YEAST STRAINS AND THEIR EFFECTS DURING FERMENTATION

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OUTLINE

- Examine the yeast associated with the winemaking process
- General overview of the compounds they can produce that can help drive wine style
- Known factors that influence yeast involved

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GENERAL COMPOSITION OF WINE

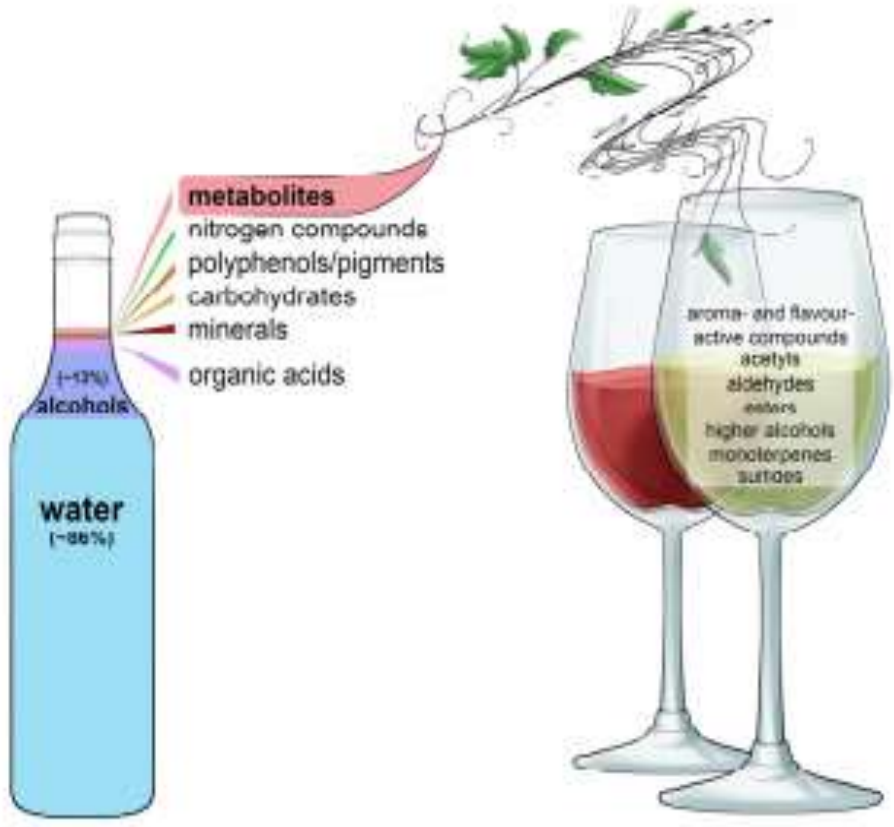

Image from Pretorius I. S. **Beverages** 2, 36, 2016

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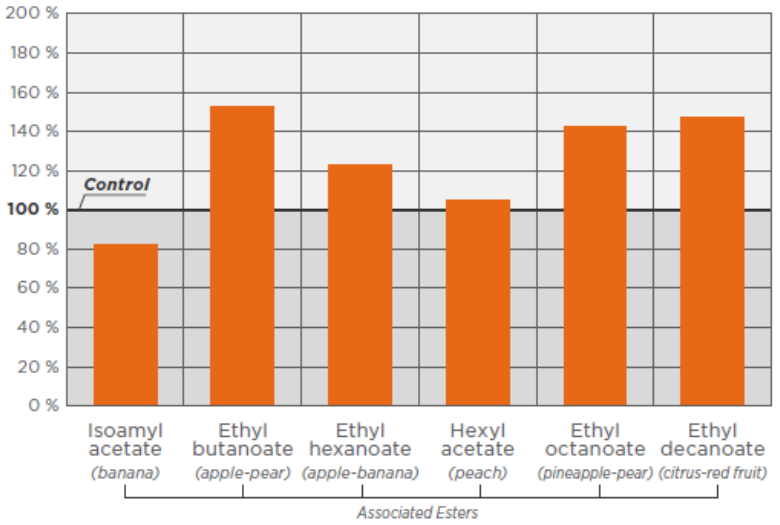
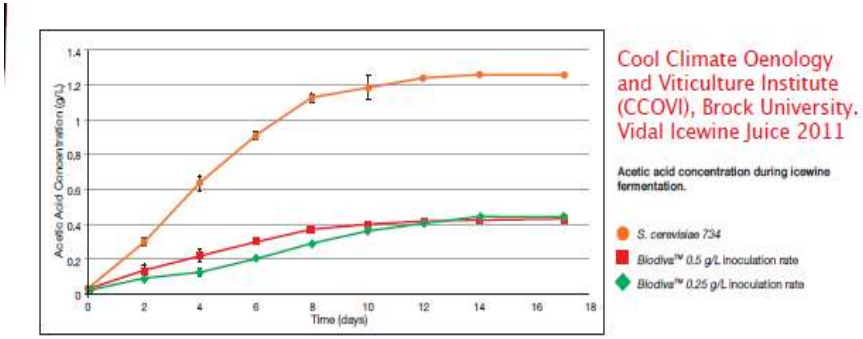
YEAST DIVERSITY DURING FERMENTATION

Pretorius I. S. **Beverages** 2, 36, 2016

TORULASPORA DELBRUECKII

- Interesting in:
  - Aromatic whites, late harvest wines
- Metabolites of interest
  - Linalool (sweet-floral)
  - Succinic acids (sweet-bitter)
  - Enhanced esters
- Additional points
  - Osmotolerant
  - Low production of negative compounds (VA, Sulfides, Vinyl Phenols)





Images courtesy of Lallemand, Inc.

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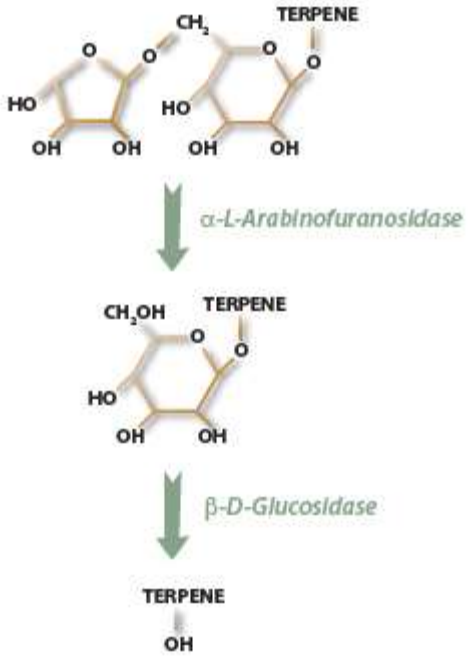
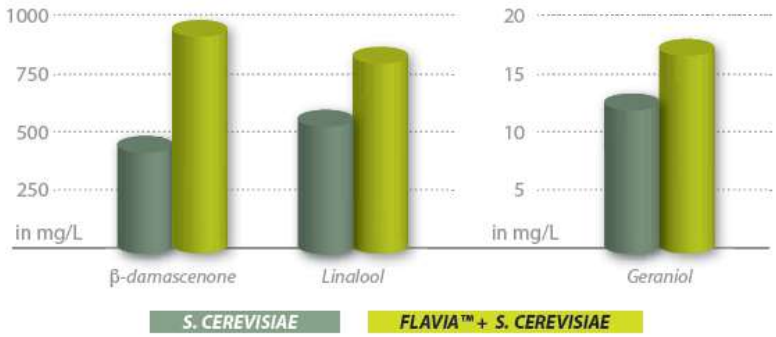
METSCHNIKOWIA SPP.

- Interesting in:
  - Pre-fermentation maceration bio-control
  - Aromatic whites and roses due to enzyme activity
- Metabolites of interest
  - Release of glycosylated terpenes (fruity aromas)
  - Release of volatile thiols
  - Varietal aromas
  - Esters (esp. pear)
  - Polysaccharides to build volume
- Additional points
  - No/ low fermentative capacity
  - Can be incompatible with Saccharomyces





Images courtesy of Lallemand, Inc.

