Malolactic fermentation 2005

R-e-s-p-e-c-t
the bacteria in your wines
and
manage your MLF

Geneva on the Lake, February, 2005
BACTERIA IN MUST & WINE

- Only a few bacteria are adapted to survive in wine

  alcohol
  acidity
  Low pH
  $SO_2$
  Nutrient deficiency
Microflora in must and wine

Brettanomyces
Lactobacillus
Pediococcus
Acetobacter e Oenococcus
Saccharomyces
THE CHEMISTRY...

HOOC · CH₂ · CHOH · COOH

134
malic acid

→ CO₂ + CH₃ · CHOH · COOH

44
carbon dioxide

90
lactic acid
THE CHEMISTRY

- L (-) malic acid converted into L(+) lactic acid (commercial additions of D(+)) will remain untouched).
- Not really a ‘fermentation’ as no energy is produced
- Reduction of acidity by 1-3 g/L
- Addition of diacetyl: good or bad?
Metabolism in heterofermentative Lactic Acid Bacteria

- **CITRATE**: 100-700 ppm
- **GLUCOSE**
  - 300-1000 ppm
- **FRUCTOSE**
- **MALATE**: 1000-4000 ppm
- **Pyruvate**
- **ATP**
- **Acetaldehyde-TPP**
- **Acetyl-CoA**
- **Acetyl-P**
- **ATP**
- **pH, temp**
- **Fatty acids**
- **LIPIDS**
- **L-LACTATE**: 670-2680 ppm
- **D-LACTATE**: 100-200 ppm
- **DIACETYL**: 2-8 ppm
- **acetoin**
- **ACETATE**: 100-200 ppm
Bacteria found in must and in wine

<table>
<thead>
<tr>
<th>LACTIC ACID BACTERIA</th>
<th>ACETIC BACTERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation:</td>
<td></td>
</tr>
<tr>
<td><em>Oenococcus oeni</em> (ex <em>Leuc. oenos</em>)</td>
<td>hetero</td>
</tr>
<tr>
<td><em>Leuconostoc mesenteroides</em></td>
<td>hetero</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em></td>
<td>homo</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>homo</td>
</tr>
<tr>
<td><em>Lactobacillus brevis</em></td>
<td>hetero</td>
</tr>
<tr>
<td><em>Pediococcus damnosus</em></td>
<td>homo</td>
</tr>
<tr>
<td><em>Pediococcus pentosaceus</em></td>
<td>homo</td>
</tr>
<tr>
<td><em>Gluconobacter oxydans</em></td>
<td>sugars</td>
</tr>
<tr>
<td><em>Acetobacter aceti</em></td>
<td>ethanol</td>
</tr>
<tr>
<td><em>Acetobacter pasteurianus</em></td>
<td>ethanol</td>
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</table>
BACTERIA EVOLUTION
UNDER DIFFICULT CONDITIONS

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>Lactobacillus</th>
<th>Pediococcus</th>
<th>Gluconobacter</th>
<th>Oenococcus</th>
<th>Acetobacter</th>
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</thead>
<tbody>
<tr>
<td>10^8</td>
<td>10^5</td>
<td>10^3</td>
<td>10^2</td>
<td>10^1</td>
<td>10^2</td>
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<td>10^7</td>
<td>10^5</td>
<td>10^3</td>
<td>10^2</td>
<td>10^1</td>
<td>10^2</td>
</tr>
<tr>
<td>10^6</td>
<td>10^5</td>
<td>10^3</td>
<td>10^2</td>
<td>10^1</td>
<td>10^2</td>
</tr>
<tr>
<td>10^5</td>
<td>10^3</td>
<td>10^2</td>
<td>10^1</td>
<td>10^1</td>
<td>10^2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cells/ml</th>
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</thead>
<tbody>
<tr>
<td>10^8</td>
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<tr>
<td>10^7</td>
</tr>
<tr>
<td>10^6</td>
</tr>
<tr>
<td>10^5</td>
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<tr>
<td>10^4</td>
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<tr>
<td>10^3</td>
</tr>
<tr>
<td>10^2</td>
</tr>
<tr>
<td>10^1</td>
</tr>
</tbody>
</table>

HARVEST ALCOHOLIC MALOLACTIC STORAGE
DELIVERY FERMENTATION FERMENTATION
Most important parameters

