CONTAMINATION OF FROZEN VEGETABLES BY COAGULASE-POSITIVE STAPHYLOCOCCI

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SUMMARY

The incidence of coagulase-positive staphylococci was determined in 112 samples of peas, green beans, and corn collected during different stages of processing for freezing. The highest staphylococcal counts were found in peas, 7.3 per g, while the greatest percentage of staphylococcal contaminated samples, 64%, was obtained with corn. The data indicated that although the hands of employees were a major source of these organisms, other factors were also involved. The gravity separator, used in the processing of peas, was found to be a potential area for staphylococcal build-up.

Contamination of foods by coagulase-positive strains of Staphylococcus aureus is of concern because of the organism's potential to produce enterotoxins. While in our present stage of technology it is difficult to prevent consistently contamination of foods with these organisms (2), only low numbers should be permitted.

Various studies have dealt with the numbers and sources of staphylococci in precooked frozen foods (6, 7). Little attention has been given to their presence in commercially frozen vegetables, probably because these foods are thought to present slight hazard to the consumer. Frozen vegetables, however, are often ingredients of precooked frozen foods and have been reported to be a major source of microorganisms in these products (3).

The purpose of this investigation was to estimate the incidence of coagulase-positive staphylococci in certain frozen vegetables and to determine whether specific processing conditions were responsible for their presence.

METHODS

Vegetables in different stages of processing were collected from the lines of area factories. The samples were transported to the laboratory over ice and then frozen in a -23 C room. Eleven-gram samples were blended, diluted, and plated the following day for "total" counts (8) and again when the vegetables were cultured for staphylococci. The lengthy procedures for identification and enumeration of staphylococci resulted in some samples being stored as long as six months at -23 C before being examined for these organisms. Since no significant decrease in the average "total" counts was observed during this storage, it is probable that there also was little decrease in the numbers of the relatively freeze-resistant staphylococci.

A most probable number (MPN) technique was used for estimating numbers of staphylococci. One ml of a given decimal dilution was inoculated into each of 10 tubes containing 2 ml of an enrichment medium. This medium consisted of brain heart infusion broth (Difco) to which had been added 20 g of d-mannitol and 80 g of sodium chloride per liter (9). Following 48-hr incubation at 35 C on a rotary shaker, tubes showing growth were streaked on staphylococcus-110 agar (Difco). The plates were incubated 24 hr at 35 C and then colonies resembling the staphylococcal group were picked and stabbed into d-mannitol agar (5). The catalase-positive cocci that attacked mannitol either oxidatively or fermentatively were subsequently propagated in brain heart infusion broth 18 hr and then tested for free coagulase using the tube method recommended for the commercially prepared plasma (Difco).

The efficiency of the above methods was evaluated by determining the MPN of staphylococci after blended vegetables were inoculated with known numbers of these organisms as reflected by plate counts. The results of these trials indicated that approximately 50% of the staphylococci was being counted.

RESULTS

During the 1962-64 processing seasons, samples of frozen peas, green beans, and whole-kernel corn were examined for staphylococci. The vegetables were collected in 42 separate surveys conducted at three processing factories. A summary of the results (Table 1) shows that 31 to 64% of the samples contained coagulase-positive staphylococci, and that the vegetables yielding these organisms were not contaminated with large numbers. The highest staphylococcal counts were found in peas, 7.3 per g, while the greatest percentage of positive samples, 64%, was obtained with corn. The staphylococcus-positive samples showed higher average total counts than the negative samples in three out of four instances. These differences, however, were not statistically significant.

Since staphylococci have been shown to be common contaminants of the human hand (1), it seemed likely that the hands of employees were the source of the small numbers that were being recovered. This was investigated by comparing the incidence of staphylococci in peas and beans subjected to varying degrees of human contact. These vegetables provided an excellent opportunity for this study because
## Table 1. The Incidence of Coagulase-Positive Staphylococci in Frozen Vegetables

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>No. of samples</th>
<th>% staph. positive</th>
<th>Av. MPN staph./g</th>
<th>Av. &quot;total&quot; count/g x 10^6</th>
<th>Staph. positive samples</th>
<th>Staph. negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peas</td>
<td>51</td>
<td>39</td>
<td>7.3 ± 2.4</td>
<td>75 ± 15</td>
<td>95 ± 25</td>
<td></td>
</tr>
<tr>
<td>Green beans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>French</td>
<td>31</td>
<td>39</td>
<td>1.7 ± 0.2</td>
<td>530 ± 130</td>
<td>260 ± 66</td>
<td></td>
</tr>
<tr>
<td>Cut green beans</td>
<td>16</td>
<td>31</td>
<td>1.4 ± 0.2</td>
<td>370 ± 87</td>
<td>230 ± 70</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>14</td>
<td>64</td>
<td>1.9 ± 0.3</td>
<td>600 ± 290</td>
<td>360 ± 140</td>
<td></td>
</tr>
</tbody>
</table>

*± standard error of the mean.

