Growth of *Staphylococcus* and *Salmonella* on Frankfurters With and Without Sodium Nitrite

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Conventional and nitrite-free frankfurters in loosely wrapped packages were compared as to their ability to support growth of *Salmonella*, *Staphylococcus*, and their naturally occurring spoilage flora at 7 C (simulating refrigerated storage) and 20 C (simulating possible temperature abuse). At 7 C *Salmonella* did not grow in either type of frankfurter; *Staphylococcus* and the natural spoilage flora sometimes grew more rapidly in the absence of nitrite, but the difference was not significant. At 20 C growth of *Salmonella*, *Staphylococcus*, and of the spoilage flora was, at most, only slightly faster on nitrite-free frankfurters. *Salmonella* was not suppressed in broth culture experiments at the pH and nitrite content found in frankfurters. Although either type of frankfurter can become hazardous due to growth of *Salmonella* or *Staphylococcus*, no unusual or additional hazard resulted from the omission of nitrite from frankfurters.

That the incorporation of sodium nitrite in cured meats inhibits the growth of some food-poisoning bacteria has long been known (16, 22). Under some conditions it is also bactericidal (23, 24). Nitrite also plays a role in the flavor and heat stable color of processed meats and originally may have been used primarily to impart these characteristics.

In recent years there has been an increasing public consciousness of the use of additives in foods. Results of toxicological (17, 18) and other investigations (1, 9, 27) have caused concern about the effect of additives, including nitrates, on the health of consumers. In response to this public interest, one large local food chain arranged for a meat processor to provide both nitrite-containing (cured or conventional) and nitrite-free (uncured) frankfurters for its retail meat counters. The cured frankfurters are vacuum-packed in plastic by the processor, whereas the uncured are shipped in bulk and loosely wrapped at the retail level. The uncured frankfurters have a gray appearance and slightly different taste. These unusual characteristics, and the lack of nitrite, prompted our interest in their microbiological safety.

Our objective was to observe the growth characteristics of *Staphylococcus* and *Salmonella* to determine whether they present a greater health hazard on nitrite-free frankfurters, if exposed to mishandling, than on similarly treated conventional frankfurters. A secondary objective was to determine whether deterioration caused by harmless spoilage organisms takes place more rapidly in nitrite-free frankfurters.

**MATERIALS AND METHODS**

The National Canners Association Laboratory, Berkeley, and the California State Public Health Laboratory, Berkeley, supplied the cultures of *Staphylococcus aureus* 413 and *Salmonella enteritidis* 2179, respectively. *S. typhimurium* Tm-1 was from our own collection. Experimental inocula of these strains were grown on a rotary shaker at 28 C for 60 h in Trypticase soy broth (BBL) containing 2% yeast extract (Difco). The cells were harvested by centrifugation, washed twice in distilled water, and resuspended in 100 ml of sterile demineralized water, giving a count of 10^9 or 10^10/ml. They retained their viability during several months of storage at 7 C.

Nitrite-free and conventional frankfurters were obtained from a local market or directly from the processor. Their pH ranged from 5.2 to 5.8, but was usually about 5.5. They were inoculated either internally, by injecting 0.5 ml of a 10^9/ml cell suspension of the test organism into each end of the frankfurter, using a 2.5-ml syringe with a 8-cm needle, or externally, by immersion in a water suspension containing 10^9 cells/ml for 2 min and draining until almost dry. The inoculated frankfurters were placed in plastic pallets, which were then loosely wrapped with plastic film and incubated at either 7 or 20 C.

Sampling times were 0, 2, 3, 4, 7, 10, and 15 days for 7 C incubated sausages and 0, 2, 4, 6, and 8 days for 20 C incubated sausages. At each sampling date, two whole frankfurters (about 45 g each) were aseptically removed. Separate counts were made on each as follows.
Staphylococcus. The samples were blended in Trypticase soy broth (BBL) containing 2% yeast extract (Difco), diluted in the same medium with 9.5% sodium chloride added, spread plated on Vogel Johnson Tellurite agar (BBL), and incubated at 37°C for 48 h (3).

