Control of Mites and Insects in Pet Food Packages Using Controlled Atmospheres

Bhadriraju Subramanyam (Subi), PhD
Professor
Department of Grain Science and Industry
Kansas State University
Manhattan, KS 66506, USA
Controlled Atmospheres for Mite and Insect Control

- Controlled or modified atmospheres
  - Use of the inert gases, N₂ and CO₂
  - Reduce atmospheric O₂ from 20.9 to ≤1%
  - Increase CO₂ from 0.03 to >40%

- Advantages
  - Increases shelf life of perishables/dry durables
  - Pesticide-residue free
  - Kills insects and mites and suppresses progeny
  - Replacing traditional IPM approaches

Controlled Atmosphere Research Projects

Project I
- Control of mites in pet food packages
- Package susceptibility to mite invasion

Project II
- Control of insects in low protein, grain-based pet product
- Suitability of product for insect reproduction
- Effect of various controlled atmosphere treatments on insects
- Package susceptibility to insect invasion and/or penetration
Project 1: Controlled Atmospheres and Mites

• Mites cultured from infested samples originating in Thailand

• Identified as *Suidasia medanensis* (=*pontifica*) Oudemans (Acari: Suidasiidae)*

• Reported from pet food at supermarkets in Recife, Brazil (de Sousa et al. 2005)

• Reported from grain stores in Greece (Palyvos et al. 2008)

• Found in house dust (Fernández-Caldas et al. 1993)

*Thanks to Nickolas E. Palyvos, Agricultural University of Athens, Greece, for identification of the species*
Scanning Electron Micrographs of *Suidasia medanensis*
Biology of *Suidasia medanensis*
(Mercado et al. 2002)

- 26°C and 86% RH (on tetramin fish food + yeast)
- Egg-to-adult development: 11-13 days
- Reproductive period: 10 – 36 days
- Eggs/female: 62 – 177
- Adult longevity: 15 – 75 days
Laboratory Rearing

• Reared on pet food (26-28% moisture) in 0.45-L glass jars at 25°C and 70% RH, in a small growth chamber

• Pet food has 26-28% moisture

• Mixed ages of mites were used in experiments
Trial I: Effectiveness of Three O₂ Levels on Mite Mortality

- 10 ml vials (4.8 cm long & 1.7 cm diam) with 0.19 ± 0.03 g (n = 4) of diet or 0.45 g of bleached flour
- Each vial had either 20 S. medanensis or 10 T. castaneum adults
- 0.5, 1.0, and 2.0% O₂, remainder N₂ in cylinders from Praxair company
- Pouches (45 g) were filled within 3-4 seconds
- Untreated pouches served as the control treatment (~20% O₂)
- Survival of mites and insects checked in the laboratory 3, 6, and 9 d after purging pouches
Mites and Insects for Exposure

- Pet food vials with 20 *S. medanensis*
- Flour with 10 adults of *T. castaneum*
Cylinders and Certified Gases

- Gas cylinders with certified gases (0.5, 1.0, and 2.0% of O₂, remainder N₂) were purchased from Praxair company.
Purging Packages with Controlled Atmospheres

- A foot pedal initiates gas flow
- 3-4 seconds per pouch
- Pouch is then heat-sealed
Heat-Sealing of Pouches
## Expected and Observed O₂ Levels in Pouches

<table>
<thead>
<tr>
<th>Expected O₂ (%)</th>
<th>Observed O₂ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.58 ± 0.01</td>
</tr>
<tr>
<td>1.0</td>
<td>1.03 ± 0.01</td>
</tr>
<tr>
<td>2.0</td>
<td>1.98 ± 0.00</td>
</tr>
</tbody>
</table>

\( n = 4 \) replications for observed values

Control packages had an O₂ level of 19.95 ± 0.04 \((n = 3)\)
### Survival of Mites and Insects in Control and Treated Pouches

