Botulism and Heat-Processed Seafoods

JOSEPH J. LICCIARDELLO

Introduction

From 1899 to 1973 there have been 688 reported outbreaks of botulism in the United States, involving 1,784 cases and 978 deaths (Center for Disease Control, 1974a). Seventy-two percent of the outbreaks were associated with home-canned foods, whereas commercially processed products were involved in only 9 percent of the outbreaks. In Japan, Canada, Sweden, Denmark, and Russia, the majority of outbreaks have been due to improper home preservation of fish and fish products. Since 1940 there has been a decline in the number of botulism cases in the United States. This is believed attributable to an improvement in canning procedures both in the home and industry.

When one considers the total number of cans of commercially processed food produced in this country since the inception of the canning industry, on the whole the overall safety record pertaining to botulism is remarkable. Out of 775 billion cans of food sold between 1926 and 1971, only three cans have produced (four) fatal cases of botulism (Borgeson, 1971).

Most botulism cases associated with commercially processed foods have not involved fully sterilized products, but semi-preserved products (i.e., smoked, brined, pickled, pasteurized) which must be refrigerated and which rely upon salt content, pH, etc., for semi-preservation. In such products, the commensal microbial flora has been either reduced or inhibited, whereas the spores of *C. botulinum* may be unaffected. Under suitable conditions, germination and outgrowth may occur without competition from other bacterial types, producing toxins—and sometimes with no outward sign of spoilage.

Nevertheless, when botulism from commercially produced foods does occur, the sensational publicity generated by the insidious nature of the disease arouses great concern and accusations which diminish public confidence in the food industry involved. This causes serious economic loss to the affected industry. Rebuilding public confidence in such situations may take 2 years or more (Anonymous, 1978).

The author is with the Gloucester Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Emerson Avenue, Gloucester, MA 01930.

Commercial Seafood Products Involved

The principal habitat of *C. botulinum* is in soil; therefore, it is not surprising that vegetables have been implicated in the majority (17.4 percent) of U.S. outbreaks (Center for Disease Control, 1974a). Fish and fish products have been implicated in 4.4 percent of all outbreaks, whereas meat products were involved in 1.7 percent. However, in 66.7 percent of all outbreaks, the vehicle for the intoxication was either not known or not reported (Center for Disease Control, 1974a).

Botulism associated with commercially prepared fish products in the United States has involved canned clams, clam juice, crab, salmon, sardines, sprat, tuna, and smoked or vacuum-packed ciscoes and whitefish (Meyer and Eddie, 1965; Center for Disease Control, 1974a). Most of these products, however, are usually consumed directly from the container at ambient temperature with no further culinary preparation which could inactivate any toxins.

Distribution and Occurrence in Fish

There are seven known strains or types of *C. botulinum*, designated type A to G, which produce pharmacologically identical but serologically distinct toxins. Types A, B, E, and F are responsible for human botulism, whereas types C and D are usually associated with birds and animals (Smith and Holdeman, 1968). The role of type G has not yet been established.

The predominant strain in fish-related botulism has been type E, although types A and B have been occasionally implicated. Type E *C. botulinum* is ubiquitously distributed in nature and has been isolated from terrestrial soils, rivers and freshwater lakes, marine muds and waters, warm waters (i.e., Gulf of Mexico), and cold waters (i.e., Baltic Sea) (Graikoski, 1971; Hobbs, 1976). It has been found in such principal U.S. fishing areas as the Great Lakes, Gulf of Mexico, and the Atlantic and Pacific coasts (Dolman, 1957; Bott et al., 1966; Ward and Carroll, 1965; Nickerson et al., 1967; Presnell et al., 1967; Ward et al., 1967; Craig et al., 1968; Eklund and Poysky, 1970; Laycock and Longard, 1972).

Type E *C. botulinum* seems to have its greatest concentrations in bays,

estuarine areas, and the surrounding land masses. These areas act as catch basins for rivers draining the contiguous land masses and thus maintain reservoirs of the organisms in the immediate environs (Graikoski, 1971). However, type E habitat is not confined to littoral waters. Dolman (1957) isolated the organism from benthic mud off the coast of British Columbia at depths ranging from 153 to >765 yards (140 to >700 m). Laycock and Longard (1972) detected it in waters 150 miles (241 km) off the northeast coast of Newfoundland, and the spores are probably disseminated throughout the marine environment by underwater currents.

