Potassium Sorbate as a Fungistatic Agent In Country Ham Processing

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ABSTRACT

Sixty, seventy and ninety-day-old country cured hams were used to evaluate potassium sorbate as a fungistatic agent during aging and holding for market. A 1 min spray of 5% (w/v) potassium sorbate offered the lowest effective level for inhibition of fungal growth. Mold and yeast colony counts 30 days post-treatment were significantly lower than initial numbers but protection was lost by the 60th day under conditions conducive to fungal outgrowth (21 ± 5°C and 70 ± 5% relative humidity). Greater mold inhibition was noted when a 10% potassium sorbate spray was used under identical conditions. Less than 65% relative humidity inhibited mold growth on 120-day-old ham slices held at 7°C. Mold and yeast counts tended to be lower on hams treated after 60 days of processing than on hams treated after 90 days of processing. Residual concentrations of sorbic acid required to inhibit mold growth and yield an acceptable ham after 30 days storage were within the limit approved by the Food and Drug Administration for other food products.

Country cured ham has been a traditional meat product of the Southeastern United States for more than 300 years, and its commercial value has been realized for many generations. Currently, large scale commercial production is found in much of the Southeastern region. One of the recurring problems in production and distribution of country cured hams is the growth of molds and yeasts on ham surfaces during conditions of high relative humidity. Such conditions are fairly common in the Southeast.

Fungal growth on aged country ham was at one time thought to be indicative of proper aging and flavor development. However, recent studies have revealed that development of flavor in dry-cured ham is the result of enzymatic and chemical changes rather than the result of microbial growth (9,11).

For several years, larger processors have been expressing interest in expanding their sales to locations beyond local and regional markets, including overseas markets. Yet a consumer from outside the "country ham belt" may regard a moldy country ham as spoiled. More important is a possibility of the potential presence of mycotoxins. Several studies have revealed that a number of mold species isolated from country ham possess the capability of producing mycotoxins when grown in pure culture on laboratory media. These toxins include ochratoxin, sterigmatocystin, citrinin and aflatoxin (6,10,14,15,17). A need, therefore, exists for an effective fungistatic agent for use on country cured hams and other dry-cured meats.

Sorbic acid and its potassium salt have long been used in foods as effective non-toxic inhibitors of fungal growth, including those genera that possess a mycotoxin-producing capability. Many investigations on use of sorbates in food products have resulted in their broad approval as antimicrobial agents in dairy, bakery and fruit preparations. The only meat products where use of sorbate is presently permitted are dry sausages (2.5% dip) and dog food patties (3). Studies conducted during the past 23 years have demonstrated the lack of toxicity of sorbates to rats, mice and dogs (4,5,8,12).

The purpose of this work was to determine the effectiveness of potassium sorbate as a fungistatic agent on country hams under conditions conducive to fungal growth.

MATERIALS AND METHODS

Treatment of ham slices

Ham slices were obtained from four country hams (long shank variety) that had been cured 30 days, held 30 days at 55°F to allow even distribution of salt (equalized) and aged 205 days under ambient conditions. Each ham was cut in half and the butt half was discarded. Ten 1.3-cm slices from the shank half of each ham were randomly paired within hams. One-liter sorbate solutions of 0, 2.5, 5, and 10% concentration were prepared at ambient temperature (25°C). One pair of slices from each ham was successively placed into each solution for 1 min. Another pair was dipped into the 10% sorbate solution for 2 sec. One slice from each pair was placed in storage at 22 ± 5°C and 70 ± 10% relative humidity to be examined at 60 days for mold growth and sorbate residuals.

Treatment of whole hams

Twenty-eight 70-day-old country cured (papper-style cut) hams were used for studying the inhibitory properties of potassium sorbate applied by a 1 min spray of ambient temperature aqueous solutions at 0, 2.5, 5, and 10% concentrations. Analyses were as follows: (a) one ham per treatment level was analyzed on the day of treatment for sorbate residual; (b) three hams at each treatment level were analyzed at 30 and 60 days for sorbate residue, mold and yeast colony counts and visible mold.

Before spray treatment, the black pepper coating applied by the commercial processor was removed by scrubbing and washing to prevent it from masking mold development. Hams were air-dried overnight, bagged in cotton stockinettes, and hung shank end down. The hams were then sprayed for 1 min with appropriate concentrations of potassium sorbate using a stainless steel hand sprayer operated by compressed air (garden type). The hams were stored in a room held at 21.1 ± 0.5°C and 70 ± 5% relative humidity until removed for testing.

