

# Antibotulinal Activity of Methyl and Ethyl Fumarates in Comminuted Nitrite-Free Bacon

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## ABSTRACT

Mono- and dimethyl and ethyl esters of fumaric acid were evaluated for their antibotulinal efficacy in cans of comminuted nitrite-free bacon. At 0.125%, all were equal or superior to 120 ppm nitrite in preventing toxin formation in cans incubated at 30°C for 8 wk. No cans swelled or became toxic when mono- or dimethyl fumarate was added. With monoethyl fumarate, two cans out of twenty swelled but were nontoxic. The least effective ester was diethyl fumarate but its activity was equal to that of 120 ppm nitrite.

## INTRODUCTION

THE NITRITE IN BACON is singularly suspect as a carcinogenic precursor, since it can nitrosate amines during frying (NAS, 1982). Nitrite however, acts as an inhibitor of *Clostridium botulinum* toxin formation in bacon. Decreasing or eliminating it could increase the risk of botulism from temperature-abused bacon (Hauschild, 1982) although decreasing the potential for nitrosamine formation. Although the risk of botulism from bacon is probably less than that from other cured meat products, a number of substitutes have been proposed. Among them are irradiation (Rowley et al., 1982), sorbate/sorbic acid (USDA, 1979; Ivey et al., 1978; Huhtanen and Feinberg, 1980), sodium hypophosphite (Pierson et al., 1982), and natural acidification by lactic acid bacteria (Tanaka et al., 1980).

Our laboratory has been involved in a program to find a replacement for nitrite for inhibiting *C. botulinum* in bacon. A number of alkynoic and alkenoic acids and esters were included in this program; some exhibited antibotulinal activity (unpublished results). The most promising compounds were esters of fumaric acid. This paper is a report of their activity against *C. botulinum* in a comminuted bacon system.

## MATERIALS & METHODS

### Comminuted bacon

Bacon was prepared in a commercial processing plant using a nitrite-free curing brine which contained 12.5% salt, 0.86% sodium tripolyphosphate, 0.39% erythorbate, and 0.11% liquid smoke. The target pump was 14% with processing back to 4% above green weight. After processing, the bacon was frozen and comminuted by grinding in a Hobart model 84-145 bowl cutter (3/16-in. plate). It was then mixed in a "Butcher Boy" model B52 food chopper. The bacon was distributed in 2-kg quantities, heat sealed in plastic pouches, and frozen at -23°C until use. Packages were thawed under running tap water. The meat was spread out in a thin layer (1-1.5 cm) and spores, esters, or NaNO<sub>2</sub> (H<sub>2</sub>O solution) were added. These were spread out on the surface with gloved hands followed by hand mixing. Spore suspensions were added by pipetting 0.1 ml onto each 75g portion of meat. Water was added where necessary to maintain equivalent liquid additions. The bacon was distributed in 70-75g amounts into 208 x 107 aluminum tab cans which were sealed under vacuum in a Rooney canner. The cans were heated for 30 min at 68°C (center temperature) and rapidly cooled in tap water. Temperature abuse was at 30°C; cans were observed

daily (once on weekends) and were removed when swollen or at predetermined intervals (1, 2, 4, and 8 wk).

### Spores

Heat-shocked spores of 20 strains (12 type A, 8 type B) of *C. botulinum* were used as previously reported by Huhtanen and Feinberg (1980), with the exception that strain 5 was omitted. Stock spore suspensions ( $3 \times 10^5$ /ml) were kept frozen and were thawed when needed. The final concentration was 400 spores/gm, which was determined by serial dilution (in triplicate) in a fluid thioglycolate medium (Huhtanen and Feinberg, 1980).

### Fumarate esters

The monomethyl and dimethyl esters of fumaric acid were purchased from Pfalz and Bauer (Stanford, CT); the monoethyl and diethyl esters were purchased from Aldrich Chemical Company (Milwaukee, WI). The purity of the first two esters is not given by the distributor, but the monethyl ester is listed as being 95% pure, while the diethyl is 99%. These were added at a concentration of 0.125% (2 oz/100 lb).

### Extract preparation

Swollen cans (refrigerated after removal from the incubator) or those removed at predetermined time intervals, were opened while cold in a fume hood and approximately 15g of bacon were placed in a 40 ml polypropylene centrifuge tube. Two volumes of gelatin phosphate buffer (0.2% gelatin, 0.4% Na<sub>2</sub>HPO<sub>4</sub>, pH 6.2) were added and the samples mixed with wooden tongue depressors. The tubes were centrifuged at 3000g for 20 min at 5°C. The supernatants were placed in serum bottles which were sealed and kept frozen until tested.

