Bacteria timing of selected wine bacteria inoculation: – Diacetyl management and more

Dr. Sibylle Krieger-Weber

ML SCHOOL STELLENBOSCH 2016
Long-established winemaking protocols for MLF induction generally involve:
- waiting of indigenous lactic acid bacteria populations to work
or
- inoculation of selected bacteria at the end of post alc. fermentation

More recently, there is an increasing acceptance to introduce selected wine bacteria starter cultures earlier in the fermentation process.
CO-INOCULATION- TEMPERATURE AND SO$_2$ CONSIDERATIONS

<table>
<thead>
<tr>
<th>Potential Alcohol Content of wine (v/v%)</th>
<th>Maximum Temperature during MLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;14.5%</td>
<td>28°C</td>
</tr>
<tr>
<td>&gt;14.5%</td>
<td>23°C</td>
</tr>
</tbody>
</table>

- **SO$_2$ management**
  - Inoculation of the bacteria 24, 48 or 72 hrs after the yeast addition depending on the SO$_2$ addition at crush:
    - ≤ 50 ppm SO$_2$ added: wait 24 hrs before ML bacteria inoculation.
    - 50 – 70 ppm of SO$_2$ added: wait 48 hrs
    - > 70 – 90 ppm of SO$_2$ added: wait 72 hrs

- The lower the pH the more moderate the SO$_2$ addition!
INOCULATION AFTER ALCOHOLIC FERMENTATION

The advantages

- No increase in volatile acidity
- An easier control of MLF after alcoholic fermentation

The risks

- No start or stuck MLF due to the alcohol concentration
- Nutrient deficiency after alcoholic fermentation
Advantages

- Absence of alcohol
- Presence of nutrients
- Faster MLF performance, faster completion of MLF and the possibility for earlier stabilization of the wine

Risks

- In case of a stuck alcoholic fermentation in a high pH situation – production of acetic acid and D-lactic acid out of residual sugar
Bacteria timing of inoculation practices – Diacetyl management
Citric acid

Oxaloacetic acid

Aspartic acid

CO₂

Pyruvic acid

Acetylphosphate

Lactic acid

TTP

Acetaldehyde-TTP

α-aceto-lactic acid

Acetolactate synthase

Non-enzymatic decarboxylation

Diacetyl reductase

NAD(P)

NAD(P)H

Acetoin

2,3-Butanediol

Co-inoculation

Eveline Bartowsky, AWRI, 2004
Diacetyl – strain specific production

Inoculation post AF

Diacetyl (mg/L)

<table>
<thead>
<tr>
<th>Citric acid</th>
<th>Diacetyl</th>
</tr>
</thead>
</table>

Clare Valley

Adelaide Hills

Eveline Bartowsky, AWRI,
Diacetyl production in sequential inoculation

<table>
<thead>
<tr>
<th>MT01</th>
<th>O-MEGA</th>
<th>VP41</th>
<th>ALPHA</th>
<th>31</th>
<th>PN4</th>
<th>Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Attack of citric acid with a delay</td>
<td>Very late attack of</td>
<td>Medium attack of citric acid during</td>
<td>medium to early attack</td>
<td>Early attack of citric acid</td>
<td>Very Early attack of citric acid</td>
</tr>
<tr>
<td>build-up</td>
<td>after MLF</td>
<td>very low attack of citric</td>
<td>citric acid during the MLF</td>
<td>of citric acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>culture</td>
<td>culture</td>
<td>acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No producer</td>
<td>No production of diacetyl</td>
<td>Very low - to no</td>
<td>Medium producer of diacetyl</td>
<td>Medium producer of</td>
<td>High producer of diacetyl</td>
<td>High producer of diacetyl</td>
</tr>
<tr>
<td>of diacetyl</td>
<td></td>
<td>production of diacetyl</td>
<td></td>
<td>diacetyl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Diacetyl management**

**O. oeni strain**
Aromatic impact of our selected wine bacteria

**ALPHA**
- Battery impact (Diacyetyl production):
  - Moderate in Sequential inoculation
  - Low in Co-inoculation
- Reduction of green and vegetative flavors
- Mouthfeel:
  - Respect varietal aromas
  - High in ethyl propionate
- Velvet red fruit
- Roundness
- Mouthfeel

