Growth of *Salmonella typhimurium* and *Staphylococcus aureus* in Retail Pumpkin Pies[1]

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ABSTRACT

The ability of pumpkin pies as prepared and distributed in the food distribution system to support growth of selected food pathogens was studied. Products were puchased from a cross-section of retail outlets. Microbial quality of the products was determined. One of four samples contained high levels of coliforms and *Staphylococcus aureus*. *Salmonella* was not detected in any of the samples. Samples were inoculated with *Salmonella typhimurium* and *S. aureus* and incubated at 4, 25 and 35 C. Water activity (a_w), pH and *S. aureus* enterotoxin were measured. Pumpkin pies supported growth of the pathogens at 25 and 35 C. Data revealed if contaminated and held at room temperature, pumpkin pies could present a public health hazard. Growth of pathogens is inhibited at refrigeration temperatures. Enterotoxin was present in samples containing *S. aureus*. Potassium sorbate (0.25%) inhibited growth of *S. typhimurium* but not *S. aureus*. Refrigeration is recommended for pumpkin pies to eliminate the possible health hazard.

Meat and poultry are commonly identified as vehicles of salmonellosis. These foods are most vulnerable because *Salmonella* associated with infected animals are spread to foods during processing, and the foods are inadequately cooked or stored, or both, in a manner that allows this bacterium to survive or multiply.

With modern food distribution systems, widespread outbreaks could occur before a recall of contaminated food could be made. In 14 or more outbreaks stemming from kosher "ice cream" manufactured from eggs, processed in one plant, it was estimated that over 9000 cases of salmonellosis occured in four eastern states within 13 days (1).

Salmonella is sometimes found in the intestinal tract of man and animals. The optimum growth temperature of *Salmonella* is 35 - 37 C but it can grow between 5 and 47 C. Salmonellae are killed by mild heat, most are significantly inactivated at 60 C within 1 to 15 min. Salmonellae grow between pH 4.5-9.0, with an optimum

of 6.5-7.5, and a_w between 0.999 - 0.945. Salmonellae are sensitive to many treatments commonly employed to preserve foods. As conditions in foods become suboptimal, survival or growth of the organism diminishes. If, however, handling of food changes the environmental conditions to favor growth or if salmonellae are reintroduced into the food, *Salmonella* could become a serious threat to public health (6).

The annual incidence of reported isolation of salmonellae has remained relatively constant at about 20,000 to 25,000 per year since the surveillance program was started in the United States in 1963 (12,13).

Staphylococcal enterotoxin is a major cause of foodborne illness, not only in the United States but in other countries as well. The true incidence of staphylococcal food poisoning is unknown. In the United States from 1969-1973, a total of 499 outbreaks (including unconfirmed) were attributed to staphylococci, which represented approximately 30% of the foodborne diseases reported. The staphylococci are ubiquitous in nature, with man and animals as their primary reservoir. In most staphylococcal food poisoning outbreaks, the cause is usually improper food handling. This is particularly true of foods that have been heated during preparation to destroy the organism but recontaminated by humans. Many people believe baked food is safe and do not realize the need for adequate refrigeration (5).

It is safe to assume that all strains of *Staphylococcus aureus* are potential pathogens. There are no particular characteristics or substances produced by the staphylococci that could reveal their pathogenicity. These organisms produce many substances, a number of which are toxic to one animal or another. Some suggest coagulase-positive and DNAse-positive organisms produce enterotoxins, but the relationship appears to be coincidental (8).

Factors that affect growth of the organism, in general, affect production of enterotoxins. The temperature range for optimum growth of staphylococci is 35 - 37 C. Most strains of staphylococci grow at pH values between 4.5 - 9.3, with the optimum being 7.0 - 7.5, and water

activity reported as low as 0.86.

Any time a food is exposed to human handling, there is a good possibility the food will be contaminated with staphylococci. Heating of the food after handling normally would insure against food poisoning unless the food was held unrefrigerated for several hours before eating. If enterotoxins were formed in the food, heating might not be sufficient to destroy them. In many instances, foods are not heat-processed further after handling and unless proper care is taken, the organism may multiply and elaborate enterotoxins. The course of action recommended is to keep susceptible foods refrigerated at all times except while being prepared and served. Most food poisoning outbreaks could be prevented if this simple precaution were taken.

