

Effect of pH and Oxygen Tension on Staphylococcal Growth and Enterotoxin Formation in Fermented Sausage

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Commercial fermented sausages that contained significant numbers of viable coagulase-positive staphylococci were found to have the growth localized in the outermost areas of the sausage where oxygen tension was highest. Staphylococci were found to be more acid-tolerant aerobically than anaerobically. With chemical acidulation of sausage, growth could be controlled both aerobically and anaerobically with approximately 1.5% glucono delta lactone. Biological acidulation with a high inoculum of *Pediococcus cerevisiae* inhibited anaerobic staphylococcal growth but failed to suppress aerobic growth completely. A staphylococcal count of approximately 4×10^7 cells/g of sausage appeared to be necessary to produce detectable enterotoxin A within 24 hr in sausage. A minor difference existed in the relative rates of production of the different types of enterotoxin. Detectable enterotoxin A was produced in 24 hr in sausage held in atmospheres containing 10, 15, and 20% oxygen. In an atmosphere containing 5% oxygen, toxin was detected after 48 hr of incubation. No toxin was detected after 120 hr under anaerobic conditions. Most staphylococcal strains tested initiated growth and produced detectable enterotoxin aerobically at a pH of 5.1 in broth media. Anaerobically, however, most strains failed to produce detectable enterotoxin below pH 5.7.

The incidence of staphylococcal food poisonings associated with fermented sausage has been sporadic over the past decade. Although a few outbreaks have been documented (1), fermented sausage is not a significant item in the overall incidence of this type of food poisoning. Some years ago, during a study involving the development of a starter culture for this type of sausage (5), a peculiar localization of staphylococcal growth was noted in some marketed products. It was observed that, when extensive staphylococcal growth occurred, it always involved the outermost part of the sausage or areas of highest oxygen tension. The importance of this observation for industry control and public health laboratories is evident. In addition, the relationship of oxygen tension and pH value on staphylococcal growth and toxin formation in these products merits elucidation so that corrective measures can be instituted. Most staphylococcal food poisonings have involved type A enterotoxin. This is also true in most instances in which fermented sausage has been implicated; for this reason, we

focused our attention especially on strains producing type A enterotoxin.

MATERIALS AND METHODS

Quantitation of staphylococci in sausage. Sausage samples were weighed, diluted 1:10 in 0.1% peptone, and blended in a Waring blender for about 1 min. Further dilutions were made in 0.1% peptone, and 0.1-ml amounts from the dilution tubes were spread plated on Vogel-Johnson agar (Difco). After 48 hr at 37 C, colonies were counted; 10 randomly chosen isolates were subcultured in brain heart infusion broth (BHI; Difco) and incubated at 37 C. The cultures were tested for coagulase production, followed by a calculation of the *Staphylococcus aureus* count.

Lactobacillus selective agar (LBS; BBL) was used to estimate lactic acid-forming bacteria in commercial sausage products.

Laboratory-formulated sausage mix preparation. Sausage was made in the laboratory by the formulation and method described previously (5). When added, *S. aureus* was diluted in 0.1% peptone from a 24-hr BHI culture. Glucono delta lactone (GDL) was added to sausage in the powdered form for acidulation (2). After a final mixing, the sausage was placed in a beaker, loosely covered with Parafilm,

tempered in a water bath at the desired temperature (usually 37 C), and incubated. All pH values in the study were estimated by use of a model 26 radiometer pH meter (Copenhagen).

Enterotoxins and antisera. Purified enterotoxins and their specific antisera were obtained from M. S. Bergdoll, Food Research Institute, University of Wisconsin, Madison. Most of the *S. aureus* strains used in this study were also obtained from him.

