Use of Antioxidant-Coated Salts as N-Nitrosamine Inhibitors in Dry- and Brine-Cured Bacon¹


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ABSTRACT

Several experiments were completed to further evaluate use of α-tocopherol-coated salts as inhibitors of N-nitrosamine formation in fried bacon. Studies with dry-cured bacon prepared with various levels of α-tocopherol indicated that the chemical did not contribute to formation of N-nitrosodimethylamine (NDMA). N-Nitrosopyrrolidine levels for the α-tocopherol-treated bacon samples were generally below 5 µg/kg, which represents an average reduction of approximately 70%. Experiments were also done to evaluate the role of lecithin as a possible precursor of NDMA in brine-cured bacon. At concentrations used to disperse α-tocopherol in the curing brine, lecithin did not contribute to NDMA formation in bacon prepared with α-tocopherol-coated salts.

N-Nitrosamine formation in fried bacon can be effectively reduced by the inclusion of α-tocopherol as a curing adjunct. Fiddler et al. (3) demonstrated that this compound, when used at concentrations ranging from 250 to 500 mg/kg and when dispersed with polysorbate emulsifiers in the curing brine to obtain adequate distribution in cured pork bellies, significantly reduced N-nitrosamine formation in bacon. Similarly, Gray et al. (7) showed that α-tocopherol, when coated on the surface of salt in combination with lecithin, was an effective inhibitor of N-nitrosopyrrolidine (NPYR) formation in brine-cured bacon. Recently, d-α and dl-α-tocopherols have been approved for use as inhibitors of N-nitrosamine formation in pumped (brine-cured) bacon (2).

However, some questions remain regarding the efficacy of α-tocopherol-coated salts as curing adjuncts. Reddy et al. (11) reported that dl-α-tocopherol increased formation of N-nitrosodimethylamine (NDMA) in dry-cured bacon, even though NPYR concentrations were greatly reduced. It was suggested that further studies be conducted to delineate reasons for differences between brine-cured and dry-cured bacon with regard to NDMA formation (2). There has also been some discussion of the possible involvement of lecithin in N-nitrosamine formation in bacon (14). Lecithin is included as part of the coated salt system to increase dispersibility of α-tocopherol in the brine. Model system studies revealed that NDMA could be formed from lecithin when heated in the presence of nitrite (6,10). Therefore, further studies are necessary to establish if N-nitrosamine formation in brine-cured bacon is enhanced by use of lecithin in coated-salt.

Objectives of this study were: (a) to investigate the apparent increase in NDMA concentrations in dry-cured bacon prepared with various levels of α-tocopherol, and (b) to determine the possible contribution of lecithin to N-nitrosamine formation in brine-cured bacon when used as an integral part of coated salt.

MATERIALS AND METHODS

Effect of α-tocopherol levels on inhibition of N-nitrosamine formation in dry-cured bacon

Dry-cured bacon was manufactured using the procedure described by Reddy et al. (11). Pork bellies (4-5.5 kg) were obtained from a local supplier within 24 h of slaughter and divided into four groups, each group consisting of five randomly selected bellies. All bellies were rubbed with dry curing mixtures to obtain target levels of 2.5% salt, 0.83% sugar, 120 mg of sodium nitrite/kg and 550 mg of sodium ascorbate/kg in the finished bacon. α-Tocopherol-coated salts were used to give ingoing α-tocopherol concentrations of 250, 500 and 750 mg/kg in three groups of cured bellies. The fourth group contained no α-tocopherol and served as control bacon samples. Rubbed bellies were held in a curing room at 2°C for 10 d, smoked, tempered, sliced and packaged as reported previously (11). Packaged bacon was held at 2°C for 7 d before analyzing for residual nitrite (1) and α-tocopherol (16). At the same time, portions of the bacon samples were fried and analyzed for N-nitrosamine formation using the mineral oil distillation procedure described earlier by Reddy et al. (11). Percent recoveries of the N-nitrosamines from the fried bacon samples were determined by spiking the distillation flask containing 25 g of fried pork bellies with known amounts of NPYR and NDMA. Average values for recovery from spiked samples were 80±5% and 88±8% for NPYR and NDMA, respectively.

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A second experiment was done to evaluate the effect of storage at 2°C on the degree of inhibition of N-nitrosamine formation in dry-cured bacon by α-tocopherol-coated salts. Ten fresh pork bellies (i.e., less than 48 h after slaughter) were randomly allotted into two groups and processed into dry-cured bacon as described previously. One group of bellies had an ingoing α-tocopherol concentration of 500 mg/kg, while the other group served as the control (no α-tocopherol). The five bellies per treatment were sliced and packaged as described by Robach et al. (12). After each belly was sliced, it was packaged in 20 packages with the first slice going into the first package, the second slice into the second package and so on, until the belly was completely packaged. Thus, each of the 20 packages could be considered representative of an entire belly. Two packages were randomly selected from each belly immediately after packaging and the bacon was fried and analyzed for NPYR and NDMA. These analyses were repeated after 1, 2, and 3 weeks of storage of the packaged samples. The N-nitrosamine data were analyzed as an incomplete block design with repeat measurement (4), with two treatments (five bellies per treatment) being analyzed over four time periods.