Since the organism inhabits marine and freshwater environments, it has been detected in or on the associated fauna. In infected fish or shellfish, it is usually found on the slime or exoskeleton, the gills, and in the intestinal tract (Huss, 1981). Among salmon taken in Alaskan waters (coastal and rivers), the number of positive gill samples exceeded the number of positive viscera specimens, particularly with the fish caught in rivers (Houghtby and Kaysner, 1969). The authors believed the gills were acting as filtering agents.

While much research has been directed toward establishing the qualitative incidence of type E *C. botulinum* in various fishes, there remains a paucity of information on the concentration of viable cells or spores, particularly in edible portions. A survey by Goldblith and Nickerson (1965) for the quantitative contamination by type E *C. botulinum* of commercially produced haddock fillets in Boston, Mass., revealed that about 24 percent of the samples taken from five different processing plants were contaminated, but the highest level found in any of the samples was 17 organisms per 100 g of fillets. No attempt was made to differentiate between cell and spore content. The incidence of type E spores among herring taken from Norwegian fishing grounds was estimated at ≤1 spore per 16 g of fish (Cann et al., 1966). The highest level of type E organisms found in Gulf of Maine groundfish intestines was 10 per 100 g (Nickerson et al., 1967). These limited data suggest a low natural level of type E botulism spores on finfish; however, the fact that a significant part of the raw material is infected provides the potential for introduction and buildup of the organism in a processing plant unless strict safeguards and good manufacturing practice (GMP) are maintained.

Recent Cases With Commercially Packed Fish

Botulism concerns were greatly stimulated in 1963 when commercially prepared foods were responsible for more cases of the disease in the United States than foods processed in the home (Dowell et al., 1970). Three cases and two deaths in Detroit, Mich., resulted from commercially canned tuna containing type E toxin. Seventeen cases and five deaths due to type E botulism in some southeastern states were associated with vacuum-packed smoked whitefish chubs which had been inadvertently held at room temperature (Dack, 1964; Center for Disease Control, 1974a).

In 1974 a recall of canned tuna was issued by the Food and Drug Administration (FDA) after botulinum toxin type C was detected in one can (Center for Disease Control, 1974b). Packed in Samoa, the can was reportedly an "obvious leaker." In Great Britain during 1978 four cases of botulism with two deaths were attributed to a single can of salmon packed in Alaska (Corwin, 1980), but damaged sometime after processing. This was the first such case in the long history of canning which resulted from commercially canned salmon produced in a U.S. plant. FDA attempts to reproduce the specific can damage in the canning plant were unsuccessful.

More recently, in early 1982, another incident with one associated death involving canned salmon from Alaska was reported in Belgium (Anonymous, 1982a; 1982b). A tiny hole was found in the sidewall of the can near the bottom seam. Unlike the earlier cases, it was speculated that the defect may have been caused in the plant by the can reforming equipment which may have caused tears in the edges of the can. For economy in shipping, some canneries receive formed but collapsed can bodies and use a reforming machine to restore these flattened bodies to a cylindrical shape, after which the body is flanged at both ends and a cover attached to one end.

Determination of a Safe Commercial Process

To appreciate the inherent protection from botulism in commercially canned foods, a brief discussion follows of the principles involved in determining process times. Thermal process requirements for commercially canned low-acid foods were originally based on a mathematical integration of heat penetration data and thermal resistance of the most heat-tolerant pathogen, *C. botulinum*. The heat resistance data were derived from a thermal death time (TDT) curve established by Esty and Meyer (1922) for an initial inoculum of 60 billion spores of the most heat-resistant strains of types A and B *C. botulinum* in M/15 phosphate buffer pH 7. An extrapolation of that curve indicated a TDT of 2.78 minutes at 250°F (121°C).

