Changing Paradigms in Wine Fermentation Management

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Yeast Nutrition Research Initiative

Laffort Pillars for Growth

Denis Dubourdieu
Virginie Moine
Philippe Marullo
Marina Bely

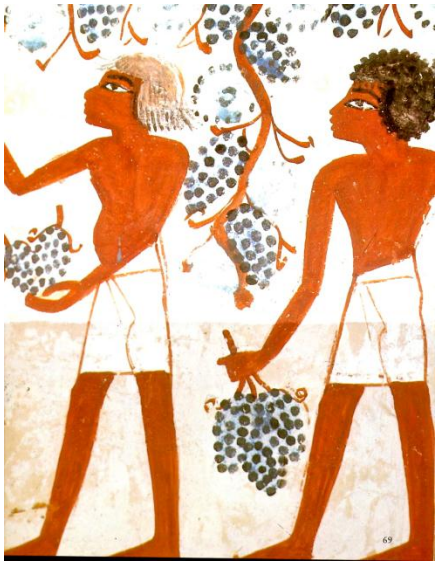
- Research
- Innovation
- Quality

T.V.d. Westhuizen
Maryam Ehsani
Gal Winter
Chris Curtin

Todays Focus

- ADY - Yeast Production
- Yeast Nutrition in Primary Fermentation
- Primary Fermentation Impacts on MLF
- ADY Rehydration Protection

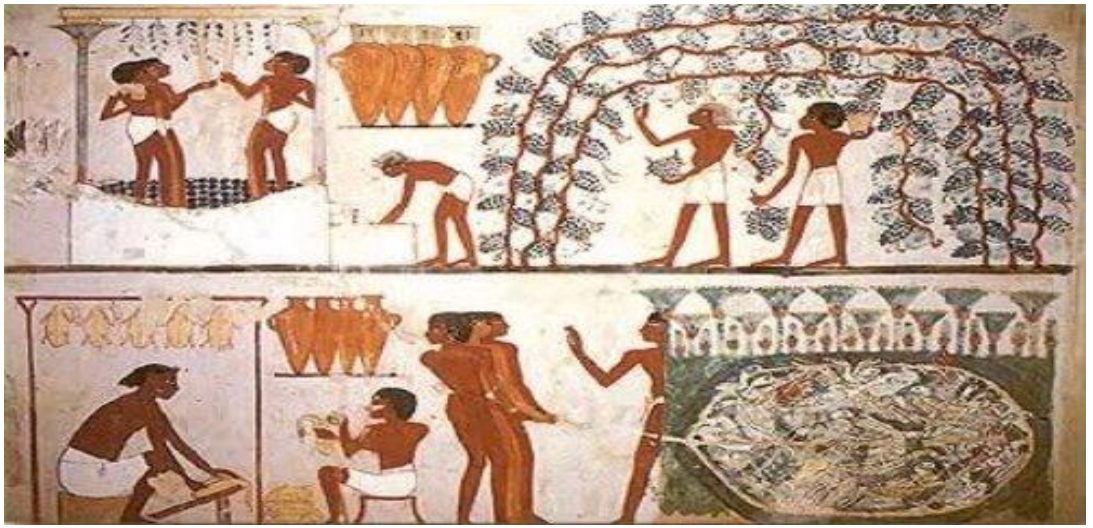
Native Ferments to ADY Development



Ancient Macedonia – 4000BC
Old World – New World

Louis Pasteur mid 1800's

Fleischman's Active Dry Yeast - WWII



Active Dry Yeast vs 'Wild' Yeast
Cultured vs Native Ferments

Captured from the Wild

Born to be Wild

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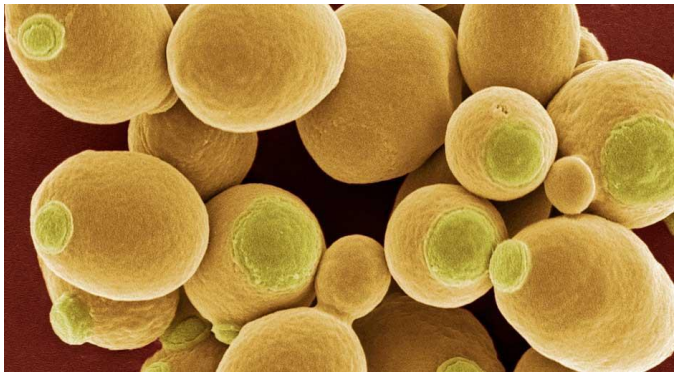
Benefits of Cultured Fermentations

- ✓ Predictable , dependable, complete fermentation
- ✓ Characteristic production of flavor/aroma compounds
- ✓ Reliable start to fermentation to outcompete microbial flora
- ✓ Complete utilization of sugars
- ✓ Predictable sugar to alcohol conversion
- ✓ High Ethanol resistance - over 15%
- ✓ High SO2 resistance with low H2S production
- ✓ Produce a minimum of pyruvate, acetic acid and acetylaldehyde
- ✓ Minimum foaming during fermentation
- ✓ Good flocculation properties for lees compaction

Yeast Protection vs Nutrition

PROPER YEAST REHYDRATION



Yeast Survival Factors

- Fatty Acids
- Sterols

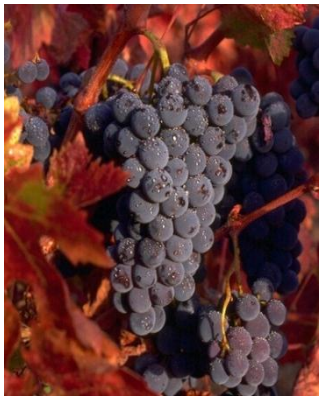
PROPER YEAST NUTRITION

Yeast Growth Factors

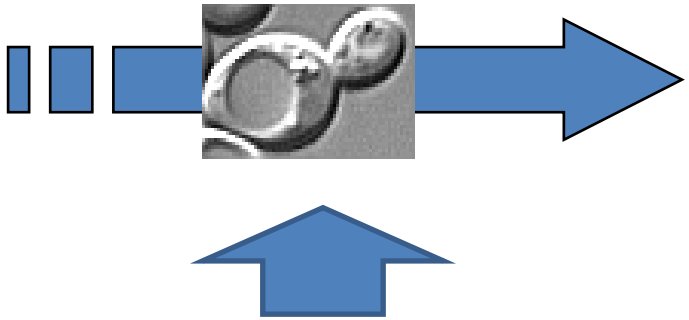
- Nitrogen
  - Inorganic source
  - Organic source
- Vitamins
  - 8 B vitamins
- Minerals
  - 9 Minerals

Fermentation Nutrition and "The Perfect Fermentation"

Clean Grapes **Good Yeast** **Clean Wine**



"Happy Yeast"