PICHIA KLUYVERI

- Interesting in:
  - Aromatic whites , reds and roses
- Metabolites of interest
  - Release of volatile thiols
- Additional points
  - Must have a compatible Saccharomyces strain

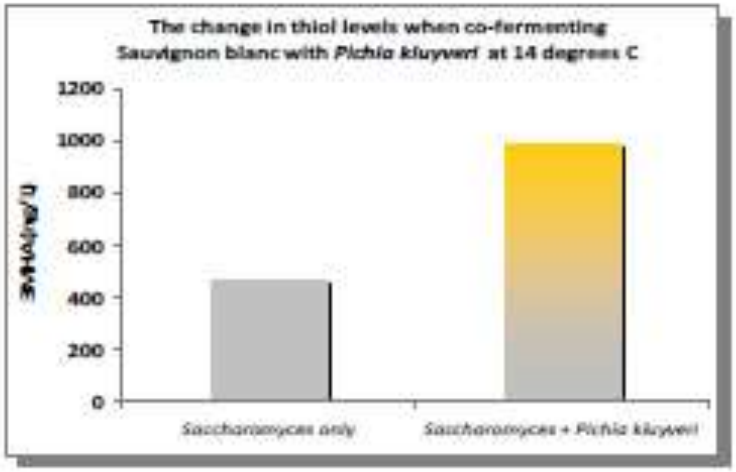


Image courtesy of Gusmer Enterprises

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NON-SACCHAROMYCES

- Can produce a range of flavor compounds
  - Terpenes
  - Esters
  - Higher alcohols
  - Glycerol
  - Acetaldehyde
  - Acetic Acid
  - Succinic Acid
- Must consider organism compatibility

The enological yeast of choice!

SACCHAROMYCES CEREVISIAE

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FERMENTATION GOALS

- Goal
  - Sugar to Ethanol + CO_2
  - Sugar to Ethanol + CO_2 with no sensory deviations
  - Sugar to Ethanol + CO_2 with an influence on:
    - Aromatic production and enhancement
    - Mouthfeel
    - Stability
    - Acid chemistry

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S. CEREVISIAE- INFLUENCE ON SENSORY PROFILE

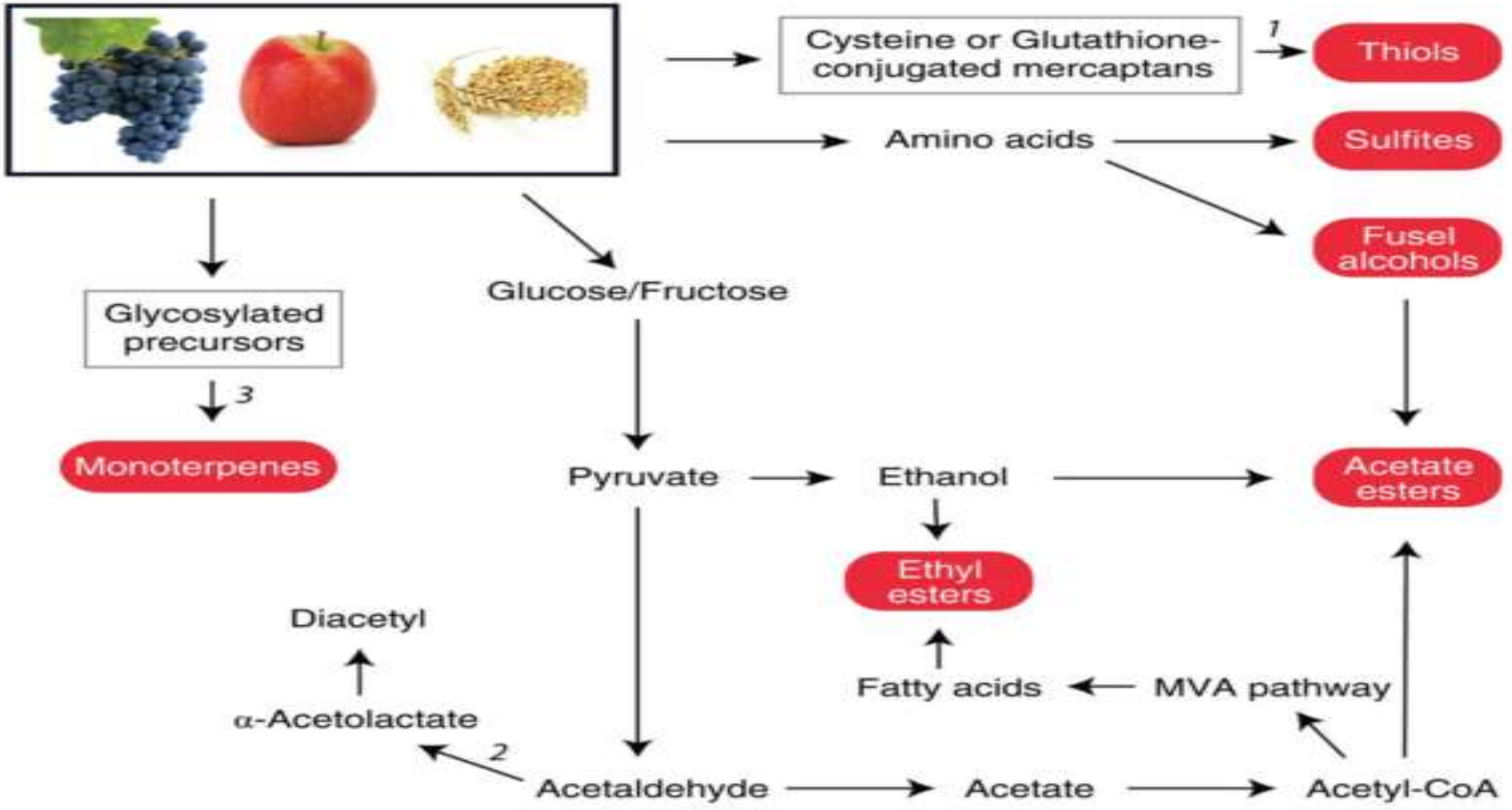

Image adapted from: Hirst M.B. and Richter C.L. AJEV67:4 (2016)

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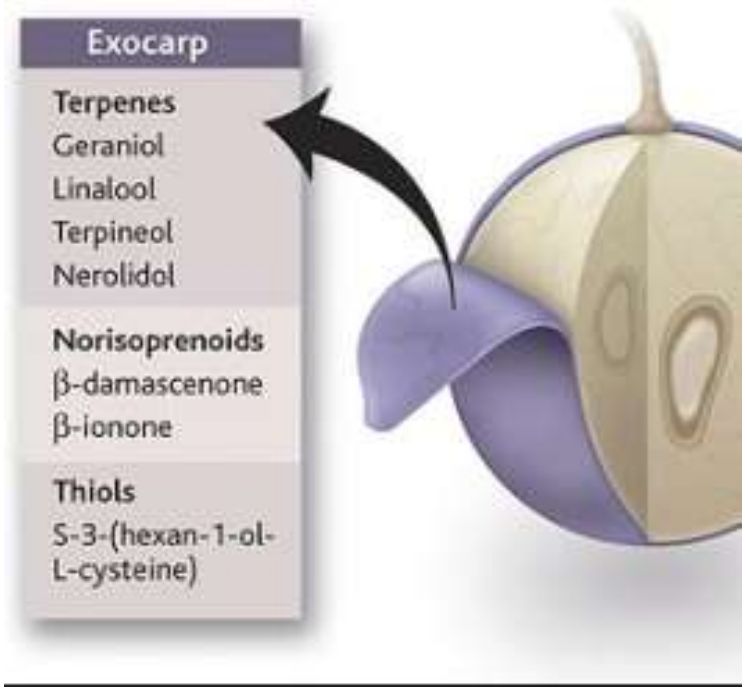
VOLATILE THIOLS

- Sulfur based compounds
- Located in the skin
- Aromatic whites and reds
- Need to be elaborated from their odorless form