Townsend et al. (1938) applied a correction to the data of Esty and Meyer for heating lag time and recalculated the TDT at 250°F to be 2.45 minutes. The number of minutes required at any given temperature to inactivate the above spore load of *C. botulinum* is designated the *F* value for that particular temperature. Thus, by this definition $F_{250} = 2.45$, and this value may be used as the reference for safety when calculating the sterilizing value of a canning process. Other time-temperature combinations, i.e., 24.5 minutes at 232°F (111.1°C), 245 minutes at 214°F (101.1°C), etc. obtained from the TDT curve would also provide a sterilizing value equivalent to 2.45 minutes at 250°F. For the sake of conformity, 250°F has been designated the reference temperature in thermal process calculation and a

process which receives a sterilizing value equivalent to 2.45 minutes at 250°F would satisfy the requirement for a minimum botulinum cook; however, for safety reasons industry commonly considers the "minimum botulinum cook" as equal or equivalent to $F_{250} = 3.0$. This sterilizing value is applied in the absence of satisfactory TDT data which would show that a lower sterilizing value is appropriate.

Another parameter often used in thermal process evaluation is the *D* value, referred to as the death rate constant, or decimal reduction time. This is defined as the time required at a given temperature to effect a 90 percent reduction in numbers of the organism during heating, or the time required for the rate-of-destruction curve to traverse one log cycle when numbers of survivors are plotted as a function of heating time on semilog paper. The D_{250} for *C. botulinum* spores in phosphate buffer was determined to be 0.20 minutes (Schmidt, 1964). If the thermal death time value of 2.45 minutes (at 250°F) is divided by the D_{250} value of 0.20 minutes, a value of 12 is obtained. This indicates that the 60 billion spores in the study of Esty and Meyer were reduced by 12 log cycles or a factor of 10^{-12} after heating for 2.45 minutes at 250°F. Consequently, a sterilizing process designed to effect a minimum botulinum cook is also termed a 12 *D* process.

One perspective for perceiving the safety factor inherent in a minimum botulinum cook is to envision 60 million cans of product each arbitrarily containing 1,000 *C. botulinum* spores. Theoretically, after a minimum botulinum heat process, only one among all these cans would contain a viable spore. Today commercial heat processes for low-acid canned foods are usually not designed principally for the destruction of *C. botulinum,* but rather for the spores of more heat-resistant nonpathogens such as Putrefactive Anaerobe 3679, a strain of *Clostridium sporogenes* which could cause putrefactive spoilage if they survived the process (Lewis and Hall, 1970).

Stumbo et al. (1975) recommended a sterilizing value (F_{250}) of 5-6 for commercial heat processes for low-acid conduction-heating canned foods at a retort temperature of 240°F to obtain sterility with a moderate degree of assurance that only minor economic losses from spoilage by spore-forming mesophilic bacteria more heat resistant than *C. botulinum* would result. However, when good thermal resistance data are available, thermal processes may be based on sterilizing values that are somewhat lower than 5 or 6. Commercial processes today are generally based on these recommended *F* values of 5-6. This is twice the lethal requirement of *C. botulinum* and thus provides a wide margin of safety.

One must caution, however, that these sterilizing values are founded on moist-heat inactivation kinetics where bacterial spores are relatively heat-labile. Bacterial spores are more resistant with dry heat, i.e., in a hot-air oven or in oil. Lang and Dean (1934) found *C. botulinum* spores to be more resistant in fish products packed in oil. Neufeld (1971) recovered viable microorganisms from some cans of organoleptically sound, commercially heat-processed fish products, with survival attributed to fat protection since the isolated cultures were not found to be heat resistant.

Canned foods are not generally considered absolutely sterile but rather "commercially" sterile. This term implies that a few viable or injured cells may be present in a product, but growth and multiplication are suppressed by environmental conditions in the substrate. In low-acid canned foods, spores of heat-resistant thermophiles such as *B. stearothermophilus* are the only ones which are expected to survive the processes used.

Another factor which contributes to the prevention of botulism is the heat lability of the toxin. Simply heating the food to the boiling temperature before eating should be sufficient for detoxifying a product (Licciardello et al., 1967). Most outbreaks with commercially packed seafoods involve products which may be served cold and directly from the can (i.e., tuna, crab, and sardines).

Factors Responsible for a Botulism Hazard

In contrast to type A and type B spores, the spores of type E *C. botulinum* are relatively heat sensitive (Angelotti, 1970), and would be easily destroyed by a conventional commercial heat process as designated by the National Food Processors Association (1966). It has been stated that all outbreaks of botulism from U.S. commercially canned foods in recent years have been due to technical errors and not to the recommended thermal process itself (Lewis and Hall, 1970).