Extraction of enterotoxin from sausages. The method of extraction was based on an unpublished modification (Bergdoll, *personal communication*) of Zehren's procedure (18), which was further modified to detect toxin in laboratory-formulated sausage. A 1- μ g amount of enterotoxin was added to an uninoculated sausage and blended just prior to extraction. This served as a control in each experiment to determine whether the extraction procedures were adequate. Initially, three extractions of a 100-g sausage sample were made with 0.2 M NaCl (pH 7.5) to yield a total volume of 600 ml of solution containing the extracted toxin from 100 g of sausage. After filtration of extracts through Miracloth (Robert Schinner Co., New Berlin, Wis.), about 20 ml of chloroform was shaken with the extract. After centrifugation, the chloroform phase was discarded. The pH of the aqueous phase was adjusted to 4.5 with 6 N HCl. The mixture was stirred and centrifuged again. The pH of the aqueous phase was adjusted to 7.0 with 5 N NaOH, and the extract was placed in dialysis sacs. The sacs were immersed in polyethylene glycol (PEG) overnight and held at 2 to 4 C.

The concentrated extract was centrifuged, and the supernatant fluid was heated to 56 C in a water bath followed by chilling in ice. This heating step was performed only with extracts from the laboratory-formulated sausage. The heat treatment helped to remove substances which interfered in the serological tests to be described. After the heating and cooling steps, 2 ml of chloroform was added, and the preparation was shaken vigorously and centrifuged. After two additional chloroform extractions, the pH was adjusted to 4.5. The sample was centrifuged, and the pH of the supernatant fluid was adjusted to 6.5. The extracted sample was added to 12 to 15 ml of wet carboxymethylcellulose CM 22 (Reeve Angel and Co., Clifton, N.J.), which had been adjusted to pH 6.5, and the mixture was stirred at 4 C for 30 min. It was then poured into a column (size 234, Kontes Glass Co., Vineland, N.J.) over a glass-wool plug, and washed with 0.01 M phosphate buffer (pH 6.5). The toxin was eluted from the column with 150 ml of 0.2 M sodium phosphate containing 0.2 M NaCl at pH 7.5 (elution conducted in a cold room at 4 C). The sample was concentrated in PEG overnight.

The sample was then rinsed down to one end of the dialysis sac which was again tied off, and the sample was dialyzed for 4 hr against water. This was followed by three further chloroform extractions and concentration in PEG. After rinsing from the sac, the sample was lyophilized and rehydrated to 0.2 ml. This extract was used on microslides and compared with a known reference enterotoxin (1 μ g/ml) in the serological procedure. As noted by Zehren (18), 0.4 μ g of en-

terotoxin A/ml (in the final concentrate) is detectable by this method.

Enterotoxin detection. Enterotoxin was detected by immunodiffusion on slides prepared by the method of Casman et al. (4) with the following modifications: (i) the slide was initially coated with a mixture of equal volumes of 1 N NaOH and 95% ethanol, (ii) a layer of 0.2% agar (Difco) was placed on the slide and allowed to air-dry, and (iii) melted Ouchterlony agar was prepared with 1.2% Epiagar (Colab Laboratories, Inc.). Quite often, the immunodiffusion slides that were used to test for enterotoxin from laboratory-formulated sausages were obscured with nonspecific protein precipitations from the meat. After the 3-day incubation period for immunodiffusion, the slides were immersed in 0.2% Haemo-Sol (Haemo-Sol, Inc., Baltimore, Md.) for 2 hr. This treatment removed much of the haze without adversely affecting the precipitin lines.

RESULTS

Growth of staphylococci in commercial fermented sausage. In initial studies, it was observed that if commercial fermented sausage contained significant numbers of viable staphylococci these organisms occurred in the outermost areas where the oxygen tension was highest (Table 1). Insignificant numbers of *S. aureus* cells were detected in the core samples. Although the LBS counts were generally high, the pH values varied, and no correlation could be made between the LBS count and pH of the sausage. All samples were purchased in local markets and constituted approximately a 20- to 25-cm piece (not sliced). Usually labels were not available to ascertain the use of a starter culture. In one instance (sample A1), a label was observed and no starter was used. No significant difference in pH values of core and surface samples could be detected.

Several lines of experimentation were followed to ascertain the reason for the high *S. aureus* counts in the peripheral area of the sausages and their relative absence in the core area. Nine samples of raw, natural casings were examined for staphylococci; none contained detectable numbers of the organism.