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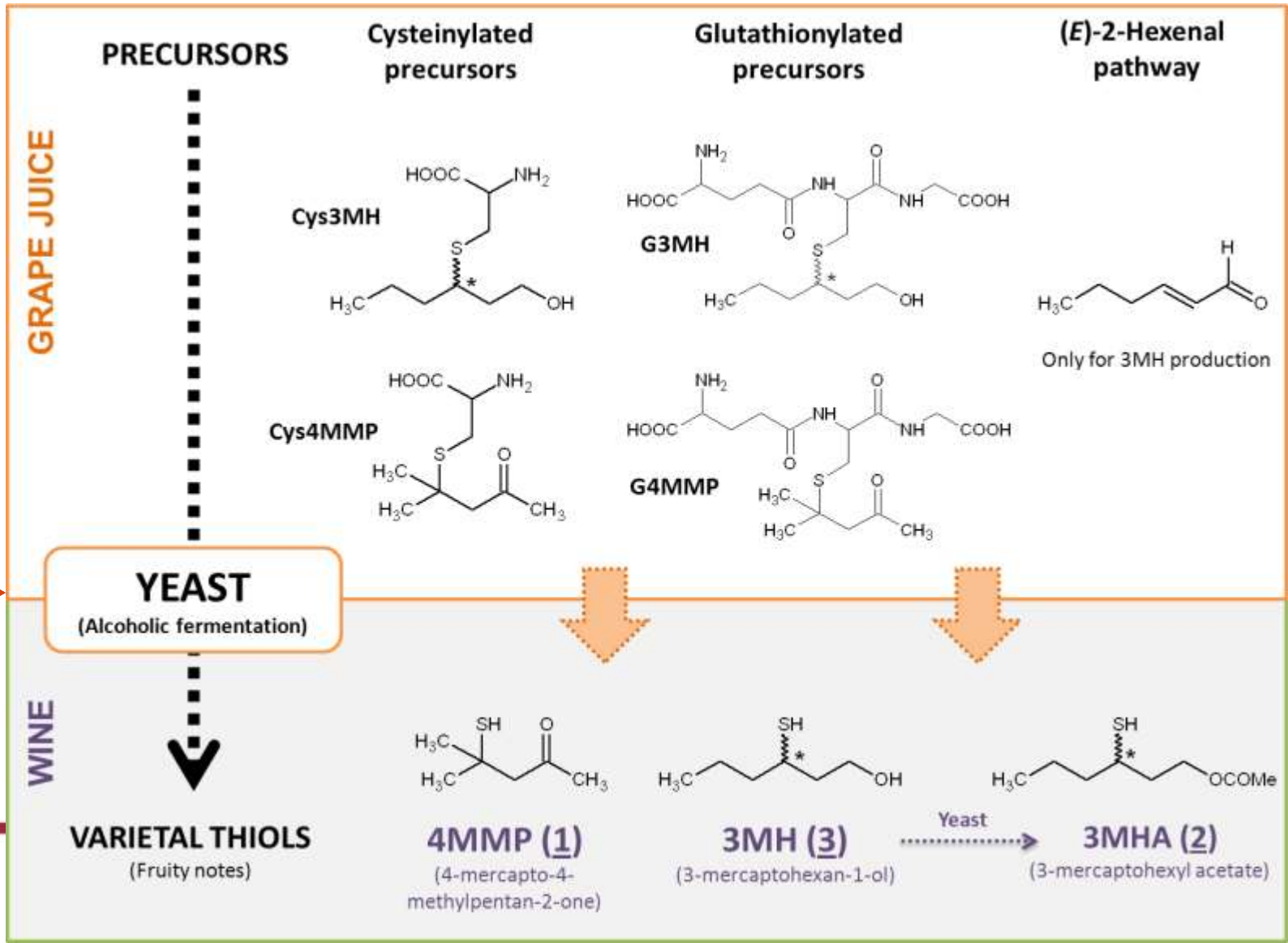
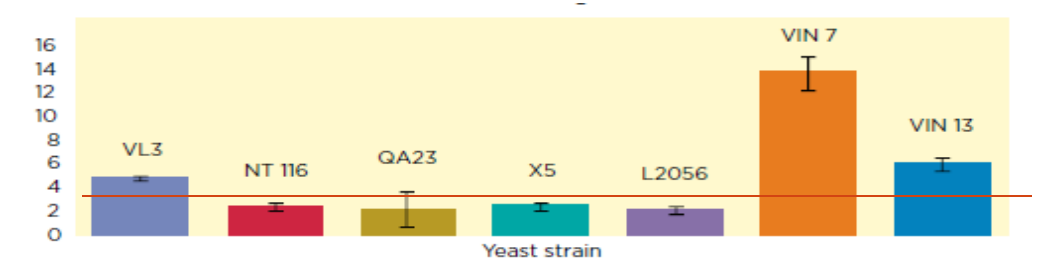
TROPICAL VARIETAL THIOLS

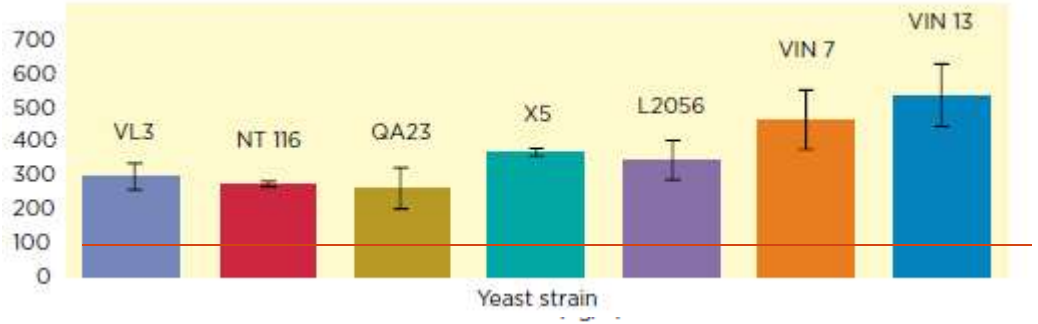
Rémi Guerin-Schneider (IFV) , 2012



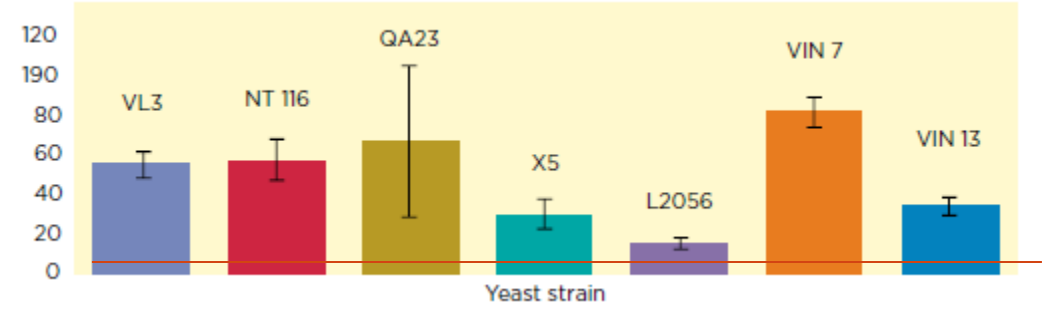
YEAST STRAIN INFLUENCE- SAUVIGNON BLANC



- 4MMP
  - Sensory threshold 3ng/L
  - Boxwood, passion fruit, blackcurrant



- 3MH
  - Sensory threshold 60ng/L
  - Passion fruit, grapefruit



- 3MHA
  - Sensory threshold 4ng/L
  - Passion fruit, boxwood, sweaty

Swiegers, J.H., Francis, I.L., Herderich, M.J. and Pretorius, I.S. *Meeting Consumer Expectations Through Management in Vineyard and Winery: The Choice of Yeast for Fermentation Offers Great Potential to Adjust the Aroma of Sauvignon Blanc Wine.* Australian and New Zealand Wine Industry Journal 21:34-42 (2006).

