A Research Note

Evaluation of Unacidified Products Bottled in Oil for Outgrowth and Toxin Production by *Clostridium botulinum*

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ABSTRACT

A variety of unacidified products bottled in oil or water were investigated for their ability to support growth and toxin production by *Clostridium botulinum*. The products were inoculated with a mixture of five strains of *C. botulinum* type A spores (about 50 spores/g or ml) and incubated at room temperature (23°C). At monthly intervals the organoleptic acceptability of the products, as determined by appearance, odor, and texture, was evaluated and a portion of each sample was removed, diluted 1:2 in gel-phosphate buffer, and injected intraperitoneally into mice. At the end of 4 months the drained solids of each sample were macerated with a minimal amount of buffer and centrifuged; the clear extracts were then injected into mice. None of the products tested supported growth and toxin production by *C. botulinum*.

Two recent outbreaks involving 40 cases of botulism poisoning were caused by unacidified chopped garlic bottled in oil and commercially produced in the United States (1-4,8). After these incidents, the Food and Drug Administration (FDA) ordered all producers to stop making any garlic-in-oil products that relied solely on refrigeration for safety. To be safe, garlic-in-oil products must contain a second barrier, such as an acidifying agent, in addition to refrigeration, because *Clostridium botulinum* spores capable of outgrowth and toxin production under proper conditions can be found on all types of vegetables that grow in soils where these spores occur naturally (6,7).

Earlier studies (6) showed that unacidified garlic in oil supported *C. botulinum* outgrowth and toxin production while the product remained organoleptically acceptable, as determined by appearance, odor, and texture. FDA’s action regarding this product stimulated inquiries about the safety of similar products on the market. The objective of the present study was to determine whether the large variety of unacidified products bottled in oil (or water) would support outgrowth and toxin production by *C. botulinum* at room temperature before the products became organoleptically unacceptable.

MATERIALS AND METHODS

Cultures

A mixture of *C. botulinum* type A spores of five strains isolated primarily from vegetables was used as inoculum (Table 1). Individual spore suspensions were prepared as described previously (7). Before mixing, the five individual strains were tested for purity and toxicity.

Products bottled in oil

Ten or 12 samples of each of the following products bottled in oil (or water) were investigated: oil extract of garlic, black beans in oil, chili-garlic in oil, chopped shallots in oil, walnuts in oil, garlic in water, sun-dried tomatoes in oil, dried tomatoes in olive oil, dried tomatoes in sunflower oil, “Romance” pesto sauce, and “Contadina” pesto sauce. Only the dried tomatoes in oil and the pesto sauces came in 7-oz containers and were used as such. The other products came in bulk quantities and were distributed into 7-oz portions in wide-mouth screw-capped bottles. The pH of all but one product was 6.0. The exception was chopped shallots in oil with a pH of 4.7. Likewise, the pH of tomatoes in oil was 4.0-4.5.

Inoculation

The pH of the products was determined by means of a Fisher Accumet No. 925, with the electrode dipped directly into the

<table>
<thead>
<tr>
<th>Strain-type</th>
<th>Origin</th>
<th>Date of isolation</th>
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<tbody>
<tr>
<td>426-A</td>
<td>Green peppers in jars</td>
<td>1971</td>
</tr>
<tr>
<td>Clovis-A</td>
<td>Potato salad, Clovis, NM</td>
<td>1978</td>
</tr>
<tr>
<td>OS3-A</td>
<td>Onion skins, Peoria, IL</td>
<td>1983</td>
</tr>
<tr>
<td>51-A</td>
<td>Fresh garlic skins</td>
<td>1987</td>
</tr>
<tr>
<td>CS2-A</td>
<td>Coleslaw outbreak</td>
<td>1988</td>
</tr>
</tbody>
</table>

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product, and/or by Sigma pH measuring strips. The products were then inoculated with the five-strain mixture of *C. botulinum* type A spores at a level of about 50 spores/g or ml. The inoculum consisted of a 1-ml suspension of the spores and was delivered to the bottom of the jar. Wheaton storage bottles (125 ml) containing 100 ml trypticase-peptone-glucose-yeast extract broth were similarly inoculated to serve as positive controls (5). The jars containing the inoculated products plus the positive controls were incubated at room temperature (23°C).

**Product testing**

At monthly intervals a portion from each sample was removed and diluted 1:2 in gel-phosphate buffer (5), and 0.5 ml was injected intraperitoneally (i.p.) into each of two Swiss-Webster mice (18-20 g). Products were tested over a 4-month period. At the end of that time, they were drained of the oil or water and macerated in a stomacher with either a minimal amount of buffer or no buffer added. The centrifuged extracts were injected i.p. into mice: 0.5 ml/mouse; two mice/dilution.

**RESULTS AND DISCUSSION**

None of the 120 samples inoculated with *C. botulinum* type A spores exhibited toxicity after 4 months of incubation at room temperature. Since FDA banned further production of unacidified garlic in oil, questions arose about the safety of similar products bottled in oil or water and whether the regulatory action against garlic in oil should be expanded to include these products.

The results of our study showed that the products tested did not support outgrowth and toxin production by *C. botulinum* type A spores under our test conditions. However, new and unusually potent strains of *C. botulinum*, or a combination of optimum conditions, might induce their growth in these products and could be a potential hazard. Therefore, caution in the manufacture and distribution of these items is still urged.

**REFERENCES**