Clostridium botulinum and Acid Foods

THERON E. ODLAUG* and IRVING J. PFLUG

Department of Food Science and Nutrition
University of Minnesota
1334 Eckles Avenue, St. Paul, Minnesota 55108

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ABSTRACT

Outbreaks of botulism involving acid foods are rare. Of the 722 total botulism outbreaks reported from 1899 to 1975, only 34 (4.7%) involved acid foods. Home-canned acid foods were implicated in 34 of the 35 acid food outbreaks. Clostridium botulinum cannot grow at a pH of < 4.6; therefore, for a botulism hazard to exist in an acid food, a condition must exist where in a macro- or micro-environment the pH is raised. A botulism hazard in acid food will be the result of several contributing factors: (a) contamination with C. botulinum, (b) contamination with other microorganisms due to a process delivery failure and/or post-process contamination, (c) favorable composition of the food and storage conditions which are particularly conducive to C. botulinum growth and toxin production, and (d) metabolism. The way each factor affects the botulism hazard in acid foods is discussed in this report. An acid food is safe from C. botulinum if the heat process kills all organisms capable of growth at a pH of < 4.6 and there is no post-process contamination.

Clostridium botulinum food intoxication (botulism) has been recognized as a public health problem since 1793 (20). Compared to outbreaks caused by other food poisoning microorganisms, outbreaks of botulism are rare but the fatality rate is high (6). In 1974 the fatality rate was 23%.

Seven types (A, B, C, D, E, F, and G) of C. botulinum are currently recognized on the basis of antigenically distinct toxins (53). Types A, B and E are the principal causes of the disease in man. Botulism caused by type A or B toxins is associated with canned vegetables, fruits and meats. Botulism caused by Type E toxin is associated with fish or various types of marine products.

Botulism is primarily considered a hazard of low-acid canned food; however, an often overlooked fact is that botulism occurs at a low rate in acid foods. Home-canned acid foods account for 97% of these acid food outbreaks. Home-canned acid foods were implicated in 34 (4.7%) of the 722 reported outbreaks of food-borne botulism from 1899 through 1975 (6,9,10,35). This number is too large to be neglected or written off to faulty diagnosis. Accordingly, C. botulinum must be regarded as a potential public health hazard in acid foods.

Outbreaks of botulism attributed to home-canned acid foods, especially tomato products, are a concern of consumers, governmental agencies and academic institutions (23,42,45,46,50).

This is a review, analysis and discussion of the problem of botulism in canned acid foods. The study leading to this report was carried out with the hope that if the conditions responsible for C. botulinum growth and toxin production in acid foods can be identified and put in perspective, then we will be better able to develop a positive control program.

OUTBREAKS OF BOTULISM INVOLVING ACID FOODS

In Table 1 are listed the number of botulism outbreaks that have been recorded in the United States from 1899 through 1975. Since 1899, 9% of the botulism outbreaks have been traced to commercially processed foods (Table 1). The products involved in these outbreaks have all been low-acid foods except for one outbreak involving tomato catsup in 1915 (27,35). This is a very good record for the canning industry considering that probably more than 775 billion cans of commercially canned foods have been consumed since 1930 (33).

Most botulism outbreaks have been traced to home-processed foods (Table 1). These foods were implicated in 72% of all outbreaks from 1899 through 1975. Home-processed low-acid foods accounted for 67.3% of all the outbreaks. During the past 76 years, 34 botulism outbreaks have been associated with consumption of home-canned acid foods. The 34 outbreaks attributed to acid foods are listed individually in Table 2. Some type of tomato product was the vehicle for botulism toxin in 17 (50%) of the 34 home-canned acid food outbreaks.

