Cool Climate Oenology & Viticulture Institute
Brock University

Targeting wine balance using biological deacidification methods and by monitoring polyphenolic maturity

George Kotseridis

2012 CCOVI Annual Lectures Series
Wednesday, February 15th

Wine balance

Balance in wine refers to the interaction and harmony between two or more of the wine's constituents

By far the most straightforward balance is that between alcohol/sugar and acidity

Not all wines, of course, have residual sugar, though all have some acidity and of course alcohol

Wine balance

A wine which has a good acid-sugar/alcohol balance tastes neither too sweet nor too acidic:

the alcohol/sugar exists in the right quantity for the acid, and vice versa

Wine balance

- A wine with too little alcohol/sugar for its acid will taste harsh, sharp and acidic

- A wine with too much sugar will taste cloying, sugary and flabby, and will not refresh the palate.

Peter Bell, Finger Lakes

Balance Between Acidity and Astringency

- the less tannic a wine is, the more acidity it can support
- the higher a red wine is in tannins, the lower should be its acidity
- the combination of high acid and high tannins make for the hardest and most astringent wines

Emile Peynaud, The Taste of Wine

Balance Between Alcohol and Acidity/Astringency

- Too little alcohol will cause acidity and astringency to dominate, making the wine harsh and thin

- Too little acid and astringency will cause a wine to taste overly soft, heavy and flabby

Balance Between Acidity and Astringency

- a wine tolerates acidity better when its alcohol content is higher

- a considerable amount of tannin is more acceptable if acidity is low and alcohol is high

Juice and wine acidity

- Juice / Wine acidity

essential parameter of juice and wine that <u>preserves</u> it, <u>shapes its flavors</u> and helps <u>prolong its aftertaste</u>. Acidity is identifiable by the crisp, sharp character it imparts to a wine

[Glossary of Wine Terms from THE WINE SPECTATOR]

Juice and wine acidity

Why are acid & pH important ?

Acids:

- Wine acidity balances alcohol & residual sugar

- Quality wines are well-balanced wines

Intro-Juice and wine acidity

Why are acid & pH important ?

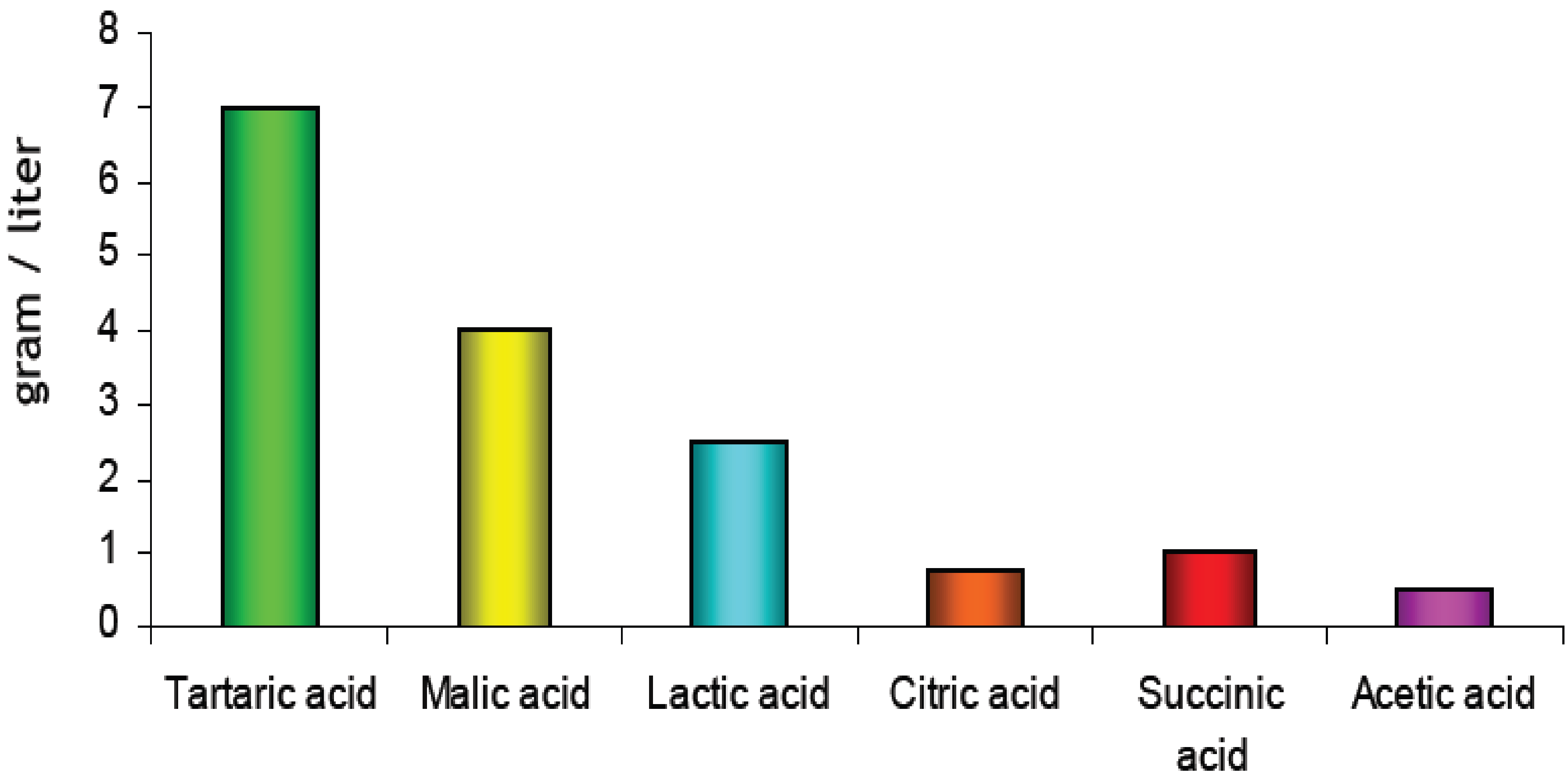
Acids:

- A wine with low acidity has a distinctly flat taste, whereas a wine with high acidity has a sour taste

  J. R. Munyon, C. W. Nagel *(1977)*

Juice and wine acidity

Acids in wine:



Juice and wine acidity

Cool Climate Oenology & Viticulture Institute
Brock University

Why are acid & pH important ?

pH:

- Low pH in juice and wines
  - Increase antimicrobial action of SO_2
  - Increase colour expression in young red wines
  - Selection of desirable micro-organisms
  - Enhance clarification of juices and wines
  - Enhance expression of fruit character
  - Promote balance of wine colour

Intro-Juice and wine acidity

How do we measure acidity?

pH & titratable acidity:

- pH – the equilibrium measure of hydrogen ion concentration in a juice or wine

- Titratable acidity (TA) measures the total amount of protons available in a juice or wine, expressed as g/L tartaric acid equivalent

Intro-Juice and wine acidity

pH:

- is the negative logarithm of the concentration of free Hydrogen ions (protons) in a juice or wine

$$pH = -\log[H+]$$

ie pH 3 is 10x more acidic than pH 4

- the pH of juice can be very different at similar levels of TA depending on the amounts and proportions of tartaric acid, potassium bitartrate, di-potassium tartrate & malic acid

Deacidification

Reduction of TA and increase of pH:

- cold year, unripe grapes, cool climate viticulture areas

Methods of deacidification:

1. Biological
2. Physicochemical

Biological Methods of Deacidification

a) Malic acid degradation through alcoholic fermentation

- The standard wine yeast *Saccharomyces cerevisiae* is consuming some malic acid,
- but negligible amounts as compared with bacteria

- This yeast species does not have an enzyme for transporting the malique acid into their cells

Biological Methods of Deacidification

- But malic acid passes through the yeast cellular membrane by <u>simple diffusion</u> where it is metabolized to ethanol, through a malo-ethanolic pathway (Pretorius 2000)

- Low pH also favors malic acid consumption, as only the undissociated form of the acid is able to pass through the cellular membrane (Ramon-Portugal et al. 1999)

Biological Methods of Deacidification

Reminder HA $\longrightarrow$ $A^{-} + H^{+}$

The undissociated form of malic acid ranges from:

64% at pH 3.2 to

41% at pH 3.6

- Malic acid consumption by yeast should increase at lower pH values and with higher malic acid concentrations

Biological Methods of Deacidification

- The ability of commercial strains of *S. cerevisiae* to consume malic acid varies from 0 to 40%

- *S. cerevisiae* strain 71B consuming about 30% of malic acid as compared with strain EC1118 (Pilone and Ryan 1996)

Biological Methods of Deacidification

Cool Climate Oenology & Viticulture Institute
Brock University

b. The yeast strain ML01

- This yeast has been engineered to have an active transport system to enable malic acid to enter the yeast cell and an efficient enzyme system within the cell to convert malic acid to lactic acid
- No MLF using malolactic bacteria needed any more
- speeds the winemaking process

Biological Methods of Deacidification

Briefly:

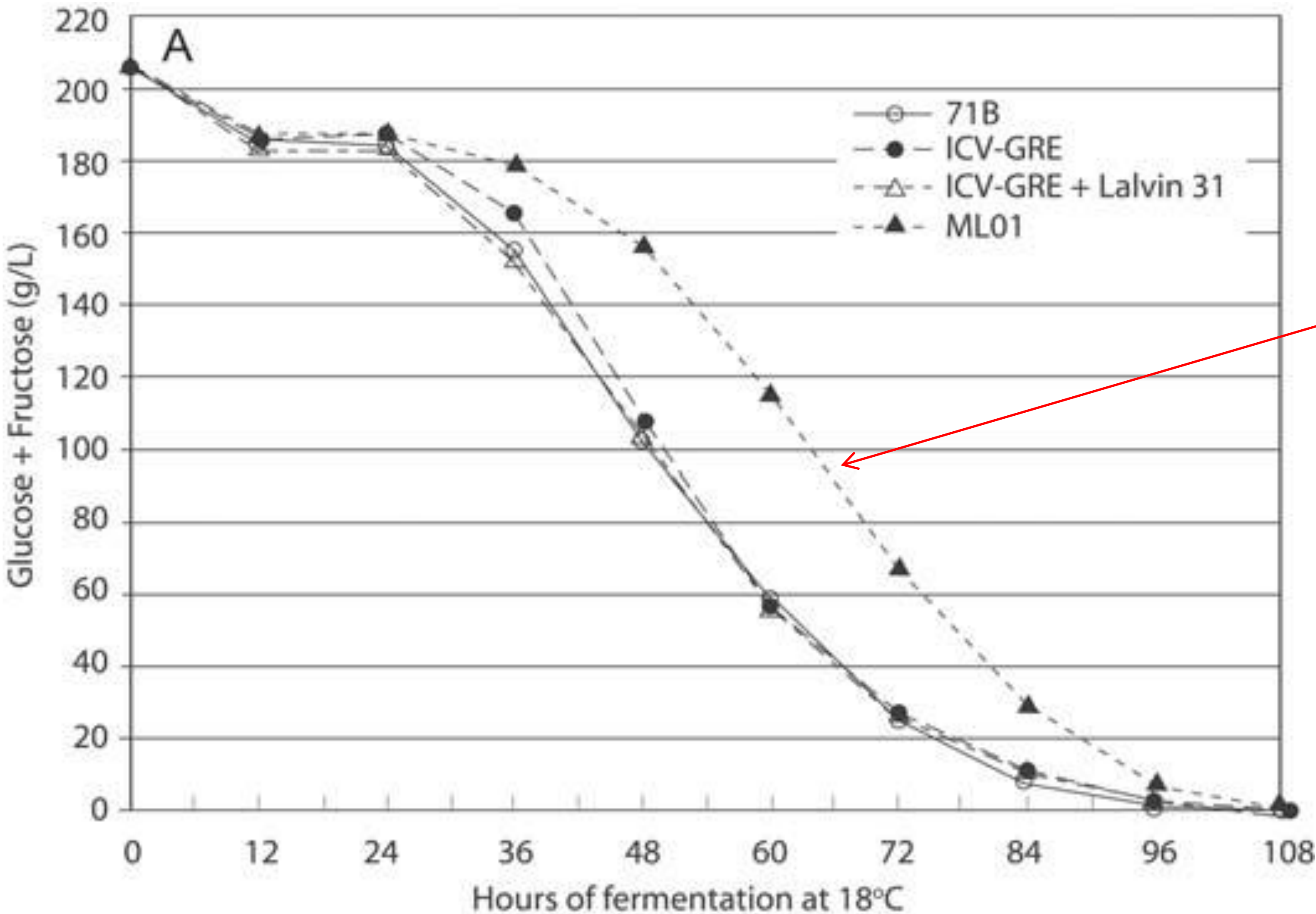
- Contains two type of enzymes

  1) A malic acid transporter (from *Schizosaccharomyces pombe*)

