Enzymes: Natural Tools for the Modern Winemaker

Russell Robbins M.S.
Enologist, Laffort
Indiana Presentation 2009
Tools for Winemakers

- Yeast and Bacteria
- Enzymes
- Nutrients
- Tannins
- Fining
- Filtration
- Stabilization
What is an Enzyme?

- A complex protein produced by living cells that promotes specific biochemical reaction by acting as a catalyst

- A tool that a winemaker can use to accomplish specific tasks at a specific time
What are Enological Enzymes?

- Most commercial enzymes are a mixture of many different enzymes which typically contain:
  - Pectin esterase
  - Polygalacturonase
  - Pectin Lyase
  - Hemicellulase
  - Cellulase
- Many commercial enzymes contain other side activities that can be detrimental
  - Anthocyanase
  - Cinnamyl esterase
- Early enzyme preparations were developed for the fruit (apple) industry for one sole purpose- increase yield
- Derived from Aspergillus niger – a fungus
Enzymes as Tools

- Naturally present in grapes and yeast
- Designed to break down cell walls and pectinase quickly
- Cuts at very precise and specific points
- Designed to make winemaking easier and more predictable
- Each preparation designed for a specific task
- You must understand what your specific need is
Enzymatic hydrolysis of pectin substances: Depectinization

Winemaking basics

Polygalacturonase

Arabinase

Pectin esterase

Pectin lyase

Rhamnogalacturonase

Rhamnogalacturonan acetyltransferase

Galactanase

Galactose

Arabinose

Galacturonic acid

Rhamnose

Methyl group

Acetyl group
Enological enzymes work on grape skin cells

Grape skin cell wall structure

- Cellulose fiber
- Xyloglucane
- Pectin substances
Why use enzymes?

• Originally used to increase yield (Bulk=Cheap)
  – Extremely effective but can harm wine quality
  – Very effective on Labrusca varieties
  – Also good for hybrid varieties
• Increase clarification
• Improve filterability
• Increase color and tannin extraction
• Improve complexity, mouthfeel and stability
• Release aroma compounds
• Break down yeast cell walls
Purification and Side-activities

- Bulk enzymes contain many “activities”
- Many activities can be detrimental
  - Cinnamyl esterase can lead to phenolic characters (band-aid, pharmaceutical) (4-vinyl phenol)
  - Anthocyanase can lead to color break down
  - Too much activity can lead to complete degradation of skins and excess sludge
- Purification can remove these bad side-activities
- High quality enzyme preparations designed to be gentle and specific for grape extraction
White winemaking

“In the winemaking of the dry white wines, important choices are made before alcoholic fermentation; then, there are no possible corrections or adjustments. Once alcoholic fermentation has started, potentially the taste of white wines are already largely given.”

Extract from Traité d’œnologie 1998.
A well-planned extraction should limit:

- the phenomena of oxidation
- the extraction of phenolic compounds from the skin
- a pH increase mainly linked to the extraction of potassium from the solid parts of the grape
Effects of high quality enzyme preparation

- Improve the yield extraction of free run juice
- Optimize pressing (lower pressure and shorter cycles)
- Limit skin tearing and harsh treatment
- Efficient at low temperature
- Very little variation in pH
- Facilitate settling, decrease turbidity
- Limit aromatic deviations (volatile phenols) and preserve the aromatic freshness
Aroma results

Aromatic profile analysis of Sauvignon Blanc wines and determination of the chromatic characteristics – Trial New Zealand

<table>
<thead>
<tr>
<th></th>
<th>[3MH] in ng/L</th>
<th>[A3MH] in ng/L</th>
<th>[4MMP] in ng/L</th>
<th>[IBMP] in ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grapefruit, passion fruit</td>
<td>Passion fruit</td>
<td>Box tree, broom</td>
<td>Green pepper</td>
</tr>
<tr>
<td>Lafazym Press</td>
<td>870</td>
<td>126</td>
<td>nd</td>
<td>3</td>
</tr>
<tr>
<td>Enzyme B</td>
<td>598</td>
<td>79</td>
<td>nd</td>
<td>2</td>
</tr>
</tbody>
</table>