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2Part of a thesis submitted by Theron E. Odlaug to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
TABLE 1. Outbreaks of foodborne botulism attributed to commercially-processed or home-processed foods, 1899-1975

<table>
<thead>
<tr>
<th>Interval</th>
<th>Home-processed Acid</th>
<th>Low acid</th>
<th>Commercially-processed Acid</th>
<th>Low acid</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>1899-1909</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1910-1919</td>
<td>5</td>
<td>42</td>
<td>1</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>1920-1929</td>
<td>4</td>
<td>73</td>
<td>0</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>1930-1939</td>
<td>6</td>
<td>129</td>
<td>0</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>1940-1949</td>
<td>9</td>
<td>111</td>
<td>0</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>1950-1959</td>
<td>3</td>
<td>47</td>
<td>0</td>
<td>2</td>
<td>51</td>
</tr>
<tr>
<td>1960-1969</td>
<td>3</td>
<td>39</td>
<td>0</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>1970-1975</td>
<td>4</td>
<td>42</td>
<td>0</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>485</td>
<td>1</td>
<td>64</td>
<td>137</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td>4.7</td>
<td>67.3</td>
<td>0.1</td>
</tr>
<tr>
<td>of Total (%)</td>
<td></td>
<td></td>
<td>8.9</td>
<td>19.0</td>
<td></td>
</tr>
</tbody>
</table>

Total Number of Outbreaks 722

aData from 6, 9, 10, 35.

CONTAMINATION OF FOOD WITH CLOSTRIDIUM BOTULINUM AND OTHER MICROORGANISMS

C. botulinum spores are widely distributed in soil in the United States (34, 53). Riemann (53) summarized the many reports on the prevalence of C. botulinum in soils. C. botulinum was found to be widespread in the many soil and sediment samples tested. These reports indicate that C. botulinum is ubiquitous in nature.

Fruits and vegetables, because of their intimate contact with soil, probably will be contaminated with spores of C. botulinum. There have been only a few studies on the incidence of botulinum spores on raw fruits and vegetables. Meyer and Dubovsky (34) found 6 to 33% of vegetable and fruit samples to be positive for C. botulinum spores. Hauschild et al. (24) found 15 C. botulinum spores per 100 g of unwashed mushrooms and 41 C. botulinum spores per 100 g of washed mushrooms. The National Canners Association (40) found 100 to 4000 bacterial spores per gram of harvested tomatoes. It can be assumed that C. botulinum spores were some fraction of this total.

Based on the studies just cited, it is probable that a large percentage of the raw fruits and vegetables in the home or at the processing plant will have C. botulinum spores associated with them. Undoubtedly some of these spores will still be present on the product when it is placed into containers.

When fruit and vegetable products are placed into containers, they will also be contaminated with other

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TABLE 2. Outbreaks of botulism attributed to acid foods, 1899-1975