  2) A malolactic enzyme (from *Oenococcus oeni*)

- Efficiently converts malate to lactate during alcoholic fermentation

Biological Methods of Deacidification

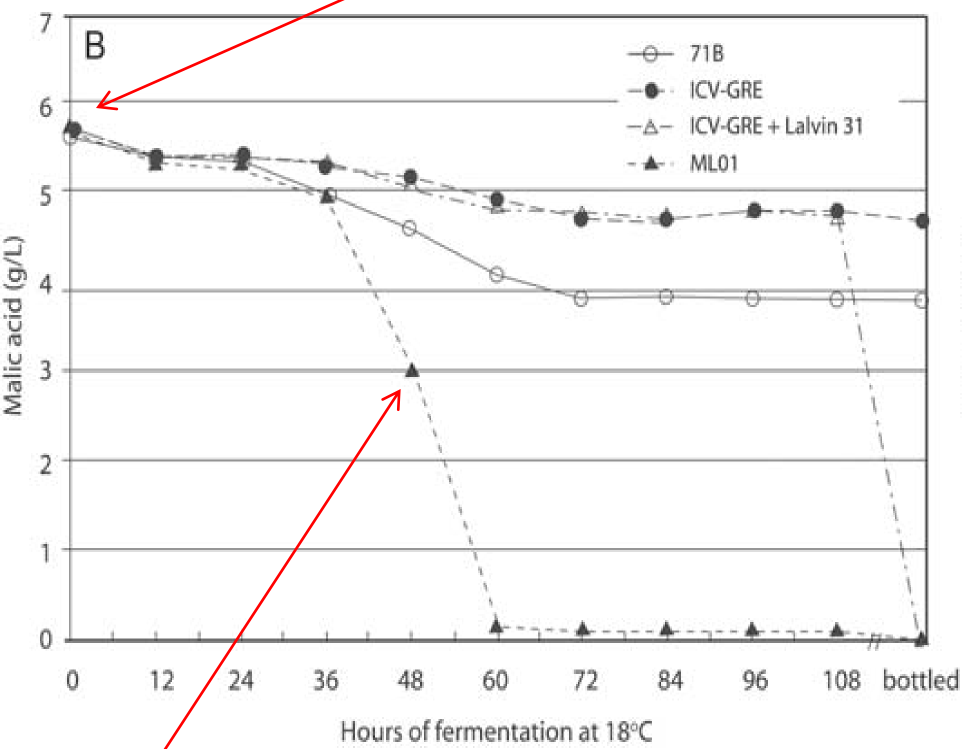


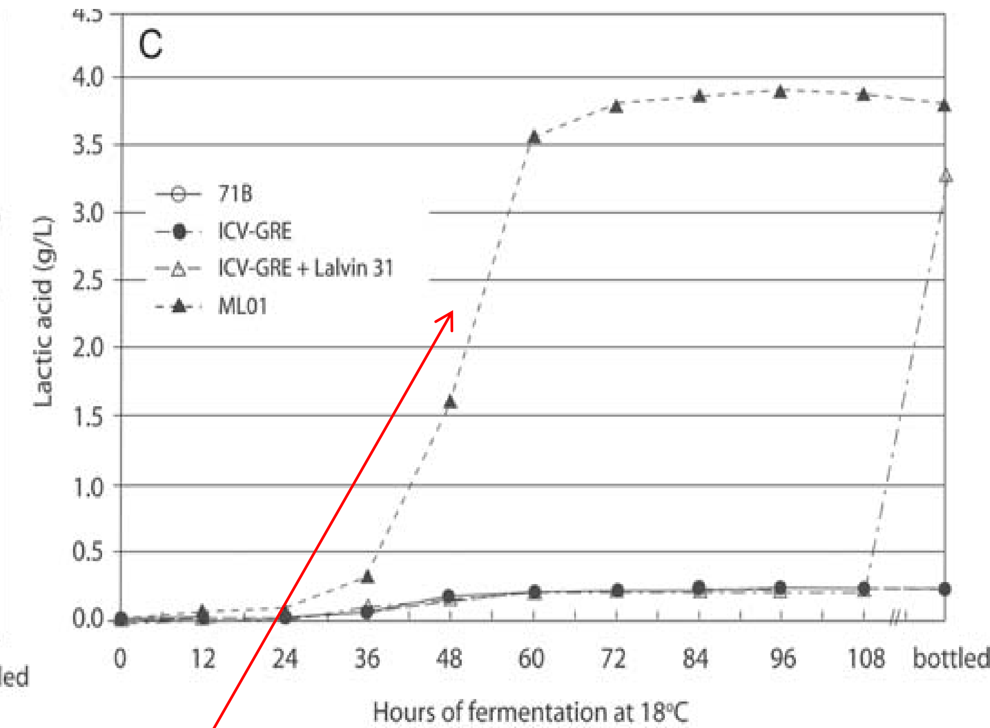
ML01

Main et al. 2007

Biological Methods of Deacidification

Initial Malic acid content = 5.7 g/L





ML01

ML01

Biological Methods of Deacidification

Initial Malic acid content = 5.7 g/L

Table 1 Effect of natural yeast (71B and ICV-GRE), genetically enhanced yeast (ML01), and natural malolactic bacteria (Lalvin 31) on Vignoles wine.

Treatment	Glucose + fructose (g/L)	Titratable acidity[a] (g/L)	Tartaric acid (g/L)	Citric acid (g/L)	L-malic acid (g/L)	L-lactic acid (g/L)	Succinic acid (g/L)	Glycerol (g/L)	Ethanol (% v/v)	Total SO_2 (mg/L)
71B	0.88 b[b]	8.1 b	1.53 b	0.38 c	3.81 b	0.03 c	0.50 a	6.06 a	12.3 a	82 b
ICV-GRE	0.65 c	8.6 a	1.56 ab	0.41 b	4.67 a	0.03 c	0.43 b	5.37 d	12.2 b	92 ab
ICV-GRE + L31[c]	0.57 c	6.7 d	1.74 ab	0.18 d	0.06 c	3.11 b	0.44 b	5.44 c	12.3 a	73 b
ML01	1.48 a	7.1 c	1.81 a	0.44 a	0.05 c	3.64 a	0.35 c	5.95 b	12.0 c	121 a

[a]As tartaric acid.
[b]Means within column with the same letter(s) are not significantly different at the $p \leq 0.05$ level of significance.
[c]ICV-GRE + Lalvin 31.

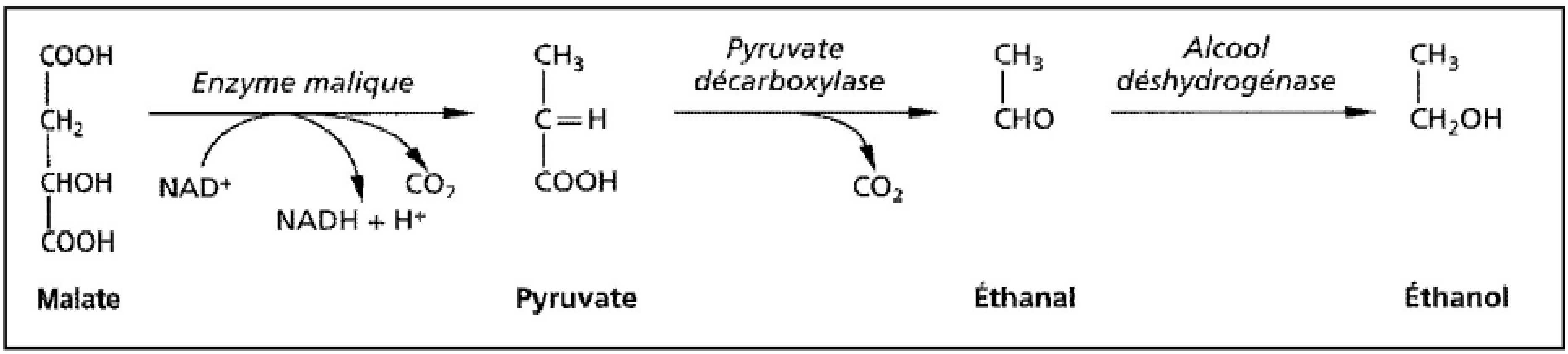
Main et al. 2007

Biological Methods of Deacidification

c) Scizosaccharomyces pompe

Metabolizes malic acid to ethanol – no lactic acid produced

Malo-Ethanol Fermentation



2.33 g/L malic acid ⟶ 0.1% ethanol

Biological Methods of Deacidification

shows a number of attractive properties, such as :

high acid tolerance

SO_2 tolerance

good growing capability under conditions of must and wine

easy cultivation and storage of strains

Biological Methods of Deacidification

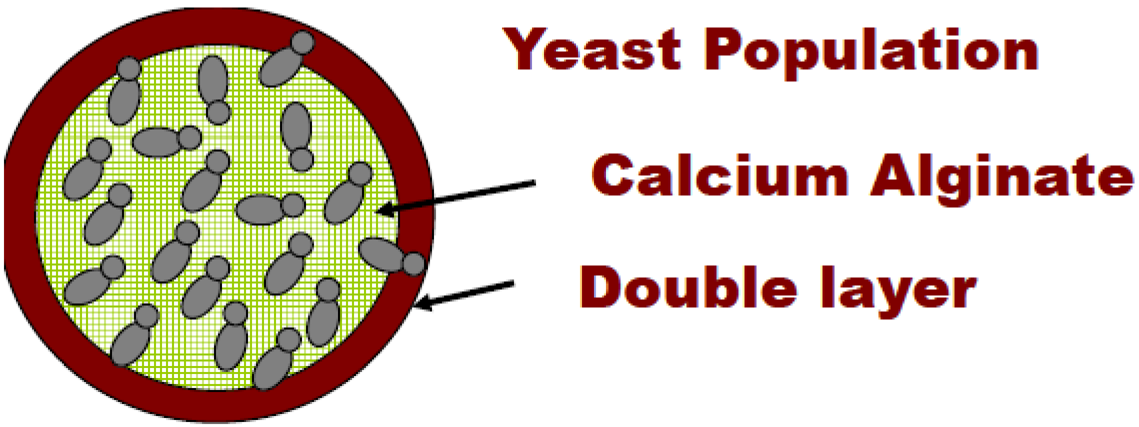
However

S. pombe was considered a spoilage yeast – can product off odors during late alcoholic fermentation

- H_2S or atypical aroma and flavor (Gallander, 1977)

- Scientists were trying to find solutions for removing *S. pompe* from the must during fermentation / or co-inoculate with S. cerevisae

Biological Methods of Deacidif ication - ProMalic



- 2.5 million living yeast cells per gram
- 2 millimeter average diameter of each encapsulated bead

