Interrelationship of Heat and Relative Humidity in the Destruction of Clostridium botulinum Type E Spores on Whitefish Chubs

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Heat destruction of types B and E *Clostridium botulinum* spores on whitefish chubs was observed to be dependent upon the relative humidity (RH) in the chamber in which fish were heated. Experimental conditions were designed to simulate those attainable in commercial fish-smoking plants. Low numbers of type E spores were destroyed with regularity, within 30 min, on fish which were held at an internal temperature of 77 C (170.6 F) in an atmosphere of at least 70% RH. However, an internal temperature of 82 C (179.6 F) and a minimum RH of 70% were required to destroy several hundred thousand type E spores. Quantitative estimates of spore destruction were arrived at with a modified most probable number procedure. Type E spore populations were reduced by 2 to 4 logarithms at 77 C (170.6 F), by 5 to 6 logarithms at 82 C (179.6 F), and by more than 6 logarithms at 88 C (190.4 F) when fish were heated in an atmosphere of 70% RH. A 5 to 6 logarithm reduction of spores was also observed when fish inoculated with type B spores were processed at 82 C (179.6 F) in an atmosphere of 70% RH.

Type E botulism, resulting from ingestion of smoked whitefish chubs, attracted national concern following outbreaks reported in 1963 (19). The gravity of these outbreaks focused attention on an alarming lack of information concerning the biology of type E botulism in this country. Therefore, a symposium, the proceedings of which were subsequently published (9), was convened in 1964 to establish guidelines for the control of botulism, particularly as it related to the smoked-fish industry. A smoked-fish processing ordinance embodying these guidelines was adopted in the city of Milwaukee early in 1964 (Ordinance No. 735, Part 1, Sect. 70-55 through 70-71 of the Milwaukee Code). Subsequent evaluation of the ordinance revealed that slightly more than 1% of whitefish chubs smoked in compliance with it harbored *Clostridium botulinum* (13, 14; P. J. Pace et al., Bacteriol. Proc., p. 10, 1966).

The reason for the occurrence of *C. botulinum* in approximately 1% of the fish smoked at 180 F (82.2 C) for 30 min was not entirely discernible. Persistence of *C. botulinum* type E spores through the process had not been expected because of the organism's widely reported low tolerance to heat (6, 11, 12, 16, 17).

Therefore, the possibility of (i) incomplete heating and (ii) postprocessing contamination also had to be considered (13).

C. botulinum was not detected in smoked whitefish chubs commercially heat-processed in a chamber in which they were engulfed with live steam. This observation led to speculation when considered with respect to the role of water activity (a_w) in heat resistance of bacterial spores. Murrell and Scott (10) demonstrated that *C. botulinum* type E spores were most resistant to heat at an a_w of 0.2 to 0.4.

If one accepts the definition of wet heat as heating in a medium of 100% relative humidity (RH), it would follow that dry heat occurs in an environment where the RH is an undefined value less than 100%. Desiccation, which certainly occurs during dry-heat smoking of fish, would cause a gradient in a_w to occur at the surface of the fish. The a_w in this gradient should progressively diminish as it moves out into space from the surface of the fish, until it equilibrates with the RH of the air. Thus, evaporation of water from the surface of fish smoked in an atmosphere of low RH could result in an a_w, at that location, of considerably reduced magnitude. Furthermore, the

evaporation rate and magnitude would be controlled by the capacity of the ambient air to accept water vapor, a function of the RH. Therefore, the foundation which became the basis of investigations reported herein was established. The question of what, if any, relationships exist between temperature, time, and RH in destruction of *C. botulinum* spores during heat processing of whitefish chubs in conjunction with smoking was posed.

MATERIALS AND METHODS

Spore suspensions. S-32,7 (type E) and M-17,5 (type B) strains of *C. botulinum* were employed. The former was isolated from commercially smoked whitefish chubs and the latter, a nonproteolytic type B, from commercially brined whitefish chubs (13, 14). Stock spore suspensions were prepared and assayed by a procedure described previously (15). These were employed in qualitative tests of *C. botulinum* survival in and on fish exposed to controlled temperatures and RH. The same suspensions were also employed in evaluation of a most probable number (MPN) technique adapted for quantitative estimation of the number of *C. botulinum* spores on commercially brined whitefish chubs. In the course of these studies, the biphasic production method described by Bruch, Bohrer, and Denny (3) was found to yield higher numbers of clean type B and type E *C. botulinum* spores. Therefore, this method was adopted for the production of spores used in experiments designed to provide quantitative estimates of spores surviving on experimentally inoculated fish exposed to controlled temperature and RH. Viable spore counts of these stock suspensions were made by use of the tube procedure described by Crisley et al. (6).