<table>
<thead>
<tr>
<th>Year</th>
<th>State</th>
<th>Product</th>
<th>Toxin type</th>
<th>cases</th>
<th>Deaths</th>
<th>pH</th>
<th>Other references</th>
</tr>
</thead>
<tbody>
<tr>
<td>1910</td>
<td>California</td>
<td>Pears</td>
<td>NA</td>
<td>12</td>
<td>11</td>
<td></td>
<td>49</td>
</tr>
<tr>
<td>1915</td>
<td>California</td>
<td>Apricots</td>
<td>NA</td>
<td>5</td>
<td>5</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>1915</td>
<td>Illinois</td>
<td>Tomato catsup c</td>
<td>NA</td>
<td>2</td>
<td>0</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>1918</td>
<td>California</td>
<td>Pears</td>
<td>NA</td>
<td>1</td>
<td>0</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>1918</td>
<td>California</td>
<td>Apricots</td>
<td>A</td>
<td>8</td>
<td>6</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>1918</td>
<td>California</td>
<td>Apricots</td>
<td>NA</td>
<td>2</td>
<td>2</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>1922</td>
<td>California</td>
<td>Tomato relish</td>
<td>A</td>
<td>2</td>
<td>2</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>1924</td>
<td>Washington</td>
<td>Pickles</td>
<td>A</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1927</td>
<td>California</td>
<td>Pears</td>
<td>A</td>
<td>2</td>
<td>2</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>1929</td>
<td>Colorado</td>
<td>Tomatoes, green</td>
<td>NA</td>
<td>4</td>
<td>2</td>
<td>3.86</td>
<td>22</td>
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<tr>
<td>1931</td>
<td>Oregon</td>
<td>Applesauce</td>
<td>NA</td>
<td>2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1933</td>
<td>Canada</td>
<td>Tomatoes</td>
<td>NA</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1935</td>
<td>California</td>
<td>Tomato juice</td>
<td>NA</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1936</td>
<td>California</td>
<td>Tomatoes</td>
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<td>1</td>
<td></td>
<td></td>
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<tr>
<td>1938</td>
<td>California</td>
<td>Tomatoes, green</td>
<td>NA</td>
<td>2</td>
<td>2</td>
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<td></td>
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<tr>
<td>1939</td>
<td>California</td>
<td>Okra, sourgrass</td>
<td>NA</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1940</td>
<td>California</td>
<td>Tomatoes</td>
<td>A</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1940</td>
<td>California</td>
<td>Apricots</td>
<td>A</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>1940</td>
<td>Tennessee</td>
<td>Tomatoes</td>
<td>B</td>
<td>2</td>
<td>2</td>
<td>4.0</td>
<td>58</td>
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<tr>
<td>1941</td>
<td>Nebraska</td>
<td>Tomatoes</td>
<td>NA</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1942</td>
<td>California</td>
<td>Pickles, dill</td>
<td>A</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1943</td>
<td>California</td>
<td>Tomatoes</td>
<td>NA</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1947</td>
<td>New Mexico</td>
<td>Peaches</td>
<td>A</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1948</td>
<td>California</td>
<td>Tomatoes</td>
<td>NA</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1948</td>
<td>California</td>
<td>Pears</td>
<td>A</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1951</td>
<td>California</td>
<td>Tomatoes</td>
<td>NA</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1953</td>
<td>California</td>
<td>Huckleberry</td>
<td>A</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1954</td>
<td>California</td>
<td>Peaches</td>
<td>A</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1954</td>
<td>Kansas</td>
<td>Pickles</td>
<td>A</td>
<td>7</td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1965</td>
<td>Alabama</td>
<td>Tomato juice</td>
<td>NA</td>
<td>1</td>
<td>0</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>1969</td>
<td>Kentucky</td>
<td>Tomato juice</td>
<td>B</td>
<td>1</td>
<td>0</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>1973</td>
<td>Kentucky</td>
<td>Blackberries</td>
<td>B</td>
<td>2</td>
<td>1</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1974</td>
<td>Alabama</td>
<td>Tomatoes</td>
<td>B</td>
<td>1</td>
<td>0</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>1974</td>
<td>Idaho</td>
<td>Tomato juice</td>
<td>A</td>
<td>1</td>
<td>0</td>
<td>4.2</td>
<td>8</td>
</tr>
<tr>
<td>1975</td>
<td>New Jersey</td>
<td>Applesauce</td>
<td>B</td>
<td>1</td>
<td>0</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

aData from 6, 9, 10, 35.

bNA, information not available.
cCommercially canned.
dNumbers refer to references cited.
microorganisms. These organisms are from several sources: normal microflora associated with the fruit or vegetable as it grows in the field, microorganisms that are deposited onto the product during food handling operations and microorganisms that are present on the container when the product is packaged (18,30,56,57).

EFFECT OF pH ON CLOSTRIDIUM BOTULINUM

A pH of 4.6 is generally considered to be the dividing line between low-acid and acid foods. Foods with pH values > 4.6 are considered low-acid and foods with a pH of < 4.6 are considered to be acid foods.

