Use of Antimycotic Agents to Control *Listeria monocytogenes* in RTE Meats

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MIRC
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Common Listeria Interventions

- **Ingredient based interventions**
  - Examples (lactate/diacetate, bacteriocins, plant extracts)
  - Application methods (formula, spray, dip, applied to casings/packaging, added to packaged product)

- **Process based interventions**
  - Examples (post package pasteurization, high pressure processing)

- **Other**
  - Example (irradiation)

- **Combinations**
  - (takes advantage of hurdle technology)
Some criteria for selecting an intervention system

- Review of current processing system
- Review of current product and new product possibilities
- Efficacy of proposed intervention(s)
- Impact of intervention(s) on product quality attributes
- Other manufacturer specific criteria
Antimycotics (organic acid salts) as Interventions in RTE meats

American Meat Institute Foundation funded research at the University of Wisconsin demonstrated that benzoate, propionate and sorbate could inhibit Lm growth in RTE meat products


Influence of Antimycotics in Ham (Glass and Claus, 2006)

Figure 1. Changes in populations of *L. monocytogenes* on cured ham prepared with various levels of sodium benzoate, sodium propionate, or potassium sorbate, and stored at 4°C for 12 weeks (averages for duplicate trials, standard deviations not shown).
AMI Bologna & Turkey Data

Bologna produced with 156 ppm sodium nitrite
Turkey contained no added nitrite

Days at 4C

Log cfu/package
**Antimycotics**

Antimycotic agents have been used in various food products for a long time and are considered GRAS by FDA.

Under USDA regulations, potassium sorbate is approved for use as a mold inhibitor for dry sausages (dipping in 10% solution) and in margarine (0.1% singly or 0.2% in combination).

Benzoic acid approved to protect flavor in margarine and oleomargarine.

Sodium propionate is approved for use as a mold inhibitor in pizza crust.

\[ C_{16}H_{17}KNa_2O_6 \]
\[ \text{Mol. Wt.: 390.38} \]
\[ C, 49.23; H, 4.39; K, 10.02; Na, 11.78; O, 24.59 \]

\[ C_7H_5NaO_2 \]
\[ \text{Mol. Wt.: 144.1} \]
\[ C, 58.34; H, 3.50; Na, 15.95; O, 22.21 \]

\[ C_3H_5NaO_2 \]
\[ \text{Mol. Wt.: 96.1} \]
\[ C, 37.51; H, 5.25; Na, 23.93; O, 33.31 \]
Effectiveness of Combinations of Antimycotics

A mixture design of combinations of benzoate, sorbate, and propionate in ham

Estimated Response Surface

Comments:
• Benzoate was more effective than the others
• Benzoate was more effective even in combinations totaling 0.1%
Modeling overview

Preparation
- Identify experimental factors
  - Set factors and ranges
  - Make test products

Execution
- Inoculation studies
  - Gather data

Modeling & validation
- Analyze data and build model
  - Validate model by comparing with independent samples
Experimental Design

Central composite design for four continuous variables:
- Center point replicated 6 times resulting in 30 treatments
- Products were made, inoculated, stored at 4°C, and assessed every 2 weeks for Lm count.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Low</th>
<th>Center</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl, %</td>
<td>0.2</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>45</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>Na diacetate, %</td>
<td>0</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>Na Benzoate, %</td>
<td>0</td>
<td>0.08</td>
<td>0.165</td>
</tr>
</tbody>
</table>


Modeling approach

• **Boundary model:**
  1. Define growth threshold as sustained 1 log increase and measure time-to-growth (TTG).
  2. Develop generalized regression model for time-to-growth as a function of predictor variables *(Minitab, Regression With Life Data).*
  3. Predict time before growth occurs.
Establish time to growth

Inspect all growth data

- Growth (sustained 1 log_{10} increase): y/n?
  - Evaluated by 5 observers for each treatment
  - If yes, TTG? If no, censored @ 18 weeks.

- Calculate mean for each treatment

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**Graph:**
- X-axis: Weeks
- Y-axis: Log_{10} cfu/g
- Data points indicating growth over time, with a notable increase after 8 weeks.
Model building

- Life regression analysis in Minitab (Weibull hazard distribution model):
  1. **Fit** a full model with all main factors (4), two-way interactions (6) and quadratic terms (4) and 3 way interactions (4).
  2. **Remove** the least significant term
  3. **Fit** the remaining reduced model.
  4. **Repeat** 2-3 until all remaining terms are significant.
  5. Final model has main factors, 6 two way interactions, 4 three way interactions.
Regression coefficients for Weibull distribution

