Wine Making Workshop

Wednesday afternoon 2:00 pm

Where: Grand Gallery (lower level) Room D

CCA Credits: PD(2.0)

Moderator: Nancie Corum, St Julian Wine Company, Paw Paw, MI

2:00 p.m. Cellar Methods to Reduce Methoxypyrazine Levels in Cabernet Franc & Cabernet

Jon Treloar, Horticulture Dept., MSU

2:20 p.m. Wine Production of Clone Trials, Cabernet Franc Training and Shoot Density

Jon Treloar, Horticulture Dept., MSU

2:40 p.m. Mastering the Microbes of Wine Spoilage

Ellen Butz, Enology Specialist, Purdue University

3:20 p.m. Methods of Winery Sanitation

Les Bourquin, Food Science Dept., MSU

4:00 p.m. How to Discover What is Living in Your Winery

Ellen Butz, Enology Specialist, Purdue University

4:30 p.m. Good Manufacturing Practices for Wineries

Linda Jones, Executive Director, Michigan Grape and Wine Industry Council
Influence Of Yeast And Malolactic Bacteria Strain Choice On 3-Isobutyl-2-Methoxypyrazine Concentration In Cabernet Sauvignon And Franc Wines

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Michigan State University, Dept. of Horticulture,
A16 PSS Bldg¹., East Lansing, MI 48824

Introduction: The increased demand for wines with emphasized varietal character has made it necessary to understand the compounds responsible for the flavors and aromas producing that character, and how they may be controlled to produce a consistent product. In cool climate regions that produce Bordeaux style wines from the grapes Cabernet Sauvignon, Cabernet Franc, Merlot and Sauvignon blanc this understanding is crucial. One of the aromas that can be associated with the above varietals is the herbaceous character associated with green bell pepper. It has been well reported that 3-isobutyl-2-methoxypyrazine (IBMP) is the specific compound most responsible for these characteristics (Augustyn, et al. 1982, Harris et al. 1987, Lacey et al. 1991, Allen et al. 1994, 1995).

The detection threshold for IBMP in red wine is reportedly as low as 10-16 ng/L (Kotseridas et al. 1998, Roujou de Boubée et al. 2000). Detectable levels of IBMP in red wine is typically considered unacceptable, while detectable levels in Sauvignon blanc can increase the quality of the wine when balanced by other compounds (Allen et al. 1991, Marais et al. 1999).

Little research has been done to understand the influence of wine production techniques on IBMP concentration. However, the results from these few studies seem to be in agreement suggesting that the majority of IBMP in grape must is leached from fruit in the first 24 hrs of maceration, prior to the onset of alcoholic fermentation. The same reports suggest that first, IBMP concentration increases during fermentation, although the increases were small, and second, malolactic fermentation has limited affect on final IBMP concentration (Sala et al. 2002, 2004, Roujou de Boubee et al. 2002). However, to date there have been no published comparisons of yeast or ML affects on IBMP concentration in finished wine.

Press fractions in red wine have had higher levels of IBMP than free run, and pumping over has little affect (Roujou de Boubée et al. 2002). Oak and phenolic additions to spiked model wine solutions did not substantially affect IBMP concentration, even after 24 days exposure (Hartmann et al. 2002), while grape rachi have been identified as having a dramatic impact on increasing wine IBMP concentrations if left in the fermenting wine. However steam treatment (100°C for 60 min.) of the de-fruited rachi reduced IBMP concentration by 95% compared to wines fermented with non-steamed stems (Hashizume et al, 1997, 1998). Unfortunately, their have been no reports on the affects of heat treatment (hot-pressing) of must, on IBMP concentration in the resulting wine.

The purpose of these investigations was to gain understanding of the practical wine production techniques that can be utilized to influence the occurrence and concentration of 3-isobutyl-2-methoxypyrazine in finished wines. The focus of this effort is on selected strains of commercially available yeasts and ML bacteria applied to uniform grape must and wine of Cabernet Sauvignon and Cabernet Franc.
Source material: All Cabernet Sauvignon and Cabernet Franc grapes used in these experiments were grown at Michigan State University’s Southwest Michigan Research and Extension Center. After harvest the grapes were immediately transported to Michigan State University’s pilot winery at the main campus in East Lansing, MI. After crushing/destemming, must analyses was completed (Table 1.).

Table 1. Fruit composition of source grapes at time of crush.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Harvest Date</th>
<th>Soluble Solids °Brix</th>
<th>pH</th>
<th>Titratable Acidity (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004 Cabernet Sauvignon</td>
<td>10/20/2004</td>
<td>21.3</td>
<td>3.44</td>
<td>8.33</td>
</tr>
<tr>
<td>2004 Cabernet Franc</td>
<td>10/20/2004</td>
<td>22.2</td>
<td>3.56</td>
<td>6.41</td>
</tr>
</tbody>
</table>

2003 & 2004 Yeast Experiments: Wines were produces utilizing different commercially available yeast strains. These wines were bottled and then analyzed for IBMP concentration.

Yeast choice had a significant affect on IBMP concentration in Cabernet Sauvignon wines in both 2003 and 2004. Wines produced using D-21 yeast contained the highest IBMP concentration, statistically higher than Pasteur Red, Fermicru VR5, MI-24, ICV-D80, ICV-D80 and BM-45 (Table 2). More importantly, BM-45 and ICV-D80 were both significantly lower than ICV-D21 and ICV-GRE. There were subtle differences in IBMP concentration among the majority of yeast strains compared in this study, however the differences between the highest concentration (ICV-D21) and the lowest (BM-45) is 35%.

Table 2. Effect of yeast choice on IBMP concentration in Cabernet Sauvignon wines produced in 2003.

<table>
<thead>
<tr>
<th>Yeast Selection</th>
<th>Mean IBMP (ng/L)</th>
<th>Std. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lalvin ICV-D21</td>
<td>4.45a</td>
<td>1.33</td>
</tr>
<tr>
<td>Lalvin ICV-GRE</td>
<td>4.19ab</td>
<td>0.69</td>
</tr>
<tr>
<td>Cepage Cabernet</td>
<td>3.91abc</td>
<td>1.59</td>
</tr>
<tr>
<td>Lalvin W15</td>
<td>3.74abc</td>
<td>0.42</td>
</tr>
<tr>
<td>NT-50, Anchor</td>
<td>3.43abc</td>
<td>0.50</td>
</tr>
<tr>
<td>Lalvin ICV-D254</td>
<td>3.35abc</td>
<td>0.43</td>
</tr>
<tr>
<td>Pasteur Red, Red Star</td>
<td>2.99bc</td>
<td>0.30</td>
</tr>
<tr>
<td>Fermicru VR5</td>
<td>2.98bc</td>
<td>0.41</td>
</tr>
<tr>
<td>MI-24, Mitech</td>
<td>2.98bc</td>
<td>0.52</td>
</tr>
<tr>
<td>Lalvin ICV-D80</td>
<td>2.93c</td>
<td>0.31</td>
</tr>
<tr>
<td>Lalvin BM-45</td>
<td>2.92c</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* p < 0.05

The 2004 yeast study provided additional significant differences in IBMP concentration based on yeast choice (Table 3). Cabernet Sauvignon wines fermented with Enoferm CSM proved to have a significantly lower IBMP concentration then those that were fermented solely by the native flora. In this experiment there was a 37% difference in IBMP concentration in the wines with the highest and lowest IBMP levels.
Table 4. Effect of yeast choice on IBMP concentration in Cabernet Sauvignon wines produced in 2004.