Experiments with broth cultures indicated the ability of several of the *S. aureus* isolates to initiate growth at a pH value of approximately 5.0 when the cultures were incubated aerobically. Anaerobically, no growth occurred at this pH value. These results supported the conclusion that under aerobic conditions the staphylococci were significantly more acid-tolerant and that this characteristic accounted for the large number of staphylococci occasionally encountered in fermented sausage.

Staphylococcal growth with chemical acidulation. Portions of 100 g of the laboratory-

TABLE 1. Occurrence of coagulase-positive staphylococci in commercial fermented sausage

Sample source	Sausage type	Sample no.	pH	Plate count/g of sausage			
				Surface ^a		Core	
				<i>S. aureus</i>	LBS ^b	<i>S. aureus</i>	LBS
A	Genoa	1	5.3	3.0×10^7	4.2×10^6	<100	9.2×10^6
		2	5.3	6.8×10^6	5.1×10^7	<100	1.4×10^6
		3	5.1	3.9×10^6	6.3×10^7	<100	7.2×10^7
B	Unknown	1	4.9	4.6×10^5	3.8×10^6	<100	7.1×10^6
		2	4.9	8.2×10^6	6.2×10^6	<100	4.9×10^6
C	Genoa	1	5.2	3.6×10^4	4.9×10^6	230	8.9×10^6
		2	4.9	8.1×10^6	3.1×10^6	<100	4.6×10^5

^aThe outer 6 mm of sausage was sampled.

^b*Lactobacillus* selective agar (BBL).

formulated sausage mix were prepared with various concentrations of GDL to ascertain the extent of acid production from the hydrolysis of the lactone. Levels of 0, 0.25, 0.5, 0.75, 1.0, and 1.5% GDL resulted in pH values of 5.75, 5.65, 5.45, 5.20, 5.05, and 4.85, respectively, after an 8-hr incubation period at 37 C. Approximately 1.0% GDL, therefore, lowered the pH value to 5.0, which is the average pH for this type of sausage. In separate experiments, it was demonstrated that incubation temperatures from 10 to 44 C did not appreciably affect the pH attained at 8-hr readings. Moreover, acid production was not affected by oxygen tension.

An experiment was performed to determine the effect of chemical acidulation on staphylococcal growth. The laboratory-formulated sausage mix was prepared, and a diluted preparation of *S. aureus* was incorporated to yield a viable count of approximately 10^3 cells/g. Various amounts of GDL were added, and 100-g portions were placed in a 100-ml beaker, loosely covered to retard evaporation, and incubated at 37 C. After 24 hr, pH values and viable staphylococcal counts were estimated for samples obtained from the surface and deep areas of the sausage. Significant growth of staphylococci was observed aerobically at GDL concentrations up to 1.3% (Table 2). Under anaerobic conditions, growth was decreased at least 10-fold as compared to growth under aerobic conditions, and thus a much lower concentration of GDL was necessary to decrease the ultimate counts significantly. From this experiment, it appeared that approximately 1.4 to 1.5% GDL was necessary to eliminate significant aerobic growth in sausage.

Further experiments were performed to decrease the pH value more rapidly by the direct addition to the sausages of 0.1% citric or tartaric acids as well as GDL. There was little

additional effect of these organic acids on either pH value or staphylococcal growth. Attempts were made to control aerobic staphylococcal growth by acidulation with the sausage starter, *Pediococcus cerevisiae*. Kao and Frazier (12) demonstrated that some lactic acid bacteria can inhibit the growth of *S. aureus*. Various mixtures of inocula of staphylococci and pediococci were made in the laboratory-formulated sausage, and viable counts were estimated after 24 and 48 hr of incubation at 37 C. In the anaerobic areas of the sausage, staphylococci were not detected. In the surface, or aerobic, areas, the pediococci failed to suppress the staphylococcal growth completely. An inoculum ratio of $10^6:10^1$ of pediococci to staphylococci still allowed the staphylococci to attain a population level of 10^3 to 10^4 cells/g of surface sausage.

