Staphylococci in Competition[1]

I. Growth of Naturally Occurring Mixed Populations in Precooked Frozen Foods during Defrost

A. C. Peterson, J. J. Black, and M. F. Gunderson

Department of Bacteriological Research, Campbell Soup Company, Camden, New Jersey

Received for publication July 11, 1961

Abstract

Peterson, A. C. (Campbell Soup Company, Camden, N. J.), J. J. Black, and M. F. Gunderson. Staphylococci in competition. I. Growth of naturally occurring mixed populations in precooked frozen foods during defrost. Appl. Microbiol. **10**:16–22. 1962.—In chicken pot pies, it was not possible to promote the growth of appreciable numbers of staphylococci under any condition of defrost. The pies spoiled under the same conditions, however. In macaroni and cheese dinners, tremendous numbers of saprophytic bacteria developed, but only after extended incubation at room temperature in which even spoilage was carried to an extreme. Under these conditions, staphylococci also multiplied vigorously. At the extreme temperature of 37 C, rank spoilage of such an advanced state as to render the product completely inedible was reached in less than 24 hr. Staphylococci grew very well under these extreme conditions.

Little attention has been directed to how well staphylococci grow in competition with other microorganisms, despite the frequent publications concerned with the many facets of the activities of this genus. Elek's (1959) comprehensive monograph and Dack's (1949) authoritative book on food poisoning say nothing of this ecological aspect. With the development of pure culture techniques, there has been an intense preoccupation with single strains of bacteria and, only very recently, a renewed interest in bacterial ecology and in microbial behavior and interactions in natural situations.

Brian (1957) cited the importance of microbial antagonisms in determining microbial populations in the mixed flora of the soil. Gibson (1957) noted the pronounced microbial population changes when the pH of the rumen of sheep was abruptly lowered by indigestion induced by a sudden change in diet from hay to grain. The protozoa were killed, and there was a very large increase in the proportion of gram-positive organisms, especially *Streptococcus bovis*.

[1] Presented in part to the 61st Annual Meeting of the American Society for Microbiology, Chicago, Ill., April 23–27, 1961.

Mickelson and Flippin (1960) reported use of an *Escherichia coli* strain to eliminate *Salmonellae* from egg white. Rahn (1945) in his monograph mentioned the inhibition of *Staphylococcus* and typhoid organisms by *Pseudomonas aeruginosa*. The inference can be drawn from the papers of Dearing and Heilman (1953) and Dearing and Needham (1960), who reported acute toxemias due to the growth of large numbers of staphylococci in the gut as a result of receiving an antibiotic capable of altering the intestinal bacteria, that massive growth of *Staphylococcus aureus* in mixed populations is only possible when growth of the other bacteria is suppressed.

Although no knowledge of the exact number of staphylococci required to be present in a food product to cause food poisoning exists, some inferences can be drawn from the literature. Elek (1959) wrote that abundant growth of *Staphylococcus* was required for food to become poisonous. Frazier (1958) indicated that necessary levels of enterotoxin were produced only after a considerable growth of staphylococci to a population level of several millions per gram. Tanner and Tanner (1953) in discussing food poisoning mentions staphylococcal populations of 600 million and 1 billion organisms per g in an experiment with inoculated ham and bread, 18 to 36 million per g in goat's cheese, 5 million per g in ice cream, 93 million per g in a custard pastry, and 25 to 30 million in cakes. Dack (1949) has said that foods involved in staphyloccal food poisoning usually contain hundreds of millions of staphylococci per g and verified this by experiments with inoculated foods. Miller (1958) observed that, with the exception of predation, grazing, and parasitism, one individual affected another through the environment and not directly from individual to individual. Thus individual microbial species do not exist or grow in mixed cultures in a food product, without affecting or being affected by the other bacterial species present, presumably through modification of the environment. The purpose of this investigation was to determine how well staphylococci grow in competition with the normal flora in precooked frozen foods and to determine if it was possible to so optimize conditions to favor the growth

of staphylococci sufficiently to constitute a food poisoning hazard.