<table>
<thead>
<tr>
<th>Yeast Selection</th>
<th>Mean IBMP (ng/L)</th>
<th>Std. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural/Native Flora</td>
<td>5.48a</td>
<td>0.83</td>
</tr>
<tr>
<td>Pasteur Red</td>
<td>4.47ab</td>
<td>0.86</td>
</tr>
<tr>
<td>Enoferm CSM</td>
<td>3.99b</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* p < 0.05

It is problematic to compare different vintages as well as yeasts responses to two different wines. However, Pasteur Red yeast was utilized in both experiments and provides a base data point to which possible relationships might be calculated. Using the 2004 results, the percentage of difference between the Pasteur Red and CSM (-10.5%), and Pasteur Red and Native (18.5%) can be compared to the 2003 data, assuming the relative response be equal in another wine. Given that the wines fermented with Pasteur Red in 2003 had an average of 2.99 ng/L IBMP the calculated IBMP concentration for CSM and Native flora would be 2.68 ng/L and 3.54 ng/L respectively. Figure 1 illustrates the likely relationship of the response in IBMP concentration based on yeast choice from 2003 & 2004 experiments.

![Fig. 1. Influence of yeast on IBMP concentration. A comparison of 2003 and 2004 Cabernet Sauvignon wines. Shaded data points represent theoretical IBMP concentration based on 2003 and 2004 yeast/IBMP relationships using Pasteur Red as a benchmark.](image)

2004 Malolactic (ML) Bacteria Experiment: The Cabernet Franc must was inoculated with Pasteur Red (RedStar) yeast, and fermented until dryness. After pressing the wine was then separated into 21 vessels (10 liters each) for ML bacteria strain addition. Each vessel was randomly assigned a ML bacteria treatment and replicated three times.
The IBMP concentration in Cabernet Franc wines resulting from different malolactic bacteria fermentations is summarized in Table 4. Lalvin VP41 produced wines with the lowest IBMP concentration, and Lalvin 31 produced wines with the highest. However, the malolactic bacteria did not have a statistically significant effect on the resulting wine’s IBMP concentration.

Table 4. Effect of Malolactic choice on IBMP concentration in Cabernet Franc wines produced in 2004.

<table>
<thead>
<tr>
<th>MLF Selection</th>
<th>Mean IBMP (ng/L)</th>
<th>Std. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lalvin 31</td>
<td>3.08</td>
<td>0.82</td>
</tr>
<tr>
<td>Lalvin Elios 1</td>
<td>2.77</td>
<td>0.46</td>
</tr>
<tr>
<td>Lalvin Pro Vino</td>
<td>2.75</td>
<td>0.44</td>
</tr>
<tr>
<td>Enoferm Beta</td>
<td>2.71</td>
<td>0.34</td>
</tr>
<tr>
<td>Enoferm Alpha</td>
<td>2.51</td>
<td>0.27</td>
</tr>
<tr>
<td>Viniflora Oenos</td>
<td>2.48</td>
<td>0.39</td>
</tr>
<tr>
<td>Lalvin VP41</td>
<td>2.31</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Conclusions: Yeast choice clearly has an impact on the IBMP concentration of wines (Tables 2 & 3). Of the yeast strains included in this study, CSM, BM-45 and ICV-D80 produced wines with the lowest IBMP concentration. ICV-GRE and D-21 produced wines with the highest IBMP concentrations. Significant differences in IBMP concentration of as much as 37% (1.53 ng/L) were shown, however the fermentation of wines having differing initial IBMP concentrations would be necessary to determine whether a 37% difference would result in a wine with a considerably higher initial IBMP concentration.

Malolactic bacteria choice did not appear to have a clear effect on IBMP concentration in the resulting wine, this is consistent with previous studies (Sala et al. 2002, 2004, Roujou de Boubee et al. 2002). Lalvin VP41 produced wines with the lowest IBMP concentration (2.31 ng/L), and Lalvin 31 produced the wines with the highest IBMP concentration (3.08 ng/L) although these differences were not statistically significant (Table 4).
Literature cited:
Wine Production Assessment of Clonal Trials, Cabernet Franc Training, IBMP and Experimental Breeding Program Selections

Jon D. Treloar¹ and G. S. Howell,
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A16 PSS Bldg¹, East Lansing, MI 48824

Objectives:

a) Produce experimental wines from each clone, selection or cultural trial using standardized methods to evaluate the wine quality in association with the viticultural assessments.
b) Create a library of wines available for future evaluation and enological research.
c) Provide specific opportunities for industry persons to evaluate specific cultivars grown under Michigan conditions.

Accomplishments:

Wines were produced for the following Viticultural Experiments

1. Clonal Evaluations – Riesling (Table 1.), Pinot Blanc (Table 2.), Cabernet Sauvignon (Table 3.), Pinot Noir (Table 4.)
2. Hybrid and Advanced Breeding Program Selections – Hibernal (GM322.58), Frontenac, Vignoles, and Marechal Foch (Table 5.)
3. Cabernet Franc Training System & Shoot Density Assessment – Midwire Sylvoz, Scott Henry, Low Cordon, Fan, Guyot and Hudson River Umbrella (HRU). Each system had four different shoot density treatments, Control – no thinning, 10 shoots/m, 15 shoots/m, Handwidth (Table 6.)
4. Impact of Leaf Removal Timing on IBMP concentration in Merlot (Table 7.), Cabernet Franc (Table 8.) and Cabernet Sauvignon (Table 9.). Treatments at Pea-size, Berry-touch, Veraison. 2-weeks post veraison and control - no leaf removal.

Results:

Riesling – The Riesling clones at Fenn Valley were harvested slightly early (10/5/05) due to excessive Botrytis, and sour rot infection. The fruit was underripe, although the concentration effect of the Botrytis infection did result in fruit with a °brix of 20.5-20.9 (Table 1.). During vinification higher sulfur addition was required, and the alcoholic fermentations were stopped prematurely in an attempt to reduce the oxidative affects of the laccase activity associated with the Botrytis infection. Although most of the laccase activity was inhibited by the R.S. in the wines, some oxidation occurred. The low fruit quality also resulted in excessive volatile acidity.

Pinot Blanc – Both clones ripened adequately although cl.152 achieved 1.6 °brix higher than cl.159. The wines from the two clones both have very nice balance and did not require any cellar modifications.

