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System for Evaluating Clostridial Inhibition in Cured Meat Products

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A method for evaluating inhibition of *Clostridium botulinum, C. sporogenes,* and *C. perfringens* in cured meat products was developed. This system can easily be used in the microbiology laboratory using aluminum ointment tubes as the product container. Swells caused by gas production by the organism are easily observed by using the aluminum tubes. Results obtained confirmed earlier work on the inhibitory effect of sodium nitrite and sorbic acid against the clostridia in cured meat products.

In most studies evaluating inhibition of clostridial species in meat products, it has been common to use vacuum-seamed cans for packaging the product (2, 8, 10) because this is one of the ways in which the normal product is packed and because swollen cans can be easily observed. This, however, normally requires large, expensive equipment not common to microbiology laboratories and uses large quantities of product per variable tested. Vacuum packaging of inoculated products has also been frequently used (3, 4, 6) which also requires large quantities of product and special equipment. Furthermore, gas production indicative of organism outgrowth may be difficult to observe (F. J. Ivey, K. J. Shaver, R. B. Tompkin, and L. N. Christiansen, J. Food Protection, in press).

Studies have been conducted in model systems with glass bottles or glass tubes (7, 9), but heat transfer is slow when processing the product, and, again, swells may be difficult to judge because of visible air bubbles trapped during processing.

In an attempt to find a more suitable container for use during clostridial outgrowth studies, aluminum ointment tubes with a 30-g capacity were evaluated. These tubes were easily filled with inoculated product and closed by using a small, hand-operated closing device. Several meat products were formulated, inoculated with different clostridial spores, and packed into tubes in an effort to evaluate this method of packing the product.

Spores of *Clostridium sporogenes* (P.A. 3679) were prepared by the method of Anellis et al. (1). A portion of these spores were heat shocked and dried on sand over P_2O_5 by the method of Christiansen et al. (3). Both sand-dried spores and aqueous spores were used to inoculate products as a comparison. *C. perfringens* NCTC 8798 spores were prepared in Duncan and Strong sporulation medium (5). *C. botulinum* spores (strains 52A, 36A, 77A, 10755A, 41B, 7949B, 53B, 213B, and Lamana B) were a gift from Virginia Polytechnic Institute and State University.

Pork product was prepared by mixing the ingredients for 5 min in a Hobart mixer. The product contained pork, which was thawed and ground through a ⅛-inch (ca. 0.32-cm) plate; 2.5% salt; 0.5% dextrose; 300 ppm of sodium ascorbate with 0.1 and 0.2% sorbic acid; 40 ppm of sodium nitrite, with 0, 0.1, and 0.2% sorbic acid; and (for one lot) 156 ppm of sodium nitrite. The pH of the product was 6.2 ± 0.1 in all cases. The product from each test lot was inoculated to 1,000 spores per g with heat-shocked aqueous P.A. 3679 spores and stuffed into 30-g aluminum tubes (Prescription Packaging, St. Louis, Mo.). Another set of test lots was inoculated with sand-dried spores to 1,000 spores per g and stuffed into the tubes. The tubes were closed by using a hand-operated sealing machine (Per Machine Manufacturing Co., Inc., New York, N.Y.). Forty tubes of each test lot were sealed and heated in a 70°C water bath to an internal temperature of 68.5°C, and then chilled and incubated at 27°C.

A third set of 20 tubes per test lot was packed with the pork macerate after inoculating with heat-shocked *C. perfringens* spores to a level of 10 spores per g. The tubes were sealed, heated, and incubated as described above.

Frozen, deboned poultry meat was thawed and mixed with 2.5% salt, 2% corn syrup solids, 1.5% dextrose, and 200 ppm of sodium ascorbate. The same seven test lots were formulated as in

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the pork experiment containing 0% $NaNO_2$ with 0, 0.1, and 0.2% sorbic acid; 20 ppm of $NaNO_2$ with 0, 0.1, and 0.2% sorbic acid; and (for one lot) 156 ppm of $NaNO_2$. The pH of the product was 6.2 ± 0.1 in all lots. Each test lot of 40 tubes was inoculated with about 500 spores of a heat-shocked mixture of the four type A and five type B strains of *C. botulinum* per g. The test lots were stuffed into 30-g aluminum tubes, sealed, heat processed as described above, and incubated at 27°C.

The aluminum tubes proved to be a totally satisfactory packaging system for incubation of inoculated meat products. Ease of filling and sealing were major benefits, although good heat penetration, ease of swell observation, and reduction of incubation space were also observed.

Whereas toxin assay is undoubtedly the best detection method for inhibition studies with *C. botulinum*, gas production has been observed as a reasonably satisfactory indicator, particularly in a canned, comminuted cured meat (10). Toxin assay is unavailable in nontoxic clostridial species, such as P.A. 3679, and observation of gas production is, then, one indication of outgrowth.

Tables 1 and 2 show that similar results were obtained in different meat-type products inoculated with different clostridial species. Sodium nitrite is known to act as an inhibitor of clostridial outgrowth (2). Sorbic acid, alone or in combination with nitrite, has been reported to also inhibit clostridial outgrowth (11; Ivey et al., J. Food Protection, in press). These inhibitory effects were confirmed in these tests by using the sealed aluminum tubes.

TABLE 1. *Average swelling time for inoculated cured pork product packed in aluminum tubes and incubated at 27°C*

Test lot	Swelling time (days) for:		
	P.A. 3679[a] (1,000 spores/g, sand dried)	P.A. 3679[a] (1,000 spores/g, aqueous)	*C. perfringens*[b] (10 spores/g)
1. 0 N[c]	4.88	4.53	5.10
2. 0 N-0.10% S	6.98	5.35	7.15
3. 0 ppm of N-0.20% S	7.90	7.90	8.45
4. 40 ppm of N	6.68	4.45	5.90
5. 40 ppm of N-0.10% S	7.60	7.68	8.35
6. 40 ppm of N-0.20% S	8.35	8.58	9.85
7. 156 ppm of N	7.08	7.00	7.75

[a] $n = 40$ tubes.
[b] $n = 20$ tubes.
[c] N, $NaNO_2$; S, sorbic acid.

TABLE 2. *Average swelling time of cured chicken product inoculated with 460 C. botulinum spores per g and incubated at 27°C in aluminum tubes*

Test lot[a]	$\bar{X}$ (days)
1. 0 ppm of N	4.30
2. 0 ppm of N-0.10% S	5.75
3. 0 ppm of N-0.20% S	8.15
4. 20 ppm of N	6.0
5. 20 ppm of N-0.10% S	6.80
6. 20 ppm of N-0.2% S	15.25
7. 156 ppm of N	12.70

[a] N, $NaNO_2$; S, sorbic acid.

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