Fate of *Salmonella newport* and *Salmonella typhimurium* Inoculated into Summer Sausage[1]

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ABSTRACT

Various fermentation schedules and finishing temperatures used in the summer sausage industry were investigated for their ability to eliminate *Salmonella newport* or *Salmonella typhimurium* inoculated into raw ingredients during the formulation phase of production. Summer sausage was produced with and without the aid of a starter culture. Use of the starter culture, a *Lactobacillus plantarum* strain, provided for a more rapid fermentation and subsequent decrease in pH. Elimination of *Salmonella* was dependent upon the initial contamination level, serotype involved, rate of fermentation and processing temperature.

Isolation of salmonellae from raw beef and pork is not uncommon. Various investigators have indicated that beef samples are usually relatively free of salmonellae contamination; the percent found to be salmonellae-positive is generally less than that of pork. Studies conducted with pork often result in positive results ranging upwards to 20 or 30% or higher *(1,6,8)*.

Both meat types can be used in the production of the fermented meat product referred to as summer sausage. It is possible, therefore, that salmonellae might be incorporated into summer sausage formulations. Such conditions as reduced water activity (a_w), increased hydrogen ion concentration (pH), elevated processing temperatures and a variety of spice and cure mixtures have all been thought to contribute to the reduction and eventual elimination of viable salmonellae that might be present in summer sausage formulations. Recently, results of studies by a number of authors have demonstrated the ability of salmonellae to survive some of the conditions encountered during the production of

similar fermented meat products *(2,3,4,5)*. However, no salmonellosis outbreaks have been attributed to consumption of summer sausage. The knowledge that salmonellae can be contaminants of the ingredients used to produce this product prompted these investigations.

A wide variety of fermentation schedules and finishing temperatures are used in the summer sausage industry. In addition, some producers use bacterial starter cultures while others depend on the growth of naturally occurring lactic acid producing bacteria to ferment their product. Therefore, this study was undertaken with the following goals: (a) to evaluate the effectiveness of a wide range of fermentation schedules and finishing temperatures used in the summer sausage industry in the elimination of *Salmonella* and (b) to ascertain the effect a starter culture has on the survival of *Salmonella*.

MATERIALS AND METHODS

Bacterial cultures

Salmonella cultures used in these studies were *Salmonella typhimurium* and *Salmonella newport*. Both were chosen because of their frequent role in foodborne salmonellosis outbreaks. The *S. newport* used had been isolated from a foodborne (beef) salmonellosis outbreak and was obtained from the Food Safety and Quality Service, USDA, Beltsville, MD. *S. typhimurium* was a laboratory strain that had been maintained for a number of years in our laboratory. Starter culture *Lactobacillus plantarum*, strain T, was isolated from a fermented sausage by American Bacteriological and Chemical Research Corporation personnel. It has been developed as an USDA-approved starter culture for use in fermented sausage.

Stock cultures of *Salmonella* were maintained on nutrient agar, and *L. plantarum* cultures were maintained on APT agar slants. All media and reagents were products of Difco Laboratories, Detroit, MI. Working cultures of each were prepared by daily transfers into Brain Heart Infusion broth (BHI) or APT broth, respectively.

All inocula were prepared by diluting a 24-h-old culture of the appropriate organism in 100 ml of sterile 0.85% saline solution to obtain the desired concentration of cells for each experimental batch. *Salmonella* cultures were diluted to yield inoculum levels of

TABLE 1. *Inoculum levels of Salmonella[a] strains used in artificially contaminated summer sausages.*

	S. newport			S. typhimurium		
Schedule	High	Intermediate	Low	High	Intermediate	Low
I	16000	120	24	6000	90	2
II	7400	120	7	15000	120	17
III	7700	450	ND[b]	3100	90	ND

[a]Plate count/100 g of sausage product.
[b]ND, Not determined.

approximately 5000, 50 and 5 *Salmonella*/100 g of sausage batter. *L. plantarum*, strain T, was diluted to yield approximately 10^6 Colony Forming Units (CFU)/g of sausage batter. Actual levels of inocula used in all schedules were determined by plating in triplicate serial dilutions of each inoculum before use and are given in Table 1. Plate count and APT agar, respectively, were used to accomplish this.

