Sur Lie Science –
Wine Character Revealed

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Presented at Brock University – CCOVI   February 6 2013

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

<u>Sur Lie</u> is the French term for leaving the wine in contact with its lees

<u>Batonnage</u> is the term for stirring the lees back up into the wine

Classical French Burgundian schedule for <u>sur lie</u> cellar ageing

Rack off gross lees – “<u>debourbage</u>” – Nov/Dec
Rack again in March
Rack again in June – SO_2 add
Rack in Sept followed by cellar ageing/bottling

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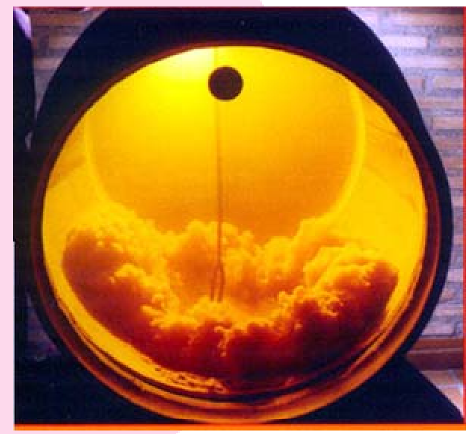
Using Lees to Drive Wine Style

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 – H_2S, mercaptans
- ✓ wine oxidation from frequent stirring
- ✓ microbial sanitation
- ✓ inhibition of MLF

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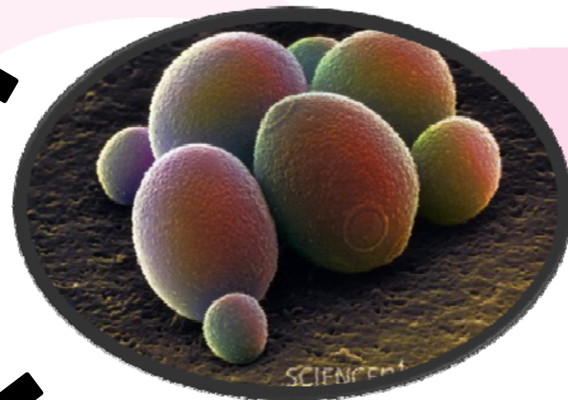
Yeast Autolysis

PEPTIDES – FATTY ACIDS

- flavor-aroma
- sweetness
- nutrients
- anti-oxidation

AMINO ACIDS

- flavor-aroma
- nutrients



POLYSACCHARIDES

- stability
- mouthfeel

- Anti-oxidation
- fining

NUCLEOTIDES

- flavor-aroma
- nutrients

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

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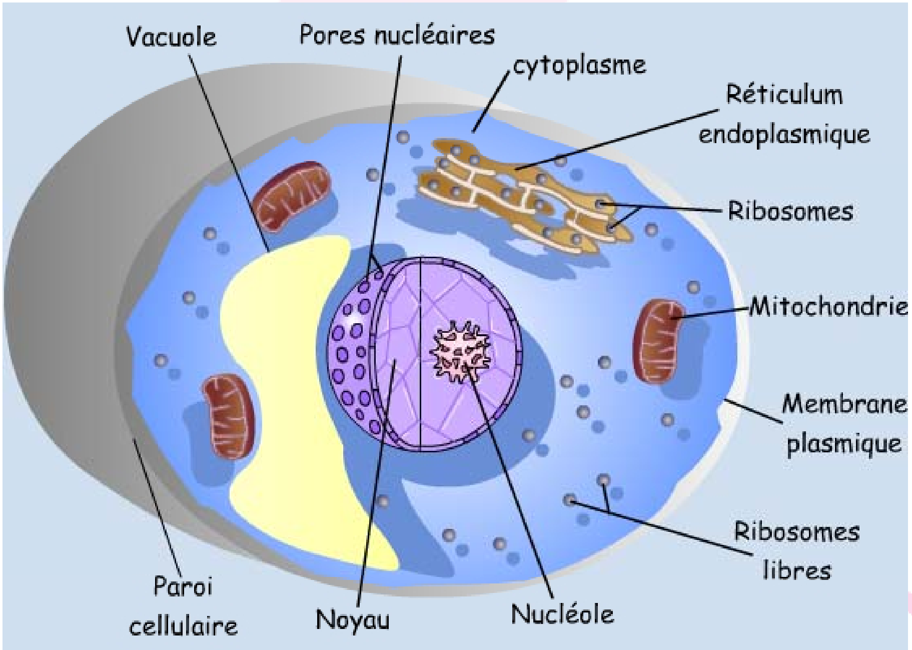
Yeast Derived Molecules from Sur Lie

Yeast Schematic Diagram



Cell Wall Mannoproteins

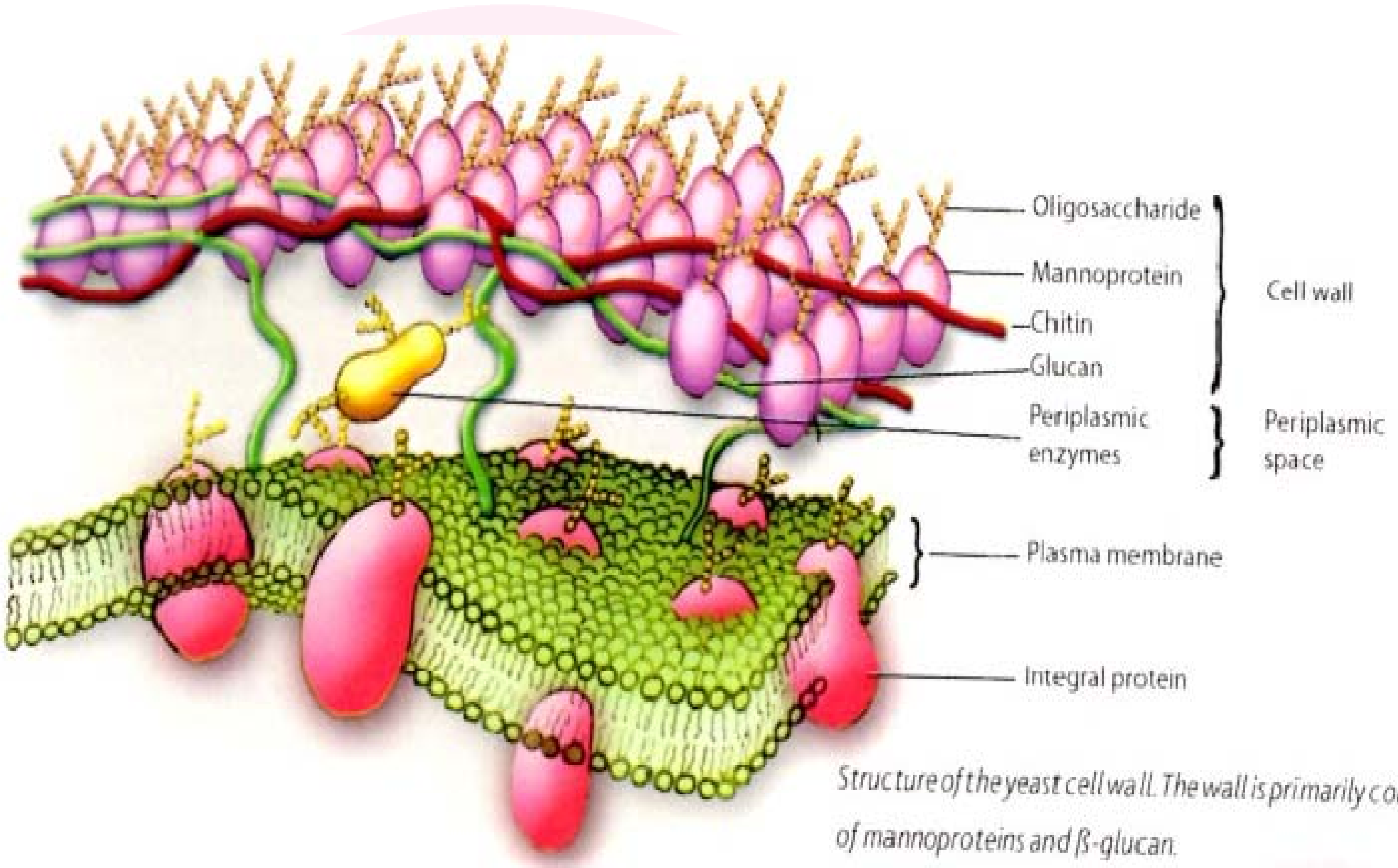
Cell Membrane Associated Peptides

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 ß-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

Todays Focus

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

Peptides in Wine

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Influence of Yeast Macromolecules on Sweetness in Dry Wines: Role of the *Saccharomyces cerevisiae* Protein Hsp12

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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

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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

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Table 2. Modalities Used for Sensorial Tests

factor studied	test	modality 1	modality 2	modality 3	modality 4
			Effect on Sweetness		
ethanol effect	ranking (n = 38)	red wine	red wine + 0.5% (v/v)	red wine + 1% (v/v)	red wine + 1.5% (v/v)
glycerol effect	ranking (n = 38)	red wine	red wine + 1 g/L	red wine + 3 g/L	red wine + 5 g/L
yeast lees effect	ranking (n = 38)	red wine[a]	red wine + 2 × 10^8 cells/mL[a]	red wine + 4 × 10^8 cells/mL[a]	red wine + 8 × 10^8 cells/mL[a]

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

factor studied	R_1[a]	R_2[a]	R_3[a]	R_4[a]	L	L'[b,c]
ethanol	98	88	94	100	956	0.34 ns
glycerol	89	93	99	99	968	1.01 ns
yeast lees	67	71	106	123	1019	3.87**

[a] R_1, R_2, R_3 and R_4 are the sums of ranks for modalities 1 to 4. [b] L and L' were calculated as described in ISO 8587:2006:[31]

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

(n is the number of panelists and p the number of modalities). [c] Significativity: ns, nonsignificant; (*) significant at 5%; (**) significant at 1%.

