APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1978, p. 59-61

Vol. 35, No. 1
Printed in U.S.A.

Enhancing Nitrite Inhibition of *Clostridium botulinum* with Isoascorbate in Perishable Canned Cured Meat

R. B. TOMPKIN,* L. N. CHRISTIANSEN, AND A. B. SHAPARIS
Swift & Company, Research and Development Center, Oak Brook, Illinois 60521

Received for publication 1 August 1977

Addition of sodium isoascorbate to the formulation for perishable canned comminuted cured meat markedly enhanced the efficacy of nitrite against *Clostridium botulinum*. This effect was reproducible through a series of three tests. In one test it was found that the initial addition of 50 μg of sodium nitrite per g plus isoascorbate was as effective as 156 μg of sodium nitrite per g alone.

The use of nitrite in cured meats is being challenged due to concern over the possible formation of nitrosamines. In the event that permissible nitrite levels are lowered, it will be necessary to supplement, or enhance, the antibotulinal effect of nitrite by some means. One product category of major concern is perishable canned cured meat, which is processed to a minimum internal temperature of 65.6°C and is dependent upon refrigeration for stability and safety. Products in this category have a history of temperature abuse after manufacture.

A data base has been established for evaluating additives that might replace or enhance the effect of nitrite (11). In the course of that research it was observed that increasing levels of nitrite were much more inhibitory to *Clostridium botulinum* than had been previously reported. To determine the reason(s) for this difference, tests were conducted comparing the model system of Rhodes and Jarvis (8) and Roberts et al. (9) with our test product. Differences in fat-to-lean and meat-to-water ratios were examined without success. The major reason for the difference in inhibition appears to be the addition of sodium isoascorbate as described herein.

The value of ascorbate and its stereoisomer isoascorbate (erythrobate) for increasing the rate of cure color development, providing a more stable color, and retarding oxidative rancidity are well known. Borenstein (2) lists the functions of isoascorbate as follows: (i) acts as an oxygen scavenger, (ii) shifts the redox potential of the system to a reducing range, and/or (iii) reduces undesirable oxidation products.

The use of isoascorbate in the mid-1950s was one factor permitting modern, rapid processing of cured meats. At the time ascorbate and isoascorbate were being introduced into the meat industry, research was conducted showing that ascorbic acid is of value for microbial control in canned shelf-stable cured meat while using a reduced thermal process (W. R. Schack and R. E. Taylor, U.S. Patent 3,258,345, June 1966). Although isoascorbate is now widely used in the U.S. meat industry, its value for enhancing the antimicrobial effect of nitrite has gone unrecognized.

It is now believed that isoascorbate can help reduce the formation of nitrosamines, particularly in bacon. It is not clear whether the optimum level of isoascorbate for reducing nitrosamine formation is compatible with the level desirable for botulinal inhibition. Limited data (3) suggest an inverse relationship between botulinal inhibition and high ascorbate levels at a given level of added nitrite in bacon.

MATERIALS AND METHODS

Inoculum. The *C. botulinum* inoculum consisted of a mixture of five type A (33A, 36A, 52A, 77A, and 12885A) and five type B (ATCC 7949, 41B, 53B, 213B, and Lamanna B) strains prepared as previously described (4). The mixed spore suspension was heat shocked at 80°C for 15 min and added to the meat during formulation, using a target level of 100 spores/g of product. The spore levels present in the product after cooking were 78 (mean of five cans), 14 (mean of eight cans), 16 (mean of ten cans), and 64 (mean of eight cans) for tests 1 through 4, respectively.

Formulation and processing. Perishable canned comminuted cured pork was formulated with salt, water, and sugar, inoculated, processed, and chilled as previously described (4). Sodium isoascorbate (Pfizer, Inc., New York, N.Y.) was included at a level of 0.02% on the basis of the weight of meat in the formulation, except where it was omitted for purposes of comparison. Sodium nitrite was added at levels of 0, 50, or 156 μg/g on the basis of the weight of meat in the formulation.

Analysis of product from the variables in the tests showed the following ranges: salt, 2.3 to 2.4%; moisture, 57.9 to 59.6%; brine, 3.8 to 4.0%.

Holding conditions. Twenty-five cans of inoculated product per test variable were placed at 27°C

for up to 110 days. Cans were removed from incubation as they swelled.

Microbiological and chemical analyses. Spore levels, toxin assays, and chemical analyses were determined as previously described (4). The first five cans to swell from each test variable were tested for botulinal toxin. Five years' experience of inoculated packs with this product has shown more than 90% of swelled cans to contain toxin. For example, 39 of 40 samples from test 2 were toxic.