Whitefish chubs. Chubs were obtained from a commercial smoked-fish processor in the Milwaukee area. Fish which have been brined, washed, and smoked at low temperature are referred to as "cured" in local industry parlance. An internal temperature of 135 F (57.2 C) to 160 F (71.1 C) is attained in these fish during the low-temperature smoke. Exposure in this temperature range for an empirical time (1.5 to 2.5 hr) causes the fish flesh to acquire desirable firmness and color. Local dry-heat processors then stoke up the wood fires to achieve a fish internal temperature of 180 F (82.2 C) for the required 30 min.

Brined whitefish chubs were commercially prepared by immersing eviscerated, tap water-rinsed fish in tanks of salt water. Brine solutions of 40° to 50° salometer values were employed. Fish soaked in brine solution overnight, 12 to 14 hr, were found to contain 1.5 to 3.0% NaCl in the water phase of the loin muscle.

"Cured" chubs were used as substrate in qualitative spore destruction experiments, whereas brined chubs were employed in quantitative spore destruction studies.

Heat and humidity exposure. An electronically controlled environmatic test chamber (model no. 1240, Hotpack Corp., Philadelphia, Pa.) was used to expose fish to controlled temperatures and humidities. Wet-bulb and dry-bulb temperature values of the chamber were tracked on a continuous recorder chart. RH values of the chamber atmosphere were calculated from these readings with the aid of published tables (7). This equipment, although lacking a smoke generator, is similar in design to an automated smoke house manufactured for the meat industry (2). Temperature-sensing probes were inserted into the loin muscle of each inoculated fish as well as into an uninoculated control. The probes were connected to a six-circuit thermistor-thermometer (model no. 41, Yellow Springs Instrument Co., Yellow Springs, Ohio) by means of leads passed through a port in the top of the heating chamber. Temperature values were recorded manually at 5-min intervals.

C. botulinum spore suspensions were assayed for population estimates just prior to each experiment. Inoculum dilutions were adjusted relative to the most recent spore viability assay. However, the reported concentration of spores per inoculum was derived from the actual number found viable by assay set up concurrently with each experiment. Spore concentrations were based on the number surviving mild heat treatment (60 C for 15 min). In the case of type E spores, heat-treated suspensions repeatedly yielded viable counts ranging from 40 to 60% less than nonheated suspensions, as previously reported (15). Viable counts of type B spores, in similarly heated suspensions, were increased by a factor of 20 to 50% over counts of nonheated suspensions. Although inoculum levels were calculated from counts of heat-treated suspensions, nonheated suspensions were inoculated on or into fish employed in heat exposure experiments. Consequently, inoculum levels cited represent that portion of each spore suspension resistant to, and includes that portion activated by, mild heat (60 C for 15 min).

Spore inocula were introduced into the loin muscle of fish by means of a 25-gauge hypodermic needle attached to a disposable syringe. Surface inoculations were made with the same equipment. One half of the inoculum per fish (0.1 ml) was introduced onto the outer surface of the fish and the other half onto the surface of its abdominal cavity. The fish were supported on metal shelves, fabricated of 0.25-inch (0.64-cm) mesh, situated in the heating chamber.

Five inoculated fish and one uninoculated control were used in each experiment designed to detect qualitative destruction of spores. Two inoculated fish and one uninoculated control were employed in experiments directed at assaying quantitative destruction of spores.

Exposure timing commenced when the desired internal temperature was attained in each fish included in the experiment. Fish were removed from the chamber directly upon completion of the experimental exposure time. They were held in individual sterile containers at room temperature and processed for enrichment culture within 30 min.