The purpose of this study was to examine the food handling and distribution practices of the food retailing industry as it relates specifically to bakery products. *Salmonella typhimurium* and *S. aureus* were inoculated into pumpkin pie, which possessed pH, a_w, and ingredients conducive to growth of the organisms and could present a potential health hazard.

MATERIALS AND METHODS

Samples were collected and examined as described previously (*14*). *Salmonella* were enumerated according to methods described by FDA (*7*), Sperber and Tatini (*11*) and Poelma and Silliker (*9*).

S. aureus was enumerated by the selective enrichment procedure outlined by Speck (*10*). Isolated colonies were obtained on S-110 medium incubated at 35 C for 48 h. Colonies from each plate were transferred to Brain Heart Infusion Broth (BHI) and incubated overnight at 35 C. The coagulase test was made with 0.5 ml of coagulase plasma EDTA (Difco) and two drops of broth culture incubated at 35 C and the mixture was examined periodically for clot formation. Tubes with a firm clot were considered positive (*11*). The MPN of *S. aureus* per gram of sample was reported.

Pure cultures of *S. aureus* 265-1 A strain and *S. typhimurium* were prepared by inoculating BHI broth and incubating overnight at 35 C. The broth was then centrifuged for 10 min at 2300 rpm. The supernatant fluid was discarded and cells were suspended in sterile 0.067 M phosphate buffer.

Growth was determined by inoculating the cooked filling with known amounts of the pure cultures. After the organisms were well dispersed in the mix, portions were incubated at 4, 25 and 35 C. The organisms were enumerated at regular intervals.

S. aureus was enumerated by taking duplicate 25-g portions of the product inoculated with the organism, blended with 225 ml of 0.1% sterile peptone water for 1 min. Ten-fold dilutions were made in 90-ml peptone blanks. For each dilution, 1 ml was spread on S-110 plates, incubated at 35 C for 48 h (*2*). Each colony was tested for coagulase production.

S. typhimurium in the inoculated products was enumerated using the 3-tube MPN procedure. Duplicate 25-g portions taken at regular intervals were blended for 1 min in 225 ml of sterile 0.1% peptone water and subsequent ten-fold dilutions were made. For each dilution, 1 ml was transferred to 3 tubes containing 10 ml of selenite cystine broth (SC). Tubes were incubated for 24 h at 35 C and then a portion from each was streaked onto XLD agar and incubated for 24 h at 35 C. Typical colonies were isolated and confirmed by differential tests (*9,10*).

Samples were tested for the presence of staphylococcal enterotoxin A. After inoculation with 1×10^4/g, the samples were incubated at 25 C for 24, 48 and 72 h. At the end of the incubation period, samples were placed in sterile Whirl-Pak bags, frozen and shipped by air freight to the testing laboratory (FDA, Washington, D.C.). Extraction of the enterotoxin was carried out using the Cassman-Bennett method (*4*). Sample elutes were tested serologically by the procedure outlined by

Bennett and McClure (*3*).

Potassium sorbate (0.25%) was added to the pumpkin pie mix before baking and inoculation with the pure cultures. The sample was incubated at 25 C for up to 3 days and samples were taken at regular intervals for enumeration of organisms.

RESULTS AND DISCUSSION

Table 1 shows the results of *S. aureus* and salmonellae survey of the pumpkin pies as purchased from retail stores and held at 25 C for 72 h. In only one out of four samples, was *S. aureus* detected and it seemed to have multiplied rapidly during storage at 25 C, reaching over 2400 organisms per g after 72 h. Salmonellae were not detected in any of the samples. The internal baking temperature of the pie reaches 108 C for 1 min (*14*); this temperature was sufficient to destroy both salmonellae and *S. aureus*.

