Effect of post-harvest starvation and rinsing on microbial numbers in mealworm larvae

Annette Nygaard Jensen
& Esben Bragason
Division for Microbiology and Production, National Food Institute, Technical University of Denmark

S47_04 Abstract no. 29709

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Background: mealworm production

Eggs from mealworm bettle (*Tenebrio molitor*) are grown to pre-pupae larvae stage.

At harvest, larvae are separated from the remaining substrate (frass).

→ Killing of larvae
  - freezing
  - heating or
  - chopping

Starvation 1-2 days
  - to empty the gut i.e.
    - remaining substrate
    - gut bacteria
Aim:

To test the microbial numbers in mealworm larvae at harvest after

➢ Starvation 1-2 days
➢ Rinsing with tap water
➢ Feeding with sterile substrate 1-2 days
Experimental set-up - I:

Day 0
Separation

Day 1
24 h Starvation
No wash
Rinsed, 200 ml water, 1 min

Day 2
48 h Starvation
No wash
Rinsed, 200 ml water, 1 min

Experimental set-up - II:

Day 0
Separation

Day 1
24 h Starvation
No wash
Rinsed, 200 ml water, 1 min

Day 2
48 h Starvation
No wash
Rinsed, 200 ml water, 1 min
Experimental set-up - II:

Day 0

Separation

Day 1

24 h Starvation
24 h Sterile feeding

Day 2

48 h Starvation 48 h Sterile feeding 48 h Substrate
larvae +/- rinse 40 ml water, 1 min
**Method:**

*Enumeration of bacteria in mealworms (& substrate)*

- Homogenization of 1 g sample with a mortar pestle
- Preparation of 10-fold dilution series
- Plate-spreading on agar plates
- Incubation
- Counting of colonies

→ Colony forming units (CFU)/gram
Method:
Bacterial groups enumerated

- **Total aerobic count**
  
  *Plate count agar (PCA) at 37°C, 24h*

- **’Psychrotrophs’** (II)
  
  *PCA at 6.5°C, 10 days*

- **Enterobacteriaceae**
  
  *Enterobacteriaceae count plate (ECP, Petrifilm, 3M) at 37°C for 24h*

- **Lactic acid bacteria (LAB)** (II)
  
  *de Man-Rogosa-Sharpe agar (MRS) at 30°C (anaerobic) for 48h*

- **Bacterial endospores** (II)
  
  *100°C for 5 min → PCA at 30°C, 3 days*
### Results:

**Starvation of larvae – rinsing I**

<table>
<thead>
<tr>
<th>Sampling:</th>
<th><strong>Total aerobic count</strong></th>
<th>Log CFU/g</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No rinse</td>
<td>Rinse</td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>8.36</td>
<td>8.20</td>
<td>8.32±0.08</td>
</tr>
<tr>
<td></td>
<td>8.36</td>
<td>8.35</td>
<td></td>
</tr>
<tr>
<td>Starvation 24 h</td>
<td>7.64</td>
<td>7.85</td>
<td>7.85±0.29</td>
</tr>
<tr>
<td></td>
<td>7.65</td>
<td>8.26</td>
<td></td>
</tr>
<tr>
<td>Starvation 48 h</td>
<td>7.78</td>
<td>7.92</td>
<td>7.91±0.10</td>
</tr>
<tr>
<td></td>
<td>7.93</td>
<td>8.02</td>
<td></td>
</tr>
</tbody>
</table>

No significant effect of rinsing

Some effect of starvation 0.4 log ~ 60% reduction
# Results:

## Starvation of larvae – rinsing II

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Total aerobic count (II)</th>
<th>Log CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No rinse</td>
</tr>
<tr>
<td>Start</td>
<td>7,86±0,11</td>
<td>7,83±0,06</td>
</tr>
<tr>
<td>Starvation 24 h</td>
<td>8,13±0,29</td>
<td>7,81±0,27</td>
</tr>
<tr>
<td>Starvation 48 h</td>
<td>8,10±0,35</td>
<td>7,74±0,18</td>
</tr>
</tbody>
</table>

~ Opposite tendency in experiment II compared with I

i.e. no effect starvation &

– some effect rinsing!? (not significant)
Results:
Starvation of larvae – rinsing

