THE GROWTH OF STAPHYLOCOCCI IN CONDENSED SKIMMILK ¹

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SUMMARY

The following variables were studied from the standpoint of their effect on growth of staphylococci in condensed milk: (a) precondensing heat treatments, (b) milk solids concentrations, and (c) growth temperatures. Growth also was studied under conditions simulating vacuum-condensing operations.

Preheat treatment of skim milk at 165 or 185 ° F. for 30 min. before condensing did not affect the growth of two cultures studied. However, growth of one of these cultures was retarded somewhat when the preheat treatment was 150 ° F. for 30 min.; growth of the other culture was not retarded.

Cultures of staphylococci grew optimally, or nearly so, at temperatures ranging from 90 to 113 ° F. in condensed skimmilks having solids concentrations ranging from approximately 30 to 50%. Three of six cultures grew, although slowly, in 40% milk solids at 116 ° F. and one grew under similar conditions at 118 ° F.

Under conditions of simulated vacuum condensing, growth was less extensive than under normal atmospheric pressure. However, growth was sufficiently rapid to exclude the possibility that subatmospheric pressures, likely to prevail in a vacuum-condensing operation, would afford sufficient retardation of growth to be of any practical significance in controlling growth of staphylococci.

During recent years, outbreaks of staphylococcal food poisoning have implicated nonfat dry milk. In England, a series of eight outbreaks, all associated with foods prepared with dried milk, were reported by Anderson and Stone (2). In 1956, a series of 19 outbreaks of gastroenteritis among school children participating in the school lunch program in Puerto Rico was reported by Armijo et al. (3). Although bacteriological and toxicological tests for metallic poisons were negative, clinical and epidemiological evidence indicated that staphylococcal enterotoxin was the probable agent.

Various opinions have been expressed as to the probable sources of staphylococci and conditions for their growth in milk and condensed milk for drying. Anderson and Stone (2) and Hobbs (7) have reported that raw milk, heated milk, or condensed milk may show considerable staphylococcal growth and probable enterotoxin production. Hobbs (7), Hawley and Benjamin (5), Crossley and Campling (4), and Heineman (6) were of the opinion that extensive growth and consequent enterotoxin formation most likely would occur during the manufacturing process following preheating of the milk.

Heineman (6) has reported that pasteurized milk and condensed skim milk (29.02–42.77% solids) served as excellent media for the growth of Staphylococcus aureus. He pointed out that whether enterotoxin formation occurred could only be speculative, since no tests for enterotoxin were made. Crossley and Campling (4) reported that S. aureus always proliferated in condensed whole milk (38–40% solids) following inoculation of the product after the condensing

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process. When the milk was inoculated before condensing, they observed that the population of *S. aureus*, in most trials, was reduced during the vacuum-condensing process. However, the condensing process was relatively short, being 4 to 5 hr. Upon further incubation of this condensed milk at 37° C., the staphyloccocal count was further reduced. They offered no explanation for the failure of growth in the inoculated milk during and after condensing.

The present study was undertaken to determine (a) the effect of precondensing heat treatment of skimmilk on growth of *S. aureus* in condensed skimmilk; (b) the effect of various incubation temperatures and skimmilk solids concentrations on the growth of *S. aureus* in condensed skimmilk, and (c) the effect of conditions simulating those which may occur during vacuum condensing on the growth of *S. aureus*.

**MATERIALS AND GENERAL METHODS**

One of the cultures of *S. aureus* designated as 196E in the figures presented herein was enterotoxigenic and was obtained from the Robert A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati, Ohio. Other cultures of this species used in these studies were obtained from various sources; all were coagulase-positive but may or may not have been enterotoxigenic.

Milk used was heated to 110° F. and separated. Following separation, the skimmilk was immediately heated to 185–188° F., held for 30 min., and cooled to 40° F. In preliminary studies, the skimmilk was heated to 150, 165, and 185° F. for 30 min. to determine the effect of precondensing heat treatment on growth of staphyloccoci in the condensed product. In all experiments, the skimmilk was condensed to the desired milk solids concentration in a "Flash-Evaporator." To prevent foaming and loss of product during condensing, 0.01 ml. of sterile Antifoam B (Dow Corning) was added per liter of skimmilk. The vapor temperature in the evaporating flask during condensing ranged between 36 and 54° F.