**BETA**
- Battery impact (Diacyetyl production):
  - Moderate to high in Sequential inoculation
  - Low in Co-inoculation
- High in botanicals
- Increase volume and softness
- Enhance fruity aromas

**V22**
- Very low buttery impact
- Reduction of green notes and mint
- Very high in dark and red fruit

**Lalvin 31**
- Enhance fruity aromas
- Complexity and sweet tannins
- Structure
- Spiciness

**PN4**
- Battery impact (Diacyetyl production):
  - Moderate to high in Sequential inoculation
  - Low in Co-inoculation
- Structure
- Increases general perception of fruitiness

**VP41**
- Very low buttery impact
- Complexity and sweet tannins
- Structure
- Floral notes

- Enhance tropical and passion fruits
- Red young fruit finish forward
France – Val de Loire - Chardonnay 2010

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars g/l</td>
<td>196</td>
</tr>
<tr>
<td>Total acidity (g/l H2SO4)</td>
<td>4.72</td>
</tr>
<tr>
<td>pH</td>
<td>3.21</td>
</tr>
<tr>
<td>VA (g/l H2SO4)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>L malic acid (g/l)</td>
<td>5.1</td>
</tr>
<tr>
<td>Assimilable nitrogen (mg/l)</td>
<td>160</td>
</tr>
<tr>
<td>Free SO2 (mg/l)</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Total SO2 (mg/l)</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

**Malic acid degradation**

- CY3079-Beta 48h
- CY3079-Beta 2/3 AF
- CY3079-Beta Post AF

**In co-inoculation,**
very low production of diacetyl
**vs sequential inoculation**
with our « highest diacetyl producer »

Under the reductive conditions generated by active yeast cells (consuming the oxygen available), diacetyl is immediately reduced to acetoain and then 2,3-butanediol (diacetyl reductase)
Diacetyl management

Key points:

• Choice of the bacteria
• Bacteria timing of inoculation

<table>
<thead>
<tr>
<th>Buttery aroma</th>
<th>Fruit driven style</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequential inoculation with strains Beta® or PN4™</td>
<td>Co-inoculation with e.g. Alpha®, VP41®, Beta®, O-MEGA™</td>
</tr>
<tr>
<td>Eliminate as much as possible yeast lees</td>
<td>Sequential inoculation with Lalvin 31®, VP41®, O-MEGA™</td>
</tr>
<tr>
<td>Lower inoculation ratio</td>
<td>18-20°C</td>
</tr>
<tr>
<td>Lower temperature during MLF</td>
<td>Co-inoculation</td>
</tr>
<tr>
<td>Quick stabilization with SO₂ at end of MLF</td>
<td>Yeast lees contact &amp; stirring</td>
</tr>
<tr>
<td></td>
<td>Delayed SO₂ addition (minimum 1 week if pH allows two weeks)</td>
</tr>
</tbody>
</table>
Summary

It is possible to create the different styles of Chardonnay implication selected wine LAB strains?

- Choice of a strain: diacetyl producer or no producer
- Timing of inoculation: co-inoculation or sequential inoculation
- MLF in oak or in stainless steel
- Combination with a selected wine yeast strain
COINOCULATION

=>

for fruity rosé or fresh young red wines
How can co-inoculation be used?

Clare Valley (2008)

Shiraz

1 kg ferments

9 kL tanks

Caroline Abrahamse

Co-inoc

Mid-AF

Pressing

Sequential

AF

Eveline Bartowsky, AWRI, Int. ML School Toulouse 2014
MLF kinetics

Eveline Bartowsky, AWRI,, Int. ML School Toulouse 2014
Volatile fermentation-derived compounds

Lab scale (1.5 kg)

Winery scale (9 kL)

PC1 (65%)

PC2 (24%)

PC1 (54%)

Co-inoculation
Mid alcoholic
Pressing
Post alcoholic
No MLF

ML SCHOOL
Bartowsky & Abrahamse GGWM (2012)
Barbera d’Asti 2008:

Coinoculation vs Inoculation post AF

- **COINOCULATIONS**
  - WB 24 h after inoculation with ADY

- Inoculation post AF: SWB inoculated at the end of AF

- Pot. Alc. 13 %vol
- pH 3,19
- T-SO2 in must 25 mg/l
Sensorial Analyses (triangle test)