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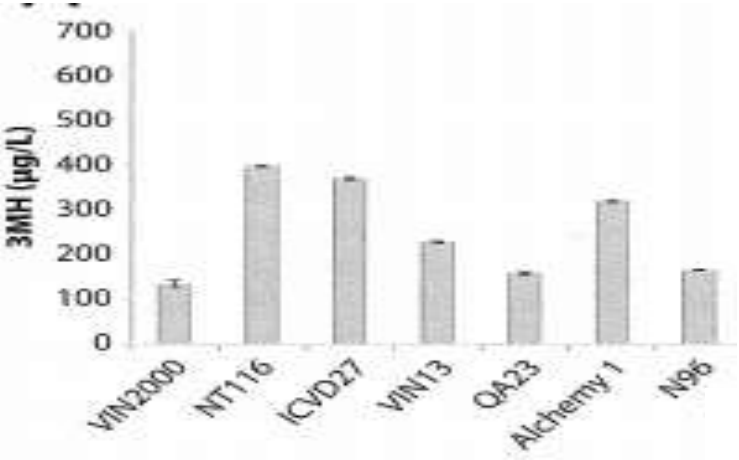
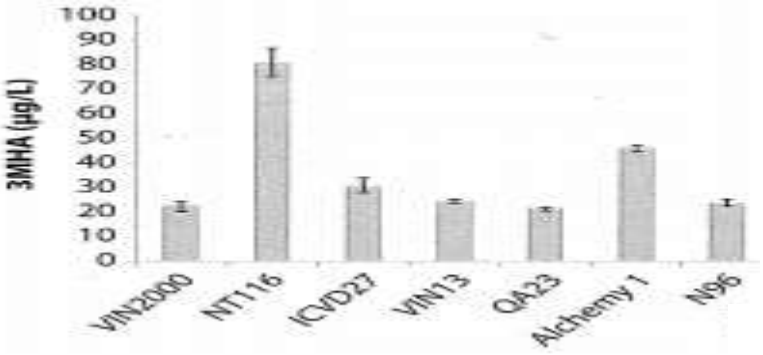
YEAST STRAIN INFLUENCE- CHARDONNAY

- 2008 Margaret River Chardonnay
  - 12.7 Baume (~23brix)
  - pH 3.4
  - TA 7.0g/L
  - Ferm temp. 60-65°F
- Interesting that these compounds are demonstrated in cool climate Chd.
- Compounds can be reduced by O2 => Protect, protect, protect!!!





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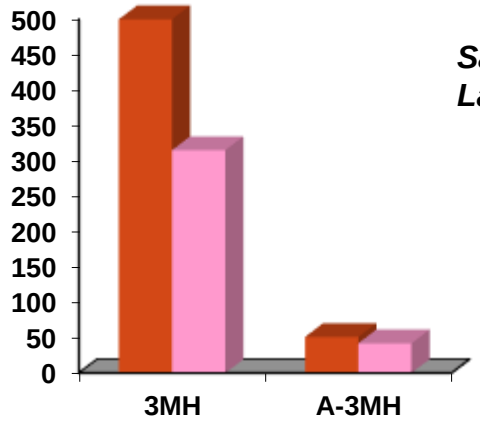
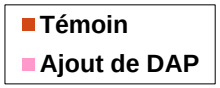
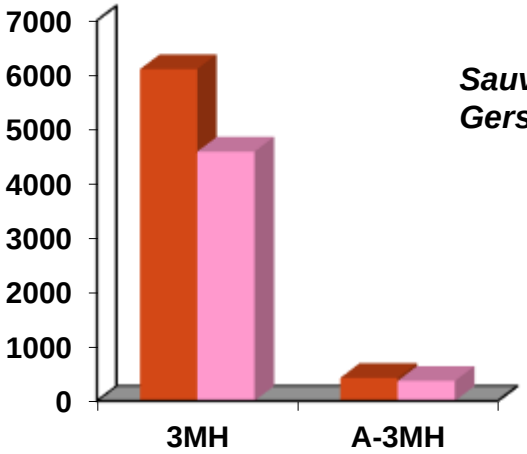
THIOL PRECURSORS AND THE INFLUENCE OF YEAST NUTRITION

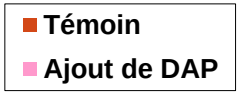


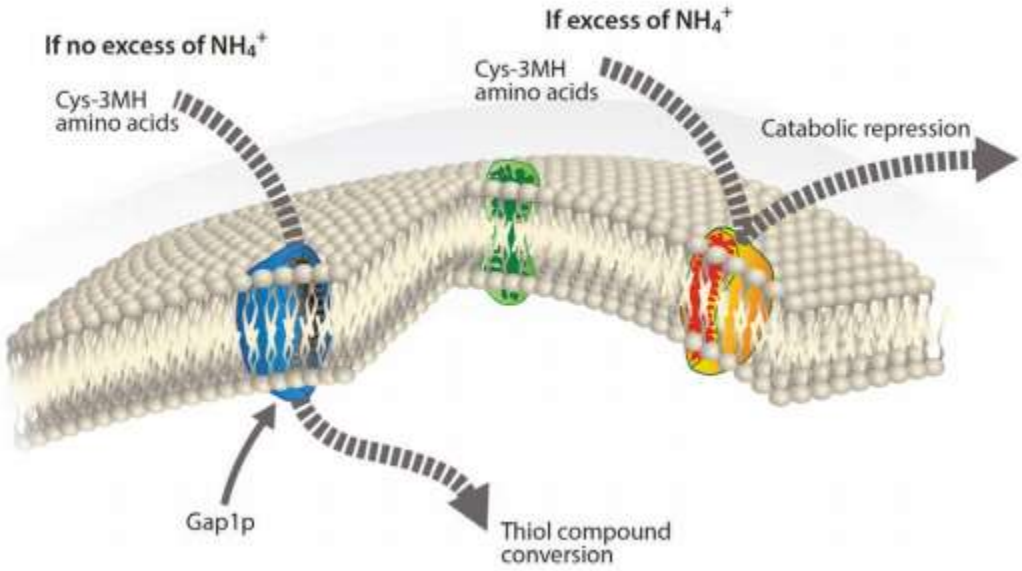
Sauvignon blanc
Languedoc 2004





Sauvignon blanc
Gers 2006





Fermentations at 70°F

Subileau, et. al 2008

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S. CEREVISIAE- INFLUENCE ON FUSEL ALCOHOLS

- A.K.A. Higher alcohols or, aliphatic and aromatic alcohols
  - Compounds with more than 2C units
  - Produced via a series of reactions
    - Ehrlich pathway
    - Sugar metabolism
  - Can have a positive, negative or effect
    - >400ppm = pungent, solvent
    - <300ppm = fruity

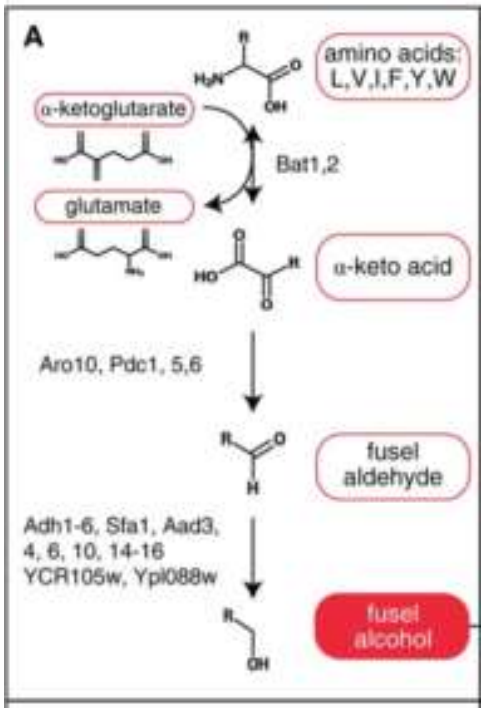

Image adapted from: Hirst M.B. and Richter C.L. AJEV67:4 (2016)
Leucine (L), valine (V), isoleucine (I), phenylalanine (F), tyrosine (Y), and tryptophan (W).

