Effect of Acid and Salt Concentration in Fresh-Pack Pickles on the Growth of Clostridium botulinum Spores

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The addition of various amounts of acetic acid to pureed cucumbers inoculated with Clostridium botulinum spores has shown that outgrowth is inhibited at pH 4.8 but not at pH 5.0. Inoculation experiments with whole cucumbers showed that as little as 0.9% acetic acid in the brine was sufficient to prevent outgrowth from spore inocula as high as 10⁶/cucumber. It was further shown that the rapid rate of acetic acid penetration into fresh-pack pickles prevents the growth of any C. botulinum spores that may be present.

Historically, the cucumber pickle has been thought of as the properly fermented cucumber to which vinegar, spices, salt, and perhaps some sugar has been added. The use of heat for preservation was not required. In recent years fresh-pack pickles, made by the addition of vinegar, salt, and spices to the fresh cucumbers and preserved by pasteurization, have come to represent a substantial portion of the pickle business. Monroe et al. (8) in 1969 estimated that about 40% of the pickling cucumbers produced were used in fresh-pack pickles.

The preservation of fresh-pack pickles is dependent upon proper application of acid and salt and the utilization of proper pasteurization techniques (8). Pasteurization and acidification are used to preserve the product by destroying the normal spoilage organisms (4, 5, 6) and to control the growth of organisms of public health significance.

Although commercially packed pickles have never been implicated in botulism food poisoning, Clostridium botulinum appears capable of growing in pickles and cucumbers. Meyer and Eddie (7) cite three cases of botulism from home-canned pickles and one incident from home-canned cucumbers. The reason for C. botulinum growth in these instances was not thoroughly investigated. It was the purpose of this investigation to determine the pH levels necessary to inhibit the growth of C. botulinum spores in cucumbers and to determine the effect of fresh-pack pickling procedures upon the growth of C. botulinum spores.

MATERIALS AND METHODS

Spore suspensions. The organisms used were C. botulinum strains 33A, 62A, 73A, 78A, 32B, 113B, 169B, and 213B (from K. F. Meyer, G. W. Hooper Foundation for Medical Research, San Francisco, Calif.) and strains 44A, 12033B, and 13983B (from National Canners Association, Berkeley, Calif.).

The spores of C. botulinum 62A and 213B were prepared in polyepetone broth (5%) and were washed and harvested by the methods of Tsuji and Perkins (11). The spores of the remaining strains were prepared in Wheaton beef heart-casein broth and were harvested by the methods of Denny et al. (2).

Prior to subsequent use, equal numbers from each of the spore suspensions were mixed together to form a single composite spore suspension.

Studies in cucumber puree and cucumbers. Whole, pickling cucumbers (0.75 to 1.25 inches [ca. 1.9 to 3.17 cm] in diameter and 3 to 4.5 inches [ca. 7.6 to 11.4 cm] in length) were obtained from California fields. Upon receipt, the cucumbers were refrigerated and processed within 48 h.

The cucumbers were cleaned by washing in several changes of tap water. The cleaned cucumbers were blanched in hot water for 20 min at 180°F (82.2°C). The blanched cucumbers were air cooled and then thoroughly comminuted by using a Waring blender. The resultant cucumber puree was placed in beakers and sterilized at 250°F (121.1°C) for 20 min. After sterilization and subsequent cooling, the pH of the pureed cucumbers was adjusted, using either acetic acid or sodium hydroxide, to a range of values starting with pH 4.5 and rising at approximately 0.2 pH unit intervals to pH 6.2. The pH of the pureed cucumbers before adjustment was 5.65.

Each of the samples of pureed cucumbers, adjusted to various pH values, was transferred to eight culture tubes (16 by 150 mm). The air was exhausted from the product by heating the tubes in a hot water bath. After air exhaustion, one-half of the tubes were inoculated with 0.1 ml of spore inoculum, which had been previously heat shocked in sterile distilled water at 180°F (82.2°C) for 10 min. Two inoculum levels (10² and 10⁶ spores/tube), each in
duplicate, were used. The remaining tubes were used as uninoculated control tubes and were inoculated with 0.1 ml of sterile distilled water. All tubes were overlaid with sterile vaseline and incubated at 30°C.

For inoculation experiments with whole cucumbers, 0.1 ml of the spore suspension, containing $10^6$ or $10^8$ spores, was injected with a syringe and needle through the blossom end into the centers of cucumbers. The holes made in the cucumbers from the inoculation were sealed with paraffin or plastic cement.

All of the cucumbers (inoculated and uninoculated) were blanched for 30 to 60 min in a kettle containing 20 salometer brine at 120°F (48.9°C). The blanched cucumbers were hand packed into 27-oz (ca. 0.8-liter) glass jars (9 to 11 cucumbers/jar) so as to have about 40% brine by volume in the jar. An inoculated pickle was placed in the center of the jar. The cucumbers were covered with a brine containing 12° Brix sugar and various amounts of acetic acid and salt. The jars were closed with a screw-cap lid, using a vacuum sealing device, and were processed under water at 185°F (85°C) for 26 to 36 min or until the center of the pickle in the slowest heating zone of the jar reached 165°F (73.9°C) for 15 min (5); then the jars were immediately water cooled. The temperature was monitored with a thermocouple placed in the center of the pickle in the slowest heating zone of several jars. Four-jar replicates were made for each variable tested. The processed pickles were incubated at 30°C.