1 – Color intensity
2 – Aromatic intensity
3 – Fruity aromas (red fruit)
4 – Fruit expression
5 – Acidity
6 – Morbidity
7 – Equilibration/harmony
8 - Persistence
Summary

• MLF can influence wine aroma
  – Buttery character
  – Fruity aromas
  – Oak character

• Changes are dependent upon several factors
  – O. oeni strain
  – Wine composition
  – MLF conditions

• Increase in total fruit berry compounds translates into an increase in berry related sensory descriptors
  – Cabernet Sauvignon, and other red varieties behave similarly
  – Persistence with storage

• Co-inoculation of MLF can influence wine aroma
  – Red and white wines

➢ MLF is more than just deacidification & wine stabilisation
COINOCULATION

=>

WHAT ABOUT HIGH pH WINES???
Potential risk of spontaneous MLF - The influence of the pH

- Risk, that MLF does not occur
- Difficulties to induce a spontaneous MLF
- Increasing risk of spontaneous MLF induced by *Lactobacillus* & *Pediococcus* sp.

OFF-FLAVORS

Lack of sugar degradation after MLF by *O. oeni* in low pH conditions

*Oenococcus oeni*

3.0 3.3 3.5 3.7 4.0

Wine pH

Potential risk of sugar degradation after MLF by *O. oeni* at high pH

Peter Costello, AWRI, 2003
Evolution of bacteria under favorable conditions

- **Yeast**
- **Lactobacillus**
- **Pediococcus**
- **Gluconobacter**
- **Oenococcus**
- **Acetobacter**

** Timeline:**
- Harvest/Transport: 0 - 3 days
- Alcoholic Fermentation: 3 - 24 days
- Malolactic Fermentation: 24 days - 3 months
- Aging: > 3 months
Trials in France 2006-2008: impact of timing of inoculation on biogenic amines content

Biogenic amines content after MLF

Concentration (mg/L)

Control, Inoc. Post-AF, Co-Inoc., Control, Inoc. Post-AF, Co-Inoc., Control, Co-Inoc., Control, Co-Inoc., Control, Co-Inoc.

Trial 1, Trial 2, Trial 3, Trial 4, Trial 5
Days to complete MLF and *Brett.* population

From V. Renouf et al., 2005, *J. Int. Sc. Vigne Vin.*, 39, 179-190
Survival and growth of a complex O. oen population after MLF at different pH and res. glucose levels
Evolution of acetic acid in a Pinot Noir in dependence of pH and residual sugar levels

- **Residual sugar ca. 1 g/l**
- **Residual sugar ca. 3 g/l**

**pH Values and Acetic Acid Levels**
- pH 3.3
- pH 3.5
- pH 3.7

**Acetic Acid Levels**
- End MLF
- MLF + 1 month
- MLF + 2 months
Evaluation of technological effects of yeast-bacterial co-inoculation in red table wine

100 WINES *(VINTAGE 2008)*

**COINOCULATION IN MUST**

**GRAPES FROM DIFFERENT VARIETIES**

**MUST WITH DIFFERENT CHEMICAL COMPOSITIONS**

**VINIFICATION 100-200 LITERS**
100 WINES (VINTAGE 2008)

Duration of MLF (days)

Final acetic acid levels from 0.2 to 0.6 g/l
Co-inoculation instructions - 1

1. Selected, rehydrated and protected yeast.*
2. Choose a yeast with low nitrogen requirements adapted to the style of wine desired.
3. Bacteria addition timing depends on SO₂ added:
   - <50 ppm of SO₂ added: wait 24 hours
   - 50 to 80 ppm of SO₂ added: wait 48 hours
   - >80 ppm of SO₂ added: wait 72 hours

Note: If measuring free SO₂ 24 hours after addition, at pH > 3.3, the free SO₂ should be <25 ppm and <10 ppm when pH is below 3.3 pH.