Salmonella. For a three-tube most probable number method, lactose broth was used as a blending, diluting, and pre-enrichment medium, brilliant green tetrazionate broth was used as an enrichment medium, and brilliant green sulfadiazine agar was used for a confirmatory test (10). Incubation was at 37°C.

Total aerobic counts. These were done simultaneously with counts of Staphylococcus or Salmonella; thus, the blending media were as stated above. Subsequent dilutions were made immediately in Trypticase soy broth with 2% yeast extract, followed by spread plating on agar of the same composition and incubating at 28°C for 2 days.

When the population increased, typical growth curves for Staphylococcus and for the total population (total counts) were drawn from the counts after various storage periods. The time required to reach an arbitrarily chosen population was taken as an end point for comparison of conventional with nitrite-free frankfurters. The counts on Salmonella were irregular because the most probable number method lacks precision, but it was possible to determine the approximate storage time at which the count first surpassed 10⁶/g. When no growth occurred, the counts decreased and we compared the numbers of surviving organisms in the conventional and nitrite-free frankfurters.

The direct effect of nitrite on aerobic growth of Salmonella in broth was determined in 250-ml nephelometer flasks. The broth contained Tryptone (Difco), 1%; yeast extract (Difco), 0.5%; NaCl, 0.3%; dextrose, 1%; and KH₂PO₄, 0.5%. The pH was adjusted to several levels in the range 4.6 to 7.5 with NaOH or phosphoric acid. A 10% stock solution of sodium nitrite sterilized through a Morton filter apparatus was added to give several final concentrations up to 8,000 μg/ml. Each flask, containing 50 ml of broth, was inoculated and incubated at 28°C for 48 h. Growth was measured with a Klett-Summerson photoelectric colorimeter equipped with a red filter (640 to 700 nm).

Nitrite concentrations were determined by the method of Greiss (2). This method does not detect reaction products of the nitrite with the meat, although these may have physiological activity.

RESULTS

Staphylococcus. When inoculated frankfurters were incubated at 20°C, their count increased logarithmically to 10⁹/g to 10⁶/g within 2 to 8 days. Their count reached 10⁹/g in 2 to 6 days without nitrite and in 2 to 8 days when cured (Table 1). There was no consistent difference between the conventional and the nitrite-free frankfurters. Thus, omission of the nitrite did not permit any spectacular increase in the growth rate of the Staphylococcus on nitrite-free as compared with conventional frankfurters. In one experiment (b, Table 1), growth on the conventional frankfurters was very slow and never reached 10⁶/g. The reason for this is unknown, but a dependable inhibiting action of nitrite is not indicated as it was not seen in the other experiments.

In similar experiments incubated at 7°C, growth was logarithmic after a lag period but much slower. As the count did not always reach 10⁶/g during the 15-day storage period, a count of 10⁶/g was taken as the end point for comparison. In three experiments (Table 2) growth was found on nitrite-free but not on the conventional frankfurters during the 15-day incubation period. In the three other experiments, growth was found both in the presence and the absence of nitrite. The lack of growth in the presence of nitrite probably reflects the fact that this temperature is very near the minimum growth temperature for Staphylococcus. In general, bacteria become unusually fastidious at temperatures near their minima (20); thus, the nitrite could prevent growth if it con-

| Method of inoculation | Days to reach 10⁶ cells/g With nitrite | Without nitrite |
|-----------------------|--------------------------------------|-----------------|
| Injected              |                                      |                 |
| Expt a                | 5.3                                  | 2.5             |
| Expt b                | 2.0                                  |                 |
| Immersed              |                                      |                 |
| Expt a                | 3.5                                  | 4.0             |
| Expt b                | 2.5                                  |                 |
| Expt c                | 5.6                                  |                 |
| Expt d                | 3.3                                  |                 |

| Method of inoculation | Days to reach 10⁶ cells/g With nitrite | Without nitrite |
|-----------------------|--------------------------------------|-----------------|
| Injected              |                                      |                 |
| Expt a                | Slight growth                        | 9               |
| Expt b                | No growth                            | 10              |
| Immersed              |                                      |                 |
| Expt a                | 15                                   | 9               |
| Expt b                | No growth                            | 20*             |
| Expt c                | 12                                   | 9               |
| Expt d                | 9                                    | 14              |

* By extrapolation.
fers even a slight additional physiological burden on the experimental organism.

