Sur Lie Science –
Wine Character Revealed

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Laffort USA

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Sur Lie Ageing - Batonnage

The Roman historian Cato is credited with observing that wines left on their lees developed different flavors than those racked clean.

**Sur Lie** is the French term for leaving the wine in contact with its lees.

**Battonnage** is the term for stirring the lees back up into the wine.

Classical French Burgundian schedule for sur lie cellar ageing:

- Rack off gross lees – “debourbage” – Nov/Dec
- Rack again in March
- Rack again in June – SO2 add
- Rack in Sept followed by cellar ageing/bottling
Using Lees to Drive Wine Style

Observed Benefits of Sur Lie Ageing
✓ enhance structure and mouthfeel
✓ extra body, decreased astringency
✓ increase aromatic complexity
✓ flavor-aroma depth and length
✓ increase perception of sweetness
  ✓ increased color stability
  ✓ increased protein stability
  ✓ increased tartrate stability
  ✓ oxidation protection
✓ improve nutrition for MLF
✓ improved fining and clarity

What Risks are Involved?
✓ reductive aromas – H2S, mercaptans
✓ wine oxidation from frequent stirring
  ✓ microbial sanitation
  ✓ inhibition of MLF
Yeast autolysis occurs at the end stage of alcoholic fermentation and beyond when physical pressure, hydrolytic enzymes and oxidative damage degrade yeast cell integrity releasing cellular components into the wine.
Yeast Derived Molecules from Sur Lie

**Yeast Schematic Diagram**

- Cell Wall Mannoproteins
- Cell Membrane Associated Peptides
- Cytosolic Peptides and S-amino acids
- Cell Wall-Membrane Fragments

*Other molecules will probably be very interesting for winemaking as well...*
Yeast Cell Wall and Membrane

Structure of the yeast cell wall. The wall is primarily composed of mannoproteins and β-glucan.
Sur Lie Research Initiative

Laffort Pillars for Growth

- Research
- Innovation
- Quality

Virginie Moine
Alex Marchal
Ann Hebert
Paul Boyer
Charlotte Gaurroud

Denis Dubourdieu
Philippe Marullo
Marie-Laure Murat
T. Van der Westhuizen
Maryam Ehsani

Today's Focus

- Peptides in Wine
- Mannoprotein Characteristics
- Anti-Oxidation and Fining

LAFFORT
The aims of the present investigation were first to validate the role of yeast lees on the increase of sweetness empirically observed during the autolysis process and then to identify the chemical or biochemical origin of this phenomenon.
Perception of Sweetness in Lees

Validation of the observation of sweetness in lees

Wine base was red wine 12.2% alc, 6.9 g/l glycerol, 0.37 g/l g+f
Lees generated by yeast harvest and placement in red wine base

Forced Ranking Sensory Test

✓ Comparison of ethanol concentrations
✓ Comparison of glycerol concentrations
✓ Comparison of increasing amounts of lees
Validation of Sweetness in Lees

Table 2. Modalities Used for Sensorial Tests

<table>
<thead>
<tr>
<th>factor studied</th>
<th>test</th>
<th>modality 1</th>
<th>modality 2</th>
<th>modality 3</th>
<th>modality 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol effect</td>
<td>ranking (n = 38)</td>
<td>red wine</td>
<td>red wine + 0.5% (v/v)</td>
<td>red wine + 1% (v/v)</td>
<td>red wine + 1.5% (v/v)</td>
</tr>
<tr>
<td>glycerol effect</td>
<td>ranking (n = 38)</td>
<td>red wine</td>
<td>red wine + 1 g/L</td>
<td>red wine + 3 g/L</td>
<td>red wine + 5 g/L</td>
</tr>
<tr>
<td>yeast lees effect</td>
<td>ranking (n = 38)</td>
<td>red wine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>red wine + 2 × 10&lt;sup&gt;6&lt;/sup&gt; cells/mL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>red wine + 4 × 10&lt;sup&gt;8&lt;/sup&gt; cells/mL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>red wine + 8 × 10&lt;sup&gt;8&lt;/sup&gt; cells/mL&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Effect on Sweetness