Cabernet Sauvignon- All clones achieved at least 21.7°brix, some reaching 22.5 °brix (Table 4.) The wines produced all exhibit strong varietal character with IBMP levels below human detection threshold. UCD 21 contained the highest level of IBMP, with UCD 5 containing the lowest.
Pinot Noir – All of the clones ripened exceptionally well, with cl.113 & cl.115 reaching 24.0 °brix (Table 3.) As typical, these two clones produced the most desirable wines, although all of the clones produced exceptional quality wines as a result of the extraordinary growing season.

Hybrid/Breeding selection – Once again the Hibernal fruit was exceptional. Although it had slightly less aromatics than previous years, the fruit chemistry was near perfect for a dry white wine. This variety deserves closer attention due to its ability to produce very balanced wines in very different years. Frontenac ripened up very well, with an average of 26.2 °brix among the four shoot densities. Notorious for having extremely high T.A., this year Frontenac averaged below 8.0g/L.

Cabernet Franc Training – All of the fruit from this trial were ripe, with an average of 22.85 °brix. Wines produced from the training system/shoot density trial were very good examples of Cabernet Franc, and showed very little difference among the treatments. *See MGWIC Final Report for tables 7,8 &9.

IBMP Leaf Removal - The fruit was harvested slightly early in hopes of retaining some of the IBMP in such an outstanding year. Although the fruit was harvested 2-4 weeks early, the Merlot, and Cabernet Franc already had an average of 22.0°brix, while Cabernet Sauvignon had an average of 20.0°brix (Tables 7,8,9*). The IBMP concentration was below human perception in all of the treatments. *See MGWIC Final Report for tables 7,8 &9.

Funding Partnerships:
The wines from the Cabernet Franc clonal trial, Cabernet Franc training system/shoot density trial, and leaf removal timing trial were all utilized in studies measuring impacts on 2-Isobutyl-3-methoxypyrazine concentration funded by the Eastern Viticultural Consortium.

The 2005 Vignoles, Hibernal, Frontenac and M. Foch wines were all produced in coordination with the Hrt 434, and Hrt 434 Wine production courses. The students interacted with the entire production of these wines. They have also been evaluated by industry newcomers at Spartan Cellars, as examples of different stylistic approaches for these varietals.
### Table 1. Wine data of Riesling Clones, Treatments and Tons/Acre grown at Fenn Valley during 2005.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Harvest Date</th>
<th>SS</th>
<th>pH</th>
<th>TA</th>
<th>HARVEST</th>
<th>Residual Sugar (mg/100mL)</th>
<th>pH</th>
<th>%alcohol</th>
<th>BOTTLING</th>
<th>Volatile Acidity (g/100mL)</th>
<th>%MLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riesling 21</td>
<td>10/5/05</td>
<td>20.5</td>
<td>3.14</td>
<td>9.50</td>
<td>279.08</td>
<td>8.7</td>
<td>3.05</td>
<td>11.96</td>
<td>0.91</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Riesling 198</td>
<td>10/5/05</td>
<td>20.9</td>
<td>3.17</td>
<td>9.10</td>
<td>1993.13</td>
<td>8.7</td>
<td>3.72</td>
<td>10.40</td>
<td>1.61</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Riesling 110</td>
<td>10/5/05</td>
<td>20.9</td>
<td>3.17</td>
<td>8.90</td>
<td>3192.84</td>
<td>8.5</td>
<td>3.08</td>
<td>9.77</td>
<td>1.63</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Wine data of White grape varieties grown at the Southwest Michigan Research and Extension Center during 2005.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Harvest Date</th>
<th>SS</th>
<th>pH</th>
<th>TA</th>
<th>HARVEST</th>
<th>Residual Sugar (mg/100mL)</th>
<th>pH</th>
<th>%alcohol</th>
<th>BOTTLING</th>
<th>Volatile Acidity (g/100mL)</th>
<th>%MLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinot Blanc 152</td>
<td>9/13/05</td>
<td>21.9</td>
<td>3.22</td>
<td>6.08</td>
<td>54.83</td>
<td>6.4</td>
<td>3.20</td>
<td>12.24</td>
<td>0.71</td>
<td>0</td>
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<tr>
<td>Pinot Blanc 159</td>
<td>9/13/05</td>
<td>20.3</td>
<td>3.21</td>
<td>5.79</td>
<td>55.29</td>
<td>5.6</td>
<td>3.10</td>
<td>11.73</td>
<td>0.37</td>
<td>0</td>
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<tr>
<td>Pinot Gris 3309</td>
<td>9/13/05</td>
<td>21.6</td>
<td>3.31</td>
<td>6.45</td>
<td>70.39</td>
<td>6.6</td>
<td>3.30</td>
<td>12.88</td>
<td>0.57</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pinot Gris 5C</td>
<td>9/13/05</td>
<td>21.7</td>
<td>3.22</td>
<td>6.41</td>
<td>95.14</td>
<td>7.4</td>
<td>3.20</td>
<td>11.80</td>
<td>0.50</td>
<td>0</td>
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</tbody>
</table>