Detection of C. botulinum on fish. A nonheat shock enrichment culture technique (15) was used for qualitative detection of *C. botulinum* spores. An MPN technique was adapted for quantitative estimation of the number of viable spores remaining in inoculated fish. In this procedure, individual fish were comminuted in a sterile, home-type food chopper. Ground flesh was collected into sterile beakers and the weight was determined by tare subtraction. Five samples of one-tenth and five of one-hundredth of the total mass were transferred into tared screw-cap bottles containing 100 ml of Trypticase, peptone, glucose (TPG) medium (18) with 0.1% soluble starch. The remaining mass of ground flesh, roughly one-half of the total, was made up with sufficient TPG broth to yield a 1:6 dilution. Five 0.6-ml samples (approximately one-thousandth of the ground flesh) of the latter culture were transferred to individual screw-cap jars containing 100 ml of TPG broth. Each of the 16 cultures was capped with sterile Vaspar and incubated at 28 C for 7 days. Toxin testing and neutralization tests in mice, and upon occasion reisolation procedures, were performed as previously described (15). MPN values were taken from a published table (1).

RESULTS

Qualitative detection of C. botulinum spores on fish. Preliminary experiments were designed to determine the minimal temperature and RH combination which might render "cured" whitefish chubs free from *C. botulinum* spores. Fish internal temperatures of 60 C (140 F), 66 C (150.8 F), and 70 C (159.8 F) were each employed with controlled RH levels at 10-unit increments from 20 through 90%. The exposure time at the specified temperature and RH combination was standardized at 30 min. Spore inocula ranged from as few as 134 per fish to 2,021 per fish. In spite of the low spore levels, each of five fish tested at each temperature-RH combination repeatedly produced type E toxin-containing cultures.

Thermal destruction of spores, by qualitative assay, was first noted at an internal temperature of 77 C (170.6 F). However, fish inoculated with several hundred thousand type E spores required exposure at an internal temperature of 82 C (179.6 F) for 30 min to render them consistently spore-free. At either of these temperatures, the sporicidal effect was evidenced only in those experiments conducted in an atmosphere of 70% or greater RH. The minimal RH required for spore destruction was decreased to 50% by increasing the internal temperature to 88 C (190.4 F). No difference was noted between experiments which employed surface-inoculated fish and those in which spores were placed within the loin muscle. These data are presented in Tables 1–3.

TABLE 1. *Effect of relative humidity (RH) during heating upon qualitative destruction of C. botulinum type E spores in fish held at an internal temperature of 77 C (170.6 F) for 30 min*

Total time in chamber	No. of spores/fish[a]	RH of chamber (%)	No. of fish yielding toxic cultures[b]	
			Experimental	Control
2 hr 50 min	823	27	5/5	0/1
1 hr 55 min	355	30	5/5	0/1
2 hr	260	39	5/5	0/1
1 hr 55 min	40	51	4/5	0/1
1 hr 50 min	25	61	3/5	0/1
1 hr 15 min	327	69	0/5	0/1
50 min	85	79	1/5	0/1
55 min	72	92	0/5	1/1
2 hr 17 min	7.9×10^5	28	5/5	0/1
1 hr 37 min	4.9×10^5	39	5/5	1/1
1 hr 47 min	3.6×10^5	51	5/5	0/1
1 hr 20 min	7.7×10^5	60	5/5	0/1
1 hr 20 min	1.9×10^5	69	5/5	0/1
1 hr 5 min	1.5×10^5	79	4/5	0/1
55 min	3.0×10^5	92	5/5	0/1

[a] Spores were inoculated onto the surface of the fish.

[b] Number positive/number inoculated (experimental results) or number employed (controls).

MPN evaluation. Brined whitefish chubs were inoculated with 1.0-ml amounts of non-heat-treated spore suspensions. MPN values of spores determined to be in these fish were compared to inoculum concentrations calculated by averaging 10 replicate plate counts (Table 4). Of a total of 20 experiments, 10 (50%) gave MPN values whose 95% confidence limits included the inoculum concentration and nine gave results whose lower limit of the 95% confidence interval was above the inoculum value. In one experiment, the MPN value was indeterminant owing to failure to reach a negative end point. The fish employed in these experiments were randomly collected from a commercial processing plant. It is clear that in none of the experiments did constituents of commercially brined fish or their normal microflora appear to affect adversely the detection of *C. botulinum* type E.

