Use of Preservatives to Delay Toxin Formation by Clostridium botulinum (Type B, Strain Okra) in Vacuum-Packed, Cooked Potatoes

S. NOTERMANS1*, J. DUFRENNE1 and M. J. H. KEYBETS2

Laboratory for Water and Food Microbiology, National Institute of Public Health and Environmental Hygiene, P.O. Box 1, 3720 BA Bilthoven, The Netherlands and Institute for Storage and Processing of Agricultural Produce (IBVL), P.O. Box 18, 6700 AA Wageningen, The Netherlands

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ABSTRACT

Storage at temperatures below 4°C prevents growth and toxin production by Clostridium botulinum in vacuum-packed, cooked potatoes. The use of preservatives as an additional, built-in safety factor has been investigated. Dipping potatoes in a solution of ascorbic and citric acid before vacuum-packing and cooking (95°C for 50 min) inhibited growth and toxin production by proteolytic C. botulinum type B at an incubation temperature of 15°C for 70 d and at 20°C for at least 14 d. This preservative treatment also resulted in an organoleptically acceptable product with a prolonged shelf life. Risk analysis showed that the presence of C. botulinum in vacuum-packed, cooked potatoes may be expected, i.e., one spore in each 1585 kg of product. A preservative treatment with a combination of ascorbic and citric acid will limit the public health risk even if the potato product is accidently stored for a short time at a temperature higher than 4°C.

In several European countries vacuum-packed, cooked potatoes are produced commercially. The product is semi-preserved with a limited shelf life even under refrigeration. Vacuum-packed, cooked potatoes can be consumed after heating, or without heat treatment in a salad. Notermans et al. (7) showed that there is a potential risk of toxin production by Clostridium botulinum in vacuum-packed, cooked potatoes. Spores of both proteolytic and non-proteolytic C. botulinum strains may survive the cooking process. Since it has been shown that vacuum-packed, cooked potatoes are an ideal substrate for growth and toxin production by C. botulinum, outgrowth of this bacterium must be prevented. Only storage of the potato product at or below 4°C prevented outgrowth of C. botulinum and subsequent toxin production (7). On the other hand, industry has claimed a shelf life for such a product of 2 to 3 months at ambient temperature (1), which would certainly cause a public health risk.

To prevent botulism, it is advisable to incorporate an additional safety factor. In the case of vacuum-packed, cooked potatoes, such a safety factor may be a preservative. Preservatives often used to control C. botulinum include sodium nitrite usually combined with sodium chloride (12,16), potassium sorbate (5,14), sulfites (4,11,17) and acidulants (3,9). The disadvantages of the use of nitrite as a preservative are well-documented because it is a potential precursor of carcinogenic nitrosamines (6,18). The use of other preservatives is often limited because their taste becomes noticeable (11) or because of legal limitation, as in the case of sulfites.

We describe in this paper investigations on the effect of preservatives not having these disadvantages, including ascorbic acid, citric acid and sorbate, on the shelf life of and on the inhibition of growth of C. botulinum in vacuum-packed, cooked potatoes. The necessity of good preservation of vacuum-packed, cooked potatoes is underlined by estimation of the incidence of C. botulinum in the processed product.

MATERIALS AND METHODS

Organism and production of spores

The proteolytic C. botulinum type B strain Okra was used. From earlier experiments (7) it was evident that this strain produces relatively large amounts of toxin in potatoes as the substrate. Spores were obtained by surface inoculation of brain heart infusion agar (BHI; Oxoid). After incubation at 30°C for 4 d in anaerobic conditions (80% N2, 10% CO2, 10% H2), spores were collected by first rinsing them with sterile physiological saline, subsequently centrifuging the suspension, then washing spores once with distilled water. The washed spores were suspended in distilled water, heated at 70°C for 10 min, and stored in small portions at -20°C. Each portion was only used once.

Preparation, inoculation and storage of vacuum-packed, cooked potatoes

Several lots of potatoes were used. These lots had been grown on experimental farms or had been purchased directly.
from farmers. The tubers (variety Bintje; dry matter 20.7 to 22.7%) were stored at the IBVL at 7 to 8°C for 1 to 6 months.