pH > 3.3
Use 1-Step cultures wine bacteria without acclimatization
pH > 3.6
Also a co-inoculation with *Lb. plantarum ML-Prime* (homo-fermentative for hexose sugars) is recommendable
*Lactobacillus plantarum* a new generation and a new concept for co-inoculation and to control malolactic fermentation in wine
<table>
<thead>
<tr>
<th>Name</th>
<th>Lactobacillus plantarum</th>
<th>Oenococcus oeni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation of sugars (hexoses)</td>
<td>Homo-fermentative = 2 x lactate</td>
<td>Hetero-fermentative = Lactate + acetate + CO2</td>
</tr>
<tr>
<td>Wine parameter for best performance</td>
<td>pH &gt; 3.5 Alcohol &lt; 15.5%vol Total SO2 &lt; 50 ppm Temperature &gt; 17°C</td>
<td>pH &gt; 3.1 Alcohol &lt; 15.5%vol Total SO2 &lt; 50 ppm Temperature &gt; 17°C</td>
</tr>
</tbody>
</table>
BACTERIAL METABOLISM

Optional heterofermentative metabolism
(MLPrime™ metabolism)

GLUCOSE-FRUCTOSE

\( \text{CO}_2 \) → Lactic acid

Acetic acid → Ethanol

Obligatory heterofermentative metabolism
(Standard metabolism of selected enological bacteria)

GLUCOSE-FRUCTOSE

\( \text{CO}_2 \) → Lactic acid

Acetic acid → Ethanol
A new approach

- Developed by a new production process that optimizes the activity of the malolactic enzyme system of the bacterial culture. This high activity of malolactic enzyme reduces the lag phase and allows a very quick malolactic fermentation
  - Fast malic acid degradation due to a non-proliferating LAB biomass = MLF can be achieved during AF
  - Important malolactic enzyme pool
  - Wine can be stabilized right after AF
Others properties

- No production of biogenic amines
  - No citric acid degradation
  - No diacetyl production

- Bacteria cinnamyl esterase negative
cannot degrade coutaric acid to coumaric acid, which is a precursor for volatile phenol formation by *Brettanomyces*
Examples of application: France 2015: Grenache

**Kinetics of malolactic fermentation**

Too fast to be measured!
<table>
<thead>
<tr>
<th>Analysis end of MLF</th>
<th>ML prime co-inoc</th>
<th>O-Mega co-inoc</th>
<th>VP41 co-inoc</th>
<th>O-Mega sequential</th>
<th>VP41 sequential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of sampling</td>
<td>29-sept</td>
<td>06-oct</td>
<td>29-sept</td>
<td>12-oct</td>
<td>12-oct</td>
</tr>
<tr>
<td>Days after start of trial</td>
<td>J+13</td>
<td>J+20</td>
<td>J+13</td>
<td>J+26</td>
<td>J+26</td>
</tr>
<tr>
<td>TAV (% vol.)</td>
<td>14,28</td>
<td>14,21</td>
<td>14,22</td>
<td>14,27</td>
<td>14,25</td>
</tr>
<tr>
<td>Total acidity (g/L H2SO4)</td>
<td>4,42</td>
<td>4,22</td>
<td>4,14</td>
<td>4,12</td>
<td>4,14</td>
</tr>
<tr>
<td>Sugars (g/L)</td>
<td>0,02</td>
<td>0,01</td>
<td>0,01</td>
<td>0,02</td>
<td>0,02</td>
</tr>
<tr>
<td>Volatile acidity (g/L H2SO4)</td>
<td>0,15</td>
<td>0,19</td>
<td>0,17</td>
<td>0,13</td>
<td>0,13</td>
</tr>
<tr>
<td>pH</td>
<td>3,57</td>
<td>3,64</td>
<td>3,60</td>
<td>3,58</td>
<td>3,59</td>
</tr>
<tr>
<td>Potassium (mg/L)</td>
<td>1266</td>
<td>1201</td>
<td>1238</td>
<td>1307</td>
<td>1280</td>
</tr>
<tr>
<td>Free SO2 (mg/L)</td>
<td>6</td>
<td>13</td>
<td>11</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Total SO2 (mg/L)</td>
<td>24</td>
<td>26</td>
<td>23</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>TPI</td>
<td>39,4</td>
<td>36,4</td>
<td>39,8</td>
<td>38,6</td>
<td>38,4</td>
</tr>
<tr>
<td>DO420 (yellow)</td>
<td>2,33</td>
<td>2,14</td>
<td>2,22</td>
<td>2,3</td>
<td>2,29</td>
</tr>
<tr>
<td>DO520 (red)</td>
<td>5,17</td>
<td>4,57</td>
<td>4,79</td>
<td>4,82</td>
<td>4,8</td>
</tr>
<tr>
<td>DO620 (purple)</td>
<td>0,72</td>
<td>0,65</td>
<td>0,7</td>
<td>0,72</td>
<td>0,7</td>
</tr>
<tr>
<td>IC</td>
<td>8,22</td>
<td>7,36</td>
<td>7,71</td>
<td>7,84</td>
<td>7,79</td>
</tr>
<tr>
<td>Teinte</td>
<td>0,45</td>
<td>0,47</td>
<td>0,46</td>
<td>0,47</td>
<td>0,47</td>
</tr>
<tr>
<td>Anthocyanes (mg/L)</td>
<td>478,3</td>
<td>392,4</td>
<td>488,8</td>
<td>430,8</td>
<td>435,8</td>
</tr>
</tbody>
</table>