Materials and Methods

A local brand of commercial frozen chicken pies was obtained in specially selected case lots. Pies in case lots frequently represent consecutive pies in production and thus are usually from the same batches of ingredients. Variations from one pie to another were presumably minimized in this manner. The particular cases were selected because they had total counts somewhat higher than those usually encountered among most commercial precooked frozen foods. They also contained staphylococci which gave typical colonies on mannitol salt agar and on Tellurite glycine agar. Thus, easily usable techniques for following the fate of this portion of the microbial population were available.

Using aseptic technique, 11-g samples were removed from the pie, added to a sterile 99-ml water blank containing a teaspoonful of glass beads and shaken on a mechanical shaker for 3 min to break up the solids. Duplicate plates were made of appropriate dilutions and duplicate series of plates of Tryptone-glucose-meat extract (TGE) agar (BBL)[2] were incubated at 37 C for 48 hr, or 20 C for 5 days. Appropriate note has been made in the tables of the incubation temperature used. One of the series of TGE plates was incubated at 5 C for 21 days for the psychrophilic count.

Both Mannitol salt (MS) agar (Difco)[3] and Tellurite glycine (TG) agar (BBL) were used to enumerate staphylococci. Plates were incubated at 37 C for 24 to 36 hr for TG agar and 48 hr for MS agar. During a preliminary survey of frozen foods, colonies which gave "typical" staphylococcal reactions on MS agar and TG agar were found. Although these colonies gave typical reactions on differential media when first isolated, they immediately lost this characteristic and were or became coagulase negative on further subculturing. Attempts made as a part of this study to secure coagulase-positive staphylococci from either of these media were negative. Staphylococci with stable characteristics on selective media and particularly with ability to coagulate plasma were needed in a succeeding part of the study; none were available from the frozen products tested, in spite of the high staphylococcal counts. In experiments with known pathogenic staphylococci, better correlation was found between the total count and staphylococcal count on TG agar than between total count and staphylococcal count on MS agar. Some of these cultures also lost both the ability to give typical staphylococcal reactions on differential media and ability to coagulate plasma on storage in the frozen state. For some cultures, these characteristics were regenerable by passage on enriched, nonselective media; for others, it was not. Steward and Kelly (1959) reported significantly reduced or no bound coagulase activity in 16 known coagulase-positive strains of *S. aureus* on storage in the frozen state. Although there was a decrease in pigmentation, it was not always associated with loss of bound coagulase activity. These workers found that bound coagulase activity could be restored in some of the cultures by subculturing, but not in others. The need for appraisal of the effect of freezing and freezing storage on the indices of pathogenicity of staphylococci is manifest.

Coliforms were counted on Violet red bile (VRB) agar (Difco) after incubation at 37 C for 48 hr.

One group of pies was brought to an approximate internal temperature of 30 C from the frozen state by rapid defrosting for 24 min in a 218 C oven. The actual temperature of the pies was not ascertained, but was approximated from some previous studies made in this laboratory.[4] Lots of four pies each were sampled before and immediately after heating and at four different intervals during incubation at 37 C.

In the experiments with the chicken pies, a case was

[2] Baltimore Biological Laboratory, Inc., Baltimore, Md.

[3] Difco Laboratories, Inc., Detroit, Mich.

[4] Unpublished data of the Department of Bacteriological Research, Campbell Soup Company, Camden, N. J.

TABLE 1. *Bacteriological analysis of one brand of commercial frozen chicken pies, intact case lots sampled*

No. of samples	Sample	Bacteria per g of sample			Standard deviation	Coefficient of variation
		Range	Mean	Median		
						%
24	Total[a]	25,800–800,000	172,000	93,500	183,000	106
	Staphylococci[b]	840–39,000	8,140	4,000	9,700	121
20	Total	6,200–77,000	32,000	29,000	20,000	63
	Staphylococci	10–240	90	80	68	76
20	Total	1,800–5,700,000	1,100,000	270,000	1,880,000	171
	Staphylococci	10–330	110	75	128	99
Summary	Total	1,800–5,700,000	418,000	43,000	1,020,000	246
	Staphylococci	10–39,000	3,120	210	5,940	190

[a] Total count was carried out on Tryptone-glucose-meat extract (BBL) agar and incubated at 20 C for 5 days.

[b] The *Staphylococcus* count was carried out on Tellurite-glycine (BBL) agar and incubated at 37 C for 24 to 36 hr.

sampled immediately prior to defrosting to establish the initial population levels and subsequently divided so that groups of pies were incubated at several different temperatures. These were then sampled at arbitrarily selected times. In several instances, an entire case was incubated at a selected temperature and sampled at various time intervals.

