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Evaluation of Potential Risk of Botulism from Seafood Cocktails

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Clostridium botulinum E could not be detected in 35 samples of commercial seafood cocktails, ranging in pH from 4.10 to 4.85. At 30 C, toxinogenesis in homogenates acidified with a citric-acetic acid mixture was prevented at pH 4.86 or lower for crabmeat and at 5.03 or lower for shrimp. Measurements of the rate of acid penetration into the centers of large pieces of flesh indicated that the already small risk of botulism from seafood cocktails could be completely eliminated by using a cocktail sauce at a maximum pH of 3.70 and by cooling the final product to at least 10 C for 24 h.

The increasing production of semipreserved convenience-type foods has again focused attention on *Clostridium botulinum*, even though the overall incidence of this organism in such foods appears to be quite low (for example, see reference 10). Type E has been found in an appreciable proportion of certain raw seafoods: in 13% of whitefish chubs (7), in 10% of vacuum-packed frozen flounder (4), and repeatedly in marine food species off the Pacific Coast of the United States (1, 3). Crab and shrimp provide a good growth medium for *C. botulinum* (5). When cooked, peeled, and used in the preparation of cocktails in sealed glass jars, these seafoods are not sterilized but are preserved primarily by the bacteriostatic action of acetic acid and secondarily by refrigeration, which, in commercial channels, is not always reliable. In California, the method of preservation has evolved empirically and, while no cases of botulism attributed to such seafood cocktails are known, neither is experimental evidence of their safety available. The purpose of the present investigation was to assess risks of botulism from seafood cocktails.

MATERIALS AND METHODS

The isolation procedure used for *C. botulinum* has been described previously (5).

Preparation of homogenates. The freshly cooked and peeled meat of Pacific Coast crab (*Cancer magister*) or the coastal species of shrimp (*Pandalus jordani*) was homogenized in a blender with an equal weight of water containing 0.5% each of NaCl and sucrose. The final NaCl concentration was 1.25%. A 1:1 mixture of 0.1 N citric and 5% acetic acids was used to adjust pH. Citric acid was selected because it occurs naturally in tomatoes, acetic acid because it is added as vinegar during the commercial preparation of cocktail sauce. The homogenate was then distributed in test tubes, autoclaved, and cooled. The final pH was determined on several tubes of each batch. A Corning Model 10 meter was used for pH determinations.

C. botulinum strains and spore production. *C. botulinum* E strains Beluga and VH, isolated by C. E. Dolman, University of British Columbia; Saratoga, isolated from canned tuna in 1963; and E-8, one of Kushnir's original strains, were used. Because differences in their behavior with respect to pH inhibition were both slight and inconsistent, only Saratoga and VH were used in the later experiments.

Spores were produced in the TPG medium of Schmidt et al. (8) modified to contain 0.2% glucose and 0.1% yeast extract (final pH 7.0). Screw-capped jars (8-oz size) filled to within 2 cm of the top were heated for 20 min in boiling water, cooled rapidly, inoculated with 15 ml each of an actively growing culture, and incubated at room temperature (22 to 24 C). The progress of sporulation was observed twice daily by phase-contrast microscopy, and the spores were harvested when about 90% of the cells showed refractile spores. The crop was washed five times and finally suspended in sterile, deionized water. The viable spore count of the stock suspension was made in deep tubes of heart infusion agar (Difco) after heat shocking for 15 min at 60 C. The tubes were overlaid with vaseline and incubated at room temperature (22 to 24 C), and the colonies were counted after 72 and 96 h.

Inoculation of crab and shrimp. Tubes of crab meat homogenates were inoculated with *C. botulinum* E in six runs. Four strains were used in the first two runs and two strains in the last four. Similarly, two strains were used for each of four runs done on shrimp

meat. For each run, sets of tubes were prepared containing homogenates at two or three different pH values; each set was divided into either two or four 36-tube subsets, depending on the number of strains used. The tubes were inoculated with approximately 10,000 heat-shocked spores, sealed with vaseline, split into three groups of 12, and incubated at three different temperatures. Tubes at 5.5 C were observed weekly; at 10 C, daily; and at 30 C, twice daily, and were tested for toxin at the first appearance of gas. At the end of each run, tubes of the highest pH step that had remained negative were also tested for toxin.

Crab leg muscles were inoculated in the center with about 10,000 spores of *C. botulinum* E VH; placed in beakers containing sauces of different pH values; stored at 10, 24, and 30 C; and periodically tested for toxin.

Detection of toxin. Homogenates were tested by adding to 1 ml of press juice 1 ml of 0.5 N phosphate buffer (pH 6.1) containing 0.2% trypsin (Difco, 1:250). The pH values of all samples were thus brought to between 6.00 and 6.10, depending on initial acidity. After incubation at 37 C for 75 min, 2 ml of sterile deionized water was added, the mixture was centrifuged, and 0.4 ml of the supernatant fluid was injected intraperitoneally into each of two mice. Type E toxin was confirmed periodically by neutralization with specific antitoxin. The mice were observed for 96 h after inoculation; none died after more than 24 h.

