SODIUM HYPOPHOSPHITE INHIBITION OF CLOSTRIDIUM BOTULINUM IN BACON

M. D. Pierson,[1] K. M. RICE[1] and J. F. JADLOCKI[2]

[1]Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA
[2]FMC Corporation, Princeton, New Jersey 08540, USA

INTRODUCTION

An important function of nitrite in meat curing is the protection nitrite provides against Clostridium botulinum growth and toxin production. It is well known that C. botulinum is widely distributed in nature and its presence in meats may be expected (Lechowich et al., 1978). Recently, nitrite has become the source of some very serious concerns. The foremost concern has been that nitrite may react with secondary amines present in meats to which it has been added, leading to the formation of carcinogenic nitrosamines (Lijinsky and Epstein, 1970; Fazio, et al., 1971). Also, there have been studies which have implicated nitrite in the development of cancer in laboratory animals (Newberne, 1979). However, studies suggesting that nitrite per se is a carcinogen have been termed inconclusive by the USDA and FDA. As a result of this concern over the possible health hazards associated with nitrite, there has been an effort to either eliminate nitrites from cured meats or to reduce the level of nitrite added to cured meats in hope of reducing the potential for nitrosamine formation. Another result of this concern has been a major effort to discover a substitute or sparing agent for nitrite. To date, no one compound possessing all of the useful properties of nitrite has been identified.

One approach to solving this problem has been the investigation of processing meats using low levels of sodium nitrite in combination with another compound which is known to inhibit C. botulinum and which is known to be safe for human consumption. A cure combination such as this would allow the production of a meat product which would retain the typical characteristics of a cured meat, have a low potential for nitrosamine formation, and have protection against C. botulinum. Several antibotulinal additives for cured meats have been proposed (Sweet, 1975; Goodfellow, 1979; and Ivey, et al., 1978). The number of possible nitrite sparing agents is rather limited due to toxicological considerations, cost, practicality in application, concentrations necessary for inhibition and effects on the sensory quality of the product.

Manganese hypophosphite is listed as an approved nutrient or dietary supplement (Title 21, U.S. Code of Federal Regulations 182.5458) and Food Chemicals Codex lists specifications for food-grade manganese hypophosphite. The calcium, potassium and sodium salts, although not listed, are presumed to be "Generally Recognized as Safe." There has been limited use of hypophosphorus acid as a preservative or stabilizer in pharmaceutical preparations. Hypophosphorus acid has been proposed as an accelerator for meat curing (Woidich, 1933). Sodium hypophosphite is very soluble in water and has possible antioxidant properties. The purpose of this study was to determine the effectiveness of sodium hypophosphite (NaH_2PO_2) in inhibiting C. botulinum growth and toxin production in bacon.

MATERIALS AND METHODS

Experiments with ground pork. Fresh, boneless pork hams were ground and placed in beakers (99 g each). The cure ingredients were dissolved in water and added (11 g) to each beaker to give the 11 variables listed in Table 1. The color, odor and pH of the meat was noted after the addition of ingredients. The beakers were covered with aluminum foil and heated in a water bath at 55°C for seven hours. After heating, the color, odor and pH of the meat in each beaker was recorded. This general procedure was carried out three times. The first trial involved the 11 variables in Table 1 with 1.5% NaCl, 0.3% Curafos and 550 ppm sodium erythorbate in each variable. The second trial utilized all variables except ON-.3H and 40N-.3H. In this trial, 10 g of cure solution was mixed with 57.5 gm of ground pork. The cure was adjusted to give 0.1% sucrose, 2.0% NaCl, 0.3% Curafos and 550 ppm sodium erythorbate in the product. The mixture was heated for 6 hr at 55°C. The third trial was the same as the second except that the meat slurry contained 30% added fat.