### Table 3. Wine data of Pinot Noir clones grown at the Southwest Michigan Research and Extension Center during 2005.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Harvest Date</th>
<th>SS</th>
<th>pH</th>
<th>TA</th>
<th>HARVEST</th>
<th>Residual Sugar (mg/100mL)</th>
<th>pH</th>
<th>%alcohol</th>
<th>BOTTLING</th>
<th>Volatile Acidity (g/100mL)</th>
<th>%MLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>#115</td>
<td>9/19/05</td>
<td>24.0</td>
<td>3.51</td>
<td>5.00</td>
<td>55.29</td>
<td>6.4</td>
<td>3.50</td>
<td>13.18</td>
<td>0.54</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>#113</td>
<td>9/19/05</td>
<td>24.4</td>
<td>3.42</td>
<td>4.81</td>
<td>59.40</td>
<td>6.4</td>
<td>3.38</td>
<td>13.65</td>
<td>0.47</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>UCD 1A</td>
<td>9/19/05</td>
<td>22.8</td>
<td>3.63</td>
<td>7.28</td>
<td>59.06</td>
<td>5.3</td>
<td>3.80</td>
<td>11.50</td>
<td>0.60</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>UCD 4</td>
<td>9/19/05</td>
<td>23.7</td>
<td>3.78</td>
<td>6.88</td>
<td>77.56</td>
<td>5.6</td>
<td>3.80</td>
<td>13.51</td>
<td>0.74</td>
<td>100</td>
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<tr>
<td>UCD 9</td>
<td>9/19/05</td>
<td>23.3</td>
<td>3.76</td>
<td>8.68</td>
<td>59.05</td>
<td>5.8</td>
<td>3.70</td>
<td>12.78</td>
<td>0.51</td>
<td>100</td>
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</tr>
<tr>
<td>UCD 13</td>
<td>9/19/05</td>
<td>22.4</td>
<td>3.50</td>
<td>6.34</td>
<td>49.05</td>
<td>5.6</td>
<td>3.40</td>
<td>12.39</td>
<td>0.58</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>UCD 23</td>
<td>9/19/05</td>
<td>22.9</td>
<td>3.50</td>
<td>6.74</td>
<td>65.06</td>
<td>4.9</td>
<td>3.60</td>
<td>12.43</td>
<td>0.56</td>
<td>100</td>
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<tr>
<td>UCD 29</td>
<td>9/19/05</td>
<td>22.9</td>
<td>3.76</td>
<td>9.01</td>
<td>52.78</td>
<td>5.6</td>
<td>3.70</td>
<td>12.14</td>
<td>0.62</td>
<td>100</td>
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</tr>
<tr>
<td>UCD 33</td>
<td>9/19/05</td>
<td>22.3</td>
<td>3.65</td>
<td>8.04</td>
<td>62.03</td>
<td>5.5</td>
<td>3.70</td>
<td>11.98</td>
<td>0.47</td>
<td>100</td>
<td></td>
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</table>
Table 4. Wine data of Cabernet Sauvignon clones grown at the Southwest Michigan Research and Extension Center during 2005.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Harvest Date</th>
<th>SS</th>
<th>pH</th>
<th>TA</th>
<th>Residual Sugar (mg/100mL)</th>
<th>Total Acidity (g/L)</th>
<th>pH</th>
<th>%alcohol</th>
<th>Volatile Acidity (g/100mL)</th>
<th>%MLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCD 2</td>
<td>10/11/2005</td>
<td>22.3</td>
<td>3.48</td>
<td>6.70</td>
<td>61.92</td>
<td>6.2</td>
<td>3.45</td>
<td>12.00</td>
<td>0.52</td>
<td>100</td>
</tr>
<tr>
<td>UCD 4</td>
<td>10/11/2005</td>
<td>21.8</td>
<td>3.32</td>
<td>7.80</td>
<td>63.57</td>
<td>5.7</td>
<td>3.57</td>
<td>12.54</td>
<td>0.55</td>
<td>100</td>
</tr>
<tr>
<td>UCD 5</td>
<td>10/11/2005</td>
<td>22.5</td>
<td>3.40</td>
<td>6.40</td>
<td>64.93</td>
<td>6.6</td>
<td>3.51</td>
<td>13.10</td>
<td>0.40</td>
<td>100</td>
</tr>
<tr>
<td>UCD 8</td>
<td>10/11/2005</td>
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<td>72.04</td>
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<td>UCD 10</td>
<td>10/11/2005</td>
<td>21.7</td>
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<td>5.9</td>
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<td>12.88</td>
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Table 5. Wine data of Hybrids and Experimental Breeding program wines made during 2005.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Harvest Date</th>
<th>SS</th>
<th>pH</th>
<th>TA</th>
<th>Residual Sugar (mg/100mL)</th>
<th>Total Acidity (g/L)</th>
<th>pH</th>
<th>%alcohol</th>
<th>Volatile Acidity (g/100mL)</th>
<th>%MLF</th>
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<tr>
<td>Foch Sparkling</td>
<td>9/8/05</td>
<td>19.0</td>
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<td>3.20</td>
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<td>3.25</td>
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Mastering the Microbes of Wine Spoilage

Ellen Harkness
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W. Lafayette, IN  47907-1160

The production of wine is still a magical and moderately unpredictable endeavor. Since grape must is not sterilized to kill all microorganisms, nor is a production facility ever sterile, no matter how thorough the sanitation process, every fermentation is a mixed bag - every wine a zoo of yeasts and bacteria fighting for available nutrients, each beast adding his own particular character to the final brew. The gist of this lurid description it to reassure you that every winemaker has made wines which smell of elephant droppings; but, the winemaker dedicated to quality control, with a good basic understanding of the behavior of the organisms involved, should rarely experience such a disaster.

Microbes are living organisms that can only be seen with the aid of a microscope. The ones that play a part in the final character of wine can be grouped into two separate kingdoms of the Five Kingdom Classification System, the Fungi (yeasts and molds) and the Procaryotes (bacteria). Bacteria are very primitive in that they usually require less complex nutrients, they have a simple reproductive system. Yeasts and molds are structurally more complex, and generally they are more tolerant of inhospitable environments. Fungi are active at low pH, they tolerate high sugar or salt concentrations, lower water content, less nitrogen and lower temperatures. These are generalizations; however, they are fairly accurate when limiting our microbial discussion to those associated with wine.

FACTORS AFFECTING MICROBIAL GROWTH

As with all living things, the ability of microbes to thrive is directly dependent on their environment.

Moisture is absolutely essential to cellular activity. In extremely dry environments, actively growing cells will eventually die or go into a dormant state if they have that capability. As the moisture content of the environment increases, cellular activity increases. In situations where there is a very high concentration of dissolved solids (sugars or salts) living cells cannot utilize the moisture in solutions and are not able to multiply, - thus jellies, and concentrates, and brine solutions are resistant to microbial spoilage. Juice concentrates should be greater than 68 Brix for long term storage, and still should be held at refrigeration temperatures.

Temperature is a controlling factor for microbial activity. Optimum growth rates for wine related organisms is in the range of 60-85 F; however, some yeasts are quite capable of fermenting slowly at temperatures as low as 28 F. Most microbial growth ceases at temperatures above 100 F, and viable cells (actively growing cells) die at temperatures above 155 -160 F, especially if they are exposed to that temperature for 3-10 minutes.
Nutrients are required by all cells, but each organism has specific and differing requirements. Wine provides carbohydrates in the form of sugars, ethanol and organic acids. Amino acids and ammonia are available in wine and juice as sources of nitrogen and juices contain vitamins and other complex factors required for bacterial growth.

pH, a measure of acid strength, is a major factor controlling microbiological activity, and low pH is one of the main reasons so few microbes will grow in wine. Yeasts and a few Leuconostoc SP. will tolerate pH as low as 2.8. Wine organisms are increasingly active as pH increases to 4.0. Since wines experience serious non-microbial problems at pH higher than 4.0, high pH is never a factor in inhibiting microbial growth in wines or juices.

THE FUNGI

Filamentous fungi or molds will not grow in bottled or properly stored bulk wine, but they are often associated with vineyard bunch rot and sour rot. They will happily grow in any damp place with a bit of grape juice or wine left behind, such as hoses, barrels, air locks, and musty/moldy aromas have never been appreciated, whether in tennis shoes or wine. The main offenders have names like Penicillium, Aspergillus, Mucor, Rhizopus, Botrytis, and they are all detrimental to wine quality. Increased levels of glycerol, acetic acid, ethanol and gluconic acid are noticeable in musts extracted from rotten fruit. Botrytis is the fungus responsible for the condition we call Noble Rot. Infection occurring late in the season, followed by DRY conditions, may produce concentrated berry components by a wicking action. Since the mold produces gluconic acid, glycerol, galacturonic acid, attacks malic and even tartaric acid, the resulting wine will have very different flavor/aroma characteristics. Often the line between "rotten" rot and "noble" rot is very fine indeed. Under the best conditions, it is the only laudable mold associated with wine.