NUTRIENTS

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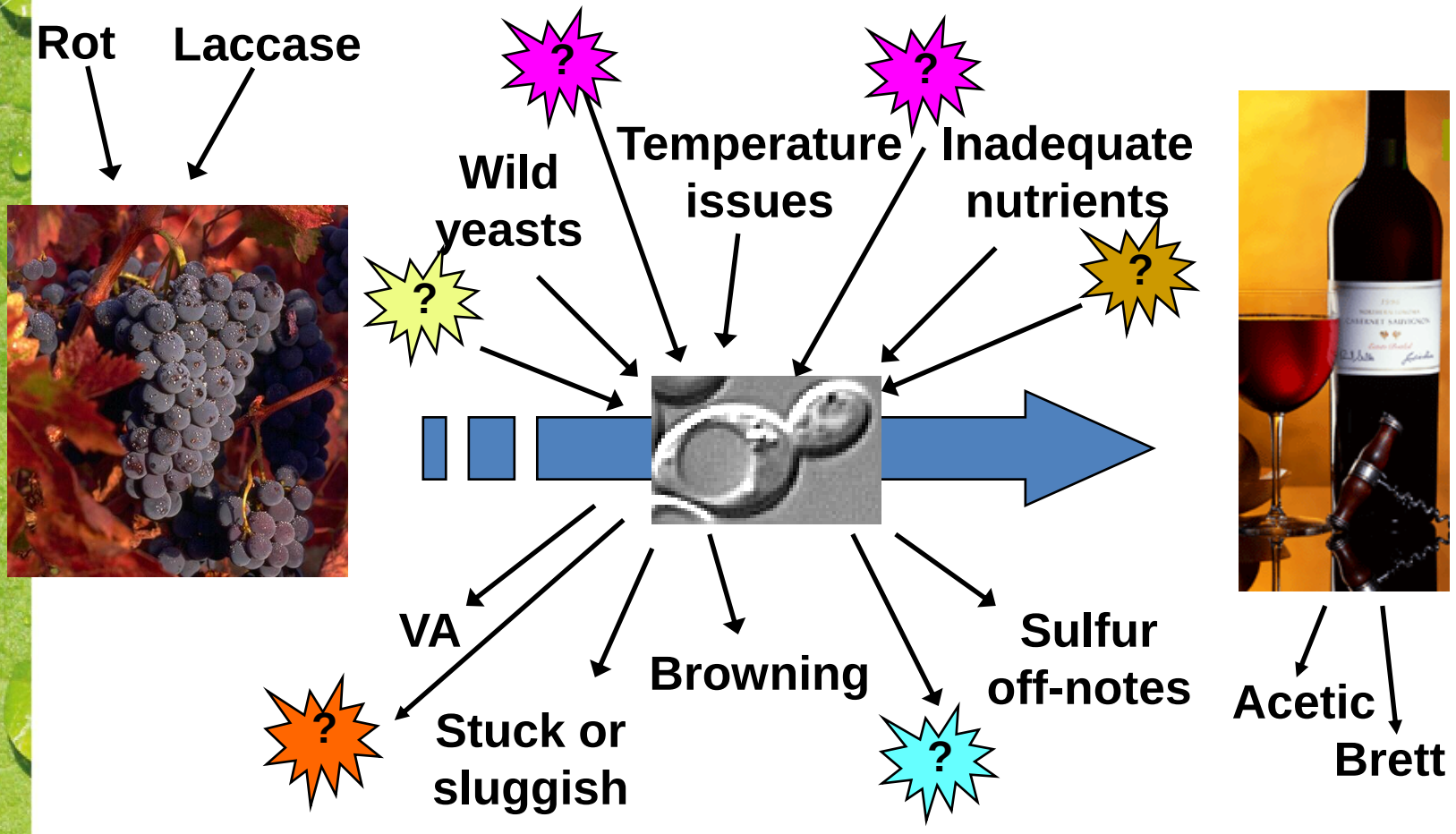
Yeast nutrition and protection

Reality
"Managing the Microbial Milieu"



"UnHappy Yeast"

Where to Look ?

- **<u>Poor Fermentation Causes – No Shortage!</u>**
  - Low Population of Viable Yeast
  - Fermentation Temperature Spikes – Heat, Cold
  - Microbial Competition – Early, Late
  - Toxins – Microbial, Al, Pb, Pesticide Residues
  - Yeast Genetic Mechanisms – Prions - Epigenetics
  - Nutrition
    - C / N Balance - Brix / YAN
    - Macro nutrient shortage
    - Micro nutrient shortage
    - Trace nutrient shortage

Consequences of Unhappy Yeast

- Stuck or Sluggish Fermentations
- H2S – Other Aroma Consequences
- Elevated C8 + C10 Fatty Acids
- High SO2 Production by Yeast
- Costly Fermentation Restart
- Negative Impact on Subsequent MLF

Yeast Nutrition Paradigm

Yeast Growth Factors

Nitrogen

YAN = Ammonia + alpha amino acids

Vitamins

8 B complex vitamins -

- **B1 – thiamine**
- **B2 – riboflavin**
- **B3 – niacin**
- **B5 – pantothenic acid**
- **B6 – pyridoxine**
- **B7 – biotin**
- **B9 – folic acid**
- **B12 - cobalamin**

Minerals

8 Major Minerals

Ca, Co, Cu, Fe
Mn, Mo, Ni, Zn

Macro nutrients >100 ppm

Micro nutrients <1 ppm

Trace Nutrients

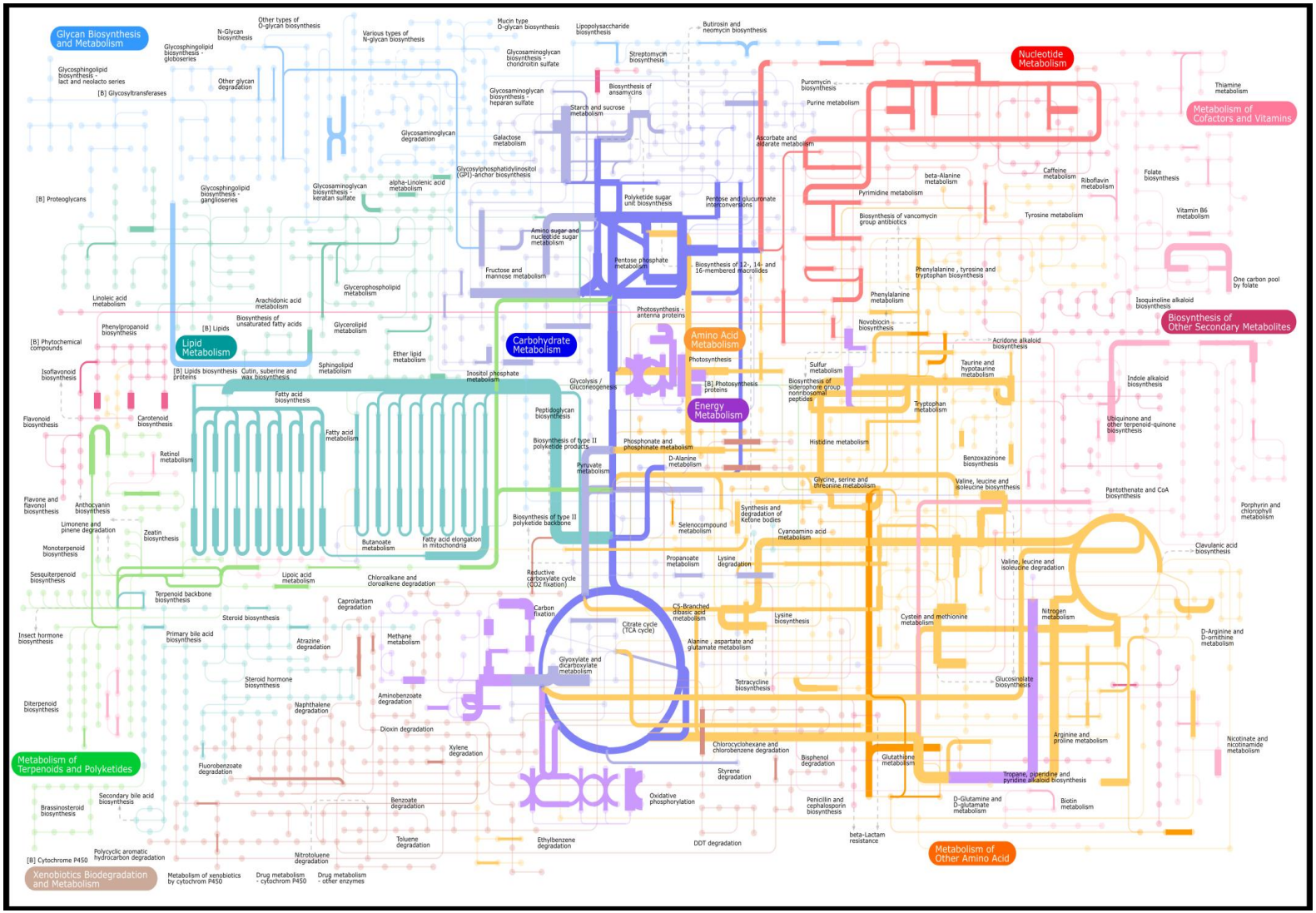
– Se, B, Na, Inositol, ???