These beads keep the yeast from spreading into the wine, but are permeable enough to let sugar and nutrients in

and ethanol and CO_2, out

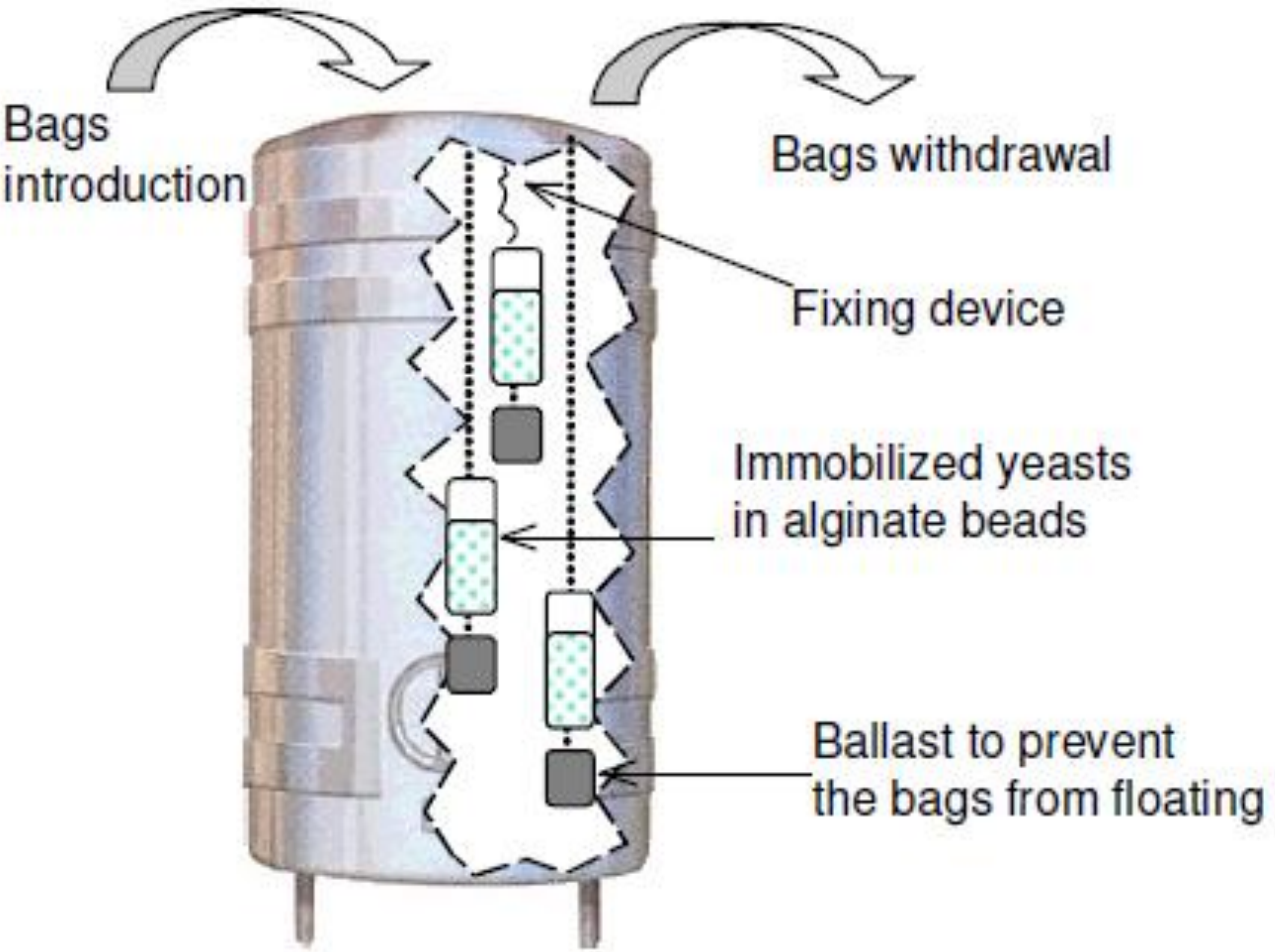
Biological Methods of Deacidification - ProMalic

ProMalic is added to the juice at the beginning of the alcoholic fermentation and removed once the desired malic level is achieved

- Can be used up to 5 times (5 differents tanks)
- Storage up to 20 months at 4°C

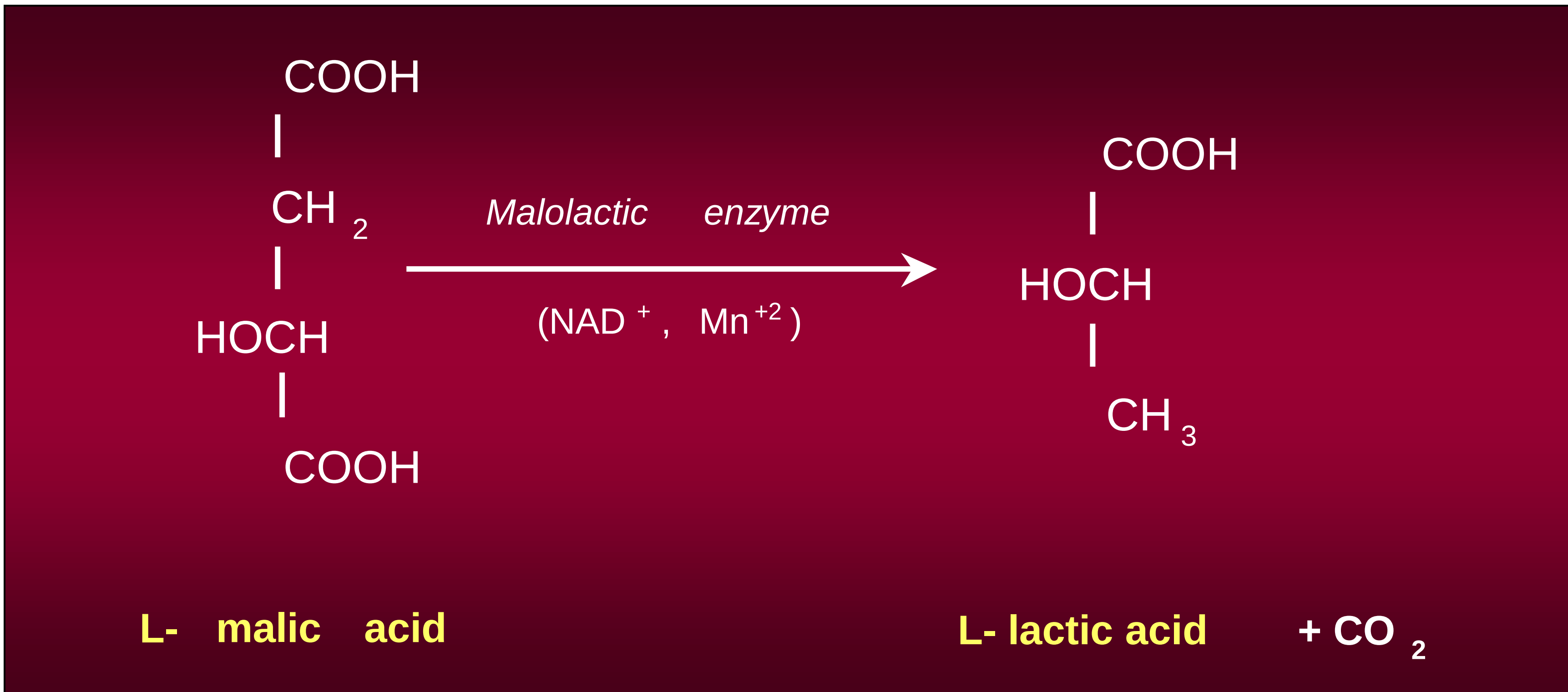
Biological Methods of Deacidification - ProMalic

Biological Methods of Deacidification - ProMalic



Biological Methods of Deacidification

d. Malolactic fermentation



Biological Methods of Deacidification

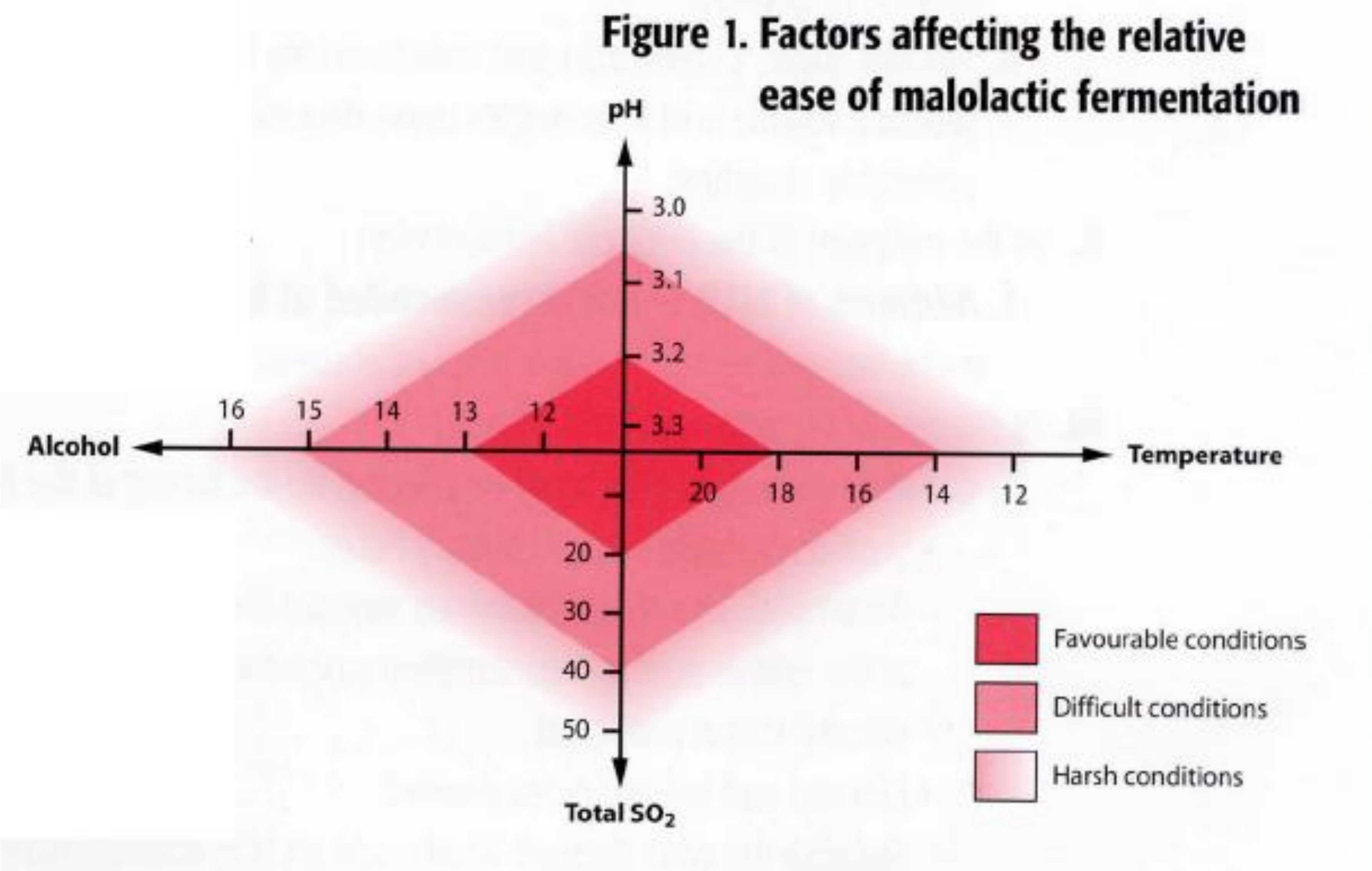
Three reasons for MLF:

- Lowering of acidity (deacidification)
- Creation of aroma compounds
- Microbiological stability