In the various experiments, growth under conditions described was measured by the agar plate method (1), using Plate Count Agar (Difco), Staphyloccocus Medium No. 110 (Difco), and an incubation temperature of 37° C. for 48 hr. The two media revealed essentially the same information; consequently, only the results obtained through use of the Staphyloccocus Medium No. 110 are presented.

**EXPERIMENTAL METHODS AND RESULTS**

**Effect of precondensing heat treatment of skimmilk on growth of staphyloccoci in condensed skimmilk.** Condensed skimmilks of 40% solids prepared from skimmilks given three different precondensing heat treatments were placed in 8-oz. bottles containing 40–60 glass beads. The condensed milks were inoculated with the respective culture, using sufficient inoculum to obtain a population of approximately 10,000 per milliliter in the inoculated product. The bottles were then incubated at 99° F. At 3-hr. intervals, the bottles were removed from the incubator, shaken vigorously and plated.

*Laboratory Glass and Instrument Corporation, New York, N. Y.*
Figure 1 illustrates the growth of Cultures 196E and 1363 at 99°F. in condensed milks prepared from skimmilks subjected to precondensing heat treatments of 150, 165, and 185°F. for 30 min. The data indicate that rapidity of growth of Culture 196E in the three products was essentially the same. Culture 1363 responded similarly, except for a noticeable retardation of growth, which occurred in the condensed product prepared from skimmilk preheated to 150°F. for 30 min. The higher precondensing heat treatments more closely approximate those which are used commercially. Consequently, the skimmilks used in subsequent experiments were all heated to 185°F. for 30 min. before condensing.

Growth of staphylococci in condensed skimmilks of various milk solids concentrations and at various incubation temperatures. Condensed skimmilks containing 30, 41.5, and 47% milk solids were placed in 8-oz. bottles containing glass beads. The condensed products were inoculated as indicated in the previous experiment. The bottles were incubated at 90, 99, and 108°F. Platings were made at 3-hr. intervals.

In Figure 2, the growth curves for Culture 196E at the three temperatures indicated above and in condensed skimmilk containing 47% milk solids are shown. Essentially the same data were obtained for this culture when grown in condensed skimmilk containing 30 and 41.5% milk solids and, therefore, are not presented. The data represented by these curves show that growth was rapid at the three temperatures used and they indicate that the optimum growth temperature was at least 108°F. The data further show that the concentration of milk solids up to 47% had little effect on the growth of this culture.

A similar study was made using another strain of *S. aureus*. Data obtained are not presented, because they were essentially the same as those obtained for Culture 196E, except that growth at 90°F. was somewhat slower than that for Culture 196E at this temperature. As in Culture 196E, the concentration of milk solids had little effect on growth.

In additional experiments, condensed milk was standardized at 40% milk solids.

Figure 3 shows the results obtained with Culture 196E when incubated in water baths at 113, 116, 118, and 120 ± 0.5°F. Growth was rapid at 113°F. At 116°F., death of many cells occurred after 3 hr., but an initiation of limited growth occurred after 9 hr. and continued until 18 hr. Growth was completely inhibited at 118 and 120°F.

Following the studies with Culture 196E, growth of five additional strains of *S. aureus* in condensed milk was studied at 90, 99, 113, 116, and 118°F. Two of these five cultures also were grown at 108°F. A portion of the data obtained using these cultures as well as Culture 196E (indicated as No. 2) is presented in Figure 4. A comparison of the populations reached in 12 hr. at each of the six temperatures is shown. Three cultures failed to grow at 116°F., while three, including Culture 196E, showed limited growth. One of the five cultures grew at 118°F.; all others failed to grow.

Growth of staphylococci in condensed milk during the vacuum-condensing process. In these experiments, the growth of Culture 196E in condensed milk
FIG. 1. Effect of precondensing heat treatments on the growth of Cultures 196E and 1363 at 99°F in condensed skimmilk of 40% solids.