Experiments with bacon. Approximately 33 lbs of bacon for each of 9 variables was manufactured in a commercial meat processing plant. The pickle solutions were pumped into 10-12 lb pork bellies to give approximately a 13% weight gain. After pumping, the bellies were smoked for 7 hours to an internal temperature of 53°C. The average yield after smoking was about 101%. The desired concentration of ingredients in the finished product for each of the variables is given in Table 2. The product in each variable was targeted to contain 550 ppm sodium erythorbate, 0.3% sodium tripolyphosphate, 1.5% sodium chloride and 0.11% sucrose. The following combinations of sodium nitrite (in ppm-N) and sodium hypophosphite (as %-H) were used to give 9 variables: ON-OH, ON-.05H, ON-.1H, ON-.3H, 40N-OH, 40N-.05H, 40N-.1H, 40N-.3H, 120N-OH. The 120N-OH variable represented bacon as it is currently marketed. The cured, smoked bellies were cooled and sliced for sampling and analysis.

Slices of bacon for each test variable were randomly picked from the sliced, processed bellies. The slices were uniformly inoculated (0.25 ml for 100 gm of bacon) with a heat-shocked (80°C, 10 minutes) suspension of C. botulinum spores. The inoculum consisted of spores of 4 type A strains and 5 type B strains (36A, 52A, 77A, 10755A, ATCC 7949, 41B, 53B, 213B and Lamanna B). The target inoculum level was 100 to 500 spores per gram of bacon. Fifty-five vacuum packages (Cryovac P850S) of inoculated bacon were prepared for each variable. Each package contained 100 gm of bacon (approximately 4 slices per package). One package from each variable was examined immediately for toxicity, C. botulinum count (heat-shocked and unheated), and pH.

The packages for each variable were divided into two groups: group A (28 packages) and group B (25 packages). All packages were then incubated at 27°C. The experiment was designed so that 4 unswollen packages from each variable in group A were to be tested for C. botulinum toxin after 4, 7, 10, 14, 24 and 56 days storage at 27°C. However, as swells occurred in this group, the time to swell formation was recorded and toxicity determined. Group B was incorporated into this experiment to determine swell rate and toxicity without removing nonswells from the variables. Group B samples were observed for swells and toxicity of bacon in swollen packages.

Microbiological procedures and toxin assay. Spore crops of the nine strains of C. botulinum were prepared and harvested by the biphasic culture methods of Anellis, et al. (1972). The spore crops were kept frozen (-20°C) until used. Viable spore counts of the nine different crops were determined by the roll tube methods of Pierson, et al. (1974) using prereduced peptone yeast extract agar (PYA) and prereduced 0.1% peptone dilution media prepared as described by Holdeman, et al. (1977). The spore inoculum was prepared by mixing equal numbers of spores from each strain of C. botulinum in sterile distilled water (4°C).

Sample homogenates for C. botulinum counts were prepared by mixing 100 gm of inoculated bacon for two minutes in 200 ml of sterile peptone water with a Model 400 Stomacher (Cooke Laboratory, Alexandria, VA). Inoculated sample homogenates were examined for total C. botulinum counts using prereduced chopped meat glucose (PCMG) and a three-tube most probable number procedure (MPN). Total C. botulinum spore counts were determined using the same PCMG three-tube MPN procedures except that heat shocked (80°C, 10 min) samples of inoculated bacon homogenates were examined. All PCMG tubes positive for growth were tested for toxicity to confirm the presence of C. botulinum.

To assay for C. botulinum toxin in bacon, samples were blended with 0.05M sodium phosphate-gel buffer (0.1% gelatin; pH 6.2) for two min at room temperature. The sample homogenates were then transferred to sterile centrifuge tubes and centrifuged at 27,000 x g for 20 min at 4°C. The supernatant fluid was examined immediately for C. botulinum toxin. A modification of the mouse bioassay described by Christiansen, et al. (1974) was used for toxin detection. All mice used in the assay were Dub:(ICR) white, males weighing 16 to 22 g. For each sample, three mice were injected interaperiotneally with unheated sample extract (0.5 ml per mouse) and two mice were injected with heated (100°C) sample extract. Evidence for the presence of C. botulinum toxin occurred when typical symptoms of death from botulinal toxin were observed in mice receiving the unheated extract and the two mice receiving heated extract survived.

Chemical procedures. Uninoculated product was randomly sampled (500 gm per variable). The uninoculated product was used for pH, fat, protein, salt, and sodium nitrite determinations according to standard AOAC (1975) procedures for meat products.

