Effect of Salt Content and pH on Toxigenesis by 
Clostridium botulinum in Caviar

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ABSTRACT

Bottled lumpfish caviar was prepared with different salt (NaCl) concentrations and pH, and injected with spores of Clostridium botulinum. Under abusive storage conditions (30°C), outgrowth and toxigenesis occurred at concentrations <3.95% salt in the water phase and pH >5.2, and ≤4.67% salt and pH ≥5.6. No toxin was formed at salt concentrations ≥5.56% or at pH ≤5.0. A survey of commercial caviar products showed that most of these had salt-pH combinations which would effectively inhibit C. botulinum at abusive temperatures during storage.

Commercial caviar receives only a mild heat process (<70°C), insufficient to control growth or toxigenesis of Clostridium botulinum. This control is generally obtained by a combination of adequately low levels of water activity and pH. Most caviar products contain 0.1% sodium benzoate for control of yeasts and molds, but it is unlikely that above pH 5.0 it has a significant effect on clostridia (2, 5, 23). The safety record of caviar is good, though not without blemish. Imported caviar was incriminated as the cause of one type B botulism outbreak in Japan (6), and an isolated case of type E botulism from caviar was mentioned by Sebald (20). In a recent report (10) we listed an incident of suspected botulism in Montreal, Canada, with imported lumpfish caviar as the most likely cause.

On the other hand, numerous incidents of botulism have been caused by raw, fermented fish eggs, a traditional Indian food on the North American West coast (4), and by home-prepared or semi-commercial raw salted fish eggs around the Caspian Sea (16).

The present study was undertaken to determine the minimum requirements of salt (NaCl) and pH for the control of C. botulinum in caviar.

MATERIALS AND METHODS

One lot (C-93) of imported black lumpfish caviar in glass jars, 50-g content, was obtained directly from warehouses and kept at 4°C until used. Caviar of this lot had been the most likely cause of a suspected case of botulism (10). A few 100-g jars of the same lot were also obtained.

For toxin assays, 10-g samples were homogenized in a stomacher (J.A. Seward, London, England) with 10 ml of phosphate-gelatin (3) for 2 min. The homogenates were centrifuged at 15,000×g for 10 min and the supernatant fluids separated from the surface layer of fat. Toxin assays and neutralization were as described (8).

The pH was measured by placing a combination electrode directly in the product. The readings were essentially identical to those obtained after addition of a minute amount of water to ensure an adequate aqueous phase for immersion of the electrode, and about 0.1 unit higher than after grinding the product in a mortar. Sodium chloride was determined by AOAC method 18.030 (AOAC, 1975) and the water content by AOAC method 7.003 (1).

The brine concentration was calculated by the equation % brine = g salt × 100/g salt + g H₂O. Water activities were measured in Luft a_w meters (G. Luft Metallbarometerfabrik, Stuttgart, Germany) at 25°C. The instruments were standardized with slurries of different salts (7). However, in the a_w range ≥0.94, the units indicated by the instruments were not identical to the actual a_w values. Standard curves were prepared by adjusting the instruments with either KNO₃ (a_w = 0.936) or K₂SO₄ (a_w = 0.973) and plotting the a_w readings of 0.8-2.0 molal concentrations of NaCl against the known a_w values of these solutions (8).

Spores of C. botulinum were prepared by the method of Schmidt et al. (19) and enumerated by a pour plate method (9). Lot C-93 was tested for its ability to allow toxigenesis by C. botulinum. Twelve 50-g jars each were incubated with 0.05 ml of a spore suspension containing 6.5×10⁷ spores each of type A (A-6) and Type B (13983-II B); 12 100-g jars were inoculated with 0.1 ml of the same spore mixture. The jars were heated to an internal temperature of 62°C, cooled, incubated anaerobically at 30°C, and tested for toxin after 2 weeks. In each experiment, four uninoculated jars were incubated as controls.

For adjustments of salt concentrations and pH, the brine of groups of three jars (150 g) were emptied into 1.5 liters of filter-sterilized 0.01 M Na citrate at various combinations of pH and salt concentrations. The buffer concentration was kept low to minimize its effect on the water activity. At the critical brine concentration of 5% it accounted for about 1% of the total molar salt content. The caviar-brine mixture was kept at 4°C for 48 h. The pH was adjusted to desired levels at the start; readjustments were made after 24 h and, if necessary, after 48 h. The solution was then decanted and the slurry of eggs filtered off with gentle suction and returned to the three jars. The jars were injected with 0.05 ml of one of the following spore suspensions containing (a) 10⁸ spores of C. botulinum type A (A-6), (b) the same number of type B (13983-II B) and (c) of type E (Gordon strain), and (d) a mixture of 3.3×10⁸ spores each of type A strains A-6, A-62 and CK2-A and of type B strains 13983-II B, 368-B and 426-B. The jars were heated to 62°C at the center, cooled, incubated at 30°C, and tested for toxin after 2 and 4 weeks.