## Table 2. Effect of Human Contact with the Product on the Amount of Contamination by Coagulase-Positive Staphylococci

<table>
<thead>
<tr>
<th>Staphylococcus-positive</th>
<th>Degree of human contact</th>
<th>Sampling areas</th>
<th>% Staph. per 10^5</th>
<th>Av. MPN/g</th>
<th>&quot;total&quot; count/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peas</td>
<td>Slight</td>
<td>Flumes, belts, quality graders</td>
<td>12</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>Inspection belts</td>
<td>80</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>After areas of heavy contact</td>
<td>Hoppers, filling machines, final package</td>
<td>45</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Green beans</td>
<td>Slight</td>
<td>Conveyors, slicers</td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>Inspection belts, weighing reserves</td>
<td>28</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>After areas of heavy contact</td>
<td>Hoppers, final package</td>
<td>65</td>
<td>2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

during a number of operations following the blanch only incidental human contact occurred. Contrast was provided by later stages, such as inspection and filling, where the vegetables were subjected to considerable handling by employees.

An increase in the incidence of staphylococcus-positive samples appeared to result from human contact (Table 2). The percentage of staphylococcal contaminated pea samples rose from 12 to 80% at the inspection belts, the first point of human handling. The percentage of beans contaminated with staphylococci also increased but not to the extent found with the peas. Fourteen out of 18 of these bean samples were "weighing reserves" which were beans used to adjust the cartons to the correct weight. Since the weighing reserves were exposed to repeated contact with ungloved hands, staphylococcus contamination had been expected to be most heavy in those samples.

Vegetables collected from points following the areas of heavy human contact continued to show elevated numbers of staphylococci. Additional contamination of peas by other organisms occurred which accounts for the lower ratio of staphylococci to total count even though the MPN figures increased. The doubling of the percent of staphylococcus-positive beans is not understood but may merely reflect variations in sampling and counting procedures.

Peas were the least heavily contaminated vegetable according to the total counts (Table 1). In spite of this, however, the percentage of staphylococcus-positive pea samples was not appreciably lower, and the average staphylococcal count was actually higher than obtained with beans and corn (Tables 1 and 2). A possible explanation for this was obtained during the 1963 season when line surveys were made twice a week at one factory. The results showed a significant build-up of microorganisms in the gravity separator as the processing season progressed (Figure 1). Since this unit contained NaCl brine at a concentration of 8-10%, a study of the contaminants was made after the increase in total counts was noted. Forty isolates, randomly selected from plates cultured on the 25th processing day, were studied. Catalase-positive cocci that grew well on media containing 10% NaCl made up 85% of the isolates. Although all
The results of this study indicate that frozen peas, beans, and corn should contain only low numbers of coagulase-positive staphylococci when prepared under normal commercial conditions. Even though the total counts of many samples were in the hundred-thousands per g, none yielded staphylococcal counts as high as 100 per g. It is of interest that this level of staphylococcal contamination appears to be considerably lower than that reported for vegetables processed in a pilot plant (4). These workers found over 12% of the organisms enumerated by plate count to be toxin producing micrococci.

The data support the idea that the hands of employees are a major source of staphylococci. The fact that the greatest numbers were not recovered from French-style beans, the vegetable receiving the most human contact, suggests that other factors influence growth and survival of these organisms.

The build-up observed in the gravity separator illustrates that certain processing conditions may favor the growth of undesirable organisms such as staphylococci. The fact that only chance human contact occurred between the blanch and the gravity separator may explain why no coagulase-positive staphylococci were found in the sample examined. One can speculate about the microflora that might predominate had the inspection belts preceded the gravity separator.

**ACKNOWLEDGMENT**

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**REFERENCES**