Table 3. Ethanol, Glycerol, and Yeast Lees Effect on Perceived Sweetness

<table>
<thead>
<tr>
<th>factor studied</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>L</th>
<th>L&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td>98</td>
<td>88</td>
<td>94</td>
<td>100</td>
<td>956</td>
<td>0.34 ns</td>
</tr>
<tr>
<td>glycerol</td>
<td>89</td>
<td>93</td>
<td>99</td>
<td>99</td>
<td>968</td>
<td>1.01 ns</td>
</tr>
<tr>
<td>yeast lees</td>
<td>67</td>
<td>71</td>
<td>106</td>
<td>123</td>
<td>1019</td>
<td>3.87**</td>
</tr>
</tbody>
</table>

<sup>a</sup> R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are the sums of ranks for modalities 1 to 4. <sup>b</sup> L and L<sup>'</sup> were calculated as described in ISO 8587:2006:<sup>31</sup>

\[
L = \sum_{i=1}^{p} iR_i \text{ and } L' = \frac{12L - 3np(p + 1)^2}{p(p + 1)\sqrt{n(p - 1)}}
\]

(n is the number of panelists and p the number of modalities). <sup>c</sup> Significance: ns, nonsignificant; (*) significant at 5%; (**) significant at 1%.
Yeast Lees Autolysis Medium

YLAM prepared to simplify purification

1) Saccharomyces grown in defined medium
2) Cells harvested, washed and resuspended
3) Autolysis for 10 days at 32°C in dark
4) Autolysate subjected to ultrafiltration
Membrane Filtration of YLAM

✓ Fractionation protocol

1. Fermented model medium

2. Retentate >10 KDa

3. Retentate 10-3 KDa

4. Retentate 3-0.5 KDa

5. Retentate < 0.5 KDa

Ultrafiltration vs 10 Kda filter

Ultrafiltration vs 3 Kda filter

Nanofiltration vs 0.5 Kda filter

5 Fractions for testing
Sensory Analysis of UF Fractions

Table 4. Evaluation of Molecular Weight and Biochemical Nature of Sapid Fractions. Confirmation of the Role of Hsp12 Protein

<table>
<thead>
<tr>
<th>modality</th>
<th>fraction name</th>
<th>no. of &quot;correct&quot; answers ($n = 23$)</th>
<th>$P^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>autolysis medium before UF</td>
<td>YLAM</td>
<td>14</td>
<td>0.006**</td>
</tr>
<tr>
<td>retentate after UF 10 kDa</td>
<td>YLAM &gt; 10</td>
<td>4</td>
<td>0.974 ns</td>
</tr>
<tr>
<td>retentate after UF 3 kDa</td>
<td>YLAM 3–10</td>
<td>9</td>
<td>0.349 ns</td>
</tr>
<tr>
<td>retentate after UF 0.5 kDa</td>
<td>YLAM 0.5–3</td>
<td>14</td>
<td>0.006**</td>
</tr>
<tr>
<td>filtrate after UF 0.5 kDa</td>
<td>YLAM &lt; 0.5</td>
<td>8</td>
<td>0.519 ns</td>
</tr>
</tbody>
</table>

\(a\) The expression “correct answers” designates the expected answer, i.e. when the taster has chosen the sample of different composition. \(b\) $P$ was calculated using binomial law. Significativity: ns, nonsignificant; \(\ast\) significant at 5%; \(\ast\ast\) significant at 1%.

In triangle testing only YLAM preparation and 0.5-3.0 kDa retentate showed significant differences in sweetness perception.
Proteinase K Digestion

**Enzymatic treatment investigating the peptide nature of the sapid effect**

1) Concentrated solution of sapid fraction

2) Treatment with Proteinase K

3) Sensory evaluation
## Proteinase K Digest Evaluation

### Table 2. Modalities Used for Sensorial Tests

<table>
<thead>
<tr>
<th>Nature</th>
<th>Triangular</th>
<th>Synthetic Soln</th>
<th>Synthetic Soln + Retentate After Digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical</td>
<td>(n = 23)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Evaluation of Molecular Weight and Biochemical Nature of Sapid Fractions. Confirmation of the Role of Hsp12 Protein