Data in the literature indicate that C. botulinum is not capable of growth and toxin production at a pH of < 4.7. Townsend et al. (60) conducted an extensive study on the effect of pH on growth and toxin production of C. botulinum. Tubes containing various foods at different pH levels were inoculated with 2.0-2.5 x 10⁶ spores of a mixture of C. botulinum types A and B. The tubes were incubated at 30 C until growth was evident, or for as long as 1 year if no growth was evident. The lowest pH at which toxin was formed was 4.8. In tubes that contained a tomato substrate, 4.96 was the lowest pH at which growth and toxin production was observed. Recently, Huhtanen et al. (25) using an inoculum of 10³ spores/ml, reported 5.24 as the lowest pH for toxin production in tomato juice for 11 strains of C. botulinum types A and B. Ito et al. (27) were able to demonstrate C. botulinum toxin production in cucumber puree with a spore concentration of 10⁶/tube at pH 5.0, but not at pH 4.8.

Lechowich (32) presented data showing that the lowest pH at which C. botulinum could grow was greater than 4.6. Dozier (15), Ingram and Robinson (26), Bever and Halvorson (1), and Kadavy and Dack (28) have all shown in studies with C. botulinum that no growth or toxin production occurred at a pH of < 4.6.

Thompson and Tanner (65) inoculated cans of apricots, apples, blackberries, cherries, gooseberries, peaches, pears, plums, raspberries, strawberries, pickles, and sauerkraut with 2 x 10⁶ to 2 x 10⁷ C. botulinum spores. These cans of food were incubated at 37 C for 10 months; no botulinum toxin was detected.

Lagarde and Beerens (31) inoculated spores of a type A botulinum strain (grown from an isolate from canned peaches) into pear syrup; no toxin was produced when the pH was < 4.6. Odlaug (43) found that the spores grown from a C. botulinum type A strain, isolated from home-canned tomato juice in the outbreak of botulism in Idaho in 1974 (9), grew in tomato juice at pH 4.9 but not at pH 4.8. Odlaug (43) also found that spores grown from the C. botulinum type B strain, implicated in the outbreak of botulism in Alabama in 1974 (7) involving home-canned tomatoes, grew in tomato juice at pH 5.2 but not at pH 5.1. The inoculum in these experiments was 10⁵/spore ml.

Results of these studies indicate that the minimum pH at which C. botulinum will grow and produce toxin is dependent on the initial pH, incubation temperature and time, and food substrate, as well as the concentration, type and strain of C. botulinum spores. However, in none of these studies was C. botulinum able to grow and produce toxin when the substrate pH was < 4.7. If the pH of all of the substrate remains below 4.6 then there will be no botulism hazard.

SURVIVAL OF CLOSTRIDIUM BOTULINUM SPORES IN ACID FOOD

The process that is used to preserve a food in a container is based on pH and other attributes of the product and container. In the heat preservation of low-acid canned foods, the sterilization process is designed to kill all the C. botulinum spores in the food product since surviving spores can grow and produce toxin. In the heat preservation of acid canned foods, the process is designed to kill those microorganisms that can grow and spoil the product, and not necessarily to kill sporforming organisms such as C. botulinum because it cannot grow at pH < 4.6.

Organisms that are of concern in acid foods are: Bacillus coagulans, butyric anaerobes (e.g., Clostridium pasteuriannum), mesophilic non-spor-forming bacteria (e.g., Lactobacillus sp.), yeasts, and molds. The spor-forming bacteria that are normally capable of growth in acid foods are characterized by D(100 C)-values of 0.1 to 0.5 min and the non-spor-forming bacteria, yeasts and molds by D(65 C)-values of 0.5 to 1.0 min (62). Heat processes for acid foods are usually designed to kill these organisms.

There has been little work on the heat resistance of C. botulinum in acid foods because of its inability to grow at a pH < 4.6 (Ito, personal communication). Xezones and Hutchings (68) investigated extensively the effect of pH on the heat resistance of C. botulinum spores suspended in food. They found that there is a significant decrease in the spore D-value as the pH becomes more acid. The extrapolated D(100 C)-value for 62A spores in a tomato sauce-spaghetti-cheese product was approximately 6 min at pH 4.0 and 42 min at pH 7.0. The heat resistance of a type A strain, isolated from tomato juice in the outbreak of botulism in Idaho in 1974 (9) was shown by Odlaug and Pflug (45,47) to have an extrapolated D(100 C)-value in pH 7.0 buffer of 47 min and in pH 4.2 tomato juice of 18 min. These results indicate that C. botulinum spores have lower heat resistance in acid foods than in low-acid foods. However, even at the acid pH values, C. botulinum spores still have 10 to 200 times greater heat resistance than the spore-formers that normally grow in and spoil such foods.