MPN of C. botulinum spores in commercially brined whitefish chubs. Of 101 fish examined, 4 (4%) were found to contain *C. botulinum* type E (Table 5). Three of the four produced MPN values of one spore each, and the fourth was found to contain two spores. Weights of the fish containing 1 spore each were 115.0, 128.4, and 144.4 g; the fish which contained two spores weighed 168.0 g.

Brined whitefish chubs previously had been found to yield a contamination rate of 20% (13).

TABLE 2. *Effect of relative humidity (RH) during heating upon qualitative destruction of C. botulinum type E spores in fish held at an internal temperature of 82 C (179.6 F) for 30 min*

Total time in chamber	Inoculum site	No. of spores/fish	RH of chamber (%)	No. of fish yielding toxic cultures[a]	
				Experimental	Control
1 hr 50 min	Surface	302	30	4/5	0/1
1 hr 45 min		400	40	3/5	1/1
1 hr 55 min		226	50	1/5	0/1
2 hr		305	61	1/5	0/1
1 hr 40 min		137	70	0/5	0/1
1 hr 40 min		127	80	0/5	0/1
1 hr 15 min		62	92	0/5	0/1
1 hr 50 min		1.7×10^5	30	5/5	0/1
1 hr 35 min		2.3×10^5	40	5/5	0/1
1 hr 35 min		2.0×10^5	50	5/5	0/1
1 hr 22 min		1.2×10^5	61	2/5	0/1
1 hr 20 min		6.0×10^5	70	0/5	0/1
1 hr 15 min		6.3×10^5	80	0/5	0/1
1 hr 5 min		3.8×10^5	92	0/5	0/1
1 hr 27 min	Intramuscular	1.5×10^4	31	5/5	0/1
1 hr 45 min		4.1×10^4	41	5/5	0/1
1 hr 45 min		4.5×10^4	50	2/5	0/1
1 hr 40 min		7.1×10^4	61	1/5	0/1
1 hr 40 min		5.3×10^4	69	0/5	0/1
1 hr 40 min		7.0×10^4	80	0/5	0/1
1 hr 10 min		8.8×10^4	90	0/5	0/1

[a] Number positive/number inoculated (experimental results) or number employed (controls).

TABLE 3. *Effect of relative humidity (RH) during heating upon qualitative destruction of C. botulinum type E spores in fish held at an internal temperature of 88 C (190.4 F) for 30 min*

Total time in chamber	Inoculum site	No. of spores/fish	RH of chamber (%)	No. of fish yielding toxic cultures[a]	
				Experimental	Control
1 hr 58 min	Surface	212	31	1/5	0/1
1 hr 48 min		171	39	0/5	0/1
1 hr 55 min		118	50	0/5	0/1
2 hr 15 min		455	60	0/5	0/1
1 hr 45 min		722	70	0/5	0/1
1 hr 25 min		658	80	0/5	0/1
1 hr 8 min		716	92	0/5	0/1
1 hr 30 min		6.1×10^5	30	5/5	0/1
1 hr 33 min		1.2×10^5	39	5/5	0/1
1 hr 26 min		1.6×10^5	50	0/5	0/1
1 hr 32 min		2.5×10^5	61	0/5	0/1
2 hr 5 min		7.0×10^5	70	0/5	0/1
1 hr 28 min		2.9×10^5	81	0/5	0/1
57 min		3.7×10^5	92	0/5	0/1
2 hr	Intramuscular	6.4×10^4	31	5/5	0/1
2 hr 10 min		1.2×10^5	40	1/5	0/1
1 hr 33 min		1.6×10^5	51	0/5	0/1
1 hr 36 min		1.0×10^5	61	0/5	0/1
1 hr 25 min		5.3×10^5	72	0/5	0/1
1 hr 15 min		4.3×10^5	80	0/5	0/1
1 hr 33 min		3.0×10^5	92	0/5	0/1

[a] Number positive/number inoculated (experimental results) or number employed (controls).