All factors are important but…

Supplement additions are usually based on YAN and Brix So…

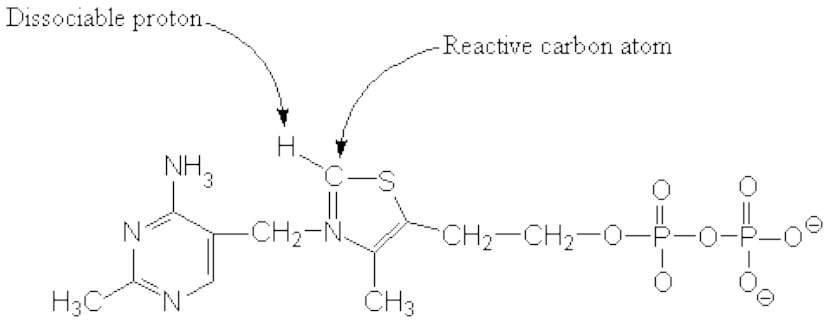
Balancing Nitogen does <u>not</u> always balance total nutrition!

Global Cellular Metabolic Network

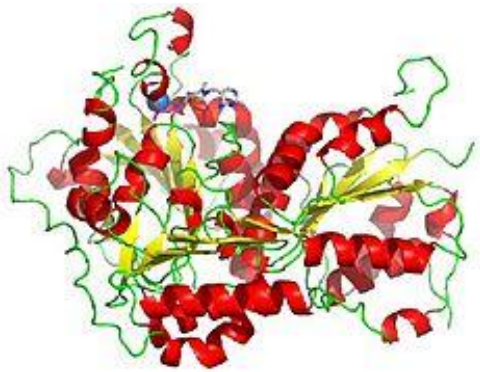


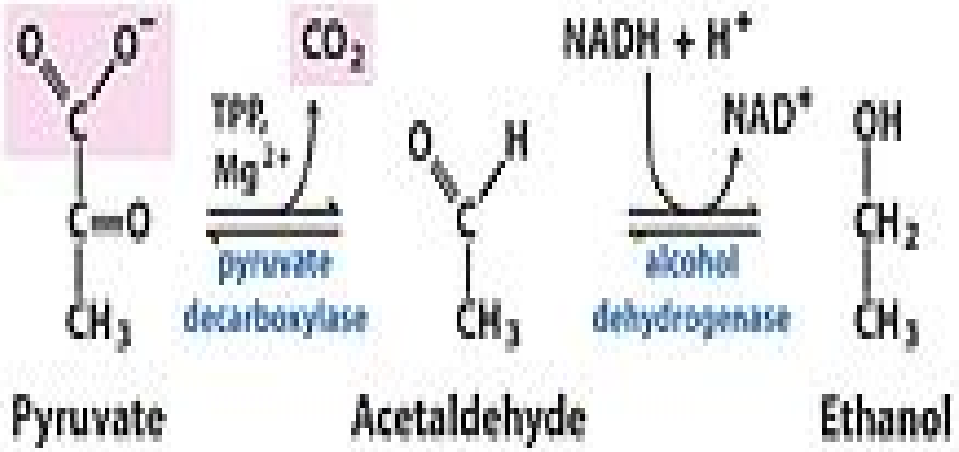
Complicated and Interrelated

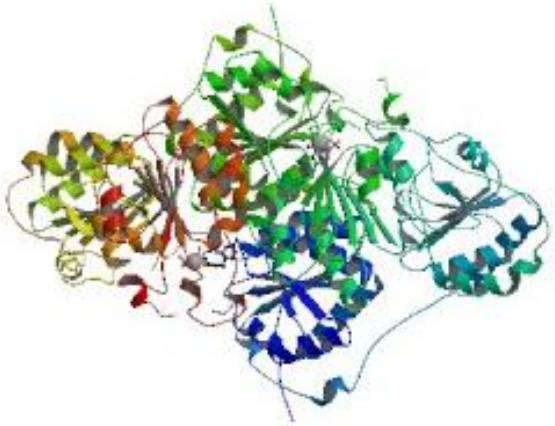
Thiamine – Vitamin B1
Role in Fermentation



Thiamine pyrophosphate
(TPP)





