STAPHYLOCOCCI IN COTTAGE CHEESE[1]

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Considerable attention has been directed to staphylococci in food. Cases of staphylococcal food poisoning associated with spray-dried milk *(1, 2)* and cheese *(6, 8)* have been reported in recent years. In a survey of a wide variety of cheese, excluding cottage, obtained at the consumer level, 70.4% contained *Staphylococcus aureus (11)*. Walker, et al. *(14)* have reported results of a study involving the survival of staphylococci in Colby cheese. Jezeski, et al. *(7)* made Cheddar and Colby cheese with milk containing 25,000 to 100,000 *Staphylococcus aureus*, strain 196E per ml. Both showed maximum counts of *Staphylococcus aureus* within 24 hr after setting.

Mattick, et al. *(10)* in a study of multiplication of *Staphylococcus aureus* in 4 variations of Cheddar cheeses of slightly different pH, found that *Staphylococcus aureus* added to the milk increased almost 8-20 fold after stirring of curd. The number dropped during the 1st 7-10 weeks of storage and the organisms were completely eliminated in 14-22 weeks of storage. Roughley and McLeod *(12)* inoculated pasteurized milk with several strains of *Staphylococcus aureus* and manufactured it into Cheddar cheese. They found that the number of *Staphylococcus aureus* increased during the process up to hooping. During curing, counts generally showed a marked decline and no *Staphylococcus aureus* could be isolated from any samples after 10 days. Lyons and Mallmann *(9)* noticed that survival of *Staphylococcus aureus* in cottage cheese was linked with pH and the organisms could survive up to 192 hr at pH 4.6. Stadhouders *(13)* observed seasonal variation in starter activity which he associated with the seasonal variation of peroxidase activity of milk. He found that increased peroxidase activity of milk inhibited the cultures.

If pasteurized milk containing staphylococci or low heat, skim milk powder containing staphylococci were subsequently made into cottage cheese, incubation temperature and time could be conducive to considerable growth with accompanying enterotoxin development during manufacture. The high acidity encountered during manufacture of cottage cheese may inhibit staphylococcal growth. However, Foltz, et al. *(4)* isolated staphylococci from cultured butter milk. The isolation of staphylococci from cultured butter milk points out that some strains of the organism apparently tolerate acidity levels equal to or greater than those encountered in the manufacture of cottage cheese.

Cottage cheese with its long setting period likely provides environmental conditions suitable for the growth of enterotoxigenic staphylococci. In attempting to determine the possible role that the organisms play in the manufacture of cottage cheese, answers were sought to the following questions:

1. What is the incidence of staphylococcal contamination in cottage cheese at the commercial level?
2. Is there a seasonal trend in the incidence of staphylococcal contamination in commercial cottage cheese?
3. Do staphylococci increase in number during the setting period for long-time and short-time methods of cottage cheese manufacture?
4. Do staphylococci survive the customary minimum cooking period (120F for 30 min) under the acidic condition prevailing during the manufacture of cottage cheese?
5. If staphylococci survive the cooking process, do they multiply in the curd during subsequent storage?

Materials and Methods

Samples of cottage cheese were obtained from retail outlets during winter and summer months. All samples were held in the original container under refrigeration at 35F from the time of collection until analysis was started. Total elapsed time of holding never exceed 24 hr.

Bacteriological Examination

Both Tellurite-Glycine agar (TG) as recommended by Zebovitz, et al. *(16)* and Staphylococcus Medium No. 110 (S-110) (17) were used for initial isolation of staphylococci. In addition, an enrichment

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procedure was used in which 0.1 g of cheese (1.0 ml of a 1-10 water suspension) was transferred to 10.0 ml of enrichment broth consisting of Staphylococcus Medium No. 110 minus the gelatin and agar.

Packages were opened and 11 g of cheese were weighed into 99 g of sterile water in pint jars, Oster Blender heads were used to prepare a 1-10 suspension of each sample.

A 1.0-ml volume of each cheese-water sample (0.1 g cheese) was aseptically transferred to plates of TG and S-110 media. The surface plating technique of Snyder *(18)* was used to spread the sample over the plates. At the same time 1.0 ml of the cheese-water suspension was transferred to 10.0 ml of S-110 Enrichment Broth.

The TG and S-110 plates were incubated at 37C for 24 and 48 hr, respectively, at which time suspected colonies were isolated to proteose-peptone agar slants for identification.

The S-110 enrichment broth tubes were incubated at 37C for 24 hr after which time aliquots were transferred to and incubated on GT and S-110 plates as previously described. These plates were designated GTE and 110E. Suspected colonies were isolated for study and identification as were isolated from the GT and S-110 plates.