TABLE 1. *Incidence of salmonellae and staphylococci in retail pumpkin pies stored at 25 C.*

Store	Time (h)	S. aureus/g	Salmonellae[1]
A	0	< 3	ND[2]
	24	< 3	ND
	48	< 3	ND
	72	< 3	ND
B	0	< 3	ND
	24	< 3	ND
	48	< 3	ND
	72	< 3	ND
C	0	< 3	ND
	24	460	ND
	48	1100	ND
	72	> 2400	ND
D	0	< 3	ND
	24	< 3	ND
	48	< 3	ND
	72	< 3	ND

[1]Average MPN of duplicate sample.
[2]ND - None detected.

Figures 1 and 2 show growth of *S. aureus* and *S. typhimurium* inoculated in pumpkin pie filling and held at 4, 25, and 35 C. Pumpkin pie supported growth of *S. aureus* and *S. typhimurium* at 25 and 35 C; however, 4 C effectively inhibited growth of both microorganisms. The pathogens studied increased to large numbers within a relatively short time, from an initial count of less than 100 to over a million organisms/g in 24 h at 25 and 35 C. Potassium sorbate (0.25%) inhibited growth of *Salmonella* at 25 C but was not effective against *S. aureus*. The results of the potassium sorbate treatment are shown in Fig. 3.

All samples inoculated with *S. aureus* were positive for enterotoxin A (Table 2).

Results of this experiment, showed that the microbial quality of 3 out of 4 retail pumpkin pies was good. One product contained *S. aureus* and coliforms (*14*). Pumpkin pie was able to support growth of *S. aureus* and *S. typhimurium*; therefore, it can be concluded that if contaminated and stored or displayed at room temperature, the product could become a public health hazard.
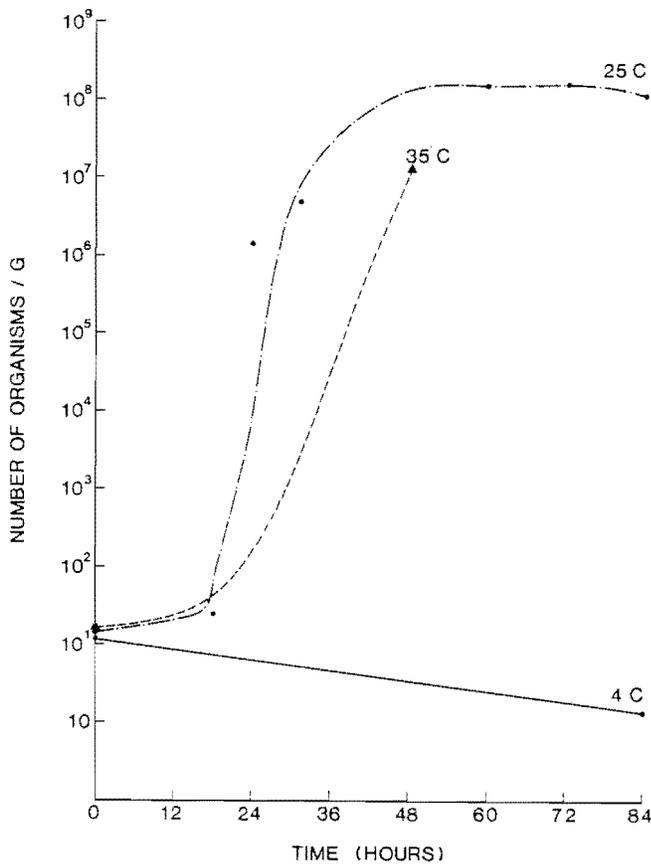
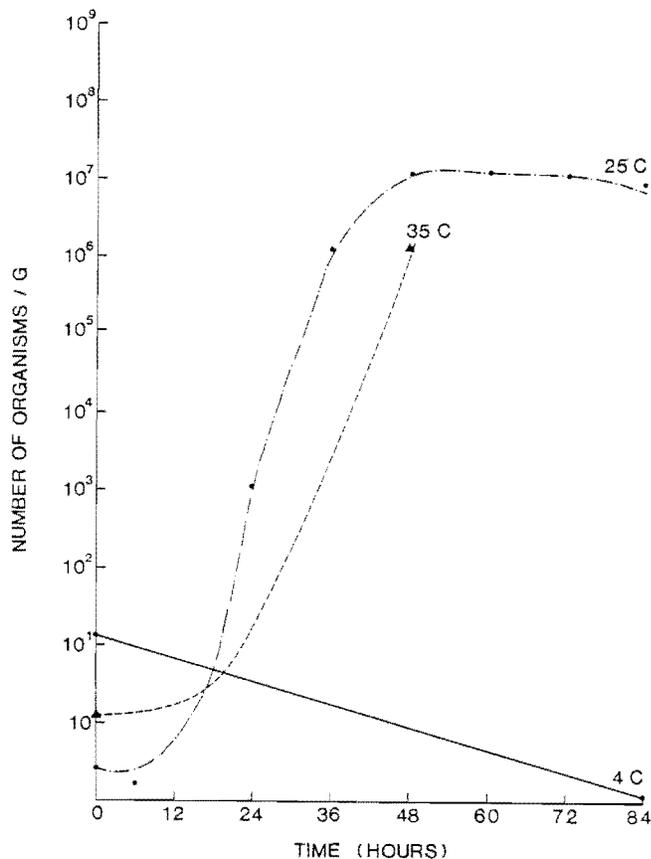Figure 1. *Growth of* S. aureus *in pumpkin pie.*