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S. CEREVISIAE- INFLUENCE ON FUSEL ALCOHOLS

- The amount of higher alcohols produced depends on:
  - Grape
    - Cultivar, maturity, skin contact
  - Microbial interactions
    - Yeast strains, yeast growth
  - Matrix considerations
    - pH, temperature, amino acid concentration, level of solids
- Subsequent interactions and reactions
  - **Higher alcohols are precursors for esters!**

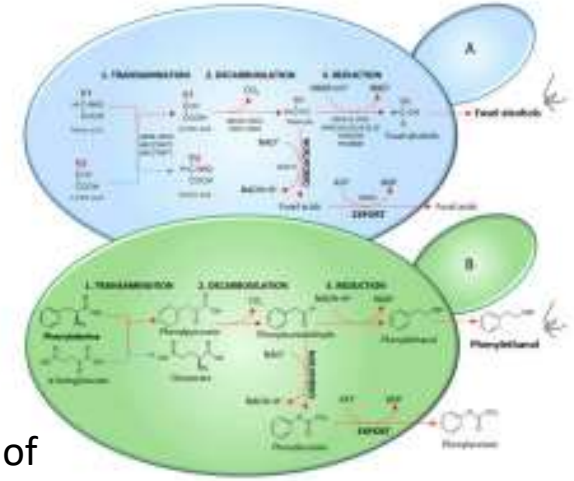

Image adapted from: Moreno-Arribas et.al. (2017) Molecules, 22:189

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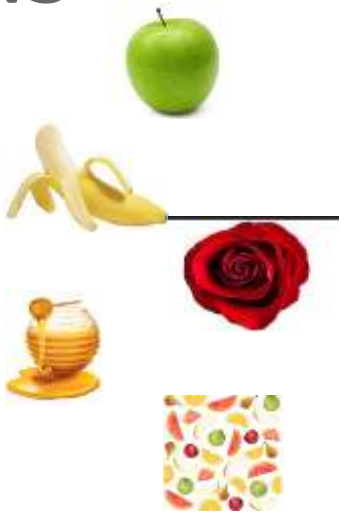
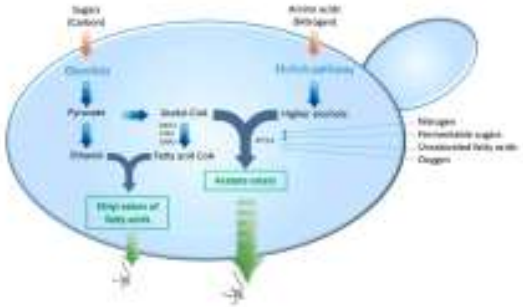
S. CEREVISIAE- INFLUENCE ON ESTERS

- Ester are volatile molecules
  - Fruity and floral
- Formed via a reaction between an alcohol and an acid
  - Ethyl esters (of fatty acids)
    - Formed via ethanol and acid
      - E.g. Ethyl hexanoate (aniseed, apple), Ethyl octanoate (sour apple)
  - Acetate esters (of higher alcohols)
    - Formed via acetate (derivative of acetic acid) and ethanol
      - E.g. Isoamyl acetate (banana), Isobutyl acetate (fruity), Phenyl ethyl acetate (rose, honey), Ethyl acetate (solvent)
- Ester formed
  - Enzymatic esterification during fermentation
  - Chemical esterification during storage





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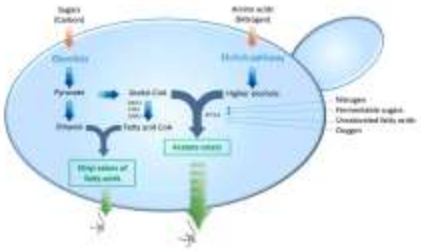

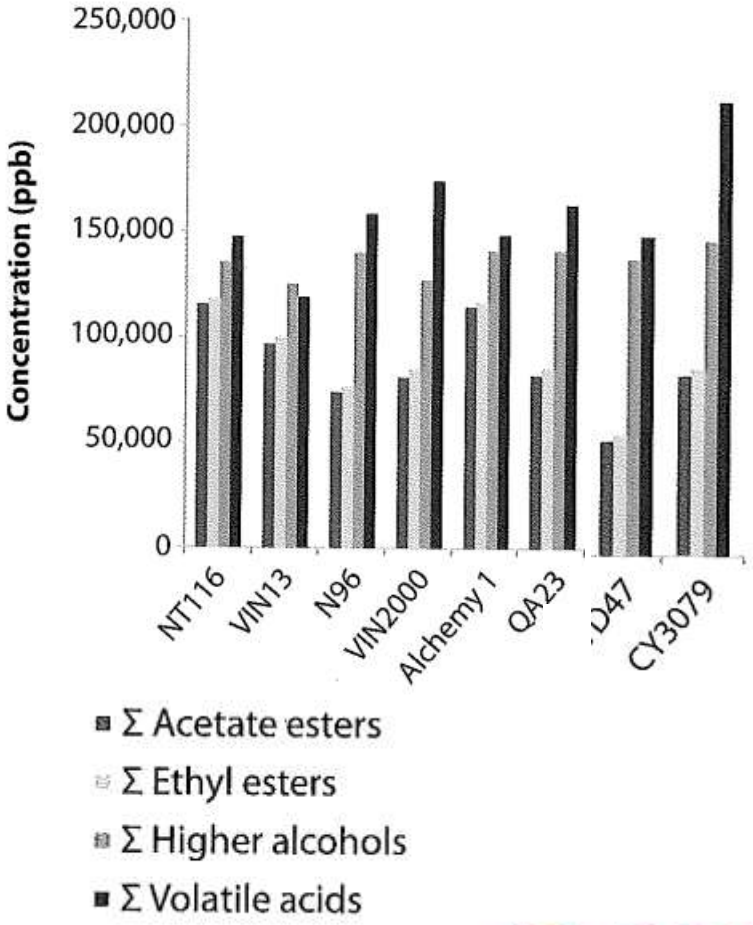

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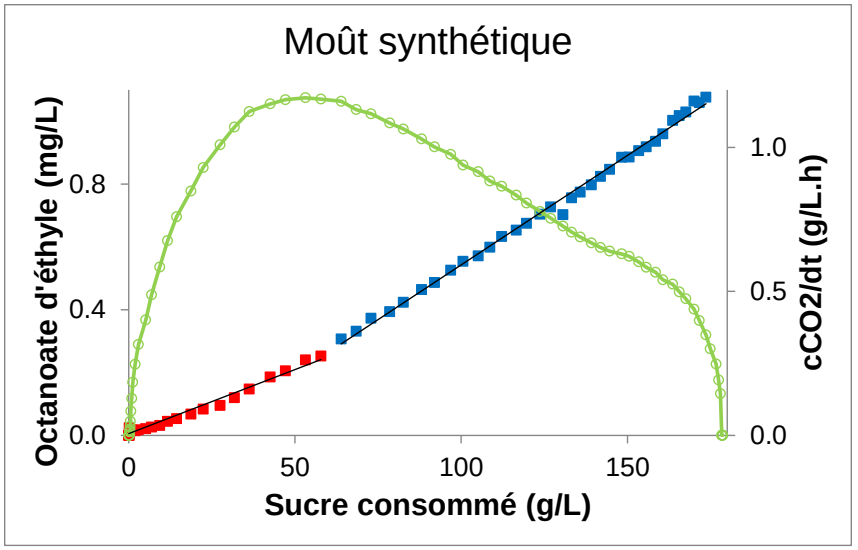
ESTER FORMATION

- Influenced by:
  - Concentration of substrates acetyl-CoA and fusel alcohol
  - Enzymatic activity
- Influenced by fermentation variables
  - Yeast strain
  - Composition of fermentation medium and conditions
    - Sugar concentration, Nitrogen composition => positive influence
    - High level of lipids => negative influence



- **NEW INFORMATON!**
- **2 phases of linear synthesis in function of the sugar consumption**
- **Yield of production of the 2nd phase always higher**