RESULTS

Results in Fig. 1 show that isoascorbate alone at a level of 0.02% does not affect botulinal outgrowth. In this test a short delay in botulinal outgrowth was observed when 50 μg of sodium nitrite per g was added. The combination of isoascorbate and sodium nitrite caused a significant delay in the observed time for the cans to swell.

Results of a second test are shown in Fig. 2. Isoascorbate alone did not retard botulinal outgrowth. Sodium nitrite alone at 50 μg/g had little or no effect. The combination of isoascorbate and sodium nitrite at 50 μg/g substantially delayed the swell time in all three replicates.

In a third test 50 μg of sodium nitrite per g alone did not retard botulinal outgrowth (Fig. 3). Isoascorbate plus 50 μg of added sodium nitrite per g caused appreciable delay. Sodium nitrite added at a level of 156 μg/g alone gave results equal to the combination of isoascorbate plus 50 μg of sodium nitrite per g. The combination of isoascorbate and 156 μg of sodium nitrite per g caused a pronounced delay in the rate of can swelling.

A fourth test (data not shown) was conducted

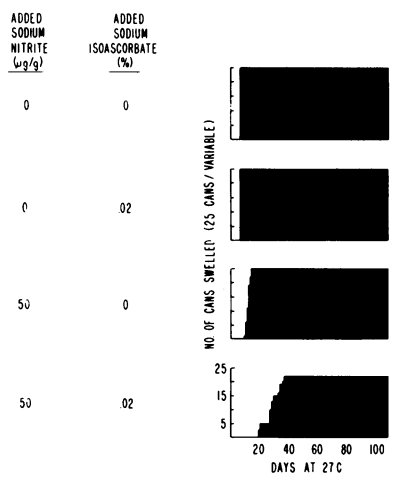

FIG. 1. *Effect of nitrite and isoascorbate on rate of swelling of cans of perishable comminuted pork containing C. botulinum and held at 27°C.*

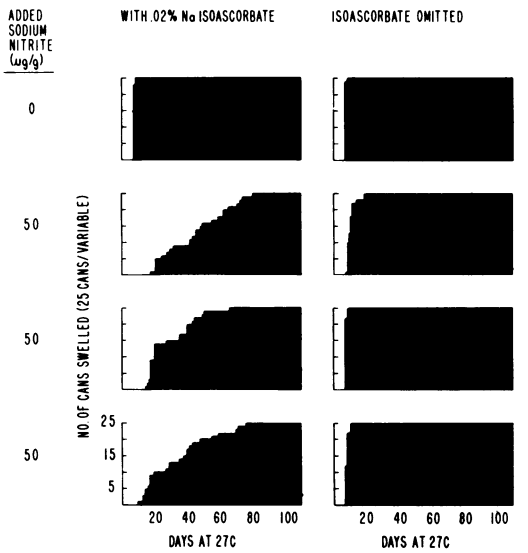

FIG. 2. *Effect of nitrite and isoascorbate on rate of swelling of cans of perishable comminuted pork containing C. botulinum and held at 27°C.*

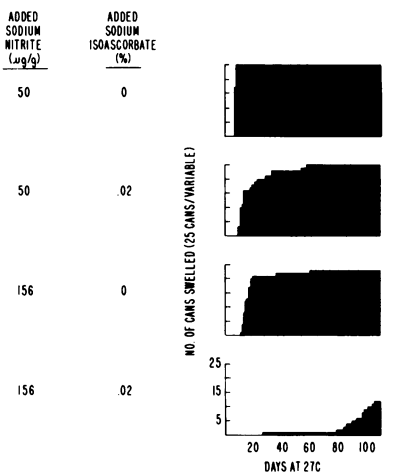

FIG. 3. *Effect of nitrite and isoascorbate on rate of swelling of cans of perishable comminuted pork containing C. botulinum and held at 27°C.*

to evaluate the method of adding isoascorbate. Three replicates of 25 cans each were prepared in which the isoascorbate (0.02%), sodium nitrite (50 μg/g), and spores were added to the meat, mixed, vacuumized, packed into the cans, and vacuum sealed. This has been our regular procedure for preparing this test product. A second set of three replicates was prepared wherein isoascorbate was mixed into the meat with the spores and then vacuumized. The nitrite (50 μg/g) was then mixed into the meat, revacuumized, and then packed into cans and vacuum

sealed. The results of the six sets of 25 cans/replicate were the same. Thus, the order of adding isoascorbate and nitrite, as described, was not a critical factor.