For example, the presence of *C. botulinum* in commercially canned mushrooms was thought to have been caused by use of a new type of filling machine which tended to pack the mushrooms into a solid mass in the container, thus altering the heat penetration characteristics. The processor unwittingly continued to maintain the same process schedule which resulted in an underprocessed product. Processing time should be redetermined whenever there is any change in product formulation involving particle size, drained weight, consistency, etc., which could affect heat transfer characteristics.

The 1963 outbreak in Detroit, Mich., resulted from recontamination of canned tuna due to a faulty seal (Stersky et al., 1980). The mechanism by which this can occur is as follows: As soon as steam pressure is released in a retort at the end of a thermal process, and while the cans are still hot, a tremendous strain is imposed on the container seams and microleakage can occur, particularly if the can had been sealed by a seaming machine that was out of adjustment or had badly worn rolls. During the cooling period a negative pressure develops within the can, and in a can with a defective seal, the cooling medium (air or water) can be sucked in. Although can sizes of approximately 4 inches diameter and smaller are normally designed to withstand internal pressures which

develop if the sterilizer steam pressure is suddenly released, can sizes greater than 4 inches diameter must be cooled under pressure to prevent straining and/or buckling of the container ends. Failure to pressure cool properly can lead to leaker spoilage problems with these large diameter cans. Post-process recontamination can be reduced by treatment of the cooling water with an effective germicide, by gentle handling of filled containers to avoid damage to sealed ends, and by proper sanitation of can runways and equipment (Bee and Hontz, 1980; Ito and Seeger, 1980).

Significance of z Value in Determining Process Times

The strains of *C. botulinum* found in fish or marine foods are usually of the nonproteolytic variety (type E and some types B and F). This is fortunate in the respect that nonproteolytic strain spores are less heat resistant than those of proteolytic strains. However, it is unfortunate because the proteolytic strains produce a putrid, recognizable spoilage pattern which would be a deterrent to the consumption of a toxic product, whereas spoilage and toxin production induced by the nonproteolytic types are usually less evident. In addition, nonproteolytic strains are capable of growing at lower temperatures, e.g., 37.4°-41°F (3°-5°C) (Hobbs, 1976).

Most type E outbreaks with commercially processed fish, particularly during the early 1960's, have been associated with inadequately refrigerated smoked fish. As a result, a code of practice was instituted for commercial production requiring smoked fish (from the Great Lakes) to be heated at least 30 minutes to an internal temperature of either 180°F (82°C) if the salt content of the aqueous phase is 3.5 percent, or 149°F (65°C) if the salt concentration is 5.0 percent (Liston, 1980). However, it has been demonstrated that this process schedule may not be adequate for complete elimination of type E spores from contaminated fish (Christiansen et al., 1968; Pace et al., 1972).

When establishing a process schedule, the objective is usually the destruction of the most heat-resistant strain of a particular bacterial species. Quite often the thermal resistance of different bacteria may be compared either through their thermal death times or *D* values at a particular temperature. This would be a valid comparison only if the slopes of the TDT curves were the same (Townsend et al., 1938). In thermal processing terminology, the slope of a bacterial TDT curve, expressed as the degrees Fahrenheit intercepted by the curve in traversing one log cycle, measures the change in thermal death time as a function of temperature. This parameter is called the z value. Another concept of the z value is that it represents the number of Fahrenheit degrees required to cause a tenfold change in heating time to achieve the same lethal effect. In the classic study by Esty and Meyer (1922) referred to previously, the z value in phosphate buffer was found to be 18. As this value becomes smaller, the effect of a processing temperature change becomes more marked. Thus, two strains of type E spores with different z values could have the same heat resistance at one particular temperature, but the spores with the smaller z value will be more resistant at lower temperatures and less resistant at higher temperatures (Perkins, 1964). A thermal process based on the heat resistance of the spores with the higher z values in this case would result in underprocessing (Perkins et al., 1975).

The z values for various strains of type E spores in either water, phosphate buffer pH 7, or trypticase peptone glucose broth have been reported to range from 7.4 to 17 (Ohye and Scott, 1957; Schmidt, 1964; Roberts and Ingram, 1965; Crisley et al., 1968; Ito et al., 1970; Lynt et al., 1977). Although the substrate is known to affect the z value, variable values were reported even in situations with the same substrate. There are essentially two different methods for determining the z value: 1) From a TDT curve constructed from end-point destruction data, and 2) from a phantom TDT curve obtained by plotting log *D* value as a function of temperature (Perkins, 1964).