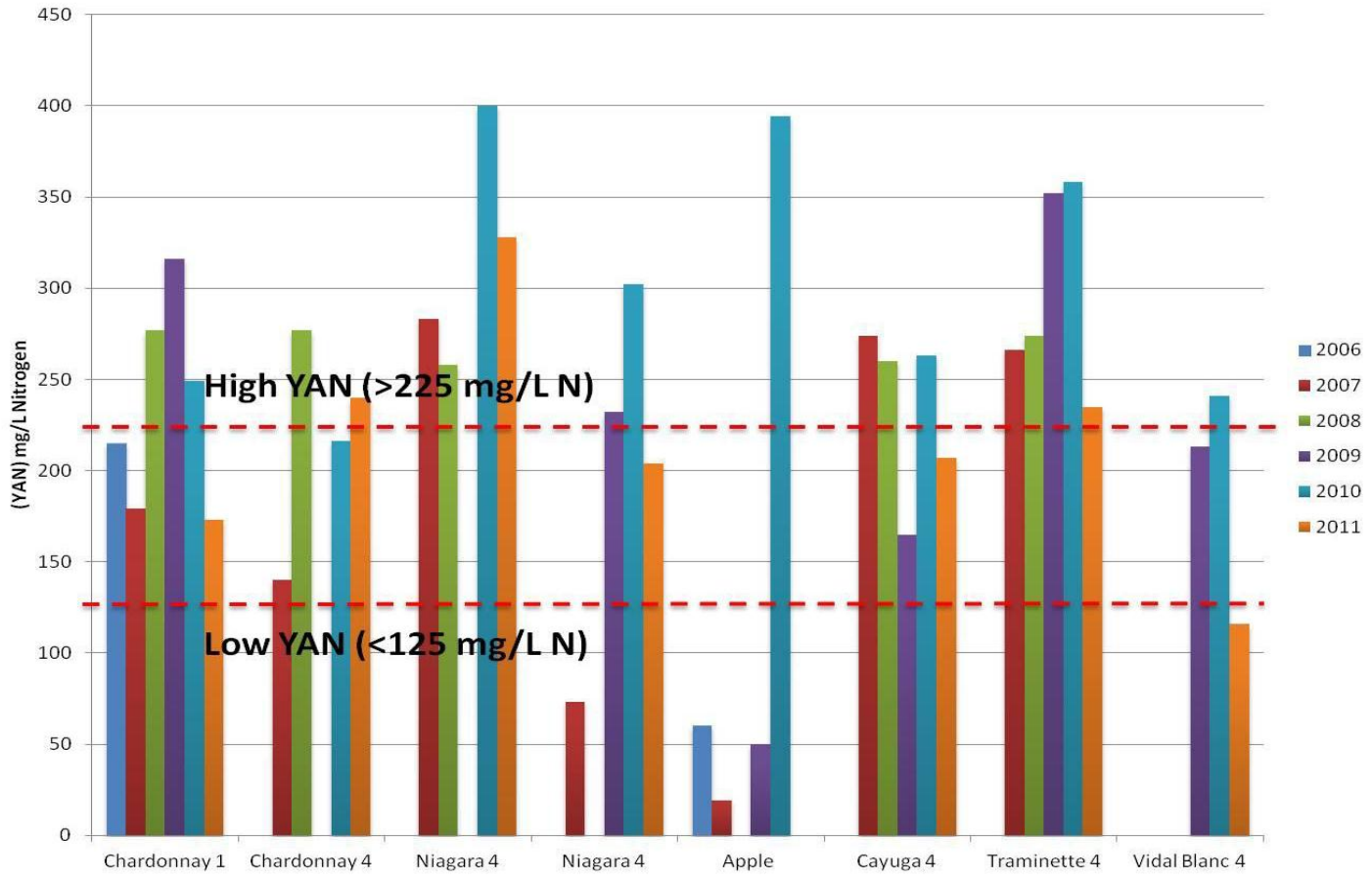


Standard Brix – YAN Ratio Targets

°Brix of must or juice	Target YAN concentration (mg/L)
21	200
23	250
25	300
27	350

Guidelines for this type of ratio table were developed using only inorganic Nitrogen (DAP) for adjustment

Incoming YAN Values Over 6 Years



Vineyard variability illustrates the need to measure every year

Negative Sulphur Compounds

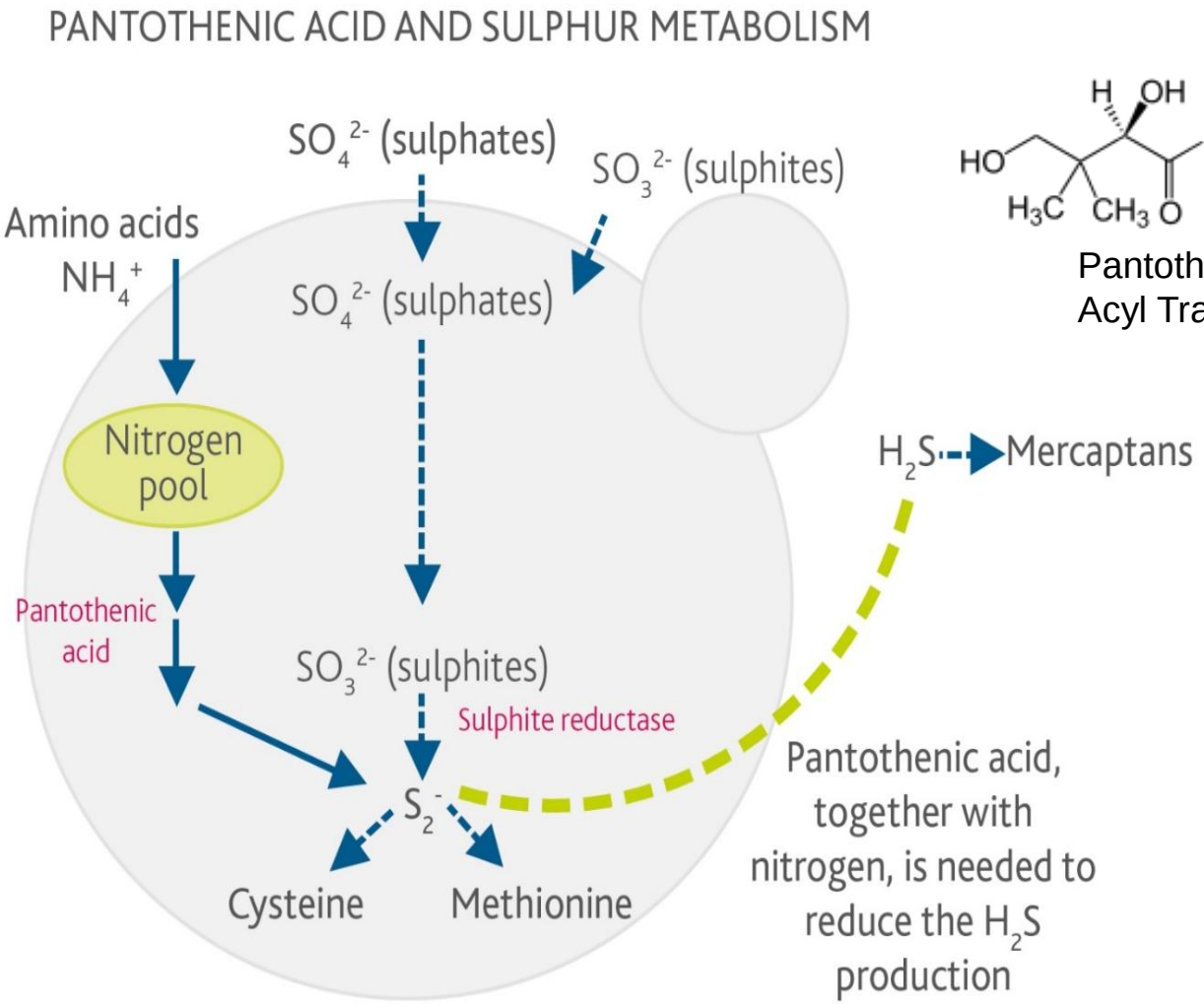
« Heavy » sulphur compounds

MOLECULE	PERCEPTION THRESHOLD (µg/L)	DESCRIPTORS
Carbonyl sulphide		ether
Hydrogen sulphide	**0,8**	**Rotten egg**
Methanethiol	**0,3**	**Stagnant water**
Ethanethiol	**0,1**	**Onion**
Dimethyl sulphide	5	Quince, truffle
Carbon disulphide		Rubber