TABLE 2. Growth response of *Staphylococcus* strain 9 in laboratory-formulated sausage acidulated with glucono delta lactone (GDL)

GDL (%)	Staphylococcus inoculum ^a	<i>S. aureus</i> count/g of sausage	
		Aerobic area	Anaerobic area
0	0	<100	<100
0.5	0	<100	<100
0	+	4.0×10^8	2.4×10^6
0.5	+	2.9×10^7	1.9×10^6
1.0	+	3.3×10^5	2.8×10^4
1.1	+	1.1×10^6	1.2×10^2
1.2	+	9.3×10^5	ND ^b
1.3	+	2.7×10^5	<100
1.4	+	<100	<100
1.5	+	<100	<100

^aSufficient cells were added to yield approximately 10^3 /g.

^bNot determined.

Numbers of *S. aureus* necessary to produce detectable enterotoxin A in sausage. A series of experiments was performed to determine the minimal population level of staphylococci in sausage that would produce detectable enterotoxin (in the usual assay procedure involving 100 g). Portions of laboratory-formulated sausage mix were prepared to which strain 9, an enterotoxin A producer, was added to yield a count of approximately 10^4 cells/g. After incubation for 24 hr at 37°C in loosely covered beakers, viable *S. aureus* counts were estimated in the surface areas of the sausages. Surface samples of 100 g were extracted and analyzed for enterotoxin A by the modified Casman microslide procedure (see Materials and Methods). This experiment was repeated eight times in an effort to estimate accurately the number of cells required to produce detectable toxin. No enterotoxin A was detected when the 24-hr *Staphylococcus* counts per g were 3.5×10^6 , 4.1×10^6 , 5.2×10^6 , or 3.8×10^7 . Weak microslide precipitin lines were observed when the counts per g were 1.2×10^7 , 1.4×10^7 , and 2.3×10^7 . Definitive precipitin lines were observed in 15 samples in which the surface counts exceeded 6.4×10^7 staphylococci/g. Thus, a *Staphylococcus* count per g ranging from 1.0×10^7 to 4.0×10^7 appeared to be the minimal number that produced toxin detectable by the described assay procedure.

Production of other staphylococcal enterotoxins in sausage. Several strains producing type B enterotoxin and a strain producing type E were tested for toxin production in sausage as described previously. As presented in Table 3, strain S-6 produced type B toxin within 24 hr if the population level exceeded approximately 9.0×10^8 cells/g. Other B-producing strains (272 and 334) did not produce

TABLE 3. Enterotoxin production in sausage by staphylococcal strains producing types B and E

<i>S. aureus</i> strain	Type of toxin produced	<i>S. aureus</i> count/g	Time (hr)	Detectable enterotoxin ^a
S-6	B	5.0×10^7	24	-
S-6	B	8.8×10^7	24	-
S-6	B	9.5×10^8	24	w+
S-6	B	8.3×10^8	48	+
272	B	4.0×10^8	24	-
334	B	8.0×10^8	24	-
326	E	2.3×10^8	24	+

^a In aerobic area of sausage; -, enterotoxin not detected; w+, microslide precipitin lines were weak; +, enterotoxin detected.

toxin within the 24-hr incubation period. Thus, there appear to be some minor differences in the relative rates of toxin production in sausage by the different strains examined.

Effect of oxygen tension on type A enterotoxin production in sausage. A series of experiments was performed to determine the effect of oxygen tension on staphylococcal enterotoxin A production in sausage. Beakers containing sausage mix were placed in desiccators, which were then flushed three times and filled with the desired gaseous mixture. Commercially prepared oxygen-nitrogen mixtures were used for the 5, 10, and 15% O₂ mixtures, and helium/CO₂ (trace) was used for anaerobic incubation. Atmospheric conditions were used for the "20%" oxygen tension. The desiccators were placed at 37°C, and the viable counts were estimated after the various incubation periods. When multiple samplings were required, the requisite number of desiccators was prepared. This obviated the introduction of oxygen during the sampling procedure. Samples of 100 g of sausage were extracted and analyzed for enterotoxin as previously stated. The results shown in Table 4 indicate a distinct correlation between detectable enterotoxin production and oxygen tension. Toxin was readily produced within 24 hr in atmospheres containing 10, 15, and 20% oxygen. In an atmosphere containing

