Effects of Potassium Sorbate on Normal Flora and on Staphylococcus aureus Added to Minced Cod

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(Received for publication December 9, 1981)

ABSTRACT

Minced cod and pasteurized minced cod, with and without 0.5% potassium sorbate, were subjected to abusive storage temperatures of 7 and 15°C. Staphylococcus aureus FRI 100 was inoculated into the cod before storage. Total aerobic plate counts (20 and 35°C), pH changes, S. aureus counts and the presence of thermonuclease were monitored throughout the studies. With the unpasteurized minced cod, potassium sorbate caused slightly lower aerobic plate counts (at 20 and 35°C) in the 7°C study over an 11-day storage period. Psychrotrophic organisms were inhibited to a slightly greater extent than were mesophilic organisms. Inoculated S. aureus was quickly outgrown by the normal microflora without or with sorbate. Similar results were obtained at the still more abusive temperature of 15°C over a storage period of 5 d, but the inhibitory effect of sorbate was less evident. Pasteurized minced cod, inoculated with S. aureus and stored at 15°C, showed a considerable difference in growth of S. aureus with and without sorbate. Potassium sorbate resulted in a markedly slower rate of growth of the pathogen and a substantial delay of several days in production of detectable levels of thermonuclease. This delay in nuclease production is indicative of a similar delay in enterotoxin production.

One way to improve upon food yield from the ocean's harvest is to use mechanical deboning technology. This technology permits a higher percentage of flesh to be removed from fish than is normally obtained, and it also allows a larger spectrum of underutilized species of fish to be used (10,14,15).

Mechanical deboning of fish, in addition to its advantages, also provides opportunity for increasing the total bacterial load of the product and for encouraging the presence of pathogenic bacteria. Not only is the environment for growth improved (17), but the mincing operation also serves to blend microorganisms, from raw materials in the deboner and previously processed fish, throughout the product. Due to the large initial numbers and fast growth of naturally occurring fish microflora, pathogenic contaminants are usually outgrown (1,3), but as the amount of processing increases, the threat from pathogens also increases, especially where processing alters the normal flora.

Potassium sorbate is an effective and safe food preservative and is used in a wide variety of foods. It is inhibitory to yeasts, molds and bacteria (20). Although the upper pH range for sorbate activity is 6.0 to 6.5 (16), which is below the normal fish flesh pH range of 6.8 to 7.4 (2), lack of other preservatives possessing the desirable characteristics of sorbate has caused researchers to investigate the potential use of this preservative in fish (3,6,19).

The present study was undertaken to determine the advantages of using potassium sorbate in minced cod. Due to the widespread occurrence of foodborne staphylococcal intoxication (4), effects of sorbate on Staphylococcus aureus inoculated into minced cod and pasteurized minced cod also were investigated.

MATERIALS AND METHODS

Minced and pasteurized cod

Frozen 7.5-kg blocks of cod were obtained from local retail outlets. The blocks were sawed into workable size pieces (0.7 kg), thawed at 6°C overnight and were then minced in a 3.8-L Waring blender at low speed for 1 min. The thoroughly mixed mince was divided into 50-g portions (patties) which were individually packed in 30 x 11-cm Whirlpak bags (American Scientific Products). When cod was pasteurized, this was done by immersing the bags, loosely held in vertical position in a wire basket, into agitating water at 85°C for 30 min followed by quick cooling in ice water. Pasteurization under these conditions consistently decreased bacterial counts to less than 10² per g of minced cod.

Staphylococcus aureus inoculum

S. aureus FRI 100, obtained from the Food Research Institute, University of Wisconsin, was inoculated into Brain Heart Infusion (Difco) broth and incubated for 24 h at 37°C. Transfer and incubation were repeated three times. This culture, before inoculation into fish patties, was centrifuged and resuspended in BH broth three times to remove all preformed enterotoxin and thermostable nuclease, according to the method of Tatini et al. (23). After the washing step, cells were resuspended in 0.1% peptone diluent (Difco) so that 1 ml of diluent added to fish patties yielded approximately 10⁶ colony forming units of S. aureus per g.

Additions to fish patties

Patties in the Whirlpak bags received 1 ml of S. aureus inoculum, and/or 2 ml of a membrane-sterilized potassium sorbate (Monsanto)
solution to yield a concentration of 0.5% (w/w) potassium sorbate per g of fish. Sterile water was added to the control and to other patties to maintain equal added fluid volumes. When patties were pasteurized, additions followed pasteurization. Following the additions, the minced fish flesh was thoroughly mixed inside its Whirlpak bag by the pounding action of a Stomacher Lab-Blender 400 (Cooke Laboratory Products, Alexandria, VA.) for approximately 20 s. After mixing, the sealed bags were held at the appropriate storage temperature.

Analytical tests

Duplicate samples of fish after mincing and before additions were analyzed for moisture, sodium chloride and total protein. Moisture was calculated from weight loss after oven-incubation at 110°C for 48 h. Sodium chloride level was determined using the A.O.A.C. method 18.031b (1). Protein was determined by converting micro-kjeldahl nitrogen level using the factor 6.25. Determinations of pH were made by blending 50 g of fish with 50 ml of distilled H2O and measuring with a Corning research pH meter.