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>Standard error</th>
<th>Z</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.91691</td>
<td>0.01835</td>
<td>158.94</td>
<td>0.000</td>
</tr>
<tr>
<td>Salt</td>
<td>0.56124</td>
<td>0.01498</td>
<td>37.46</td>
<td>0.000</td>
</tr>
<tr>
<td>Benzoate</td>
<td>1.00151</td>
<td>0.01409</td>
<td>71.10</td>
<td>0.000</td>
</tr>
<tr>
<td>Diacetate</td>
<td>0.54083</td>
<td>0.04198</td>
<td>36.10</td>
<td>0.000</td>
</tr>
<tr>
<td>Moisture</td>
<td>-0.002001</td>
<td>0.01393</td>
<td>-0.14</td>
<td>0.886</td>
</tr>
<tr>
<td>Salt x Benzoate</td>
<td>0.166310</td>
<td>0.01979</td>
<td>8.41</td>
<td>0.000</td>
</tr>
<tr>
<td>Salt x Diacetate</td>
<td>0.31550</td>
<td>0.02864</td>
<td>11.02</td>
<td>0.000</td>
</tr>
<tr>
<td>Salt x Moisture</td>
<td>-0.101357</td>
<td>0.02863</td>
<td>-3.54</td>
<td>0.000</td>
</tr>
<tr>
<td>Benzoate x Diacetate</td>
<td>-0.071808</td>
<td>0.01979</td>
<td>-3.63</td>
<td>0.000</td>
</tr>
<tr>
<td>Benzoate x Moisture</td>
<td>0.356149</td>
<td>0.02186</td>
<td>16.29</td>
<td>0.000</td>
</tr>
<tr>
<td>Diacetate x Moisture</td>
<td>-0.265609</td>
<td>0.02863</td>
<td>-9.28</td>
<td>0.000</td>
</tr>
<tr>
<td>Salt x Benzoate x Diacetate</td>
<td>-0.080852</td>
<td>0.03147</td>
<td>-2.37</td>
<td>0.018</td>
</tr>
<tr>
<td>Salt x Benzoate x Moisture</td>
<td>0.221129</td>
<td>0.02829</td>
<td>7.82</td>
<td>0.000</td>
</tr>
<tr>
<td>Salt x Diacetate x Moisture</td>
<td>-0.373129</td>
<td>0.01808</td>
<td>-20.64</td>
<td>0.000</td>
</tr>
<tr>
<td>Benzoate x Diacetate x Moisture</td>
<td>0.126852</td>
<td>0.02829</td>
<td>4.48</td>
<td>0.000</td>
</tr>
<tr>
<td>Shape</td>
<td>43.7990</td>
<td>12.2618</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Log-likelihood = -2.162

1 Variable analyzed using the coded value (-1, 0, 1).
2 Variables with $P$ values less than 0.05 were included in the model except for moisture. Moisture was included because all the interactions with moisture were significant.
Final regression model

\[ TTG = e^{(2.91691 + (0.56124(S)) + (1.00151(B)) + (0.54083(D)) - (0.002(M)) + (0.16631(SxB)) + (0.3155(SxD)) - (0.101357(SxM)) - (0.071808(BxD)) + (0.356149(BxM)) - (0.265609(DxM)) - (0.080852(SxBxD)) + (0.221129(SxBxM)) - (0.373129(SxDxM)) + (0.126852(BxDxM))} \]

Where \( TTG \) = Time to one log growth (weeks), \( S \) = the coded value for salt, \( B \) = the coded value for benzoate, \( D \) = the coded value for diacetate, and \( M \) = the coded value for product moisture.
Contour Plot of TTG by Benzoate and Moisture
(at 2% Salt, 0.1% Diacetate)

Salt content set at 2% and Diacetate content set at 0.1%
Contour Plot of TTG by Benzoate and Moisture
(at 2% Salt, 0.1% Diacetate)

Salt content set at 2% and Diacetate content set at 0.1%

Area of predicted growth
Area of no predicted growth
Contour plot of TTG by Moisture and Diacetate
(Benzoate at 0.1% and salt at 2.0%)

Area of predicted growth

Area of no predicted growth

Benzoate locked at 0.1%; Salt locked at 2%
Model performance: summary

Model is internally consistent and predicts well for the data used to create it.

Observed vs. Predicted Time to Growth using Four Factor RSM Model

- **Fail hazardous zone** - Shorter TTG than predicted
- **Fail safe zone** - Longer TTG than predicted
- **Censored zone** - No growth observed

RSM data

Predicted weeks to 1 log growth

Observed weeks to 1 log growth

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Model validation samples

Observed vs. Predicted Time to Growth using Four Factor RSM Model

Observed vs. predicted time-to-growth (TTG) of *Listeria monocytogenes* in inoculated model products stored at 4°C in low moisture products (i.e., those with less than 60% moisture – beef wieners (□), wieners (○), beef bologna (◇), and bologna (▲))
Summary of RSM modeling

- All four factors are important to Lm growth inhibition.
- Moisture effect is due to a complex interaction with the other ingredients.
- Combinations of 0.1% sodium benzoate and 0.1-0.15% sodium diacetate can prevent Lm growth in hot dogs and bologna.
- Results in regular bologna did not conform to the model: This likely due to the increased pH in the product due to its high content of mechanically separated chicken.
- Higher moisture RTE cured meats such as ham will require higher diacetate concentrations and may require additional hurdles.
### TTG in turkey and ham with additional hurdles