Yeast are classified as Fungi although they have many noticeable differences. Microscopically they are typically single celled, oval or spherical, multiply by producing a structure called a bud at some point on the mother cell that grows and finally splits off. Although some yeast may produce filamentous looking structures, they are not the true hyphae with cross membranes produced by filamentous fungi. As many as 19-20 different yeasts have been reported occurring on the skins of grapes, the vast majority of which are considered undesirable in winemaking. There is a significant difference in the alcohol tolerance of different yeast species that accounts for the "sequential" nature of yeast populations in a fermenting wine. Various reports suggest that yeast populations at the beginning of all fermentations are mixed, comprised mainly of Kloekera, Hanseniaspora as well as Candida, Pichia and Pansexual. Saccharomyces cerevisiae, the one yeast capable of fermenting sugars to appropriate alcohol levels, is rarely isolated in uninoculated musts. Saccharomyces appears as alcohol levels increase, and the less tolerant varieties are eliminated. Brettanomyces is also rarely reported in must and early fermentations but may become more prevalent as fermentation finishes.

For winemaking and quality control purposes, yeasts can be divided into two groups based on their chemical behavior.

Fermentative yeasts are responsible for the conversion of sugar solutions to alcoholic solutions, a process that does not require oxygen. The primary fermentative yeasts are:

Kloekera, a relatively small, (2-4 um), lemon shaped yeast, often the primary wild yeast in early fermentation. It may be associated with higher levels of acetic acid and aldehydes if its dominance is not challenged by the more desirable fermenter,
**Saccharomyces cerevisiae**, and *S. bayanus* are round medium to large cells (7 X 8 um), showing budding at ends. They are alcohol tolerant to 12-14% or higher, moderately tolerant to sulfur dioxide and have a wide temperature range tolerance. These strains rapidly produce alcohol from sugar without adding objectionable odor or flavor components and are the yeasts selected for nearly all of the commercial starter cultures.

*Brettanomyces* is considered an undesirable fermenter since it is capable of producing large amounts of acetic, isobutyric and isovaleric acids, yielding a pungent mousy or horsey, sometimes metallic character to the wine. In general this yeast is considered a serious spoilage organism and most wineries are careful to avoid any growth in their wines. However, since *Brettanomyces* has been credited with some of the unique characteristics of French Burgundy wines, some American wine makers are experimenting with carefully controlled populations in their wines. It is easily identified in culture by its relative resistance to actidione (cyclohexamide), tolerating concentrations in excess of 100 mg/liter. Some strains are fairly resistant to sulfur dioxide at standard use levels. Because of these tolerances this yeast can survive and slowly grow in bottled dry wines, causing increasing damage to the wine and limiting its shelf life.

**Oxidative yeasts** are those that depend on oxygen and are therefore undesirable in winemaking, unless you are a sherry producer. Low alcohol wines are more at risk, with the oxidative yeasts utilizing alcohol and sometimes organic acids as carbon sources, producing volatile esters and aldehydes. These compounds in very small amounts may add complexity to the bouquet of a wine, in larger amounts they will damage the freshness and fruit aromas. All of these are capable of producing a chalky wrinkled white surface film if undisturbed for a long enough period in containers having some head space. They reproduce by budding but are also capable of producing filament-like structures in surface films. The most common oxidative yeasts isolated from wine are:

*Hansenula*, a medium to oval or oblong shaped yeast, is fermentative as well as oxidative since it can produce alcohol from sugar. It has been studied for its potential to develop desirable bouquet characteristics in inoculated mixed cultures.

*Pichia*, is usually a short rod like yeast, often very tolerant of sulfur dioxide and sometimes benzoic acid.

*Candida*, a distant relative of the infectious species *Candida albicans*, often produces long rod shaped cells, budding usually from the ends.

**THE BACTERIA**

Of the thousands of bacteria now classified, only 6 different genera have been found growing in wine. This is due to the nearly antiseptic combination of alcohol and acids that occur. Not only do most organisms not grow in wine, they actually die off if introduced through contaminated water, human or animal contact, or soil contact. Microbiologically speaking, unknown wine is much safer than unknown water. Wine bacteria can be grouped into two camps: those that produce acetic acid, (acetic acid bacteria) and those that produce lactic acid (lactic acid bacteria).

**Acetic Acid Bacteria** are small gram negative organisms. They can utilize alcohol (ethanol) in the presence of oxygen to produce enormous quantities of acetic acid (vinegar), called volatile acidity in wine since the acid can be steam distilled. Even minute quantities of oxygen will support the production of undesirable levels of acetic acid, which may be chemically oxidized to ethyl acetate. They are capable of utilizing various sugars remaining in wines to produce gluconic acid and ketogluconic acids in small amounts, they can metabolize
glycerol to dihydroxyacetone, and they may metabolize (break down) citric, malic, succinic, and fumaric acids. Since organic acids may go on to form aromatic esters with various alcohols, the potential for aroma and flavor changes in a wine under acetic acid bacterial attack is extremely important and nearly unpredictable. Acetic acid is a component of all wines; however, the level at which it is damaging to a wine’s quality varies with the wine and with the consumer. Although yeasts and some other bacteria do produce acetic acid, levels that have a noticeable sensory effect are usually the result of acetic acid bacterial spoilage. BATF regulations set the maximum allowable limit for acetic acid at 0.12gm/100ml in white wines, and 0.14gm/100ml in red wines. Acetic acid bacteria are tolerant of pH levels as low as 3.0 but are more active at higher pH levels.

Two genera of acetic acid bacteria are found in wine.

Gluconobacter are small gram negative bacteria which may be nearly round or seen as long irregular strands. It differs from the other acetic acid bacteria in that it can only oxidize ethanol to acetic acid. Gluconobacter is the predominant acetic acid bacteria on grapes where levels of alcohol are very low. It is responsible for producing undesirable levels of gluconic acid from glucose in badly infected musts, before alcohol concentration reaches 3.5%. Gluconobacter can produce extra cellular fibrils of cellulose from glucose, and may develop a thick tough pellicle on the surface of a must of low alcohol content. Even early stages of cellulose production could affect the filterability of a wine.

Acetobacter species appear identical to Gluconobacter microscopically; however, their tolerance to ethanol, in some cases up to 15%, assures that they are the dominant acetic acid bacteria in finished wines. They are separated from Gluconobacter by their ability to oxidize ethanol to acetic acid and then, under very low ethanol concentrations, on to carbon dioxide and water. Several strains of Acetobacter can also produce cellulose fibers.