<table>
<thead>
<tr>
<th>Modality</th>
<th>Fraction Name</th>
<th>No. of &quot;Correct&quot; Answers (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autolysis Medium Before UF</td>
<td>YLAM</td>
<td>14</td>
<td>0.006**</td>
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<tr>
<td>Retentate After UF 10 kDa</td>
<td>YLAM &gt; 10</td>
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<td>Retentate After UF 3 kDa</td>
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<td>9</td>
<td>0.349 ns</td>
</tr>
<tr>
<td>Retentate After UF 0.5 kDa</td>
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<td>14</td>
<td>0.006**</td>
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<tr>
<td>Filtrate After UF 0.5 kDa</td>
<td>YLAM &lt; 0.5</td>
<td>8</td>
<td>0.519 ns</td>
</tr>
<tr>
<td>Enzymatic Digestion of YLAM 0.5–3</td>
<td>D-YLAM 0.5–3</td>
<td>7</td>
<td>0.670 ns</td>
</tr>
</tbody>
</table>

*The expression "correct answers" designates the expected answer, i.e. when the taster has chosen the sample of different composition. P was calculated using binomial law. Significativity: ns, nonsignificant; (*) significant at 5%; (**) significant at 1%.
HPLC Peptide Purification

Figure 1. Chromatographic purification of sapid fraction. Chromatograms HPLC with UV detection at 220 nm of (a) YLAM 0.5—3 on Superdex Peptide HR column and (b) collected 34 min peak on RP-18 column.
Peptide Sequencing Results

**sp|P22943|HSP12_YEAST** 12 kDa heat shock protein (Glucose and lipid-regulated protein) – Saccharomyces

- K.ADKVAGKVQPEDNK.G 1498.78600
- K.EYITDKADKVAGKVQPEDNK.G 2248.14557
- K.ASEALKPDSQK.S 1173.61099
- D.AVEYVSGRVHGEED.P 1546.71323
- K.ADVGAKQGDVDGKQVHDSAEKGKDNAEGQGESLADQAR.D 3000.40419

**sp|P00560|PGK_YEAST** Phosphoglycerate kinase (EC 2.7.2.3) – Saccharomyces cerevisiae (Baker’s yeast)

- K.RVFIR.V 690.44095
- D.KISHVSTGGGASLE.L 1342.69612
- E.VWKSSAAGNTVIIGGDATVAKK.Y 2244.25579
- K.SSAAGNTVIIGGDATVAKK.Y 1918.02400
- R.IVAALPTIK.Y 925.60808

**HYPOTHESIS**

Hsp12 peptide source of sweetness

**TEST:**

Genetic Knockout

**Online Capillary HPLC Nanospray Ion Trap MS/MS Analysis**

**BLAST Search for ID of Peptides**

Majority of isolated and identified peptides were from Hsp12

**LAFFORT**
Yeast Strains and Genetics

1) Saccharomyces strain FX-10 is a homothallic, fully homozygous diploid strain

2) Create haploid strain

3) Use Cre-Lox recombination to KO Hsp12

4) Cross ΔHsp12 with FX-10 by spore micromanipulation

5) Segregate and allow self diploid formation (HO endonuclease)

6) Verify homozygous ΔHsp12 by sporulation on selective media and PCR
# Yeast Strains and Genetics

## Table 1. Yeast Strains and Plasmids Used

<table>
<thead>
<tr>
<th>biological material</th>
<th>description</th>
<th>origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yeast Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actiflore C</td>
<td>commercial starter</td>
<td>Laffort Inc. referenced as H4-1D 27</td>
</tr>
<tr>
<td>Fx10</td>
<td>commercial starter $HO/\overline{HO}$ fully homozygous strain ($Zymaflore$ Fx10, Laffort)</td>
<td></td>
</tr>
<tr>
<td>RG1</td>
<td>$F10\ ho::HYG^R,\ Mat\ a$</td>
<td>kind gift of Pr. Richard Gardner</td>
</tr>
<tr>
<td>YPM32</td>
<td>haploid derivate of Fx10, $ho::HYG^R,\ MAT\ a$</td>
<td>this study</td>
</tr>
<tr>
<td>YPM33</td>
<td>YPM32, $hsp12::LoxP::KANMx::LoxP, ho::HYG^R,\ MAT\ a$</td>
<td>this study</td>
</tr>
<tr>
<td>YPM34</td>
<td>YPM33, $\Delta^9hsp12,\ HO::HYG^R,\ MAT\ a$</td>
<td>this study</td>
</tr>
<tr>
<td>YPM35</td>
<td>YPM34 x Fx10 spore, $HO/\overline{HO}$, $HSP12/\Delta^9hsp12$</td>
<td>this study</td>
</tr>
<tr>
<td>$\Delta^9hsp12$</td>
<td>meiotic segregant of YPM35, $HO/\overline{HO}$, $\Delta^9hsp12/\Delta^9hsp12$</td>
<td>this study</td>
</tr>
<tr>
<td><strong>Plasmid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pUG6</td>
<td></td>
<td>kindly donated by Pr. Bruno Blondin</td>
</tr>
<tr>
<td>pZEO</td>
<td></td>
<td>kindly donated by Pr. Bruno Blondin</td>
</tr>
</tbody>
</table>