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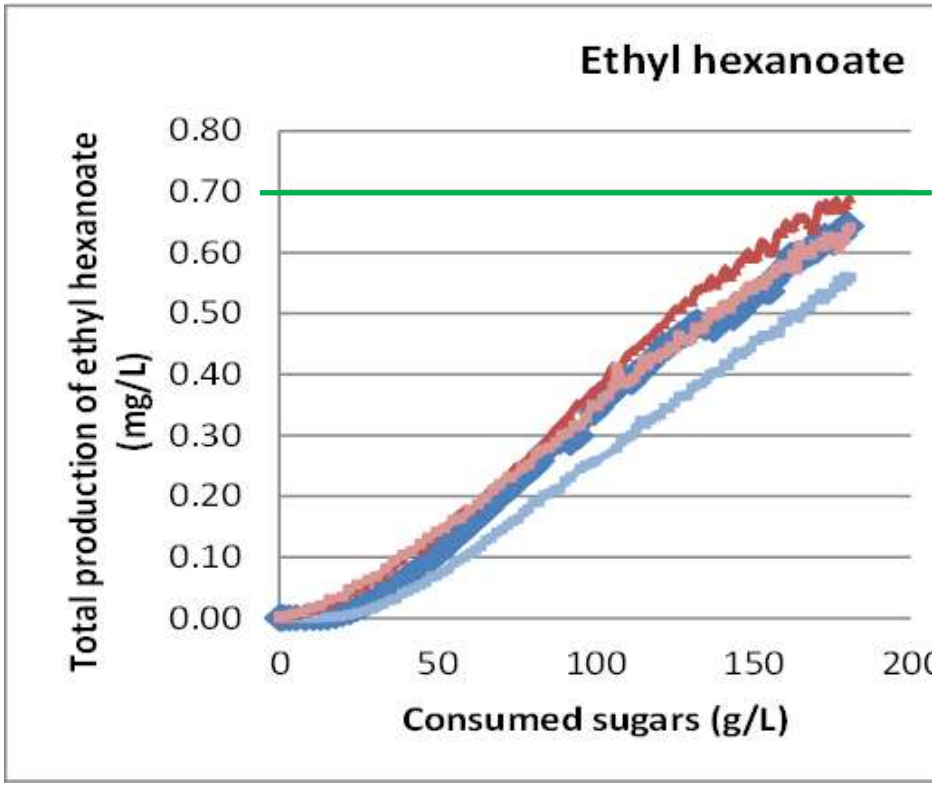
NITROGEN & LIPID INFLUENCE ON ESTER BIOSYNTHESIS



- Low Nitrogen level
  - 70ppm
- 2 yeast strains
- 2 lipid levels
  - 2mg/L = ~60ntu's
  - 8mg/L =~240ntu's

In LOW YAN : whatever the lipids & yeast :

⇨ No difference on esters production

⇨ Low esters synthesis

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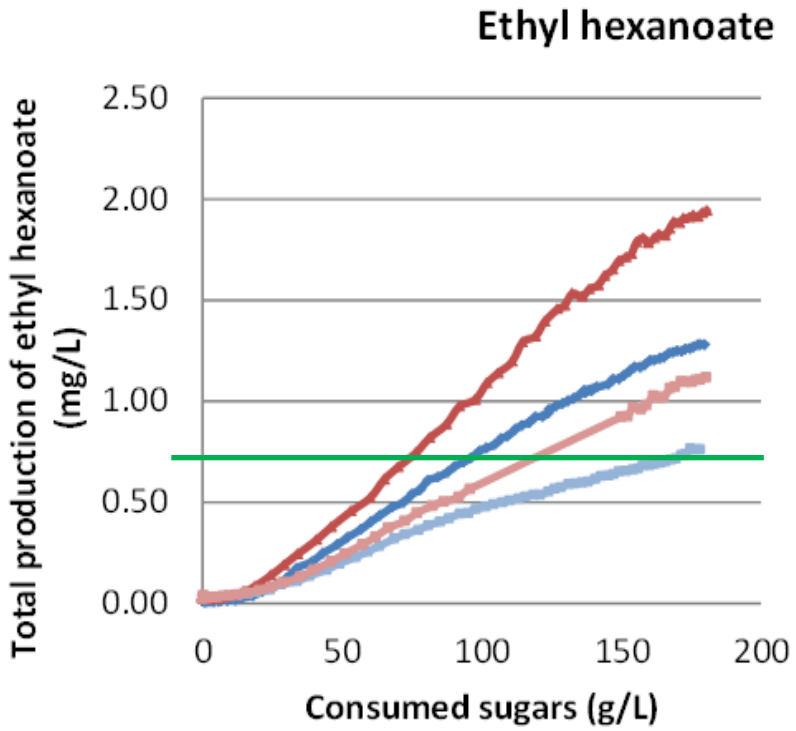
Rollero, Ortiz-Julien, Dequin, Sablayrolles, 2015

NITROGEN & LIPID INFLUENCE ON ESTER BIOSYNTHESIS



- High Nitrogen
  - 300ppm
- 2 yeast strains
- 2 lipid levels
  - 2mg/L = ~60ntu's
  - 8mg/L =~240ntu's

- **Yeast**
- High impact on esters production:
- High esters synthesis with high nitrogen, modulated by [lipids]: 2mg/l lipids : esters overproduction
- No loss of viability with 2 mg/l lipids (60 NTU)

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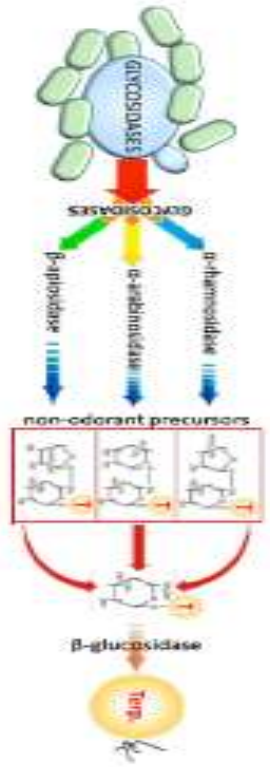
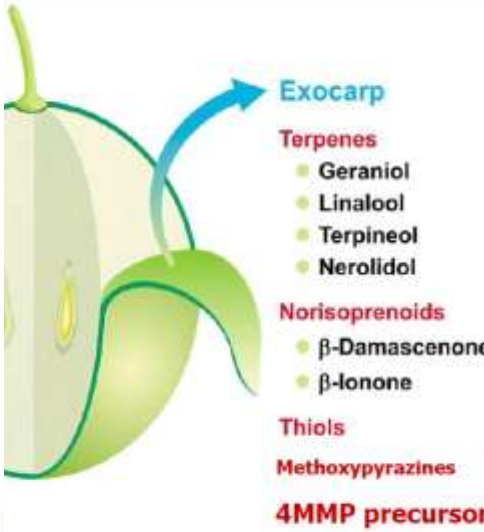
Rollero, Ortiz-Julien, Dequin, Sablayrolles, 2015

S. CEREVISIAE- INFLUENCE ON MONOTERPENES

- Free form
  - Linalool, Geraniol, Nerol, Citronellol
- Bound (Odorless) form
  - Hydrolyzed to release pleasant flavors
- Glycosidically bound form
  - Yeast enzymatic activity reveals aroma
    - Strain dependent
    - Fermentation conditions
      - Low pH, high ethanol, high sugar





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S. CEREVISIAE- INFLUENCE ON MOUTHFEEL

- Glycerol
  - Non-volatile compound
  - Contribution to mouthfeel
    - Sweetness and fullness
    - Sensory threshold of 5.2g/L wine
    - Does not contribute to viscosity (~25g/L)
  - Cellular function
    - Combat osmotic stress
    - Maintain RedOX balance