C. botulinum spores, if present in an acid food, will remain viable for a considerable period of time. Odlaug and Pflug (46) have shown that C. botulinum spores stored in acid media (pH 4.2) survived up to 180 days with little or no decrease in numbers.

It will be normal for a fraction of the C. botulinum spore population present on foods to survive the heat processes given to acid foods and remain viable during
storage. Presence of viable \textit{C. botulinum} spores in properly preserved acid foods does not constitute a public health hazard because of the inability of these spores to grow at a pH of \textless{} 4.6.

**PROCESS FAILURES IN ACID FOODS**

There are three possible areas of failure in the heat preservation of acid or acidified foods: (a) in process design or specification, (b) process delivery, and (c) post-process contamination.

A failure in process design occurs when the designed process is inadequate to preserve the specified product. Process design failures do not appear to have contributed to the botulinalm hazard in acid foods. Acid food products such as tomatoes, tomato juice, applesauce, pickles, peaches and fruit juices, jams and jellies have been produced by commercial canners for years without a botulinalm problem.

Delivery failures appear to be one cause of botulinalm in acid foods. There are two ways in which a process delivery failure can occur: (a) the product being processed is not the same as the product in the process design and (b) the scheduled (designed) process is not delivered to the specified product. Failures in process delivery may be due to human error or equipment failure.

Process delivery failures where the product was not the same as specified in the design have been a problem with some acidified foods. A product with a pH > 4.6 that does not receive a minimum botulinalm cook, and is acidified or inadequately acidified is a potential botulinalm problem. This was recently a problem with marinated mushrooms (24). In commercial acid food production a process failure is usually not equated with a botulinalm hazard. This is probably because the normal spoilage microorganisms of most acid foods tend to lower instead of increase the product pH (41).

Another possible process delivery problem, where the product was not as specified in the design, has been with low-acid tomatoes. There have been a number of studies dealing with the acidity of tomatoes that have been reviewed by Powers (50), who reported that depending on the variety, a high percentage of especially over-ripe tomatoes may have a pH above 4.6. However, the low-acid tomato problem does not account for all the botulinalm outbreaks involving acid foods especially when the epidemiological evidence is considered. Of those botulinalm outbreaks listed in Table 2, in only three outbreaks were pH values reported and in none of the foods was the \text{pH} > 4.2.

Acid food preservation using the hot-fill procedure at 85 to 90 C or heating to a center can temperature of 75 to 80 C, relies on a number of factors to effect preservation (heating time, \text{pH}, salt and/or sugar concentration, also type of acid added in acidified foods). Process failures can occur if the initial number of contaminating organisms is high, the heat process time and/or temperature is too low, or the \text{pH} is higher than in the design. A process delivery failure can occur in a hot-fill process allowing microorganisms present in the container or on the lid to survive if the container and product are cooled too rapidly after filling or the container is not inverted. Failure may also occur due to survival of encapsulated or occluded microorganisms that are not destroyed in the heat process.

The third type of process failure is post-process contamination. In a commercial operation poor quality containers, inadequate chlorination of cooling water and rough can handling can increase the probability of microorganisms leaking in and spilling canned foods (44,48,51). The homemaker may also use poor quality containers, especially in terms of the closure. The risk from contaminated water entering the container due to a poor closure is low because the homemaker will probably air cool the prepared containers. Since an acid food product is by nature non-sterile, a container failure that allows oxygen leakage and permits aerobes to grow and spoil the product may also be considered post-process contamination.