TABLE 4. Effect of oxygen tension on enterotoxin A production by *S. aureus* strain 9 in nonacidulated sausage

Oxygen (%)	<i>S. aureus</i> count/g	Detectable enterotoxin A	Incubation period (hr)	Trial no.
20	1.0×10^8	+ ^a	24	I
	1.2×10^7	+	24	III
	1.9×10^8	+	24	IV
15	1.8×10^8	+	24	I
	7.5×10^7	+	24	II
10	1.4×10^7	w+	24	I
	8.4×10^7	+	24	II
5	6.4×10^7	-	24	I
	9.9×10^7	-	24	II
	4.0×10^8	-	24	III
0	1.5×10^8	+	48	III
	5.2×10^8	+	72	III
	8.3×10^8	-	24	I
	2.5×10^4	-	24	II
	8.0×10^4	-	24	III
	5.3×10^7	-	48	III
	6.7×10^7	-	72	III
	1.1×10^8	-	48	IV
	1.1×10^7	-	96	IV
	1.3×10^7	-	120	IV

^a See Table 3 for key to symbols.

5% oxygen, growth was insufficient to produce detectable enterotoxin at 24 hr, but enterotoxin was detectable after 48 and 72 hr of incubation. Anaerobic incubation resulted in no enterotoxin production even after 120 hr, although significant growth had occurred by 48 hr. It would appear that anaerobic conditions of culture radically decrease the potential for enterotoxin production in sausage. This observation renders the potential for finding toxin in core areas of sausage a rather unlikely possibility. Thus, in the examination of sausages for the presence of toxin, the analyses should be performed on samples taken from the surface or outermost area of the sausage.

Enterotoxin production and growth in laboratory media. An extended study was undertaken to determine the effect of acidity and oxygen tension on the ability of *S. aureus* to initiate growth and to produce enterotoxin. A buffered BHI broth medium was developed to grow *S. aureus* at controlled pH values. This medium was used in attempts to define more sharply the limiting pH value on growth and enterotoxin production both aerobically and anaerobically. A medium consisting of 37 g of BHI broth powder (Difco), 3 g of K_2HPO_4 , 7 g of sodium succinate, and 1 liter of water was prepared, and 100-ml volumes were dispensed into 250-ml flasks. After the medium had been sterilized, the volume of 1 N HCl needed to increase the acidity of 100 ml of the medium to approximately the desired pH values was added (as determined in previous trials). After pH adjustment, 10-ml portions of broth were aseptically distributed into sterile 50-ml side-arm DeLong culture flasks and into sterile 18-mm test tubes. Dilutions were made from an overnight culture of *S. aureus*, and the inoculum was adjusted to yield approximately 10^4 cells/ml of final medium. Each strain to be tested was inoculated into a flask and a tube at each of the pH values. Flasks were incubated aerobically without shaking, and the metal-capped tubes were placed in a BBL anaerobic jar with a GasPak (disposable hydrogen + CO_2 generator envelope, BBL) and an anaerobic indicator. Growth was estimated by optical density readings after 72 hr of incubation at 37 C. Both aerobic and anaerobic uninoculated controls showed no change in pH value at any of the pH levels tested. Under anaerobic conditions, slight growth caused no change in the pH value, but as growth increased a decrease in pH was observed. Aerobically, an initial decrease in pH was observed, followed by an increase to a range of pH 7 to 8. After incubation, the pH of the culture was adjusted to 7.0, and the culture was

centrifuged to remove the cells. The supernatant fluid was placed in dialysis tubing and dialyzed a minimum of 4 hr in water at 4 C to remove inorganic salts. After concentration in PEG, the sample were rehydrated to the original volume and tested for enterotoxin on Casman microslides with 1 μ g of toxin/ml in the reference wells and with the specific antiserum of the toxin in question. A positive result was recorded when a precipitin line of identity with the known toxin formed between the unknown well and the specific antiserum well in at least one of the multiple (three to eight) slides prepared for each sample.