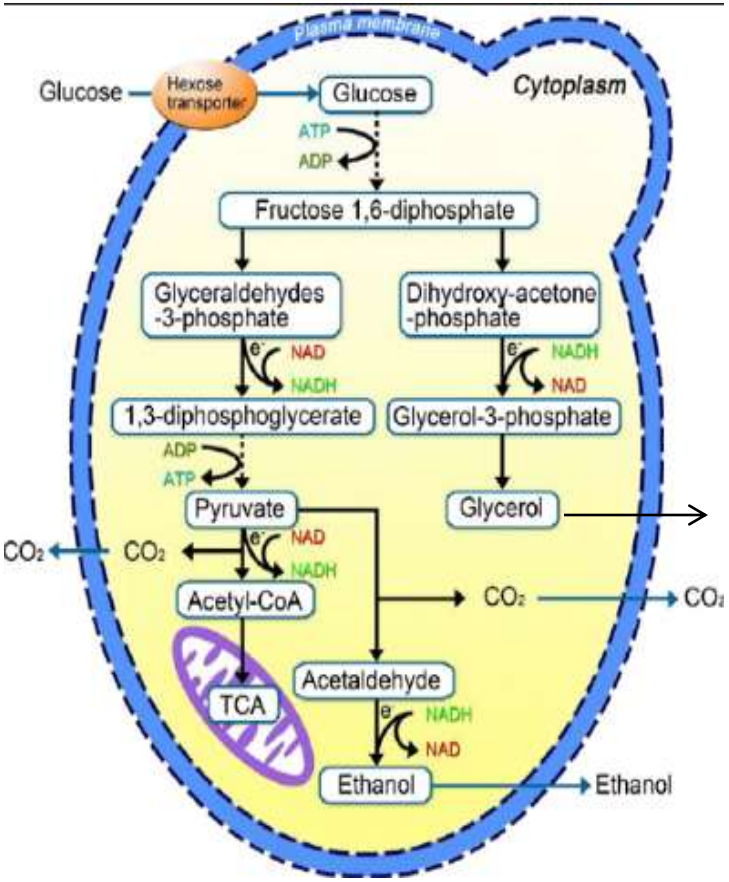

Image from Pretorius I. S. **Beverages** 2, 36, 2016

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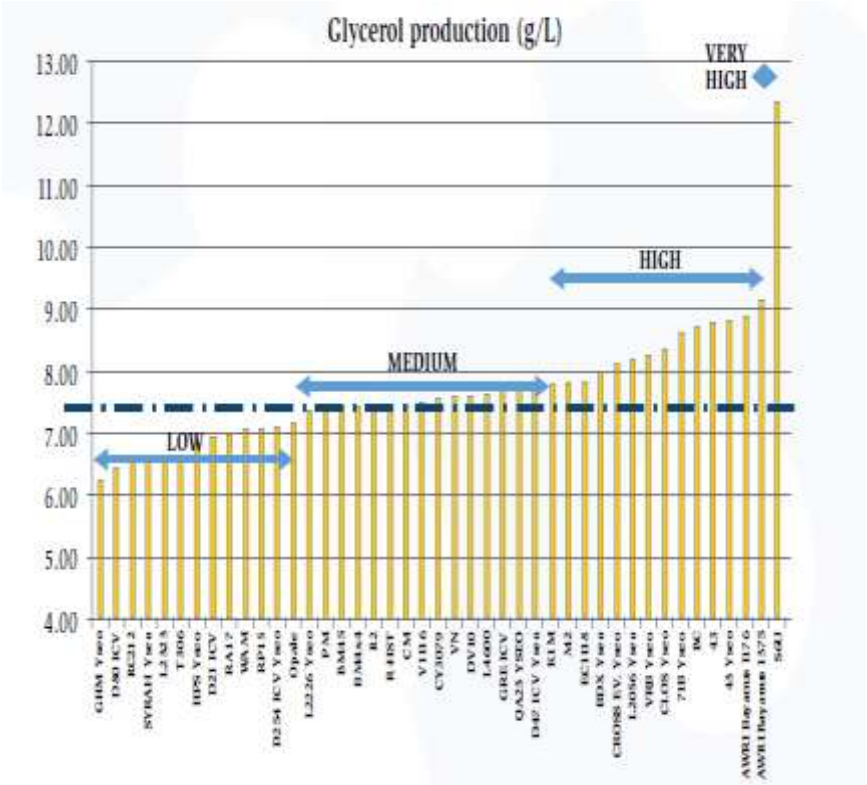
S. CEREVISIAE- INFLUENCE ON MOUTHFEEL

- Range
  - 4-9g/L
- Depends on
  - Yeast strain
  - Fermentable sugar concentration
    - Beware of increased VA
  - YAN level and composition
  - Temperature
  - SO_2 level
    - >100ppm



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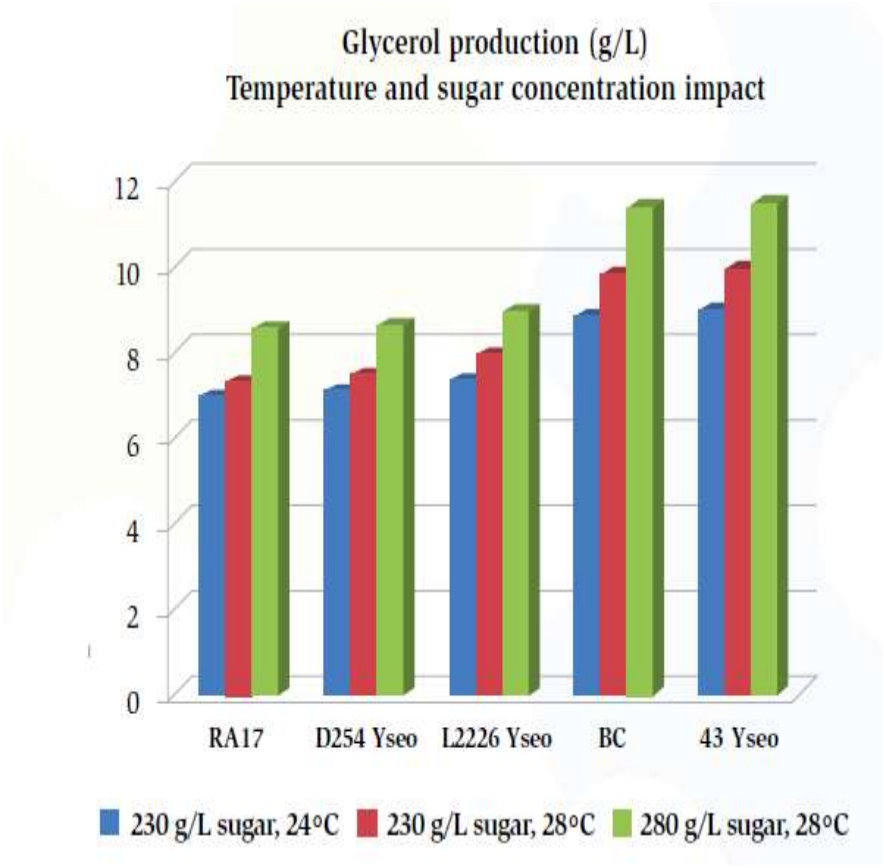
S. CEREVISIAE- INFLUENCE ON MOUTHFEEL

- High sugar => higher glycerol
- Higher temperature => higher glycerol



S. CEREVISIAE- INFLUENCE OF POLYSACCHARIDES

- Polysaccharides
  - Can be release during cell growth and during autolysis from yeast cell walls
    - A.K.A. Mannoproteins
      - Polymers of mannose & other branched monosaccharides that contain <30% peptides
- Availability
  - Depends on yeast strain
  - Lytic susceptibility of strain
- Proposed role in:
  - Stimulation of MLF
  - Increase color stability
  - Protection of color
    - Whites and roses (GSH)
  - Decreased of astringency
  - Protective effect
    - Protein and tartrate stability

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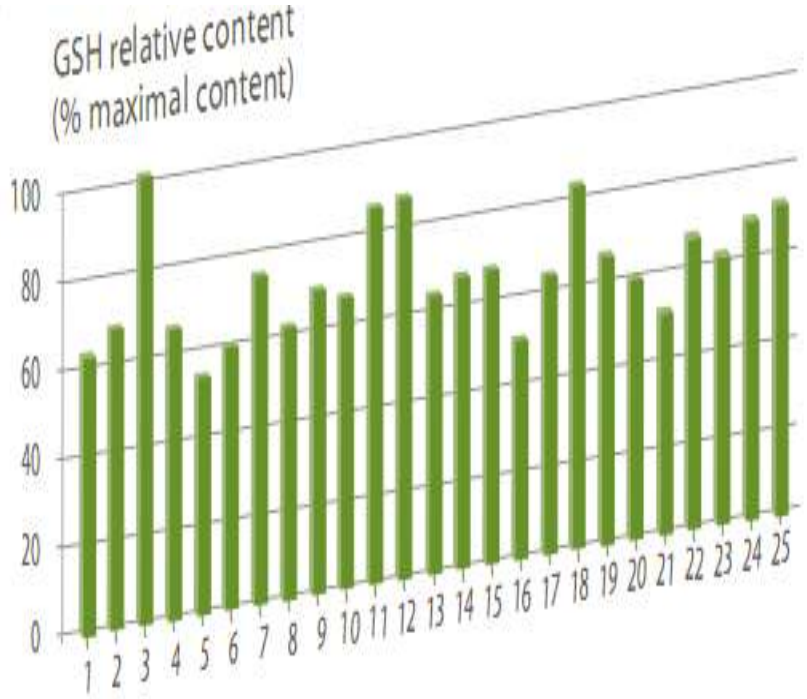
GLUTATHIONE