A single case of commercially prepared frozen macaroni and cheese dinners was sampled in a manner similar to that previously described. The dinners were then incubated at various temperatures for arbitrary periods of time.

Results and Discussion

It was desirable to estimate the initial bacterial population without actually sampling a specific pie, because an individual pie could be used for one sampling only and not carried through an entire experiment with repeated sampling. An average value obtained by sampling 4 of the 24 pies in a case before any defrost treatment was considered to be representative of the remaining 20 pies in the case. Table 1 presents data on three cases of a particular brand of commercial frozen chicken pies. All, or a majority, of the pies in these cases were sampled to determine the statistical reliability of using an average value based on a sample comprised of four pies in an intact case lot as being representative of the initial bacterial condition of the entire case. The plates for the total count recorded in this table were incubated at 20 C for 5 days. At this temperature, presumably, both psychrophiles and mesophiles grew. The psychrophilic count (5 C) was somewhat less, but was in reasonable agreement with these figures. The psychrophile count has not been included in this table. The results indicate that within a given case, great variations in both total count and staphylococcal count were present. In view of the 100% variations encountered, a sample consisting of four pies could not be considered as reliably giving the average counts for the entire case. Variations within a given case lot were lower than those from case to case.

In subsequent experiments, each individual pie was

TABLE 2. *Bacteriological analysis of one brand of commercial frozen chicken pies after incubation at various temperatures*

Incubation temp	Time incubated	No. of samples	Avg number of bacteria per g of sample			Per cent staphylococci (TG)/total
			Total tryptone-glucose-meat extract agar[c] (20 C)	Staphylococci		
				Mannitol salt agar[d]	Tellurite glycine (TG) agar[e]	
5 C[a]	0 hr	4	106,000	29,200	18,500	18
	25	4	139,000	72,800	14,600	11
	49	4	120,000	24,300	13,500	11
20 C[a]	0 hr	4	89,000	18,000	10,000	11
	6	2	287,000	29,300	6,300	2
	24	2	83,500,000	1,650,000	45,000	0.060
	30	2	99,300,000	17,000,000	80,000	0.081
	48	2	985,000,000	7,000,000	1,250,000	0.13
5 C[b]	0 days	4	188,000	53,000	30,900	16
	2	5	228,000	42,200	30,800	14
	5	5	2,350,000	415,000	44,100	1.9
	10	5	166,000,000	186,000	44,500	0.027
	16	5	978,000,000	33,600	5,400	0.0006
20 C[b]	0 days	4	390,000	106,000	44,000	11
	1	5	20,100,000	2,430,000	942,000	4.5
	2	5	228,000,000	8,260,000	3,070,000	1.3
	3	5	251,000,000	3,930,000	725,000	0.29
	4	5	199,000,000	2,160,000	532,000	0.27
37 C[b]	0 hr	4	62,000	3,280	538	0.87
	12	5	384,000,000	31,500	123,000	0.032
	24	5	399,000,000	460,000	280,000	0.070
	36	5	452,000,000	345,000	406,000	0.090
	48	5	225,000,000	483,000	300,000	0.13

[a] One-half of a case lot per temperature.
[b] One case lot per temperature.
[c] Incubation for 5 days.
[d] Incubation at 37 C for 48 hr.
[e] Incubation at 37 C for 24 hr.

analyzed after incubation and the terminal bacterial load was determined. Thus, regardless of the initial variation in staphylococcal population, Table 2 shows that at 5 C staphylococci failed to grow, whereas large numbers of saprophytic bacteria did develop. At 20 and 37 C, a slight multiplication of staphylococci took place, but these were overgrown by the tremendous numbers of saprophytes which also grew. The total count represents plates incubated at 20 C. Although a duplicate set of plates was incubated at 5 C, it has not been reported here because the results were very similar and the incubation temperature of 20 C presumably allowed the growth of both psychrophiles and mesophiles. The staphylococcal count was substantially lower on TG agar than on MS agar.

