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TITLE: GROWTH CHARACTERISTICS OF TYPE E CLOSTRIDIUM
BOTULINUM IN THE TEMPERATURE RANGE 34 TO 50 F

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TOXIN DEVELOPMENT IN FRESH MARINE PRODUCTS

About one year ago, with the cooperation of the Bureau of Commercial Fisheries Laboratory at Gloucester, an inoculated pack study was initiated to assess the public health hazard of Clostridium botulinum Type E in low dose irradiated haddock in comparison to unirradiated product. Estimates of the "spoilage rejection time", that is, the maximal product storage life based solely on odor, were determined by an untrained consumer type panel. The "spoiled rejection time", designated as "X", represents the time for unanimous rejection of a sample by this panel.

It is hoped that for each marine food product to be tested, the value of X will precede well in advance the time of first detectable toxin development. Such results would indicate a considerable margin of public health safety.

The protocol followed in the inoculated haddock study was that recommended by the Ad Hoc Committee to the AEC on botulism. For each experimental variable, triplicate samples were inoculated. Spores of the Beluga strain were inoculated into the fish to give 10^2 , 10^4 , or 10^6 viable spores/gm.

The inoculated samples were irradiated with 0.1 and 0.2 Mrad doses. After irradiation, the samples and inoculated unirradiated controls were incubated at 50 F and below. At each sampling period, triplicate samples were examined for odor, pH, total aerobic plate count, and the presence of Type E toxin. The sampling periods chosen were based on fractions of the expected storage life, on X, and on multiples of X.

The results with haddock are as follows:

With 46 F incubation (Table 1), samples containing 10^6 and 10^4 spores, both control and 0.1 Mrad irradiated, showed no toxin up to 2X. With 0.2 Mrad, samples inoculated with 10^6 spores, showed toxin at X and 2X, but no toxin at 1/2X. At an inoculum level of 10^4 spores, 0.2 Mrad samples showed no detectable toxin at each time period tested.

The results with 42 F incubation (Table 2) are even more encouraging than those at 46 F. None of the unirradiated or the irradiated samples showed toxin up to 2X, twice the expected product storage life.

The results at 50 F (Table 1) are somewhat less favorable than those at lower temperatures. Unirradiated samples showed no toxin with inocula of 10^4 and 10^2 spores up to 2X. With 0.2 Mrad irradiation and the 10^4 inoculum, toxin was present at 1/2X. The 10^2 inoculum yielded toxic samples at X and 2X, but not at 1/2X. With 0.1 Mrad irradiation, the 10^4 inoculum failed to produce any toxic samples, although toxin was detected in one out of three samples at 2X with the 10^2 inoculum level.

Besides haddock, an inoculated pack experiment on cod was conducted (Tables 3 and 4). In general, the results are similar to those just presented.

To summarize the inoculated pack data, the results at 46 F and below are quite encouraging. The results at 50 F must be interpreted in consideration of the rigorous criterion used in establishing the maximum storage life estimates, that being unanimous rejection of a sample based only on odor.

In our experience, samples often exhibit mold and slime growth well before they are judged unacceptable on the basis of odor. Certainly any consumer would consider both appearance and odor during the preparation of a irradiated marine food product. If the BCF panel had taken into account appearance as well as odor this might have caused a reduction in the maximal shelf life estimates. This might give a more favorable picture at 50 F with respect to the public health hazard of Type E.

FUTURE WORK

A continuation of the inoculated pack studies on other marine products having potential success in the AEC program is planned. The determination of the low temperature outgrowth ability of spores of nonproteolytic Type B and of Types C and F of marine origin is anticipated.

9/22/67

TABLE 1

Toxin assay results on haddock with incubation at 50 and 46 F (Spores produced at 85 F).

TEMP° F.	MRAD	INC(DAYS)	X-VALUE	PRESENCE OF TOXIN ¹			
				INOC	10 ⁶	10 ⁴	10 ²
50	0	4	1/2		-	-	-
		8	1		-	-	-
		16	2		-	-	-
	0.1	6	1/2		-	-	-
		12	1		-	-	-
		24	2		-	-	+(2)
	0.2	10	1/2			+(2,3)	-
		20	1			+	+
		40	2			+	+
	0	6	1/2		-	-	-
		12	1		-	-	-
		24	2		-	-	-
46	0.1	6	1/2		-	-	-
		13	1		-	-	-
		26	2		-	-	-
	0.2	12	1/2		-	-	-
		24	1		+(2)	-	-
		50	2		+(2)	-	-

¹ Means toxin detected; - means no toxin detected in three replicate samples.

² Toxin present in one out of three replicate samples.

³ Toxin detected only after trysin digestion.

TABLE 2

Toxin assay results on haddock with incubation at 42 F (Spores produced at 85 F).

MRAD	INC (DAYS)	X-VALUE	PRESENCE OF TOXIN ¹	
			INOC 10 ⁶	10 ⁴
0	8	1/2	-	-
	11	3/4	-	-
	15	1	-	-
	22	1 1/2	-	-
	29	2	-	-
0.1	14	1/2	-	-
	22	3/4	-	-
	29	1	-	-
	46	1 1/2	-	-
	60	2	-	-
0.2	18	1/2	-	-
	27	3/4	-	-
	36	1	-	-
	54	1 1/2	-	-
	82	2	-	-

¹ Means no toxin detected in three replicate samples.

TABLE 3

Toxin assay results on cod with incubation at 50 and 46 F (Spores produced at 50 F).

TEMP° F.	MRAD	INC (DAYS)	X-VALUE	PRESENCE OF TOXIN ¹		
				INOC	10 ⁶	10 ⁴ 10 ²
50	0	6	1		-	-
		9	1 1/2		-	-
	0.1	13	1		+	+
		18	1 1/2		+	+
	0.2	18	1		+	+
		27	1 1/2		+	+
46	0	6	3/4		-	-
		8	1		-	-
		13	1 1/2		-	-
	0.1	14	3/4		-	-
		19	1		++	-
		26	1 1/2		++	-
	0.2	17	3/4		-	-
		24	1		-	++
		35	1 1/2		+	-

¹ + Means toxin detected; - Means no toxin detected in three replicate samples.

* Toxin present in one out of three replicate samples.

TABLE 4

Toxin assay results on cod with incubation at 42 and 38 F (Spores produced at 50 F).

TEMP° F.	MRAD	INC (DAYS)	X-VALUE	PRESENCE OF TOXIN ¹		
				INOC	10 ⁶	10 ⁴
42	0	7	3/4	-	-	-
		9	1	-	-	-
		13	1 1/2	-	-	-
	0.1	19	3/4	-	-	-
		26	1	-	-	-
		39	1 1/2	-	-	++
	0.2	24	3/4	++	-	-
		31	1	++	-	-
		46	1 1/2	-	-	-
	0	8	3/4	-	-	-
		10	1	-	-	-
		16	1 1/2	-	-	-
38	0.1	25	3/4	-	-	-
		33	1	-	-	-
		48	1 1/2	-	-	-
	0.2	27	3/4	-	-	-
		38	1	-	-	-
		55	1 1/2	-	-	-

¹ + Means toxin detected; - means no toxin detected in three replicate samples.

* Toxin present in one out of three replicate samples.