FIG. 2. Growth curves for Culture 196E at various temperatures in condensed skimmilk of 47% solids.
(40% milk solids) during the condensing procedure was studied. In the various experiments, the desired temperature of the condensed skim milk during continuous operation of the evaporator was maintained by regulating the absolute pressure. A preliminary study established the relationships shown in Table 1.

The heating bath was maintained at a temperature only slightly higher than the desired boiling temperature of the condensed skim milk in the evaporating flask. Therefore, the rate of evaporation was kept to a minimum. Sterile distilled water was continuously added to the condensed skim milk to further maintain a uniform concentration throughout the growth period.

After inoculation with Culture 196E, 500 ml. of the condensed skim milk (40% solids) were placed in the evaporator flask and the apparatus was put into operation. An additional 500 ml. were placed under normal incubation. At 3-hr. intervals, samples were removed and plated.

Figure 5 illustrates the growth at 108°F. which occurred under the condi-

TABLE 1

<table>
<thead>
<tr>
<th>Boiling temperature of condensed skim milk (°F.)</th>
<th>Temperature of heating bath (°F.)</th>
<th>Absolute pressure (cm. Hg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>100–102</td>
<td>5.0</td>
</tr>
<tr>
<td>108</td>
<td>109–111</td>
<td>6.5</td>
</tr>
<tr>
<td>113</td>
<td>114–116</td>
<td>7.5–7.8</td>
</tr>
</tbody>
</table>

* Thermocouple measurements.

* Manometer readings.
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Fig. 4. Extent of growth of staphylococci in condensed skim milk (40% solids) after 12 hr. of incubation at various temperatures.

Fig. 5. Comparison of growth of Culture 196E at 108° F. in condensed skim milk of 40% solids under conditions simulating vacuum-pan operation and under conditions of quiescent incubation at normal atmospheric pressure.
tions simulating vacuum-pan operation and under conditions of quiescent incubation at normal atmospheric pressure. Rapid growth occurred under both conditions; however, under reduced pressure, growth lagged in the beginning and the final population was lower than that reached at normal atmospheric pressure. Reasons for these observed differences are yet to be determined. Growth curves also were obtained at 99 and 113°F. under the same conditions. Results were essentially the same.

DISCUSSION

Certain samples of condensed skim milk in which S. aureus had grown to various population levels have shown some evidence of toxicity by a modified kitten test (8); however, proof of the presence of enterotoxin by this method is not unequivocal. The results obtained in the studies herein reported should not be interpreted as implying that enterotoxin was formed in the various samples. On the other hand, it should be recognized that growth of enterotoxin producing staphylococci does admit of the possibility of enterotoxin production.

During the manufacture of dried milks, the fluid products involved may vary in milk solids concentration from that of normal whole milk and skim milk to approximately 50%. The temperatures to which these fluid products may be subjected may range from approximately 40 to 200°F.; temperatures intermediate within this range commonly occur. For example, it is common for condensed products in the last effect chamber of the vacuum condensing apparatus to be at temperatures ranging from 90 to 113°F. These temperatures are optimum, or nearly so, for the growth of staphylococci. Furthermore, milk solids concentrations or subatmospheric pressures likely to prevail during processing operations do not seem to retard appreciably the growth of staphylococci. Therefore, if enterotoxigenic staphylococci would be present, there may be an opportunity for growth not only within the vacuum pan system itself but also in lines and surge tanks, where condensed milk may be held prior to the final heat treatment or drying. This could result in the development of high populations of staphylococci and potentially, at least, in the formation of enterotoxin. Because of its heat stability and resistance to drying, enterotoxin once formed would, in all probability, be carried over into the dried milk.

Every precaution, therefore, should be taken to eliminate plant practices which might permit: (a) underpasteurized milk reaching or leaving the condensing apparatus, (b) the holding of condensed milk for any extended period at temperatures within the growth range of staphylococci, and (c) direct contamination of condensed milk with viable staphylococci.

REFERENCES


(8) Unpublished data obtained by the authors in cooperation with K. H. Lewis and J. E. Campbell of the R. A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati, Ohio.