In the experiments reported here, the frozen foods were subjected in part to defrost conditions much more severe than would be encountered in actual experience. The defrost at 37 C would be encountered only in extremely rare conditions because most defrost occurs at temperatures between freezing and room temperature. Spoilage proceeds by degrees and the time the product was exposed to defrost conditions was carried to an extreme and went considerably beyond the development of incipient organoleptic spoilage. Particularly at 20 and 37 C, a state of decomposition was reached.

Since the coefficient of variation of the counts within any selected case of frozen pies was less than from case to case, pies in a single case were divided among four different defrost incubation temperatures and arbitrarily sampled at times when defrost symptoms had become pronounced. Table 3 presents these data. Sufficient staphylococci to cause food poisoning did not develop, whereas sufficient saprophytic bacteria grew to cause the product to develop all of the off-odors and

TABLE 3. *Bacteriological examination of one brand of frozen chicken pies after incubation at various temperatures*

Incubation temp	Time incubated	No. of samples	Avg number of bacteria per g of sample				Per cent staphylococci (TG)/total
			Total tryptone-glucose-meat extract agar		Staphylococci		
			5 C[a]	37 C[b]	Mannitol salt agar[c]	Tellurite glycine (TG) agar[d]	
	hr						
Control	0	4	42,500	67,800	18,900	3,190	4.71
5 C	268 (11 days)	5	297,000,000	192,000,000	25,200	2,330	0.0012
20 C	77	5	112,000,000	410,000,000	4,500,000	1,060,000	0.26
30 C	51	5	44,000,000	516,000,000	10,500,000	1,100,000	0.21
37 C	26	5	32,900,000	462,000,000	8,680,000	1,560,000	0.34

A single case used for the entire experiment.
[a] Incubation for 21 days.
[b] Incubation for 48 hr.
[c] Incubation at 37 C for 48 hr.
[d] Incubation at 37 C for 24 to 36 hr.

TABLE 4. *Bacteriological examination of one brand of commercial frozen chicken pies after rapid defrost to enhance possibility of Staphylococcus growth*

Incubation temp	Time incubated	Avg number of bacteria per g of sample				Per cent staphylococci (TG)/total count (37 C)
		(Tryptose-glucose-meat extract agar) Total count		Staphylococci		
		5 C[a]	37 C[b]	Mannitol salt agar[c]	Tellurite glycine (TG) agar[d]	
	hr					
Control	0	653,000	1,800,000	85,800	23,800	1.3
After defrost to 30 C	0	45,500	311,000	32,400	9,100	2.9
37 C	14	78,000,000	830,000,000	2,780,000	823,000	0.10
	22	43,800,000	717,000,000	3,300,000	780,000	0.11
	38	38,600,000	830,000,000	2,530,000	868,000	0.11
	44	31,300,000	620,000,000	2,750,000	738,000	0.12

Each value above is the average of four pies.
One case lot used for the experiment.
[a] Incubation for 21 days.
[b] Incubation for 48 hr.
[c] Incubation at 37 C for 48 hr.
[d] Incubation at 37 C for 24 hr.

other characteristics of defrost. Plates for the total count were incubated at 37 C. The correlation of staphylococcal counts between TG agar and MS agar was poor.

As a possibility, it was considered that if the internal temperature could be elevated rapidly enough to a favorable temperature for staphylococcal growth, before the saprophytic bacterial species which could grow at lower temperatures had an opportunity to develop, sufficient numbers of staphylococci for food-poisoning

