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TITLE: GROWTH CHARACTERISTICS OF TYPE E

CLOSTRIDIUM BOTULINUM IN THE
TEMPERATURE RANGE OF 34 TO 50 F.

RELEASED

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Comparative outgrowth times with low temperature incubation of spores produced at 85, 50, and 46 F.

Spore suspensions of the Beluga Type E strain have been produced in 5% Trypticase, 0.5% Peptone, and 0.4% glucose (TPG) medium plus 1% yeast extract with incubation at 46 and 50 F. In comparison to spores produced in TPG medium at 85 F., the low temperature spores showed somewhat shorter outgrowth times in ground haddock at a comparable inoculum level (Table 1). However, the outgrowth data of low temperature spores does not appear to seriously invalidate experiments with spores produced at 85 F.

Toxin assay procedures

Ground undiluted and unsteamed haddock and cod samples were inoculated with spores of the Beluga strain, irradiated with 0.1 and 0.2 Mrad, then incubated at 46 F. and below. The samples were toxin assayed without trypsin digestion, and with trypsin digestion using the conventional phosphate buffer method and a new procedure employing a sodium acetate buffer system. An appreciably higher number of samples showed Type E toxin without trypsin digestion and with the acetate procedure than with the phosphate buffer method (Tables 2-3).

The acetate procedure involves pipetting about 2 gms of a sample into 2 ml of pH 5.5, 0.5M sodium acetate buffer. The diluted sample is held at 38 F. for 20 - 24 hours to affect extraction of possible adsorbed and intracellularly bound toxin. To each sample is added 0.2 ml of freshly prepared 10% trypsin. Samples are incubated for 3 hours at 98 F., then duplicate mice, with and without Type E antitoxin, are injected with 0.5 ml of the trypsin digest.

Toxin development in fresh marine products.

A cooperative study between the BCF laboratory at Gloucester and our laboratory is underway to determine the earliest time of Type E toxin production in unirradiated and comparable irradiated fresh fish in the refrigeration temperature zone. The object is to determine the relative time for the product to become unmistakably unacceptable based on odor. One experiment is currently in progress.

Estimates of the "spoilage rejection time", that is, the "absolute maximum storage time" are being determined by an untrained consumer panel at the BCF laboratory. The "spoilage rejection time", designated as X, is the time for unanimous rejection of a sample judged solely by its odor.

Recently, one lot of unfrozen fresh haddock fillets were shipped from the BCF laboratory to us. Circular plugs of fish were cut from the fillets with a stainless steel die mounted on a drill press. Two plugs of fish, weighing about 40 gms, were placed in 2 oz. screw cap bottles. The plugs were inoculated with 10^2 , 10^4 , or 10^6 spores/gm and irradiated with 0.1 or 0.2 Mrad at the Illinois Institute of Technology Research Institute. After irradiation, the samples and inoculated unirradiated controls were incubated at 50 and 46 F. According to the expected product storage life estimates, triplicate samples are periodically removed from each incubation temperature. They are examined for odor, pH, total aerobic plate count, and the presence of Type E toxin. The sampling periods chosen are based on one-half the expected storage life of the product, on X, and on storage periods in excess (continued on page 4)

of the maximal storage life. Sufficient data has not been accumulated to report at this time.

The difference between the "spoilage rejection time" and the time for Type E toxin production will be evaluated for several fish products. It is hoped that the "spoilage rejection time" will occur well in advance of the time of first detectable toxin production, thereby indicating a considerable margin of public health safety.

Proposed future work

Continuation of the cooperative program with the BCF laboratory that was started a few weeks ago is planned. Also, studies will be initiated during the next contract year to determine the low temperature outgrowth characteristics of spores of Cl. botulinum Type C, non-proteolytic Type B, and Type F which have been isolated from marine environments.

TABLE 1

Outgrowth times at low temperature in ground haddock of spores of the Beluga strain produced at 85, 50, and 46 F.

<u>Inc Temp °F.</u>	<u>Dose (Mrad)</u>	<u>Avg and range of outgrowth time¹ (days)</u>		
		<u>85° F spores</u>	<u>50° F spores</u>	<u>46° F spores</u>
40	0	21 (18-24)	15	15
	0.1	25 (21-27)	19 (17-21)	22 (18-24)
	0.2	31 (26-35)	22 (17-31)	24 (21-35)
38	0	59, >90	49, 49, 49, 49, >90	40, 46, 49, >90
	0.1	>90	>90	52, >90
	0.2	>90	>90	>90

¹ Substrate = undiluted haddock steamed 30 min. Inoculum level = 2×10^6 unheated spores/tube and ten replicates/variable.

TABLE 2

Toxin assay results on samples that were non-toxic by the
phosphate buffer - trypsin digestion procedure

<u>Substrate</u>	<u>Temp °F</u>	<u>Number sample toxic/number assayed</u>	
		<u>Untrypsinized</u>	<u>Acetate</u>
Haddock	46	0/22	3/22
	42	0/46	2/46
	40	0/11	0/11
Cod	46	5/30	3/30
	42	<u>3/33</u>	<u>4/33</u>
Total		8/142	12/142

TABLE 3

Toxin assay results on comparative samples of haddock and cod inoculated with spores of the Beluga strain and incubated at 40 F.

<u>Substrate</u>	<u>Dose (Mrad)</u>	<u>Inoc</u>	<u>Fraction of sample toxic</u>		
			<u>Undig</u>	<u>Ac</u>	<u>PB</u>
Haddock	0.1	10 ⁶	7/7	7/7	6/7
		10 ⁴	0/7	0/7	0/7
	0.2	10 ⁶	8/9	9/9	4/9
		10 ⁴	0/7	0/7	0/7
Cod	0.1	10 ⁶	7/12	9/12	0/12
		10 ⁴	0/12	0/12	0/12
	0.2	10 ⁶	2/12	5/12	0/12
		10 ⁴	<u>0/12</u>	<u>0/12</u>	<u>0/12</u>
Totals			24/78	30/78	10/78