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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

Ultrafiltration vs 10 Kda filter

2 Retentate >10 KDa

Filtrate

Ultrafiltration vs 3 Kda filter

3 Retentate 10-3 KDa

Filtrate

Nanofiltration vs 0.5 Kda filter

4 Retentate 3-0.5 KDa

5 Filtrate < 0.5 KDa

5 Fractions for testing

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Sensory Analysis of UF Fractions

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

modality	fraction name	no. of "correct" answers[a] (n = 23)	P^b
autolysis medium before UF	YLAM	14	0.006**
retentate after UF 10 kDA	YLAM > 10	4	0.974 ns
retentate after UF 3 kDA	YLAM 3−10	9	0.349 ns
retentate after UF 0.5 kDa	YLAM 0.5−3	14	0.006**
filtrate after UF 0.5 kDa	YLAM < 0.5	8	0.519 ns

[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%.

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

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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

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Proteinase K Digest Evaluation

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Table 2. Modalities Used for Sensorial Tests

biochemical nature	triangular ($n = 23$)	synthetic soln	synthetic soln + retentate after digestion

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

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filtrate after UF 0.5 kDa	YLAM < 0.5	8	0.519 ns
enzymatic digestion of YLAM 0.5−3	D-YLAM 0.5−3	7	0.670 ns

[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%.

HPLC Peptide Purification

A **Peptide HR column** **B** **RP-18 column**

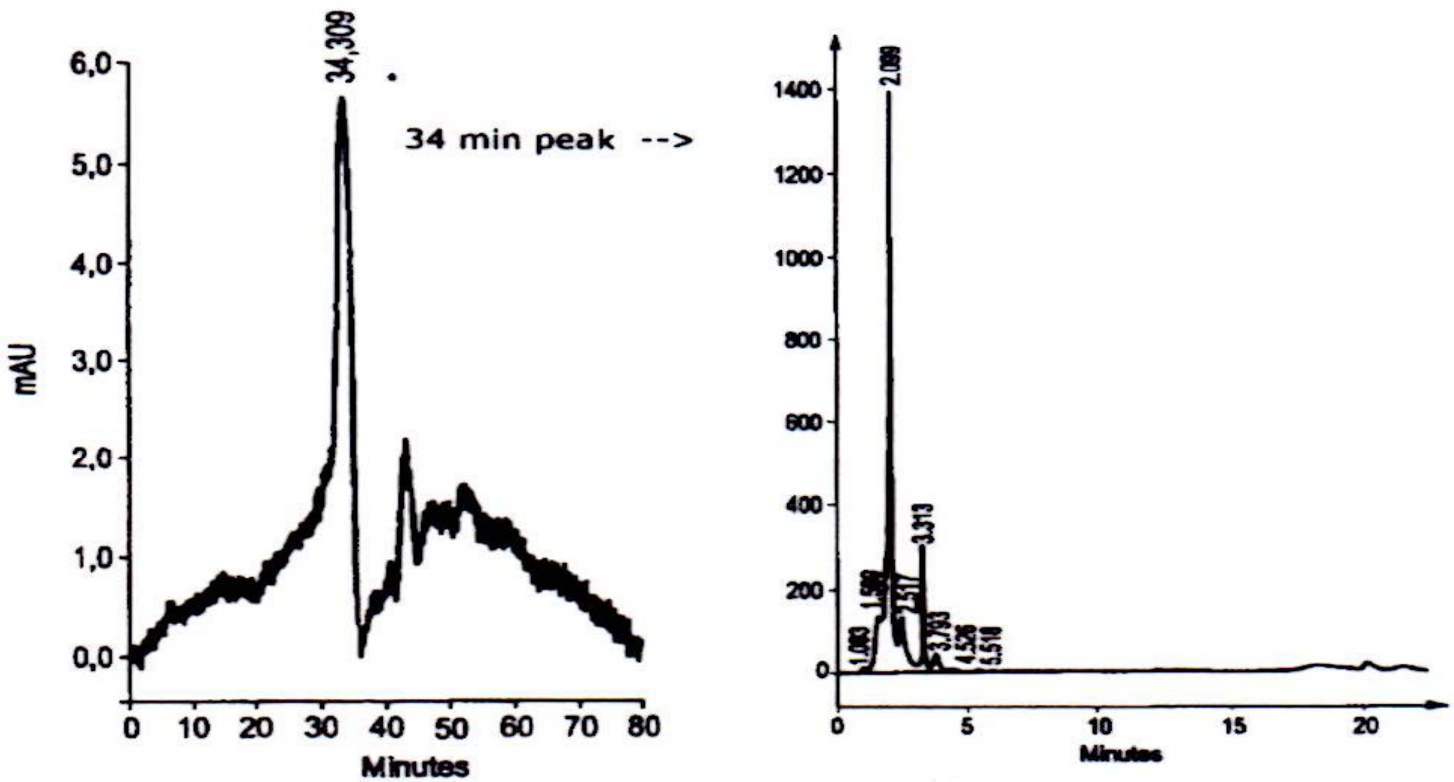

Figure 1. Chromatographic purification of sapid fraction. Chromatograms HPLC with UV detection at 220 nm of (a) YLAM 0.5−3 on Superdex Peptide HR column and (b) collected 34 min peak on RP-18 column.

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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.ASEALKPDSQKSYAEQGKEYITDK.A	2686.32063	
Y.VSGRVHGEEDPTKK.		1538.79215
K.ADKVAGKVQPED.N		1256.64811
K.ASEALKPDSQKSYAEQGK.E		1936.96106
D.AVEYVSGRVHGEEDPTKK.		2001.00359
K.ADKVAGKVQPEDNKGVFQGVHD.	S2338.17860	
K.GVFQGVHDSAEKGKDNAEGQGESLADQAR.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.VVKSSAAGNTVIIGGGDTATVAKK.Y	2244.25579	
K.SSAAGNTVIIGGGDTATVAKK.Y		1918.02400
R.IVAALPTIK.Y		925.60808
sp|P00924|EAsnO1_YEAST Enolase 1 (EC 4.2.1.11) (2-phosphoglycerate dehydratase) (2-phospho-D- glycera		
A.GENFHHGDKL.-	1153.53850	
F.AGENFHHGDKL.-	1224.57561	
Y.ARSVYDSRGNPTVE.V		1550.75576
V.SLAASRAAAAEKNVP.L		1455.79142
sp|P00950|PMG1_YEAST Phosphoglycerate mutase 1 (EC 5.4.2.1) (Phosphoglyceromutase 1) (PGAM 1) (MPGM		
D.PEAAAAGAAAVANQGKK.-		1524.81288
R.AIQTANIALEK.A	1171.66811	
Y.YLDPEAAAAGAAAVANQGKK.-		1915.98722
sp|P02994|EF1A_YEAST Elongation factor 1-alpha (EF-1-alpha) (Translation elongation factor 1A) (Euk		
K.AGVVKGKTLLEA.I	1185.72015	
Y.KIGGIGTVPVGR.V	1153.70517	
sp|P00445|SODC_YEAST Superoxide dismutase [Cu-Zn] (EC 1.15.1.1) - Saccharomyces cerevisiae (Baker's-.		
VQAVAVLKGDAGVSGVVK.F		1696.99560
sp|P32340|NDI1_YEAST Rotenone-insensitive NADH-ubiquinone oxidoreductase, mitochondrial precursor		
S.KNLYSNKRLLTSTN.T		1651.91259
sp|P05743|RL26A_YEAST 60S ribosomal protein L26-A (YL33) - Saccharomyces cerevisiae (Baker's yeast)		
R.RVLLSAPLSK.E		1083.68846
tr|Q07653|Q07653_YEAST S.cerevisiae chromosome IV reading frame ORF YDL223c - Saccharomyces cerevis		
K.ANAKVLEEDAPGYKR.E		1589.82820