The potatoes were steam-peeled, trimmed and, when necessary, cut into pieces smaller than 50 mm. Before packing, the peeled tubers were dipped for 2 min in solutions containing 2% ascorbic acid plus 1% citric acid (Treatment II), 5% potassium sorbate followed by 2% ascorbic acid (Treatment III), 2% ascorbic acid (Treatment IV), or 2% ascorbic acid plus 0.1% Na₂S₂O₅ (Treatment V). Untreated potatoes served as a control (Treatment I). Quantities of 0.7 to 1 kg were packed one layer thick in laminated pouches [50 µm polyamide, 75 µm polyethylene; sterilizable at 121°C; oxygen permeability (pouches) 18 cm³/m²/24 h, 100 kPa (at 23°C and 75 RH)] supplier Wolff Walsrode, Walsrode FRG. Approximately 7.0 log₁₀ units of spores of C. botulinum type B (strain Okra) were inoculated into pouches containing 0.7 to 0.8 kg of potatoes. Other pouches, for sensory evaluation, contained up to 1 kg of tubers and were not inoculated with spores of C. botulinum. The pouches were evacuated, until a pressure of 1 to 4 kPa was reached, sealed, heated in a water bath at 95°C for 50 min, and cooled in running tap water for 15 min.

After the outer surface of the pouches had dried, samples were stored at 15, 20 and 25°C in the dark.

Counting of C. botulinum and anaerobe-growing, spore-producing bacteria

For the quantitative assessment of C. botulinum in soil, 100 g of sample was mixed with 300 ml of sterile physiological saline solution. Serial 10-fold dilutions of the mixture were made, and 4 ml of each dilution was transferred into each of the five tubes containing 30 ml of fortified egg meat medium [150 g of egg meat (Difco), 10 g of glucose, and 10 g of ammonium sulfate per liter, sterilized at 120°C for 15 min]. Before incubation, all tubes were heated at 70°C for 20 min. After incubation of the tubes at 30°C for 5 d, the culture fluids were examined for the presence of botulinum toxinas. The number of C. botulinum present in vacuum-packed, cooked potatoes was determined as described above, however, samples were not heat treated before incubation. If >200 C. botulinum/g were expected in samples, a plate count was also done on BHI egg yolk agar. Plates were incubated anaerobically (80% N₂, 10% CO₂, 10% H₂) at 30°C for 3 d.

Anaerobe-growing spores were counted as described for assessment of C. botulinum in soil. However, tubes were only observed for growth.

Test for botulinum toxin

Potatoes. Sterile 0.05 M phosphate buffer, pH 6.0, containing 2 g of gelatin, 1 mg of streptomycin and 10⁶ U of penicillin per liter, in 500 ml volumes was added to each pouch containing 0.7 to 0.8 kg of potatoes. After shaking (100 rev/min) for 30 min and subsequent settling for 20 min, ca. 10 ml of the supernatant fluid was collected. This fluid was examined after trypsin activation for botulinum toxinas by the mouse bioassay. Activation was performed by adding trypsin (Sigma type III, Sigma Chemical Co., St. Louis, MO; at a final concentration of 0.2 mg/ml) to the culture fluid. After incubation at 37°C for 30 min, serial 5-fold dilutions were made. Three mice (18 to 20 g) were injected i.p. with 0.5 ml of each dilution and observed for 4 d. Neutralization was determined by mixing 2 ml of trypsin-activated fluid with 0.5 ml of specific anti-botulinum serum (Institut Pasteur, Paris).

Culture fluids. Culture fluids were tested for the presence of botulinum toxin by the mouse bioassay. One ml of culture fluid was mixed with 4 ml of 0.05 M phosphate buffer, pH 6.0, containing 2 g of gelatin, 1 mg of streptomycin and 10⁶ U of penicillin per liter. Trypsin activation and neutralization experiments were carried out as described above. Two mice (18 to 20 g) were injected i.p. with 0.5 ml of each dilution and observed for 4 d.