« Light » sulphur compounds

MOLECULE	PERCEPTION THRESHOLD (µg/L)	DESCRIPTORS
Dimethyl disulphide (DMDS)	2,5	Asparagus, quince
2-mercaptoethanol	130	Burned rubber
Methyl-2-tetrahydrothiophenone	90	"gaz"
2-Méthylthio-éthanol	250	Cauliflower
Ethyl methionate	300	« metalic »
Methionyl acetate	50	mushroom
Methionol	**1200**	**Cooked cabbage**
4-Methylthio-butanol	80	Earthy
Benzothioazol	50	Rubber

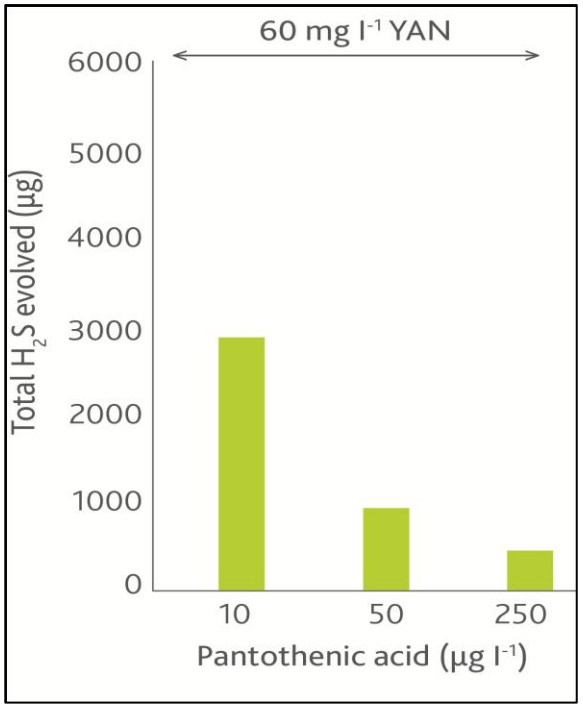
How H_2S and Negative Sulphur Compounds are Formed

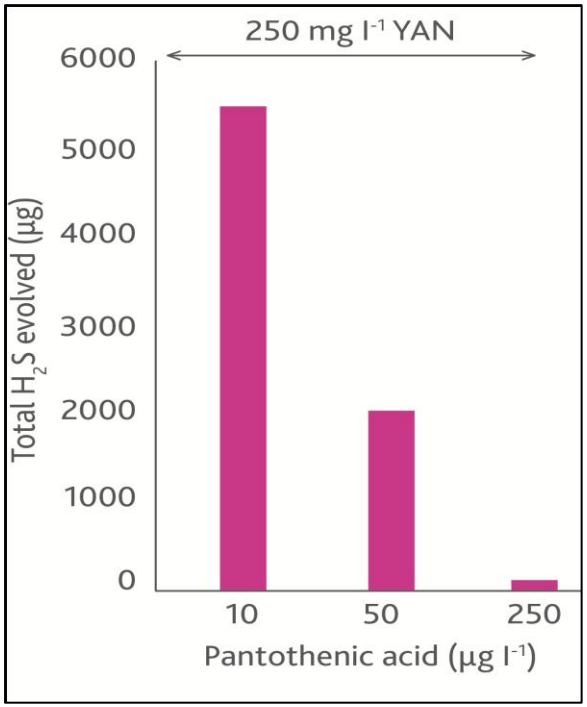


Pantothenic Acid
Acyl Transferase

Causes of “Sulphide Leakage” Nitrogen vs Nutrition

- Shortage of Nitrogen
- Shortage of Pantothenate (Vitamin B5)



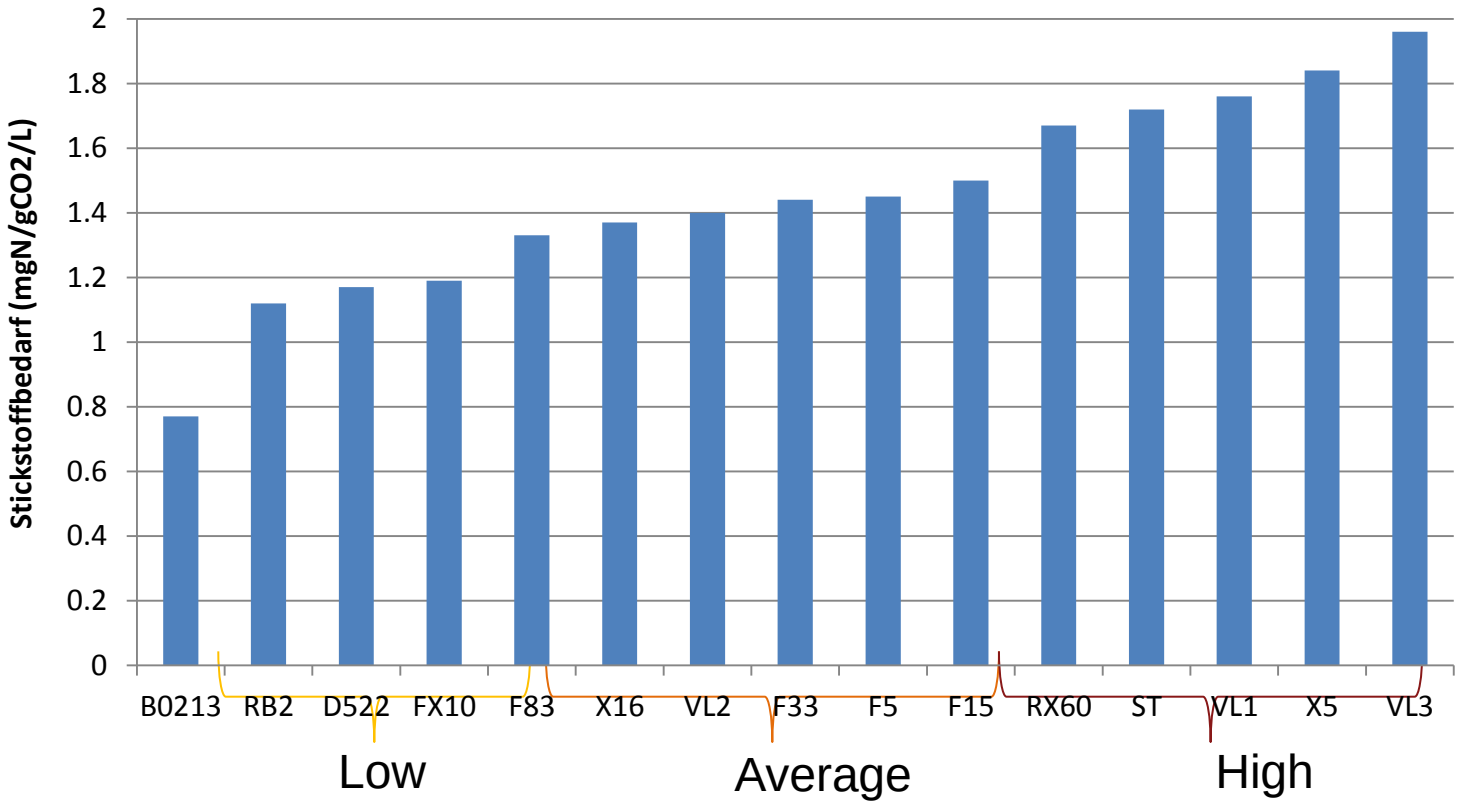


The relationship between YAN and pantothenate content. A high YAN requires an equally high pantothenate content for yeasts not to form H_2S. (Edwards, 2001)