Salmonella. After about 2 days at 20 C, the count increased and the time required to reach a population of 10⁸/g is shown in Table 3. Although variable, this time was not consistently less for the nitrite-free frankfurters than for the conventional. Growth was rapid enough on both types, however, to render them hazardous within a few days at 20 C.

When frankfurters were inoculated similarly and stored at 7 C for 15 days, Salmonella did not grow, but survived at about the original level for the duration of the experiment (Table 4). The counts were irregular and not in a pattern indicating any relationship between survival and the presence of nitrite in the frankfurters. Since Salmonella has not been observed to grow below 5.5 C (19, 20), and the pH of these frankfurters (about 5.5) is not optimal for Salmonella, it is not surprising that no growth was observed at 7 C. Comparable survival but no growth has been observed at 5 C for Salmonella on surfaces of bologna (11).

Total counts. Initial total aerobic counts on frankfurters, with and without nitrite, were between 10² and 10⁴ cells/g but usually 10³ cells/g. Throughout these experiments total aerobic counts were made simultaneously with those of surviving Staphylococcus and Salmonella, and always greatly exceeded the latter, usually by a factor of 100 or more. The total counts soon reached 10⁹/g, which was chosen arbitrarily as an end point. The time required for nitrite-free frankfurters to reach this point was compared with that for conventional frankfurters (Table 5). These data are segregated according to whether the frankfurters were inoculated with Staphylococcus. Others inoculated with Salmonella.

### Table 3. Growth of Salmonella enteridis at 20 C on conventional and nitrite-free frankfurters

| Method of inoculation | With or without nitrite | Days to reach a count of 10⁶ g/day |
|-----------------------|-------------------------|-----------------------------------|
|                       |                         | 4 to 6 | 6 to 8 | Over 8 |
| Injected              |                         |        |        |        |
| Expt a                | With                    | X      |        |        |
|                       | Without                 | X      |        |        |
| Expt b                | With                    |        | X      |        |
|                       | Without                 |        |        | X      |
| Immersed              |                         |        |        |        |
| Expt a                | With                    | X      |        |        |
|                       | Without                 |        | X      |        |
| Expt c                | With                    |        |        | X      |
|                       | Without                 |        | X      |        |
| Expt d                | With                    | X      |        |        |
|                       | Without                 |        |        | X      |

### Table 4. Survival of Salmonella enteridis at 7 C on conventional and nitrite-free frankfurters

| Method of inoculation | With or without nitrite | (Three-tube) Most probable number count/g after |
|-----------------------|-------------------------|-----------------------------------------------|
|                       |                         | 0 Days | 7 Days | 15 Days |
| Injected              |                         |        |        |        |
| Expt a                | With                    | 5      | 0.2    | 1      |
|                       | Without                 | >11    | 4      | 2      |
| Expt b                | With                    | 11     | 2      | 0.4    |
|                       | Without                 | 5      | 2      | 0.4    |
| Immersed              |                         |        |        |        |
| Expt a                | With                    | 0.4    | <0.3   | 0.4    |
|                       | Without                 | 0.2    | 0.4    | <0.3   |
| Expt b                | With                    | 0.2    | 0.2    | 0.1    |
|                       | Without                 | 0.2    | 0.04   | <0.03  |
| Expt c                | With                    | 2      | 2      | 0.6    |
|                       | Without                 | 5      | 4      | 3      |
| Expt d                | With                    | 3      | 13     | 4      |
|                       | Without                 | >11    | 7      | 27     |