Lactic Acid Bacteria are responsible for the process called malolactic fermentation by metabolizing malic acid, producing lactic acid and carbon dioxide. They do not require oxygen. The lactic acid bacteria are gram positive, extremely small, and very slow growing, compared to yeasts. They are also capable of utilizing citric acid and glucose producing important flavor compounds including acetic acid, diacetyl and acetoin. At best, their activity can increase the complexity of a wine, reduce the acidity, and reduce the varietal fruit character. At worst, a flat, mousy, sauerkraut character can develop. Three genera of lactic acid bacteria can be isolated from grapes and wine.

Leuconostoc is the most common lactic acid bacteria isolated from low pH wines. Some will tolerate pH levels of 3.0 and have been reported to induce malolactic fermentation in champagne cuvee material with pH of 2.8-2.9. Microscopically it appears as pairs of cocci (spherical cells) in chains. Since it is a heterofermentative organism ( produces more than one end product), it also produces small amounts of acetic acid. Leuconostoc oenos, now named Oenococcus oenos is the main organism used for commercial malolactic starter cultures.

Lactobacillus are more often isolated from wines with higher pH levels. Microscopically they may appear as short to medium length rods, usually a bit larger than Leuconostoc. Since Lactobacillus grows more rapidly than Leuconostoc, some starter culture work has been done with this genus.

Pediococcus appears as clusters of cocci microscopically, and is easy to recognize. It are homofermentative, producing only lactic acid and carbon dioxide from malic acid, but is not used for starter cultures because of its sensitivity to low pH and high alcohol wines. In general, both Pediococcus and Lactobacillus are
associated with off flavors and aromas - they may attack malic acid, citric acid, glucose, glycerol and produce lactic acid, diacetyl, acetic acid, polysaccharides (viscosity), acrolein (bitterness).

**UNINOCULATED YEAST FERMENTATIONS:**

Wine writers give much attention to wines that have been produced the “natural” way, allowing the yeast populations that either come in with the harvest or are in residence in the winery to perform the fermentation. Increased flavor complexity, and body are attributed to some of these wines; however, there are many reasons to take advantage of the selected yeast strains available at very reasonable prices. Stuck fermentations, increased levels of hydrogen sulfide, acetic acid, and many other unpleasant aromas and flavors are common in uninoculated fermentations. If the potential for a unique wine overwhelms your concerns about the potential for spoilage, the following considerations will improve your chances for a marketable product: perfect fruit, free of rot, hand harvested, <6 hrs from harvest to crush, healthy pH at harvest 3.2 - 3.4, CLEAN winery & equipment, daily monitoring of fermentation and of course, commercial yeast culture on hand ready to add if anything starts to smell!

**MICROBIAL SPOILAGE OF BULK WINE:**

Wine stored in bulk tanks is susceptible to microbial problems including undesired malolactic fermentation, acetic acid spoilage, surface yeast growth, and Brettanomyces growth. It is essential that tanks be monitored often in order to detect problems and remedy them before the wine is spoiled beyond repair.

**Malolactic fermentation** is accompanied by gas, decreasing total acidity and increasing pH. Diagnosis involves paper chromatography evaluation looking for the loss of malic acid, microscopic examination and culture of the wine to demonstrate the presence of lactic acid bacteria.

**Acetic acid spoilage**, vinegar spoilage, is always accompanied by an increase in the total acidity, sour taste and vinegar or ethyl acetate smell and eventually a noticeable slimy surface film. Diagnosis involves analyzing for an increase in volatile acidity by the Cash Still or enzymatic methods.

**Surface yeast growth** is noticed as a powdery, white, sometimes blistered film on wine surface that will eventually cause and oxidized or musty smell and darkening/browning of the wine. Microscopic examination of the film will quickly separate this problem from an acetic acid bacteria infection.

**Brettanomyces yeast infection** is common in wineries that import bulk wine from other facilities, it is rarely found in new wineries using only new barrels. Characteristics of Brettanomyces spoilage include increased volatile acidity, horsey, metallic, bandaid or iodine medicinal aromas and flavors. The only real diagnosis is culture of the Brettanomyces yeast on selective medium or chemical analysis of the flavor compounds, 4 ethyl phenol and 4 ethyl guaiacol.

Prevention of spoilage of bulk wines can be accomplished with vigilant attention to basic wine housekeeping practices: maintain all wine containers absolutely full at all times, store wine as cold as possible (26°F – 35°F), and most important, maintain sulfur dioxide at appropriate level for wine pH. The treatment for surface yeast and acetobacter spoilage may also require pad or sterile filtration into a sanitized container, and early bottling.
MICROBIAL SPOILAGE OF BOTTLED WINE:

Since many wines contain fermentable sugars at >0.5 %, refermentation in the bottle is one of the most common and most disruptive bottle problems. Malolactic fermentation and Brettanomyces spoilage occur in bottled wines as neither requires the presence of air. Problems include gas and haziness, bitterness, metallic or horsey flavors, reduced acidity, or crushed geranium leaf odor. A microscopic examination of the bottle sediment can quickly identify the microbial culprit.

Prevention of bottle spoilage is easily accomplished by maintenance of appropriate levels of free sulfur dioxide for wine pH. If residual sugar >0.2%, use potassium sorbate, 180-200 mg/l (1gm per gallon wine) and sulfur dioxide after sterile or tight pad filtration into sanitized filler into sanitized bottle in sanitized room. There is growing interest among producers of large volumes of sweet wines for the compound Dimethyl Dicarbonate (DMDC), a sterilant, sold under trade name Velcorin. The TTB allows the use of up to 200 mg/l in table wines. The product, added at bottling, rapidly destroys yeast and bacteria, then decomposes to CO2 and small amount of methanol within 1-5 hours. Velcorin requires expensive dosing equipment, but may be well worth the trouble and expense for lower alcohol sweet wines and fruit blends that will be shipped away from the winery.

The spoilage of a batch of your precious wine, regardless of the actual value of the product, is never acceptable. Wines are living systems – good winemakers can guide them into healthy beautiful creations that go thru their life cycles with grace and never come home to haunt you.
Microbiological Testing for Winery Sanitation

“What’s Living in Your Winery”

Ellen Butz, Indiana Wine Grape Council
Purdue University, Food Science Department, 7465 Agriculture Mall Dr. W. Lafayette, IN 47907-2009

Whether or not you like to admit it, if you are dependent on controlling the behavior of yeasts to make your living, you are a microbiologist. Fortunately most of the microbes encountered in a winery are involved in performing desired services such as making alcohol out of sugar, and turning malic acid into lactic acid. The encouragement and manipulation of these elite microbes is detailed in the literature distributed with commercial cultures and is generally well known to winemakers. However, enologists are also constantly harassed by microscopic villains, and this presentation is designed to give you some insight in dealing with their detection, identification and control. Although lack of equipment and familiarity with techniques may limit your range, many important microbial matters can be properly attended to by the layman.