Commercial starter cultures: *Oenococcus oeni*

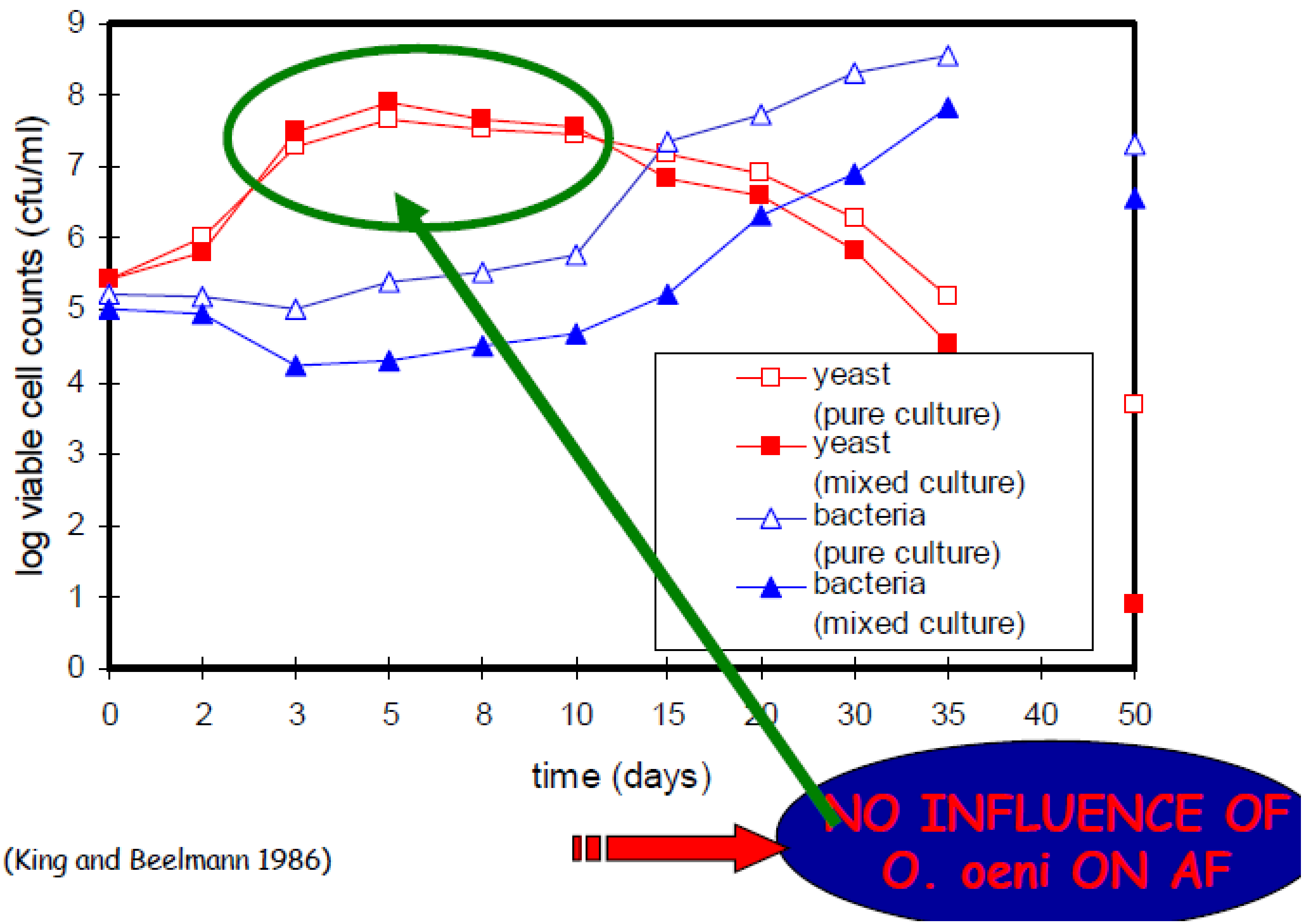
- Tolerant to lower pH, Ethanol, SO_2
- Low temperatures

Biological Methods of Deacidification



Biological Methods of Deacidification

Co-inoculation



(King and Beelmann 1986)

Biological Methods of Deacidification

Co-inoculation, no VA difference

	Simultaneous AF/MLF	Sequential AF/MLF
EQ54	0,2	0,15
ALPHA	0,19	0,17

NO DIFFERENCE

Chemical Methods of Deacidification

2. Physicochemical:

Calcium carbonate double salt precipitation

- reduces simultaneously the tartaric acid and malic acid concentrations

Chemical Methods of Deacidification

- Conditions to get a 1:1 removal of tartartic:malic
  - 2:1 ratio of malic:tartaric, **generally [malic] > [tartaric]**

- 2 moles of acid react with 2 moles of calcium carbonate, therefore 1:1 molar relationship

TARGET : LOWER ACIDITY WHILE KEEPING pH RELATIVELY LOW

Bacco noir 2011 experiment

Winemaking trials

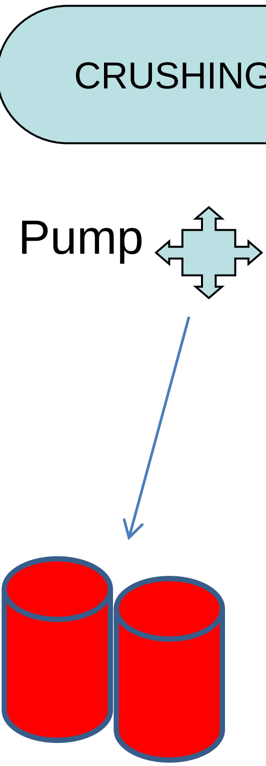
- Control
- Double salt
- 71 B
- Promalic
- Co-inoculation

Harvest date : 27/09/2011

T.A. = 10.7 g/L, pH = 3.46

Bacco noir 2011 experiment

Control sample, BU1 Contr1 and BU2 Contr2



- SO2 addition 35 gr/tn
- Enzymes 30 gr/tn

- Inoculation with K1 200 mg/L
- After 6 days separation from the skins
- Racking one week after pressing
- End of F.A. VP41 ino-cculation

Conditions

- Temperatures 25 ºC
- Punching down 3 times per day

Bacco noir 2011 experiment

Promalic, BU3 Prom1 and BU4 Prom2

CRUSHING

- SO_2 addition 35 gr/tn
- Enzymes 30 gr/tn

Conditions

- Temperatures 25 ºC
- Punching down 3 times per day

Pump

1) 40 % Juice drained and transferred to fermentor/

2) inocculation with Promalic

3) Skins and remaining juice

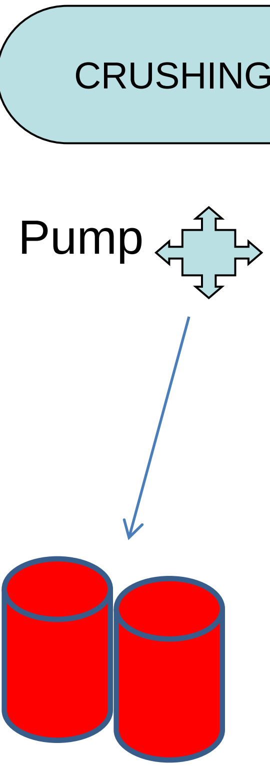
4) Inocculation with K1 200 mg/L

5) Blend back after 3 days

End of F.A. VP41 ino-cculation

Bacco noir 2011 experiment

71B, BU5 71B1 and BU6 71B2



- SO2 addition 35 gr/tn
- Enzymes 30 gr/tn

- Inoculation with 71B 200 mg/L
- After 6 days separation from the skins

- Racking one week after pressing

- End of F.A. VP41 ino-cculation

Conditions

- Temperatures 25 ºC
- Punching down 3 times per day

Bacco noir 2011 experiment

Double salt, BU7 DS1 and BU8 DS2

CRUSHING

- SO2 addition 35 gr/tn
- Enzymes 30 gr/tn

Conditions

- Temperatures 25 ºC
- Punching down 3 times per day

Pump

2) Skins and remaining juice

1) A portion of Juice was drained / transferred and treated with acidex

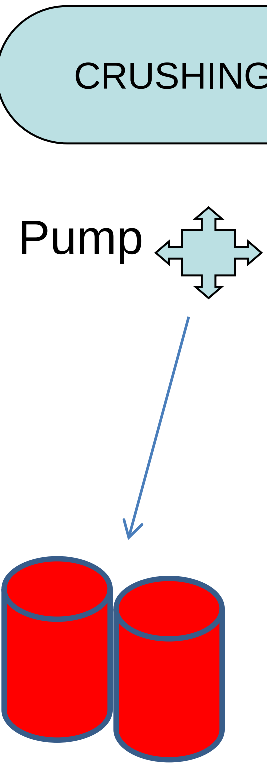
3) Filtered and blend back after 1 hour

4) Inocculation with K1 200 mg/L

End of F.A. VP41 ino-cculation

Bacco noir 2011 experiment

Co-inoculation, BU9 Coinoc1 and BU10 Coinoc2



- SO2 addition 35 gr/tn
- Enzymes 30 gr/tn

- Inoculation with 71B 200 mg/L
- Next day VP41 ino-cculation

Conditions

- Temperatures 25 ºC
- Punching down 3 times per day

Bacco noir 2011 experiment

Cool Climate Oenology & Viticulture Institute
Brock University

Fermentation Monitoring

	BU 1 (CONT1)	BU 2 (CONT2)	BU 3 (PROM1)	BU 4 (PROM2)	BU 5 (71B1)	BU 6 (71B2)	BU 7 (DS1)	BU 8 (DS2)	BU 9 (COIN1)	BU 10 (COIN2)
Date	Density	Density	Density	Density	Density	Density	Density	Density	Density	Density
28/09/2011	1.100	1.100	1.096	1.095	1.097	1.098	1.096	1.097	1.097	1.096
29/09/2011	1.100	1.087	1.083	1.111	1.095	1.115	1.086	1.089	1.092	
30/09/2011	1.050	1.048	1.052	1.050	1.060	1.070	1.056	1.056	1.067	1.088
01/10/2011	1.025	1.024	1.033	1.030	1.054	1.053				1.064
02/10/2011	1.014	1.012	1.020	1.020	1.047	1.044	1.023	1.020	1.046	
04/10/2011	0.999	0.999	1.009	1.008	1.031	1.026	1.006	1.007	1.030	
06/10/2011	0.998	0.998	0.998	0.998	1.013	1.013	0.999	0.999	1.015	1.045
07/10/2011	0.998	0.997	0.997	0.997	1.010	1.011	0.998	0.998	1.012	1.037
09/10/2011					1.006	1.006			1.006	
10/10/2011					1.004	1.004			1.004	
11/10/2011					1.008	1.002			1.002	1.018
13/10/2011					1.001	1.002			1.001	1.009
14/10/2011					1.000	1.000			0.999	1.005
16/10/2011	0.997	0.996	0.995	0.994	0.999	1.000	0.996	0.996	0.998	1.002
										1.000
										0.999

