Causes of Variation in Botulinal Inhibition in Perishable Canned Cured Meat

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Final internal processing temperatures within the range of 63 to 74°C did not alter the degree of botulinal inhibition in inoculated perishable canned comminuted cured pork abused at 27°C. Adding hemoglobin to the formulation reduced residual nitrite after processing and decreased botulinal inhibition. Different meats yielded different rates of botulinal outgrowth when substituted for fresh pork ham. Pork or beef heart meat showed no inhibition of the Clostridium botulinum inoculum even with a 156-μg/g amount of sodium nitrite added to the product. This effect appears to be one of stimulating outgrowth, since residual nitrite depletion was not measurably altered.

It was earlier reported that some variation occurred among inoculated packs of perishable canned comminuted cured meat (6). Tests have been conducted to determine the cause of this variation, thereby improving the test system for monitoring additives. Also, by determining the causes of variation, some clues might develop as to the mechanism by which nitrite inhibits Clostridium botulinum in cured meats. The factors examined in this series of tests include thermal processing and the amount of muscle pigmentation. The role of residual nitrite versus added nitrite is also discussed.

MATERIALS AND METHODS

Inoculum. The C. botulinum inoculum consisted of a mixture of five type A and five type B strains prepared as described earlier (6). The mixed spore suspension was heated at 80°C for 15 min and added to the meat during formulation, using a target level of 100 spores/g of product.

Formulation and processing. Except as otherwise stated, perishable canned comminuted cured pork was formulated, inoculated, processed, and chilled as previously described (1). Sodium nitrite was added at levels of 50 or 156 μg/g on the basis of the weight of meat in the formulation.

The effect of processing temperature was tested by placing the cans in 77°C water until internal product temperatures of 63, 65.5, 68.5, 71, and 74°C were attained. Twenty-five cans were removed from the water at each temperature and chilled immediately in ice water.

Addition of bovine hemoglobin powder, type II (Sigma Chemical Co., St. Louis, Mo.), at a level of 1% (wt/wt) of the weight of meat in the formulation was used to reduce the level of residual nitrite after processing.

Variation in pigment content was tested using different meats, namely, fresh pork hearts, beef hearts, boneless pork ham muscle, beef round, a blend of various veal cuts, turkey breast, and turkey thigh meat. The same basic formulation mentioned above was used with all the meats. Sodium nitrite was added at a level of 156 μg/g on the basis of the weight of meat. No adjustments were made for differences in fat content of the meat, since an earlier test with pork had shown fat content did not influence botulinal inhibition. Nitrite depletion was followed in these canned meats during storage at 27°C.

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 (1). The first five cans to swell from each test variable were tested for botulinal toxin. In this series of tests only two of the 80 samples tested were not toxic.

RESULTS

Processing perishable canned comminuted pork cured with 156 μg of sodium nitrite per g to final internal temperatures of 63 to 74°C did not influence the degree of botulinal inhibition (Fig. 1). Viable botulinal counts and residual levels of sodium nitrite after processing showed no differences related to processing temperature.

Increased pigment in the form of added hemoglobin decreased botulinal inhibition (Fig. 2). Addition of 1% (wt/wt) hemoglobin had the net effect of reducing residual sodium nitrite after cooking by about 25 μg/g. The response observed with 50 μg of added nitrite per g plus hemoglobin was the same as that obtained when no nitrite was added to this product (6).

The effect with different meats is apparent from Fig. 3. Pork hearts and beef hearts showed...
no inhibition. Turkey thigh meat gave a response similar to pork ham formulated with 50 μg of nitrite per g (6). Beef round gave an unclear response. A group of five cans swelled early, and then the remainder swelled at a rate comparable to that of pork ham muscle. Veal and turkey breast meat swelled at the same rate. This test system is not sufficiently sensitive to state that veal and turkey breast meat differ from pork ham on the basis of one test.

The nitrite depletion curves for the various types of meat were similar. The rates of depletion of residual nitrite showed a normal response in all meats (Table 1) and were typical of what we have observed for product made with pork ham and held at 27°C (1).

**DISCUSSION**

The data show that the variation in nitrite inhibition reported earlier (6) cannot be explained on the basis of subtle differences in the thermal processing. Rhodes and Jarvis (4) observed similar results with their meat slurry system.

The stability of shelf-stable canned cured meat has been found to be due to the low level of contaminating spores in meat and the spore-injuring effect of the thermal process in the presence of sodium nitrite (5). “Injury” was the term used to describe the inability of surviving spores to proliferate in the product, even though their viability could be demonstrated by subculture in a suitable recovery medium.

A 1962 survey of seven meat packers found the thermal process values for shelf-stable canned cured meats to range from $F_0 = 0.1$ to 0.5, with a median value of 0.2 (3). This thermal process involves retorting at process temperatures in the range of 104 to 116°C. In one study with inoculated product, an approximate 4- to 5-log reduction in viable botulinal spores occurred during the thermal process. Thus, the process for shelf-stable canned cured meats involves both thermal destruction and injury.