The products listed in Table 2 were sampled at the retail level in Ottawa. The 56 products listed in Table 3 were produced by 19 companies in eight countries. They were sampled before distribution by the Inspection and Technology Branch, Industry Services Directorate, Fisheries and Environment Canada. The analytical data were kindly supplied to us by the same agency. Some of the 56 products seemed to differ merely in size of container and/or colorant added, and a few were identical (three products with two matching jars each).

RESULTS AND DISCUSSION

The salt content of 50-g jars of lot C-93 was 4.08 ± 0.17% (mean of 10 jars ± SD); the water content was 75.7 ± 0.3%, the brine salt concentration 5.11 ± 0.21%, and the pH 5.7 ± 0.1. Aerobic colony counts (11) were of the order of 100/g, while anaerobic counts
were < 10/g. Most of the viable microorganisms belonged to the Bacillus and Clostridium genera.

Six out of 12 of the 50-g jars and 5/12 of the 100-g jars inoculated with C. botulinum spores contained toxin after 2 weeks of incubation. The toxicities were between 4 and 200 MLD/g, the contents showed a slight discoloration, and the pH was increased by about 0.1 unit, but none of the toxic products had an offensive smell. The control jars did not contain detectable toxin and showed no noticeable changes. Botulinal toxin was confirmed in all toxic samples by neutralization with specific antisera.

Toxigenesis occurred in caviar with brine concentrations of 2.27 and 3.95% at pH levels of 5.2 and higher (Table 1), but not at pH 4.6 (data not shown) to pH 5.0. With 4.67% brine, toxin was produced at pH ≥ 5.6 but not at pH ≤ 5.4. No toxin was produced at any pH level with brine concentrations of 5.56% or higher. The corresponding aw values are also shown in Table 1. Most of the means were about 0.003 unit higher than the minimum values calculated from the brine concentrations (14), and it is apparent that only the salt contributed significantly to the water activity. No toxin was produced in any of the jars inoculated with C. botulinum type E spores.

Brine concentrations, water activities and pH of 11 commercial products are given in Table 2. On the basis of the results in Table 1, only one of these (a lumpfish caviar from country G) appears to have a pH-aw combination that might permit toxigenesis. The mean water activity of the product would not indicate a potential hazard, but the variation in the aw measurements was relatively large (see SD, Tables 1 and 2). Two other products (salmon and lumpfish caviar from country N) are close to the critical salt content, particularly in view of their high pH. Unfortunately, we were unable to obtain the same products later for further challenge with C. botulinum spores.

Table 3 shows a summary of the survey data supplied by Fisheries and Environment Canada. Water activities

TABLE 1. Effect of salt content and pH on production of toxin by C. botulinum.

<table>
<thead>
<tr>
<th>Brine (%)</th>
<th>aw</th>
<th>pH</th>
<th>Type</th>
<th>2 weeks</th>
<th>4 weeks</th>
</tr>
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<tbody>
<tr>
<td>2.27</td>
<td>0.986</td>
<td>5.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.27</td>
<td>0.986</td>
<td>5.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>0.986</td>
<td>5.4</td>
<td>ND</td>
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<td>0</td>
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<tr>
<td>2.27</td>
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<td>5.6</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2.27</td>
<td>0.986</td>
<td>5.8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.95</td>
<td>0.978</td>
<td>5.0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>3.95</td>
<td>0.978</td>
<td>5.2</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>3.95</td>
<td>0.978</td>
<td>5.4</td>
<td>ND</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3.95</td>
<td>0.978</td>
<td>5.6</td>
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<td>0</td>
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<tr>
<td>3.95</td>
<td>0.978</td>
<td>5.8</td>
<td>3</td>
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<td>0</td>
</tr>
<tr>
<td>4.67</td>
<td>0.974</td>
<td>5.4</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>4.67</td>
<td>0.974</td>
<td>5.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.67</td>
<td>0.974</td>
<td>5.8</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>5.56</td>
<td>0.968</td>
<td>5.6</td>
<td>0</td>
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</tr>
<tr>
<td>5.56</td>
<td>0.968</td>
<td>5.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

aSD of 6 measurements at each brine concentration varied from 0.004 to 0.005.

bNot determined.

TABLE 2. Brine concentration, water activity and pH of some commercial caviar products.