Effect of lecithin on N-nitrosamine formation in brine-cured bacon

Brine-cured bacon was prepared using the procedure described by Gray et al. (7). Ten fresh pork bellies were obtained from a local supplier and randomly divided into two groups. One group (control) was processed into bacon by pumping the bellies to 110% of their green weight with a brine containing 20% salt, 5% sucrose, 3.5% sodium tripolyphosphate, 1200 mg of sodium nitrite/kg and 5500 mg of sodium ascorbate/kg. The second group was processed with a brine containing salt which was coated with lecithin (1% w/w). The target lecithin concentration in the finished bacon was 1000 mg/kg. Concentrations of other curing adjuncts were similar to those used for control bacon samples. After pumping and a 2-d equilibration period at 2°C, the bellies were smoked-cooked, chilled, sliced and packaged. After 1 week at 2°C, bacon samples were fried and analyzed for NPYR and NDMA.

RESULTS AND DISCUSSION

Inhibition of N-nitrosamine formation in dry-cured bacon

Although Reddy et al. (11) demonstrated NPYR inhibition in dry-cured bacon with α-tocopherol-coated salts, they also reported an apparent increase in NDMA concentrations with increasing ingoing concentrations of α-tocopherol. It was suggested that further studies be carried out to determine whether these increases were artifactual (2). Results of such studies are reported in Table 1. NPYR concentrations in the range of 3.2 to 18.3 μg/kg (average 11.4 μg/kg) were obtained for control samples (no α-tocopherol). These values are much lower than those reported previously by Reddy et al. (11), but are consistent with values cited in a recent USDA report on a survey of dry-cured bacon (17). α-Tocopherol, when added at three concentrations to the pork bellies, significantly (p<0.05) reduced NPYR formation (Table 1). The amounts of inhibition were not as great as previously reported (11), but were consistent with results obtained by Fiddler et al. (3) and Skrypec et al. (13) for brine-cured bacon. The lower levels of inhibition can be explained, in part, by decreased concentrations of NPYR present in the fried control bacon samples (average 11.4 μg/kg) as compared to the average figure of 42 μg/kg reported by Reddy et al. (11).

Results of the NDMA analyses also indicated that α-tocopherol at all three concentrations generally appeared to reduce NDMA concentrations in dry-cured bacon. Although the data, when subjected to one-way analysis of variance (4), showed only statistically significant differences (p<0.05) between the control and the 500 mg of α-tocopherol/kg treatment, the overall results are in direct contrast to those of Reddy et al. (11), who reported much higher levels of NDMA in α-tocopherol-treated bacon relative to control samples. Reasons for the discrepancy in these results are not known, although concentrations of NDMA reported for dry-cured bacon in the present study are much more consistent with those cited in a recent USDA survey of dry-cured bacon (17).

To confirm the inhibitory effect of α-tocopherol on NDMA formation in dry-cured bacon, or the lack of a catalytic effect as reported by Reddy et al. (11), an additional study was done in which dry-cured bacon was prepared with an ingoing α-tocopherol level of 500 mg/kg, and analyzed initially, and after 1, 2, and 3 weeks of storage. Statistical treatment of the N-nitrosamine data

<table>
<thead>
<tr>
<th>Sample treatment</th>
<th>NPYR (μg/kg)</th>
<th>Inhibition (%)</th>
<th>NDMA (μg/kg)</th>
<th>Inhibition (%)</th>
<th>Residual nitrite (mg/kg)</th>
<th>α-Tocopherol (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.4±6.3x</td>
<td>---</td>
<td>1.3±0.7x</td>
<td>---</td>
<td>55</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>(3.2-18.3)</td>
<td></td>
<td>(0.7-2.5)</td>
<td></td>
<td>(48-68)</td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>4.0±1.4x</td>
<td>65</td>
<td>0.6±0.15x</td>
<td>50</td>
<td>38</td>
<td>151</td>
</tr>
<tr>
<td>(250 mg/kg)</td>
<td>(2.3-5.3)</td>
<td></td>
<td>(0.4-0.8)</td>
<td></td>
<td>(37-40)</td>
<td>(127-183)</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>2.6±0.7x</td>
<td>77</td>
<td>0.3±0.3x</td>
<td>77</td>
<td>35</td>
<td>390</td>
</tr>
<tr>
<td>(500 mg/kg)</td>
<td>(1.8-3.6)</td>
<td></td>
<td>(0.1-0.7)</td>
<td></td>
<td>(29-44)</td>
<td>(362-410)</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>3.7±1.7x</td>
<td>67</td>
<td>0.9±0.4x</td>
<td>28</td>
<td>29</td>
<td>580</td>
</tr>
<tr>
<td>(750 mg/kg)</td>
<td>(2.7-6.5)</td>
<td></td>
<td>(0.4-1.4)</td>
<td></td>
<td>(25-31)</td>
<td>(490-672)</td>
</tr>
</tbody>
</table>

aValues in parentheses represent the range of N-nitrosamine concentrations in 5 bellies per treatment. Bacon samples were fried 7 d after packaging and analyzed in duplicate and values averaged.

bNitrite and α-tocopherol analyses were done 7 d after packaging.