71 B, a lazy yeast strain ?

Bacco noir 2011 experiment

Cool Climate Oenology & Viticulture Institute
Brock University

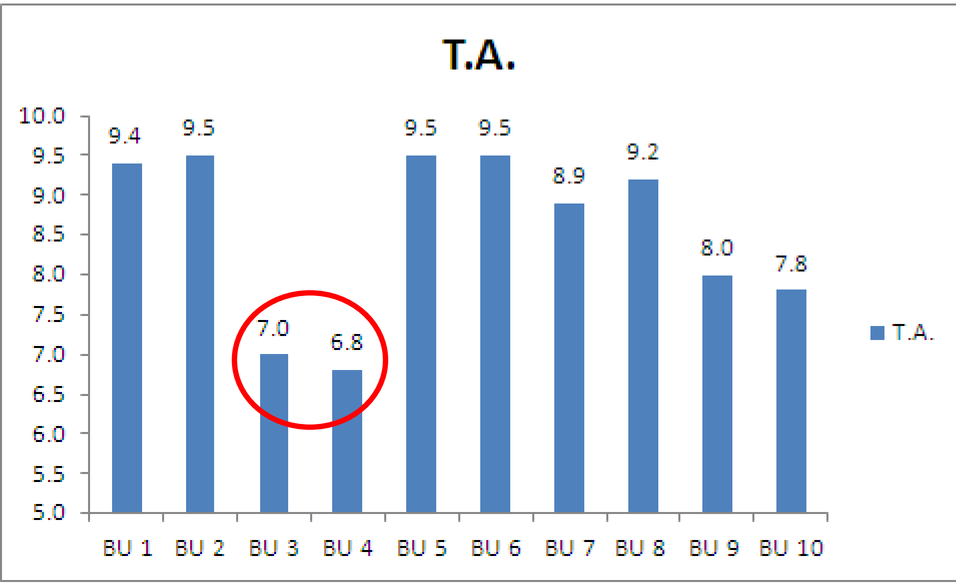
Malic acid fate

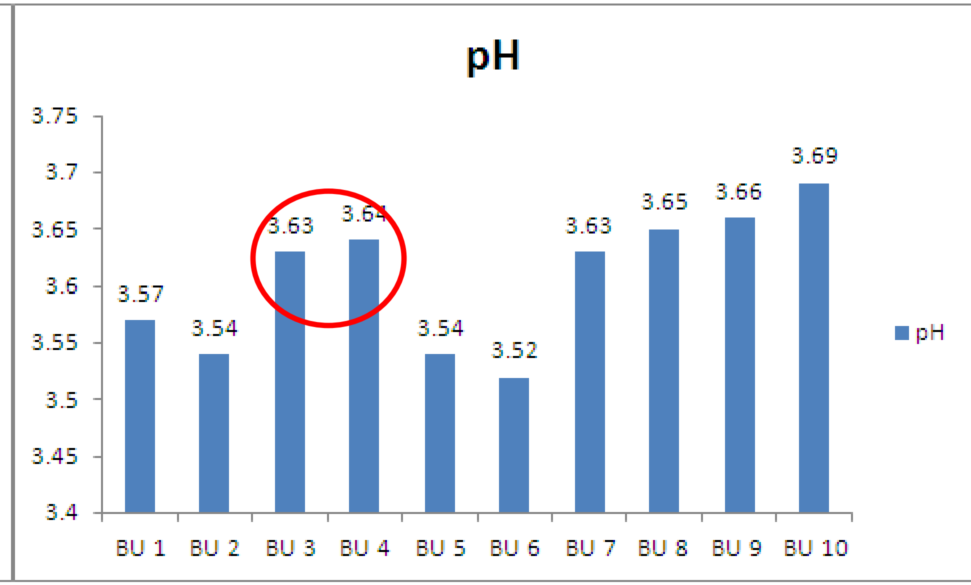
Date	BU1Control	BU2Control	BU3Promal	BU4Promal	BU5_71B	BU6_71B	BU7DoublSalt	BU8DoublSalt	BU9Coinoc	BU10Coinoc
30-Sep	3.1	3.0	2.5	2.9	3.0	2.9	3.4	3.3	2.6	1.9
07 Oct / aft press	3.1	3.0	1.9	2.1	3.0	3.0	3.4	3.3	0.3	0.1
End of Primary 17 Oct	3.1	3.0	1.0	0.9	3.0	3.0	3.4	3.3	0.0	0.0
24-Oct	2.4	2.2	0.5	0.6	2.2	2.3	2.5	2.5		
01-Nov	0.7	0.5	0.1	0.0	0.3	0.3	0.3	0.3		
08-Nov	0.01	0.01	0.0	0.0	0.01	0.01	0.01	0.01		

21 days faster

- Co-inoculation : achieving faster the biological stability
- The same for Promalic

Bacco noir 2011 experiment





BU1Control	BU2Control	BU3Promal	BU4Promal	BU5_71B	BU6_71B	BU7DoublSalt	BU8DoublSalt	BU9Coinoc	BU10Coinoc

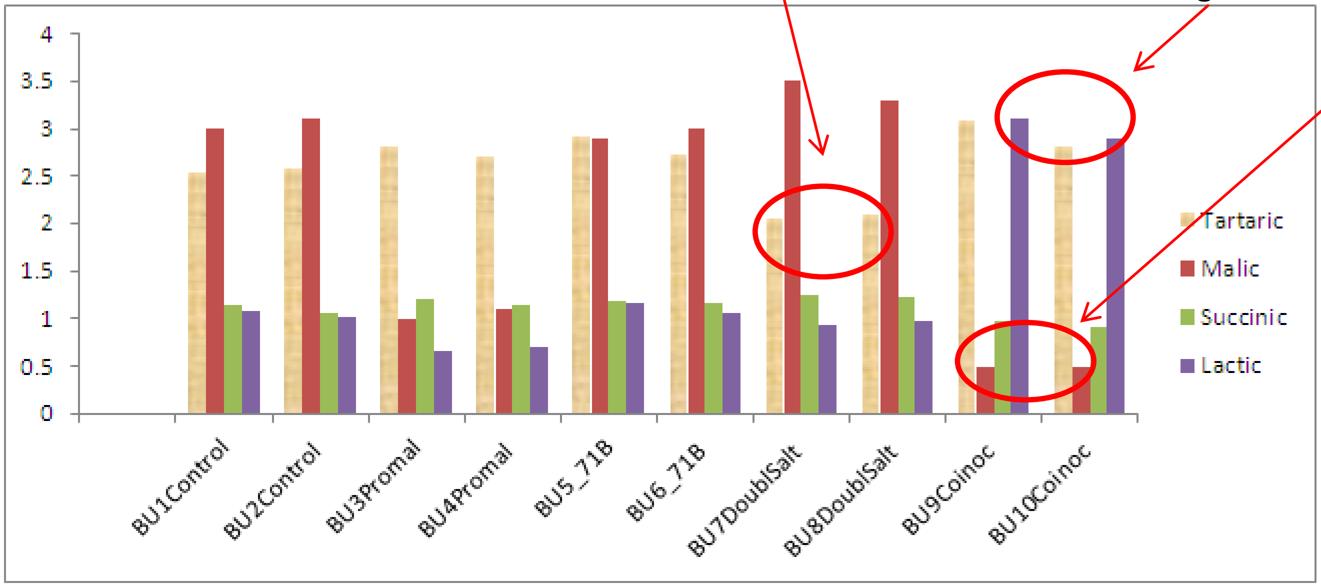
End of F.A.

- BU3 and BU4 present low T.A. and adequate pH, the winemaker could add sulfites

Bacco noir 2011 experiment

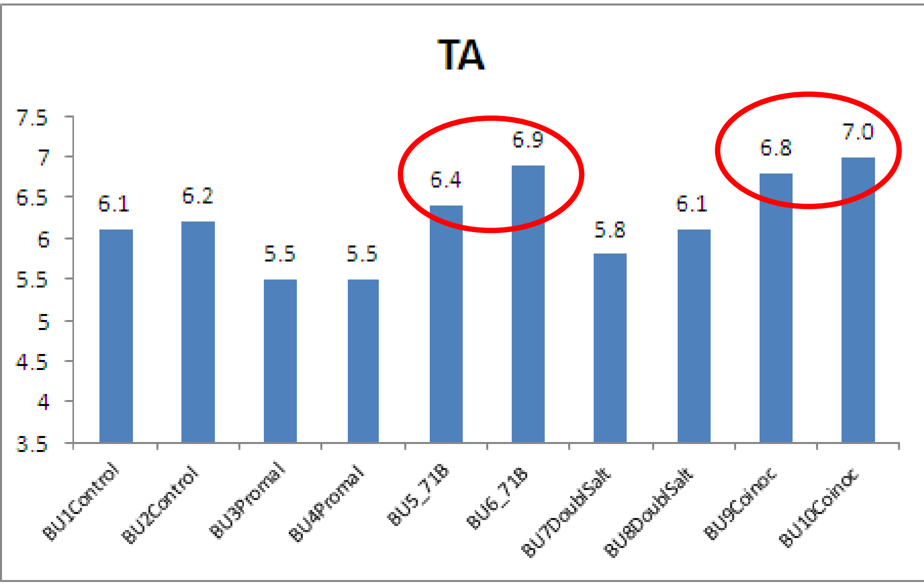
Double Salt samples - lower tartaric

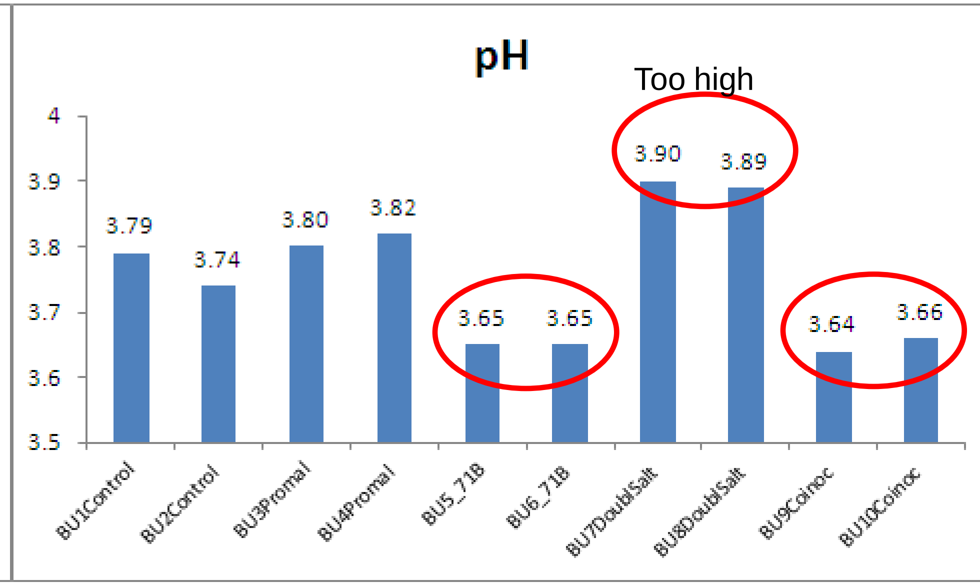
Co-inoculation samples – Low Malic, High Lactic



End of F. A.

Bacco noir 2011 experiment

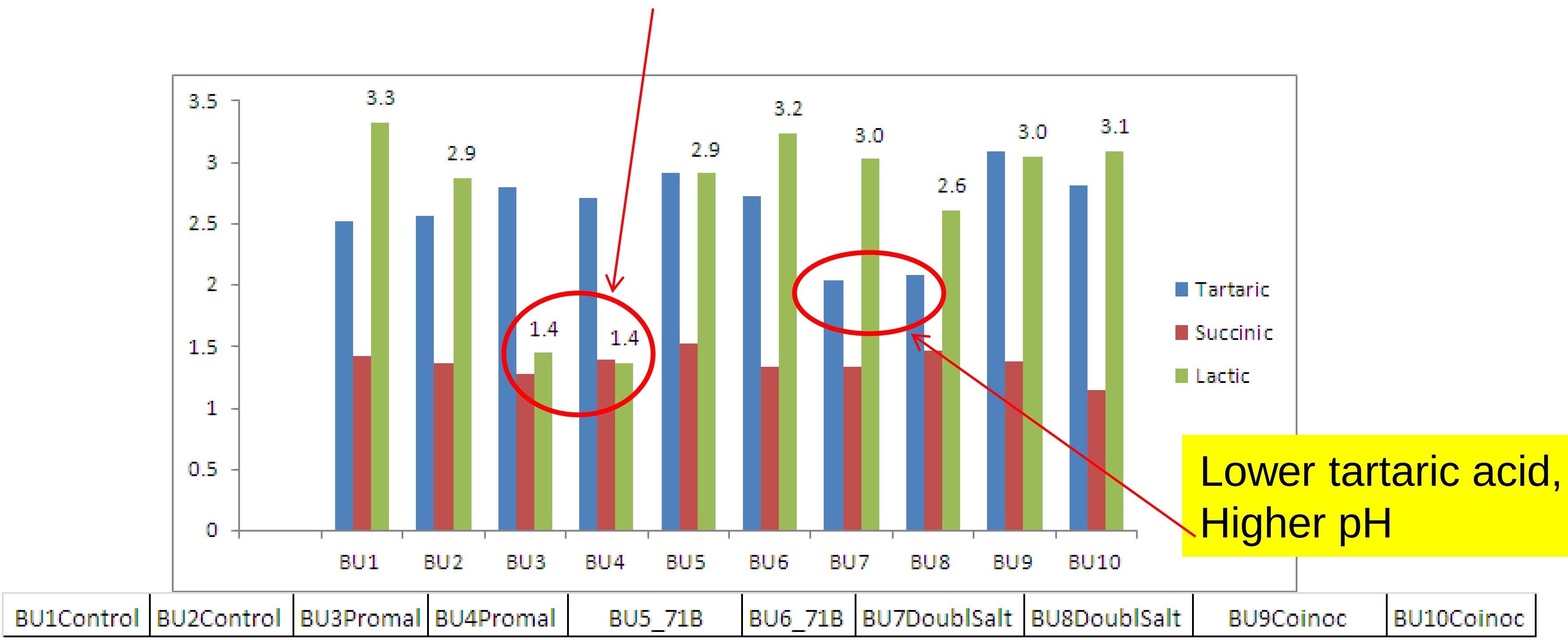




End of M.L.F.

- The samples fermented with 71B and the co-inoculated ones present lower T.A. but adequate pH too !!!