TABLE 5. *Macaroni and cheese dinners incubated at various temperatures*

Incubation temperature	Time incubated	Avg number of bacteria per g of sample					Per cent staphylococci (TG)/total (37 C)
		Total (Tryptone-glucose-meat extract agar)		Staphylococci		Coliform Violet red bile agar[e]	
		5 C[a]	37 C[b]	Mannitol salt agar[c]	Tellurite glycine (TG) agar[d]		
		Peas Component					
5 C	0 days	870	6,500	470	150	15	2.3
	3½	14,900	9,300	400	50	20	0.54
	9½	75,000,000	30,000,000	1,700	1,400	700	0.0047
	16½	4,400,000,000	1,310,000,000	1,300	700	<10	0.000053
20 C	0 hr	870	6,500	470	150	15	2.3
	26	5,600,000	10,800,000	7,500	13,600	13,500	0.13
	50	780,000,000	1,930,000,000	103,000	30,000	37,000	0.0016
	74	1,530,000,000	3,900,000,000	25,000	12,000	48,000	0.00031
37 C	0 hr	870	6,500	470	150	15	2.3
	25	630,000,000	1,420,000,000	23,500,000	2,310,000	20,000,000	0.16
	49	126,000,000	680,000,000	4,500,000	3,200,000	1,700,000	0.47
	73	100,000,000	970,000,000	5,400,000	2,350,000	890,000	0.24
		Carrot Component					
5 C	0 days	140	370	80	15	<10	4.1
	3½	100	400	100	<10	<10	2.5
	9½	13,000,000	284,000	50	50	1,800	0.018
	16½	1,530,000,000	350,000,000	120	<10	60	0.0000029
20 C	0 hr	140	370	80	15	<10	4.1
	26	5,000	25,000	150	200	3,500	0.80
	50	26,900,000	71,000,000	145,000	27,000	620,000	0.038
	74	700,000,000	990,000,000	30,000	9,000	80,000,000	0.00091
37 C	0 hr	140	370	80	15	<10	4.1
	25	4,200,000,000	28,000,000	2,000,000	155,000	32,000,000	0.0037
	49	12,000,000	510,000,000	84,000	17,000	71,000,000	0.0033
	73	10,000,000	480,000,000	15,000	2,000	70,000,000	0.00042
		Macaroni and Cheese Component					
5 C	0 days	140	1,180	930	530	<10	45
	3½	1,200	2,300	1,110	1,100	50	48
	9½	530,000	18,000	1,200	140	350	0.78
	16½	400,000,000	25,100,000	2,700	65	120	0.00026
20 C	0 hr	140	1,180	930	530	<10	45
	26	16,000	70,000	17,500	25,000	1,400	36
	50	19,000,000	56,000,000	13,000,000	3,200,000	150,000	5.7
	74	240,000,000	680,000,000	37,000,000	23,600,000	2,300,000	3.5
37 C	0 hr	140	1,180	930	530	<10	45
	25	64,000,000	600,000,000	200,000,000	90,000,000	24,000,000	15
	49	164,000,000	670,000,000	134,000,000	42,000,000	39,000,000	6.3
	73	45,000,000	820,000,000	30,000,000	7,200,000	250,000,000	0.88

One determination per time and temperature.

[a] Incubation for 21 days.

[b] Incubation for 48 hr.

[c] Incubation at 37 C for 48 hr.

[d] Incubation at 37 C for 24 to 36 hr.

[e] Incubation at 37 C for 48 hr.

conditions might be obtained. Table 4 shows these data. Pies were defrosted for 24 min in a 218 C oven. According to some unpublished data, this exposure would have been just sufficient to elevate the internal temperature of the pie to 30 C. The pies were then immediately transferred to a 37 C incubator. Although tremendous numbers of saprophytic organisms did develop, the tens or hundreds of millions of staphylococci usually believed necessary to constitute a food-poisoning hazard did not develop. The pie had, of course, become completely organoleptically unacceptable before 44 hr at this temperature. Total count was determined by incubating plates at 37 C for 24 to 36 hr. In chicken pies, it was not possible to promote the growth of staphylococci in sufficient numbers as to be a potential cause of food poisoning. Tremendous numbers of saprophytic bacteria were obtained in the pies, however.

Table 5 shows a similar type of experiment carried out with a case of macaroni and cheese dinners. Inasmuch as only a single sample was available for each determination, these results can best be viewed as indicative and in need of additional confirmation. The dinner consisted of three parts: buttered peas, buttered carrots, and the macaroni and cheese main portion. Each component has been arranged separately in Table 5. In the peas, very large saprophytic bacterial populations developed only on very extended storage at 5 C, whereas staphylococci failed to develop at all. Staphylococci grew very poorly at 20 C and, at 37 C, appreciable numbers of staphylococci were reached only after the dinners had become completely inedible. Surprisingly, coliforms grew as well, or better, than the staphylococci at these temperatures. A similar situation existed for the carrot component. Staphylococcal multiplication was less than in the peas, whereas coliform multiplication was quite marked. In the macaroni and cheese main item in the dinner, very large saprophytic bacterial populations developed only on very extended storage at 5 C, and staphylococci failed to develop at all. At 20 C, an excessive saprophyte population developed after 2 days of incubation and the product had become inedible. Appreciable numbers of staphylococci developed only when incubation was carried to inedibility. At the improbable defrost temperature of 37 C, spoilage took place in less than 1 day and the revolting odor and appearance of the product completely precluded consumption. The saprophyte count reached enormous numbers. Staphylococci reached considerable numbers to an apparent maximum and began to die off on carrying the incubation to a further extreme. Coliforns also multiplied well after conditions of extreme spoilage had been reached. Thus, in the defrost of macaroni and cheese dinners, as in the defrost of chicken pies, appreciable numbers of staphylococci did not develop during the edible life of the product.