<table>
<thead>
<tr>
<th>Day</th>
<th>Species</th>
<th>Untrt. (Control)</th>
<th>0.5% O₂</th>
<th>1.0% O₂</th>
<th>2.0% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td><em>T. castaneum</em></td>
<td>10.0±0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0±1.0</td>
</tr>
<tr>
<td></td>
<td><em>S. medanensis</em></td>
<td>5.3±0.9</td>
<td>0.8±0.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td><em>T. castaneum</em></td>
<td>10.0±0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td><em>S. medanensis</em></td>
<td>3.5±1.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td><em>T. castaneum</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td><em>S. medanensis</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Independent samples were examined over time. Each mean is based on *n* = 4 replications.
Tests with High Mite Density
25°C and 24% RH

Purged packages
## Trial II. Survival of High Density of Mites at Three $O_2$ Levels

<table>
<thead>
<tr>
<th>Day</th>
<th>Untrt (Control)</th>
<th>0.5% $O_2$</th>
<th>1.0% $O_2$</th>
<th>2.0% $O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>356.0±132.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>172.0±3.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>130.0±15.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>160.0±22.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Independent samples were examined over time
Each mean is based on $n = 3$ replications
**Trial IIIa. Use of O₂ Scavengers Inside Pouches**

**Oxygen levels inside pouches with ascorbic acid**

<table>
<thead>
<tr>
<th>Day</th>
<th>1 g Ascorbic acid</th>
<th>5 g Ascorbic acid</th>
<th>10 g Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.23 ± 0.19</td>
<td>1.26 ± 0.03</td>
<td>1.47 ± 0.14</td>
</tr>
<tr>
<td>13</td>
<td>0.61 ± 0.03</td>
<td>0.66 ± 0.11</td>
<td>0.66 ± 0.04</td>
</tr>
</tbody>
</table>

**Two-way ANOVA:**
- No significant differences among treatments ($F=0.94; \text{df}=2, 6; \text{P}=0.441$)
- Significant differences between days ($F=57.87; \text{df}=1, 6; \text{P}=0.0003$)
- Interaction of treatment x days ($F=0.60; \text{df} = 2, 6; \text{P}=0.5807$)

Ascorbic acid was placed in vials of 4.9 cm long and 2.6 cm diam (24 ml volume)
Trial IIIb. Commercial Iron Powder Sachets

- Bulk (5 kg) dog bone pet product intended for Japan
- 3 commercial sachets per 5 kg
- O₂ levels over time

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>O₂ level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar. 26, 08</td>
<td>0</td>
<td>20.00</td>
</tr>
<tr>
<td>Mar. 27, 08</td>
<td>1</td>
<td>0.19</td>
</tr>
<tr>
<td>Mar. 28, 08</td>
<td>2</td>
<td>0.32</td>
</tr>
<tr>
<td>Mar. 31, 08</td>
<td>5</td>
<td>0.10</td>
</tr>
</tbody>
</table>

n = 1 replication

Mitsubishi Chemical Company
Incorporating Scavengers Into Packaging Structure

Ciba Multisorb Technologies
Trial IV. Susceptibility of Pouches to Mite Infestation

- Pouches were purged with 1% O$_2$ level
- Control pouches consisted of unpurged pouches
- Each pouch was placed in a Rubbermaid™ cereal container ($n=5$ per treatment)
- Into each container, mite diet containing $767.3 \pm 201.3$ mites ($n=4$) were released
- Pet product inside pouches was examined under a stereo-microscope for infestation after 16 d at 25°C and 24% RH.
- A similar procedure was used with individually wrapped dog bone product from the same company, and these packages were not treated with controlled atmospheres
  - Packages were placed individually in 0.95-L glass jars with lids
  - There were 20 packages in total
Package Susceptibility Tests

Mites failed to invade packages
Project II. Controlled Atmospheres for Control of Insects in Low Protein Grain-Based Pet Product