Managing H_2S Formation

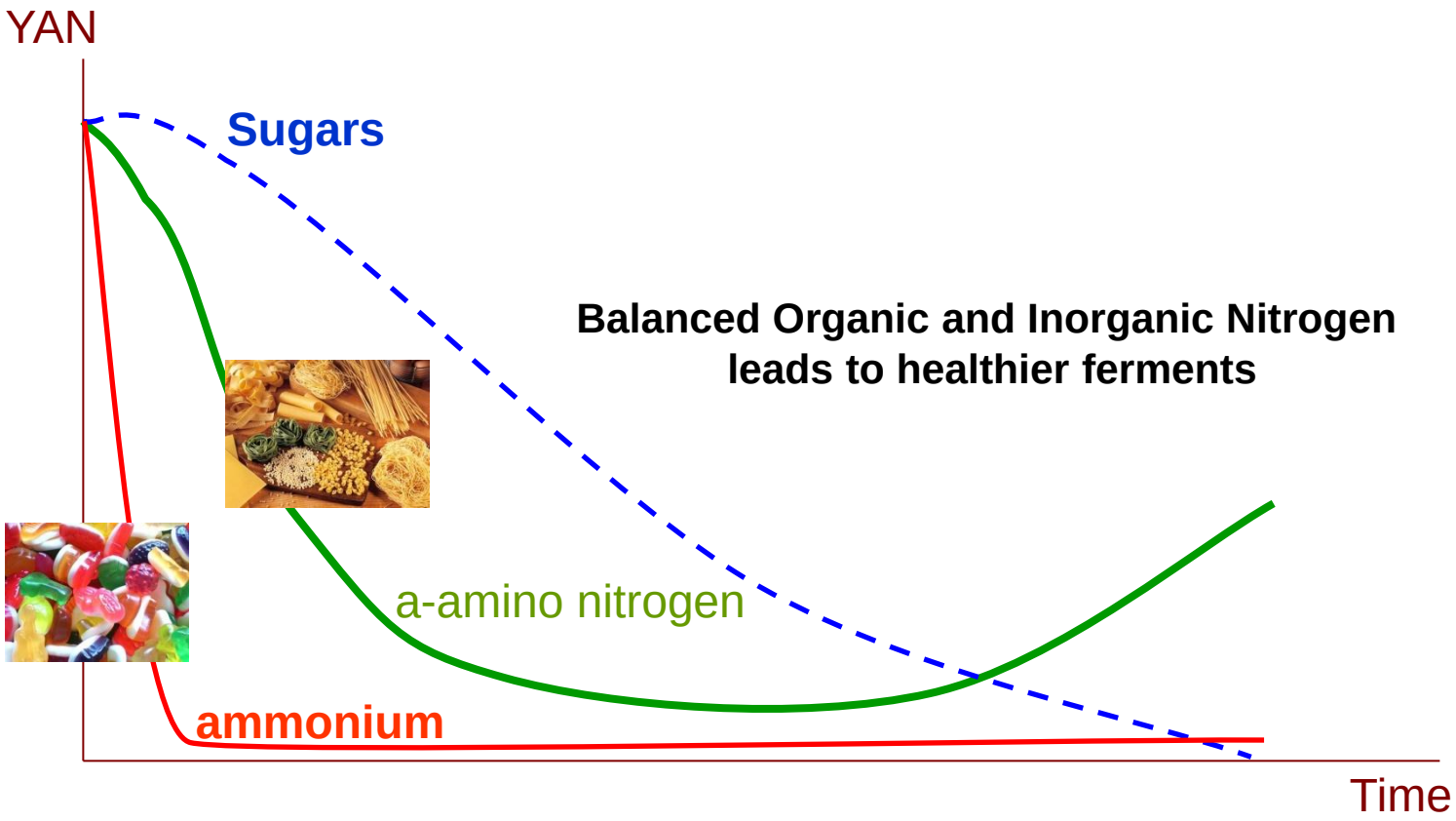
- Choose the correct yeast strain
- Know your yeast needs and characteristics
- Measure YAN and adjust accordingly
- Use appropriate yeast nutrition for the prevailing conditions
- Make sure pantothenate levels are in balance with nitrogen levels
- Control must turbidity
- Make sure the yeast will maintain good viability throughout fermentation
- Aeration during fermentation (reds)

Specific Yeast Nitrogen Needs



Under the same conditions, some yeast have nitrogen needs up to 2 times more than other strains.

Nitrogen Assimilation Kinetics



Better managed fermentation kinetics can lead to:

Slower rise in temperature	Better extraction in red fermentation
Less foaming issues	Clean fermentation completion
Less H2S	Flavor/Aroma development

Nutritional Product Categories

ORGANIC

Micronutrition +++

Organic nitrogen (aa)