**CONDITIONS NECESSARY FOR CLOSTRIDIUM BOTULINUM GROWTH IN AN ACID FOOD WITH A PROCESS FAILURE**

A process failure in acid foods either in delivery and/or post-process contamination will result in unwanted microorganisms being present in the product. If both \textit{C. botulinum} and other microorganisms are present in a canned acid food because of a process failure, a botulinalm hazard may exist. Whether or not \textit{C. botulinum} will grow and produce toxin will depend on several factors. These factors are the chemical composition of the food, physical state of the food, conditions of storage, and microorganisms interacting in the food.

\textbf{Chemical composition of the substrate}

The chemical composition of an acid food has an important effect on growth of \textit{C. botulinum} and other microorganisms in the acid medium. The pH level, nutrients, redox-potential and antimicrobial constituents must be at levels in the food to permit germination of spores, outgrowth and toxin production. Nutrients in acid foods have been shown to be sufficient to allow growth of \textit{C. botulinum} if the pH is changed to greater than 4.6 (25,31).

The redox potential in a food must also be at a level that allows growth. Spores of \textit{C. botulinum} will not germinate when the redox-potential (Eh) is above a certain critical level. The redox-potential in a food is determined by the Eh\textsubscript{p} poisoning capacity of the food itself, and the O\textsubscript{2} tension in the atmosphere and its access to the food (38). Smith (59) reported that the Eh\textsubscript{p} of canned tomato sauce was \(-399.1\) mv; tomato juice, \(-309.3\) mv; peeled tomatoes, \(-295.7\) mv; tomato sauce with bits, \(-271.1\) m; and tomato bisque soup, \(-193.5\) mv. Rapid growth of \textit{C. botulinum} will take place in a suitable medium when the Eh\textsubscript{p} is between \(-6\) mv and \(-436\) mv (37). Rowley and Amelis (54) indicate that an Eh\textsubscript{p} of
greater than −150 mv is needed to inhibit *C. botulinum* types A and B.

**Physical state of the substrates**

Water activity (aw) is a critical physical factor in food. Aw values of < 0.93 are inhibitory to all types of *C. botulinum* (53,60). The water activity in canned acid food products such as tomatoes and tomato juice would not be a factor in preventing *C. botulinum* growth and toxin production. However, there are acid foods that have low aw values (e.g., syrup-packed peaches, jams, jellies).

**Conditions of storage**

Storage temperature of a canned food and exposure of the contents to O2 are factors that will affect the growth of *C. botulinum* and other microorganisms.

The temperature will affect the growth of microorganisms. If storage is at <10 F, growth of *C. botulinum* types A and B will not take place (54). The optimal growth temperature for *C. botulinum* is between 30-37 C (53).

Sugiyama and Yang (63) suggested that *C. botulinum* types A and B have an O2 tolerance that allows growth in the presence of 1 to 2% O2. Mossel and Ingram (38) described how molds and aerobic bacteria can develop in canned foods at very low oxygen levels. *Byssochlamys fulva* has been shown to grow on Potato Dextrose Agar at O2 levels as low as 0.27% (29).

**Microbial interaction**

The organisms present in a canned acid food because of a process failure will affect one another through differences in rates of development, antagonism or synergism (38). These are called implicit factors, a term suggested by Mossel and Westerdijk (39). In an acid food, if *C. botulinum* spores and other organisms are present, these factors will determine if growth and toxin production of *C. botulinum* will take place.

There have been three outbreaks in acid foods where organisms other than *C. botulinum* have been isolated. Table 3 lists these outbreaks and the organisms isolated. In all three cases the pH recorded for the food was below 4.6.

Meyer and Gunnison (36) did experiments with the organisms isolated from the canned pears in the 1927 outbreak (Table 3). They found toxin in tubes inoculated with the yeast, the *Lactobacillus* sp., and 5 x 10⁴ spores of *C. botulinum*. They did not find toxin production when the *Lactobacillus* sp. was present along with *C. botulinum*. However, they did find toxin when the yeast was present along with the *C. botulinum* in the food. In tubes where no toxin was present, viable spores were still present. The pH values of the toxic syrups varied from 3.33 to 4.22. No other report on this project is known. Slocom et al. (59) inoculated organisms isolated from the canned tomatoes in the 1940 outbreak (Table 3) into 10 cans of commercially canned tomatoes and found no toxin after 32 days of incubation at room temperature.