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**Sorbate analysis**

The method of analysis for sorbate residuals was developed from the methods of Bokus (1) and Wilamowski (16). Ham slices were trimmed of fat and skin and then were ground in a Waring blender for 15 sec. Samples for the whole ham study were obtained by surface trimming the entire lean ham face at a depth of 2.5 mm to obtain approximately 15 g of sample. The 15 g from each ham were then dry blended in a Waring blender for 15 sec. Ten grams of the ground ham were transferred to a second blender jar containing 100 mL of an alcoholic solution of metaphosphoric acid (5 g of HPO₃ in 250 mL of H₂O and diluted to 1 liter with alcohol). The mixture was blended for 1 min and allowed to stand for 10 min. Contents of the blender jar were then vacuum-filtered through Whatman No. 3 filter paper. Five ml of the filtrate were transferred to a 250-mL separatory funnel and 100 mL of 1:1 petroleum ether-ethyl ether were added. This final ether solution contained the sorbic acid extracted from 500 mg of ham.

The mixture was shaken for 1 min, after which the ether layer was recovered and dried with 5 g of anhydrous sodium sulfate. Absorbance of the ether layer was determined spectrophotometrically at 250 nm using the extracts from untreated ham samples as a blank.

**Preparation of the standard curve**

The standard curve was prepared from stock solutions containing 0.134 g of potassium sorbate (equivalent to 0.1 g of sorbic acid) in 100 mL of deionized water. Aliquots of 1 to 6 mL were made up to 100 mL with the alcoholic metaphosphoric acid solution. Five mL of each flask were then shaken for 1 min with 100 mL of 1:1 petroleum ether-ethyl ether. The ether layer was recovered and dried with 5 g of anhydrous sodium sulfate.

Absorbance was determined at 250 nm versus a blank prepared with 5 mL of metaphosphoric acid solution and was plotted against mg of sorbic acid/100 mL of ether. A linear curve was obtained.

**Calculations: spectrophotometry**

Concentrations of sorbic acid in ham were calculated by the following formula:

\[(\text{mg sorbic acid/100 mL ether}) \times 2000 = \text{ppm sorbic acid in ham}\]

\[500 \text{ mg}\]

**Visible mold evaluations**

Subjective evaluations of visible mold on the ham slices were made at 8, 16, 24, 36 and 60 days. Whole hams were similarly evaluated at 30 and 60 days. Intensity of mold growth was rated on a scale of 0 to 5 where: 0 = no growth, 1 = very slight growth, 2 = slight growth, 3 = moderate growth, 4 = marked growth and 5 = intense growth.

**Enumeration of mold and yeast colonies**

A 25.8-cm² area of lean meat was swabbed using a 5.08-cm² template. The swab was placed in 10 mL of 0.85% saline solution and mixed. Aliquots of the solution, 1 mL and 0.1 mL, were surface-plated on acidified Czapek agar. Plates were incubated at 22°C for 5 days (7). Mold and yeast colonies were counted using a Quebec colony counter.

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**RESULTS AND DISCUSSION**

**Mold growth evaluation (slices)**

Mold growth was subjectively evaluated on the 0 to 5 scale at specific times following treatment and at various treatment levels (Table 1). Using ham slices as a test system, growth values between 0 and 1 represent optimum control; levels between 1 and 2 were acceptable; values above 2 were considered unacceptable.

Greater fungal growth was observed with increasing time and with decreasing levels of potassium sorbate. Factorial analysis of variance indicated significant differences at the P = .01 level for all three parameters: potassium sorbate level, days following treatment and source of the ham slice (Table 1), with the largest F value for the source of the slice. Slices that exhibited the most prolific fungal growth were obtained from Ham #52. The arithmetic growth mean of 2.32 was almost twice that of any other ham and about five times the minimum level, suggesting slices from Ham #52 were initially heavily contaminated with mold, and that sorbate treatment would be considerably less effective under these circumstances.

**Sorbate residuals (slices)**

Residual studies were conducted to help elucidate the relationship between sorbate concentration and fungistasis over the 60-day study. Table 2 summarizes data from 32 ham slices taken from four cured hams. Sorbic acid residuals were determined immediately after dipping and after 60 days of storage. In some instances, wide variations were observed between samples subjected to identical treatment levels. A significant difference between the sample means was noted at the P = .05 level (Table 2). Variation within a treatment was attributed to the inherent irregularities of the ham slices and uneven absorption of sorbate.