### Table 5. Total counts on frankfurters during storage

| Storage temp | Method of inoculation | Days to reach 10⁶ cells/g | Reduction of over 25% in absence of nitrite |
|--------------|-----------------------|---------------------------|-------------------------------------------|
| 7 C          | Injected              |                           |                                           |
| Expt a       | 9.0                   | 8.0                       | +                                         |
| Expt g       | 13.0                  | 6.8                       | +                                         |
| Expt h       | 2.3                   | 6.5                       |                                           |
| Immersed     |                       |                           |                                           |
| Expt a       | 10.5                  | 7.5                       | +                                         |
| Expt e       | 9.8                   | 8.0                       |                                           |
| Expt f       | 9.8                   | 12.0                      |                                           |
| Expt g       | 12.5                  | 9.5                       | +                                         |
| Expt h       | 9.8                   | 9.0                       |                                           |
| Expt k       | 13.3                  | 8.8                       | +                                         |
| Expt l       | 10.5                  | 12.3                      |                                           |
| Avg          | 10.1                  | 8.9                       |                                           |

| 20 C         | Injected              |                           |                                           |
| Expt c       | 3.5                   | 1.3                       | +                                         |
| Expt d       | 1.5                   | 1.0                       | +                                         |
| Expt i       | 2.3                   | 2.0                       |                                           |
| Expt j       | 2.3                   | 1.8                       |                                           |
| Immersed     |                       |                           |                                           |
| Expt c       | 2.8                   | 1.3                       | +                                         |
| Expt d       | 1.5                   | 1.3                       |                                           |
| Expt e       | 3.8                   | 3.0                       |                                           |
| Expt f       | 2.0                   | 2.3                       |                                           |
| Expt i       | 0.3                   | 1.8                       |                                           |
| Expt j       | 2.0                   | 1.8                       |                                           |
| Expt k       | 2.5                   | 2.5                       |                                           |
| Expt l       | 2.0                   | 2.0                       |                                           |
| Avg          | 2.2                   | 1.84                      |                                           |

a Also, inoculated with Staphylococcus. Others inoculated with Salmonella.
ulated internally or externally, but there seems to be no difference between these two treatments. The storage time before spoilage at 7 C is short compared with commercial practice. However, these frankfurters were not packed or treated as they would be commercially. Also, it has been reported (8) that organoleptic spoilage is not reached in frankfurters until the microbial population reaches 10^6/g.

At both 7 and 20 C, the storage time required to reach a total count of 10^6/g was often less in the nitrite-free frankfurters, but the time difference was usually small and only exceeded 25% in 7 of the 22 trials (Table 5). It is concluded that the nitrite did not enhance the microbiological stability of the frankfurters to an important degree.

**Effect of thermal process on flora.** It is known that the raw meat components of frankfurters bring to the sausage mix a rich and varied flora (21). Our total counts on two samples of the raw emulsion were 3 x 10^9 and 7 x 10^9 cells/g. The frankfurters were cooked for 1.25 h, with a maximum internal temperature of 70 C. After cooking, counts were from 3 x 10^9 to 10^10 cells/g in conventional frankfurters and 3 x 10^9 to 6 x 10^9 cells/g in nitrite-free frankfurters. This is a reduction in count of two to five orders of magnitude during cooking.

**Residual nitrite in frankfurters.** The maximum legally allowable nitrite concentration is 156 µg/g but this diminishes rapidly. In our tests, 39 µg/g remained after processing, 27 µg/g after 5 days of additional storage at 7 C, and 5 µg/g after 15 days at 7 C. This agrees with the more comprehensive data of Hustad et al. (14).

**Effect of pH on effective nitrite concentration.** To observe the effect of nitrite on growth of *Salmonella* in a tryptone broth medium, *S. enteritidis* and *S. typhimurium* were inoculated into 50 ml of broth at levels of 8 and 40 cells/ml, respectively, and incubated at 28 C for 48 h. The nitrite concentration varied from 0 to 8,000 µg/ml and the pH varied from 4.6 to 7.5. The inhibitory effect of nitrite was strongly dependent on pH and, at the concentration permitted in meat, nitrite was ineffective above pH 6 (Fig. 1). The nitrite concentration in the broth remained approximately constant during the course of the experiment, in contrast to that in the frankfurters. That pH influences the bacteriostatic effect of nitrite has been shown for *Staphylococcus* by others (5, 6, 23).

