Depletion of Sodium Nitrite by Lactic Acid Bacteria Isolated from Vacuum-Packed Bologna

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ABSTRACT

Different strains of lactic acid bacteria isolated from bologna were inoculated into APT broth and vacuum-packed bologna sterilized by gamma radiation. Broth cultures of Leuconostoc mesenteroides reduced nitrite levels at 5 and 15°C at a greater rate than Lactobacillus plantarum and Lactobacillus viridescens. Brochothrix thermosphacta and Lactobacillus brevis reduced nitrite at rates similar to the uninoculated broth. Similar nitrite reduction rates were noted with the same organisms in the inoculated packs of bologna at 5°C. Residual nitrite losses in the bologna by action of the lactic acid bacteria was estimated to be 30%.

Information on the contribution of the spoilage lactic acid bacteria to nitrite depletion in cured meats is sparse. Most reviews on nitrite losses in meats have tended to ignore losses by bacterial action (12). Studies by Fournaud et al. (2,3) indicated that certain lactic acid bacteria possess a nitrite reductase enzyme system which reduces nitrite under anaerobic conditions. This ability of the lactobacilli to reduce nitrite in meat products was also noted by Ingram (5), but he made no mention of published work in this area. Other authors have also reported several types of bacteria in foods which are capable of nitrite reduction (14,15).

The importance of residual nitrite levels in cured meat products was outlined in a recent review (12) with respect to antibotulinal activity. Thus any factors which change or alter nitrite levels should be understood since they may relate not only to the safety of the product but also its keeping quality.

This paper presents data on the influence and ability of specific lactic acid bacteria isolated from vacuum-packed bologna to reduce nitrite levels both in broth cultures and inoculated packaged meats.

MATERIALS AND METHODS

Isolation, identification and culturing of bacterial strains

Cultures of lactic acid bacteria were isolated from nine commercial samples of vacuum-packed bologna. These samples represented products from three different companies. Identification procedures and culturing of bacterial strains have been described previously (1).

Nitrite studies (broth cultures)

To determine the degree of microbial depletion of nitrite in broth cultures under anaerobic conditions, duplicate flasks each containing 100 ml of APT (Difco) broth were prepared. Sodium nitrite was added to each flask from a filter-sterilized solution to give a concentration of 100 µg/ml of broth. Flasks were inoculated with 0.1 ml of the selected lactic acid bacteria cultures to give an initial level of 10³ cells per ml. One set of flasks was incubated at 15°C for 3 days and the other was incubated at 5°C for 6 days. Nitrite levels were determined after 4 days for cultures incubated at 15°C, and after 3 and 6 days for cultures incubated at 5°C, using the colorimetric method 2 of Ito (6).

The degree of nonmicrobial depletion of nitrite was determined in duplicate uninoculated flasks containing 100 ml of APT broth. Sodium nitrite was added to give a concentration of 100 µg/ml, as indicated previously. Flasks were incubated at 5 and 15°C, respectively. Nitrite analysis was carried out using the method described earlier.

Nitrite studies (inoculated meat)

Twelve commercial samples of vacuum-packed processed bologna were aseptically opened, and evenly distributed to give 72 samples. Each sample was vacuum-packed in separate vinyl chloride copolymer bags (Cryovac S. 0.002 in thick, 0.2 permeability 10-30 cm²/m² per atmosphere per 24 h, W.R. Grace and Co., Duncan, South Carolina), using a Clipper Vac (Model F, Rhee Manufacturing Co.) and heat sealed. Duplicate samples were analyzed to determine the initial population, nitrite content, and pH of the meat at the beginning of the storage period.

With the exception of 10 samples for the non-irradiated controls, the rest of the samples were irradiated from a 24,000 curie cobalt-60 source with a dose rate of 0.19753 x 10⁶ Megarads (Mrad)/h at ambient temperature. The total dose given to the meat samples was 0.3 Mrad.

Survival after irradiation

Duplicate irradiated samples were tested for sterility and nitrite levels immediately after irradiation. Bacterial counts were made on streptomycin-thallous acetate agar (4) APT and on the MRS (9) medium.

Ten samples of the irradiated bologna were stored at 5°C for 4 weeks as controls. The rest of the samples were uniformly surface-inoculated by spraying 10⁵ cell/ml of each Brochothrix thermosphacta, Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum or Lactobacillus viridescens. After inoculation, the irradiated vacuum-packed bologna samples were stored at 5°C for 4 weeks and then analyzed for nitrite, pH and bacterial...
counts. The bacteriological analysis for \textit{B. thermosphacta} and for the lactic acid bacteria was performed using the medium described above and according to the methods of Paradis and Stiles (10).

**RESULTS AND DISCUSSION**

Nitrite depletion by several lactic acid bacteria grown in the presence of 100 \( \mu \text{g} \) of sodium nitrite/ml of APT broth at 5 and 15 \( \text{C} \) is shown in Table 1. The greatest reduction of nitrite under anaerobic conditions was observed with \textit{L. mesenteroides}, followed by \textit{L. plantarum} and \textit{L. viridescens}. Both \textit{L. brevis} and \textit{B. thermosphacta} showed nitrite depletion similar to that of the uninoculated broth. These losses were much higher at 15 \( \text{C} \) and in some instances were 10 times the rate observed at 5 \( \text{C} \). Reports by Fournaud et al. (3) on reduction of nitrite by \textit{Lactobacillus lactis} and other lactic acid bacteria showed this reduction to be pH- and temperature-dependent. The optimum temperature was found to be about 20 \( \text{C} \) at pH 6.5. They showed that bacteria such as \textit{Lactobacillus leichmannii} and \textit{Lactobacillus buchneri} were capable of reducing nitrite at a rapid rate to either nitrogen dioxide, nitrous oxide or nitrogen. At 30 \( \text{C} \), \textit{L. leichmannii} degraded 220 \( \mu \text{g} \) of nitrite/ml of broth in 15 h. Nitrite reduction also occurred only under anaerobic conditions, a result we observed during this study.