Experimental Comparison of z Value for *C. botulinum* Type E by Two Different Methods

Because of the reported variability in z value for type E *C. botulinum* spores, the following study was conducted to determine the influence of the particular method by which the value was derived. Thermal destruction rates were determined for two strains of type E *C. botulinum* spores (8E and Detroit) in either M/15 phosphate buffer pH 7.0, clam liquor, or a haddock slurry over a temperature range of 140°-180°F (60.0°-82.2°C). The spore suspensions were heated in a thermostatically controlled mineral oil bath in sealed melting-point-determination capillary tubes which allowed rapid heating or cooling to ambient temperature (Licciardello and Nickerson, 1962). The number of survivors was counted by aseptically crushing the acetone/chromic acid cleaned tubes in sterile diluent and inoculating appropriate dilutions into Miller-Prickett[1] tubes containing 0.5 ml of 5 percent filter-sterilized sodium bicarbonate solution and then adding 10 ml of a molten agar medium (50 g trypticase, 5 g peptone, 10 g glucose, 0.5 g sodium thioglycollate, 0.5 g ferric citrate, 1 g K_2HPO_4, 1 liter distilled water, pH to 7.2). Tube cultures were incubated 48 hours at 85°F (29.4°C) before counting colonies.

A typical set of survivor curves is shown in Figure 1 for strain 8E in a haddock slurry. The nonlinear survival curve with heat-resistant tail exhibited by 8E in haddock slurry also occurred with the other substrates and with the Detroit strain. Lynt et al. (1977) also reported a tailing effect for thermal destruction of type E spores. The *D* value was calculated for each heating temperature from the reciprocal linear regression slope of the survival data over the first three to

[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

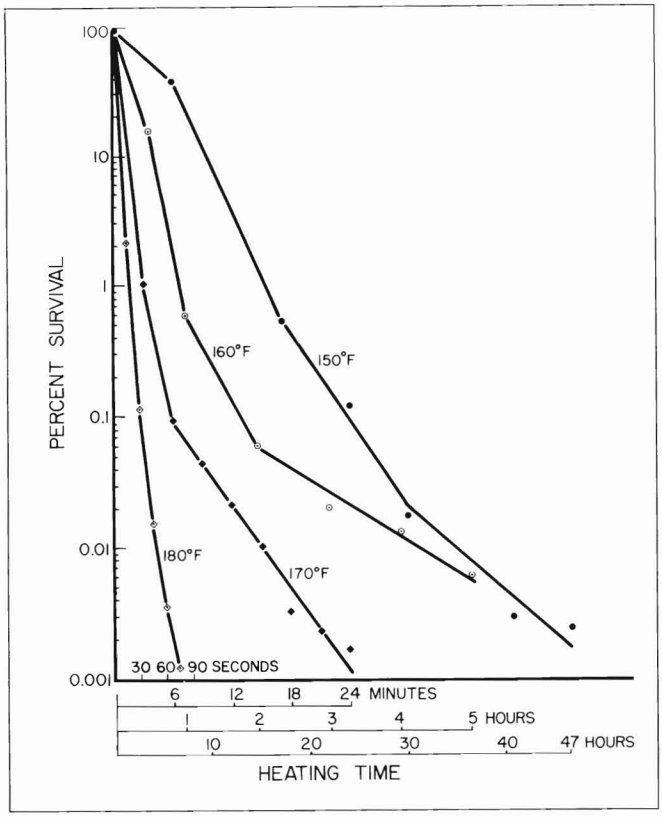

Figure 1.—Survivor curves for spores of *C. botulinum* type E (8E) heated in a haddock slurry. Each point represents the average count of duplicate tubes.

