How fall and winter temperature during ensiling and storage influence microbial populations

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Lactic acid bacteria and temperature

- Like most organisms, growth of lactic acid bacteria is temperature dependant
- Frequently grown at 37°C on synthetic culture media
- Maximum growth temperature: ~ 45°C
- Optimum: ~ 30°C
- Usually not good performer at low temperatures
Temperature related studies on silage

- Influence of the temperature on the silage fermentation processes was studied previously
  - Mainly in relation with high temperature
  - High temperature impact on aerobic stability
  - Kim (2006) - low aerobic stability
  - Weinberg (1998) - poor quality
  - McDonald (1966) - clostridial fermentation
- Most ensiling experiments conducted at moderate temperatures ~ between 20 and 30 °C
Harvested corn
- 1/3 milk line
- 31.0% dry matter
- 126 g kg\(^{-1}\) WSC

Initial fermentation for 60 days

- 10 °C (44 silos)
- 20 °C (44 silos)

Storing at low temperature for 60 days

- 5 °C (4 silos)
- 10 °C (4 silos)
- 15 °C (4 silos)
- 20 °C (4 silos)
- 25 °C (4 silos)

Incubation temperature was slowly increased over a 60 days period (2.5 °C per week)
Harvested corn
- 1/3 milk line
- 31.0% dry matter
- 126 g kg WSC

Initial fermentation for 60 days
- 5 °C (4 silos)
- 10 °C (44 silos)
- 15 °C (4 silos)
- 20 °C (4 silos)
- 25 °C (4 silos)

Incubation temperature was slowly increased over a 60 days period (2.5 °C per week)

Storing at low temperature for 60 days
- 10 °C (4 silos)

Experimental design

Measurements for each silo
- LAB counts
- Yeast & molds counts
- DNA extraction
- DGGE fingerprinting:
  - bacterial (357FGC/517R - 200 pb)
  - fungal (ITS1FGC / ITS2 - 280 pb)
- pH
- VFA concentration
- WSC concentration
Final pH

- 20 °C
- 10 °C

SEM 20 °C = 0.003
SEM 10 °C = 0.022
Water soluble carbohydrates

Storage temperature

SEM 20 °C = 0.943
SEM 10 °C = 5.211
# Microbial counts (log\textsubscript{10} CFU g FM\textsuperscript{-1})

<table>
<thead>
<tr>
<th>Storage temperature (°C)</th>
<th>LAB</th>
<th>Enterobacteria</th>
<th>Yeasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before storage</td>
<td>9.12</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>5</td>
<td>7.85</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>10</td>
<td>7.82</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>15</td>
<td>7.67</td>
<td>4.02 ± 0.07</td>
<td>3.93 ± 0.08</td>
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<td>20</td>
<td>7.81</td>
<td>4.71 ± 0.47</td>
<td>4.85 ± 1.21</td>
</tr>
<tr>
<td>25</td>
<td>8.19</td>
<td>4.78 ± 0.10</td>
<td>4.44 ± 1.11</td>
</tr>
<tr>
<td>SEM</td>
<td>0.49</td>
<td>0.517</td>
<td>0.504</td>
</tr>
</tbody>
</table>

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<tbody>
<tr>
<td>Before storage</td>
<td>8.15</td>
<td>n.d.</td>
<td>4.43</td>
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<tr>
<td>5</td>
<td>5.80</td>
<td>nd</td>
<td>nd</td>
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<td>10</td>
<td>5.85</td>
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<td>nd</td>
</tr>
<tr>
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<td>5.85</td>
<td>nd</td>
<td>nd</td>
</tr>
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<td>20</td>
<td>7.43</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>25</td>
<td>8.54</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>SEM</td>
<td>0.255</td>
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</tbody>
</table>

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Fermentation temperature

- 20 °C
- 10 °C
Bacterial diversity

![Graph showing Shannon diversity index (H') for different storage temperatures.]

- **Weissella koreensis**
- **Leuconostoc citreum**
- **Lactobacillus brevis**
- **Chryseobacterium sp.**
- **Lactobacillus buchneri**

**Shannon diversity index (H')**

- **SEM 20 °C = 0.286**
- **SEM 10 °C = 0.031**
Fungal diversity

Davidiella spp.
uncultured Basidiomycota
(Bulleromyces albus - 99%)
Candida humilis

Shannon diversity index ($H'$)

Storage temperature

SEM 20 °C = 0.028
SEM 10 °C = 0.021
## Main VFA and ethanol (g kg$^{-1}$ DM)

<table>
<thead>
<tr>
<th>Storage temperature (°C)</th>
<th>Lactic acid</th>
<th>Acetic acid</th>
<th>Ethanol</th>
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</thead>
<tbody>
<tr>
<td>Before storage</td>
<td>63.3</td>
<td>80.0</td>
<td>3.16</td>
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<tr>
<td>5</td>
<td>59.24</td>
<td>80.01$^{AB}$</td>
<td>10.66</td>
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<td>10</td>
<td>55.92</td>
<td>70.64$^A$</td>
<td>7.50</td>
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<td>59.01</td>
<td>93.22$^{BC}$</td>
<td>8.10</td>
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<tr>
<td>20</td>
<td>50.98</td>
<td>107.63$^C$</td>
<td>13.45</td>
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<td>25</td>
<td>61.33</td>
<td>107.86$^C$</td>
<td>15.25</td>
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<tr>
<td>SEM</td>
<td>1.91</td>
<td>4.08</td>
<td>0.90</td>
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<tr>
<td>NS</td>
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<td>***</td>
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<th>Acetic acid</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before storage</td>
<td>32.4</td>
<td>41.0</td>
<td>3.96</td>
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<tr>
<td>5</td>
<td>34.99</td>
<td>78.41$^A$</td>
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<td>10</td>
<td>40.49</td>
<td>80.96$^A$</td>
<td>2.79</td>
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<td>36.08</td>
<td>80.17$^A$</td>
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<tr>
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<td>44.39</td>
<td>81.16$^A$</td>
<td>2.52</td>
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<tr>
<td>25</td>
<td>45.41</td>
<td>139.17$^B$</td>
<td>4.08</td>
</tr>
<tr>
<td>SEM</td>
<td>1.15</td>
<td>5.57</td>
<td>0.21</td>
</tr>
<tr>
<td>NS</td>
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Fermentation temperature 20 °C

Fermentation temperature 10 °C

SEM: Standard Error of the Mean

NS: Not Significant

***: Significant at the 0.001 level
Conclusion

- Temperature at ensiling contributes to different microbial and biochemical dynamics as silage warm-up after storage at low temperature.
- Corn silages initially fermented at higher temperature was not stable and allow growth of enterobacteria and yeasts after storage.
- Low temperature at fermentation phase restrict biochemical activities - but fermentation resume as temperature warmed-up.
Thanks to:
Marcelle Mercier
Marie-France Thibault

Questions

see also poster ID-151:
Impact of low temperature on lactic acid bacteria diversity in corn silage