Proof of isolation

Isolates from the TG, S-110, TGE and S-110E plates were first purified by sub-reisolation on proteose-peptone agar plates by streak-plate isolation. Reisolation was made to proteose-peptone agar slants. Purified cultures were subjected to Gram's stain. Morphologically typical isolates were retained and subjected to the following examination:

1. *Anaerobic growth*: Melted and cooled tubes of glucose yeast extract agar were inoculated by needle-stab and immediately cooled. Growth in the bottom of the tube was necessary to retain the culture as a member of the genus *Staphylococcus.*

2. *Mannitol fermentation*: Sugar-free peptone agar base in deep tubes to which 1% mannitol was added was inoculated similarly to the method for determining anaerobic growth. Anaerobic fermentation of mannitol was considered a positive finding in describing an organism as *Staph. aureus.*

3. *Coagulase test*: Citrated plasma (Difco) was used to carry out the tube coagulase test using 0.5 ml plasma, 1 loop of culture from a 24-hr slant and a maximum incubation time of 4 hr at 37C.

4. *Fraziers gelatin Agar,* in plates, was used to determine gelatin hydrolysis. Hydrolysis was determined by flooding the plates with Frazier's developer after incubation.

5. *Action on blood*: Proteose-peptone agar base to which 5% defibrinated sheep blood had been added was the medium employed. Incubation was for 48 hr at 37C. Hemolysis was described as typical of beta or a combination of beta and alpha.

6. *Pigmentation* was determined from growth on Frazier's gelatin agar before testing for gelatin hydrolysis.

7. *Bacteriophage typing*: All *Staph. aureus* cultures were sent to the Regional Typing Center, Public Health Laboratories, Kansas State Board of Health, Topeka for typing. This laboratory follows the recommendations for bacteriophage typing as established by the National Reference Laboratory, Laboratory Branch, Communicable Disease Center, U. S. Public Health Service, Chamblee, Georgia.

Source of known culture

A known culture (196E) of a potentially pathogenic *Staphylococcus aureus* was obtained from Dr. G. M. Dack of the Food Research Institute, University of Chicago. The cell suspension of the organisms was prepared by washing a 24-hr proteose-peptone agar plate with 15 ml of sterile saline solution, filtering and adjusting to 50% transmittance at 500 mμ as measured on a Bausch and Lomb Spectronic 20.

Inoculation of milk

Small batches of cottage cheese were made by both long and short-set procedures described by Hales *(5).* When starter was added, 0.5 ml of cell suspension was added to 5 quarts of skim milk to give a "light" staphylococcal contamination (approximately 10,000 per ml). Twenty-five ml of the cell suspension in 5 quarts of skim milk gave a "heavy" staphylococcal contamination (approximately 500,000 per ml). Samples of the milk or curd were collected at the beginning of setting, at the end of cooking, and at the end of the 3rd rinse and were plated on S-110 and TG agar as described.

The washed curd was divided into three portions; one portion was frozen, a second maintained at 40-50F, and the 3rd held at 70F. The curd was surface plated on S-110 and TG agar at 48-hr intervals for six days.

RESULTS AND DISCUSSION

The results of the analyses of 24 samples of commercial cottage cheese obtained during winter months and 42 samples obtained during summer months are presented in Table 1. Incidence of samples containing staphylococci, *Staphylococcus aureus* and *Staphylococcus epidermidis* was higher in summer than in winter. A chi-square test indicated that the difference in incidence of *Staphylococcus epidermidis* and *Staphylococcus aureus* between summer

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TABLE 1. THE INCIDENCE OF STAPHYLOCOCCI IN COTTAGE CHEESE OBTAINED IN CONSUMER MARKETING CHANNELS

Season	No. of samples examined	Samples containing staphylococci		Samples containing Staph. epid[a]		Samples containing Staph. aureus[b]		Samples containing both Staph. epid & Staph. aureus		Samples containing phage typeable Staph. aureus	
		no.	%	no.	%	no.	%	no.	%	no.	%
Winter	24	11	45	11	45	1	4	1	4	1	4
Summer	42	24	57	24	57	8	19	8	19	3	7
	66	35	53	35	53	9	14	9	14	4	6

[a]*Staphylococcus epidermidis* (Mannitol negative; Coagulase negative; anaerobic growth positive)
[b]Staphylococcus aureus (Mannitol positive; Coagulase positive; Anaerobic growth positive)
Note: Three of the 8 summer samples contained *Staph. aureus* which were phage typeable; one winter sample contained *Staph. aureus* which was phage typeable.

and winter samples was not significant even at .10 level. The exact test by 2 x 2 contingency table under the hypothesis of equality of proportions also showed nonsignificant differences between summer and winter samples containing staphylococci. Thus, no significant seasonal trend of staphylococcal contamination in cottage cheese was found.