**CHEMICAL/PHYSICAL**

$SO_2 \succ pH \succ \text{temperature} \succ \text{alcohol}$

**NUTRIENTS**

(achtung! O. Oeni cannot use ammonia)

**MICROBIOLOGICAL**

Influence of the wine yeast from AF
POSSIBLE INTERACTIONS BETWEEN YEAST & ML BACTERIA
INHIBITION OF OENOCCCUS OENI BY THE YEAST
STIMULATION OF OENOCCCUS OENI BY THE YEAST
NO INFLUENCE = INDIFFERENT
IN SOME CASES INHIBITION OF SACCHAROMYCES CEREVISIAE BY WILD LACTIC ACID BACTERIA WAS REPORTED.
IN SOME CASES THEY EVEN CAN HAVE MUCH FUN

Ha Ha Ha Ha... stop it.
Bello... hehehehe...
ITV 99 - Tasting
Pinot noir – Qualitative differences

Blackcurrant

Cherry

Pepper

Caramel

Earty

Hay

Control

EQ 54

Lalvin 31

Bacteria X
**ML Properties based on organoleptic properties**

**WHITE WINES**
- Tropical fruit,
- Vanilla in barrel fermentation
- Peach and melon characteristics

**RED WINES**
- Reduction of vegetal aromas

**MOUTHFEEL:** volume and balance in mouth

- Mature fruit and jam
- Increase
Uncontrolled Malolactic Fermentation...

**THE MASKS**...

(MLF Sensory Defects Kit) presented by...

Dr. Sibylle Krieger
Didier Theodore
Dr. Antonio Palacios
Winemakers are concerned with:

• Limiting chemical inputs (optimizing the SO₂ dosage).
• Limiting health risk concerns and spoilage (low biogenic amines).
• Avoiding heavy curative treatments of clarification, filtration and stabilization.
• Developing and stabilizing positive aromas and tannin perception.
• Maintaining quality through the winemaking process.
Wine bacteria: The drawbacks...

- Volatile Acidity
- Too much Diacetyl
- Undesirable Aromas & Flavors
- Varietal character loss
- Color loss
- Ethyl Carbamate
- Biogenic Amines
- Geranium Aromas

The Usual Suspects:
- Some Oenococcus
- Many Lactobacillus
- Many Pediococcus
Wine Bacteria: The positive side...

- Lowering acidity
- Ethyl Lactate / Diacetyl
- Varietal aroma enhancement
- Reducing Vegetative notes
- Rounding the mouthfeel
- Lowering astringency
- Lowering bitterness
- Increasing complexity
- Lowering overall SO$_2$

The Usual Suspects: Some Oenococcus
Ethyl Lactate
Ethyl Lactate Aroma

- Formed mainly during MLF.
- Low levels contribute mouthfeel volume.
- High concentrations add milk and yogurt aromas.
- Above 15 mg/L is usually considered negative in wines.
Acetaldehyde
ACETALDEHYDE

- Threshold detection ~ 100 mg/L.
- Odor: overripe apple, fish in vinegar, “oxidation”.
- Sometimes formed during MLF, and other times its levels are reduced.
- Normally the levels are reduced by binding with free SO₂.
Geranium Odor
Geranium odor

- Derived from the metabolism of sorbic acid, which hydrogenates to SORBINOL, which isomerizes to 3,5-Hexadien-2-ol, then reacting with the ethanol part of 2-ethoxy-hexa-3,5-dien, resulting in the spoilage odor.
- Perception threshold is 0.1 µg/L.
- *O. oeni* has low production.
- Not a problem in beverages without ethanol.
Diacetyl Impact

Bacteria Strain Diacetyl Production

- 5-14 mg/L Butter
- 2-4 mg/L nutty caramel yeasty honey (threshold > reds)

The final concentration depends on the bacteria strain used for the MLF & on its citric acid metabolism.
Metabolism of citrate by Oenococcus oeni and the transformation of diacetyl by yeasts

- **Citrate**
  - Oxalacetate
  - Pyruvate
  - Acetate

The transformation of diacetyl to butandiol lowers the buttery aromas and transforms them into mouthfeel volume and fattness.
Biogenic Amines
Biogenic amines are unhealthy (histamine) and also contribute negative sensory compounds (putrescine & cadaverine)

Decarboxylation of amino acids, Ex.: histidine decarboxylase for histamine
# Biogenic Amine Formation: Examples