Length of storage definitely influenced residual concentrations (P < .01). After 60 days, sorbic acid concentrations decreased by about 50% (583.5 to 250.0 ppm). These results differ from those of Boyd and Tarr (2) who found that sorbic acid residuals in smoked fish did not decrease appreciably over a period of 60 days at 25°C and 75% relative humidity.

**Mold and yeast evaluation (whole hams)**

The application of potassium sorbate to whole hams gave pronounced effects on levels of mold and yeast. In ham sprayed for 1 min with potassium sorbate (2.5, 5 or 10%), the initial yeast population was reduced to less than
1 colony forming unit/cm² (CFU/cm²), while the control hams sprayed only with plain water maintained a viable population of 1,100 yeasts CFU/cm² (Table 3). In contrast, mold counts on zero day were not significantly different at all treatment levels (0, 2.5, 5, and 10%) (Table 3); yet a slight reduction in numbers was noted. Reduction in yeast population appeared related to the washing of spores from the ham surface.

In a preliminary study, the spray (until visibly wet) applied to a lot of hams, identical to those in above study, did not significantly reduce the numbers of yeast or mold at all treatment levels (0, 2.5, 5, and 10%). Thus, the 1-min spray exposure appears to be the best method of application and one that is feasible in most commercial curing operations.

**TABLE 3. Statistical summary of mold and yeast colony counts on 70-day-old country hams at 0 days.**

<table>
<thead>
<tr>
<th>Potassium sorbate (%)</th>
<th>Mold Colony count (Mean)</th>
<th>Range</th>
<th>Yeasts Colony count (Mean)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.3b</td>
<td>0-3.9</td>
<td>1.100</td>
<td>496-1,400</td>
</tr>
<tr>
<td>2.5</td>
<td>0.7</td>
<td>0-1.6</td>
<td>0.3</td>
<td>0-0.8</td>
</tr>
<tr>
<td>5</td>
<td>0.6</td>
<td>0-1.6</td>
<td>0.7</td>
<td>0.2-1.2</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0-0.4</td>
</tr>
</tbody>
</table>

*All values expressed as colony forming units/cm². Mean of 4 observations from one ham. F = 29.3**d

After 30 days of storage, the mold and yeast colony counts increased. Mold on control hams (0% potassium sorbate) were too numerous to count while yeast counts ranged from 2,500 to 39,000/cm² (averaging 11,700 yeasts/cm²). The 10% potassium sorbate treatment showed mold counts ranging from 16 to 240 CFU/cm² with an average of 81 CFU/cm². Yeast counts at the 10% level ranged from 150 to 490 CFU/cm² with an average of 290 CFU/cm².

Table 4 gives growth means obtained by visual observation. At the end of the 30 days, the effect of sorbate treatment was significantly different at each level. The 5% sorbate concentration showing a 1.3 growth value (mean) would be acceptable but marginal for production of marketable hams. The 10% level produced a 0.7 growth value which is below the “very slight” category and would represent a very acceptable level in the market place. The fungistatic effect of this level is very desirable.

**TABLE 2. Sorbic acid residuals from 60-day ham slice study.**

<table>
<thead>
<tr>
<th>Potassium sorbate (%)</th>
<th>Residual (Mean)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>224-270</td>
<td>247c</td>
</tr>
<tr>
<td>5</td>
<td>400-444</td>
<td>422</td>
</tr>
<tr>
<td>10d</td>
<td>590-660</td>
<td>625</td>
</tr>
<tr>
<td>10</td>
<td>680-1400</td>
<td>1040</td>
</tr>
</tbody>
</table>

*All values expressed as ppm sorbic acid. Mean of two observations. F = 584 Average c

At the 60 days evaluation, fungal growth was approaching the intense level (Table 4), and there was no significant difference in the effects of treatment (0-10%). Thus, the inhibitory property of potassium sorbate was lost sometime between 30 and 60 days of storage in an ambient humid environment.

**Table 4. Summary of visible mold evaluation of 70-day-old country hams.**

<table>
<thead>
<tr>
<th>Potassium sorbate (%)</th>
<th>30 Days Growth (Mean)</th>
<th>Range</th>
<th>60 Days Growth (Mean)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.3</td>
<td>(4-5)</td>
<td>4.7</td>
<td>(4-5)</td>
</tr>
<tr>
<td>2.5</td>
<td>3.3</td>
<td>(3-4)</td>
<td>4.0</td>
<td>(4)</td>
</tr>
<tr>
<td>5</td>
<td>1.3</td>
<td>(1-2)</td>
<td>4.0</td>
<td>(4)</td>
</tr>
<tr>
<td>10</td>
<td>0.7</td>
<td>(0-1)</td>
<td>4.0</td>
<td>(3-5)</td>
</tr>
</tbody>
</table>