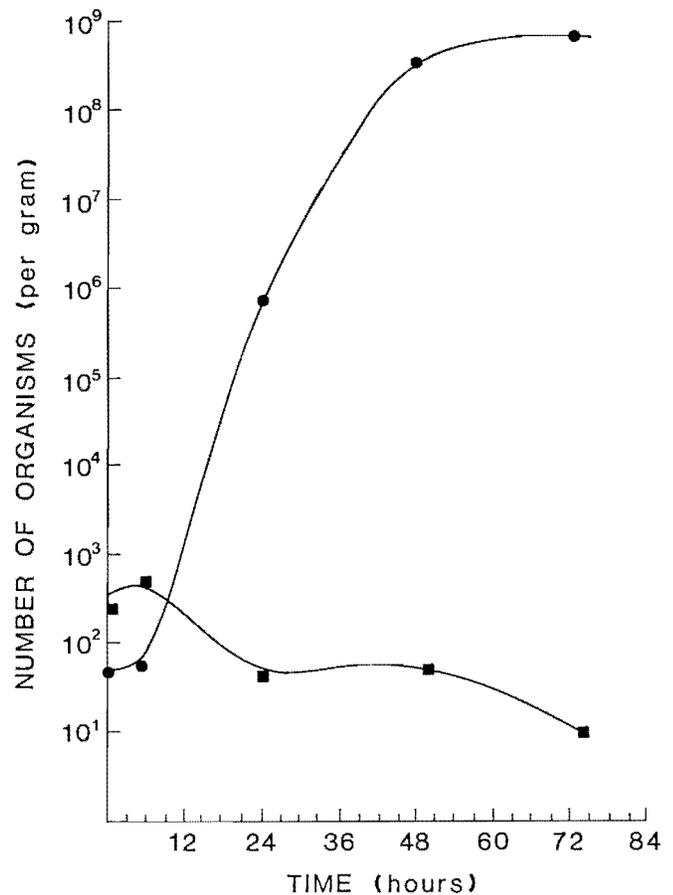Figure 2. *Growth of* S. typhimurium *in pumpkin pie.*

Figure 3. *Growth of* S. aureus (●) *and* Salmonella (■) *in pumpkin pie mix, treated with potassium sorbate (0.25%) incubated at 25 C.*

TABLE 2. *Enterotoxin A in pumpkin pie inoculated with* S. aureus.

Time (h)	Temp (C)	S. aureus/G[1]	Enterotoxin A
24	25	1.4×10^8	+
48	25	9.1×10^8	+
72	25	5.1×10^8	+
48	35	4.3×10^8	+

[1]Inoculum: 1.1×10^4 cells/g.

The baking temperature is normally sufficient to destory the pathogens, which places importance on the handling and distribution practices after baking. Pumpkin pies are not usually refrigerated but, if so, it is an effective way to control the growth of *S. aureus* and *S. typhimurium*. Potassium sorbate (0.25%) inhibited growth of salmonellae but was not effective against *S. aureus*.

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