 37 C was inhibited by gradually reducing pH to $<$5.1 with lactic acid.

Casman and Bennett (2) estimated staphylococci able to produce enterotoxin A should form 1 to 4 μg of toxin per 100 g of most foods. Up to approximately 12 μg enterotoxin A per 100 g was encountered by Zehren and Zehren (14) in highly toxic cheeses. Based on this information and our data, under certain conditions cream is satisfactory for production of enterotoxin.

BEHAVIOR OF STAPHYLOCOCCI IN BUTTER

Our results indicate that the fate of

staphylococci in butter can be affected by a) salt concentration, b) storage temperature, and c) whipping. Probability of staphylococcal growth is greater when butter contains $<1.5\%$ salt. Organisms can survive in butter containing $\geq 1.5\%$ salt, but survival is markedly less at low temperatures.

Survival of staphylococci in butter at 10 C was greatly enhanced by whipping. Whipped butter with 1.5% salt did not support growth of staphylococci at 23 C. However, adding milk to butter when it is whipped lowers salt and increases moisture, thereby increasing the probability of growth.

BEHAVIOR OF STAPHYLOCOCCI AND ENTEROTOXIN DURING BUTTERMAKING

If cream contains enterotoxin and is churned, most of the toxin will appear in the buttermilk but detectable amounts remain in the butter after washing.

Bergdoll (1) believes that <1 μg of enterotoxin A may evoke symptoms of staphylococcal food poisoning in humans. If this belief is correct and butter from toxic cream contained 0.5 μg enterotoxin A per 100 g, approximately 100 to 200 g of the butter would have to be consumed within a short time for enterotoxemia to occur. Since this quantity of butter is seldom consumed at one time, either butters involved in staphylococcal food poisoning contained >0.5 μg enterotoxin A per gram or the effective dose required for onset of enterotoxemia is <1 μg.

If adequate sanitary practices are followed during the manufacture of butter, the probability of staphylococcal food poisoning should be low. However, a practice in the dairy industry which might increase the risk is the manufacture of whey-cream butter which utilizes fat in whey recovered from cheesemaking. If staphylococci grow in milk during cheesemaking, it is reasonable to expect that butter from whey might be enterotoxic. Another potential public health hazard, if staphylococci grew in cream, is when buttermilk from buttermaking, is collected, dried, and marketed as an ingredient for other foods. Since most of the toxin in cream would be in the buttermilk, this product could be potentially more hazardous than the butter.

Acknowledgments

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