Online Capillary HPLC Nanospray Ion Trap MS/MS Analysis

BLAST Search for ID of Peptides

Majority of isolated and identified peptides were from Hsp12

HYPOTHESIS
Hsp12 peptide source of sweetness

TEST:
Genetic Knockout

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Yeast Strains and Genetics

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

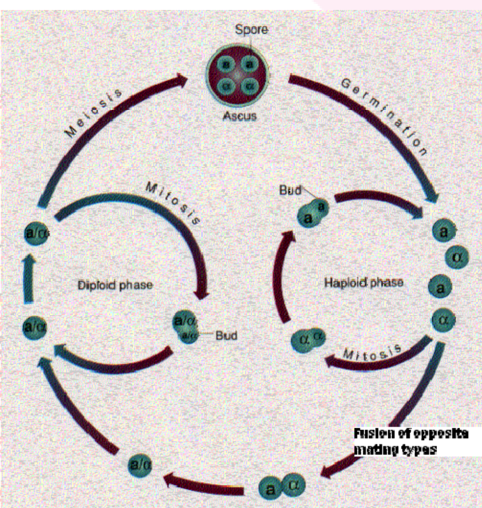
2) Create haploid strain

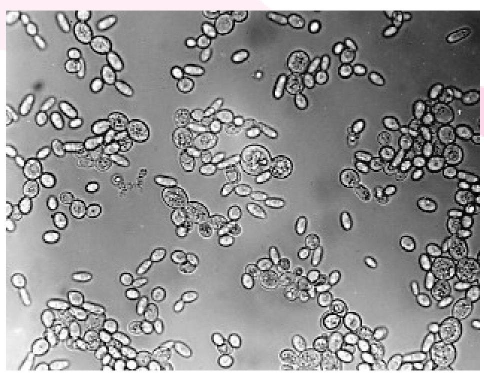
3) Use Cre-Lox recombination to KO Hsp12

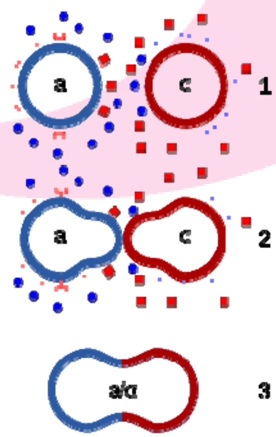
4) Cross ΔHsp12 with FX-10 by spore micromanipulation

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

6) Verify homozygous ΔHsp12 by sporulation on selective media and PCR







Micromanipulator

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Yeast Strains and Genetics

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Table 1. Yeast Strains and Plasmids Used

biological material	description	origin
	Yeast Strains	
Actiflore C	commercial starter	Laffort Inc.
Fx10	commercial starter *HO/HO* fully homozygous strain (Zymaflore Fx10, Laffort)	referenced as H4-1D 27
RG1	F10 *ho::HYG*^{R}, *Mat a*	kind gift of Pr. Richard Gardner
YPM32	haploid derivate of Fx10, *ho::HYG*^{R}, *MATa*	this study
YPM33	YPM32, *hsp12::LoxP::KANMx::LoxP, ho::HYG*^{R}, *MATa*	this study
YPM34	YPM33, Δ°*hsp12, HO::HYG*^{R}, *MATa*	this study
YPM35	YPM34 x Fx10 spore, *HO/ho::HYG*^{R}, *HSP12/*Δ°*hsp12*	this study
Δ°hsp12	meiotic segregant of YPM35, *HO/HO*, Δ°*hsp12/*Δ°*hsp12*	this study
	Plasmid	
pUG6		kindly donated by Pr. Bruno Blondin
pZEO		kindly donated by Pr. Bruno Blondin

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Evaluation of a ΔHsp12 Strain

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Table 2. Modalities Used for Sensorial Tests

Hsp12 effect	triangular (n = 23)	red wine + Fx10 (2×10^8 cells/mL)[a]	red wine + Δ°hsp12 (2×10^8 cells/mL)[a]

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

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

modality	fraction name	no. of "correct" answers[a] (n = 23)	P^b
autolysis medium before UF	YLAM	14	0.006**
retentate after UF 10 kDA	YLAM > 10	4	0.974 ns
retentate after UF 3 kDA	YLAM 3−10	9	0.349 ns
retentate after UF 0.5 kDa	YLAM 0.5−3	14	0.006**
filtrate after UF 0.5 kDa	YLAM < 0.5	8	0.519 ns
enzymatic digestion of YLAM 0.5−3	D-YLAM 0.5−3	7	0.670 ns
autolysis of Fx10 and Δ°hsp12 yeast strains in red wine (Hsp12 effect)		13	0.019*

[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 Vinitech 2006 Bordeaux - France**

VINITECH
TROPHEE OR
INNOVATION 2006

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

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Mannoproteins in Wine

US006139891A

United States Patent [19]
Dubourdieu et al.

[11] **Patent Number: 6,139,891**
[45] **Date of Patent: Oct. 31, 2000**

[54] **BIOLOGICAL SUBSTANCE FOR THE PHYSICO-CHEMICAL STABILIZATION OF WINES**

[75] Inventors: **Denis Dubourdieu**, Beguey; **Virginie Moine**, Pessac, both of France

[73] Assignee: **Faculte d'Oenologie**, Talence, France

[21] Appl. No.: **08/817,937**

[22] PCT Filed: **Oct. 27, 1995**

[86] PCT No.: **PCT/FR95/01426**

§ 371 Date: **Apr. 30, 1997**

§ 102(e) Date: **Apr. 30, 1997**

[87] PCT Pub. No.: **WO96/13571**

PCT Pub. Date: **May 9, 1996**

[30] **Foreign Application Priority Data**

Oct. 31, 1994 [FR] France 94 13261

[51] **Int. Cl.**[7] **C12G 1/10**; C12G 1/12; C12H 1/10

[52] **U.S. Cl.** **426/330.4**; 426/60; 426/424

[58] **Field of Search** 426/330.4, 60, 426/424

[56] **References Cited**

PUBLICATIONS

Cameron et al, The Mannoprotein of Sacch. cer. is an Effective Bioemulsifier, Applied Environmental Micro., Jun. 1988, pp. 1420–1425.

Bouton et al, Principles and Practices of Winemaking, Chapman & Hall Enology Library, 1986, pp. 90–91.

Wucherpfennig et al, Effect of Colloidal Substances Originating from Yeast on Wine Filterability, Zeitschrift fuer Lebensmittel–Untersuchung und–Forschung 1984, 179 (2) pp. 119–124.

Vine, R., Commercial Winemaking, AVI Publishing Co., Wesport Conn., 1981, pp. 161–164.

Villettaz et al, Am. J. Enol. Vitic., vol. 35, No. 4, 1984, pp. 253–256.

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

[57] **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

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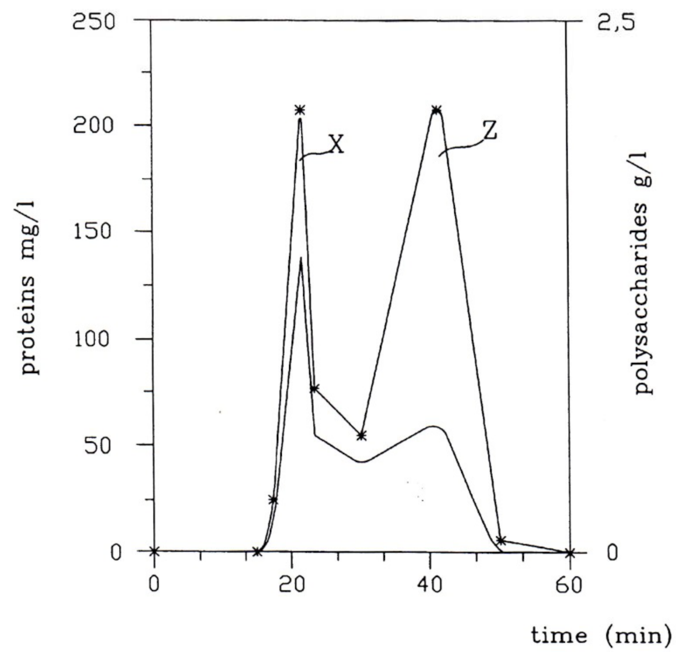
HPLC Analysis of MP Extracts