**DISCUSSION**

Each of the growth-survival experiments (Tables 1 through 5) was repeated several times with varying results. Nevertheless they present consistent patterns showing little difference between the conventional and the nitrite-free frankfurters. Each lot of frankfurters was from a different commercial batch although from the same manufacturer. Also the conventional and nitrite-free frankfurters within an experiment were never from the same batch of meat emulsion. For these reasons, variation is to be expected.

*Staphylococcus* and *Salmonella* are known to be present in the raw meat used in sausage manufacture (13, 21, 25, 26). They are not usually found in frankfurters because they cannot survive the heat process (21). Also our data, showing that cooking reduces the total count by several orders of magnitude, indicate that *Staphylococcus* and *Salmonella* would suffer similar depletion and so could survive only after gross contamination of the raw meat emulsion.

After cooking, the frankfurters can be contaminated on the packaging line, during warehousing and distribution, in the retail storage and preparation area, in retail supply cabinets, or in the home. Vacuum packaging only eliminates part of these chances for contamination. *Salmonella* is known to be carried by frankfurters (7).

Since nitrite-free frankfurters are being sold locally and their sale and use is being advocated nationally, it is important to know whether the omission of nitrite will make them a more suitable substrate for *Staphylococcus* and *Salmonella*, and therefore more likely to carry these organisms to the consumer. Our data (Tables 1 and 3) show that these bacteria grow about equally on the conventional and the nitrite-free frankfurters when loosely wrapped for storage; hence nitrite does not inhibit growth under these conditions.

There is other evidence that nitrite is not strongly inhibitory against *Staphylococcus* in frankfurters. *S. aureus* can multiply in the presence of extremely high concentrations of sodium nitrite under aerobic conditions and, even at a pH as low as pH 5.4, it can grow in the presence of 140 µg of nitrite per g (6). In our experiments, the plastic wrappings used during incubation did not exclude oxygen, and the nitrite concentration was far below 140 µg/g. Also, meat itself provides some protection to the microorganisms against the inhibitory effect of nitrite (16), thus minimizing any difference between growth on nitrite-free and on conventional frankfurters.

Our broth culture experiments also showed that growth of *Salmonella* was not prevented
by the residual nitrite concentration (39 μg/ml) and pH (about 5.5) of our frankfurters (Fig. 1).

Thus the nitrite concentration present in conventional frankfurters is insufficient to prevent growth of Staphylococcus and Salmonella, either in this type of frankfurter or in inoculated nutrient broth. Therefore, the omission of the nitrite does not present any new or unusual hazard from these organisms. Either type of frankfurter, if subjected to contamination by Staphylococcus or Salmonella followed by temperature abuse, can become hazardous.

Frankfurters can be spoiled by nonhazardous spoilage organisms if they are subjected to temperature abuse. These organisms can survive the heat treatment in moderate numbers, and they can also be present as a result of post-processing contamination. However, they do not grow faster to an important degree on the nitrite-free frankfurters as compared with the conventional (Table 5); hence, the omission of the nitrite will not result in a product that requires extra precautions to prevent spoilage.

In experiments with Clostridium botulinum in bacon and frankfurters, the inhibitory action of nitrite depended on its level at the time of processing rather than its residual level during storage (3, 12, 14). This suggests that nitrite reacts with the meat to form other antibacterial compounds (22), and the effect of such compounds has been investigated (15). In our studies, frankfurters were inoculated with Salmonella and Staphylococcus after processing, thus simulating the probable route by which contamination could occur in commercial and home food handling. Under these conditions we found no evidence that nitrite, added at the maximum permitted level before processing, prevented growth of these organisms by itself or through the formation of antibacterial reaction products.

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