The relationship of the two variables (pH value and O_2 tension) is demonstrated in these experiments (Table 5). With strains 100 and 196E, no toxin was detectable under aerobic conditions when the initial pH was below 5.0. The significant growth response of strain 196E below pH 5.0 but lack of toxin formation should be noted. In striking contrast, under anaerobic conditions, growth was not initiated at pH 5.4. Enterotoxin was produced at pH 6.0 by both strains but not at pH 5.7.

Enterotoxin production as a function of pH and oxygen tension was considered to be of significance. Therefore, these studies were extended by use of a larger collection of *S. aureus* strains. Emphasis was placed on type A-producing strains, as they account for the majority of food-poisoning outbreaks.

Eight strains producing enterotoxin type A, three producing type B, one producing type C₂, and one producing type E were tested for aerobic and anaerobic growth and for detectable enterotoxin production over a range of initial pH values from 4.8 to 7.0. These experiments were performed as described in the preceding paragraphs, with the use of the buffered BHI medium.

The results again indicated a significant interrelationship among growth, enterotoxin production, pH value, and aerobiosis (Table 6). Under aerobic conditions, some strains produced toxin at pH 4.9, but under anaerobic conditions no toxin was produced by any strain at a pH of 5.4. Some strains failed to produce toxin at pH 5.7.

Significant variation among the strains occurred in the minimal pH values for enterotoxin production. One strain (334) failed to produce any toxin anaerobically, but anaerobic growth was initiated at pH 5.7, and growth proceeded to the same extent as in those strains that did produce toxin. Another strain (118) evidenced extreme sensitivity to pH with respect to toxin production. Thus, the combined inhibitory ef-

TABLE 5. Growth and enterotoxin A production in buffered BHI after 72 hr at 37 C

Initial pH value	Aerobic ^a				Anaerobic			
	Strain 100		Strain 196E		Strain 100		Strain 196E	
	OD ^b	Toxin	OD	Toxin	OD	Toxin	OD	Toxin
7.0	>1.4	+	>1.4	+	0.7	+	0.9	+
6.5	>1.4	+	>1.4	+	0.7	+	0.8	+
6.0	>1.4	+	>1.4	+	0.7	+	0.7	+
5.7	>1.4	+	>1.4	+	0.4	-	0.6	-
5.4	>1.4	+	>1.4	+	0.0	-	0.0	-
5.1	1.2	+	>1.4	+	0.0	-	0.0	-
5.0	0.8	w+	>1.4	+	0.0	-	0.0	-
4.9	0.6	-	1.4	-	0.0	-	0.0	-
4.8	0.1	-	1.0	-	0.0	-	0.0	-

^a Static incubation of 50-ml Delong culture flasks containing 10 ml of broth.

^b Optical density at 600 nm.

TABLE 6. Lowest pH values for enterotoxin production in buffered BHI after 72 hr at 37 C

Initial pH value	Strain no. and type of enterotoxin produced												
	9, A	95, A	107, A	118, A	119, A	166, A	167, A	169, A	S-6, B	272, B	334, B	361, C ₂	326, E
Aerobic													
5.7	+ ^a	+	+	÷	+	+	+	+	+	+	+	+	+
5.4	+	+	+	- ^b	+	+	+	+	w+	+	+	+	+
5.1	+	+	+	- ^b	+	w+	+	+	- ^b	- ^b	+	+	+
5.0	- ^b	+	+	- ^b	+	- ^b	+	- ^b	- ^c	- ^b	+	+	+
4.9	- ^b	- ^b	+	- ^b	+	- ^b	+	- ^b	- ^c	- ^b	- ^b	+	+
4.8	- ^c	- ^c	- ^b	- ^c	- ^b	- ^b	- ^b	- ^c	- ^c	- ^c	- ^b	- ^c	w+
Anaerobic													
7.0	+	+	+	+	+	+	+	+	+	+	- ^b	+	+
6.5	+	+	+	- ^b	+	+	+	+	+	+	- ^b	+	+
6.0	+	+	+	- ^b	+	+	+	+	+	+	- ^b	+	+
5.7	+	- ^c	+	- ^b	- ^b	+	+	+	+	+	- ^b	+	- ^b
5.4	- ^c	- ^c	- ^b	- ^b	- ^b	- ^b	- ^b	- ^b	- ^c	- ^c	- ^c	- ^c	- ^c

^a Symbols: +, toxin detected; w+, weak positive reaction for toxin; -, no toxin detected.