<table>
<thead>
<tr>
<th>Sampling:</th>
<th>I (No rinse)</th>
<th>I (Rinse)</th>
<th>II (No rinse)</th>
<th>II (Rinse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>7.06 ± 0.06</td>
<td>6.65 ± 0.61</td>
<td>6.71 ± 0.32</td>
<td>6.80 ± 0.21</td>
</tr>
<tr>
<td>Starvation 24 h</td>
<td>5.73 ± 0.40</td>
<td>6.09 ± 0.17</td>
<td>7.18 ± 0.33</td>
<td>7.07 ± 0.19</td>
</tr>
<tr>
<td>Starvation 48 h</td>
<td>6.73 ± 0.31</td>
<td>7.22 ± 0.22</td>
<td>7.13 ± 0.41</td>
<td>6.70 ± 0.30</td>
</tr>
</tbody>
</table>

Generally a high level of bacteria from the Enterobacteriaceae family
- an apparent decrease at 24 h (exp. I)
not supported by exp. II
## Results:
### Sterile feeding of larvae (II)

<table>
<thead>
<tr>
<th>Sampling:</th>
<th>Total aerobic count</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log CFU/g</td>
<td>Log CFU/g</td>
</tr>
<tr>
<td></td>
<td>No rinse</td>
<td>Rinse</td>
</tr>
<tr>
<td>Start</td>
<td>7,86 ± 0,11</td>
<td>7,83 ± 0,06</td>
</tr>
<tr>
<td>Sterile feed 24 h</td>
<td>8,05 ± 0,24</td>
<td>7,83 ± 0,12</td>
</tr>
<tr>
<td>Sterile feed 48 h</td>
<td>8,22 ± 0,39</td>
<td>8,08 ± 0,31</td>
</tr>
</tbody>
</table>

- For total aerobic count / *Enterobacteriaceae* - no clear effect of
  - starvation 24 - 48 h
  - rinsing with tap water
  - feeding with sterile substrate
Results:
Other bacterial groups in larvae

- Level of other bacterial groups in larvae
  - Again no clear effect of ‘treatment’ i.e. starvation/rinsing/sterile feeding
  - Psychrotrophs i.e. growing at 6.5°C
    - 3.5-5.5 log CFU/g
  - Lactic acid bacteria
    - 5-7 CFU/g
  - Bacterial endospores
    - close to or below detection limit <2 log CFU/g
**Level of bacterial groups in substrate (log CFU/g*)**

<table>
<thead>
<tr>
<th>Bacterial group:</th>
<th>Substrate 0 h</th>
<th>Substrate 48 h</th>
<th>‘Sterile’ substrate 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic count</td>
<td>4.19±0.17</td>
<td>7.28±0.37</td>
<td>8.35±0.58</td>
</tr>
<tr>
<td>Psychrotrophs’</td>
<td>4.39±0.04</td>
<td>4.50±0.45</td>
<td>2.93±0.41</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>3.84±0.54</td>
<td>7.02±0.57</td>
<td>7.61±0.15</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>2.35±0.08</td>
<td>5.90±0.19</td>
<td>5.86±0.09</td>
</tr>
<tr>
<td>Bacterial endospores</td>
<td>0.67±1.15</td>
<td>0.67±1.15</td>
<td>1.65±1.43</td>
</tr>
</tbody>
</table>

*Mean 3 replicates ±SD

Start at 0 h: Bacterial levels <4.5 Log CFU/g

After 48 h larval feeding: Bacterial levels increases in both normal / sterile substrate - depending on bacterial group! ~ reflects larval content...
Summary: Mealworm post-harvest practices

Neither, rinsing with tap water nor feeding with sterile substrate seemed to reduce bacterial load in mealworm larvae markedly → starvation to remove substrate from gut may still be desirable!!?

Generally high bacterial numbers in larvae → application of heat treatment necessary

Bacterial numbers in substrate apparently ‘reflects’ the level in the larvae (~balance)
Thanks to:

Esben Bragason
Freja Lønborg Fabricius
Sisse Nygaard Rasmussen

inVALUABLE - INsect VALUe Chain in a CircuLAR BioEconomy

Funded by Innovation Fund Denmark
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Bacterial numbers in substrate apparently ‘reflects’ the level in the larvae (~balance)