### Toxin testing

Two mice (15-20g) were each injected i.p. with 0.5 ml of each supernatant. If either mouse died, another pair of mice were injected with a heated sample (100°C, 10 min) and the sample was classified as positive if the latter injection failed to produce botulism symptoms (respiratory distress followed by death). If the original injection produced no symptoms in either mouse the sample was classified as negative. In practice, all samples producing symptoms of botulism were negative when the extracts were heated and the injections repeated.

## RESULTS

A PRELIMINARY EXPERIMENT using monomethyl fumarate at concentrations of 0.200%, 0.175%, 0.150%, 0.125%, 0.100%, and 0.075% showed complete inhibition of *C. botulinum* spore germination and outgrowth (based on can swelling) at levels of 0.125% or above. At 0.100% and 0.075%, four out of five cans swelled before the termination of the experiment (60 days). The level of 0.125% was therefore chosen for the experiment whose results are shown in Table 1.

A comparison of the antibotulinal activity of the fumarate esters with that of nitrite is shown in Table 1. All 10 control cans swelled and were toxic in 7 days. In addition, one nonswollen can of bacon treated with 120 ppm nitrite was toxic at 7 days. There were, however, no additional toxic cans from this treatment until after 4 wk. Four of the nitrite cans swelled between 36 and 43 days, one at 56 days; all were toxic.

Cans containing bacon treated with 0.125% mono- or

Table 1—Comparison of fumarate esters and nitrite for antbotulinal activity in comminuted bacon

Addition	Abuse period (days) <sup>a</sup>									
	7 <sup>b</sup>		14		28		56		Cumulative	
	Cans <sup>c</sup>		Cans		Cans		Cans		Cans	
	Swollen	Toxic	Swollen	Toxic	Swollen	Toxic	Swollen	Toxic	Swollen	Toxic
none	10	10	ND <sup>f</sup>	ND	ND	ND	ND	ND	10	10
120 ppm NaNO <sub>2</sub>	0	1	0	0	0	0	5 <sup>d</sup>	5	5	6
0.125% MMF <sup>e</sup>	0	0	0	0	0	0	0	0	0	0
0.125% DMF	0	0	0	0	0	0	0	0	0	0
0.125% MEF	0	0	0	0	0	0	2	0	2	0
0.125% DEF	0	0	1	0	0	0	4	5	5	5

<sup>a</sup> Abuse temperature 30°C.

<sup>b</sup> Ten cans per treatment interval; all the rest had five cans.

<sup>c</sup> No. of cans swollen or toxic between the abuse periods shown.

<sup>d</sup> The nitrite treated cans of meat swelled at 36, 39, 40, 43, and 56 days; the two MEF treated cans swelled at 49 days; the DEF treated cans swelled at 11, 33, 47, 47, and 49 days.

<sup>e</sup> MMF = monomethylfumarate; DMF = dimethylfumarate; MEF = monoethylfumarate; DEF = diethylfumarate.

<sup>f</sup> ND = not done.

dimethyl fumarate did not swell or become toxic, even after 8 wk of temperature abuse. Two cans of bacon treated with monoethyl fumarate swelled at 49 days but neither was toxic. With diethyl fumarate, there was one nontoxic, slightly swollen can at 11 days and four more toxic, swollen (these swelled between 33 and 49 days) cans before the termination of the experiment. There was one nonswollen toxic can at 8 wk in the group treated with diethyl fumarate.

### DISCUSSION

THE RESULTS indicate that fumarate esters at 0.125% were at least as effective as 120 ppm nitrite in preventing toxin formation by *C. botulinum* in the comminuted bacon. To be useful for bacon produced under commercial conditions, however, it would have to be demonstrated that these compounds are safe and free of properties which may adversely affect the organoleptic properties of the bacon. Very little is known about the toxicity of these esters. According to the "Registry of Toxic Effects of Chemical Substances" (DHEW, 1975), the oral LD<sub>50</sub> for rats of the fumaric acid diethyl ester is 1780 mg/kg; for the dimethyl ester it is 2240 mg/kg. By comparison, the LD<sub>50</sub> of sodium nitrite is 85 mg/kg. More comprehensive studies of their acute and chronic toxicity would need to be done before their commercial use could be contemplated.

Several of these esters, particularly the dimethyl and diethyl fumarates, have a "perfumey" odor which, if carried through to the finished products, would make these compounds organoleptically undesirable.

In addition, to be commercially feasible, nitrite substitutes should be usable without major changes in processing conditions. Solubility in the curing brine is an important consideration in this respect. Of the four esters, the most soluble in water at 25°C was monomethyl fumarate (4%) followed by the monoethyl ester (1.8%). The dimethyl

and diethyl esters were less than 1% soluble. Because of their inhibitory activity and favorable solubilities, the most promising esters for further studies would be the mono-methyl and monoethyl fumarates.

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Reference to a brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.