^b Growth occurred but no toxin was detected.

^c No growth.

fects of low pH value and anaerobiosis in broth resulted in the most effective reduction or elimination of toxin production. Essentially, these results verify those obtained with the commercially fermented sausage observed in the initial phase of this study.

DISCUSSION

Our results regarding the approximate number of staphylococci needed to produce detectable enterotoxin A in sausage are in keeping with those observed by Donnelly et al. (6) in milk (enterotoxin A) and Genigeorgis (11) in other cured meat products (type B). Caution must be exercised in interpreting the significance of exact population levels. These are only approxi-

mations, and the type of enterotoxin produced, oxygen tension, and strain of *S. aureus* are critical parameters to be considered.

Since the pioneering studies of Lechowich et al. (13), many investigators have observed the effect of oxygen tension, pH value, and curing ingredients on staphylococcal growth (8, 11, 17). These studies have been extended by subsequent investigators to the effect of oxygen tension and pH value on enterotoxin production (3, 7, 9, 14, 16). Generally, high oxygen tension favors both growth and toxin production. Type B toxin production has been observed at pH values of 5.0 to 5.1 (10, 15), but the observation that type A-producing strains can initiate growth and produce toxin under aerobic

conditions at pH 4.9 reflects the necessity for rapid and extensive acidification in sausage. In no instance did we detect toxin production in sausage under anaerobic conditions. In contrast, in most instances in which aerobic growth by a toxin-producing strain was initiated, toxin was found.

There appears to be a low level of food-poisoning risk associated with fermented food products such as cheese and sausage. Although these products have been consumed for many years, in more recent times they have been occasionally associated with staphylococcal food poisoning. In cheese manufacturing, excessive staphylococcal growth generally reflects faulty acidulation. This is probably true in fermented sausage production, and the rate of acidulation may be an approach to the control of staphylococcal growth. If the acidulation is very rapid and an extensive pH reduction results, as seen in the experiments with 1.5% GDL, then aerobic staphylococcal growth is curtailed. However, biological acidulation did not afford a comparable degree of control.

In certain ripened cheeses, where starter cultures definitively influence flavor, the sole employment of chemical starters is not feasible. However, in fermented sausage, where sugar, salt, spice and smoke are the major organoleptic factors, it would appear that biological acidulation can and ultimately will be replaced by chemical acidulation. In recent years, frozen starter cultures have been introduced commercially to enhance the rate of acid production in sausage. However, the relatively absolute nature and exacting control of acidification are extremely appealing characteristics of the chemical approach. Moreover, the relative ease of shipping, storage, and in-plant handling of a chemical acidulating agent over a starter culture are important factors for future consideration.

In comparing cheese and sausage manufacture, the sausage maker is at a distinct disadvantage in controlling staphylococcal growth because he cannot pasteurize his raw product. Moreover, the sausage maker cannot control the moisture content in his product to the same extent as the cheese maker. After removal of the whey, the decreased water activity, combined with increased acidity, can aid in the control of microbial growth. A comparable decrease in water activity of sausage requires considerably more time, thus resulting in a greater chance for staphylococcal growth.

The cheese maker effects a rapid decrease in pH value by a massive inoculation of the milk. This cannot be practiced by the sausage maker,

as he cannot grow his starter culture in a sausage mix and add it to a fresh batch. The high cost of a comparable massive inoculum for sausage if purchased precludes its employment. However, the possible use of chemical acidulants or a combination of chemical and biological acidulants may provide an approach to effect a rapid pH reduction. In reality, a combined biological and chemical acidulation could be used in both the cheese and sausage industries.

Industry control and public health laboratories should incorporate a sampling procedure involving only the aerobic areas of the individual sticks of sausage. This is true not only for viable staphylococcal counts but also for the detection of toxin. Samples taken by a cross sectioning of the sausage will have the effect of diluting the critical aerobic areas, and, in the detection of toxin, this could be a cardinal consideration.

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