Detection of thermonuclease and enterotoxin A in fish

The metachromatic agar-diffusion slide method of Laechica et al. (11) was used to assay for thermonuclease. This was the same procedure as used in the confirmation test for S. aureus. The procedure of Chesbro and Auburn (5) was used to extract thermonuclease from the fish. Purified micrococcal nuclease (Sigma) was used in preliminary experiments to test the efficacy of the extraction procedure.

The procedure used to extract enterotoxin A from the cod was that of Reiser et al. (4). Enterotoxin A was determined with the microslide method (5). Preliminary experiments were made to test the efficacy of the extraction and assay methods using crude enterotoxin A and its antiserum obtained from the Food Research Institute, University of Wisconsin and also from the FDA, Washington, DC.

Experimental design

Three major storage studies were conducted. Storage study 1 consisted of four groups of fish patties held at 7°C for 11 d. Groups A and C had no preservative added while groups B and D received potassium sorbate. Groups A and B further were uninoculated while groups C and D were inoculated with S. aureus. Storage study 2 was identical to the first except that it was conducted at 15°C for 5 d. Storage study 3 had two groups of fish patties that were held in storage at 15°C for 10 d. Both groups were pasteurized. After pasteurization and cooling, both groups were inoculated with S. aureus while only one group received potassium sorbate.

RESULTS AND DISCUSSION

Preliminary experiments

Experiments conducted to test the efficacy of the Baird-Parker Agar medium for its differentiating capabilities revealed a high correlation between typical S. aureus colony morphology and S. aureus confirmation. Of 30 colonies that gave typical colony morphology, 29 were confirmed as being S. aureus. All organisms that gave atypical morphologies were found not to be S. aureus. The non-S. aureus organism that gave a colony typical of S. aureus was found to be a gram positive coccus that formed tetrads, was benzidine negative, cleared egg yolk, was non-motile, did not liquefy gelatin, produced no gas from glucose, fermented glucose both aerobically and anaerobically, and grew well at 35 but not at 53°C. It gave a negative coagulase score and a negative thermonuclease result.

Problems in identifying S. aureus colonies on crowded plates were encountered throughout this study. This was due to S. aureus being overgrown by highly competitive microflora and to the difficulty of distinguishing features of S. aureus on such plates. Similar problems have been reported by others (21).

Preliminary attempts to extract inoculated thermonuclease from cod were positive. Freshly minced cod (pH 6.9 and APC at 35°C of 104 per g) and minced cod with severe proteolysis (pH 7.4 and APC at 35°C of 104 per g) were inoculated with several levels of thermonuclease. All nuclease levels above and including 5 µg per 50 g of fish were detected. Tatini et al. (23) found that while every system was different, enterotoxin production in detectable levels often was observed soon after nuclease levels reached 5 µg per 50 g of food. This indicates that in the minced cod, when the thermonuclease extraction resulted in a positive metachromatic slide reaction, there was likelihood that enterotoxin also was present.

A great deal of difficulty was encountered with extraction of enterotoxin A from cod. Due to repeated inconsistencies associated with the extraction of numerous inoculated samples, it was decided not to conduct this assay in the subsequent storage studies.

Analytical tests

The values for minced cod moisture, sodium chloride, protein and initial pH are in Table 1. The variations between the protein and NaCl values of the first two experiments and those of the third are due to the frozen cod of the latter being obtained from a different retailer. All values for moisture, NaCl, protein and pH are in agreement with the results of Gordon and Roberts (7). The high salt values in the third study are most likely due to the fish muscle having undergone a light salt brining before freezing. Some manufacturers use this to help prevent the free liquid drip that occurs when frozen fish are thawed (22).
TABLE 1. Moisture, sodium chloride, protein and initial pH values of minced cod used in the three storage studies.

<table>
<thead>
<tr>
<th>Storage study</th>
<th>Moisture (%)</th>
<th>NaCl (%)</th>
<th>Protein (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83.39</td>
<td>0.066</td>
<td>16.48</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>81.60</td>
<td>0.161</td>
<td>17.98</td>
<td>6.9</td>
</tr>
<tr>
<td>3</td>
<td>78.14</td>
<td>0.605</td>
<td>20.32</td>
<td>6.7</td>
</tr>
</tbody>
</table>

a All values are averages of duplicate samples.

b NaCl and protein values are calculated on a wet basis.

Effects of storage on minced cod at 7 and 15°C

Results of storage study 1 at 7°C are in Fig. 1 and 2 and in Table 2. Throughout this study the total aerobic plate counts, both at 20 and 35°C, were lower for the cod with 0.5% potassium sorbate than with no preservative added. The difference was slightly greater for the psychrotrophic organisms (APC at 20°C, Fig. 1). These results are in agreement with the findings of Chung and Lee (6) in their study using English sole.