Heat extraction profile - MEC

void eluent

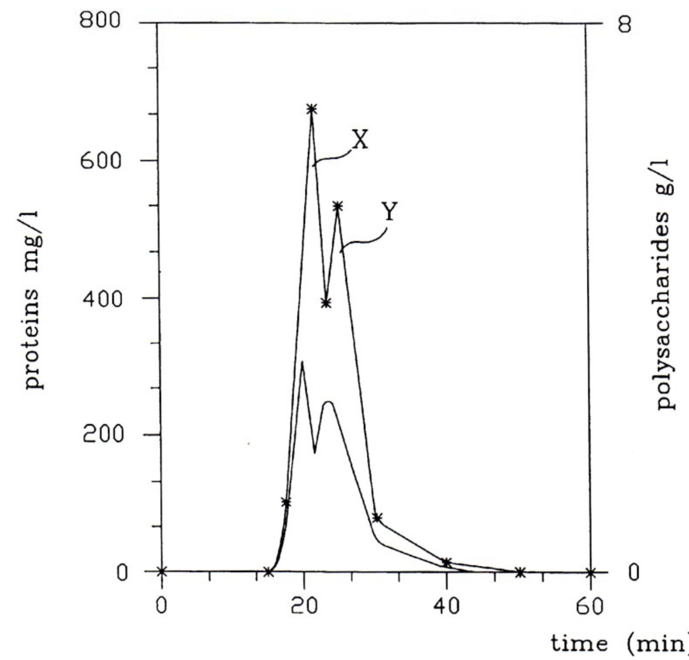


⁎ spectrophotometric detection at 225 nm (proteins)

• refractometric detection (polysaccharides)

Enzyme digestion profile - MEE

void eluent



⁎ spectrophotometric at 225 nm (proteins)

• refractometric detection (polysaccharides)

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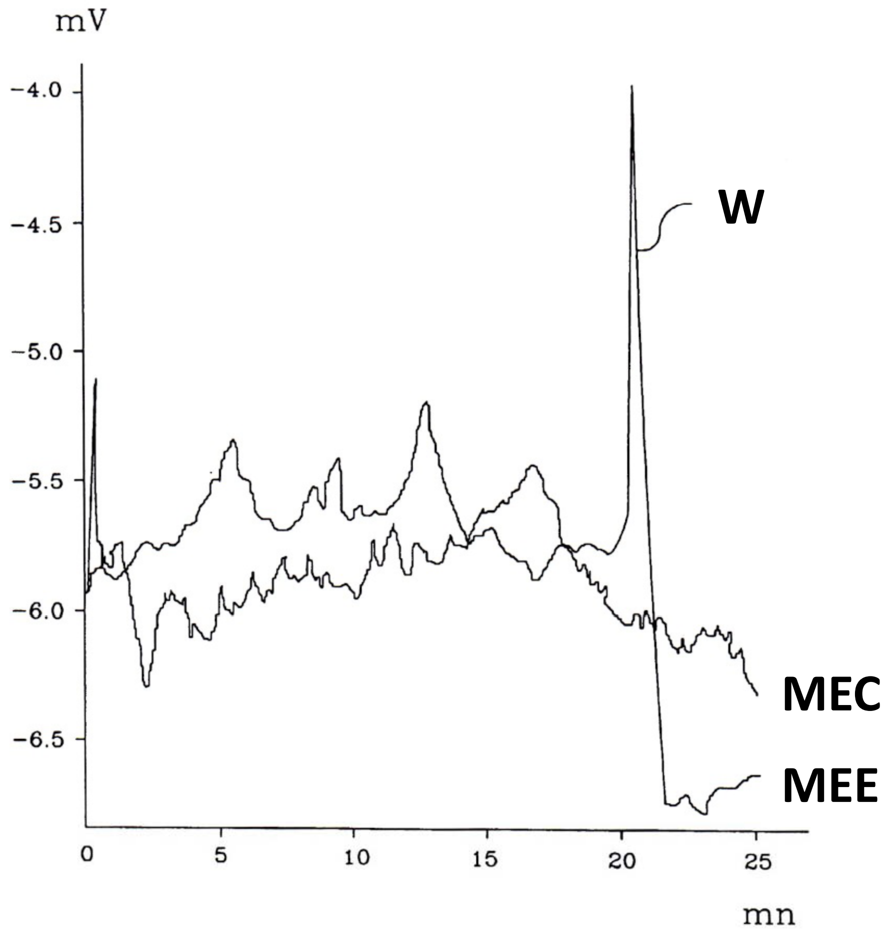
Capillary Electrophoresis Separation



minutes

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.

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

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%.

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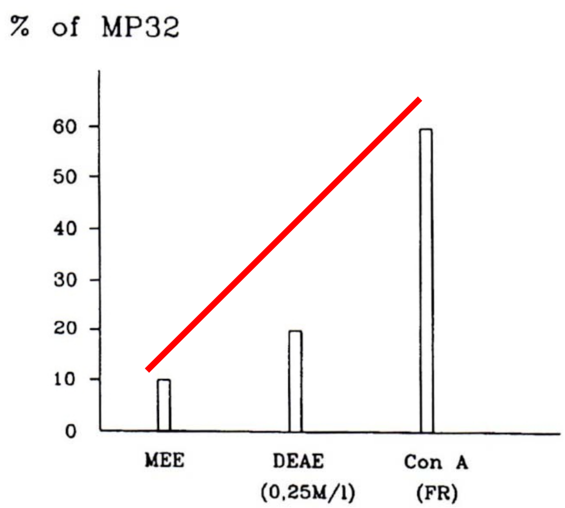
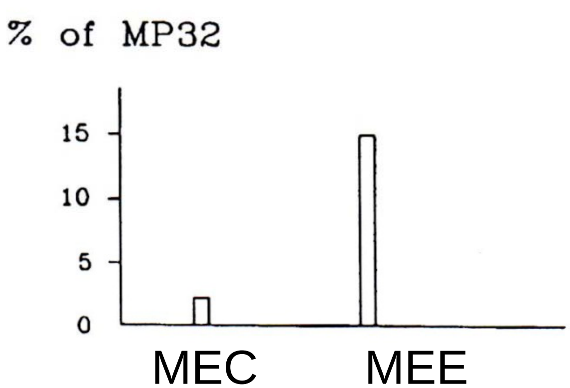
Analysis of MP32



Molecular weight kda		
MEE	DEAE (0.25 mole/l)	Con A (FR)
77.8	77.8	
	53	
44.1	44.1	
41.6		
35.2	35.2	
31.8	31.8	31.8
30.3		
27.5		
25.2		
23.2		
21.3		
19.8	19.8	19.8
18.4	18.4	
17.2	17.2	17.2
16	16	16
15.2	15.2	15.2

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

Only MP32 increased in concentration



Mannoproteins	% of proteins	% of polysaccharides	% of mannose	% of glucose
extracted with heat	4.2	93.8	92	8
extracted enzymatically	15	83.2	100	0

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Specific Mannoprotein Effects

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

Wine	White 1	Rosé 1	Red 1
Control	***	****	***
MEC 25 g/hl	**	****	**
MEE 25 g/hl	0	0	0

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Differential Specificity of MP

***: crystallization
ND: not determined
O: no crystallization

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

Wines	Reference	Meso. acid 10 g/hl	MEC 25 g/hl	MEE1 25 g/hl
White 1	***	O	***	O
White 2	***	O	***	O
White 3	***	ND	***	O
White 4	***	ND	***	O
White 5	***	ND	***	O
White 6	***	ND	***	O
Rosé 1	***	ND	***	O
Rosé 2	***	O	***	O
Red 1	***	***	***	O
Red 2	***	***	***	O
Red 3	***	***	***	O

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.

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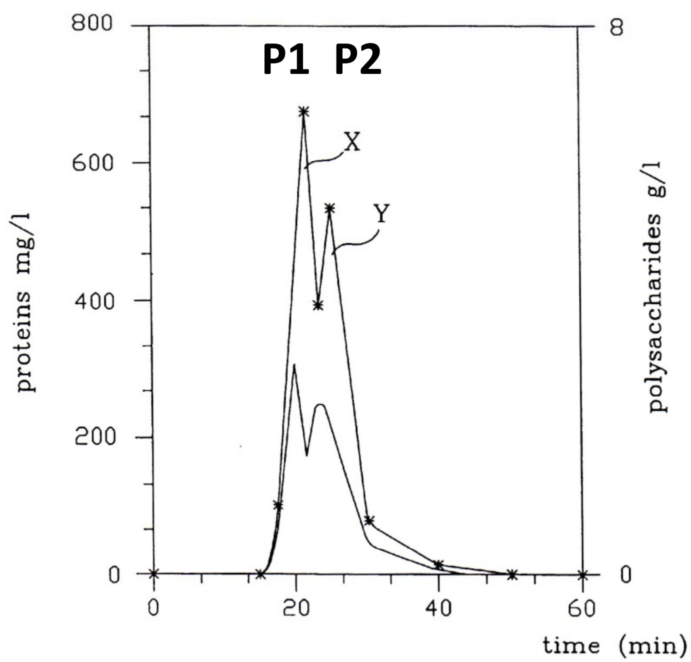
Analysis of MP40