DISCUSSION

The addition of isoascorbate markedly enhances the antibotulinal effect of nitrite in perishable canned cured meat. This effect was reproducible through a series of three tests. The patent of Schack and Taylor (U.S. Patent 3,258,345, June 1966) describes a similar effect in a similar canned cured meat. In our attempt to simulate the order and method of adding isoascorbate and nitrite as described in the patent, a vacuum rather than a positive pressure was applied as the final step before canning. Also, isoascorbate, not ascorbic acid, was used. The significance of these deviations from the patent is not known.

Other reports in the literature show that reducing agents enhance the effect of nitrite in shelf-stable, canned cured meat. Johnston and Loynes (7) demonstrated that adding reducing agents to an underprocessed meat slurry greatly reduced the amount of nitrite required to inhibit spore outgrowth. Ashworth and Spencer (1) reported similar findings when reductants and nitrite were heated together in minced pork at 115°C for 20 min. Our findings expand upon this information and show isoascorbate to be very effective in perishable canned cured meat prepared similarly to commercial practice.

Omission of isoascorbate from the formulation might explain the differences observed among laboratories during the past few years. Collins-Thompson et al. (6) did not find a relationship between increasing nitrite levels and botulinal inhibition in bacon stored at 30°C. This is in marked contrast to the data of Christiansen et al. (5).

Tjaberg and Kvale (10) reported no relationship between increasing the nitrite level and botulinal inhibition in shelf-stable or perishable canned cured meat. Christiansen et al. (4) and Tompkin et al. (11) reported that increasing the level of added nitrite reduced the number of cans in which toxin was produced and the length of time for toxin development.

Research into the question of nitrite and protection against botulism in cured meats reflects current practice in those countries where the research has been conducted. Isoascorbate has been included in our formulations because it is common industry practice in the United States. Presumably, isoascorbate was omitted from the formulations tested in other laboratories because it either is not commonly used or is not permitted.

Our tests have been based upon using a level of 0.02% sodium isoascorbate. This level was selected as being representative of industry practice and was agreed upon by those involved in designing the protocol of the initial cooperative study (4). Information on the effect of other levels of isoascorbate in this product should be developed.

LITERATURE CITED

1. **Ashworth, J., and R. Spencer.** 1972. The Perigo effect in pork. J. Food Technol. 7:111-124.
2. **Borenstein, B.** 1965. The comparative properties of ascorbic acid and erythroic acid. Food Technol. (Chicago) 19:1719-1721.
3. **Bowen, V. G., and R. H. Deibel.** 1974. Effects of nitrite and ascorbate on botulinal toxin formation in wieners and bacon, p. 63-68. *In* Proceedings of the Meat Industries Research Conference. American Meat Institute Foundation, Arlington, Va.
4. **Christiansen, L. N., R. W. Johnston, D. A. Kautter, J. W. Howard, and W. J. Aunan.** 1973. Effect of nitrite and nitrate on toxin production by *Clostridium botulinum* and on nitrosamine formation in perishable canned comminuted cured meat. Appl. Microbiol. 25:357-362 (Erratum 26:653).
5. **Christiansen, L. N., R. B. Tompkin, A. B. Shaparis, T. V. Kueper, R. W. Johnston, D. A. Kautter, and O. J. Kolari.** 1974. Effect of sodium nitrite on toxin production by *Clostridium botulinum* in bacon. Appl. Micribiol. 27:733-737.
6. **Collins-Thompson, D. L., P. C. Chang, C. M. Davidson, E. Larmond, and H. Pivnick.** 1974. Effect of nitrite and storage temperature on the organoleptic quality and toxinogenesis by *Clostrium botulinum* in vacuum-packaged side bacon. J. Food Sci. 39:607-609.
7. **Johnston, M. A., and R. Loynes.** 1971. Inhibition of *Clostridium botulinum* by sodium nitrite as affected by bacteriological media and meat suspensions. Can. Inst. Food Technol. J. 4:179-184.
8. **Rhodes, A. C., and B. Jarvis.** 1976. A pork slurry system for studying inhibition of *Clostridium botulinum* by curing salts. J. Food Technol. 11:13-23.
9. **Roberts, T. A., B. Jarvis, and A. C. Rhodes.** 1976. Inhibition of *Clostridium botulinum* by curing salts in pasteurized pork slurry. J. Food Technol. 11:25-40.
10. **Tjaberg, T. B., and O. Kvale.** 1972. The effect of sodium nitrite on germination and growth of spores from *Clostridium botulinum* type B in a meat product, p. 119-129. *In* Proceedings of the 18th Meat Research Workers. University of Guelph, Guelph, Ontario.
11. **Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis.** 1977. Variation in inhibition of *C. botulinum* by nitrite in perishable canned comminuted cured meat. J. Food Sci. 42:1046-1048.