The earlier surveillance employed five fish per culture as opposed to the present which employed one per sample. Perhaps the probability of finding one or more contaminated fish in a five-fish sample is five times as great as when a single fish is employed. The results suggest that the frequency and magnitude of *C. botulinum* contamination of brined whitefish chubs are not sufficient to distort thermal destruction data reported herein.

Quantitative destruction of C. botulinum spores by MPN assay. Qualitative studies gave indication that a minimal RH of 70% was necessary for consistent destruction of spores on fish processed at 82 C (179.6 F). Spore re-

TABLE 4. *Most probable number (MPN) values of C. botulinum type E spores derived from whitefish chubs inoculated with known numbers of spores*

Spore inoculum per fish	MPN index per fish[a]	95% confidence interval	
		Lower	Upper
1,000	918	300	3,200
1,000	1,609	640	5,800
500	>1,609		
500	240	68	750
100	221	57	700
100	348	120	1,000
10	49	17	130
10	49	17	130
1	7	1	17
1	24	8	75
3	17	5	46
3	11	2	25
3	24	8	75
3	8	2	19
3	3	<0.5	8
3	13	4	31
3	5	<0.5	13
3	3	<0.5	8
3	33	11	93
3	49	17	130

[a] Calculated from number of cultures producing neutralizable type E toxin, of 16 prepared from each fish. One culture of one-half the total mass of a given fish and five replicate cultures each of one-tenth, one-hundredth, and one-thousandth of the mass.

duction assays by an MPN procedure provided additional insight into the relationship between heat and RH.

Type E spores were reduced by 2 to 4 logarithms with a temperature of 77 C (170.6 F), between 5 and 6 logarithms at 82 C (179.6 F), and more than 6 logarithms at 88 C (190.4 F). Averages of the finite log reduction values observed at each temperature are plotted in Fig. 1. These data were derived from experiments in which fish were held at the indicated internal temperatures for 30 min in an atmosphere of 70% RH.

MPN values of from 1 to 5 survivors each were detected in 8 of 10 fish which had been inoculated with approximately 5×10^5 type E spores prior to exposure at 82 C (179.6 F). Ten fish which had been inoculated with 2×10^6 to 5×10^6 type E spores were exposed to 88 C (190.4 F). Only 1 of these 10 produced a toxic culture. It was found to yield an MPN survival level of one.

Spore reduction values of 5 to 6 logarithms occurred when fish were exposed to 82 C (179.6 F) for 30 min in a chamber having RH levels varying at 10-unit increments from 60 through 90% (Fig. 2). Five replicate experiments at 30% RH and five at 40% RH failed to reach a negative end point. The 16 positive cultures per experiment generated MPN values of greater than 1,609 per fish. The largest spore inoculum was 1.6×10^6 per fish. Therefore, spore reduction values at these lower RH levels were in all cases less than 3 logarithms.

Only a slight increase in type E spore destruction resulted from varying the time of exposure from 5 through 40 min (Fig. 3). An average time of 40 min was required to attain an internal temperature of 82 C (179.6 F). However, no more than 15 min lapsed in raising the dry-bulb temperature and wet-bulb temperature from an ambient to a processing level. The wet-bulb temperature was set at 88 C (190.4 F) and the dry-bulb temperature at 79 C (174.2 C), thereby providing approximately 70% RH.

Fish inoculated with 1.3×10^6 spores, upon

TABLE 5. *Quantitative estimate of C. botulinum type E spores on commercially brined whitefish chubs*

No. of specimens	No. of toxic cultures in MPN[a] series				MPN index per fish	95% confidence interval	
	0.5 of fish	0.1 of fish	0.01 of fish	0.001 of fish		Lower	Upper
97	0/1	0/5	0/5	0/5	<1	—	—
3	0/1	1/5	0/5	0/5	1	<0.5	4
1	0/1	2/5	0/5	0/5	2	<0.5	6

[a] Most probable number. Toxic cultures confirmed by specific toxin neutralization and isolation of *C. botulinum* type E.