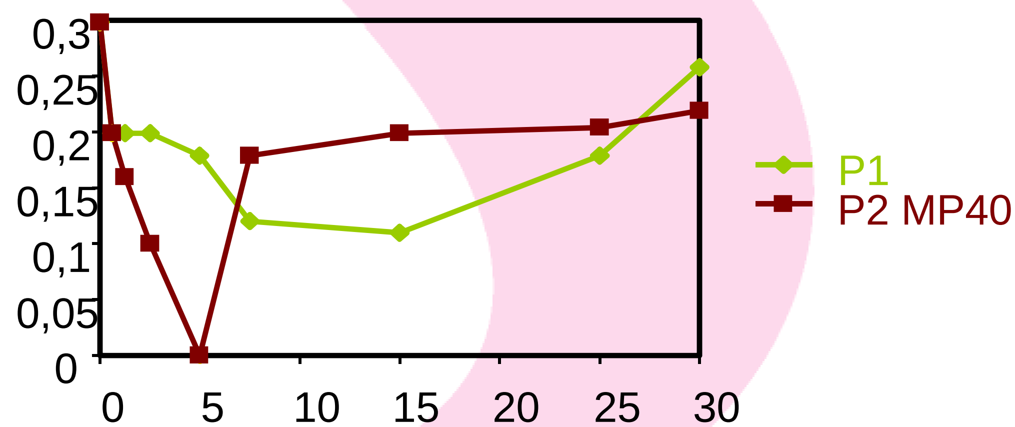
HPLC separation of MEE



⁎ spectrophotometric at 225 nm (proteins)

• refractometric detection (polysaccharides)

variation of potassium (g/l)
after cold treatment



Purified P1 and P2 fractions by HPLC g/hl

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Analysis of MP40

Molecular weight in kda (kilo dalton)			
MEE	P1	P2	FR con A
77.8	77.8		
		53.3	
44.1	44.1		
41.6		41.6	41.6 ⬆ conc.
35.2		35.2	
31.8	31.8	31.8	31.8
30.3	30.3	30.3	
27.5	27.5	27.5	
25.2	25.2	25.2	
23.2		23.2	
21.3		21.3	
19.8		19.8	
18.4		18.4	
17.2	17.2	17.2	17.2
16	16	16	
15.2	15.2	15.2	15.2

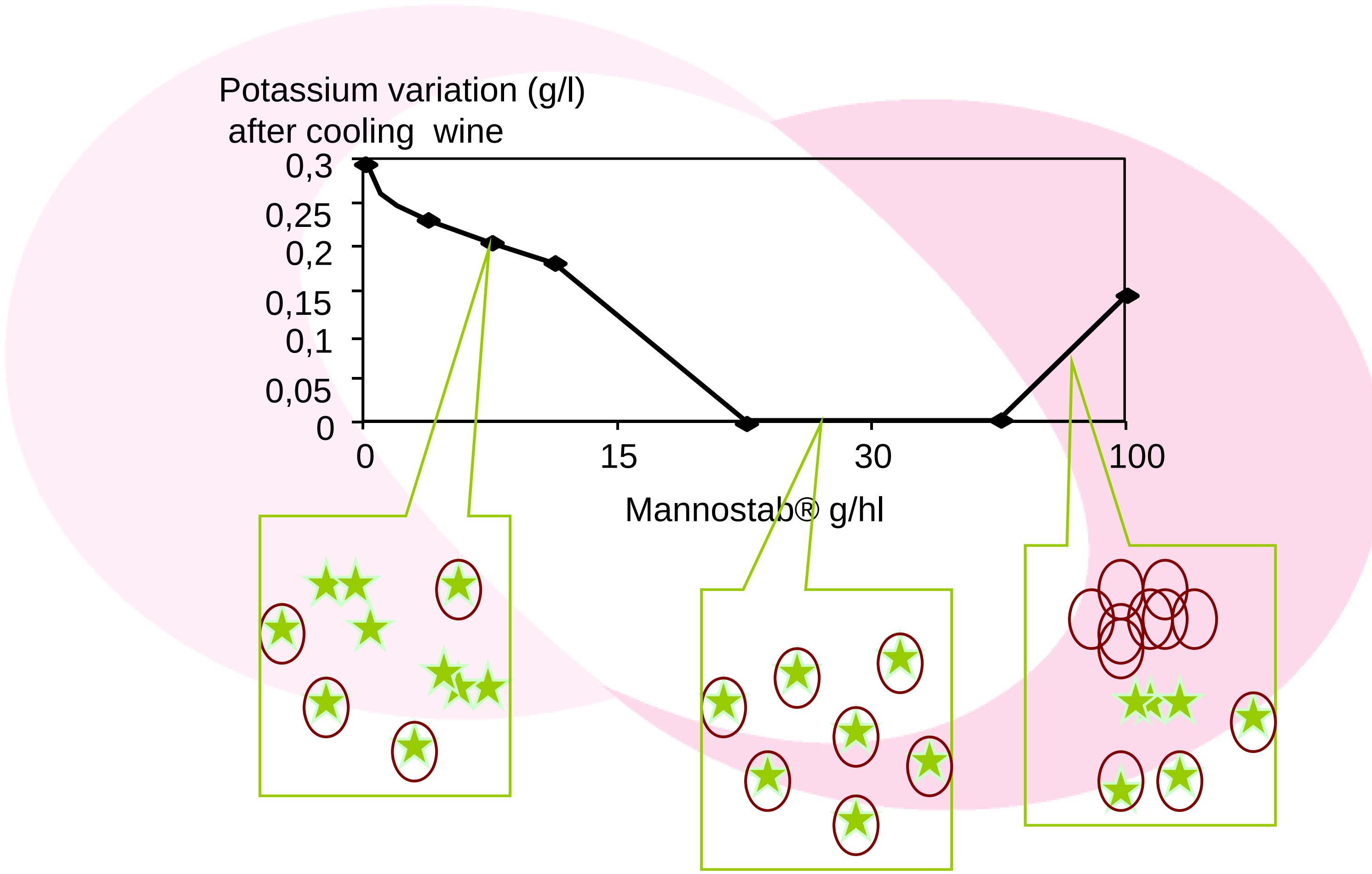
The active fraction thus contains only four mannoproteins, the molecular weights of which are 41.6; 31.8; 17.2; 15.2 kda.

The only protein which increases in concentration is the 41.6 kda. Accordingly, this is the mannoprotein responsible for the tartaric stabilization.

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

Only fraction P2 including MP40 allows a stabilization.

Colloidal Behavior of Mannoproteins



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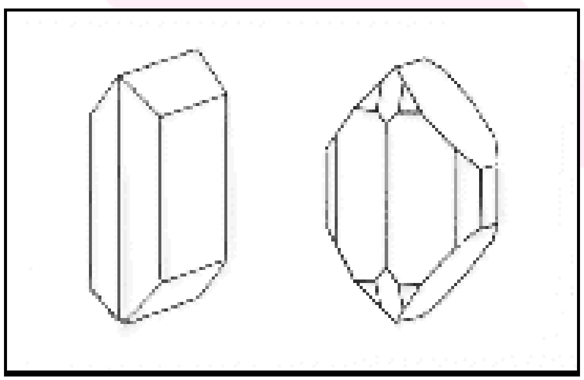
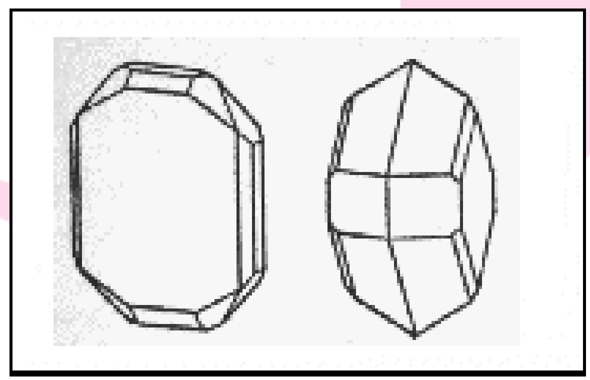
Crystalization of Potassium Bitartrate

Mechanism of Crystallization:

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

Structure of the crystal: orthorhombic geometry





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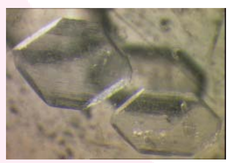
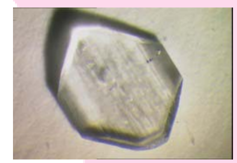
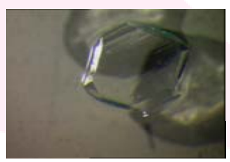
Microscopic Observation of the Crystallization of Potassium Bitartrate

Date of obs.	27/06	30/06	2/07	4/07	7/07
Control					
MP40					

With MP40 crystals are flat - undeveloped

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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