Chemical analysis

The concentration of ascorbic acid, sorbate and sulfite taken up by the potato tubers was determined after heating the vacuum-packed samples. Ascorbic acid was determined by HPLC using the procedure described by Rückemann (13). However, for extraction, oxalic acid was used instead of meta-phosphoric acid. Sorbate was determined by GLC (10) after an extraction procedure in which dichloromethane was used instead of methyl isobutylketone. Sulfite was analyzed according to the procedure of Zonneveld and Meyer (19).

In order to determine the influence of dipping on pH, 200 g of whole potatoes was immersed in 200 ml of demineralized water and pH was measured after 10 min.

Shelf life

Shelf life was determined by periodic sensory evaluation and microbiological analysis of uninoculated pouches. A panel of 7 to 11 experienced members evaluated color and flavor of the stored samples, after they were prepared for consumption by steaming for 25 min, according to a 7-point scale (8 = good to 2 = bad). The limit of acceptability was arbitrarily set at a score of 4 (4 = moderate).

Aerobic mesophilic bacterial count

The aerobic mesophilic bacterial count of vacuum-packed, cooked potatoes was determined after different storage times as described previously (7). This count was only done on uninoculated pouches.

RESULTS

Estimation of the number of C. botulinum naturally present in vacuum-packed, cooked potatoes

To estimate the presence of C. botulinum in vacuum-packed, cooked potatoes (heated at 95°C for 50 min), the anaerobic spore count and count of C. botulinum spores of soil samples were made. Soil was obtained from stored potatoes originating from different regions of The Netherlands. In addition, the anaerobe-growing spore count of vacuum-packed, cooked potatoes was enumerated immediately after potatoes were processed.

In soil samples, an average of 6.4 log₁₀ anaerobe-growing spores per gram was present (Table 1). C. botulinum was found in all samples with an average of −0.32 log₁₀ spores/g.

In processed potatoes, the average count of anaerobe-growing spores was 0.50 log₁₀ spores/g. This finding indicates a 5.9-log₁₀ spores/g reduction from soil to processed potatoes. If the count of C. botulinum was reduced by the same factor, an average of −6.2 log₁₀ C. botulinum spores/g would be present in the processed product.
TABLE 1. Calculation of the number of Clostridium botulinum spores in vacuum-packed, cooked potatoes (without additional contamination) by comparing the number of C. botulinum spores and anaerobe-growing bacteria in soil samples originating from different potato fields, and the number of anaerobe-growing spores in vacuum-packed, cooked potatoes.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Type of soil</th>
<th>Anaerobe-growing spores ((\log_{10}/g))</th>
<th>C. botulinum ((\log_{10}/g))</th>
<th>Immunological type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clay</td>
<td>5.8</td>
<td>0.65</td>
<td>E</td>
</tr>
<tr>
<td>2</td>
<td>Clay</td>
<td>6.7</td>
<td>0.40</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>Clay</td>
<td>6.5</td>
<td>-1.00</td>
<td>E</td>
</tr>
<tr>
<td>4</td>
<td>Clay</td>
<td>6.3</td>
<td>-1.00</td>
<td>E</td>
</tr>
<tr>
<td>5</td>
<td>Loss</td>
<td>6.7</td>
<td>-0.40</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>Sand</td>
<td>6.3</td>
<td>-0.70</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>Sand</td>
<td>6.5</td>
<td>-0.19</td>
<td>B</td>
</tr>
</tbody>
</table>

Avg. count in soil with standard deviation: 6.4±0.3
Avg. count in vacuum-packed, cooked potatoes \((n=10)\): 0.5±0.9

TABLE 2. \(\log_{10}\) counts of C. botulinum type B and presence of botulinal toxin in vacuum-packed, cooked potatoes after prolonged incubation at different temperatures.