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*Note:* The table above lists various yeast strains and plasmids used in the study, along with their origins. The table includes information about the commercial starter strains, haploid derivatives, and plasmids, with details on their genetic modifications and sources.
Evaluation of a ΔHsp12 Strain

Table 2. Modalities Used for Sensorial Tests

<table>
<thead>
<tr>
<th>Hsp12 effect</th>
<th>triangular</th>
<th>red wine + Fx10</th>
<th>red wine + Δ°hsp12</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 23)</td>
<td>(2 × 10⁸ cells/mL)ᵃ</td>
<td>(2 × 10⁸ cells/mL)ᵃ</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ These wines and solutions were kept at 32 °C for 10 days before sensory analysis was performed.

Table 4. Evaluation of Molecular Weight and Biochemical Nature of Sapid Fractions. Confirmation of the Role of Hsp12 Protein

<table>
<thead>
<tr>
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<th>fraction name</th>
<th>no. of “correct” answers (n = 23)</th>
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</tr>
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<td>D-YLAM 0.5–3</td>
<td>7</td>
<td>0.670 ns</td>
</tr>
<tr>
<td>autolysis of Fx10 and Δ°hsp12 yeast strains in red wine (Hsp12 effect)</td>
<td>13</td>
<td>0.019*</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ The expression “correct answers” designates the expected answer, i.e. when the taster has chosen the sample of different composition. ᵇ P was calculated using binomial law. Significativity: ns, nonsignificant; (*) significant at 5%; (**) significant at 1%.
Summary of Investigation

✓ Sensory Validation of Sapid Effect of Lees
   not ethanol or glycerol

✓ Biochemical Determination of Sapid Molecule
   protein nature shown by digestion

✓ Purification and Identification of Sapid Peptide
   2 HPLC separations, LC-MS ID, BLAST

✓ Genetic Validation of Sapid Peptide Source
   ΔHsp12 Saccharomyces constructed
Mannoproteins in Wine

Role of Yeast Mannoproteins in Tartrate Stability of Wines
Dubourdieu, D., Moine-Ledoux, V.
1997 Rev. Oenol., 85:17

December 2005 OIV Regulatory Approval

- Gold Innovation Trophy Vinítech 2006
  Bordeaux - France

Mannostab: The Award Winning New Potassium Bitartrate Stabilisation Product
Boyer, P.K., Moine-Ledoux, V.
Australia & New Zealand Grapegrower & Winemaker
June 2007; 57-62
Mannoproteins in Wine

United States Patent [19]
Dubourdieu et al.

BIOLOGICAL SUBSTANCE FOR THE PHYSICO-CHEMICAL STABILIZATION OF WINES

Inventors: Denis Dubourdieu, Beguey, Virginie Moine, Pessac, both of France
Assignee: Faculte d'Oenologie, Talence, France
Appl. No.: 08/817,937
PCT Filed: Oct. 27, 1995
PCT No.: PCT/FR95/01426
§ 371 Date: Apr. 30, 1997
§ 102(e) Date: Apr. 30, 1997
PCT Pub. No.: WO96/13571
PCT Pub. Date: May 9, 1996

Foreign Application Priority Data

Int. Cl.7 ........................................ C12G 1/10; C12G 1/12; C12H 1/10
U.S. Cl. .................. 426/330.4; 426/60; 426/424
Field of Search .......................... 426/330.4, 60, 426/424

Patent Number: 6,139,891
Date of Patent: Oct. 31, 2000

References Cited
PUBLICATIONS

Primary Examiner—Curtis E. Sherrer
Attorney, Agent, or Firm—Young & Thompson

ABSTRACT
A treatment for stabilizing wine against tartaric acids and proteins by adding mannoproteins extracted from yeast walls by enzymatic digestion, is disclosed. A method for carrying out the treatment by extracting mannoproteins from yeast by enzymatic digestion, and the resulting mannoprotein, are also disclosed.