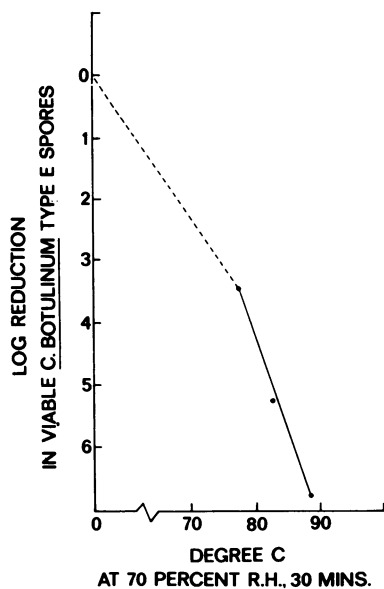FIG. 1. *Destruction of C. botulinum type E spores on fish held at indicated internal temperatures for 30 min in an atmosphere of 70% relative humidity (RH).*
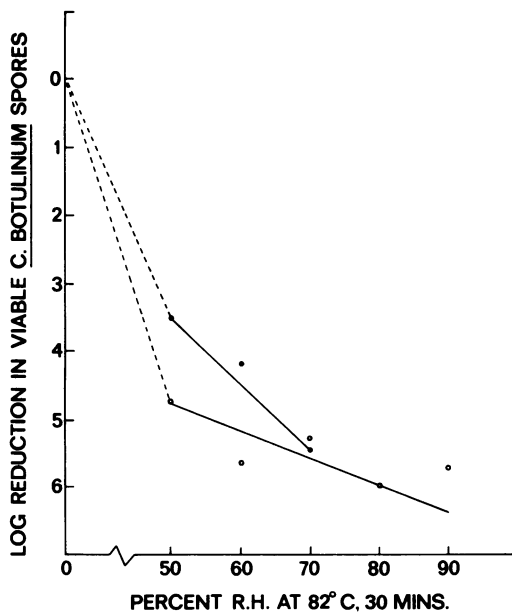

FIG. 2. *Destruction of C. botulinum spores on fish held at an internal temperature of 82 C (179.6 F) for 30 min in atmospheres of varying relative humidity (RH). Symbols:* ●, *type B;* ○, *type E.*

analysis prior to heat exposure, yielded an MPN of greater than 1,609 per fish, a reduction of less than 2.9 logarithms. Therefore, it appears that considerable type E spore destruction occurs during the time the internal temperature of fish is being raised to, and within the first 5 min of exposure at, 82 C (179.6 F) in an atmosphere of 70% RH.

A nonproteolytic *C. botulinum* type B organism, isolated from a whitefish chub at this laboratory, was also employed in temperature-RH studies. Data obtained from these experiments are depicted in Fig. 2. An average reduction of 5.4 logarithms in type B spores was obtained at a fish internal temperature of 82 C (179.6 F), provided that the RH was controlled at 70%. Ten fish processed at this temperature-RH combination had been inoculated with 3.4×10^6 to 5.0×10^6 spores each. These populations were reduced to a level of 3 to 33 per fish by heat exposure for 30 min. Unlike the experiments with fish bearing type E spores, uninoculated controls were frequently observed to produce toxin (type B) upon enrichment culturing. Four of five controls were positive at 50% RH and one each at 60 and 70% RH. It is probable that the uninoculated controls became contaminated in the chamber during heat exposure.

DISCUSSION

There is a paucity of data relevant to the

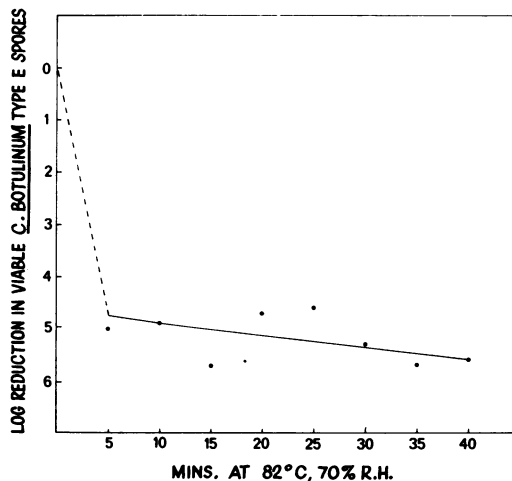FIG. 3. *Destruction of C. botulinum type E spores on fish held at an internal temperature of 82 C (179.6 F) in an atmosphere of 70% relative humidity (RH) for various periods of time.*

concentration of *C. botulinum* spores which one might expect to occur in or on naturally contaminated fish. Cann et al. (4), in the examination of six fish by an MPN method, reported one to contain one type E spore per 16.0 g and two to contain one spore each per 200.0 g. These values are in good agreement

with those reported herein. However, the maximal concentration of *C. botulinum* spores which can be expected to occur in or on naturally contaminated fish is not known. This fact complicates the design of studies intended to assay the effectiveness of commercial fish-smoking processes. The question of whether a process should be designed to destroy 1 spore, 10^6 spores, or 10^{12} spores per fish remains moot.