France 2015: Grenache (INRA Pech-Rouge)
## Pilot trials Italy 2015 - example Sangiovese

### Instituto Agrario Ciuffelli Todi

<table>
<thead>
<tr>
<th>Modality</th>
<th>Inoculation yeast</th>
<th>Inoculation wine bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>Saccharomyces</em> Lalvin CLOS** 25 g/hL</td>
<td><em>O. Oeni</em> VP41 dopo 24 ore *</td>
</tr>
<tr>
<td>B</td>
<td><em>Saccharomyces</em> Lalvin CLOS** 25 g/hL</td>
<td><em>O. Oeni</em> Elios ALTO dopo 24 ore *</td>
</tr>
<tr>
<td>C</td>
<td><em>Saccharomyces</em> Lalvin CLOS** 25 g/hL</td>
<td><em>Lb. Plantarum</em> ML-Prime dopo 24 ore *</td>
</tr>
<tr>
<td>D</td>
<td><em>Saccharomyces</em> Lalvin CLOS** 25 g/hL</td>
<td><em>O. Oeni</em> PN4 dopo 24 ore *</td>
</tr>
</tbody>
</table>

**Kinetics of malic acid degradation after co-inoculation in a 2015 Sangiovese (I)**

### Wine analyses 14/4/2016

<table>
<thead>
<tr>
<th></th>
<th>VP41</th>
<th>Elios Alto</th>
<th>ML-Prime</th>
<th>PN4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (% vol.)</td>
<td>14.04</td>
<td>13.73</td>
<td>14.16</td>
<td>14.68</td>
</tr>
<tr>
<td>Res. Sugar (g/L)</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Total Acid (g/L tartaric)</td>
<td>5.19</td>
<td>4.88</td>
<td>5.45</td>
<td>5.11</td>
</tr>
<tr>
<td>VA (g/L)</td>
<td>0.27</td>
<td>0.26</td>
<td>0.22</td>
<td>0.34</td>
</tr>
<tr>
<td>pH</td>
<td>3.62</td>
<td>3.6</td>
<td>3.59</td>
<td>3.66</td>
</tr>
<tr>
<td>Ac. malico (g/L)</td>
<td>&lt;0,2</td>
<td>&lt;0,2</td>
<td>&lt;0,2</td>
<td>&lt;0,2</td>
</tr>
<tr>
<td>Ac. Lactic (g/L)</td>
<td>1.72</td>
<td>1.55</td>
<td>1.93</td>
<td>1.66</td>
</tr>
<tr>
<td>Ac. Tartaric (g/L)</td>
<td>2.35</td>
<td>2.3</td>
<td>2.3</td>
<td>2.22</td>
</tr>
<tr>
<td>Glycerol (g/L)</td>
<td>9.9</td>
<td>9.6</td>
<td>9.96</td>
<td>10.25</td>
</tr>
<tr>
<td>SO2 total (mg/L)</td>
<td>53</td>
<td>29</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>SO2 free (mg/L)</td>
<td>12</td>
<td>7</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>IC</td>
<td>4.97</td>
<td>5.172</td>
<td>6.041</td>
<td>6.61</td>
</tr>
<tr>
<td>Tonality</td>
<td>0.77</td>
<td>0.765</td>
<td>0.77</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Winery trials NH Hemisphere
Production of volatile acidity – co-inoculation practice
(24H after yeast addition)