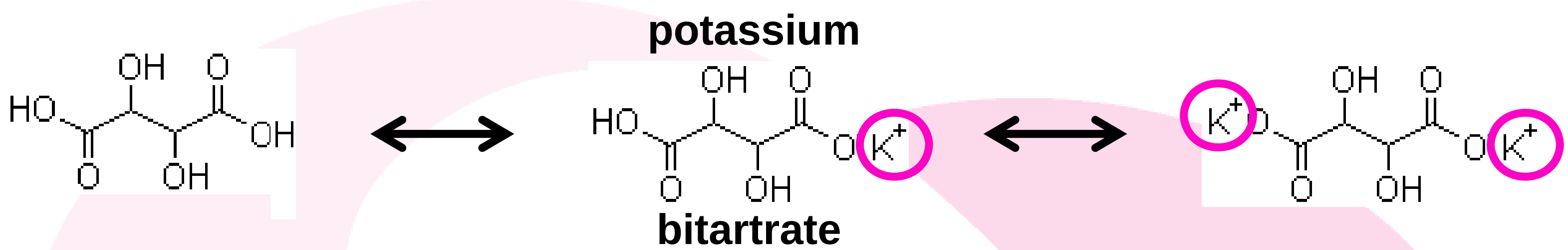
Environmental Benefits

- ✓ 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

SUSTRACTIVE TECHNIQUES

- Traditional Cold Stabilization
  -Refrigeration

- Membrane Based Technique
  (Electrodialysis)

NON-SUBTRACTIVE INHIBITORS

- Yeast Mannoprotein
  (Natural Inhibitor– MP40)

-Carboxymethyl Cellulose
(CMC – Cellulose Gum)

-Metatartric acid

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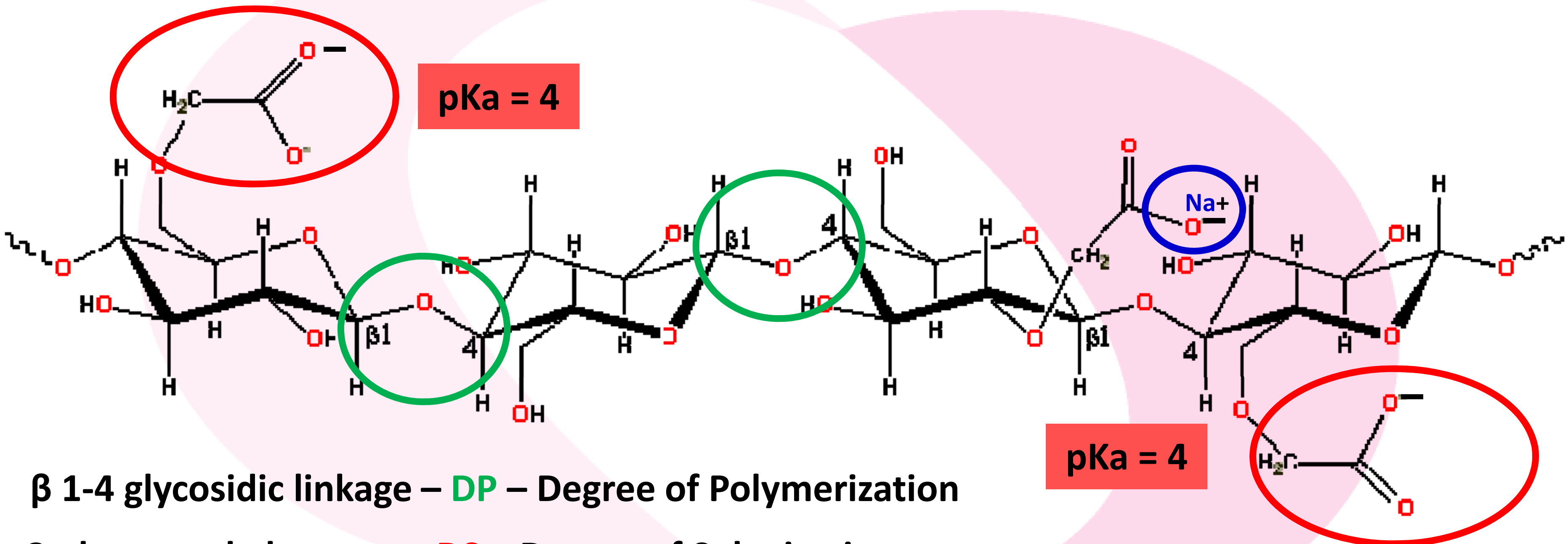
CMC Molecular Structure Characteristics



β 1-4 glycosidic linkage – DP – Degree of Polymerization

Carboxymethyl groups – DS – Degree of Substitution

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

DP – Degree of Polymerization **Influences - Viscosity, Fluidity**

DS – Degree of Substitution **Influences - Solubility, Efficiency**

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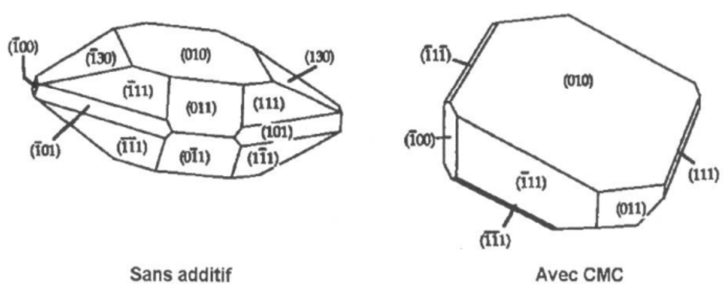
CMC Oenological Properties

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

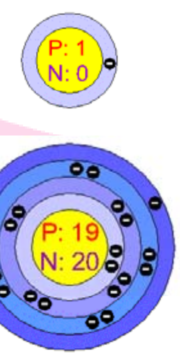
The negatively charged ionized form of CMC interacts with the (010) face of a bitartrate crystal, specifically the positively charged layer of K^+ on the crystal face

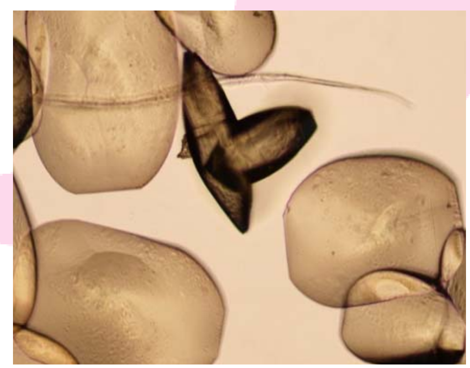


THK crystal shape without and with CMC

H^+

K^+





Inhibition of KHT crystal growth by CMC

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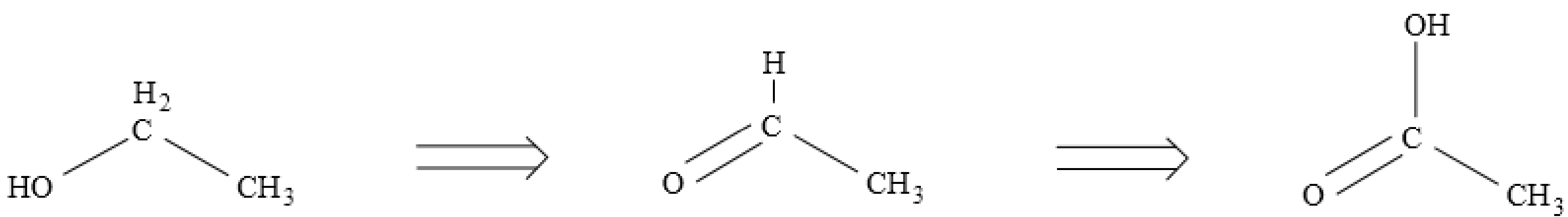
Chemical Oxidation

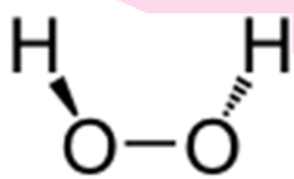
HYDROXYL (alcohol) → **CARBONYL (aldehyde)** → **CARBOXYLIC ACID**

