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MASTER

GROWTH CHARACTERISTICS OF TYPE E

CLOSTRIDIUM BOTULINUM IN THE

TEMPERATURE RANGE 34 TO 50 F

Contract No: DBM AT (11-1) - 1183

Contractor: CONTINENTAL CAN COMPANY, INC.
Metal Division Research & Development
1350 West 76 Street
Chicago, Illinois 60620

Principal Investigators: W. P. Segner
C. F. Schmidt

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PROJECT TITLE

Growth Characteristics of type E Clostridium botulinum in the Temperature Range 34 to 50 F.

OBJECTIVE

To assess the public health aspects of possible growth and toxin production of C. botulinum type E in radurized marine food products with respect to their expected refrigerated storage life.

PROGRESS AND RESULTS

Additional inoculated pack studies on fresh haddock were conducted at 50 and 46 F. Triplicate samples per variable were inoculated with 10^6 , 10^4 , or 10^2 spores of the Beluga strain per gram. Each inoculum level was preheated at 140 F for 15 min. The inoculated samples were given 0.1 and 0.2 Mrad irradiation and incubated along with comparable inoculated unirradiated control samples. Assays for type E toxin were conducted at fractions and multiples of the maximal expected product storage life. Storage life values were

previously determined by an untrained consumer type panel at the Gloucester Bureau of Commercial Fisheries Laboratory.

Samples were assayed for toxin without and with trypsin digestion by the mouse toxin-antitoxin neutralization test. The antibiotics Chloromycetin and Terramycin were used to avoid nonspecific toxicities.

At 46 F, samples inoculated with 10^6 spores and given 0.1 Mrad irradiation showed toxin at X (12 days), but not at $3/4X$ (9 days).

The control samples failed to show toxin development up to 4X (48 days). Samples given 0.2 Mrad irradiation showed toxin at $1/2X$ (12 days) the earliest time tested. With 10^4 spores, the controls showed no toxin up to $3\ 1/3X$ (40 days). Samples receiving 0.1 Mrad irradiation showed no toxin up to 2X (26 days), the longest time tested. Samples given 0.2 Mrad irradiation contained toxin in one out of three replicates at $1/2X$ and $3/4X$ (12 and 18 days, respectively).

At 50 F, control samples inoculated with 10^4 spores showed toxin at $1\ 1/2X$ (12 days), but not at X (8 days). However, the controls failed to show toxin at 24, 30, and 40 days suggesting the possibility of toxin destruction. With 0.1 Mrad irradiation, none of the samples showed toxin up to 2X (24 days). In contrast, with 0.2 Mrad irradiation, toxin was present at $1/2X$ (10 days). With 10^2 spores, the control samples failed to show toxin up to 4X (30 days) the longest

time tested. Corresponding samples given 0.1 Mrad irradiation showed toxin in one out of three replicates at 1 1/2X (18 days); all of the other 0.1 Mrad irradiated samples assayed were nontoxic. Samples given 0.2 Mrad irradiation contained toxin at 3/4X (15 days), but not at 1/2X (10 days).

Our toxicity results on unirradiated versus irradiated haddock in most cases are not based on equivalent incubation times. However, the irradiated samples tended to show toxin development somewhat earlier than comparable unirradiated samples. When compared on the basis of supposedly equivalent X-values, 0.2 Mrad samples definitely showed toxin formation earlier than either 0.1 Mrad irradiated samples or the unirradiated controls. In general, the results with 0.1 Mrad irradiation appear quite encouraging for haddock, although the data with 0.2 Mrad irradiation are less favorable.

Since little is known concerning the low temperature outgrowth ability of spores of C. botulinum type C, work was initiated to obtain pure type C cultures from marine sediment samples collected by Dr. B. Q. Ward. The samples were taken during a survey for C. botulinum type E along the Atlantic and Gulf coastal areas. Two type C isolations were made from these

sediments, one from a sample taken at New Dorp Lane, Staten Island, New York and the other from a sample collected at Yacht Club, Edgewood, Maryland.

PLAN FOR NEXT PERIOD

The inoculated pack concept will be extended to determine the public health safety of other marine food products of interest in the AEC program. Work will be started to determine the ability of spores of C. botulinum type C isolates to show outgrowth at temperatures below 50 F.

REPORTS PUBLISHED

1. Segner, W. P., and C. F. Schmidt. 1968. Nonspecific toxicities in the mouse assay test for botulinum toxin. J. Appl. Microbiol. 16: 1105-1109.
2. Segner, W. P. and C. F. Schmidt. 1968. Inoculated pack studies on Clostridium botulinum type E in unirradiated and irradiated haddock. Proc. of UJNR Conference on Toxic Microorganisms. Honolulu, Hawaii, October 7-10.

ESTIMATED DATE OF FINAL REPORT

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