Data in Table 2 show counts of S-110 and TG agar obtained during the manufacture of 9 batches of short-set and long-set cheese. Counts when the curd was cut in the short-set method, were less than the initial staphylococcal count on both S-110 and TG agar. The staphylococci were almost entirely confined to the curd portion. During the cooking operation, the staphylococcal population decreased nearly 100%. No evidence of multiplication of the organisms was found during the storage at different temperatures for 6 days.

TABLE 2. NUMBER OF STAPHYLOCOCCI PRESENT DURING VARIOUS STEPS OF COTTAGE CHEESE MANUFACTURE AND STORAGE

Trial no.	Mfg. method	Plating media used	Initial staph. count	Staph count at cutting curd & whey	Staph count at cutting whey	Staph count at cutting curd	Staph count at end of cooking whey	Staph count at end of cooking curd	Staphylococcal count per g after 48 hr at 32	48 hr at 40-50 (°F)	48 hr at 70	96 hr at 32	96 hr at 40-50 (°F)	96 hr at 70	144 hr at 32	144 hr at 40-50 (°F)	144 hr at 70
			(per ml)	(per ml)	(per ml)	(per g)	(per ml)	(per g)									
1	Short	S-110	15,450	7,650	0	66,550	0	40	10	0	0	10	0	0	0	0	0
	set	TG	13,450	9,700	0	144,500	0	0	0	0	0	0	0	0	0	0	0
2	Short-	S-110	460,000	93,800	0	75,400	0	30	100	55	1,550	0	0	10	0	0	0
	set	TG	810,000	111,900	0	59,900	0	0	0	0	0	0	0	10	0	0	0
3	Short-	S-110	1,225,000	270,250	1,050	260,000	20	10	0	0	0	0	0	0	10	0	0
	set	TG	2,664,000	162,450	1,000	137,000	0	0	0	0	0	0	0	0	0	0	0
4	Short-	S-110	400,300	198,500	0	14,000	0	0	55	0	0	200	305	290	0	0	0
	set	TG	460,000	304,000	0	288,000	0	0	0	0	0	0	0	135	0	0	0
5	Short-	S-110	16,950	10,500	0	11,495	0	0	220	0	0	10	0	0	0	0	0
	set	TG	21,100	3,100	0	8,000	0	10	0	0	0	0	0	0	0	0	0
6	Long-	S-110	17,250	4,600	0	3,600	0	0	10	10	10	0	0	0	0	0	0
	set	TG	20,050	1,700	100	2,650	0	0	0	0	0	0	0	0	0	0	0
7	Long-	S-110	18,650	10,400	0	2,440	0	0	0	0	0	105	0	405	10,500	4,590	Cont
	set	TG	6,600	80,350	2,100	1,840	0	0	0	0	0	0	0	0	305	0	Cont
8	Long-	S-110	4,900	8,000	200	1,285	0	0	100	0	125	0	0	900	0	0	0
	set	TG	8,250	8,000	300	1,110	310	0	0	0	40	0	10	30	10	0	0
9	Long-	S-110	247,000	2,120,000	5,550	56,000	0	0	110	65	0	155	60	0	0	0	0
	set	TG	206,500	555,000	4,700	220	0	0	0	10	60	0	0	0	0	0	0

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The staphylococcal count at curd cutting time, with long-set cheese, generally increased except in the 6th trial. The total population increased twelve fold in 7th trial on TG agar and eight times in 9th trial on S-110. Comparing counts in curd and in whey showed the staphylococcal population greater in the curd than in the whey. Unlike in the short-set method, significant numbers of organisms were present in the whey. Most were in the curd and a few in the whey. In two cases (7th and 9th trials on TG agar) organisms were more numerous in the whey than in the curd. Organisms present in curd and whey were fewer than at cutting. The cooking operation almost completely destroyed the organisms. No clear evidence of multiplication of staphylococci during storage was noticed. Staphylococci present in some cases may have resulted from sampling error or contamination.

Particularly noteworthy was the wide variation in counts on the two media. The inhibitory effect of S-110 on heat-shocked staphylococci has been reported earlier *(3)* but whether TG agar also inhibits heat or acid-shocked organisms is not definitely known.

No correlation between the pH and the counts on either medium or between pH and percentage of coagulase-positive organisms could be determined.

Wilson, et al. *(15)* have suggested that a minimum population of 500,000 coagulase-positive staphylococci per g is required to produce sufficient toxin to cause food poisoning. In the work reported here only one lot of cheese (Trial 9) showed multiplication exceeding 500,000 per g. The initial count in this batch was more than 200,000 per ml. Such a heavy contamination is not likely to be encountered during the normal processing of cottage cheese. However the organisms are capable of multiplying in the long-set method.

The organisms might multiply to produce enough enterotoxin even from light contamination. Subsequent population reduction during cooking and storage could leave enough organisms in the cheese to make it unsafe.

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