## Amino Acids – Biogenic Amines

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Biogenic Amine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Histamine</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyramine</td>
</tr>
<tr>
<td>Lysine</td>
<td>Cadaverine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Putrescine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Espermine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Epermidine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Ethanolamine</td>
</tr>
<tr>
<td></td>
<td>Phenylethylamine</td>
</tr>
<tr>
<td></td>
<td>Isopentylamine</td>
</tr>
</tbody>
</table>
Lactic Acid Bacteria Strain Influence on Concentration of Biogenic Amines after MLF

Putrescine, a putrid “dirty sponge” aroma, is the highest contributor to Biogenic Amines in this trial.
Lactobacillus can produce volatile phenols

Volatile phenols are formed from the grape precursors by contaminating microorganisms...

Activity of Lactobacillus plantarum
Brettanomyces Contamination...

The Problem

- Contaminant yeast, responsible for the formation in wine of volatile phenols resulting in very negative aromas

  Ethylphenols  4-ethyl phenol
  4-ethyl guaiacol
  Vinylphenols 4-vinyl phenol
  4-vinyl guaiacol

- 4-ethyl phenol results in the descriptors ("poorly cured leather", "horse sweat", "used socks", "horse stables").

Threshold perception:
Ethyl phenol: 600µg/L
Sum of ethyl phenols: 430 µg/L
Mousy Off-Flavour
Causal compounds of Mousy off-flavour

N-heterocyclic bases: 2-ethyltetrahydropyridine, 2-acetyl tetrahydropyrididine & 2-acetyl-1-pyrroline

2-ethyltetrahydropyrididine (ETPY)

Taste threshold (wine): 150 µg/L (Craig & Heresztyn 1984)
Conc’n reported in wines exhibiting mousy off-flavour: 2.7-18.7 µg/L

2-acetyl tetrahydropyrididine (ACTPY)

Odour threshold (water): 1.6 µg/L (Teranishi et al. 1975)
Conc’n reported in wines exhibiting mousy off-flavour: 4.8-106 µg/L

2-acetyl-1-pyrroline (ACPY)

Odour threshold (water): 0.1 µg/L (Buttery et al. 1983)
Conc’n reported in wines exhibiting mousy off-flavour: Tr-7.8 µg/L

Possible formation pathways of ACTPY & ACPY from \emph{L. hilgardii DSM 20176}

\begin{itemize}
  \item Lysine
  \item Heterolactic
    \begin{itemize}
      \item D-Glucose / D-Fructose
      \item CO$_2$
      \item Acetate
    \end{itemize}
  \item Acetyl-phosphate
  \item Acetyl-CoA
  \item Acetaldehyde
  \item Ethanol
  \item Ornithine
\end{itemize}

To avoid these “Masking” components...

- Limit the duration of lactic bacteria in the wine.

- Control the winery cleanliness to limit spoilage organisms (Pediococcus, Lactobacillus…)

- Use selected ML bacteria to control the MLF and avoid wine spoilage including biogenic amines.
Thank you for your attention!

For more info please contact Sigrid@lallemand.com
Nitrogen requirements of yeasts

Different demand depending on the strains fermentation temperature and pH

Jiranek et al., 1991, Manginot et al., 1998, Julien et al., 2000
Nutrient requirements for *O. oeni*

*Oenococcus oeni*  
*(Leuconostoc oenos)*

*does not grow on*  
*malic acid only*

*it needs complex nutrients*
Improved nutrient formulations for *Oenococcus oeni*
Negative effects of the yeast on the bacteria (inhibition) could be caused by:

- Competition on nutrient level
- Production of inhibitory metabolites
  - $SO_2$
  - $CO_2$
  - Medium chain fatty acids
  - Antibacterial compounds
LALVIN MBR in difficult wine

CH '98, pH 3.13, SO2T 30, Alc 14.0

Days to complete MLF

- Spont. MLF
- MBR 1
- MBR 31
- MBR 2
- MBR 3
- MBR EQ54
- STRAIN X
- MBR 4
- OSU
- EQ54 1 STEP
- MT01 STD
**LALVIN MBR pH tolerance:**

ITV Beaune, SOFRALAB ‘98

- Pinot pH 3.7; Alc 14.5
- Syrah pH 2.97; Alc 13.0
MBR®: Tolerance at low temperatures

ITV Beaune 1998, Pinot noir pH 3.35, alc. 13.20 %