Bacco noir 2011 experiment

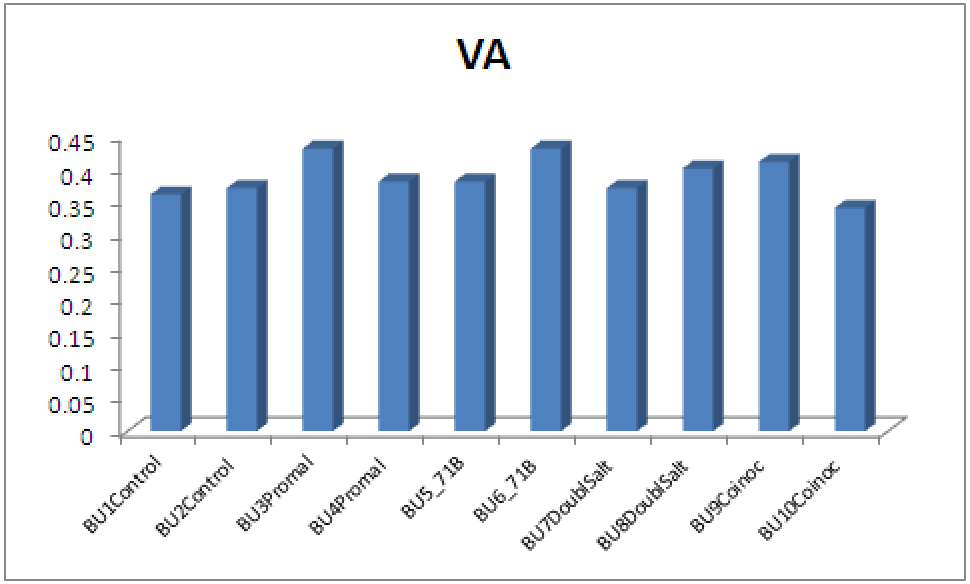


End of M.L.F.

- The very low T.A. of the samples treated with Promalic is explained by the low levels of lactic acid in comparison to all the other samples

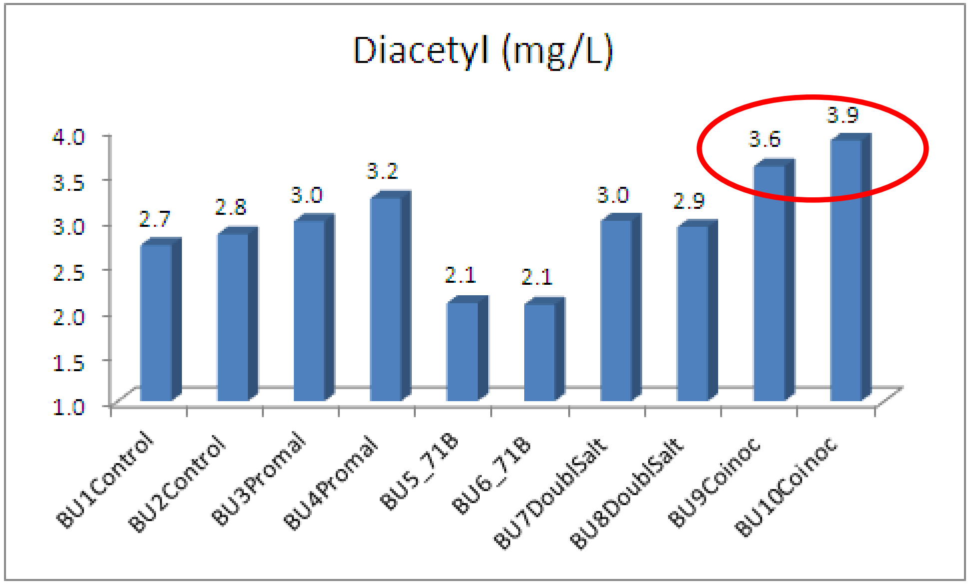
Bacco noir 2011 experiment

Cool Climate Oenology & Viticulture Institute
Brock University



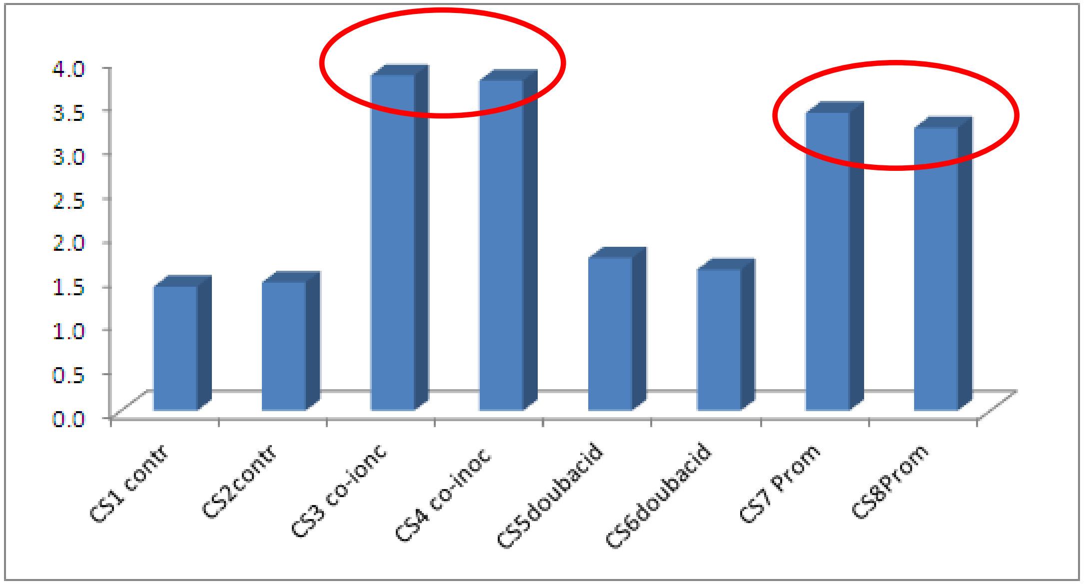
- Practically V.A. is the same between control and co-inoculation

Bacco noir 2011 experiment



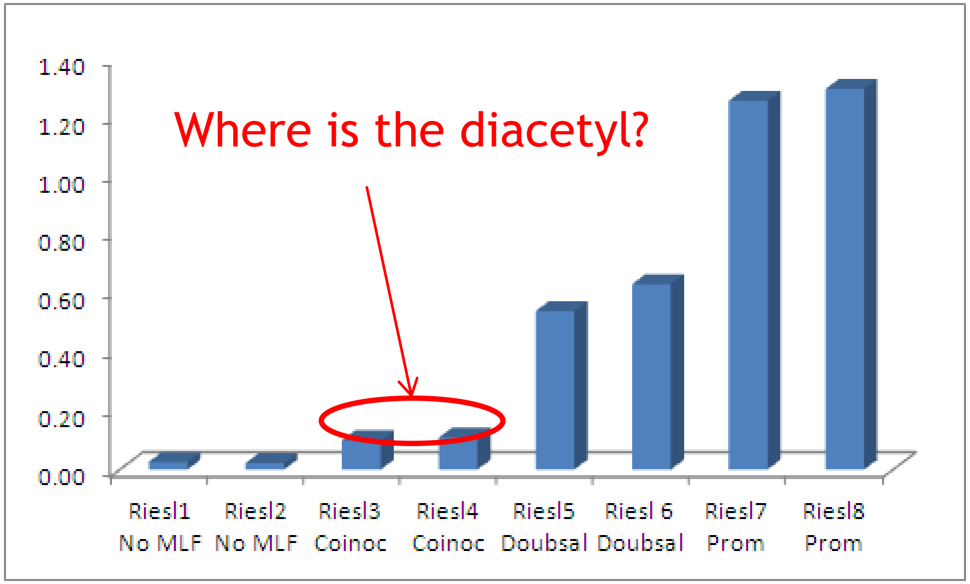
Cabernet Sauvignon 2011 experiment

Cool Climate Oenology & Viticulture Institute
Brock University



- Cabernet Sauvignon, initial pH 3.4

Riesling2011 experiment

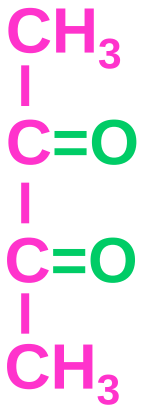


- Riesling, initial pH 2.8

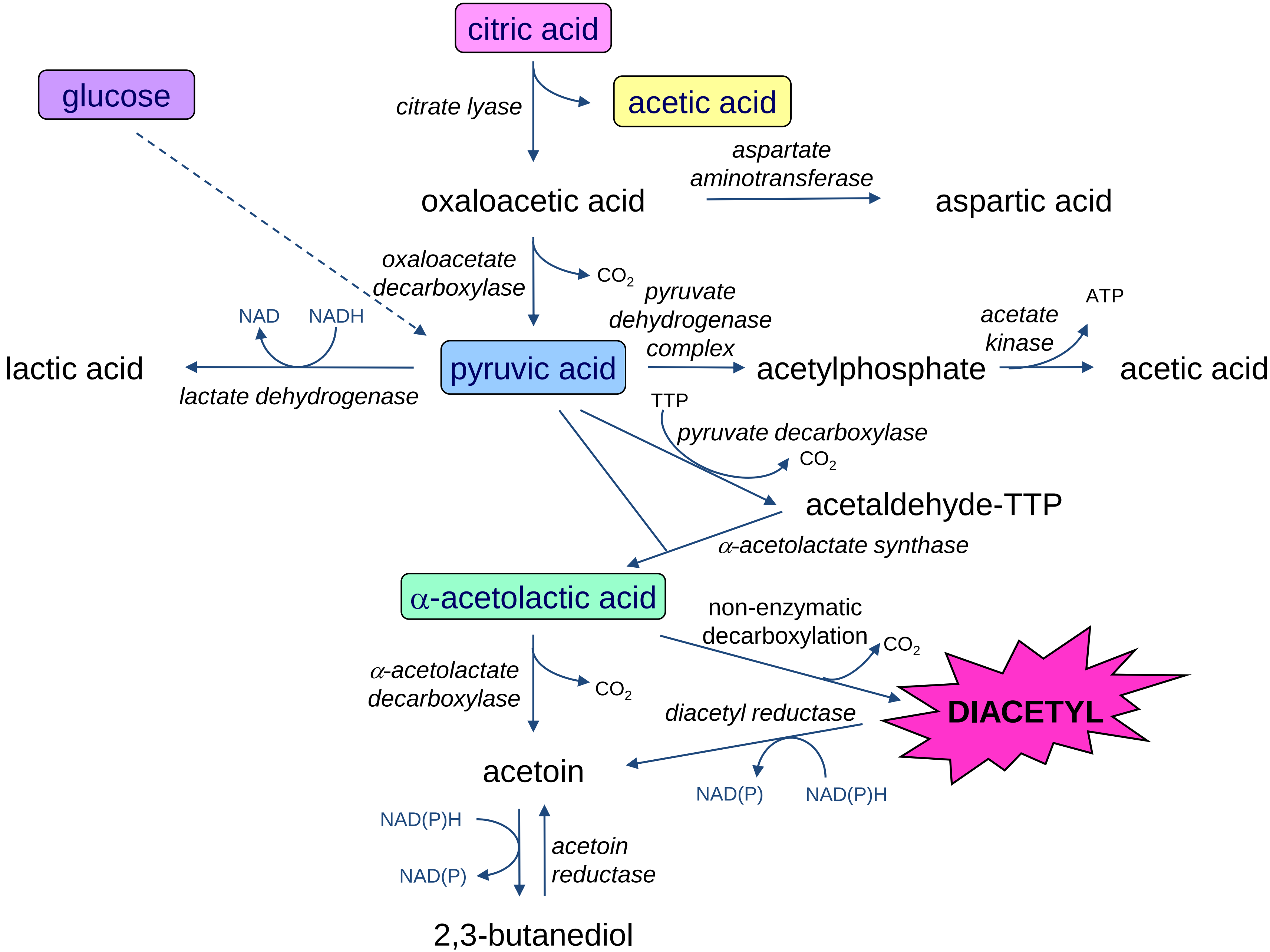
Bacco noir 2011 experiment

Diacetyl

- *O. oeni* during MLF
- Derived from citric acid metabolism
- Aroma
  - buttery, nutty, butterscotch