OMRI Listed

MACROnutrition +

COMPLEX

Micronutrition ++

Mineral and organic nitrogen

MACROnutrition ++

MINERAL

Micronutrition +

Mineral nitrogen

MACROnutrition +++

Yeast Rehydration – Survival Factors

Yeast Survival Factors

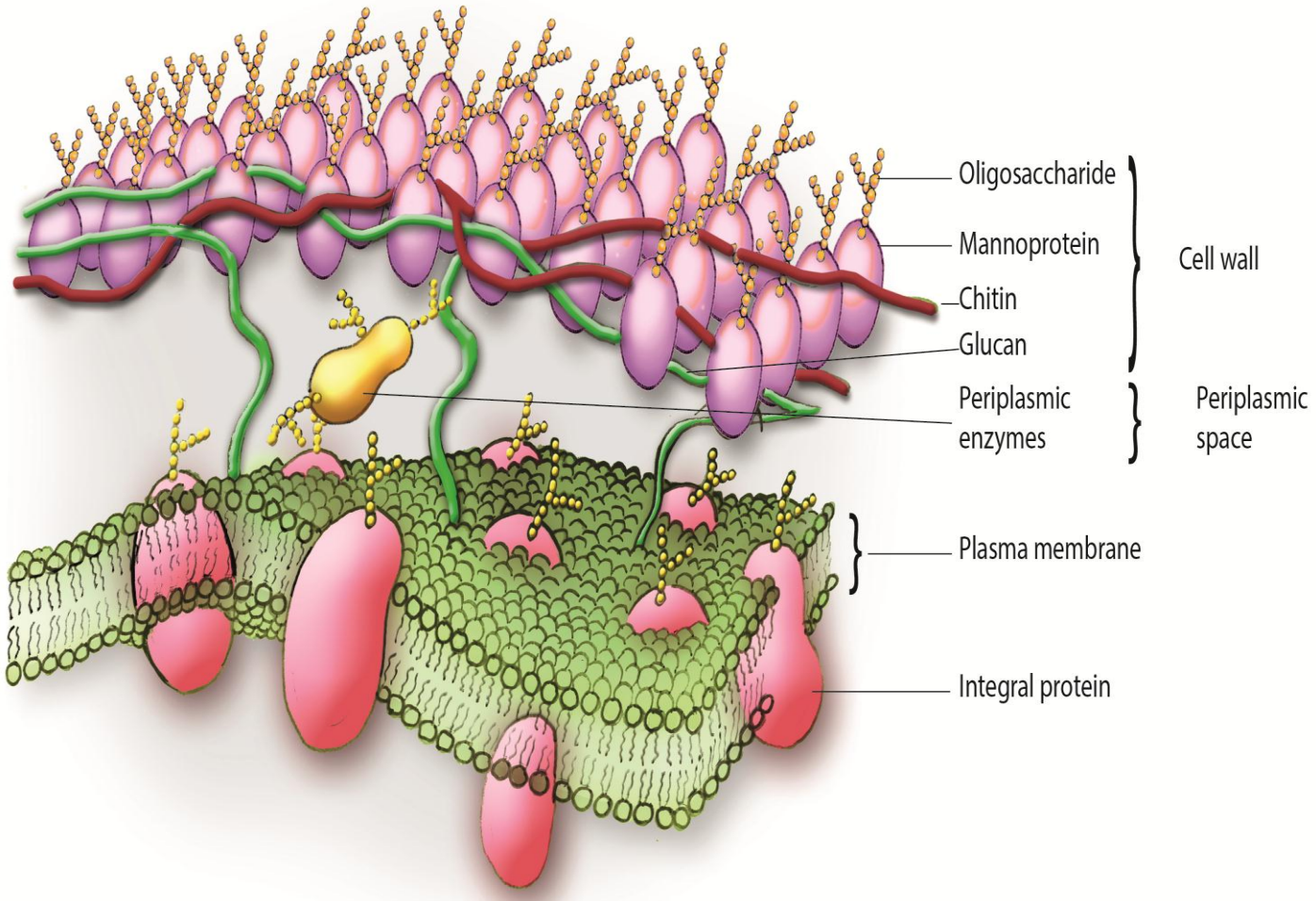
- Fatty Acids
- Sterols

DYNASTART

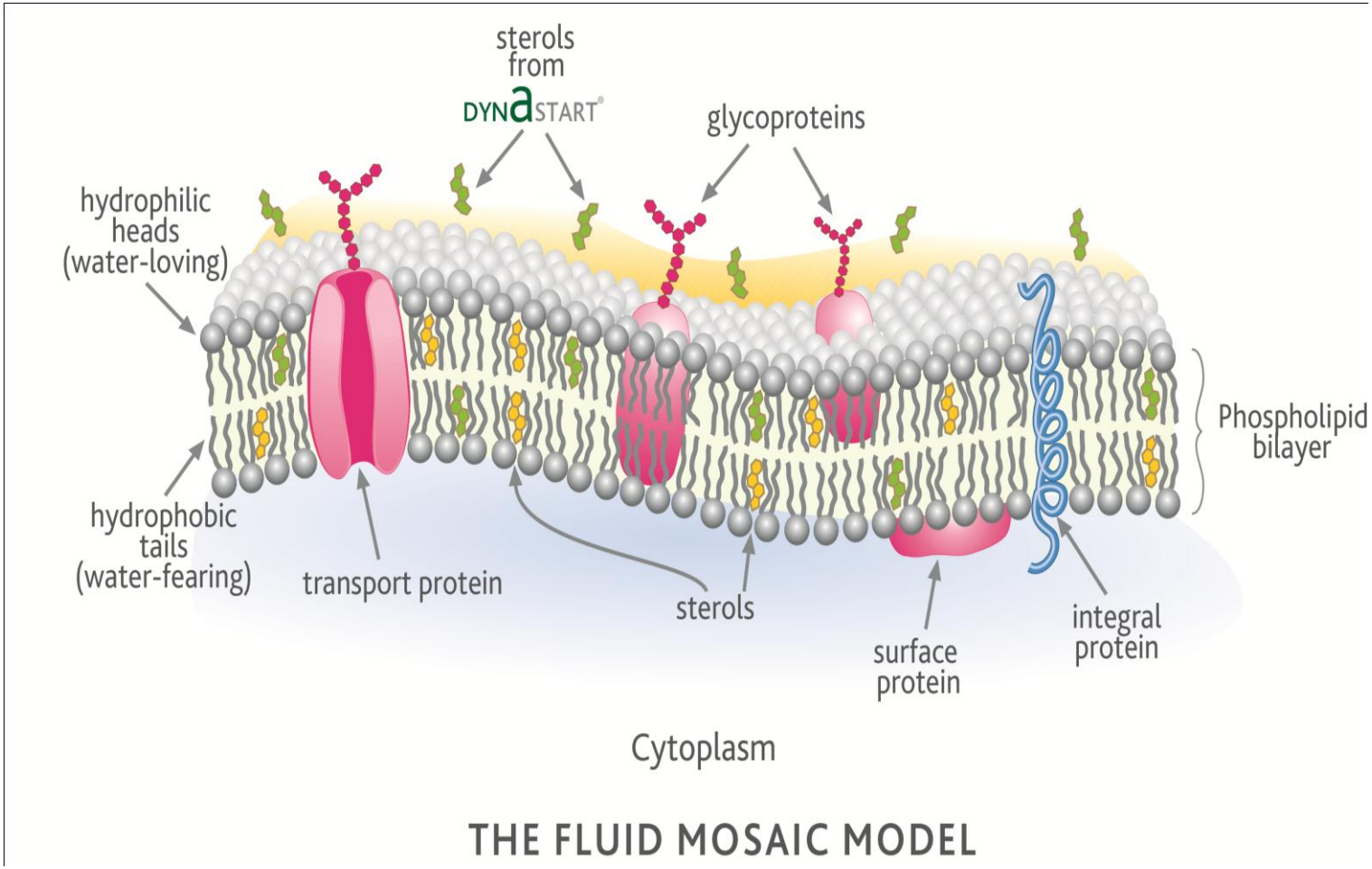
NATURAL YEAST OPTIMIZATION

Boosts Aroma and Strengthens Fermentation!

The Yeast Cell Wall and Membrane



Protection and Survival Factors



A schematic representation of the yeast cell membrane illustrating how sterols from a rehydration nutrient may get incorporated into the live yeast cell during rehydration.