Lafazym Press releases more aromas than the control, enzyme B and preserves analytical characteristics.
Lafazym Press trial: Turbidity

Turbidity (NTU)
Lafazym Press trial: Phenolics

Polyphenols extraction

Control
Lafazym Press
Lafazym Press trial: pH variations

pH variations

Control
Lafazym Press
Lafazym Press trial: K+ extraction

Level of Potassium

K+ (mg.L⁻¹)

Control
Lafazym Press
White wine extraction

• Improved aroma extraction
  • Lafazym Press significantly improves aroma extraction
• Improved yields without degrading quality
• Improved clarity in juice
• Improved filtration and settling
• Enzyme designed to sprinkle on the skins
• Improves overall quality
Clarification

• For white and rose wines
• Goal is to break down pectinase as quickly as possible to get maximum clear juice
  – Generally cool so enzyme needs to work in this environment
  – Lafazym CL designed for this purpose
  – Needs to work quickly
• Do not over clarify
• Improves quality of wine
• Add to press pan or filling tank
• Do not use with bentonite
HOW DOES THIS WORK?

**Mechanism of enzymatic settling**

Particles in suspension

- **Pectin**

Floculation, settling

- **Positive charges**

**Pectinases action**

**Electrostatic neutralisation**
Clarification

UGNI-BLANC - 20°C, pH=3,09

- Reference
- Lafazym CL
- Lafazym CL new formulation
Why use enzymes in red wine

- Enzymes function to break up skin cells quickly
  - Tannins and anthocyanins are contained in the skin cells
  - Anthocyanins are water soluble and go into solution quickly
  - Tannins needed to prevent oxidation of anthocyanins but typically need alcohol to be dissolved

- Improved pressing
  - Improves residual sugar
  - Improves free run yield

- Improves color, tannin extraction and stability
- With purified enzymes there is less phenolic acid precursors for *Brettanomyces*
- Wine is more clear and settles faster
Red wine extraction and maceration

- Selection, usage and dose depends on varietal, quality of fruit, target wine
  - Enzymes do not add color
  - Enzymes do not add tannins
- Take into consideration what your mechanical plans for the wine
  - Lower dose if thin skins
  - Lower dose if using pump-overs and have more time
  - Increase dose if colder temp or want extraction in less time
- Use high quality enzymes to improve quality and make downstream winemaking easier
  - Spray on skins as you de-stem
  - Consider using tannin addition as the effect is synergistic
- Do not add $\text{SO}_2$ directly into enzymes
- Do not add bentonite
Enzyme Activities: Lafase HE Grand Cru

Enzymatic actions in cells: Extraction

Lafase drills hole in the cell wall to create a gentle extraction of the most interesting components of the cell: free complexes of tannins in vacuole, tannins bounded to polysaccharides, polysaccharides, … at the earlier stage of the vinification.
## Harvest parameters

### Merlot

<table>
<thead>
<tr>
<th>Manual grape harvest</th>
<th>Reducing sugar (g/L)</th>
<th>Total acidity H₂SO₄ (g/L)</th>
<th>pH</th>
<th>A₂₈₀nm</th>
<th>Anthocyanin at pH3.2 (mg/L)</th>
<th>Malic acid (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept 28th</td>
<td>215 (21.5 BRIX)</td>
<td>2.8</td>
<td>3.5</td>
<td>56.8</td>
<td>852</td>
<td>1.89</td>
</tr>
</tbody>
</table>

### Cabernet Sauvignon

<table>
<thead>
<tr>
<th>Manual grape harvest</th>
<th>Reducing sugar (g/L)</th>
<th>Total acidity H₂SO₄ (g/L)</th>
<th>pH</th>
<th>A₂₈₀nm</th>
<th>Anthocyanin at pH3.2 (mg/L)</th>
<th>Malic acid (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 14th</td>
<td>218 (21.8 BRIX)</td>
<td>4.01</td>
<td>3.4</td>
<td>57.0</td>
<td>1057</td>
<td>2.71</td>
</tr>
</tbody>
</table>
Manual grape harvest