Other investigators have studied the interaction of microorganisms with *C. botulinum* in acid media. Tanner et al. (64) detected botulinum toxin in low acid fruits inoculated with *C. botulinum* spores where a *Penicillium* sp. or *Mycoderma* sp. had grown in the food and shifted the pH from 3.25 to 4.9-5.4 in raspberries, and from 3.75 to 5.1-5.3 in cherries. Lagarde and Beerens (31) observed that a contaminant such as *Trichosporon* was able to raise the pH of pear syrup from 3.8 to 4.8 where *C. botulinum* produced toxin. Huhtanen et al. (25) were able to demonstrate toxin production in tomato juice (pH 4.2) where a *Penicillium* sp. or *Cladosporium* sp. was present to raise the pH at the surface above 4.6. Odlaug (43) was able to demonstrate *C. botulinum* toxin production in pH 4.2 tomato juice if *Aspergillus* sp. was present to raise the pH at the surface above 4.6.

Both Huhtanen et al. (25) and Odlaug (43) were able to demonstrate a pH gradient in tomato juice inoculated with molds when a heavy mold mat formed on the surface. The pH values near the mat were near neutrality, and the lower portions below the mat were more acid. Odlaug (43) showed that *C. botulinum* growth was greatest at the surface and that the counts decreased with distance from the mycelial mat. Toxin was present throughout the tomato juice. A non-hermetic experimental unit was used by Odlaug (43).

Huhtanen et al. (25) suggested that the reason that botulinum toxin could be found in a food with a pH of < 4.6 is that routine mixing of the product during analysis could destroy a pH gradient formed by acid-consuming microorganisms and show the food to have a pH in the high-acid range.

**TABLE 3. Clostridium botulinum outbreaks involving acid foods where other microbial species were isolated**

<table>
<thead>
<tr>
<th>Date</th>
<th>Food product</th>
<th>Toxin type</th>
<th><em>C. botulinum</em> isolated</th>
<th>Other microbes</th>
<th>pH of food</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1927</td>
<td>Pears</td>
<td>A</td>
<td>Yes</td>
<td><em>Lactobacillus</em></td>
<td>3.86</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1940</td>
<td>Tomatoes</td>
<td>B</td>
<td>Yes</td>
<td><em>Bacillus</em></td>
<td>4.0</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Diplococcus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Streptococcus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>faecalis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1974</td>
<td>Tomato juice</td>
<td>A</td>
<td>Yes</td>
<td><em>Diplococcus</em></td>
<td>4.2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Aerobacter</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>agglomerans</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Odlaug (43) demonstrated that when a mold was present in tomato juice in a hermetic unit, the growth of the mold was restricted, probably due to lack of oxygen. The dry weight of the mycelial mat in the hermetic unit was approximately 10% of the dry weight of the mycelial mat in a non-hermetic unit. In the hermetic unit *Clostridium botulinum* growth and toxin production could be demonstrated even though the pH (recorded with a combination electrode) on the mat, below the mat and at the surface of the tomato juice, was less than 4.6. Odlaug (43) could not demonstrate any filterable of dialyzable growth factors produced by the mold that would allow production by *C. botulinum*. It was concluded that *C. botulinum* and the mold had to occupy the same microenvironment for toxin production to be demonstrated. The toxin levels under these conditions were low (< 10 MLD₅₀/ml), and the increases in the *C. botulinum* population were small. Odlaug (43) suggested that growth of the mold (*Aspergillus*) at the surface of the tomato juice in a hermetic unit created microenvironments within the mycelial mat or directly below the mycelial mat where the pH was greater than 4.6 and where *C. botulinum* spores germinated, reproduced, and produced toxin.

It is interesting that Dickson (14), when reporting on the 1917 botulism outbreak involving apricots (Table 2), noted that the food was "moldy on the surface." The pH value of this food was not reported.