Raw materials

Fresh beef chucks and beef trimmings were purchased from a local federally inspected processing plant. All meat was held at 4.4 C (40 F) and used within 48 h of purchase. Spice and cure components were also purchased commercially.

Sausage preparation

Fat and lean portions of beef were initially ground separately through a Hobart chopper, Model 4732, (Hobart Mfg. Co., Troy, OH) fitted with a 12.7-mm (½ in) plate. This ground meat was then formulated to a fat level of 25 to 30% and mixed for 30 sec, using a Keebler mixer (Keebler Engineering Co., Chicago, IL). Fat levels were determined, using the Rapid Fat Test Method described by Terrell and Hoffman (7). Sodium chloride (2.5%), glucose (1%), garlic salt (0.013%), black pepper (0.4%), sodium erythorbate (0.039%) and a nitrite cure (0.013%) were added to the meat and mixed for an additional 2 min. If the fermentation schedule to be tested required addition of a starter culture, it was added at this mixing step after all the other ingredients had been blended with the meat and then was mixed for an additional 30 sec.

Sausage batter was subdivided into seven 6.8-kg (15 lb) portions, with one set aside as "control" product. The remaining six portions (sub-batches) received appropriate dilutions of one of the two strains of the *Salmonella* inoculum and were mixed for an additional 30 sec. Each serotype was added separately with the lowest level of salmonellae being introduced first. When the *Salmonella* serotype was changed, the mixer, grinder and stuffer were cleaned and sanitized thoroughly before addition of the second serotype.

Each sub-batch was then reground through the chopper, using a 6.35-mm (1/4 in) plate and stuffed into 5.08-mm (2 in.) fibrous casings (Tee Pak Mfg. Co., Chicago, IL) with a Frey, Electro-Hydraulic Stuffer. Seven sausages, each roughly 1.94 kg (2 lb), were produced from each sub-batch. Sausages were hung in a Vortron smokehouse (Model TR, Vortron, Inc., Beloit, WI) at 35 C (95 F) with an Equilibrium Relative Humidity (ERH) of 90% (tabular measurement) until a pH of 5.0 was attained and then processed (heated) to a final internal temperature of 65.6 C (150 F). Temperatures were monitored visually by using standard meat thermometers (Kock Supplies, Inc., Kansas City, MO) placed in the center of two "sticks" of sausage located at each side of the smokehouse. Fahrenheit thermometers were used in this study; centigrade recordings are also included for clarity.

Fermentation schedules

Three fermentation schedules were studied: I) a delayed incubation/ fermentation wherein, after formulating, the product was incubated for 48 h at 4.4 C (40 F) to allow the naturally occurring lactic acid producing flora to increase in numbers, II) a fermentation process without a starter culture (no delayed incubation) and III) a fermentation process with addition of a commercial starter culture. These schedules were selected as they represent common industrial practices. All three schedules provided for heat treatment through the same temperature ranges. Sausages were analyzed for the presence of salmonellae, total aerobes/g and pH values at the same 11 processing intervals with one exception: test schedule I was analyzed after a 48-h

"lay-down" period in addition to the other 11 test intervals. The intervals selected represent the diversity of commercial processing schedules. Table 2 shows the test intervals used throughout these investigations.

TABLE 2. *Sampling intervals used for* Salmonella *detection in schedules I, II and II.*

Initial (Before fermentation)
4.4 C (40 F) 48 h (I Only)
Attainment of pH 5.0
44.4 C (112 F) Internal[a]
44.4 C (112 F) 1 h
44.4 C (112 F) 3 h
44.4 C (112 F) 5 h
48.9 C (120 F) Internal
48.9 C (120 F) 1 h
48.9 C (120 F) 3 h
54.4 C (130 F) Internal
65.6 C (150 F) Internal

[a]Temperatures refer to internal product temperature at center of sausage.

Salmonella analyses

Fifty-gram portions were preenriched, using 450 ml of nutrient broth incubated at 35 C for 24 h. This same incubation temperature/time [35 C (95 F), 24 h] period was used throughout these investigations. Selective enrichment followed, using tetrathionate broth. Resulting growth was streaked onto Bismuth Sulfite, *Salmonella-Shigella* (SS) and Xylose Lysine Desoxycholate (XLD) agar plates and multiple colonies showing typical *Salmonella* reactions on each of these plates were picked into Lysine Iron and Triple Sugar Iron Agars. Transfers were then made from each into BHI and serological analysis performed using a Poly H, A-I antiserum. Poly H negative isolates were recultured into Brain Heart Infusion broth and re-examined for Poly H agglutination after 18 h of incubation at 35 C. Such isolates also were tested for typical malonate and KCN reactions.