Oxidation of Ethanol to Acetaldehyde and Acetic Acid

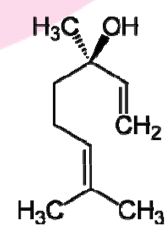


ethanol ⟹ acetaldehyde ⟹ acetic acid

- Fenton Reaction
- Fe dependent
- Peroxide reaction product



Hydrogen Peroxide



Linalool

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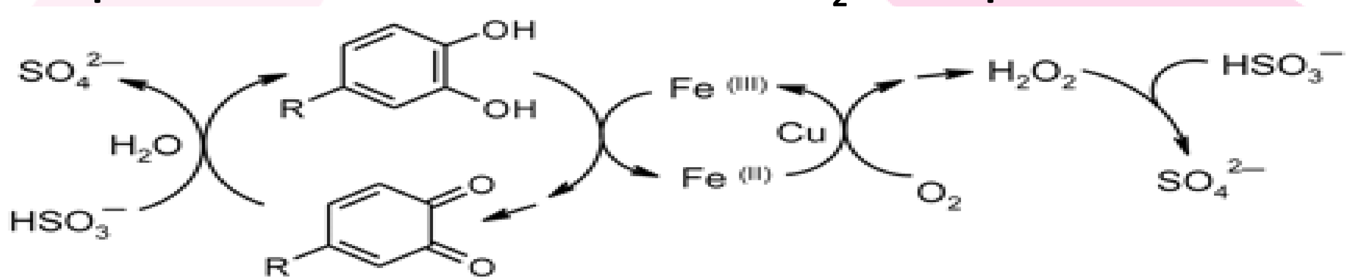
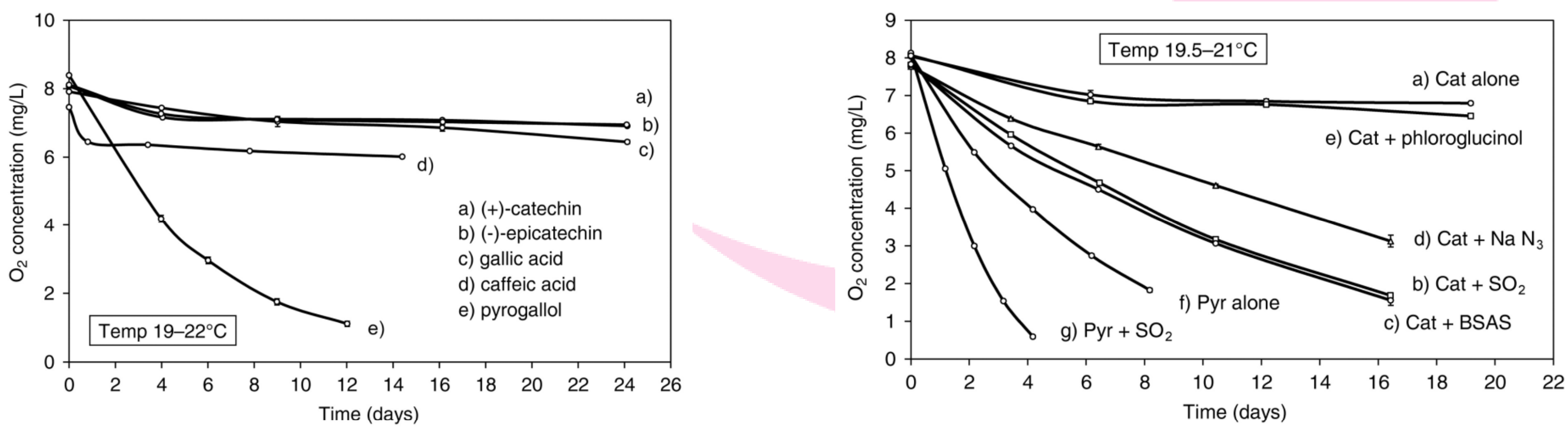
Fenton Reaction, Sulfites, Oxygen, Catechols and Quinones – Oh My!

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)

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Redox Potentials

+170 mV — SO_2

+282 mV — Ascorbic acid / Vit C

+480 mV — Tocopherol / Vit E

+600 - 750 mV — Tannins

+920 mV — Glutathione

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

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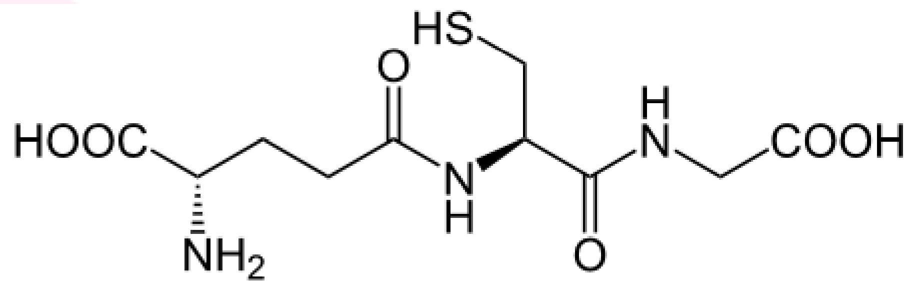
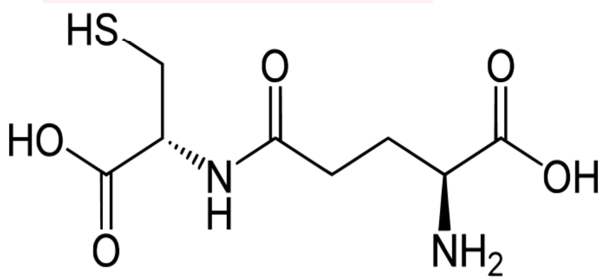
Glutathione as an Antioxidant in Wine

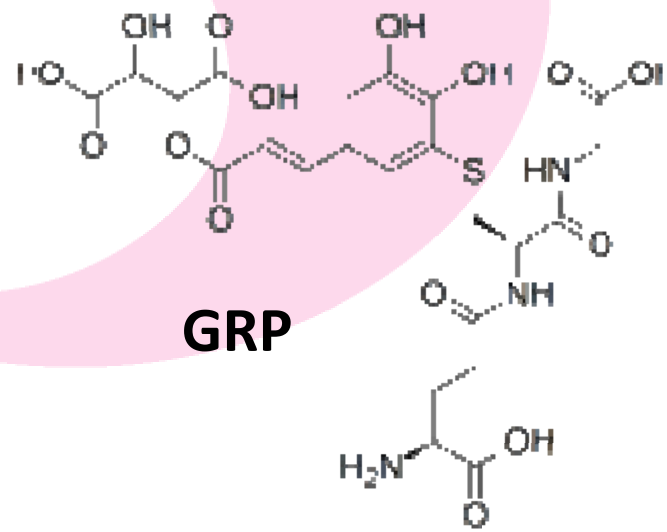
Glutathione added directly to aqueous solution or finished wine can be rapidly oxidized and with no Glutathione Reductase to recycle it loses antioxidant properties



Glutathione



ɣ - Glutamylcysteine (GGC)



GRP

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

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Glutathione in Juice and Wine

Glutathione in the juice (mg/L or ppm)	9	5	4	17	2
Glutathione in the corresponding wine (mg/L or ppm)	11	7	6	22	3

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

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Selective Adsorption-Yeast Hulls

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

Albumin | Gelatin 1 | Gelatin 2 | Yeast Hulls 1 | Yeast Hulls 2

Samples reacted with Bradford protein reagent

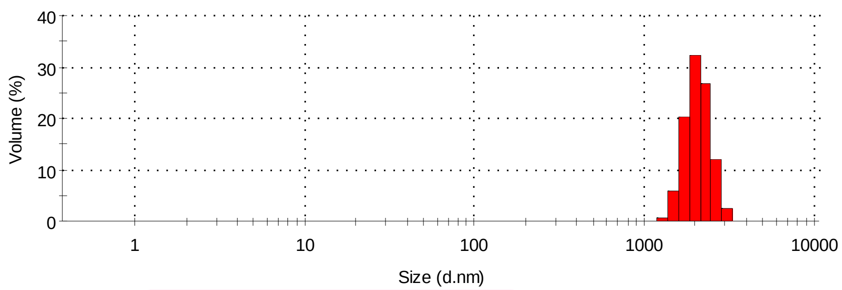
Reagent changes to blue with protein interaction

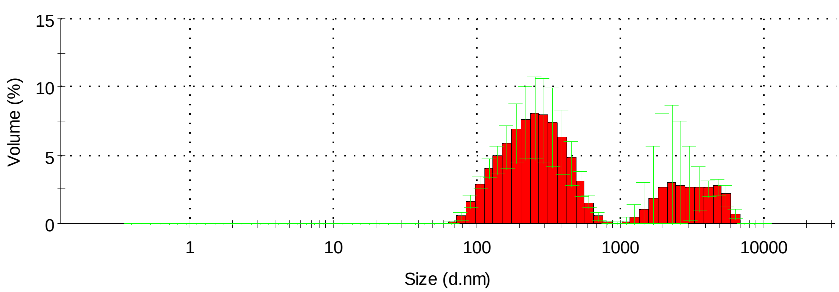
After centrifugation proteins are localized in tube bottoms, ie in the yeast hulls

Selective Adsorption-Yeast Hulls

✓ **Particles size repartition**

	Potentiel Zeta (mV)
Yeast Hull Preparation	-18,7 mV
Albumin	+ 31,1 mV





Despite a negative charge, yeast hulls react with tannins by hydrophobicity
This action mechanism is different from traditional fining agents

