Effect of Sodium Ascorbate and Sodium Nitrite on Toxin Formation of Clostridium botulinum in Wieners

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Toxin production by Clostridium botulinum was inhibited by sodium nitrite levels above 50 μg/g of wiener. Sodium ascorbate at levels of 105 and 655 μg/g of product did not decrease the effectiveness of the sodium nitrite inhibition, nor did sodium ascorbate potentiate it. The results indicate that the use of sodium ascorbate in vacuum-packaged wiener does not appreciably alter the inhibition of C. botulinum toxin formation by sodium nitrite.

Previous studies in our laboratory demonstrated the effectiveness of sodium nitrite (nitrite) in preventing the formation of Clostridium botulinum toxin in wieners that were temperature abused (1). In many cured meat items, current industry practice includes the addition of sodium ascorbate (ascorbate) to accelerate the formation and improve the stability of cured meat pigments. Little information is available, however, regarding possible changes incurred by ascorbate on the effectiveness of nitrite in controlling toxin formation in these products. Conceivably, ascorbate could enhance the growth of C. botulinum by decreasing the redox potential. Also, it could reduce the effectiveness of nitrite inhibition by reacting with the nitrite and thus lowering its concentration. On the other hand, the possibility exists that ascorbate may potentiate the inhibition by nitrite. To gain information regarding these possible effects of ascorbate, wieners were prepared as follows.

The wiener premix (without nitrite or ascorbate) was prepared according to the following formula: pork, 39.82%; beef, 34.11%; water, 3.20%; ice, 17.14%; salt, 2.52%; dried corn syrup solids, 1.80%; dextrose, 1.02%; and spice, 0.39%. Eighteen batches of product were made with six levels of nitrite (0, 15, 30, 50, 100, and 150 μg/g) and three levels of ascorbate (0, 105, and 655 μg/g). Each batch of product was inoculated with a heat-shocked preparation of C. botulinum spores. The inoculum was adjusted so that approximately 1,000 spores per g of raw premix were added. Spore levels in the premix and thermally processed product were estimated by decimal dilution and inoculation of thioglycolate broth, followed by toxicity testing (intraperitoneal inoculation of mice) of the incubated cultures. A 10-tube most-probable-number procedure was used. The spore preparation consisted of approximately equal numbers of the following strains: type A—63A, 75A, 62A, 109A, and 3A; type B—53B, 213B, 113B, 169B, and Lamanna. The total viable counts (30 C, 40 h) were made by using APT agar (Difco).

As a result of thermal processing of the wieners, an average decrease of less than 1 log unit occurred in C. botulinum spore count. The thermally processed product was vacuum packaged and incubated at 28 C. Samples were tested after 0, 7, 14, 21, 28, and 56 days for pH value and total viable count, and after 7, 14, 21, 28, and 56 days for toxicity. All general methods used in this study were reported previously (1). The average composition of the thermally processed product was: moisture, 52.3%; protein, 12.2%; fat, 29.9%; salt, 2.7%; and water, 57.3%.

The effect of nitrite and ascorbate on the incidence of toxic samples during incubation of the wieners is shown in Table 1. Samples containing 50 μg of nitrite per g of product, or less, began to show toxicity after 7 days of incubation. The incidence of toxic samples increased significantly after 14 days and remained high throughout the incubation period. Wieners containing 100 and 150 μg of nitrite per g of product failed to develop toxicity, with the exception of one sample containing 100 μg of nitrite per g of product. In the latter instance, toxicity was evident only after 56 days of incubation.

The total number of toxic samples in each series containing nitrite was not altered appreciably as the level of ascorbate was varied (Fig. 1). The incidence of toxicity declined sharply above the level of 50 μg of nitrite per g of product.
TABLE 1. Effect of nitrite and ascorbate on the incidence of toxic wiener samples during incubation at 28°C

| Days of storage | Calculated initial concn of ascorbate (μg/g) | Calculated initial concn of nitrite (μg/g) |
|----------------|---------------------------------------------|------------------------------------------|
|                | 0                                           | 0*                                        |
| 7              | 0                                           | 1 0 0 0 0 0 0                             |
| 14             | 4 5 2 4 0 0 0                               |
| 21             | 5 5 4 3 0 0 0                               |
| 28             | 4 5 1 3 0 0 0                               |
| 56             | 4 5 3 3 1 0 0                               |
| 105            | 1 3 3 1 0 0                                |
| 14             | 4 5 5 4 0 0 0                               |
| 21             | 5 5 5 5 0 0 0                               |
| 28             | 5 5 5 5 0 0 0                               |
| 56             | 5 5 5 5 0 0 0                               |
| 7              | 655                                         |
| 14             | 5 4 5 1 0 0 0                               |
| 21             | 5 4 4 1 0 0 0                               |
| 28             | 5 4 5 3 0 0 0                               |
| 56             | 5 3 5 2 0 0 0                               |

* Total number of toxic samples/five samples tested.

During the incubation period, total viable counts and pH values showed no appreciable variation among the 18 variables. The pH values dropped steadily after thermal processing through the incubation period, with the exception that at zero level of ascorbate, all nitrite levels showed a slight increase in pH at the final 56-day sampling. The total counts generally decreased in the 56-day-old samples. Apparently, acid production was not a factor in preventing toxin formation under the conditions of the experiment.

From the results obtained in this study, it can be concluded that ascorbate did not affect the efficacy of inhibition of toxin production by nitrite. Thus, the inclusion of ascorbate in the formulation of wieners does not detract from the desired inhibitory effects of nitrite on the production of botulinal toxin.

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