You will need to begin with a clean white lab coat and a few bits of micro trivia to impress your visitors. Try these:

1. Wine yeasts replicate mainly by a process called budding, which, under optimum conditions, may result in doubling the population every two hours. Therefore, single, lonely yeast may create a world of more than 16 million relatives in one wild weekend.

2. Wine may appear visibly clear while hosting a party of 100,000 bacteria or 10,000 yeasts in every milliliter. That is 50,000 yeast cells in a teaspoon of wine.

3. One gram of dry yeast contains 10 billion living yeast cells. Think about that next time the can tips over in your refrigerator.

4. No infectious microorganism, whether yeast, bacteria or even virus, can grow or even survive very long in the hostile environment of wine.

WHY SANITATION?

Since microorganisms cannot survive in wine, and food poisoning is not an issue, why do wineries need to concern themselves with the tedious and time-consuming processes involved in winery sanitation? Many reasons come to mind:

- Refuse attracts vermin and insects
- Neglected contamination is more difficult to clean when it has dried and adhered to surfaces, decreasing plant efficiency
- Contamination causes deterioration of plastic, rubber, metal and wood surfaces
- Aesthetic considerations for visitors
- Potential for wine spoilage
A WORKPLACE
The ideal place for your microbiological activities would be a small room, bathroom size, with washable walls, ceiling and floor, and containing a small sink and Formica table top. Put a sign on the door limiting use and access to certified microbiologists (wearing white coats) only.

Second choice would be a corner of a multipurpose room, away from the fermentation area, which could be isolated from visitors, workers and outside air currents when micro work is in progress.

A third, and very weak choice, would be a corner of the winery.

When microbiological tasks must be performed in a multipurpose area, a small-contained workplace called a hood, which can be sanitized and will protect the work area from dust and air currents, is necessary. If you purchase used equipment avoid chemical hoods, as they are designed for rapid air circulation in the wrong direction for micro work and are impossible to sanitize. Plans for building a small effective hood are illustrated in Figure 1. This unit made of 1/2-inch plywood, 1/4 inch Plexiglas and a few hinges, costs less than $50 to build. The plywood back and adjoining sides are cut on a bevel and hinged together to collapse for storage. The Plexiglas panels, which give the user visibility, while deflecting dust and breath aerosols, are slid into grooves made by 1/4-inch chair railing strips. The unit is covered with several coats of white, high gloss, appliance enamel paint.

A small Styrofoam incubator (Fig. 2) will provide a continuous temperature to grow yeasts and bacteria rapidly. The temperature is controlled by the wattage of the light bulb used in a socket assembly, which has been inserted through the side of the Styrofoam ice chest. This unit maintained a constant temperature of 75°F with a 5-watt bulb and cost less than $10 to assemble.

Sanitation of the workspace should be done before and after each use. Clean up spills and dry surface before wiping with 70% alcohol solution (drugstore).

The following list contains most of the equipment needed to collect if you plan to set up a small effective micro laboratory. A source, catalog number and approximate price for all items are listed; however, in most cases they are available from many different suppliers who will be glad to cross reference catalog numbers. Sources listed here are based mainly on price and availability.

EQUIPMENT

1. **Hood** - specifications listed below
2. **Incubator** - specifications listed below
4. **Alcohol Burner - glass lamp.** Fisher Cat # 04-245 AA, $4. *Used to burn off alcohol on disinfected equipment, dry microscope slides, sterilize loop, etc.*
5. **Side Arm Vacuum Flask - 1000 ml Nalgene.** Fisher Cat. # 10-f182-50B, $11. *Supports filtration unit for sterility evaluation of wine and juice.*
6. **Rubber Stopper, size 8, one hole.** Fisher Cat. # 14-135M, pkg 12/$12. *Connects filtration unit to vacuum flask.*
7. **Vacuum Tubing, black rubber.** Fisher Cat. # 14-175D, pkg. 12 ft/$35.  
*Connects vacuum flask to sink aspirator.*

8. **Millipore Yeast & Mold Swab Test Kits.** Millipore Cat. # MYSK 100 25, 25 tests/$109.  
*Used to evaluate microbial contamination of winery equipment, corks, etc.*

9. **Millipore 55 Plus Monitor.** Millipore Cat. # MHWG 05500, pkg 50/$81.  
*For analysis of yeast, mold and bacterial contamination of juice and wine.*

10. **Millipore Media in 2 ml plastic ampules:**  
*Added to 55 PLUS filter monitor after sample filtration to provide nutrient for microbial growth.*

   - **Tomato Juice Broth,** Cat. # MXOOTJ220, pkg 20 ampules/$26  
   *Best for lactic acid bacteria, also yeast and mold.*

   - **WL Nutrient Broth,** Cat. # MOOOOP2N, pkg 20 ampules/$26  
   *Yeast, mold and bacteria.*

   - **WL Differential Broth,** Cat. # MXOOWD220, pkg 20 ampules/$26  
   *Contains cyclohexamide to inhibit yeast growth, except Brettanomyces.*

11. **Paper Chromatography Kit.** Presque Isle Wine Cellars Cat. # PCK-2V, #31.  
*Detects malolactic fermentation in wine.*

12. **Methylene Blue.** Fisher Cat. # M281-25, 25 gms/ $25.  
*Dissolve one gram Methylene blue powder in 10 mls alcohol, add 90 mls water. Use to stain yeast and bacteria for microscopic observation.*

13. **Alcohol, denatured ethanol or isopropanol.** Drug store item.  
*70% solution in water good disinfectant for surfaces that will come in contact with wine. Denatured is fine for culture work, use food grade for corker jaws, filler spouts.*

*Sterile, disposable plastic bags for sample collection from tanks, barrels, etc.*

15. **Microscope, glass slides and coverslips, inoculating loop.**

**SUPPLIERS:**  
- MILLIPORE, PO BOX 255, BEDFORD, MA 01730 (800)645-5476  
- VINQUIRY, 16003 HEALDSBURG AVE., HEALDSBURG, CA 95448 (707)433-8869  
- THE WINE LAB, 477 WALNUT ST. NAPA, CA 94559 (707)224-7903  
- PRESQUE ISLE WINE CELLARS, 9440 BUFFALO RD, NORTH EAST, PA 16428 (814)725-1314

The purchase of a microscope is not within every winery's budget; however, if you do not have access to a university or medical laboratory with a good microscope to help you evaluate wine sediment problems and identify microbes, there are several sources of used microscopes. If you are fortunate enough to find a reasonably priced used microscope in good condition with built in illuminator and oil immersion (1000X) capabilities, and you can have someone test it to be sure it is working well, buy it. Many universities sell surplus microscopes, which have become obsolete, but are more than satisfactory for these purposes. Although phase contrast microscopy is often discussed and can be helpful, the microscope is very expensive and requires a thorough knowledge of microbes to differentiate them from debris in the
sample. The internet is another good source; however, be sure you are buying from a reputable source and that it is guaranteed at least until you can check the instrument out.