• Not a pet food

• The grain-based pet product is biodegradable

• Sold in packages

• Purged with inert gases prior to shipping

• Infestation complaints led to a research project to examine efficacy of 6 controlled atmosphere treatment combinations

• Common pest species reported: *Tribolium castaneum* and *T. confusum*
## Progeny Production of *Tribolium* species on the Grain-Based Pet Product

<table>
<thead>
<tr>
<th>Species</th>
<th>Diet</th>
<th>No. F₁ adults at 28 days</th>
<th>No. F₁ adults at 56 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. confusum</em></td>
<td>Lab diet</td>
<td>352.3</td>
<td>1374.3</td>
</tr>
<tr>
<td></td>
<td>Pet product</td>
<td>1.0</td>
<td>430.3</td>
</tr>
<tr>
<td><em>T. castaneum</em></td>
<td>Lab diet</td>
<td>339.7</td>
<td>987.8</td>
</tr>
<tr>
<td></td>
<td>Pet product</td>
<td>0.3</td>
<td>309.8</td>
</tr>
</tbody>
</table>

$n = 3$ replications; diet infested with 50 mixed-age adults
### Six Treatment Combinations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%CO₂</th>
<th>%O₂</th>
<th>%N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>12.0</td>
<td>0.5</td>
<td>87.5</td>
</tr>
<tr>
<td>T2</td>
<td>0.0</td>
<td>0.5</td>
<td>99.5</td>
</tr>
<tr>
<td>T3</td>
<td>0.0</td>
<td>2.0</td>
<td>98.0</td>
</tr>
<tr>
<td>T4</td>
<td>20.0</td>
<td>1.0</td>
<td>79.0</td>
</tr>
<tr>
<td>T5</td>
<td>40.0</td>
<td>5.0</td>
<td>55.0</td>
</tr>
<tr>
<td>T6</td>
<td>0.0</td>
<td>1.0</td>
<td>99.0</td>
</tr>
<tr>
<td>Control (air)</td>
<td>0.03</td>
<td>21.0</td>
<td>78.0</td>
</tr>
</tbody>
</table>

- Gases moisturized by passing through 60% glycerol solution (45-50% RH)
- Flow rate: 236 ml/min, regulated by 8 flow meters
- Insects exposed in vials (24 ml)
- Specially constructed glass tubes to confine vials during exposure
Flow meters

Specially-designed glass tubes for holding vials
Plastic vials with grain-based pet product and insects

Vials had screens on both sides

Student counting insects
Mortality of *Tribolium castaneum* at 32.2°C

[T1 treatment]

Compressed air

12% CO$_2$ + 0.5% O$_2$ + 87.5% N$_2$
Tribolium confusum Adult Mortality at Three Temperatures

![Graph showing mortality over time for Tribolium confusum at three temperatures under two different atmospheres: Compressed air and 12% CO₂ + 0.5% O₂ + 87.5% N₂. The graphs display mortality (%) on the y-axis and time (hours) on the x-axis. Different temperatures are represented by distinct lines on the graph.](image-url)
Package Susceptibility Tests

- Packages placed in large plastic containers

- Five species were released together
  - 10 *Plodia interpunctella* larvae
  - 250 *Lasioderma serricorne* adults
  - 100 *Tribolium castaneum* adults
  - 400 *Oryzaephilus surinamensis* adults
  - 400 *Trogoderma variabile* larvae

- 3 replications

- Packages (independent samples) checked after 1 and 2 weeks post-infestation

- No insects were found inside packages
Conclusions

• The examples presented illustrate the effectiveness of controlled atmospheres for managing insects in packaged stored products

• Active packaging technology exists today
  – Ensure technology is cost-competitive with controlled atmospheres
  – Use as package liners to avoid product contamination

• Use of insect-resistant packaging in conjunction with controlled atmospheres shows promise as a pest control method for packaged dry, durable products
Thank You

Questions?