Inoculated crab leg muscles were tested by triturating the meat with 1 part of buffer and using the supernatant fluid for trypsinization and injection as just described.

Acid penetration in crab leg muscles. Cocktail sauces, minus spices, were prepared from tomato paste, diluted with water to 10 to 11% solids, and adjusted with acetic acid to various pH values. Crab leg muscles measuring about 10 mm across the smallest dimension were placed in beakers of sauce. The ratio of meat to sauce (40:60) was that of commercial samples. At intervals, three pieces were removed, rinsed, blotted, and cut transversely. The pH across the section was estimated by means of indicator paper (Macherey, Nagel and Co., Düren, Germany, range 3.8 to 5.8). The calibration of the paper was checked with standard buffers.

RESULTS

Incidence of C. botulinum E in commercial seafood cocktails. Although the isolation procedure used is sensitive enough to reveal an average contamination of one spore per gram of material, no *C. botulinum* was detected in any of 35 samples from nine processors.

pH of commercial seafood cocktails. The pH of the commercial sauces ranged from 4.10 to 4.85. Of the 35 samples, seven (lobster and halibut) contained pieces of meat up to 10 mm across. The pH at the center of these chunks was always close to that of the sauce, and in all cases below 4.50.

Effect of pH and temperature on toxin formation by C. botulinum E in crab and shrimp. Table 1 shows the first appearance of detectable toxin in crab meat homogenates, combining results of tests of all strains. In no case was toxin detected in inactive tubes, whereas tubes showing gas were always toxic. Table 1 does not show the results of incubation at 5.5 C because no growth developed in any of those tubes within 127 days.

Because the method of preparation of the homogenates did not allow complete control of the final pH, the differences between some values shown in Table 1 are so small that the corresponding runs should probably be considered replicates. However, it is clear that between pH 4.86 and 5.03 is a fairly narrow grey zone, above which growth (gas formation) and toxinogenesis are apparently unimpeded, and below which both are prevented.

Results obtained with shrimp meat homogenates are quite similar and are not tabulated here. In tubes incubated at 30 C, the appearance of toxin took 2 days at pH $\geq$ 5.25 and 40 days at pH 5.06; at pH $\leq$ 5.03, no toxin could be detected within 80 days. Incubation at 10 C retarded toxin formation by about 5 days at pH 5.06; at pH $\leq$ 5.03, the tubes remained negative for 130 days.

The crab data, which also apply to the shrimp, indicate that, in order to prevent the formation of detectable toxin by *C. botulinum* E, the pH of the meat must be brought down to 4.86 or lower. Moreover, this must be done, at the latest, within 20 h at 30 C or 7 days at 10 C.

Bringing the pH down rapidly by means of a sauce of the proper acidity would seem to be a simple matter for crab cocktails, in which the meat consists mostly of separate fibers. However, in other cases, such as shrimp and lobster cocktails and seafood cocktails containing halibut, fairly large pieces of meat may be encountered, and the rate of acid penetration becomes a factor.

Rate of acid penetration from the sauce into the center of chunks of meat. Crab leg muscles were used in this phase of the work to simulate lobster or halibut chunks as well as shrimp. Table 2 shows combined results from several experiments, in which all three temperatures were not always used, accounting for the many blanks. Room temperature (24 C) was chosen rather than 30 C because it is closer to actual commercial conditions. The target pH was taken as 4.80, rather than 4.86, to allow for the coarser commercial method of measurement.

In the series of experiments summarized in Table 1, it was determined that even at pH 7.2

TABLE 1. *Days necessary for the formation of detectable toxin in crab meat homogenates inoculated with 10,000 spores of C. botulinum E*

pH	Incubation temp (C)[a]		pH	Incubation temp (C)	
	30	10		30	10
7.20	<2[b]	7	4.96	6	>127
5.68	<2	9	4.95	45	38
5.54	<2	10			
5.44	<2	9	4.86	>127	>127
5.35	<2	12	4.85	>127	>127
5.25	<2	15	4.75	>127	>127
5.23	<2	14	4.67	>127	>127
5.07	<2	20	4.58	>127	>127
5.03	5	15	4.45	>127	>127

[a] At 5.5 C, all tubes (at all pH values) were negative for 127 days.

[b] Earliest time at which toxin could be detected was 20 h.

TABLE 2. *Time (h) required to attain a pH of 4.8 or less in the interior of crab leg muscles placed in cocktail sauces of various pH values*

pH of sauce	Holding temp (C)		
	24	10	5.5
4.2	>32	>72	ND
4.1	>32	ND	ND
4.0	32	48	ND
3.97	ND[a]	ND	35
3.91	18	21	ND
3.85	ND	ND	28
3.80	18	22	ND
3.70	12.5	18	23.5
3.60	ND	ND	19.0
3.57	12.2	13.5	ND

[a] ND, No data.

and 30 C toxin could not be detected before 20 h. It would be tempting to assume that, at 24 C, likewise, detectable toxin would not be formed within 20 h, and to conclude from Table 2 that a sauce having an initial pH $\leq$ 3.91 could lower the pH in the center of a reasonably large piece of meat to a safe level within a safe time. However, the difficulty of controlling the relevant variables under commercial conditions renders this conclusion questionable.