- Glutathione
  - Available in grapes
    - Reduced form (GSH)
    - Oxidized form (GSSG)
  - GSH competes with wine thiols for o-quinones thereby protecting wine aromas
  - Available in yeast
    - Different levels
      - 0.1 – 1%



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INFLUENCE ON ACID CHEMISTRY

- Change in perception (not actual chemistry)
- Decrease in titratable acid
  - Utilization of malic acid
    - *Schizosaccharomyce pombe* and *S. cerevisiae*
- Increase in titratable acid
  - Production of acetic acid => Not desirable
  - IONYS WF

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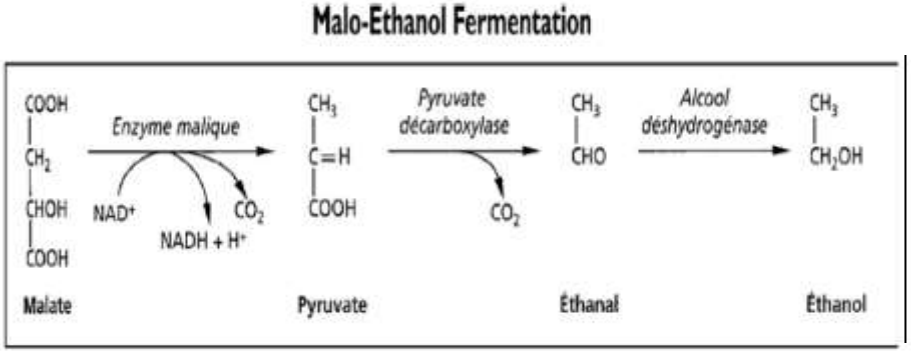
INFLUENCE OF YEAST ON MALIC ACID CONCENTRATION

Schizosaccharomyces pombe

- Very tolerant to low pH
- Not tolerant to ethanol
- Temperature influence
  - At 72F depletion is ~0.42g/L/day
  - At 50F depletion is 0.17g/L/day
    - Depletion slows once malic acid <2g/L



2.33g/L malic acid => 0.1% Ethanol

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MALATE ASSIMILATION BY SACCHAROMYCES

- Sensu stricto
  - S. bayanus, S. uvarum => cold tolerant
  - S. pastorianus => meso

  T_{opt} < 30°C
  - S. cerevisiae, S. paradoxus =>thermotolerant T_{opt} 30°C

- Cold tolerant species => synthesize L-malic acid
- Thermotolerant => can degrade L-malic acid

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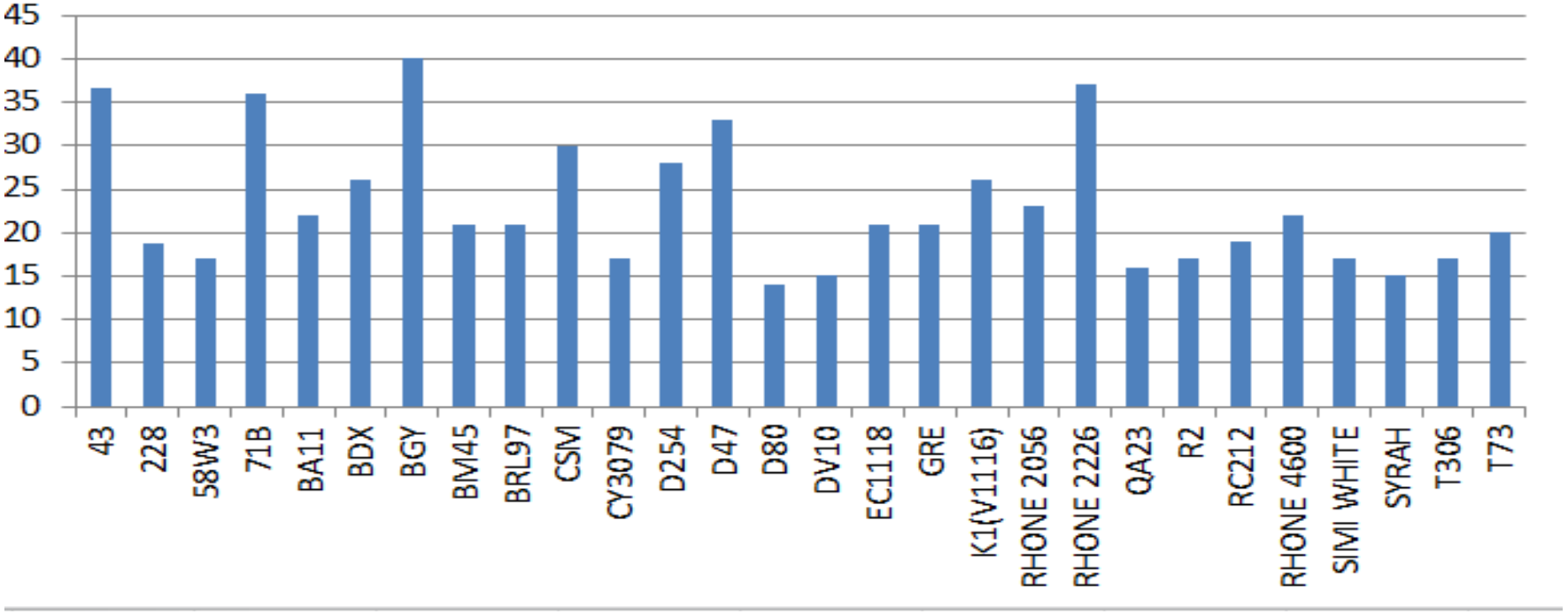
% MALIC ACID DEGRADED IN CHARDONNAY



2007 Sterile Chardonnay Juice, 240g/L G:F, 18°C pH ???
Fermentations carried out in triplicate

Richter et. al FEMS Yeast Research 13:34 2013

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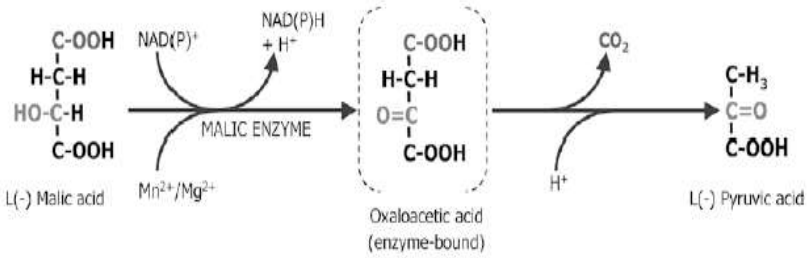
MALIC ACID DEGRADATION BY S. CEREVISIAE

	F. GRIS	LA CRESENT	FRONTENAC ROSE	MARQUETTE ROSE
INITIAL MALIC ACID	5.1 g/L	5.3g/L	4.6g/L	4.1g/L
% MALIC ACID DEGRADATION				
DV10	16	9		
LALVIN C	31		34	27
EXOTICS		19	30	20
OPALE		11		
GRE			26	18

- Genetic variability
- Phenotypic considerations
  - pH influence
    - Optimum at pH 3.0-3.5
    - Increases at end of fermentation



Summary of results published by Northern Grape Project

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INACTIVATED YEAST…

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A WORD ON YEAST SPOILAGE

- Not all yeast contribute positive notes
  - Non-Saccharomyces
    - Elevated ethyl acetate and acetic acid
  - Saccharomyces (when stressed)
    - Elevated Acetaldehyde, Acetic Acid, Sulfides
  - Brettanomyces
    - 4-ep, 4-eg, Isovaleric acid

TAKE HOME MESSAGE

- Biodiverity in the winemaking environment is astounding
  - Not every strain, or their attributes are suitable for your winemaking style
  - Yeast need **your** help to drive certain characteristics
    - They are amazing, but they are not infallible
- Microbes can and will drive style, but you need to have a style to drive towards!

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THANK YOU!

QUESTIONS?

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