*Growth expressed by visual evaluation on scale of 0 to 5: 0 = no growth. 1 = very slight. 2 = slight. 3 = moderate. 4 = marked. 5 = intense. **Denotes significance at 1% level. ***Denotes significance at 1% level. ^F = 35.07**ab

Sorbic acid residuals (whole hams)

The sorbate residual data obtained from analysis of exterior lean meat (2.5 mm in depth) trimmed from the face of the whole 70-day-old country hams are summarized in Table 5. The effectiveness of the 1-min spray exposure was substantiated by the residuals shown on zero day. Although concentrations seemed fairly high, these values were from the exterior surface of the ham and would not reflect the concentration of the overall whole ham. Presumably these high residuals resulted in part from the stockinette cover aiding in retention of sorbate.

Thirty days after treatment a reduction in the concentrations of sorbate was evident (Table 5). The difference between treatments became less after 30 days of storage, but still statistically significant. On hams treated with 2.5 and 5% sorbate, there was mold growth on 27 of 28 hams studied. Molds in sufficiently large populations can metabolize the sorbate, thereby reducing its fungistatic effect (13). This may serve to explain why we found no significant difference between 2.5 and 5% residual levels at the end of 30 days. Since the 5% level only gives marginal fungal inhibition, it would appear that 142-228 ppm sorbic acid is about the lowest level for fungal inhibition. The 10% treatment with...
residuals of 348 to 568 ppm approached the optimum concentration.

After 60 days of storage, the residual levels of sorbate became quite low, explaining why we had fungal growth on every ham. At the highest treatment level (10%), our range of residuals was from 0 to 194 ppm. As shown in Table 5, there was no significant difference between treatment levels. These results, along with the 60-day visual mold evaluation, seem to reinforce the conclusion that the ability of sorbate to inhibit fungal growth on country hams is lost sometime between 30 and 60 days of storage in an ambient humid environment.

Based on 20-day residual data, it would appear that a minimum sorbate concentration of 400-500 ppm on the surface of the hams is required to limit fungal growth to an acceptable level. Our data indicate that an initial concentration of about 2400 ppm is needed to assure an adequate concentration at 30 days. This initial level is within the range of 0.1-0.3% (by weight) already permitted by FDA in certain foods. Furthermore, we have analyzed only the exterior surface (2-5-mm depth) and the concentration in the interior of the ham would undoubtedly be much lower. Therefore, the total amount of sorbate consumed in a slice of treated ham with surface level of 2400 ppm would be well below that consumed with the same weight of processed product such as margarine where the sorbate concentration is 1000 ppm and uniformly distributed. Secondly, the level falls sharply during storage, being no more than 18% of the initial amount added after 30 days of storage on whole hams treated with 10% sorbate.

**SUMMARY**

Potassium sorbate applied as a 10% solution was effective as a fungistat on country ham slices and on whole hams. The inhibitory effect lasted at least 30 days under conditions conducive to fungal development. However, by 60 days fungistatic property was lost primarily because the residual level of sorbate dropped rapidly during storage. A 5% solution gave only marginal protection during the first 30 days. The 1-min spray exposure offered advantages for commercial use over the quick dip or spray because of its washing action to remove spores and the higher initial concentration of sorbate on the hams. The cotton stockinette used to bag the hams may have assisted in sorbate retention. The residual concentration of sorbate required to inhibit mold growth and yield an acceptable ham after 30 days storage was well within the limit approved by FDA for other food products.

**REFERENCES**


**TABLE 5. Summary of sorbic acid residuals on 70-day country hams.**

<table>
<thead>
<tr>
<th>Potassium</th>
<th>0 Days</th>
<th>30 Days</th>
<th>60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>sorbate (%)</td>
<td>Mean</td>
<td>Range</td>
<td>S.D.</td>
</tr>
<tr>
<td>2.5</td>
<td>700</td>
<td>30-148</td>
<td>60.0</td>
</tr>
<tr>
<td>5</td>
<td>2100</td>
<td>142-258</td>
<td>48.5</td>
</tr>
<tr>
<td>10</td>
<td>432</td>
<td>348-568</td>
<td>118.9</td>
</tr>
</tbody>
</table>

F = 13.82

a All values expressed as ppm sorbic acid.
b S.D. denotes standard deviation.
c One observation only.
d * Denotes significance at the 5% level for treatments (30 days).
e ** Denotes non-significant (60 days).