**Determination of the growth of C. botulinum.** Growth of microorganisms was determined by gas formation and microscope examination of the product. The presence of *C. botulinum* toxin was determined by centrifuging the brine, cucumber homogenate or cucumber puree, at 10,000 $\times g$ for 10 min and injecting 0.4 ml of the supernatant fluid intraperitoneally into mice. For neutralization, the extracts were mixed with monovalent type A or type B antiserum (Lederle Laboratories), and 0.4 ml was injected intraperitoneally into mice. When necessary, treatment with trypsin (3) was used. The mice were observed for 5 days. All positive, as well as negative, growth samples were tested for toxicity.

Study of acid penetration into pickles. Fresh-pack pickles were prepared as previously described. Acid penetration was determined by measuring pH on a pickle that was rinsed, scored at the thickest part, and broken. The tip of a Bolan microcombination electrode was inserted at the center, between the center and the periphery and just below the skin. The time of acid penetration was determined from the time the brine was poured into the jar. Whole pickles (1.5 inches [ca. 3.8 cm in diameter]) were used because acid penetration into these pickles would be slower than into slices.

**RESULTS**

**Studies in cucumber puree and cucumbers.** Results obtained on inoculated cucumber puree are given in Table 1. The pH, as measured at the time of inoculation of the product, changed slightly during incubation. This was determined by measuring the pH of the uninoculated control tube at the time that gas formation was observed in the inoculated tube. Growth and toxin formation were observed in the product at pH 5.0 with the large inoculum ($10^6$) but not with the small ($10^3$). No growth or toxin formation was observed in the product at adjusted pH values of 4.8 or below. The tubes were observed for at least 6 months for evidence of growth. The organism that grew was a type B, as determined by toxin neutralization tests.

The results obtained from inoculated whole cucumbers are shown in Table 2. The jars were observed for at least 6 months for evidence of growth. The presence of toxin was determined both in the brine and in the inoculated pickle. The inoculated pickle was homogenized and subjected to trypsinization prior to inoculation into mice. When toxin was found, both the brine and the pickle were toxic. The organism that grew was a type B, as determined by toxin neutralization tests.

Although spoilage occurred in the samples that had a cover brine with no acid, 8% salt, and 12° Brix sugar, no toxin was found in the brine or in the pickle. The spoilage was caused

| pH at time of inoculation (per tube) | Growth formation | Days of incubation for gas formation | Toxin
|----------------------------------------|-------------------|--------------------------------------|---
| 4.5 | $10^6$ | No | -
| 4.6 | $10^6$ | No | -
| 4.8 | $10^6$ | No | -
| 5.0 | $10^6$ | Yes | 18
| 5.2 | $10^6$ | Yes | 12
| 5.5 | $10^6$ | Yes | 8
| 5.7 | $10^6$ | Yes | 4
| 5.9 | $10^6$ | Yes | 4
| 6.2 | $10^6$ | Yes | 4

* pH of original cucumber puree (before adjustment), 5.65.

* Negative samples had a normal odor and no microscope evidence of bacteria. Positive samples had a slight off odor and microscope evidence of rod-shaped bacteria.
by an unidentified rod-shaped bacterium. No growth occurred when only 0.9% acetic acid was used, and no toxin was detected in either the brine or the pickles.

**Acid penetration into pickles.** Figure 1 shows the rates of acid penetration into the centers of pickles. The points shown are for the highest pH found among at least four pickles within each experimental group in two runs. It can be seen that, even with the worst performing brine, a maximum pH of 4.8 was reached within 10.5 h. With a cover brine of 8% salt and 1.8% acetic acid, this pH was reached within about 6 h.

**DISCUSSION**

The growth studies in cucumber puree indicate that *C. botulinum* is inhibited at pH 4.8 but will grow at pH 5.0, provided that a large inoculum level is present. This result is compatible with the finding of Townsend et al. (10), who found that the pH level for the inhibition of *C. botulinum* growth is not the same for all foods and for some foods may be above pH 4.6. These growth studies indicate that approximately 4 days are required for gas formation and subsequent toxin formation at pH 5.6 and above. Longer incubation times are required at lower pH values.

The rate of acid penetration is similar to the rates determined by Bell et al. (1). They determined the rate of acid equilibration and found the rate to be relatively rapid. However, total equilibration could take as long as 2 or 3 weeks. They found the penetration rate to be an exponential function and, when they plotted the percentage of acid equilibration against log time, they found the resultant line relatively straight.
In this investigation our interest was to determine the time interval for the center of the pickle to reach a level where \( C. \) botulinum spores, if present, could no longer outgrow. Thus, we focused on pH values rather than on acid equilibration. In the brine with the least amount of salt and acid, it took only 10.5 h for the center of the pickle to reach pH 4.8 or less.

Inoculation studies on fresh-pack whole pickles confirm that the rate of acid penetration is sufficiently rapid that \( C. \) botulinum spore outgrowth is prevented, even with a brine composition of 12° Brix sugar and 0.9% acetic acid. Approximately 2 days is required for gas formation and subsequent toxin production when acid is omitted and 4% or less salt is used in the cover brine.

The results would indicate that the danger of botulism from consuming fresh-pack whole pickles is rather remote, provided that acidification and pasteurization procedures such as advocated by Monroe et al. (8) are followed. However, since the only incidences of botulism in these products have occurred in home-canned pickles and relishes (7), it would appear that proper acidification of these products is not always being obtained by the home canner. Mundt et al. (9), observing samples of all types of home-canned pickles and relishes from Tennessee, found that 2.3% of the 783 submitted samples were considered unsafe for consumption because the pH was above 4.6. It would appear that continued attempts to educate the home canner on the hazards associated with improper acidification procedures are required. To this end, more popularized information, such as that available from the U.S. Department of Agriculture (12) or from University Extension Services, may be valuable to the home canner.

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LITERATURE CITED