Co-inoculation: always lower VA at the end of MLF with ML Prime
2014 Grenache (traditional vinification) INCAVI

Kinetics of malic acid degradation, volatile acid formation & viability

ML PRIME; fastest MLF in three days. 100% implantation
### Analytical data post bottling

<table>
<thead>
<tr>
<th></th>
<th>VP41 SEC</th>
<th>VP41 Col</th>
<th>ALPHA Col</th>
<th>BETA Col</th>
<th>ML PRIME Col</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Densidad relativa a 20°C</strong></td>
<td>0,9922</td>
<td>0,9922</td>
<td>0,9924</td>
<td>0,9920</td>
<td>0,9926</td>
</tr>
<tr>
<td><strong>Masa volúmica (g/L)</strong></td>
<td>0,9904</td>
<td>0,9904</td>
<td>0,9906</td>
<td>0,9902</td>
<td>0,9908</td>
</tr>
<tr>
<td><strong>Grado alc. adquirido (% v/v)</strong></td>
<td>13,51</td>
<td>13,82</td>
<td>13,69</td>
<td>14,03</td>
<td>13,59</td>
</tr>
<tr>
<td><strong>Azúcares (glucosa+fructosa) (g/L)</strong></td>
<td>&lt;0,1</td>
<td>0,18</td>
<td>0,22</td>
<td>0,22</td>
<td>0,17</td>
</tr>
<tr>
<td><strong>Acidez total (g/L ác. Tart.)</strong></td>
<td>4,8</td>
<td>4,9</td>
<td>4,8</td>
<td>4,7</td>
<td>5,0</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>3,70</td>
<td>3,66</td>
<td>3,72</td>
<td>3,74</td>
<td>3,60</td>
</tr>
<tr>
<td><strong>Acidez volátil (g/L ác. Acético)</strong></td>
<td>0,45</td>
<td>0,39</td>
<td>0,42</td>
<td>0,43</td>
<td>0,35</td>
</tr>
<tr>
<td><strong>Anhídrido sulfuroso libre (mg/L)</strong></td>
<td>24</td>
<td>22</td>
<td>25</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td><strong>Anhídrido sulfuroso total (mg/L)</strong></td>
<td>42</td>
<td>41</td>
<td>56</td>
<td>41</td>
<td>52</td>
</tr>
<tr>
<td><strong>Ác. L-Málico (g/L)</strong></td>
<td>&lt;0,1</td>
<td>&lt;0,1</td>
<td>&lt;0,1</td>
<td>&lt;0,1</td>
<td>&lt;0,1</td>
</tr>
<tr>
<td><strong>Ác. Tartárico (g/L)</strong></td>
<td>2,21</td>
<td>2,16</td>
<td>2,04</td>
<td>1,91</td>
<td>2,20</td>
</tr>
<tr>
<td><strong>Ác. Cítrico (g/L)</strong></td>
<td>0,18</td>
<td>0,16</td>
<td>0,08</td>
<td>0,05</td>
<td>0,17</td>
</tr>
<tr>
<td><strong>Glicerol (g/L)</strong></td>
<td>7,5</td>
<td>6,8</td>
<td>6,1</td>
<td>7,1</td>
<td>6,9</td>
</tr>
<tr>
<td><strong>Nitrogeno amoniaca y amínico (mg/L)</strong></td>
<td>37</td>
<td>30</td>
<td>48</td>
<td>56</td>
<td>36</td>
</tr>
<tr>
<td><strong>Nitrogeno amoniaca (mg/L)</strong></td>
<td>11</td>
<td>8</td>
<td>8</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td><strong>Nitrogeno amínico (mg/L)</strong></td>
<td>26</td>
<td>22</td>
<td>40</td>
<td>45</td>
<td>29</td>
</tr>
<tr>
<td><strong>IPT (Abs. 280)</strong></td>
<td>35,389</td>
<td>37,017</td>
<td>38,985</td>
<td>29,788</td>
<td>42,281</td>
</tr>
<tr>
<td><strong>Abs. 420 (color amarillo)</strong></td>
<td>2,4534</td>
<td>2,2900</td>
<td>3,1009</td>
<td>2,8139</td>
<td>3,1003</td>
</tr>
<tr>
<td><strong>Abs. 520 (color rojo)</strong></td>
<td>3,7910</td>
<td>4,8653</td>
<td>5,0902</td>
<td>4,4766</td>
<td>5,2249</td>
</tr>
<tr>
<td><strong>Abs. 620 (color azul)</strong></td>
<td>0,6186</td>
<td>0,7882</td>
<td>1,7014</td>
<td>0,7941</td>
<td>0,9295</td>
</tr>
<tr>
<td><strong>Intensidad colorante</strong></td>
<td>6,8630</td>
<td>8,6035</td>
<td>9,2625</td>
<td>8,0946</td>
<td>9,2628</td>
</tr>
<tr>
<td><strong>Taninos (g/l)</strong></td>
<td>1,15</td>
<td>1,32</td>
<td>1,28</td>
<td>1,26</td>
<td>1,41</td>
</tr>
<tr>
<td><strong>Antocianos (mg/l)</strong></td>
<td>224</td>
<td>261</td>
<td>217</td>
<td>246</td>
<td>250</td>
</tr>
</tbody>
</table>