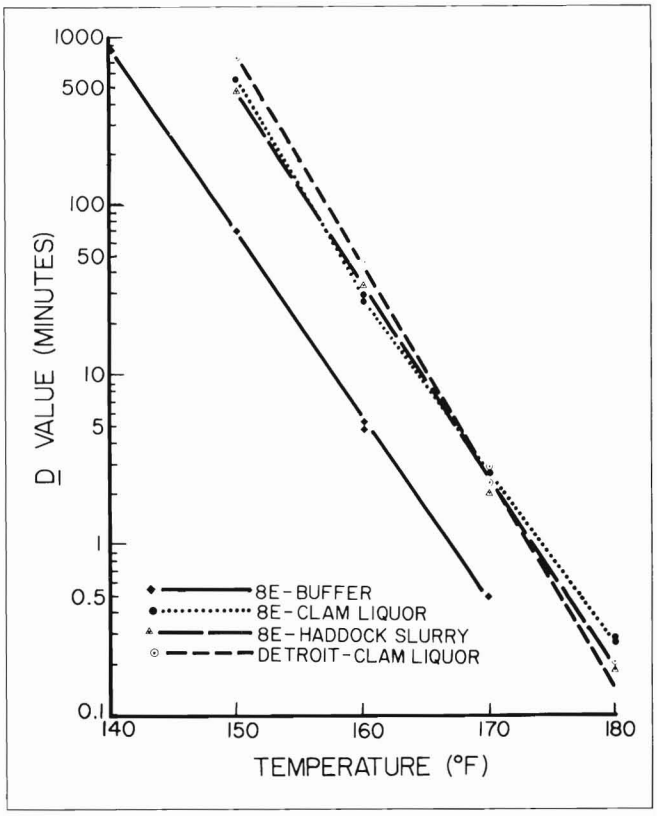

Figure 2.—Phantom TDT curves for spores of *C. botulinum* type E in various substrates. Each point represents result of separate trial.

four log cycles of destruction. Although it is acknowledged that this procedure for determining *D* value is valid only for linear survival curves, in this case the value so derived would be applicable to 99.9-99.99 percent of the population, which for all practical purposes can be considered to represent the population. The *D* values obtained in this study support the general conclusion of others that type E *C. botulinum* spores are relatively heat sensitive.

Phantom TDT curves are shown in Figure 2. The z values calculated from regression slopes were as follows: For strain 8E, 9.2 in phosphate buffer, 9.3 in clam liquor, and 9.0 in haddock slurry; for the Detroit strain, 8.2 in clam liquor.

Thermal death times were determined by the end-point method for the Detroit strain in clam liquor. A series of five capillary tubes, each containing 150×10^6 spores was heated for various time intervals at either 170, 180, 190, or 200 °F (76.7, 82.2, 87.8, or 93.3 °C) and then aseptically subcultured in TPG broth tubes and stratified with vaspar. Following a 2-week incubation period at 85 °F (30 °C), each culture tube was examined for viability by phase microscopy and for presence of type E toxin by mouse intraperitoneal injection. A TDT curve was constructed by drawing a straight line between the interval of heating times for the last set of toxin-positive tubes and the first set of five toxin-free tubes for each temperature (Fig. 3). From this TDT curve a z value of 17 was calculated, and this is to be compared with the value of 8.2 obtained from the phantom TDT curve. Lynt et al. (1977) similarly computed a higher z value from the TDT curve compared with the phantom TDT curve for strains of type E *C. botulinum* spores in crab meat. The discrepancy may be due to the fact that the TDT curve was based on the thermal resistance of a very small proportion of heat resistant spores in the population, whereas the phantom TDT curve was derived from the resistance of the major fraction of the population which was relatively heat sensitive. The question has long been debated whether the extreme heat resistance often encountered in a very small proportion of a bacterial population is due to 1) a physical phenomenon (i.e., clumping), 2) the presence of some dried spores on the inside wall of the TDT tube being subjected to dry rather than moist heat, or 3) a variation in physiological condition among the spore population. However, in this study and also in another (Graikoski and Kempe, 1964), it was demonstrated that the extreme heat resis-

tance was not an inherent trait, since spore crops produced from the resistant survivors possessed the same thermal resistance as the parent culture. That type E *C. botulinum* heat-resistant spores do exist is evidenced by the recovery by other investigators of viable type E spores from chubs which had been heated in accordance with the recommended code of practice, i.e., to an internal temperature of 180°F for 30 minutes (Pace et al., 1967; Christiansen et al., 1968). Dolman and Chang (1953) reported that some strains of type E spores withstood 30 minutes exposure to 212°F (100°C). Yet, based on *D* values generally reported for type E strains at 180°F, 30 minutes should exceed a sterility time calculated for a 12 *D* process. In our study the D_{180} for the Detroit strain in clam liquor was 0.2 minutes. A 12 *D* process based on this datum would require 2.4 minutes at 180°F. Yet, from Figure 3 the TDT at 180°F for 150 million spores is shown to be about 65 minutes. Lynt et al. (1977) similarly found that the computed process time for type E spores in crab meat at 185°F (85°C) based on 12 *D* from a phantom TDT curve was much less than the value obtained from the regular TDT curve.