The thermal process for perishable canned cured meat involves processing in hot water (e.g., 77°C) to achieve the minimum USDA internal temperature requirement of 66°C. It is reasonable to assume that domestic products of this type are likely processed with final internal temperatures in the range of 66 to 71°C. Our experience with inoculated botulinal tests indicates this thermal process causes less than a 1-log reduction in viable botulinal spores. The reduction that does occur is probably due to destruction of cells that have germinated during canning and processing. There is no apparent evidence that thermal injury has occurred. Within the range of 63 to 74°C no increased sensitivity to nitrite was observed (Fig. 1).

It was earlier reported that increasing nitrite levels from 30 to 340 μg/g showed increasing inhibition when botulinal spores were added to bacon after processing (2). This is additional evidence that the degree of botulinal inhibition is not a function of heat in lightly processed (i.e., perishable) cured meats.

Christiansen et al. (1) concluded that it is the nitrite level added at the time of formulation that is important for botulinal inhibition rather than the residual nitrite level in the product during storage. That conclusion was reached on the basis that the pattern of botulinal swells appeared to be unrelated to the levels of residual nitrite. After 4 weeks at 27°C the residual nitrite levels were all less than 15 μg/g when 300 μg of sodium nitrite per g, or less, had been added to the product. Yet, there was a definite pattern to the swell rate and toxin development which corresponded with the input level of nitrite.
### Analytical Results After Cook

<table>
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<th>Treatment</th>
<th>%H₂O</th>
<th>%NaCl</th>
<th>%Brine</th>
<th>ug/g NaNO₂</th>
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</thead>
<tbody>
<tr>
<td>50ug/g NaNO₂</td>
<td>57.2</td>
<td>2.3</td>
<td>3.87</td>
<td>30</td>
</tr>
<tr>
<td>50ug/g NaNO₂ + 1% Hemoglobin</td>
<td>55.5</td>
<td>2.3</td>
<td>3.98</td>
<td>6</td>
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<tr>
<td>156ug/g NaNO₂</td>
<td>60.2</td>
<td>2.2</td>
<td>3.53</td>
<td>112</td>
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<tr>
<td>156ug/g NaNO₂ + 1% Hemoglobin</td>
<td>57.3</td>
<td>2.3</td>
<td>3.86</td>
<td>84</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effect of addition of powdered hemoglobin on residual nitrite and botulinal inhibition in perishable canned cured pork. Product formulated with 50 or 156 ug of sodium nitrite and 10⁵ spores per g.

**Fig. 3.** Influence of meat on botulinal inhibition in perishable canned cured meat formulated with 156 ug of sodium nitrite and 10⁵ spores per g.

Reducing residual nitrite levels for cured meat products at the retail level is being proposed to reduce the possibility of nitrosamine formation at the time the products are consumed. This proposal forces the question as to whether residual nitrite plays a role in botulinal inhibition at the time of product abuse. One means of testing the role of residual nitrite would be to add some material that ties up more of the added nitrite. Nitrite reacts with myoglobin in meat to produce...
the characteristic color of cured meat. It seemed logical, then, that adding a heme-bearing pigment to the product would test the significance of added versus residual nitrite. Hemoglobin was used for this purpose. Increasing the level of pigmentation by adding hemoglobin reduced both the amount of residual nitrite remaining after processing and the degree of inhibition. This suggests, but is inadequate to prove, that the level of residual nitrite remaining after processing does influence the rate at which *C. botulinum* can initiate growth and cause swelling of the cans.

The foregoing raised the possibility that variation in muscle pigmentation might influence the efficacy of nitrite among batches of meat. The significance of pigmentation is confirmed in the test with different types of meat. The total loss of inhibition in heart meat was unexpected. Heart meat is more heavily pigmented (i.e., darker) than the other meats tested. However, the reason for the loss of inhibition is not obvious. The rate of nitrite depletion in heart meat was not different from that in the other meats. Also, both heart meats showed swells on day 5, when residual nitrite was still at a level of about 60 μg/g. One likely explanation is that some factor in heart meat stimulates botulinal outgrowth. Another possibility is that some factor is present that neutralizes or blocks the inhibitory nature of residual nitrite. The overall data suggest that this factor is in approximate proportion to the degree of pigmentation. This is apparent if the degrees of pigmentation of the meats are roughly grouped in descending order as follows: heart meat; beef round and turkey thigh meat; pork ham, veal, and turkey breast.

Muscle pigmentation is due to myoglobin and, to a lesser extent, hemoglobin remaining after carcass bleeding. We suspect that the responses obtained with the addition of hemoglobin and the use of heart meat represent two different phenomena. The inhibitory effect of residual nitrite observed in the hemoglobin test (Fig. 2) was overcome by some essential factor(s) present in the heart meats. Additional research, now in progress, suggests that it is a higher level of readily available iron in heart meat that causes the loss of inhibition. Iron bound in the form of heme pigment does not cause the same effect. We feel this is a clue to the mechanism by which nitrite is inhibitory to botulinal outgrowth in perishable canned cured meat. We offer the hypothesis that nitric oxide, which is formed from residual nitrite via nitrous acid, reacts with iron in the vegetative cells, thereby blocking some metabolic step essential for outgrowth. This reaction might involve the iron in ferredoxin or an enzyme in which iron plays an essential role.

**LITERATURE CITED**