RESULTS AND DISCUSSION

Ground pork. Prior to initiating studies with the use of sodium hypophosphite as an antibotulinal additive in bacon, it was necessary to examine whether there were unusual or undesirable changes in cured pork containing hypophosphite. This phase of the study was designed to determine the effect of sodium hypophosphite on pH, color, and odor of ground pork in the presence and absence of nitrite before and after heating at 55°C.

No differences in pH between variables both before and after heating were noted. In two additional trials with pork containing a higher salt and fat content than trial 1, similar results were obtained. In the presence of nitrite (40 and 120 ppm), unheated samples were tan, while the heated samples showed the typical pink color of cured meat. In the absence of nitrite, the heated samples showed the typical tan to gray color of uncured, cooked pork. Sodium hypophosphite did not alter the respective color reactions or odor of either the unheated or heated samples with and without nitrite in each trial.

Bacon. The average fat, protein and moisture was 57%, 8.1% and 32% respectively for the 9 bacon variables. The average salt content was 1.7% and brine 5.0%. The residual sodium nitrite level for bacon with 40 ppm ingoing sodium nitrite ranged from 7 to 13 ppm. Bacon with an ingoing sodium nitrite concentration of 120 ppm had a residual of 37 ppm after processing. The pH values ranged from 6.39 to 6.70. The average pH of inoculated samples after 4 days of storage at 27°C ranged from 5.6 to 6.0.

The geometric mean of the C. botulinum spore count for nine inoculated bacon samples was 370 per gram. The geometric mean for the total C. botulinum count in inoculated bacon was 450 per gram. This result showed that the inoculum consisted mainly of spores of C. botulinum and the level was within the desired range.

Table 3 gives the results for swell rate and toxicity of bacon that had been inoculated and stored at 27°C. As nitrite was increased from 0 to 120 ppm, the time to onset of swells was delayed and the rate of subsequent swell formation decreased. First toxic swells were obtained at 9, 12 and 24 days for variables ON-OH, 40N-OH and 120-OH, respectively. There appeared to be no difference in swell formation and toxicity of samples when comparing variable ON-OH to ON-.05H. Variables ON-.1H and ON-.3H showed an increase in time to swell in comparison to ON-OH (14 and 17 days versus 9 days). Only one toxic sample out of 25 swells was observed for variable ON-.3H. This is notable since most swells for the other no nitrite variables proved to be toxic.

All variables containing 40 ppm sodium nitrite and 0.05 to 0.3% sodium hypophosphite showed a delay in onset of swells and toxin formation in comparison to bacon containing only 40 ppm sodium nitrite. There was a notable decrease in the number of toxic swells for the 40N-.1H and 40N-.3H in comparison to 40N-OH or 40N-.05H. None of the swells in variable 40N-.1H proved to be toxic. The greatest time to first swell for all variables was the variable 120N-OH. However, the overall rate of swell formation was less for both 40N-.1H and 40N-.3H. Also, variables ON-.3H, 40N-.1H and 40N-.3H had fewer toxic swells than 120N-OH.

Often bacon inoculated with C. botulinum will become toxic before gas formation in the package is evident. Therefore, one group of packages of bacon was sampled for toxicity of nonswells during temperature abuse at 27°C. Toxic nonswells were first observed for ON-OH, 40N-OH and 120N-OH at 7, 14, and 14 days respectively (Table 4). This was an earlier formation of toxicity in variables ON-OH and 120N-OH than was found when only swollen packages were examined (9 and 24 days, respectively for swells). Variables ON-.1H and 40N-.05H also had earlier toxic samples than indicated by examination of swells (7 days for both variables for nonswells versus 14 and 17 days respectively for swells). Variables ON-.3H, 40N-.1H and 40N-.3H had very few toxic swells (Table 4). None of the nonswells in these variables were toxic over the 24 to 35 day testing period. Overall, the time to first toxic sample was greater than that of 120N-OH for variables ON-.3H, 40N-.1H and 40N-.3H.

The results of this study indicate that sodium hypophosphite has potential as a sparing agent for nitrite in cured meat products such as bacon. Concentrations of hypophosphite necessary for inhibition of C. botulinum were similar to those proposed for other nitrite alternatives (Ivey, et al. 1978).