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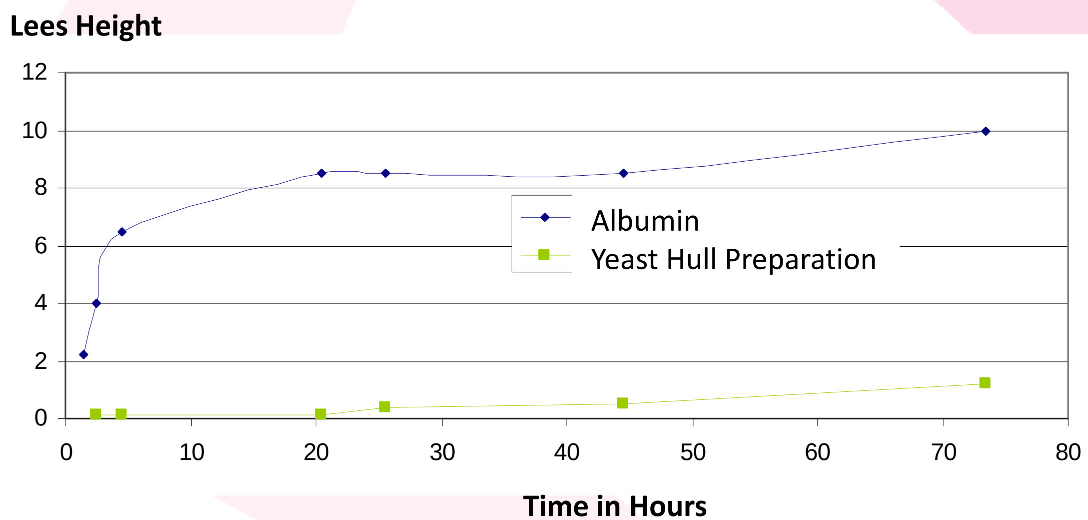
Selective Adsorption-Yeast Hulls

✓ **Comparison between albumin and yeast hull fining**



Lees 5 times more compact than with albumin

Easier racking, less wine loss

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Continuing Investigations

- Lees and Oak Interactions
- Lees and MLF Influences
- Specific Yeast Cell Wall-Membrane Components and Detoxification

WHO ARE WE?

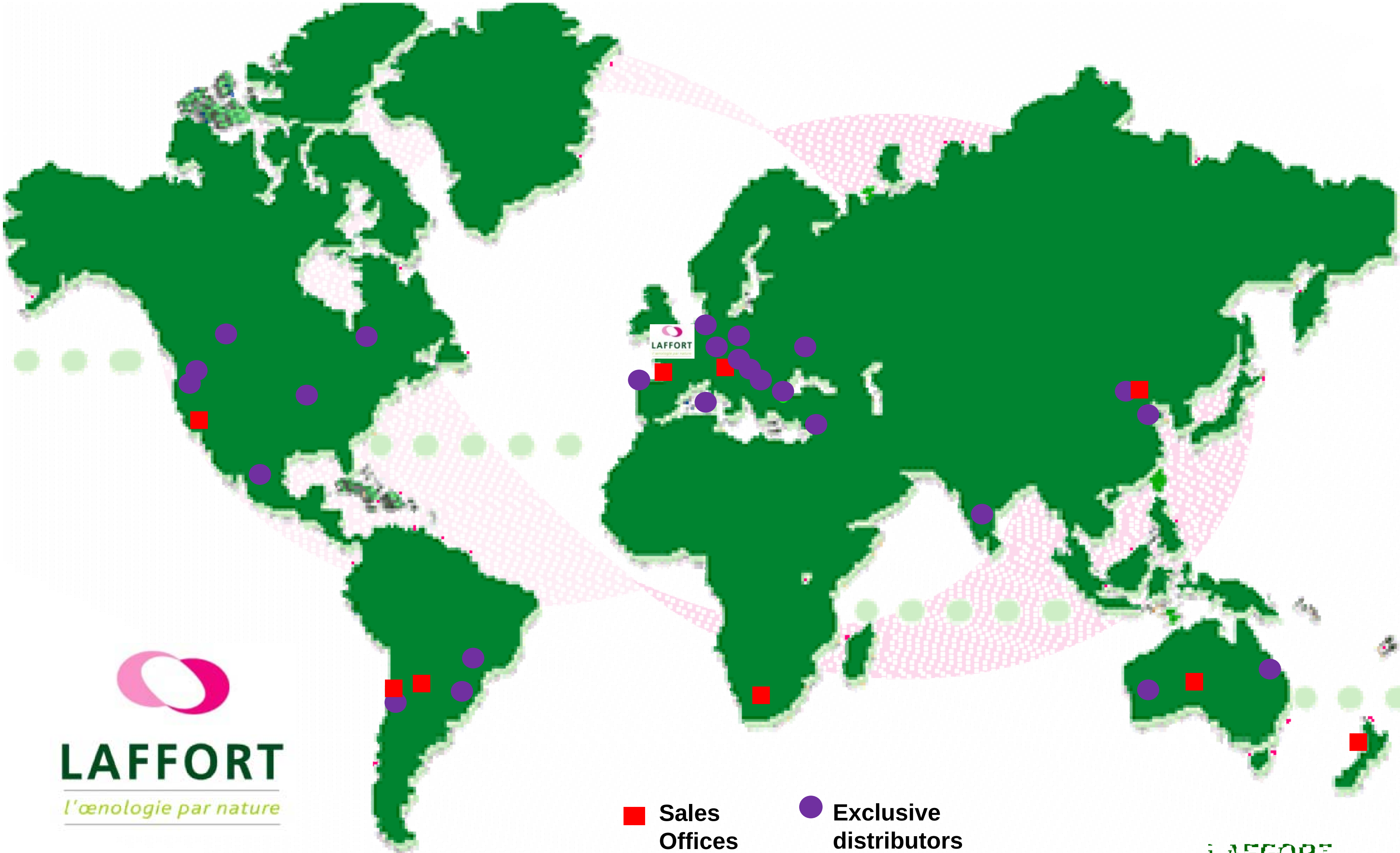
Founded in 1895, LAFFORT S.A.S. is a family-owned French company completely focused on research, production, and distribution of the highest quality and best value enological products worldwide.

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.

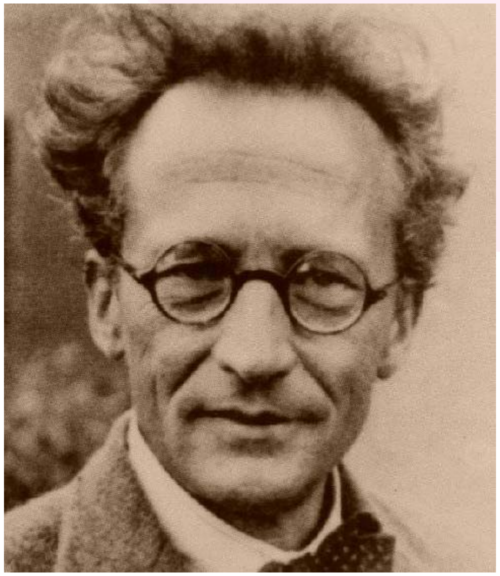
SARCO, our scientific arm, is the largest and best funded private research entity in the wine industry. We also work closely with the University of Bordeaux ISVV and wine Research Institutions around the world.

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 Schrodinger
1933 Nobel Prize for Physics

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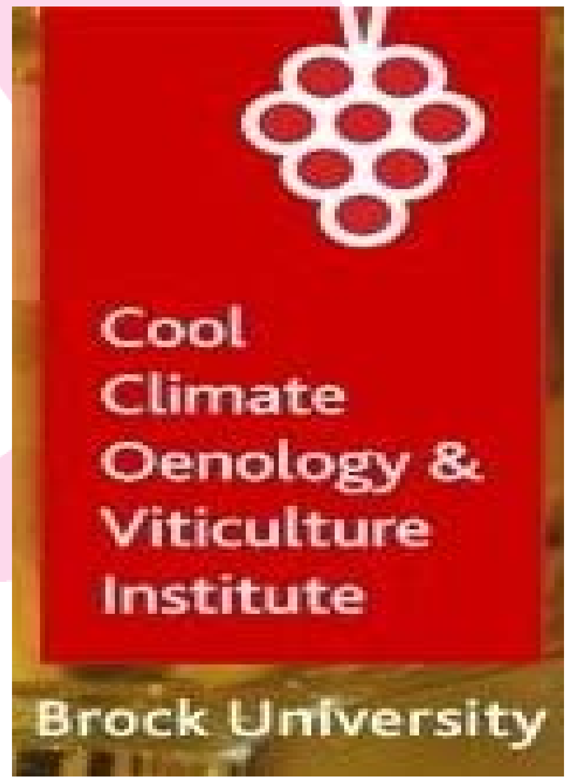
Sur Lie Science –
Wine Character Revealed

? Questions – Discussion !

Peter Salamone, Ph.D.
Technical Manager
North America

LAFFORT
l'œnologie par nature

Laffort in Ontario:
Vines to Vintages Inc.



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KHT Stability in Fined Red Wines

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/hl, and to a red wine fined with egg white at 10 g/hl.

Different modalities	Difference in concentration of potassium mg/l		
	Non-fined wine	Wine fined with gelatine	Wine fined with egg white
Reference	90	110	180
Meso. acid 15 g/hl	70	70	90
Meso. acid 25 g/hl	0	0	0
MEC 15 g/hl	90	110	130
MEC 25 g/hl	50	50	70
MEE1 15 g/hl	30	70	70
MEE1 25 g/hl	0	0	0
Gum 15 g/hl	90	70	140
Gum 25 g/hl	30	50	50

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

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

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