5 Claims, 6 Drawing Sheets
HPLC Analysis of MP Extracts

Heat extraction profile - MEC

Enzyme digestion profile - MEE

- x: spectrophotometric detection at 225 nm (proteins)
- *: refractometric detection (polysaccharides)
Capillary Electrophoresis Separation

Peak W is clearly a point of differentiation between the heat treated sample and the enzyme treated sample.

Peak W was shown to exhibit the protein and tartrate stabilization properties.
Protein Stability in Wines

The following Table shows the results obtained in respect of three white wines treated by different mannoproteins.

<table>
<thead>
<tr>
<th>Different modalities</th>
<th>Turbidity NTU</th>
<th>Quantity of bentonite g/hl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference wine 1</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>Wine 1 + MEC 25 g/hl</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>Wine 1 + MEE1 25 g/hl</td>
<td>4.4</td>
<td>30</td>
</tr>
<tr>
<td>Wine 1 + MEE2 25 g/hl</td>
<td>4.2</td>
<td>30</td>
</tr>
<tr>
<td>Wine 1 + MEE3 25 g/hl</td>
<td>4.3</td>
<td>30</td>
</tr>
<tr>
<td>Reference wine 2</td>
<td>23.1</td>
<td>120</td>
</tr>
<tr>
<td>Wine 2 + MEC 25 g/hl</td>
<td>23.4</td>
<td>120</td>
</tr>
<tr>
<td>Wine 2 + MEE1 25 g/hl</td>
<td>10.5</td>
<td>60</td>
</tr>
<tr>
<td>Wine 2 + MEE2 25 g/hl</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>Reference wine 3</td>
<td>13.8</td>
<td>90</td>
</tr>
<tr>
<td>Wine 3 + MEC 25 g/hl</td>
<td>14</td>
<td>90</td>
</tr>
<tr>
<td>Wine 3 + MEE1 25 g/hl</td>
<td>6.2</td>
<td>50</td>
</tr>
<tr>
<td>Wine 3 + MEE3 25 g/hl</td>
<td>5.8</td>
<td>50</td>
</tr>
</tbody>
</table>

In respect of the mannoproteins extracted by enzymatic digestion, the results clearly show the reduction in the quantity of bentonite required to obtain stability in the wines. The reduction in the quantity of bentonite is 50%.
Analysis of MP32

Only MP32 increased in concentration

Capillary electrophoresis confirms that the MP 32 is present at 2% in the MEC and at 14% in the MEE; see FIG.

% of MP32

<table>
<thead>
<tr>
<th>Molecular weight kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEE</td>
</tr>
<tr>
<td>DEAE (0.25 mole/l)</td>
</tr>
<tr>
<td>Con A (FR)</td>
</tr>
<tr>
<td>77.8</td>
</tr>
<tr>
<td>77.8</td>
</tr>
<tr>
<td>53</td>
</tr>
<tr>
<td>44.1</td>
</tr>
<tr>
<td>44.1</td>
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<tr>
<td>41.6</td>
</tr>
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<td>41.6</td>
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<tr>
<td>35.2</td>
</tr>
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<td>35.2</td>
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<td>31.8</td>
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<td>18.4</td>
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</tr>
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</tr>
<tr>
<td>15.2</td>
</tr>
</tbody>
</table>

% of MP32

<table>
<thead>
<tr>
<th>Mannoproteins</th>
<th>% of proteins</th>
<th>% of polysaccharides</th>
<th>% of mannose</th>
<th>% of glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracted with heat</td>
<td>4.2</td>
<td>93.8</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Extracted enzymatically</td>
<td>15</td>
<td>83.2</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
Specific Mannoprotein Effects