*S. aureus* was inoculated into the fish patties to give a count of approximately $10^4$/g. It is evident from data in Table 2 and Fig. 1 and 2 that the normal flora outgrew the pathogen and eventually caused it to die out. No difference between cod with and without sorbate was observed in regard to the rate of disappearance of *S. aureus*. All attempts to extract thermonuclease throughout this study were negative. This result was expected since *S. aureus* never entered a logarithmic growth phase where detectable amounts would be produced.

The pH of the fish were sorbate did not rise above 7.1, whereas that of fish without sorbate rose from 7.0 to 7.4. Even though bacterial growth was not greatly inhibited by sorbate in this particular system, the difference in pH is an indication that some proteolytic activity was inhibited.

Results of storage study 2 at 15°C are in Fig. 3 and 4. Fish with sorbate always had a very slightly lower total aerobic plate count at time of testing than without sorbate, but the differences were always minimal (less than one log). *S. aureus* counts were virtually identical to those in the 7°C study and similar results in the pH values also were observed. Again, all assays for thermonuclease extracts were negative.
Results of the above two studies indicate that potassium sorbate in minced cod is not necessary to inhibit S. aureus. This pathogen, were it to contaminate minced cod, would be easily controlled by the microflora naturally present. The extension of shelf-life by potassium sorbate, judged from total aerobic plate counts, also is of questionable value.

**Effects of storage at 15°C on pasteurized minced cod**

Results of study 3 with pasteurized minced cod are seen in Tables 3 and 4 and in Fig. 5. Pasteurized minced cod without S. aureus addition (Table 3) revealed that pasteurization decreased aerobic plate counts of normal flora to between 1.0 and $5.0 \times 10^4$ per g. The inhibitory effects of sorbate on subsequent growth of the normal flora also are evident from data in Table 3.

Growth of S. aureus in the pasteurized and inoculated patties which were plated using Baird-Parker medium is seen in Fig. 5. Here the effects of potassium sorbate were considerable. Counts between $10^7$-$10^8$ were reached in the fish without sorbate within 2.5-3.0 days, while with sorbate this took more than 7 days. This appreciable effect of sorbate in the pasteurized minced cod with an initial pH of 6.7, which is above the maximum pH range for sorbate activity as reported in many foods (16), is noteworthy. Further, pH values never dropped below 6.6 nor rose above 6.7 for the cod with sorbate during the 10-d storage period.

**TABLE 3. Aerobic plate counts of pasteurized minced cod without S. aureus addition and stored at 15°C.**

<table>
<thead>
<tr>
<th>Time fish patties held (days)</th>
<th>Counts/g without potassium sorbate</th>
<th>Counts/g with potassium sorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic plate counts at 20°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>$1.0 \times 10^4$</td>
<td>$5.0 \times 10^4$</td>
</tr>
<tr>
<td>4</td>
<td>$&gt;3.0 \times 10^3$</td>
<td>$8.9 \times 10^4$</td>
</tr>
<tr>
<td>8</td>
<td>$3.4 \times 10^3$</td>
<td>$4.3 \times 10^4$</td>
</tr>
</tbody>
</table>

Aerobic plate counts at 35°C

<table>
<thead>
<tr>
<th>Time fish patties held (days)</th>
<th>Counts/g without potassium sorbate</th>
<th>Counts/g with potassium sorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$1.5 \times 10^4$</td>
<td>$3.5 \times 10^4$</td>
</tr>
<tr>
<td>4</td>
<td>$&gt;3.0 \times 10^3$</td>
<td>$7.6 \times 10^4$</td>
</tr>
<tr>
<td>8</td>
<td>$2.9 \times 10^3$</td>
<td>$3.3 \times 10^4$</td>
</tr>
</tbody>
</table>

**TABLE 4. Detection of thermonuclease in pasteurized minced cod inoculated with S. aureus and stored at 15°C.**

<table>
<thead>
<tr>
<th>Time fish patties held (days)</th>
<th>Patties without potassium sorbate</th>
<th>Patties with potassium sorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+ +</td>
<td>+ +</td>
</tr>
</tbody>
</table>

*Each fish extract was assayed in duplicate for nuclease using the metachromatic agar-diffusion method.*

JOURNAL OF FOOD PROTECTION, VOL. 45, JULY 1982
Thermonuclease production over the storage period is seen from data in Table 4. Thermonuclease was detected in the cod containing no sorbate in 4 d while it could not be detected in the cod with sorbate after 7 d but appeared on the tenth day of the testing period. This is a significant difference since the detection of thermonuclease indicates the likely presence of enterotoxin. Thermonuclease levels are directly related to the size of the \textit{S. aureus} population (7). By comparing data in Fig. 5 with those of Table 4, it can be seen that in the sorbate and non-sorbate containing systems, thermonuclease was not detected in the cod until \textit{S. aureus} reached a count of $10^8$/g. Therefore it appears that potassium sorbate could have a highly desirable effect on pasteurized minced cod that might become contaminated with \textit{S. aureus}.

REFERENCES


Figure 5. Effects of potassium sorbate on \textit{Staphylococcus aureus} in pasteurized minced cod stored at 15°C.