To confirm that such reduction could also occur in vacuum-packed bologna, various lactic acid bacteria were inoculated into sterile bologna slices. The results of those studies are shown in Fig. 1. The level of nitrite in the non-irradiated pack was much higher initially than the rest of the packs under study. Loss of nitrite occurred as a result of the gamma radiation and amounted to 30-50\% of the original levels.

Over the 4-week period at 5 \( \text{C} \), the non-irradiated pack showed nitrite losses of 27 \( \mu \text{g} \) of nitrite/g of bologna (89\%). If this is compared to the sterile packs (uninoculated), an estimate of losses through chemical and bacteriological activity can be obtained. Losses in

![Loss of nitrite in irradiated vacuum-packed bologna inoculated with spoilage lactic acid bacteria](http://meridian.allenpress.com/jfp/article-pdf/44/8/593/1654410/0362-028x-44_8_593.pdf)

**Figure 1.** Loss of nitrite in irradiated vacuum-packed bologna inoculated with spoilage lactic acid bacteria. ○ = Control bologna sample, unirradiated pack. \( \times \) = Control bologna sample, irradiated sterile pack or irradiated pack inoculated with \textit{B. thermosphacta} or \textit{L. brevis}. ● = Irradiated pack inoculated with \textit{L. plantarum} or \textit{L. viridescens}. □ = Irradiated pack inoculated with \textit{L. mesenteroides}.

**TABLE 1.** Depletion of sodium nitrite by specific lactic acid bacteria grown anaerobically in APT broth at 5 and 15 \( \text{C} \).

<table>
<thead>
<tr>
<th>Culture</th>
<th>Storage time (days)</th>
<th>Temperature (( \text{C} ))</th>
<th>Residual nitrite (( \mu \text{g} )/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Lactobacillus plantarum}</td>
<td>3</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>\textit{Leuconostoc mesenteroides}</td>
<td>3</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Lactobacillus viridescens}</td>
<td>3</td>
<td>5</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>\textit{Lactobacillus brevis}</td>
<td>3</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>\textit{Brochothrix thermosphacta}</td>
<td>3</td>
<td>5</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>97</td>
</tr>
<tr>
<td>Uninoculated APT broth</td>
<td>6</td>
<td>5</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>66</td>
</tr>
</tbody>
</table>

\( ^a \)Grown in APT broth containing 100 \( \mu \text{g} \) of sodium nitrite/ml of broth.
the sterile packs through non-biological means were about 60% of the original levels over the 4-week period. Nitrite losses from irradiated packs of bologna inoculated with *B. thermosphacta* and *L. brevis* were similar to the sterile packs (60%). Neither of these two organisms possessed a nitrite reductase system (unpublished results).

Those packs inoculated with *L. viridescens* and *L. plantarum* showed rapid depletion of nitrite in the first 2 weeks of storage and an overall loss of 75% of the original levels after 4 weeks.

*L. mesenteroides* was the most active species, reducing nitrite levels from 18 µg/g of bologna to 0 µg/g in 2 weeks. Changes in pH of the bologna during these studies showed the normal decline. The pH of the meat samples at the start of the experiment was 6.5-6.6 and after 4 weeks of storage, fell to 5.3-5.5 for all inoculated packs. These results indicate that the nitrite depletion was not totally dependent on pH changes but more on the bacterial species present. In other cured meat studies, pH has been shown to be directly linked to nitrite depletion rates during storage (12). All inoculated cultures of the lactic acid bacteria (including *B. thermosphacta*) in the irradiated bologna reached levels of $5 \times 10^6$ or higher during the 4-week storage period.

Thus this study confirms the suggestion by Fournaud et al. (2) that nitrite depletion rate may well depend on types and contamination levels of lactic acid bacteria in cured meats, and that such losses are significant.

The role of the lactic acid bacteria in the ultimate spoilage of bologna has been studied extensively (7,8,10). The types of lactic acid bacteria associated with such spoilage have also been documented (7,11). It should be noted that lactic acid bacteria capable of reducing nitrite tend to dominate in the final gram-positive spoilage flora of vacuum-packed bologna. Earlier studies with vacuum-packed bologna showed that *L. viridescens, L. plantarum* and *L. mesenteroides* made up about 65-75% of the lactic population during the second, third, and fourth week of storage at 5°C (1). *L. mesenteroides*, the most active nitrite-reducing species, made up about 35% of the lactic flora throughout the 4-week period.

It is difficult to assess at this time whether or not nitrite plays an active or passive role in the selection of a specific lactic acid bacteria population during spoilage of bologna. The growth of such organisms as *L. plantarum* is stimulated in the presence of 50 µg/ml sodium nitrite at 5°C under anaerobic conditions (1). Studies by Wood et al. (7,3) with vacuum-packed bacon suggest that certain lactic acid bacteria populations are limited by low levels of nitrite.

ACKNOWLEDGMENTS

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REFERENCES