A further complication is the presence of two competing processes: (i) the elaboration of toxin by *C. botulinum* and (ii) the concurrent migration of acid, which at some point prevents further toxinogenesis. As temperature lowers, the rate of toxin formation slows substantially, whereas the rate of acid penetration slows only slightly. Consequently, at 24 C the margin of safety, namely, the difference between the rate of toxin formation and that of acid penetration, is small, a few hours at most; but at 10 C this margin is much greater. Table 3 summarizes the interaction of the two processes. At or above room temperature, sauces of pH $\geq$ 4.00, at the time of adding to the crab meat, cannot prevent toxinogenesis if *C. botulinum* E spores are present in large pieces of meat. Although a sauce at pH 3.90 appears to prevent toxin development under the same conditions, the results obtained at the next lower pH step make it prudent to consider pH 3.70 as the maximum. The negative samples were not observed beyond the times indicated because, by then, the center pH was already below 4.80.

DISCUSSION

The published data on the pH inhibition of *C. botulinum* E are somewhat conflicting. Segner et al. (9) observed growth of the Beluga strain at pH 5.03 in TPG medium, but Ohye and Christian (6) found that type E could grow at pH 6.0 but not at 5.0 in TYG medium. On the other hand, Dolman and Iida (2) reported growth and toxin production by the VH strain in pickled herring (pH 4.0 to 4.2). Little information is available on pH inhibition of *C. botulinum* E in shellfish meat. Despite differences in growth media and strains, our data on the acid tolerance of *C. botulinum* E are in general agreement with those found in the literature. As media for *C. botulinum*, crab and shrimp meat seem to possess no unusual qualities.

The risk presented by products on the market is probably minimal. Although no data are available on lobster and halibut, it has been shown here and elsewhere (5) that the principal ingredients, commercially prepared cooked crab and shrimp meat, appear to be free of *C. botulinum* spores. Furthermore, the pH of the samples that we examined was low enough to

TABLE 3. *Time (days) necessary for the formation of detectable toxin by C. botulinum in crab leg muscles held in cocktail sauces at various pH values*

pH of sauce	Storage temp (C)		
	30	24	10
4.20	1	1	>8
4.0	1	2	>8
3.90	>1	>2	>8
3.80	1[a]	>2.5	>8
3.70	>1	>2	>8
3.57	>1	>2	>8

[a] One of six pieces toxic.

prevent the growth of the organism. Nevertheless, the known association of *C. botulinum* E with raw shellfish and its ability to grow at low temperatures make it conceivable that an unusual set of circumstances might result in a hazardous situation. For example, if large pieces of meat heavily contaminated with spores were to be mixed with sauce having too high a pH, and left at room temperature, the spores could grow and produce toxin before the acid penetrated the meat. There are indications that some processors may use a sauce that is not acid enough: When crab or shrimp meat is mixed with cocktail sauce in the usual ratio of 40:60, the pH of the mixture gradually rises 0.2 to 0.55 units, depending on the original pH of the sauce and the freshness of the meat. During this survey, I encountered sauces with a final pH as high as 4.85; therefore, allowing for a rise in pH of even 0.6, I must conclude that the initial pH of the sauce was somewhere around 4.25 which, as has been shown, is not low enough under certain experimental conditions.

Based upon the findings of this investigation, I recommend two key requirements to insure the safety of seafood cocktails. (i) The initial pH of the sauce should be no higher than 3.70, and (ii) as soon as possible after preparation the product should be chilled to at least 10 C and held refrigerated for 24 h. At least with respect to potential botulism, refrigeration need not be maintained beyond this period. These two requirements are easily met and have long been part of the operating procedures of several processors.

Table 4 furnishes an estimate of safety factors in the recommended procedure under a variety of conditions. Even in the absence of refrigeration, a sauce having a pH of 3.70 is sufficient to acidify the centers of meat chunks to a safe level before any spores that may be present have had time to grow out and produce toxin. Because the minimum toxinogenesis time of 20 h was actually obtained at 30 C, the true safety factor at room temperature is probably greater than the 7.5 h shown. Nevertheless, the margin of safety is not comfortable enough when one deals with *C. botulinum*, hence the additional recommendation of rapid initial chilling to retard bacterial growth while allowing acidification to proceed. Under these conditions, any risk of botulism from seafood cocktails should be completely eliminated.

TABLE 4. *Estimated safety factors in the recommended manufacturing practice for seafood cocktails involving the use of pH 3.70 sauce with subsequent chilled storage*

Storage temp (C)	Maximum time to reach pH 4.8 (h)	Minimum time to produce toxin at optimum pH	Difference (safety factor)
24	12.5	20 h[a]	7.5 h
10	18	7 days	6.3 days
5.5	23.5	>30 days	>29 days

[a] Determined at 30 C.

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