Means with different superscript letters within the same column are significant (p<0.05). Hartley’s F max test (8) indicated heterogeneous variance for both NDMA and NPYR. Log transformation provided homogeneous variance.
showed that treatment-period interaction was not significant (p<0.01), indicating α-tocopherol inhibition was similar over all time periods (Table 2). NPYR levels were significantly (p<0.001) lower for α-tocopherol-treated samples in comparison to control samples, while NDMA concentrations in control and α-tocopherol-treated bacon could not be statistically differentiated (p<0.05). These results support findings of the first experiment, and again the N-nitrosamine concentrations are consistent with those cited in the USDA survey of dry-cured bacon (7).

Results of these studies with dry-cured bacon support use of α-tocopherol-coated salts as a means of reducing N-nitrosamine formation in fried bacon. This would be beneficial to some processors as the recent USDA study (17) indicated that 15.6% of 135 dry-cured bacon samples analyzed by the mineral oil distillation procedure had NPYR levels of 17 µg/kg or higher. The present study also refutes the results of Reddy et al. (11) which implicated α-tocopherol as a catalyst of NDMA formation in dry-cured bacon.

Role of lecithin in N-nitrosamine formation in brine-cured bacon

Lecithin is an essential component of the α-tocopherol-coated system in that it assists in dispersion of α-tocopherol in the curing brine. However, some concern has been expressed about use of lecithin as an emulsifier in curing brines because it has been implicated in NPYR formation (14) and as a possible precursor of NDMA in bacon (6,10). Thus a study was done to further clarify the precursor or catalytic role of lecithin in bacon.

Lecithin (1000 mg/kg ingoing) was introduced into pork bellies using lecithin-coated salts dispersed in the curing brine. Analysis of the fried bacon samples for volatile N-nitrosamines revealed NDMA concentrations (average 2.3 µg/kg) in the five lecithin-treated samples which were not significantly different (p<0.05) from those in the five control bacon samples (average 2.4 µg/kg). Similarly, NPYR concentrations in the control (average 11.3 µg/kg) and lecithin-treated samples (average 13.1 µg/kg) were not significantly different (p<0.05) and were comparable to the levels reported in other studies (15).

These results indicated that lecithin is not a major contributor to NDMA formation in bacon. Kuchmak and Dugan (9) reported a lecithin content of approximately 0.34 g/100 g of fresh, pork belly. Processing of bacon using α-tocopherol-coated salts with lecithin as a dispersing agent would contribute only an additional 0.1 g of lecithin per 100 g of bacon. Thus it is very unlikely that lecithin used in this manner would contribute to NDMA formation. Moreover, Spinelli-Gugger et al. (15) observed that the major precursors of NDMA in bacon were not extractable with chloroform and were thus water-soluble components. This observation would preclude lecithin as a precursor of NDMA, although free choline can exist naturally in biological systems (6).

REFERENCES


TABLE 2. Effect of storage at 4°C on inhibition of N-nitrosamine formation in dry-cured bacon processed with α-tocopherol (500 mg/kg).

<table>
<thead>
<tr>
<th>Time of analyses (weeks)</th>
<th>Control</th>
<th>A-Tocopherol</th>
<th>% Inhibition</th>
<th>Control</th>
<th>A-Tocopherol</th>
<th>% Inhibition</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>16.0</td>
<td>9.9</td>
<td>38</td>
<td>2.1</td>
<td>2.3</td>
<td>---</td>
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<tr>
<td></td>
<td>(12.8-21.8)</td>
<td>(7.2-11.4)</td>
<td></td>
<td>(1.5-2.9)</td>
<td>(1.7-3.3)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17.4</td>
<td>7.8</td>
<td>55</td>
<td>4.1</td>
<td>3.1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>(12.3-24.6)</td>
<td>(5.7-14.0)</td>
<td></td>
<td>(3.4-5.2)</td>
<td>(2.1-4.6)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12.9</td>
<td>5.5</td>
<td>57</td>
<td>3.6</td>
<td>2.9</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>(9.6-14.7)</td>
<td>(4.7-6.4)</td>
<td></td>
<td>(2.6-4.8)</td>
<td>(2.2-3.5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11.4</td>
<td>4.1</td>
<td>65</td>
<td>2.2</td>
<td>2.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(8.4-13.9)</td>
<td>(2.9-6.1)</td>
<td></td>
<td>(1.0-2.9)</td>
<td>(1.5-2.3)</td>
<td></td>
</tr>
<tr>
<td>Treatment means</td>
<td>14.45 ±4.07</td>
<td>6.83 ±2.96</td>
<td>2.96 ±1.11</td>
<td>2.58 ±0.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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