A suspicion that *C. botulinum* type E spores may persist in fish processed at an internal temperature of 180 F (82.2 C) for 30 min was evoked by data accumulated at this laboratory (13). Christiansen et al. (5), a short time later, definitively demonstrated survival of type E spores in inoculated whitefish chubs smoked at the same temperature for 30 min. The latter investigators also reported survival of spores in fish smoked in an atmosphere of high, but unmeasured, humidity. They estimated a survival level of 250 spores, of the 10^6 introduced, per fish, a reduction of 3.6 logarithms. It appears that the large inoculum level which they used was responsible for the high percentage of fish with surviving spores. Data reported herein support this conclusion. The data further demonstrate that, in an atmosphere of 70% RH and at an internal temperature of 82 C (179.6 F), the type E spore population was reduced by 5 logarithms or more. Heating the fish at an internal temperature of 88 C (190.4 F), at the same RH, reduced the spore population by more than 6 logarithms (Fig. 1).

Both type B and type E *C. botulinum* spores have been isolated from raw and smoked whitefish chubs at this laboratory (13). However, the latter type was observed to occur much more frequently.

A population of nonproteolytic type B *C. botulinum* spores was also found to be reduced by 5 to 6 logarithms in fish heated at 82 C (179.6 F) for 30 min in an atmosphere of 70% RH (Fig. 2). This laboratory has accumulated additional data, not delineated herein, which reveal that no appreciable destruction of type A spores occurs on fish processed for 30 min at 88 C (190.4 F) at RH levels of 30, 40, 50, 60, 70, 80, or 90%.

It is clear that *C. botulinum* type B and type E spores do occur naturally on whitefish chubs taken from Lake Michigan. It is equally clear that development of commercial equipment for processing a safe smoked-fish product is needed. A minimal internal temperature of 82 C (179.6 F), in an atmosphere of 70% RH, successfully eliminated several hundred thousand type E spores from fish during a simulated smoking process. However, not every *C. botulinum* type B or type E spore, if present in sufficiently high numbers on fish, was destroyed by processing temperatures and RH levels employed in these studies. The data substantiate that the rate of spore destruction is not appreciably increased by extending the time that fish are exposed to an internal temperature of 82 C (179.6 F) in an atmosphere of 70% RH from 5 min through 40 min. Therefore, the question arises of why an ordinance should require 30 min of exposure. It would appear that this provision may be subject to review. If one is to be satisfied with a spore reduction level of 5 logarithms, the processing time might well be shortened. Should a greater level of spore destruction be deemed necessary, more extensive studies on the efficiency of increased processing time are required. However, data included in this report indicate that a greater level of spore destruction is obtainable by increasing the temperature and the RH of processing above 82 C (179.6 F) and 70%, respectively, or by increasing only one of these parameters.

The incorporation of NaCl in fish, at a 3.5% level in the aqueous phase, and of 200 ppm of $NaNO_2$ have been reported to inhibit the outgrowth of type E spores (8). These additives may serve as a valuable adjunct in the processing of smoked whitefish chubs. Whether it would be advisable to rely solely on their inhibitory effect and to allow a processing temperature of 77 C (170.6 F) or lower is questionable.

Smoked whitefish chubs are in reality a mildly cooked, not a preserved, product. The effectiveness of heat in destroying or reducing types B and E *C. botulinum* spores on fish is directly related to the RH at which they are processed. Refrigeration of the finished product and shelf life limitation are feasible, enforceable, and necessary additional safety factors to protect the consumer (14, 15). An alternative to compliance with these processing requirements is the abandonment of smoked whitefish chubs as a food source.

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