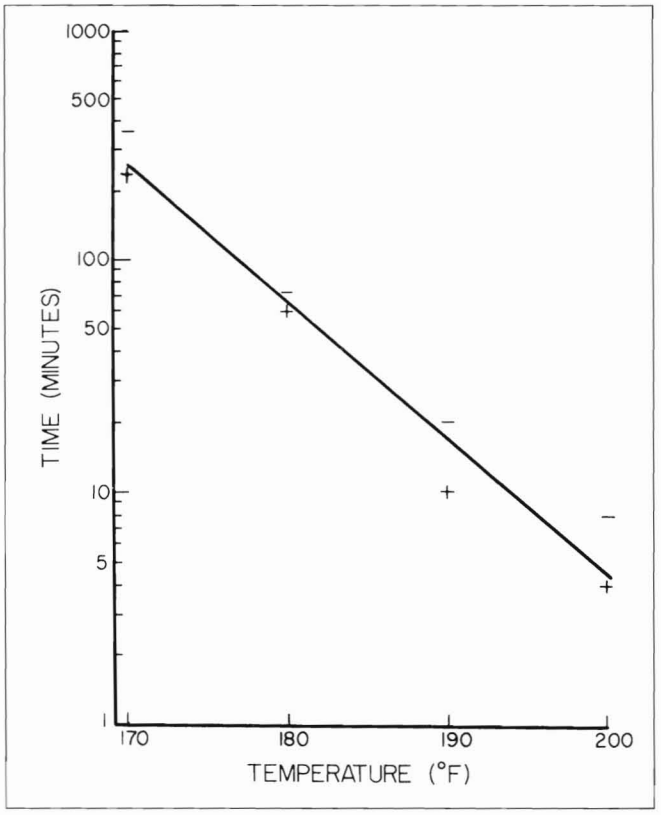

Figure 3.—Thermal death times plot for spores of *C. botulinum* type E (Detroit) in clam liquor (+ = toxin developed in at least one of five tubes after heating and − = toxin did not develop in any of five tubes after heating).

The discrepancy in processing requirement as derived from these two techniques must result from some unaccountable experimental error inherent in one of the two different methodologies. This is also reflected in the differences in z values obtained by the two methods. A possible causative factor could be the differences in incubation time after heating for recovery of viable spores or testing for toxinogenesis. In the TDT method a 2-week incubation period was allowed, whereas in the rate of destruction method the agar tubes could not be incubated beyond 2 days because of extensive gas formation which precluded counting colonies. The gas resulted from the fermentation of glucose which was considered an essential requirement for maximal recovery of type E *C. botulinum*. Also, there is a possibility of differences in recovery, particularly of heat-injured spores, resulting from subculturing in a solid nutrient medium compared with a liquid broth. Finally, enumeration of survivors in the rate of destruction method was based on colony formation, whereas in the end-point method the criterion for survival was toxin production. It is not known whether all surviving spores were capable of toxin production. The important question is which of these two methods is correct. To be realistic, thermal death times would probably be best determined by heating and incubating the spore inoculum in the same substrate for which the process time is being determined. This can be accomplished using TDT cans. It is possible that heat-injured spores may be recovered when subcultured into a nutrient medium whereas these same spores might not be able to germinate and grow out because of an unfavorable environment if left in the heated food substrate. More work is needed to establish the validity of the two methodologies reported here.

Nevertheless, if a food processor bases a process schedule on a minimum botulinum cook, it is not believed that the public health would be compromised. Pflug and Odlaug (1978) have concluded that an F_{250} of 3.0 minutes and a z value of 18 have been the standard for the minimum botulinum cook for more than 40 years and there is no epidemiological evidence to demonstrate that the standard has failed.

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