Total aerobic plate counts

Aerobic plate counts (APC) were determined using 25-g sample portions blended with 225 ml of Butterfield's diluent. Dextrose tryptone agar, supplemented with 1% yeast extract, was used to approximate the percentage of lactic acid producing organisms present. Incubation was at 35 C for 48 h.

pH

The pH was determined by using a Corning, Model 7, pH meter (Corning Glass Works Co., Medfield, MA). Twenty-five-gram portions of sausage were used in preparing 1:10 dilutions of sausage in distilled water.

RESULTS AND DISCUSSION

Before analyzing the effectiveness of the three schedules in regard to *Salmonella* survival, microbiological and chemical screening of the raw ground beef was conducted. This was done immediately after the initial grinding and preceding the mixing of fat and lean

portions. The microbiological analyses consisted of total aerobic plate counts (APC) at 35 C (95 F) for 48 h and a *Salmonella* analysis. The APC ranged from 10^4 to 10^5/g. No naturally occurring salmonellae were detected in any of the meats used for these investigations. The chemical parameters of concern were the determination of pH and fat levels. The initial pH of the stuffed sausage was 5.8. Fat levels of all test batches were between 25 and 30%.

Schedule I

No starter culture was used in this schedule; however, the delayed (48 h) incubation at 4.4 C (40 F), commonly referred to in industry as "laying down," allowed the natural lactic acid producing bacterial flora to increase markedly. After the laying down period, the sausage was placed in the smokehouse and incubated at 35 C (95 F) with a relative humidity of 90%. After 10.5 h, a pH value of 5.0 was attained, and heat was then applied. Six and one-half hours of heating was required before the product reached an internal temperature of 65.6 C (150 F).

The mean APC/g of sausage for Schedule I was higher than that encountered in Schedule II (10^7 vs 10^6), which did not use a starter culture, but lower than that encountered in Schedule III (10^7 vs 10^8), which did use a starter culture. The pH values observed among the different sub-batches remained relatively constant once the pH 5.0 sampling interval was attained. The average pH of all the sausage samples in this schedule after heat application began was 4.8.

Schedule II

Without the aid of a starter culture, this product required 46 h at 35 C (95 F) with a relative humidity of 90% before the pH decreased from an initial value of 5.8 to 5.0. When the pH reached 5.0, heat processing was initiated. Five hours in the smokehouse was required before the product reached the last sampling interval, that being an internal temperature of 65.6 C (150 F).

Within this schedule, as in the other two, there were no major fluctuations of APCs or pH values observed among the different sub-batches at the same sampling intervals. Total APCs did not change appreciably from the time the product reached a pH of 5.0 until an internal temperature of 54.4 C (130 F) was attained and ranged from 1.0 to 7.0 × 10^7/g. In addition, pH values did not change appreciably during this same period and averaged 5.0.

When the sausage was "finished" at an internal temperature of 65.6 C (150 F), APCs were reduced to approximately 1.0 × 10^3/g. Surviving bacteria were nearly all lactic acid producing bacteria in samples before finishing, but were all aerobic spore formers, i.e., *Bacillus*, in samples taken at finishing at 65.6 C (150 F).

Schedule III

After examining the results of Schedules I and II, it was decided that the inoculum level of 5 salmonellae/100 g of sausage batter could be omitted from Schedule III. This decision was made because no salmonellae had been detected past initial stages of sausage production.

Summer sausage processed directly with the addition of a bacterial starter culture required 18.5 h at 35 C (95 F), with a relative humidity of 90%, before pH 5.0 was attained. An additional 4.5 h was required to process the product to an internal temperature of 65.6 C (150 F).

APC and pH values remained relatively stable once a pH of 5.0 was reached through 3 h of processing at an internal temperature of 48.9 C (120 F). As the product reached 54.4 C (130 F), a 10-fold decrease in APC was noted. At 65.6 C (150 F), survivors had decreased to 10^3 CFU/g and were members of the genus *Bacillus*. The average pH of samples receiving heat treatment was 4.75. Table 3 indicates that the last *Salmonella*-positive sample in this schedule was found early in processing [44.4 C (112 F) internal temperature held for 1 h].