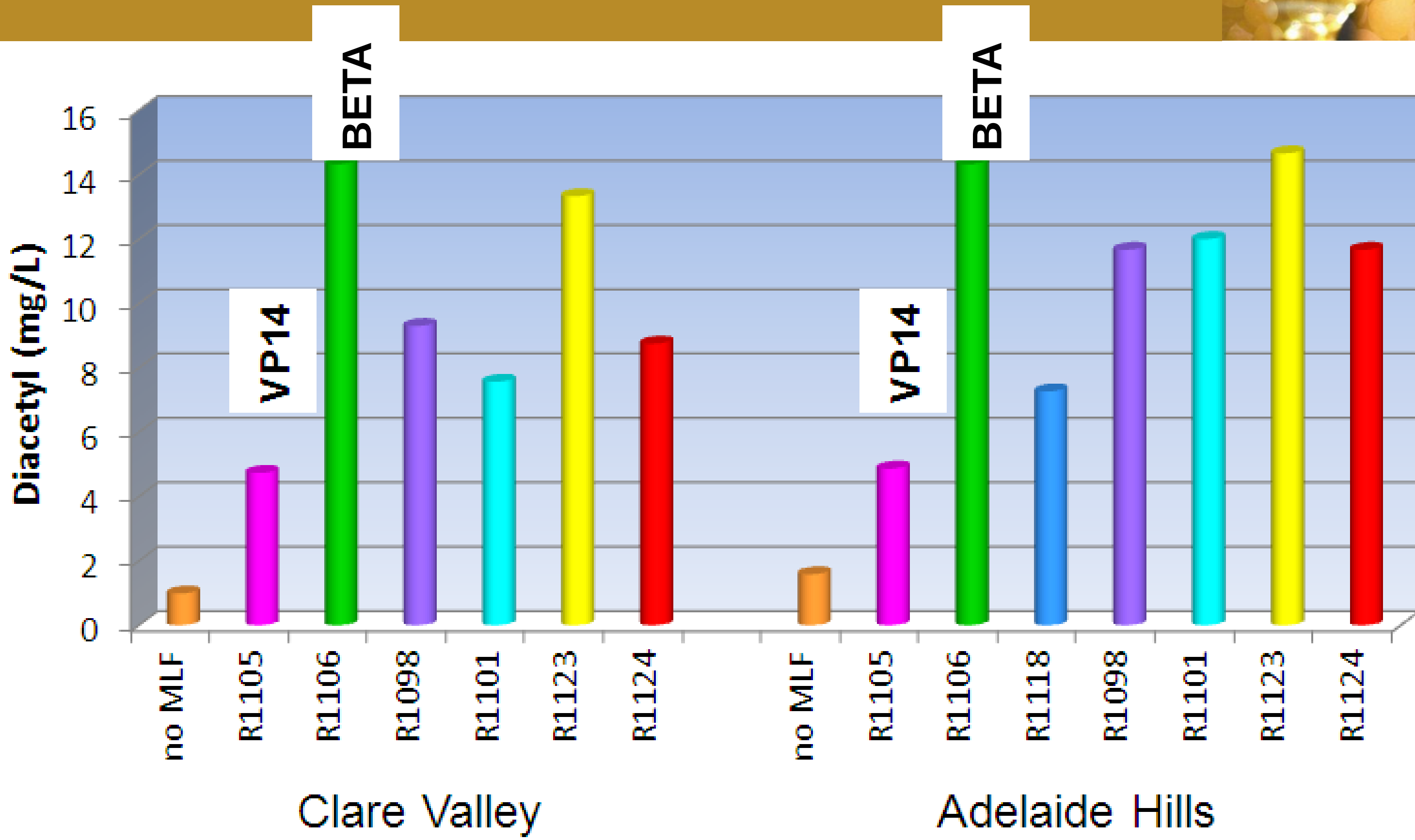


  - 1 - 4 mg/L = enhance flavour complexity
  - > 5 - 7 mg/L = undesirable buttery aroma

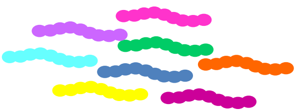
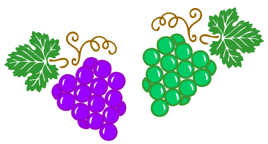
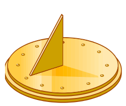


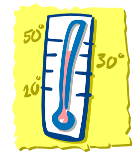
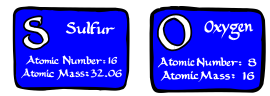
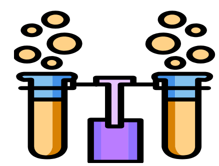
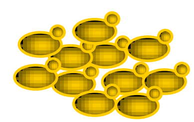
Eveline Bartowsky, AWRI, 2004

Bacco noir 2011 experiment



Diacetyl - management during winemaking

	Diacetyl conc^n
 *O. oeni* strain	variable
 wine type	white - lower red - higher
  inoculation rate	10^4 - higher 10^6 - lower
 fermentation time	longer MLF - higher

	Diacetyl conc^n
 temperature	18°C - higher 25°C - lower
 SO_2	binds to diacetyl - sensorially inactive
 aeration	air - higher anaerobic - lower
 contact with yeast lees	long contact- lower
 pH	lower pH may favour

Eveline Bartowsky, AWRI, Trier 2008

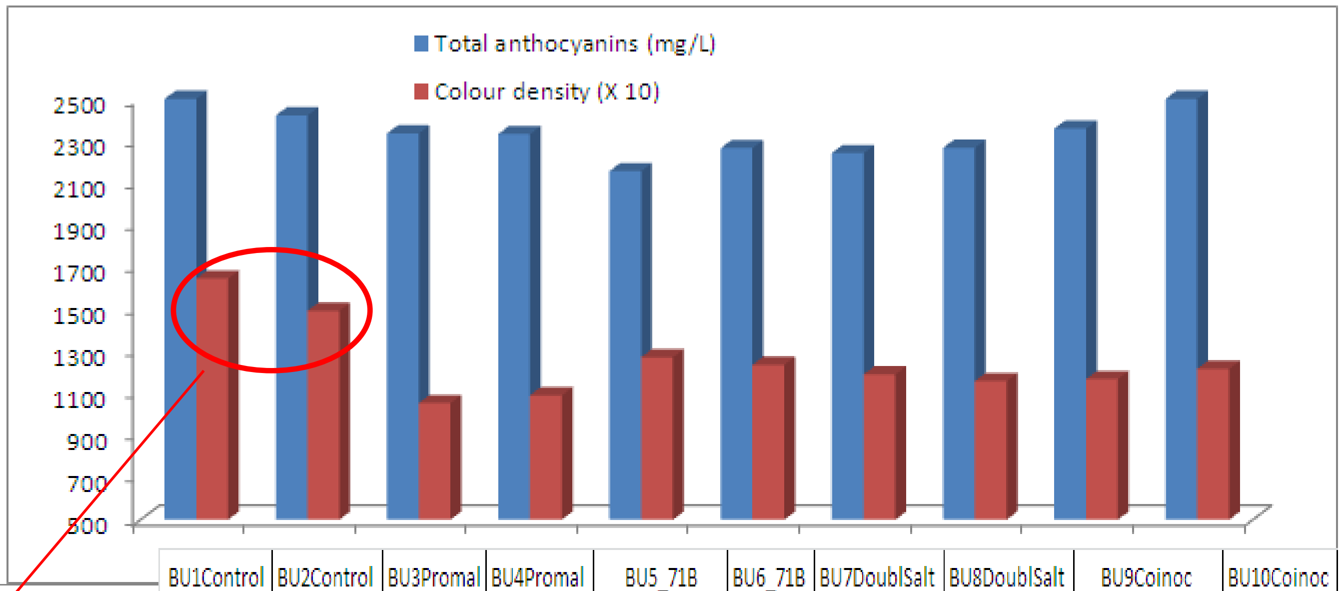
Bacco noir 2011 experiment

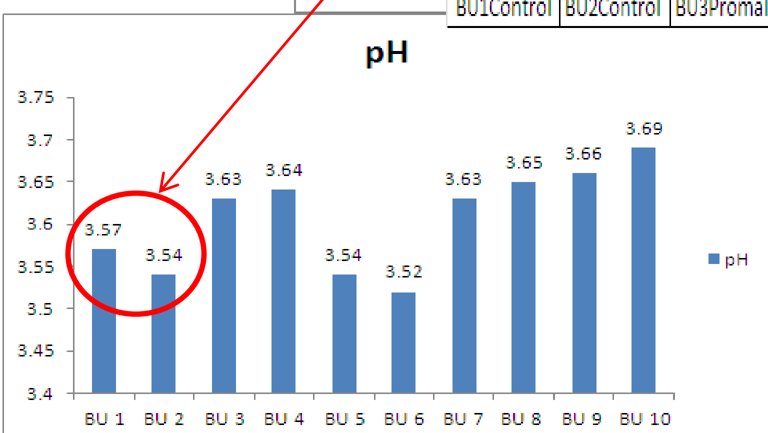
Does co-inoculation enhances diacetyl production and if yes what are the parameters ?

Could it be a method to adopt when not suficient maturity in order to cover the ‘vegetal’ aromas?

Does the Promalic enhances diacetyl production ?

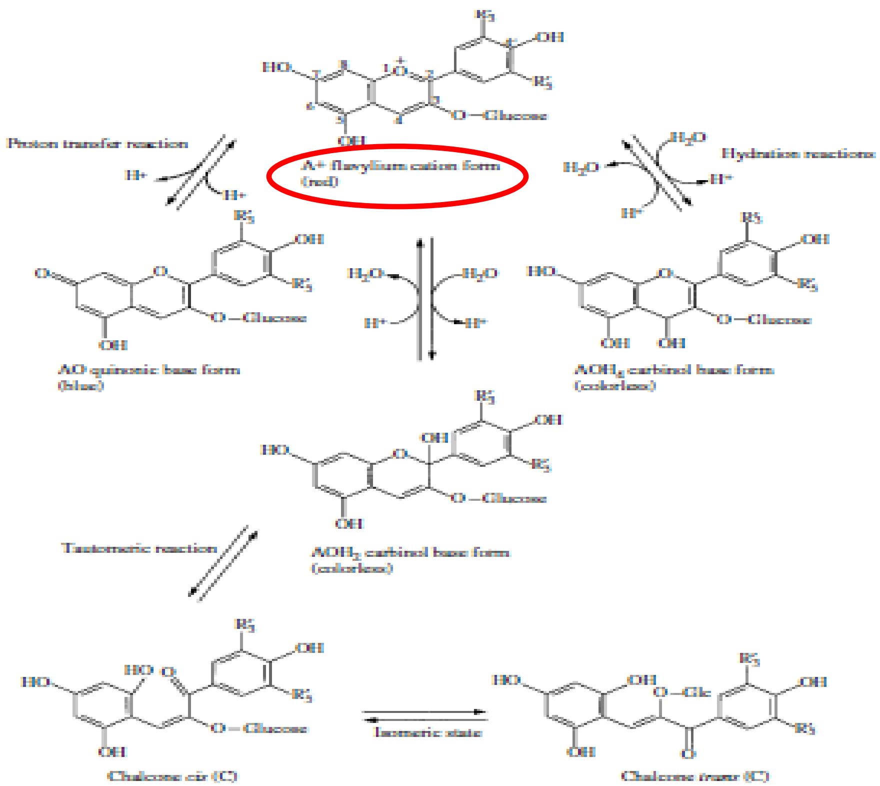
Bacco noir 2011 experiment





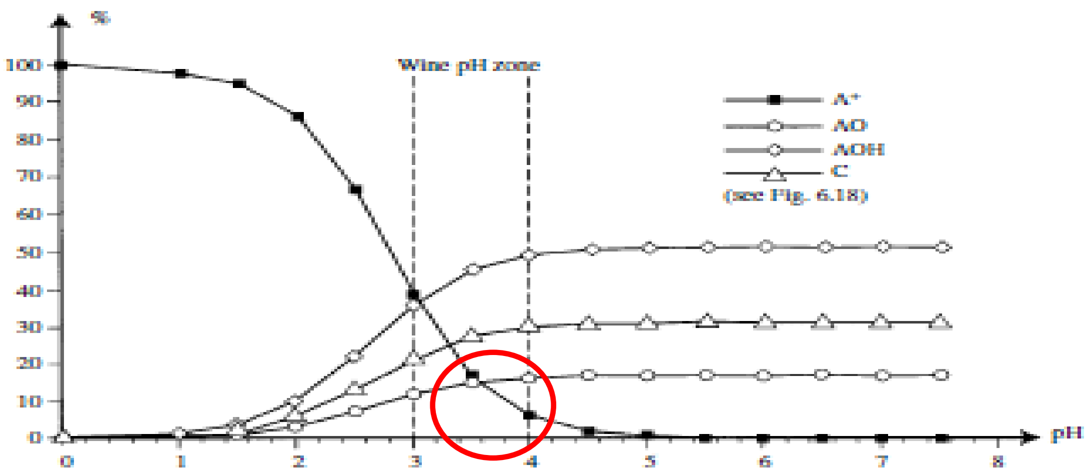
- BU1 and BU2 presented the lowest Ph at the end of F.A.
- Does that explains the higher colour densities?