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Table 1. Variables for Ground Pork

Variable	Expected Concentration of Ingredients in Product[a]	
	$NaNO_2$ (ppm)	NaH_2PO_2 (%)
0N-0H	0	0
0N-.1H	0	0.1
0N-.2H	0	0.2
0N-.3H	0	0.3
0N-.4H	0	0.4
40N-0H	40	0
40N-.1H	40	0.1
40N-.2H	40	0.2
40N-.3H	40	0.3
40N-.4H	40	0.4
120N-0H	120	0

[a]Each variable contained 1.5% NaCl, 0.3% Curafos and 550 ppm sodium erythorbate for Trial 1. Product for trials 2 and 3 contained 0.1% sucrose, 2.0% NaCl, 0.3% Curafos and 550 ppm sodium erythorbate.

Table 2. Test Variables for Bacon

Variable	Expected Concentration of Ingredients in Finished Product[a]	
	$NaNO_2$ (ppm)	NaH_2PO_2 (%)
ON-0H	0	0
ON-.05H	0	0.05
ON-.1H	0	0.1
ON-.3H	0	0.3
40N-0H	40	0
40N-.05H	40	0.05
40N-.1H	40	0.1
40N-.3H	40	0.3
120N-0H	120	0

[a]Each variable contained 550 ppm sodium erythorbate, 0.3% sodium tripolyphosphate, 1.5% sodium chloride and 0.11% sucrose.

Table 3. Effect of Sodium Hypophosphite and Sodium Nitrite on Swell Rates[a] and Toxicity of Bacon Inoculated with Clostridium botulinum

Variable	Days at 27°C											
	9	10	12	14	17	21	24	27	29	31	37	49
ON-OH	2[b] (2)[c]	6 (6)	19 (18)	25 (24)	T[d]							
ON-.05H	2 (1)	9 (8)	23 (19)	25 (20)	T							
ON-.1H	-	-	-	2 (1)	10 (8)	21 (16)	24 (18)	25 (18)	T			
ON-.3H	-	-	-	-	4 (0)	17 (0)	25 (1)	T				
4ON-OH	-	-	1 (1)	10 (7)	21 (14)	25 (15)	T					
4ON-.05H	-	-	-	-	3 (3)	17 (14)	25 (19)	T				
4ON-.1H	-	-	-	1 (0)	1 (0)	6 (0)	16 (0)	17 (0)	21 (0)	21 (0)	25 (0)	T
4ON-.3H	-	-	-	-	5 (1)	5 (1)	12 (4)	15 (4)	23 (4)	24 (4)	24 (4)	25 (4)
12ON-OH	-	-	-	-	-	4 (0)	13 (4)	18 (5)	23 (5)	25 (5)	T	

[a] Twenty-five inoculated packages per variable were stored at 27°C. Only swollen packages were tested for toxicity.

[b] Cumulative swells.

[c] Cumulative toxic swells

[d] All samples in variable swollen and variable terminated.

Table 4. Effect of Sodium Hypophosphite and Sodium Nitrite on Toxin Production by C. botulinum in Bacon. Data for unswollen packages[a]

Variable	Days at 27°C						
	0	4	7	10	14	24	35
ON-OH	0/1[b]	0/4	1/4	2/4	T[c]		
ON-.05H	0/1	0/4	0/4	0/4	T		
ON-.1H	0/1	0/4	1/4	1/4	1/4	T	
ON-.3H	0/1	0/4	0/4	0/4	0/4	0/1	T
4ON-OH	0/1	0/4	0/4	0/4	1/3	T	
4ON-.05H	0/1	0/4	1/4	2/4	2/4	T	
4ON-.1H	0/1	0/4	0/4	0/4	0/4	0/4	T
4ON-.3H	0/1	0/4	0/4	0/4	0/4	0/4	0/1
12ON-OH	0/1	0/4	0/4	0/4	1/4	T	

[a] Twenty-nine inoculated packages were stored at 27°C. At various time intervals unswollen packages were examined for C. botulinum toxin.

[b] Number of toxic nonswells over number of nonswells tested.

[c] Variable terminated since all remaining packages were swollen.