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Caviar product</th>
<th>To be kept refrigerated?</th>
<th>Salt cong. of brine (%)</th>
<th>aw</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>Lumpfish&lt;sup&gt;6&lt;/sup&gt;</td>
<td>yes</td>
<td>6.33</td>
<td>0.958</td>
<td>5.60</td>
</tr>
<tr>
<td>D</td>
<td>Lumpfish&lt;sup&gt;6&lt;/sup&gt;</td>
<td>yes</td>
<td>7.74</td>
<td>0.950</td>
<td>5.71</td>
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<tr>
<td>D</td>
<td>Lumpfish&lt;sup&gt;6&lt;/sup&gt;</td>
<td>yes</td>
<td>8.61</td>
<td>0.962</td>
<td>5.60</td>
</tr>
<tr>
<td>ID</td>
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<td>no</td>
<td>12.92</td>
<td>0.906</td>
<td>5.41</td>
</tr>
<tr>
<td>ID</td>
<td>Lumpfish&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>12.78</td>
<td>0.914</td>
<td>5.81</td>
</tr>
<tr>
<td>G</td>
<td>Lumpfish&lt;sup&gt;6&lt;/sup&gt;</td>
<td>yes</td>
<td>4.24</td>
<td>0.969</td>
<td>5.41</td>
</tr>
<tr>
<td>G</td>
<td>Lumpfish&lt;sup&gt;6&lt;/sup&gt;</td>
<td>yes</td>
<td>8.62</td>
<td>0.937</td>
<td>5.32</td>
</tr>
<tr>
<td>N</td>
<td>Salmon</td>
<td>yes</td>
<td>5.36</td>
<td>0.964</td>
<td>5.60</td>
</tr>
<tr>
<td>N</td>
<td>Lumpfish</td>
<td>yes</td>
<td>5.23</td>
<td>0.964</td>
<td>6.21</td>
</tr>
<tr>
<td>IN</td>
<td>Sturgeon</td>
<td>no</td>
<td>7.74</td>
<td>0.944</td>
<td>5.45</td>
</tr>
<tr>
<td>S</td>
<td>Cod-roe paste</td>
<td>yes</td>
<td>18.60</td>
<td>0.769</td>
<td>5.46</td>
</tr>
</tbody>
</table>

<sup>6</sup>According to label.
<sup>7</sup>SD from ± 0.03% to ± 0.50%.
<sup>8</sup>SD from ± 0.001 to ± 0.009.
<sup>9</sup>SD from 0 to ±0.06.
<sup>c</sup>Each produced by the same companies, differing in added colorants.
were not included, but the lowest brine concentration of any of the 56 samples was 6.0% indicating that all had water activities of ≤0.962. None of these products, therefore, appear to present a potential hazard.

The diversity of salt content and pH, particularly among the lumpfish and salmon caviar is striking. Many of the products had salt contents far in excess of those needed to control *C. botulinum*; the pH was often so high that it would not have contributed to product safety.

The results have shown that the suspect lot (C-93) of caviar allowed growth and toxigenesis of *C. botulinum* at an abusive temperature. On the other hand, they also indicate that this product is not particularly conducive to production of botulinal toxin. While the maximum pH for effective control of *C. botulinum* is below 5.0 in various foods (8,12,13,22), no toxin was produced at this pH in our experiments. Furthermore, no type E toxin was produced under any of the experimental conditions. Even in more suitable media, the minimum *a*<sub>w</sub> required for growth of *C. botulinum* type E appears to be 0.97 (14,15,21) which corresponds to salt concentrations of roughly 4% of the product and 5% of the brine. It is unlikely, therefore, that type E, the most common botulinal type in sea foods, would develop in commercial caviar.

Another safety factor of the product is its warning label to the effect that it has to be kept refrigerated. Since only *C. botulinum* type E and nonproteolytic strains of types B and F are capable of producing toxin at 5°C (17), it is unlikely that the product under investigation (lot C-93) would have become toxic under proper refrigeration. However, it is well known that caviar products are often displayed at the retail level with inadequate or without refrigeration. Our experimental incubation at 30°C for up to 4 weeks does not seem to be unrealistic.

Lot C-93 of the lumpfish caviar was not uniform: we noticed some significant differences in pH among jars of the same cardboard containers and also discovered that the jars of one container differed significantly from the jars analyzed earlier (see Results), both in pH (5.45 ± 0.2) and salt content (4.59 ± 0.09%). It is conceivable, therefore, that the reported illness in Montreal may have been caused by a single jar that received some temperature abuse and had, relative to the total lot, high levels of water activity and/or pH. Some heterogeneity among jars of the same lot was also noticed in some of the commercial products of Table 2, and Fukuda et al. (6) reported variations in salt content from 3.5% to 5.4% in the batch of caviar incriminated in the Japanese outbreak.

### ACKNOWLEDGMENTS

We thank the officers of the Inspection and Technology Branch, Fisheries and Environment Canada, in particular Mr. R. M. Bond and Mr. B. Lingeman, for the supply of lumpfish caviar and their permission to include a summary of their survey data in this paper. We also thank our colleagues at the Regional Health Protection Branch Laboratory in Montreal, namely Mrs. P. Ennis, Mr. H. Boisvert and Mr. R. Dufault for their most valuable cooperation.

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16. Pourtaghva, M., A. Machoun, Fatollah-Zadeh, A. Khodadoust, H. A. Lerke, A. Fukuda et al. (6) reported variations in salt content from 3.5% to 5.4% in the batch of caviar incriminated in the Japanese outbreak.
FARM METHODS REPORT,

continuation is six to ten times what the EPA has predicted (according to the American Water Works Association). For instance, Miami has predicted it will cost $40 million to $45 million, while the EPA has said it will cost Miami $3.7 million. The American Water Works Association (AWWA) has gone on record stating that "EPA is arbitrary, capricious and premature" attacking the proposed organics regulations as being pulled from a hat, based on complete research but incomplete guesswork. It also said the National Academy of Sciences did not suggest limits for organics but did call for more research.

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