Comparison of the **tartrate stabilization** effect between heat extracted (MEC) and enzyme extracted mannoproteins (MEE)

<table>
<thead>
<tr>
<th>Wine</th>
<th>White 1</th>
<th>Rosé 1</th>
<th>Red 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>***</td>
<td>****</td>
<td>***</td>
</tr>
<tr>
<td>MEC 25 g/hl</td>
<td>**</td>
<td>****</td>
<td>**</td>
</tr>
<tr>
<td>MEE 25 g/hl</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### Differential Specificity of MP

**Tartrate Stability tested at low temperature (-4°C for 6 days)**

<table>
<thead>
<tr>
<th>Wines</th>
<th>Reference</th>
<th>Meso. acid 10 g/hl</th>
<th>MEC 25 g/hl</th>
<th>MEE1 25 g/hl</th>
</tr>
</thead>
<tbody>
<tr>
<td>White 1</td>
<td>***</td>
<td>O</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>White 2</td>
<td>***</td>
<td>O</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>White 3</td>
<td>***</td>
<td>ND</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>White 4</td>
<td>***</td>
<td>ND</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>White 5</td>
<td>***</td>
<td>ND</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>White 6</td>
<td>***</td>
<td>ND</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>Rosé 1</td>
<td>***</td>
<td>ND</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>Rosé 2</td>
<td>***</td>
<td>O</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>Red 1</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>Red 2</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>O</td>
</tr>
<tr>
<td>Red 3</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>O</td>
</tr>
</tbody>
</table>

It will be noted that the mannoproteins extracted by enzymatic digestion of the yeast cell walls prevents the formation of crystals at a dose of 25 g/hl.
Analysis of MP40

HPLC separation of MEE

P1  P2

variation of potassium (g/l) after cold treatment

Purified P1 and P2 fractions by HPLC g/hl

LAFFORT
Analysis of MP40

Through HPLC and Concanavalin A Affinity Chromatography Purification the ~40kDa mannoprotein increased in concentration and effectiveness.

Only fraction P2 including MP40 allows a stabilization.

<table>
<thead>
<tr>
<th>Molecular weight in kDa (kilo dalton)</th>
<th>MEE</th>
<th>P1</th>
<th>P2</th>
<th>FR con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>77.8</td>
<td>77.8</td>
<td>53.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.1</td>
<td>44.1</td>
<td></td>
<td>41.6</td>
<td>41.6</td>
</tr>
<tr>
<td>41.6</td>
<td></td>
<td></td>
<td>35.2</td>
<td>35.2</td>
</tr>
<tr>
<td>41.6</td>
<td></td>
<td></td>
<td>31.8</td>
<td>31.8</td>
</tr>
<tr>
<td>35.2</td>
<td></td>
<td></td>
<td>30.3</td>
<td>30.3</td>
</tr>
<tr>
<td>31.8</td>
<td></td>
<td></td>
<td>27.5</td>
<td>27.5</td>
</tr>
<tr>
<td>30.3</td>
<td></td>
<td></td>
<td>25.2</td>
<td>25.2</td>
</tr>
<tr>
<td>27.5</td>
<td></td>
<td></td>
<td>23.2</td>
<td>23.2</td>
</tr>
<tr>
<td>25.2</td>
<td></td>
<td></td>
<td>21.3</td>
<td>21.3</td>
</tr>
<tr>
<td>23.2</td>
<td></td>
<td></td>
<td>19.8</td>
<td>19.8</td>
</tr>
<tr>
<td>21.3</td>
<td></td>
<td></td>
<td>18.4</td>
<td>18.4</td>
</tr>
<tr>
<td>19.8</td>
<td></td>
<td></td>
<td>17.2</td>
<td>17.2</td>
</tr>
<tr>
<td>18.4</td>
<td></td>
<td></td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>17.2</td>
<td></td>
<td></td>
<td>15.2</td>
<td>15.2</td>
</tr>
</tbody>
</table>

The active fraction thus contains only four mannoproteins, the molecular weights of which are 41.6; 31.8; 17.2; 15.2 kDa.