From inspection of Tables 3, 4, and 5 where duplicate series of total count plates were incubated at 5 C and at 37 C, it is seen that these foods had both psychrophilic and mesophilic bacterial floras. When the product was incubated below 20 C, higher counts were found on the plates incubated at 5 C than on plates incubated at 37 C. When the foods were incubated at or above 20 C, larger bacterial populations were obtained on total count plates incubated at 37 C and lower counts on plates incubated at 5 C.

If the psychrophilic and mesophilic floras were composed of exactly the same organisms (or facultative mesophiles according to one view), identical numbers of bacteria should appear in both the psychrophilic and mesophilic counts at any frozen food defrost incubation temperature. This did not occur. Thus, a saprophytic bacterial population, the temperature characteristics of which are similar to the incubation temperature of the food, arises as a defrost response to a particular defrost temperature.

This research has been concerned with the ability of naturally occurring staphylococci to grow in frozen foods in the presence of a naturally occurring mixture of other bacteria. Particular attention was directed to determine if tens or hundreds of millions of staphylococci, which are apparently necessary for food poisoning, might be produced under any condition of defrost. Since they could not, the hazard of their survival and presence is minimal. Apparently, the greater the saprophytic population, the greater the protection against staphylococcal growth through antagonism, competition for nutrients, and modification of the environment to conditions less favorable to staphylococcal growth. By rendering the food product inedible before appreciable staphylococcal populations arise, these saprophytes provide a built-in safety factor.

An acute need for reconsideration of the present indices of pathogenicity of staphylococci has been evidenced by the loss of ability to give characteristic reactions on differential media, by the loss of pigmentation and, most especially, by the loss of the ability to coagulate plasma by some cultures on frozen storage.

LITERATURE CITED

Brian, P. W. 1957. The ecological significance of antibiotic production, p. 169. *In* R. E. O. Williams and C. C. Spicer, [ed.], Microbial ecology. Cambridge University Press, London.

Dack, G. M. 1949. Food poisoning. University of Chicago Press, Chicago. 184 p.

Dearing, W. H., and F. R. Heilman. 1953. Micrococcic (staphylococcic) enteritis as a complication of antibiotic therapy. Its response to erythromycin. Proc. Staff Meetings Mayo Clinic **28**:121–134.

Dearing, W. H. and C. M. Needham. 1960. Hospitalized patients with *Staphylococcus aureus* in the intestine. J. Am. Med. Assoc. **174**:1597–1602.

ELEK, S. D. 1959. *Staphylococcus pyogenes* and its relation to disease. E. and S. Livingstone Ltd., London.

FRAZIER, W. C. 1958. Food microbiology, p. 397. McGraw-Hill Book Company, New York.

GIBSON, J. 1957. Nutritional aspects, p. 35. *In* R. E. O. Williams and C. C. Spicer, [ed.], Microbial ecology. Cambridge University Press, London.

MICKELSON, M. N., AND R. S. FLIPPIN. 1960. Use of *Salmonella* antagonists in fermenting egg white. II. Microbiological methods for the elimination of *Salmonellae* from egg whites. Appl. Microbiol. **8**:371–377.

MILLER, C. H. 1958. Science and philosophy of the community concept. Am. Scientist **46**:294–308.

RAHN, O. 1945. Injury and death of a bacteria by chemical agents. Biodynamica Monograph No. 3. Biodynamica, Normandy, Mo.

STEWARD, E. E., AND F. C. KELLY. 1959. Variation of bound coagulase of *Staphylococcus aureus*. J. Bacteriol. **77**:101–103.

TANNER, F. W., AND L. P. TANNER. 1953. Food-borne infections and intoxications, p. 306. 2nd ed. Garrard Press, Champaign, Ill.