<table>
<thead>
<tr>
<th>Model product&lt;sup&gt;1,2&lt;/sup&gt;</th>
<th>Salt (%)</th>
<th>Benzoate (%)</th>
<th>Diacetate (%)</th>
<th>Moisture (%)</th>
<th>Predicted TTG&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Observed TTG&lt;sup&gt;4,5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured turkey – 0 diacetate</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>75.5</td>
<td>0.8</td>
<td>2</td>
</tr>
<tr>
<td>Cured turkey – 0 propionate</td>
<td>1.52</td>
<td>0.1</td>
<td>0.1</td>
<td>76.7</td>
<td>6.4</td>
<td>7</td>
</tr>
<tr>
<td>Cured turkey -- 0.2 propionate, n=2</td>
<td>1.08</td>
<td>0.1</td>
<td>0.08</td>
<td>76.55</td>
<td>5.4</td>
<td>8.0</td>
</tr>
<tr>
<td>Cured turkey -- 0.2 propionate, n=2</td>
<td>1.06</td>
<td>0.1</td>
<td>0.18</td>
<td>76.60</td>
<td>8.0</td>
<td>13.3</td>
</tr>
<tr>
<td>Cured turkey -- 0.1 propionate, n=2</td>
<td>1.06</td>
<td>0.1</td>
<td>0.12</td>
<td>76.35</td>
<td>6.2</td>
<td>7.1</td>
</tr>
<tr>
<td>Cured turkey -- 0.1 propionate, n=2</td>
<td>1.07</td>
<td>0.1</td>
<td>0.17</td>
<td>76.70</td>
<td>7.8</td>
<td>11.2</td>
</tr>
<tr>
<td>Cured turkey -- 0.2 propionate, Lem-O-Fos 111®, n=2</td>
<td>1.05</td>
<td>0.1</td>
<td>0.17</td>
<td>76.65</td>
<td>7.8</td>
<td>18.0</td>
</tr>
<tr>
<td>Ham -- 0.1 propionate, n=2</td>
<td>1.71</td>
<td>0.1</td>
<td>0.14</td>
<td>76.55</td>
<td>7.5</td>
<td>11.3</td>
</tr>
<tr>
<td>Ham -- 0.2 propionate, n=2</td>
<td>1.82</td>
<td>0.1</td>
<td>0.15</td>
<td>75.85</td>
<td>8.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Ham -- 0.2 propionate + Lem-O-Fos 111®, n=2</td>
<td>1.85</td>
<td>0.1</td>
<td>0.15</td>
<td>75.90</td>
<td>8.6</td>
<td>16.8</td>
</tr>
</tbody>
</table>

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<sup>1</sup> Unless otherwise stated, samples contain no lactate.

<sup>2</sup> Sodium propionate and Lem-O-Fos 111® added as additional agents to reduce Lm growth potential.

<sup>3</sup> Predicted values do not include factors of propionate and Lem-O-fos 111®.

<sup>4</sup> TTG refers to time-to-growth in weeks.

<sup>5</sup> Maximum observed TTG is 18 weeks.
Current Legal Status

- FDA – both benzoate and propionate are GRAS.
  - Benzoate common usage is 0.1%
  - Propionate has no upper limit (good manufacturing practice)

- USDA
  - Current usage limited to surface treatment of cased sausage as a mold inhibitor.
  - The Kraft petition requests the use of benzoate up to 0.1% and propionate up to 0.2% in cured RTE meat products.
  - The petition must go through the comment/rule making process.
Thank You!
Citations on prior USDA approval for use of Benzoate in Meats

1914 USDA Bureau of Animal Industry
Order 211

Regulation 17, Section 9

Paragraph 6. Any meat or product containing any benzoate of soda shall be plainly labeled so as to show the presence and the percentage amount of such benzoate of soda.

Paragraph 10. When permitted coloring matter is used in the preparation of any meat or product the words “artificially colored” shall be set forth upon the label in a prominent manner.

Section 10. Paragraph 7. When the weight of any meat or product, prepared at an official establishment, or imported, on or after September 3, 1914, is less than the weight required by the regulations, the surplus shall be plainly marked upon the outside of the package in terms of weight, measure, or numerical count.

Section 11. Paragraph 1. No marks of Federal inspection which have been previously used shall be again used for the identification of any meat or product except as provided in paragraph 2 of this section.

Paragraph 2. All steaks, roasts, lards, and other similar products, or previously used packages, shall be removed or obliterated before such containers are used for any meat or product unless such steaks, roasts, lards, or other similar products, or previously used packages, shall be removed or obliterated before such containers are used for any meat or product except as provided in paragraph 2 of this section.

Section 12. Paragraph 1. All labeling of meat and products required to be imported by bureau employees shall be in compliance with these regulations.

Paragraph 2. No person shall apply or sell or cause to be applied or sold, any label to any article prepared or received in an official establishment or to any container thereof except in compliance with these regulations.

Regulation 18, Section 6

Paragraph 4. Sausage shall not contain cereal in excess of 2 per cent.

Paragraph 5. Sausage shall contain no coloring matter, except for the purpose of indicating grinding, chopping, cooking, or seasoning, in which case the added water or ice shall not exceed 5 per cent, except that sausages of the class which are smoked or cooked, such as Frankfurter style, Vienna style, and Bologna style, may contain added water.