The only protein which increases in concentration is the 41.6 kDa. Accordingly, this is the mannoprotein responsible for the tartaric stabilization.
Colloidal Behavior of Mannoproteins

Potassium variation (g/l) after cooling wine

Mannostab® g/hl

LAFFORT
Crystalization of Potassium Bitartrate

Mechanism of Crystallization:

1. Nucleation: formation crystal germ
2. feeding: growing of the crystal

Structure of the crystal: orthorhombic geometry
Microscopic Observation of the Crystallization of Potassium Bitartrate

<table>
<thead>
<tr>
<th>Date of obs.</th>
<th>27/06</th>
<th>30/06</th>
<th>2/07</th>
<th>4/07</th>
<th>7/07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td>MP40</td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>

With MP40 crystals are flat - undeveloped
MP40 Mannoprotein Summary

MP 40 the first natural treatment to stabilize tartrate in wines

- Naturally present in wine, MP40 is the only mannoprotein having a stabilizing effect regarding tartrate precipitation in wine

- Effective action based on the inhibition of the crystallization of potassium bitartrate
MP40 Winemaking Impact

Quality Improvements
 ✓ Natural Wine Ingredient
 ✓ Preserves Wine Balance
   ✓ Maintains Color
 ✓ Long Term KHT Stability

Ease of Use
 ✓ Direct Addition to Wine
 ✓ Rapid Dissolution
 ✓ Addition can be Automated
   ✓ Rapid Stabilization
MP40 Winery Impact

**Environmental Benefits**
- ✓ Reduced Water Use
- ✓ Reduced Processing Waste
- ✓ Reduced Carbon Footprint

**Economic Benefits**
- ✓ Increased Wine Yield
- ✓ Reduced Labor - Time
- ✓ No Capital Investment
  - ✓ Energy Savings
- ✓ Reduced Maintenance Costs
Tartrate Stabilization by Inhibitors

TARTRATE STABILIZATION

SUSTERACTIVE TECHNIQUES

- Traditional Cold Stabilization
- Refrigeration

- Membrane Based Technique (Electrodialysis)

NON-SUSTERACTIVE INHIBITORS

- Yeast Mannoprotein (Natural Inhibitor – MP40)

- Carboxymethyl Cellulose (CMC – Cellulose Gum)

- Metatartric acid LAFFORT
CMC Molecular Structure Characteristics

\[ \beta 1-4 \text{ glycosidic linkage} - \text{DP} - \text{Degree of Polymerization} \]
\[ \text{Carboxymethyl groups} - \text{DS} - \text{Degree of Substitution} \]

Polymer generated as a Sodium salt – Refinement/Processing reduces Sodium content

**DP** – Degree of Polymerization
**DS** – Degree of Substitution
Influences - Viscosity, Fluidity
Influences - Solubility, Efficiency

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CMC Oenological Properties

CMC Interaction Disrupts Bitartrate Crystal Formation

CMC action results in an inhibition of microcrystal growth by disorganization of the 010 surface of the nucleated bitartrate crystal.

The negatively charged ionized form of CMC interacts with the (010) face of a bitartrate crystal, specifically the positively charged layer of K⁺ on the crystal face.

Inhibition of KHT crystal growth by CMC
Chemical Oxidation

HYDROXYL (alcohol) → CARBONYL (aldehyde) → CARBOXYLIC ACID

Oxidation of Ethanol to Acetaldehyde and Acetic Acid

- Fenton Reaction
- Fe dependent
- Peroxide reaction product

Hydrogen Peroxide

Linalool

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Fenton Reaction, Sulfites, Oxygen, Catechols and Quinones – Oh My!

Proposed interaction of a catechol and $O_2$ in the presence of sulfite.

Uptake of $O_2$ by polyphenols in model wine in the absence and presence of sulfite

Daniliwicz, AJEV, 62:3 (2011)
Redox Potentials

SO₂
+170 mV

Ascorbic acid / Vit C
+282 mV

Tocopherol / Vit E
+480 mV

Tannins
+600 - 750 mV

Glutathione
+920 mV

SO₂ comes from Yeast as well as Winemaker addition

Tannins come from Grapes and Oak as well as Winemaker addition

Glutathione comes from Grapes as well as Yeast or by Winemaker addition
Glutathione as an Antioxidant in Wine

Glutathione added directly to aqueous solution or finished wine can be rapidly oxidized and with no Glutathione Reductase to recycle it loses antioxidant properties.