Bacco noir 2011 experiment



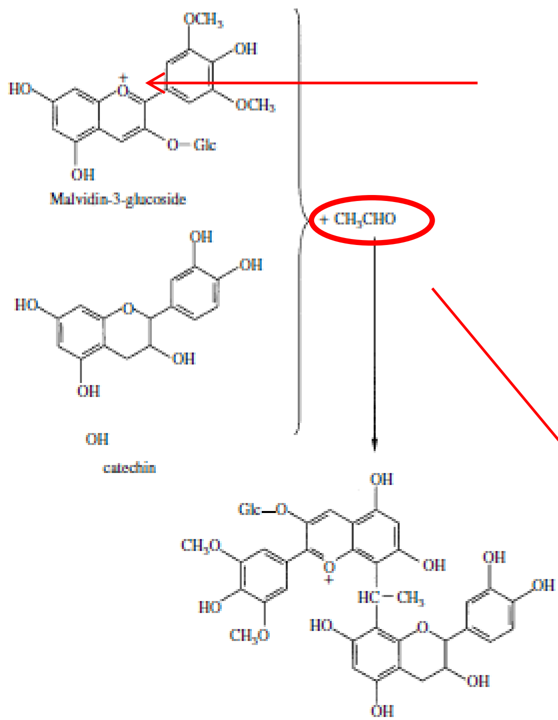
Brouillard et al. 1978

Bacco noir 2011 experiment



Glories 1984

Bacco noir 2011 experiment



- Flavylium form is the most reactive with the tannins

- Better colour stability, higher colour density

?

Timberlake and Bridle 1976

Bacco noir 2011 experiment Conclusions

- Using Biological deacidification could achieve lower T.A. while the pH remains adequate
- Co-inoculation seems to enhances diacetyl production depending on the pH
- Co-inoculation saves time (the wine turned biologically stable 21 days before)
- Co-inoculation does not enhances acetic acid production
- Promalic could be used to degrade malic acid to ethanol and thus lower significantly the T.A.
- More research is needed as also repetition of the experiments

Measuring polyphenols on grapes 2011 experiment

Tannins and anthocyanin are responsible for positive tasting characteristics, but also for negative aspects too

Body, structure, roundness and on the other hand, bitterness, roughness, harshness, astringency

Overall organoleptic impression is based on a harmonious balance between these two types of sensations, related to

- the type
- concentration of tannins and anthos
- pH and T.A. levels
- As also on ethanol levels

Measuring polyphenols on grapes 2011 experiment

One of their properties is to react with glycoproteins in saliva (mucine) and proteins in the mouth wall, modifying their condition and lubricant properties

A study of the reaction of the B3 procyanidins with synthetic, proline-rich proteins showed that three dimers were strongly bonded to the protein chains (Simon *et al*., 2003)

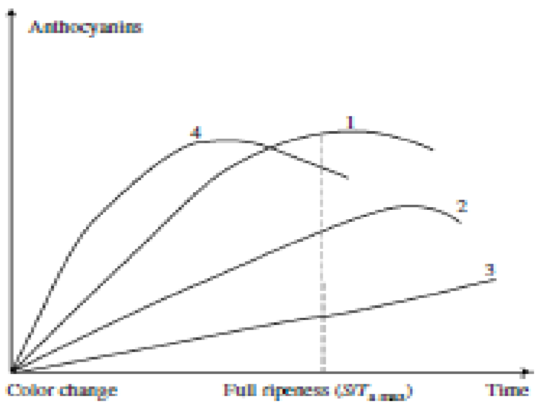
Measuring polyphenols on grapes 2011 experiment

Mainly 2 factors affects the levels of anthos and tannins in red wines

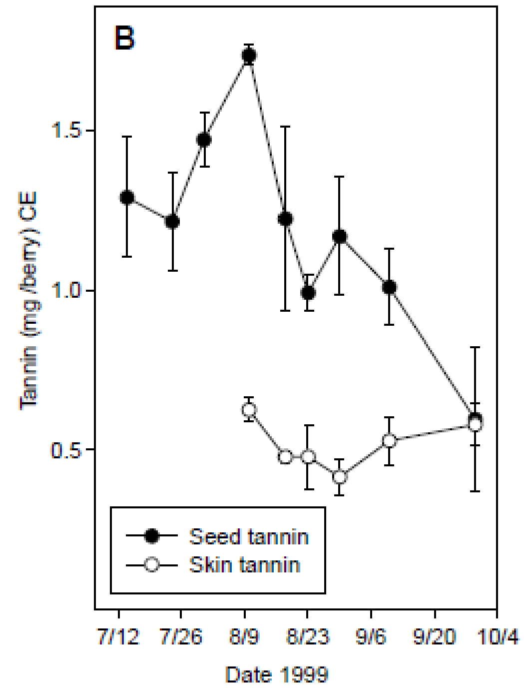
Phenolic maturity

Maceration – extraction during winemaking

Measuring polyphenols on grapes 2011 experiment



Anthos evolution during maturity
In various vineyards of Merlot



Tannins evolution during maturity in Cabernet Sauvignon

Measuring polyphenols on grapes 2011 experiment

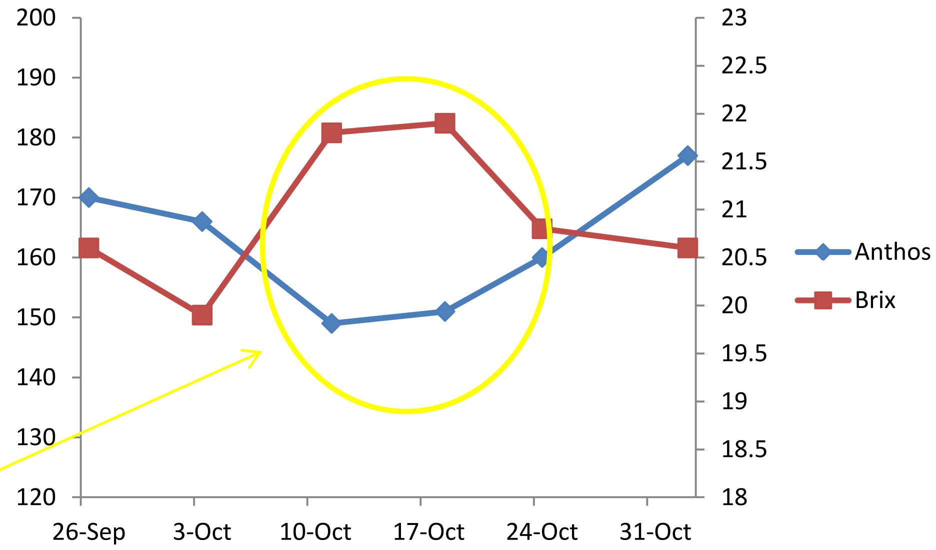
CCOVI - Grape Pre-Harvest Sampling Data 2011

Site 1

Location : East of Canal

Soil : Sandy and loamy

Variety : Cabernet Sauvignon



Higher Brix levels

does not correspond to higher anthos levels

Measuring polyphenols on grapes 2011 experiment

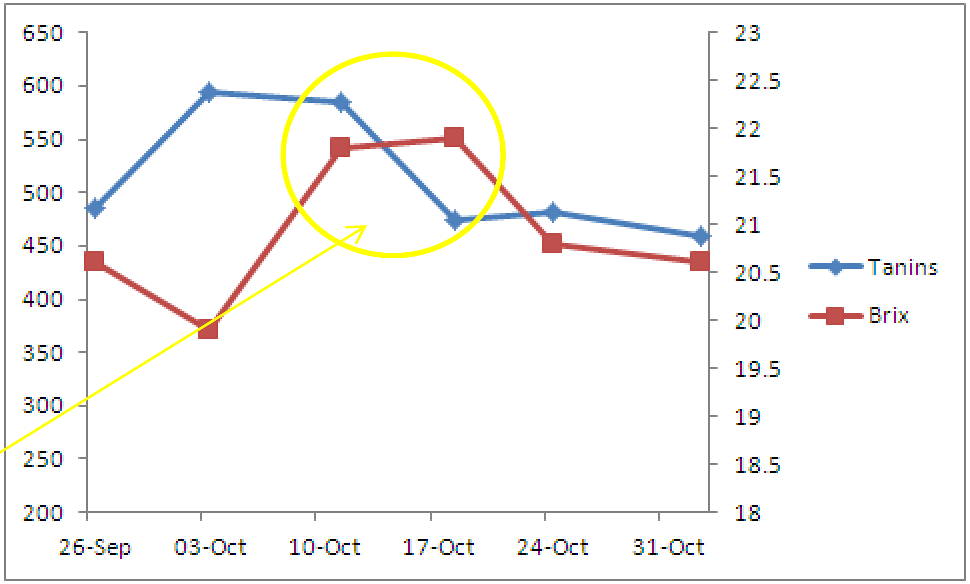
CCOVI - Grape Pre-Harvest Sampling Data 2011

Site 1

Location : East of Canal

Soil : Sandy and loamy

Variety : Cabernet Sauvignon



Grapes start being ready for harvest

Measuring polyphenols on grapes 2011 experiment

Cool Climate Oenology & Viticulture Institute
Brock University

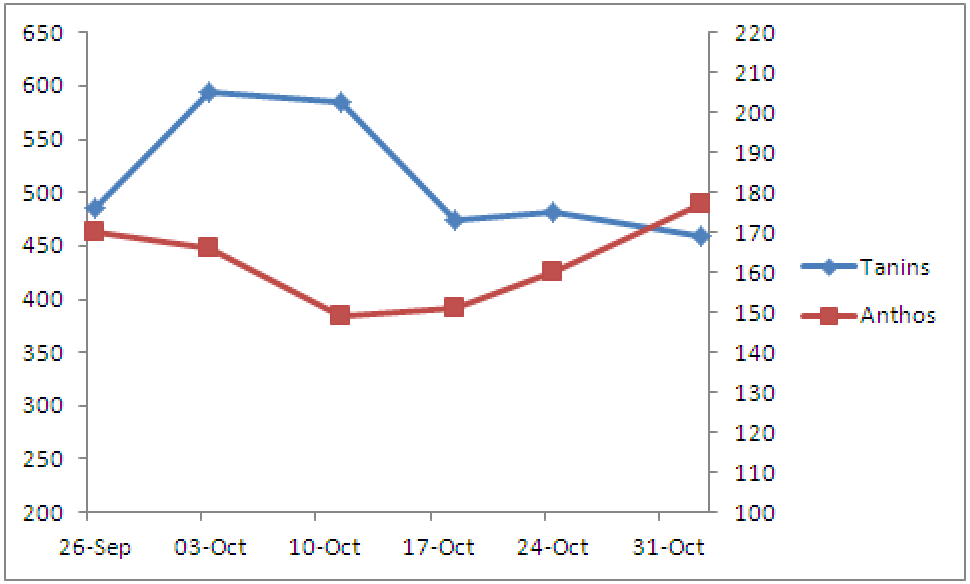
CCOVI - Grape Pre-Harvest Sampling Data 2011

Site 1

Location : East of Canal

Soil : Sandy and loamy

Variety : Cabernet Sauvignon



Conclusion

Wine balance is a very complicated task for the winemaker

Acquiring knowledge will improve Ontario wines

Further experimentation at CCOVI will be done for the biological deacidification methods

as also for the phenolic content of grapes and wines (phenolic maturity, winemaking at different maturity levels, study of various maceration methods

THANK YOU VERY MUCH

SPECIAL THANKS TO

HENRY OF PELHAM TEAM

Fed Dev ARC

LALLEMAND

STRATUS

CAVE SPRINGS

CCOVI TEAM