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Treatment of potatoes</th>
<th>I 15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>II 15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>III 15°C</th>
<th>20°C</th>
<th>25°C</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>&lt;1.0(−) &lt;1.0(−) &lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−) &lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&lt;1.0(−) &lt;1.0(−) &lt;7.6(+)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−) &lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.5(−) &gt;8.0(+)&gt;8.0(+)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−) &lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
<td>&lt;1.0(−)</td>
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<tr>
<td>28</td>
<td>3.5(−) NT NT</td>
<td>&lt;1.0(−)</td>
<td>4.3(+) &gt;8.0(+)</td>
<td>&lt;1.0(−)</td>
<td>4.1(+) &gt;8.0(+)</td>
<td>&lt;1.0(−)</td>
<td>5.3(+)</td>
<td>&lt;1.0(−)</td>
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<td></td>
</tr>
<tr>
<td>60</td>
<td>&gt;8.0(+) NT NT</td>
<td>&lt;1.0(−)</td>
<td>5.2(+) NT</td>
<td>&lt;1.0(−)</td>
<td>5.3(+) NT</td>
<td>&lt;1.0(−)</td>
<td>NT</td>
<td>&lt;1.0(−)</td>
<td>NT</td>
<td></td>
</tr>
</tbody>
</table>

*aTreatment I, potatoes not treated; Treatment II, potatoes dipped for 2 min in a solution containing 2% ascorbic acid and 1% citric acid; and Treatment III, potatoes dipped for 2 min in a solution containing 5% potassium sorbate and 2% ascorbic acid.

*b\((+/−)\), x = highest count per gram of product of one of the two pouches tested; + = botulinial toxins detected; and − = botulinial toxin not detected.

Effect of different chemical treatments of potatoes on thermal destruction of C. botulinum type B

Destruction of bacterial spores (spores of C. botulinum as well as anaerobe-growing spores naturally present) was similar for all types of treatments. From six different experiments done at approximately monthly intervals, the viable count of both added C. botulinum spores and bacterial spores naturally present was reduced by 2.6 ± 1.1 \(\log_{10}\) after heating in a water bath at 95°C for 50 min.

Effect of different treatments on outgrowth of C. botulinum type B and toxin production

The cumulative numbers of pouches showing gas production are presented in Figure 1. All pouches with gas production contained botulinum toxin. The amount of toxin present was up to 10^6 mouse LDs0/\(g\). All treatments, in particular Treatments II and III, delayed gas production.

The \(\log_{10}\) counts of C. botulinum type B and presence of toxin are presented in Table 2. At 15°C, outgrowth and toxin formation occurred in control packs of potatoes but not in those treated either with ascorbic acid combined with citric acid (Treatment II) or with potassium sorbate combined with ascorbic acid (Treatment III). At a storage temperature of 20°C, both Treatments II and III allowed outgrowth and production of botulinum toxin after incubation for 28 d. Under such conditions, production of gas was observed after 45 to 50 d of incubation (Fig. 1).

Shelf life of vacuum-packed, cooked potatoes

The shelf life of uninoculated vacuum-packed, cooked potatoes, as estimated by sensory testing, is shown in Table 3. The influence of storage temperature of the pouches on shelf life was small compared to that of the dipping treatments. The concentration of ascorbic acid in the vacuum-packed, cooked potatoes ranged from 0.59 to 0.69 g/kg (all treatments). A similar value was found for potassium sorbate, i.e., 0.66 g/kg (Treatment III). Due to off-flavor development, the shelf life of samples treated with potassium sorbate was remarkably shorter than that of the other treated samples (Table 3). The sul-
TABLE 3. Shelf life of vacuum-packed, cooked potatoes assessed by sensory tests (criterion was flavor).

<table>
<thead>
<tr>
<th>Treatment of potatoes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Storage temperature (°C)</th>
<th>Shelf life (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>&gt;58</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>&gt;58</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>&gt;58</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>&gt;58</td>
</tr>
</tbody>
</table>

<sup>a</sup>Treatments I, II and III are described in footnote "a" of Table 2; Treatment IV, potatoes dipped for 2 min in a solution containing 2% ascorbic acid; and Treatment V, potatoes dipped for 2 min in a solution containing 2% ascorbic acid and 0.1% Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>.

The potassium concentration in potatoes (Treatment V) was low, i.e., <1 mg/kg. All samples treated with ascorbic acid, and in particular those treated with the mixture of ascorbic and citric acid (Treatment II), had a somewhat sour taste. It was determined that the pH of the outside of whole potatoes dropped approximately 0.2 to 0.3 pH units after dipping in ascorbic acid solution (Treatment IV) and 0.3 to 0.5 pH units after dipping in a combination of ascorbic acid with citric acid (Treatment II).