Recently, Fields et al. (18) isolated *Bacillus licheniformis*, *Bacillus subtilis*, and other organisms from home-canned tomatoes. Some of the cultures of *B. licheniformis* and *B. subtilis* inoculated into tomato serum were able to grow and raise the pH to a level above 4.6. *C. botulinum* was not tested for growth and toxin production in the presence of these organisms in an acid medium. Wentz et al. (67) have shown *B. licheniformis* to inhibit *C. botulinum* type A toxin production in brain heart infusion by production of an antibiotic, bacitracin. Stark et al. (61) showed that *Bacillus subtilis* could destroy preformed *C. botulinum* toxin.

*Streptococcus lactis*, *Lactobacillus casei*, *Clostridium sporogenes*, *Clostridium bifermens* and *Clostridium perfringens* have all been shown to destroy botulinum preformed toxin (12,61). Saleh and Ordal (55) showed that *Lactobacillus bulgaricus* and *Streptococcus lactis* are capable of inhibiting *C. botulinum* growth and toxin production.

It is apparent that while microbes can destroy *C. botulinum* toxin or inhibit growth, they can also alter the environment to allow *C. botulinum* growth to take place where it normally would not. When one organism makes conditions favorable for growth of a second organism, the condition is known as metabolism (19).

There appear to be two ways in which microorganisms in acid foods can create favorable conditions for *C. botulinum* growth and toxin production. One is where there is gross spoilage of the product that results in gross changes of pH throughout the product to a level where *C. botulinum* can grow. Since this type of spoilage would be readily apparent, the food would probably be discarded with no harm done. The second way of producing toxin is where growth of a microorganism does not produce gross but micro-scale changes in pH. In this condition, if a *C. botulinum* spore is present in the mold growth area, growth could take place. This type of spoilage may not be readily observable and therefore the food may not be discarded and be a hazard.

**CONCLUSIONS**

A review and analysis of the literature indicate that if the pH of a food is < 4.6 and no other viable microorganisms are present, then it should be impossible for any viable *C. botulinum* to grow and produce toxin. A canned food with a pH value of < 4.6 that receives a preservation treatment that inactivates all organisms capable of growing at pH < 4.6 and is free from post-process contamination, is safe from *C. botulinum*.

For a botulinum hazard to exist in an acid food, pH < 4.6, there must be a number of contributing factors: (a) contamination of the product with large numbers of *C. botulinum* spores, (b) contamination of the food with other microorganisms due to a process failure in delivery and/or post-process contamination, (c) favorable composition of the food and storage conditions that are particularly conducive to *C. botulinum* growth and toxin production, and (d) metabolism. The probability that all of the factors necessary for *C. botulinum* growth and toxin production will occur in a single container of an acid food is undoubtedly low as is the incidence of botulism in acid food.

There has not been a reported case of botulism attributed to the consumption of a commercially canned acid food in over 50 years. The problem of botulism in home-canned acid foods is rare but still occurs occasionally. There have been two deaths attributed to consumption of home-canned acid foods containing *C. botulinum* toxin in the last 20 years. The continuing problem of botulism in home-canned acid foods and its absence in commercially canned acid foods probably indicates that non-supervised people often fail to follow recommended heat preservation methods. The problem of botulism in home-canned foods can probably never be totally eliminated because of the laws of probability and human fallibility. In view of the relatively low risk of botulism from consumption of acid foods, would a control program be of benefit in reducing the risk to an even lower level? Since home-canning is an important means of preserving food for many families, a control program that would result in less home-canning of acid foods would not be beneficial to the population as a whole. It is probable that increased consumer education regarding the proper methods of home-canning will be beneficial in reducing spoilage losses. However, changes in the processing recommendations for acid foods may
do more harm than good unless that factor is specifically identified that is most important in contributing to a botulism hazard in each type of acid food. Any control program that is initiated should be carefully examined to determine if results of the program will be a lower incidence of botulism from the consumption of acid foods.

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