**ML PRIME lower VA and significant higher color values**
While a reduction in color was observed in wines that underwent MLF with Alpha or Omega, no loss of color or polymeric pigment was noted in wines that underwent MLF with ML Prime wine that did not undergo MLF had the highest color with wines that underwent MLF with showing the lowest color loss compared to the control.

Protects the color of red wines
Linked to higher acetaldehyde content on wine inoculated with ML prime
✅ Works excellent in high pH red wines
✅ Fast start of MLF and fast malic acid degradation during AF
✅ No impact on alcoholic fermentation
✅ No increase in VA under high pH
✅ 100% implantation = dominance over wild lactic acid bacteria
✅ Early stabilization of wine
✅ No deviations – good wine quality
✅ Real trend of lower color loss
⇒ Secure and protect the wine quality against spontaneous MLF
<table>
<thead>
<tr>
<th><strong>Kind of wines</strong></th>
<th><strong>Red vinification</strong> – traditional vinification thermovinification (liquide phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing of bacteria inoculation</strong></td>
<td>24 h after yeast – only co-inoculation</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>&gt; 3.4 – (malic acid content – max 3 g/L)</td>
</tr>
<tr>
<td><strong>Temperature limitation</strong></td>
<td>20 to 26°C</td>
</tr>
<tr>
<td><strong>SO₂ addition on grapes / must</strong></td>
<td>&lt; 5 g/hL</td>
</tr>
<tr>
<td><strong>Initial malic acid</strong></td>
<td>&lt; 3 g/L</td>
</tr>
<tr>
<td><strong>Inoculation rate</strong></td>
<td>Use the right dosage (10g/hL)</td>
</tr>
</tbody>
</table>

No nutritional demand / No limitation about initial sugars content
To summarize...why introduce bacteria earlier in the winemaking process?

 ✓ To assure a fast completion of MLF (time saving, wines ready to be sold earlier)

 ✓ To reduce the risk of growth of undesired bacteria and and/or Brettanomyces (respect the work done in the vineyard and keep the quality potential of the wine)

 ✓ To achieve a successful MLF under difficult conditions to positively contribute to the wine sensory profile

 ✓ To save energy and money (no heating, MLF monitoring analysis costs)
Co-inoculation in high and low pH conditions

Co-inoculation in low pH white wines to conserve varietal fruit (diacetyl management)! OK – NO RISK

Co-inoculation in red wines to achieve an early dominance of the selected strain, to impact on the flavor profile and for an early stabilization of the wine!

Co-inoculation to reduce acetaldehyde levels and total SO2 needs

OK – BUT WE HAVE A ASSURE A GOOD AND RELIABLE ALCOHOLIC FERMENTATION = RELIABLE YEAST AND GOOD YEAST NUTRITION!
THANK YOU FOR YOUR ATTENTION & QUESTIONS ARE WELCOMED