Glutathione and its precursors added during late fermentation allows yeast to accumulate and release slowly during lees ageing - autolysis.

\[ \text{ γ-Glutamylcysteine (GGC)} \]

\[ \text{Glutathione} \]

\[ \text{GRP} \]
**Glutathione in Juice and Wine**

<table>
<thead>
<tr>
<th>Glutathione in the juice (mg/L or ppm)</th>
<th>9</th>
<th>5</th>
<th>4</th>
<th>17</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione in the corresponding wine (mg/L or ppm)</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>22</td>
<td>3</td>
</tr>
</tbody>
</table>

Valarie Lavigne, 2000

- Glutathione in juice is proportional to the initial YAN
- Grape GSH can be rapidly lost by oxidative juice handling
- Good AF Nutrition (N/C balance) allows yeast to release additional GSH
- GSH in yeast can be supplemented with a timely nutritional addition
- 20 ppm+ GSH is needed in finished wine for optimal protection
- Recent evidence of Glutathione preservation effect of SO2 in organic wines

LAFFORT
Selective Adsorption-Yeast Hulls

✔ Yeast hulls generated during autolysis with high adsorbing capacity are rich in proteins

<table>
<thead>
<tr>
<th>Albumin</th>
<th>Gelatin 1</th>
<th>Gelatin 2</th>
<th>Yeast Hulls 1</th>
<th>Yeast Hulls 2</th>
</tr>
</thead>
</table>

Samples reacted with Bradford protein reagent

Reagent changes to blue with protein interaction

After centrifugation proteins are localized in tube bottoms, ie in the yeast hulls
Selective Adsorption-Yeast Hulls

✓ Particles size repartition

Despite a negative charge, yeast hulls react with tannins by hydrophobicity. This action mechanism is different from traditional fining agents.
Selective Adsorption-Yeast Hulls

✓ Comparison between albumin and yeast hull fining

Lees 5 times more compact than with albumin

Easier racking, less wine loss
Continuing Investigations

- Lees and Oak Interactions
- Lees and MLF Influences
- Specific Yeast Cell Wall-Membrane Components and Detoxification
WHO ARE WE?

Founded in 1895, LAFFORT S.A.S. is a family-owned French company completely focused on research, production, and distribution of the highest quality and best value enological products worldwide.

Today, Laffort is the number one producer of enological products in the world. We are based in Bordeaux and export to more than 50 countries.

SARCO, our scientific arm, is the largest and best funded private research entity in the wine industry. We also work closely with the University of Bordeaux ISVV and wine Research Institutions around the world.

LAFFORT is certificated ISO 9001 – VERSION 2000 and works in conformity with the referential HACCP.
LAFFORT International Network
Research Quote

“The task is...not so much to see what no one has yet seen; but to think what nobody has yet thought, about that which everybody sees.”

Erwin Schrödinger
1933 Nobel Prize for Physics
Sur Lie Science – Wine Character Revealed

? Questions – Discussion!

Peter Salamone, Ph.D.
Technical Manager
North America

Laffort in Ontario:
Vines to Vintages Inc.
The results obtained with the red wines Nos. 1, 2 and 3 correspond to a non-fined red wine, a red wine fined with gelatine at 10 g/l, and to a red wine fined with egg white at 10 g/l.

<table>
<thead>
<tr>
<th>Different modalities</th>
<th>Non-fined wine</th>
<th>Wine fined with gelatine</th>
<th>Wine fined with egg white</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>90</td>
<td>110</td>
<td>180</td>
</tr>
<tr>
<td>Meso. acid 15 g/l</td>
<td>70</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>Meso. acid 25 g/l</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MEC 15 g/l</td>
<td>90</td>
<td>110</td>
<td>130</td>
</tr>
<tr>
<td>MEC 25 g/l</td>
<td>50</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>MEE1 15 g/l</td>
<td>30</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>MEE1 25 g/l</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gum 15 g/l</td>
<td>90</td>
<td>70</td>
<td>140</td>
</tr>
<tr>
<td>Gum 25 g/l</td>
<td>30</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

It will be noted that the mesotartaric acid produces good results starting from 25 g/l.

The mannoproteins extracted by enzymatic digestion also have an excellent effectiveness at a rate of 25 g/l.