TABLE 3. *Sampling intervals at which the last* Salmonella-*positive sausages were detected.*[a]

Schedule	Serotype	Last *Salmonella*-positive sample interval[b]
I Delayed incubation, natural flora	*S. typhimurium*	After 48 h of incubation at 4.4 C (40 F)
	S. newport	44.4 C (112 F) 5 h
II Natural flora	*S. typhimurium*	48.9 C (120 F) 1 h
	S. newport	54.4 C (130 F)
III Natural flora, with starter culture	*S. typhimurium*	Initial (before fermentation)
	S. newport	44.4 C (112 F) 1 h

[a]High inoculum level (5000 salmonellae/100 g of sausage batter).
[b]Temperatures are internal product temperatures.

Salmonella survival was greatly affected by the processing temperature and the time of sampling. Fifty-one of the 216 samples analyzed were *Salmonella*-positive. Of these positives, 27 were recovered from sausage after heat-processing had begun, i.e., at sampling intervals of 44.4 C (112 F) or greater. Twenty (74%) were recovered from sausage processed to 44.4 C (112 F), 6 (22%) from sausage processed to 48.9 C (120 F), and 1 (4%) from sausage processed to 54.4 C (130 F). Decreasing numbers of *Salmonella*-positive samples indicate that the effect of increasing processing temperature plays a major role in destruction of salmonellae in this product.

Fermentation rate also affected survival of salmonellae, as shown in Table 3. These fermentation times are defined herein as the length of time required for the sausage to attain a pH of 5.0 at 35 C (95 F) incubation temperature. Schedule I, using a 4.4 C (40 F), 48 h "lay-down", required 10.5 h of fermentation at 35 C (95 F) before a pH of 5.0 was attained. This appears to be the least time required to achieve a pH of 5.0; however, the pH of the sausage batter after the 48-h lay-down period and immediately before the start of the fermentation

phase was 5.4, as compared to 5.8 to 5.9 for the other two schedules. Fermentation apparently had begun during the 4.4-C (40 F) incubation period. *Salmonella* was isolated from samples after 5 h of processing to an internal temperature of 44.4 C (112 F), but was not found when an internal temperature of 48.9 C (120 F) was reached.

Schedule II, showing the slowest fermentation rate, was the least restrictive to *Salmonella* survival. This schedule required a fermentation time of 46 h. *Salmonella* was recovered from sausage that had been heat-processed to an internal temperature as high as 54.4 C (130 F).

Schedule III required 18.5 h of fermentation time to attain the desired pH. *Salmonella* was recovered from sausage that had been subjected to an internal temperature of 44.4 C (112 F) for 1 h. No *Salmonella* was recovered at any subsequent sampling interval which indicated that less heat-processing was required with Schedule III than with the other schedules to eliminate *Salmonella*.

Sausage batter inoculated with highest level of *Salmonella* (5000/100 g) remained *Salmonella*-positive for a longer time than those receiving the lower levels of *Salmonella*. Of the 51 samples positive for salmonellae obtained during this study, 32 (63%) had received the highest *Salmonella* inoculum. The other two levels of *Salmonella*, 50 and 5/100 g of sausage batter, resulted in 15 (29%) and 4 (8%) salmonellae-positive samples, respectively. *S. newport* appeared to be more resistant than *S. typhimurium* to conditions encountered during production of summer sausage in these investigations (Table 3), although initial inoculum levels were not identical.

As a result of these investigations, it is apparent that the fate of *Salmonella* in fermented summer sausage was affected by a combination of four factors: (*a*) the processing time/temperature at which the sausage was analyzed, (*b*) the rate of fermentation demonstrated by the three schedules, (*c*) the level of *Salmonella* contamination present and (*d*) possibly the particular serotype of *Salmonella* present as a contaminant. Under the conditions of these experiments, neither strain of *Salmonella* survived in the finished sausage products.

In these investigations, the use of a starter culture (*a*) produced a more rapid reduction of pH allowing a shorter processing time and (*b*) enhanced the destruction of added salmonellae.

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