De-stem

Maceration

Moderate Crushing

SO₂ (6 g/hL)

Enzymes (3 to 4 g/100 kg)

Dry active Yeast (20 g/hL)

Traditional winemaking

48 H cold soak
Regulated temperatures (13°C)

Nitrogen addition (180 mg/L final concentration of assimilable nitrogen)

Alcoholic fermentation

Aerations
Pump-overs 2X then 1X
Regulated temperature

Rack off

Pressing

Free run wines

Press wines

Draining: 10 minutes

Malolactic fermentation

Racking

SO₂ (4 g/hL)

AGING

Direct inoculation
Temperature: 20°C
Comparison of the pomace (Merlot)

Under the same mechanical actions, the pomace of the treated must is dryer and more extracted than the one of the control.
The modified color intensity and the total phenolic compounds were measured regularly during winemaking. Enzymes allow more extraction of color than the natural extraction.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Enzyme</th>
<th>Cold soak</th>
<th>2 days maceration</th>
<th>5 days maceration</th>
<th>8 days maceration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Color intensity</td>
<td>A280</td>
<td>Color intensity</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>Yes</td>
<td>0.04</td>
<td>10.9</td>
<td>0.81</td>
</tr>
<tr>
<td>Lafase HE « Grand Cru »</td>
<td>4 g/100 kg</td>
<td>Yes</td>
<td>0.04</td>
<td>13.3</td>
<td>1.54</td>
</tr>
<tr>
<td>Lasase HE « Grand Cru »</td>
<td>4 g/100kg</td>
<td>No</td>
<td>0.06</td>
<td>11.7</td>
<td>1.30</td>
</tr>
</tbody>
</table>
### Volumes of free run and press wines

<table>
<thead>
<tr>
<th>Samples</th>
<th>Free run wine (% total Vol)</th>
<th>Press wine (% total Vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MERLOT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>84.5</td>
<td>15.5</td>
</tr>
<tr>
<td><strong>Lafase HE Grand Cru</strong> 4 g/100 kg (no cold soak)</td>
<td>89.2</td>
<td>10.8</td>
</tr>
<tr>
<td><strong>Lafase HE Grand Cru</strong> 4 g/100 kg (with cold soak)</td>
<td>87.7</td>
<td>12.3</td>
</tr>
<tr>
<td><strong>CABERNET</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>79</td>
<td>21</td>
</tr>
<tr>
<td><strong>Lafase HE Grand Cru</strong> 3 g/100 kg (no cold soak)</td>
<td>84</td>
<td>15</td>
</tr>
<tr>
<td><strong>Lafase HE Grand Cru</strong> 3 g/100 kg (with cold soak)</td>
<td>82</td>
<td>18</td>
</tr>
</tbody>
</table>
Comparison of free run wine yields
Cabernet Sauvignon

+12% of free run wine compared to the control
Residual sugar in press wines
Cabernet Sauvignon

Press wines with enzymes contained less residual sugar (0.8 g/L) than those with no enzymes (2.1 g/L)
Polyphenolic profile - Merlot 2004

Lafase HE Grand Cru
4 g/100kg, no maceration
Polyphenolic profile - Merlot 2004

Lafase HE Grand Cru
4 g/100kg with cold soak
Other Enzymes

- β-glucosidase
  - Cleaves glucose from terpenes in wine to release aroma - *Gewurtztraminer, Traminette, Riesling*
  - Does not work as well in juice (glucose is inhibitory)

- Proteases
  - Currently none that work at wine pH
Other Enzymes

• β - 1,3 glucanase for grapes
  – Botrytis produces glucanase
  – Certain grape varietals have increased glucanase
  – Too much glucanase degrades filtration
  – Improves filtration and settling

• β - 1,3 glucanase for lees
  – Lees (dead yeast) cell walls made from glucanase
  – Lees aging improves mouth feel and stability
  – Speeds up lees aging and need less stirring

Caution

• β - 1,4 glucanase does not work in grapes
  – Usually much cheaper (use in beer)
Thanks for your attention