THE HUMAN DIRT DETECTOR
Evaluating the effects of winery sanitation procedures should begin with a careful visual and olfactory examination of the area, keeping in mind that one who lives by the sniff test may die by the sniff test. If you see dirt, cork particles, old dried mold residue in tubing, grape skins in the drain, sticky juice on filter drip trays, insects or vermin, or if you smell off odors in tubing, carboys, barrels, trash cans, the area is not sanitary, no matter how much spraying of disinfectant may have been done. If your area passes an eye and nose inspection, then it's time for a microbial culture.

MICROBIOLOGICAL EVALUATION OF WINERY SANITATION
The Millipore Company produces a 'Swab Test Kit' with 'Yeast & Mold Tester', which is effective in determining the population of living yeasts and molds on a surface.

The first step in any microbiological procedure is to eliminate yourself as a source of contamination as much as possible. Putting on a clean lab coat laundered in hot water and Clorox to cover clothing which may be spattered with wine or dirt, controlling long loose hair in a clip, and washing hands with soap, then spraying with 70% alcohol would all be effective. If the directions supplied by Millipore are followed carefully, you will have an accurate estimation of contamination of areas such as tank interiors, valves, corks, filler tubes, etc. The methodology involves rubbing the area in question with the swab, transferring the swab to a buffer solution to suspend any organisms, which have been picked up, and then dipping a media impregnated filter membrane into the buffer. The filter membrane is shaken to remove excess buffer, then replaced in its sterile housing and incubated, filter surface down, for 5-7 days at 75° F.

Look for growth after 48 hours, since mold colonies may grow fuzzy and very large - they will obscure the entire filter after a few days making it impossible to evaluate other growth. Each viable organism that lands on the filter will begin to multiply and eventually produce a small drop-like pile called a colony. Counting the number of colonies gives an estimate of how many living organisms were picked up on the swab. The presence of yeast and/or mold contamination indicates bacterial contamination also exists in the area tested, and the absence of yeast or mold growth gives reasonable assurance that the area is also free of bacteria.

In general, yeasts are smooth, shiny or creamy spots, 2-4 mm in diameter, growing in 2-5 days. Molds will begin as very small rough colonies becoming larger and fuzzier with time. They may be green or olive or black if spores form. Most bacteria will not grow on this kit. Occasionally, Bacillus sop, from dirt may grow forming yeast-like colonies, or appear as a slimy, flat, rapidly spreading white or nearly transparent film on the tester membrane. If the results of your swab culture show more than 2-3 colonies of yeast or mold, better sanitation methods would be suggested. If your tester shows no growth in 5-7 days, give your sanitary engineer a raise.

BOTTLE STERILITY EVALUATION
Although there are many different units designed for measuring living or viable organisms in a product, the Millipore 55 PLUS monitor is easy to use and designed to reduce accidental contamination. This system allows you to filter a volume of juice or wine - trapping any yeasts or bacteria on the surface of a filter membrane. When nutrient media is applied to the membrane and the system allowed to incubate at 75° F for 7-10 days, the organisms will grow into colonies, which can be enumerated and identified.
Careful washing of the bottle to be evaluated, and spraying the neck, cork and cork puller with 70% alcohol will eliminate most of the potential for contamination from the exterior of the bottle. The "Ah-So" type of cork puller with two prongs, used carefully, generally produces fewer cork crumbs than the screw type. Shake the bottle thoroughly just before removing the cork or screw cap and pour 100 ml of wine into the filter unit. If the wine is an unfiltered or very intense red, begin with 25 ml to be sure the entire sample passes through the membrane. Millipore furnishes extensive directions for the use of these units. Sterile water needed to rinse the excess wine (and preservatives such as SO₂ and sorbet) from the membrane may be obtained easily by micro waving a glass bottle with a microwave safe plastic cap half filled with water. Allow the contents of the bottle to come to a boil, and then keep it simmering for 1 minute on low power. As soon as you remove the bottle from the microwave, tighten the cap and allow the contents to cool.

Evaluation of the colonies growing on this filter system is similar to the 'Yeast and Mold Tester' in many ways; however, the WL nutrient medium does allow growth of wine spoilage bacteria and gives some color differentiation of the colonies.

In general, if a colony growing on a filter membrane is larger than 7-10mm after 7 days, slimy, fuzzy or exotically colored (red, pink, orange shades); it is not a wine spoilage organism. Since molds will not grow in bottled wines, they are not a concern in this evaluation. The presence of mold colonies or other non-spoilage organisms may indicate poor techniques during wine sampling, in which case several bottles should be selected for re-testing.

Yeast will produce visible colonies in 3-5 days as described above. Bacteria take 5-10 days; colonies will appear very small, sometimes nearly transparent requiring magnification to identify them. The presence of one or less colonies on the filter after 10 days gives you a great deal of confidence that your wine is microbiologically stable. Even in a very dry wine, <0.1% sugar, the growth of a few yeast or bacteria colonies suggests that you should hold the wine for a few weeks at ambient temperatures and do another filtration evaluation to see if the population is increasing. If you count more than 10 colonies on the filter, immediate action should be taken before bottle spoilage goes any farther.

One good system for evaluating a particular bottling would be to sample the first bottle off the line (to determine whether cleaning procedures were adequate), the last bottle (to determine the integrity of the system) and several at regular intervals during the entire run. The middle samples give some idea of extent of the problem.

Commercial laboratories charge $10-20 for this type of analysis, depending on your requirements. The Millipore kits cost about $1.50 per sample and eliminate the need to mail samples.

When information gleaned from using these simple microbiological techniques is combined with organoleptic observations and chemical analyses, the causes of most wine spoilage can be determined in the small winery. If more information is needed, wine samples or cultures can be sent to commercial labs or universities with wine research and/or extension facilities for final identification.

Although there are many more techniques which can be acquired to do thorough microbial analyses of winery problems, I hope these tips will help you feel more confident in doing some of your own evaluations and controlling the unwanted microbe activity in and around your wines.
PORTO-HOOD

PLEXIGLASS
$\frac{1}{4}'' \times 9'' \times 30\frac{1}{2}''$
$\frac{1}{4}'' \times 13'' \times 30\frac{1}{2}''$

45° BEVEL

32''
24''
16''
12''
12''
1'' DIA.

1/2'' PLYWOOD

STYROFOAM INCUBATOR

THERMOMETER
5-15 WATT

17''
12''
12''

(WATTAGE of lightbulb determines temperature)
Good Manufacturing Practices for Wineries

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Wines are licensed as Food Processing Establishments in Michigan and are subject to annual inspection by the Food and Dairy Division of the Department of Agriculture. Department of Agriculture staff in the Grape and Wine Council program and the Food and Dairy Division are working to create a document to serve as a guide for inspectors and wineries as to the sanitation expectations for wineries. This session will take the form of a discussion of the general outline of the draft recommended practices and an opportunity for winery representatives to share their experiences with Department inspections and general sanitation procedures.