Appreciable microbial growth was only detected in untreated samples (Treatment I) when stored at temperatures higher than 15°C. After 16 d at 25°C, half of the pouches showed gas production and aerobic mesophilic colony counts were >6 log<sub>10</sub> CFU/g. Microbial growth at 20°C was somewhat slower.

**DISCUSSION**

The principal habitats of *C. botulinum* are the soil and aquatic environment. All samples of soil, originating from potato fields, contained spores of *C. botulinum* in numbers varying from 0.1 to 0.5 per gram. As a consequence, *C. botulinum* enters the processing plant together with the potatoes. A reduction of the bacterial contamination of potatoes occurs during processing. In this investigation, the reduction of contamination during processing resulted in a calculated number of *C. botulinum* of -6.2 log<sub>10</sub> spores/g of processed potatoes. In other terms, one spore of *C. botulinum* may be expected in each 1585 kg of product. However, it is difficult to estimate the real extent of contamination of processed potatoes because many factors are unknown. For example, there is great variability in the level of *C. botulinum* contamination in soil and often the real numbers are underestimated due to the presence of competitive microorganisms (8,15). Also, unknown variations occur during the processing of potatoes, e.g., under identical conditions the thermal destruction of *C. botulinum* spores varied by more than 1 log<sub>10</sub>.

It is evident that *C. botulinum* is present usually in small numbers in vacuum-packed, cooked potatoes. Because potatoes are an excellent substrate for growth and toxin production by *C. botulinum*, precautions should be taken to avoid public health hazards. From an earlier study (7), it was demonstrated that growth and toxin production by *C. botulinum* was prevented at a storage temperature of 4°C. At 10°C, production of botulinal toxin occurred before the potatoes were determined to be unacceptable by sensory analysis. From a public health point of view, besides storage of the potatoes at low temperatures, an additional safety factor should be included.

![Figure 1. Cumulative number of pouches showing gas production at different storage temperatures. Ten pouches were artificially contaminated with *C. botulinum* type B at each temperature.](http://www.journaloffoodprotection.org/article-pdf/48/10/851/1651266/0362-028x-48_10_851.pdf)
results of this investigation revealed that treating potatoes with either a mixture of ascorbic acid and citric acid or potassium sorbate followed by ascorbic acid added an additional safety factor to the product. Treatment with a mixture of ascorbic acid and citric acid was preferred from the sensory perspective. With this treatment, no growth and toxin production was observed during the first 14 d of incubation.

Why the ascorbic/citric acid treatment had an antibotulinal effect is not fully understood. Ascorbic acid acts as an antioxidant and, therefore, increases the shelf life of vacuum-packed, cooked potatoes (Table 3). In addition, it has an antibotulinal effect (Table 2). Ascorbic acid alone and in combination with citric acid decreased the pH at the potato surface from pH 5.9-6.0 to 5.4-5.8, with the lowest values being reached in the presence of citric acid.

In all likelihood, the lowest values being reached in the presence of citric acid. It has an antibotulinal effect (Table 2). Ascorbic acid alone and in combination with citric acid decreased the pH at the potato surface from pH 5.9-6.0 to 5.4-5.8, with the lowest values being reached in the presence of citric acid. In general, it is accepted that C. botulinum grows equally well at these pH values (2). In all likelihood, the antibotulinal effect may be caused by a combination of different parameters, such as suboptimal storage temperature and acidification by organic acid. Treatment of food with ascorbic acid and citric acid is generally regarded as safe for the consumer. Both substances are naturally present in different food products. Additionally, citric acid represents more than 60% of all food acids used (11).

From the results obtained in this study it can be summarized that treatment of potatoes with a combination of ascorbic acid and citric acid before vacuum packing and cooking results in an organoleptically acceptable product and extends the product’s shelf life. Furthermore, this treatment delays outgrowth of C. botulinum spores. If the product is stored at low temperatures and if the storage time is limited, no public health hazards will occur even if the product is stored accidently for a short time at above 4°C. However, it was observed that toxin production may occur before gas production is observed.

REFERENCES