International Journal of Food Microbiology 141 (2010) 229–235

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Oleic acid and ergosterol supplementation mitigates oxidative stress in wine strains of *Saccharomyces cerevisiae*

Sara Landolfo [a], Giacomo Zara [b], Severino Zara [b], Marilena Budroni [b], Maurizio Ciani [a], Ilaria Mannazzu [b,*]

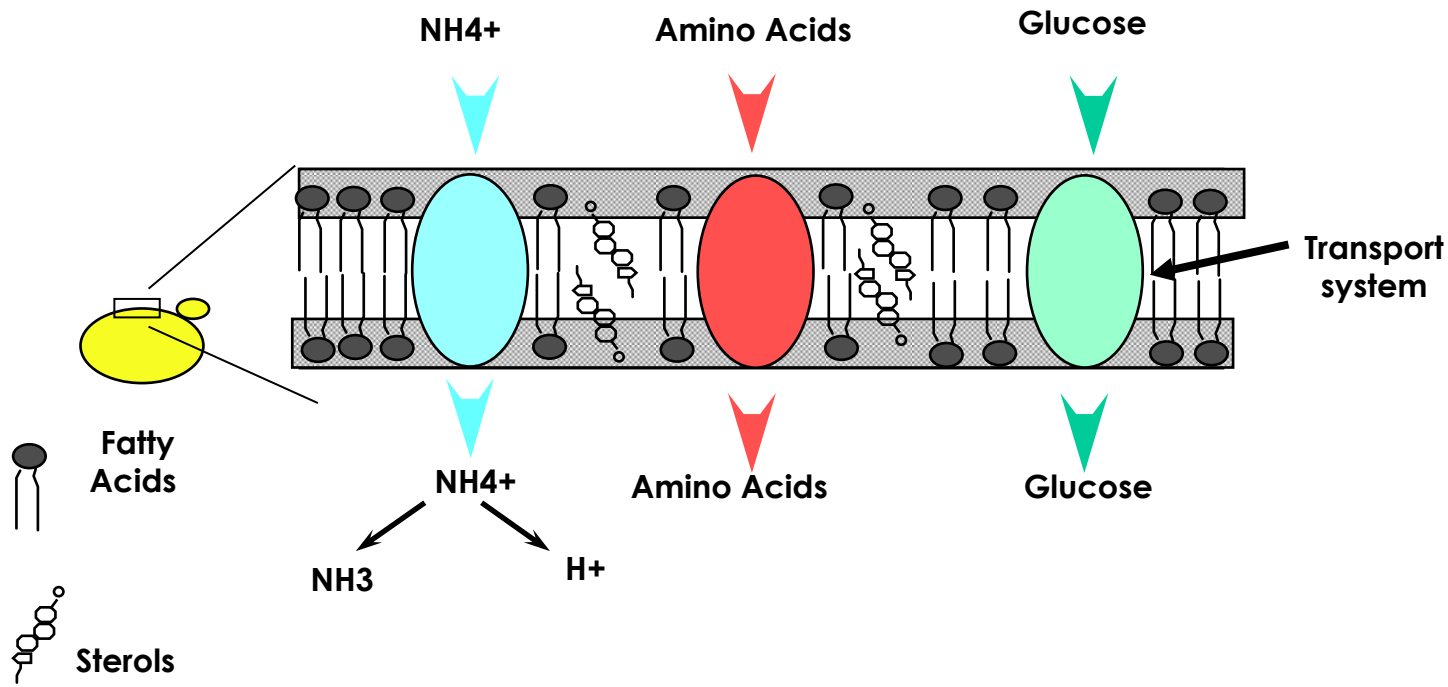
[a] Dipartimento SAIFET, Sez. Microbiologia Alimentare, Industriale e Ambientale, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy
[b] Dipartimento di Scienze Ambientali, Agrarie e Biotecnologie Agroalimentari, Università degli Studi di Sassari, Viale Italia 39, 07100 Sassari, Italy

Lipid and sterol supplementation results

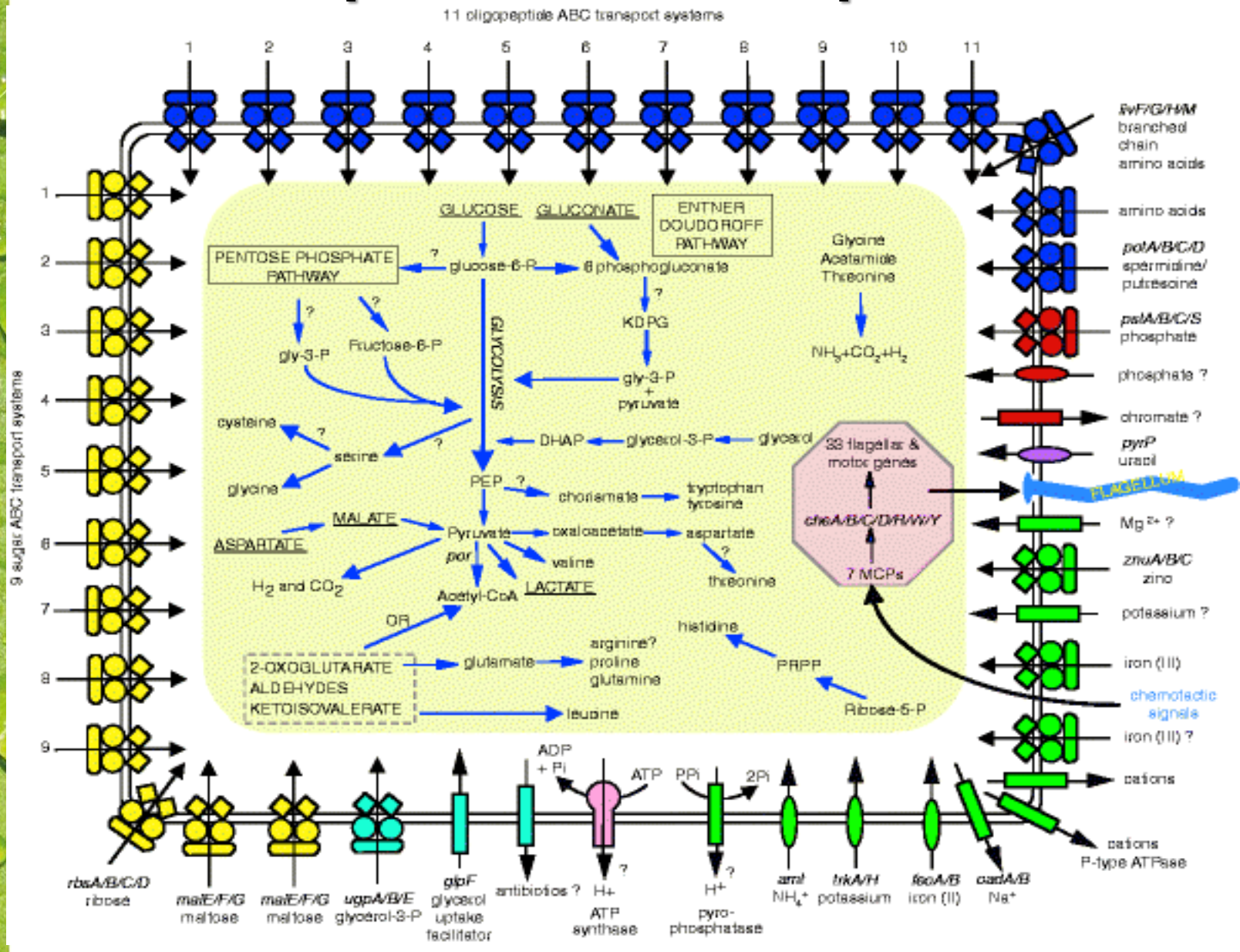
- ✓ Reduced biomarkers of oxidative stress
- ✓ Increased cellular antioxidant response – S.O.D. activity
- ✓ Increased cell viability
- ✓ Reduced production of acetic acid

Yeast Survival Factor Effects

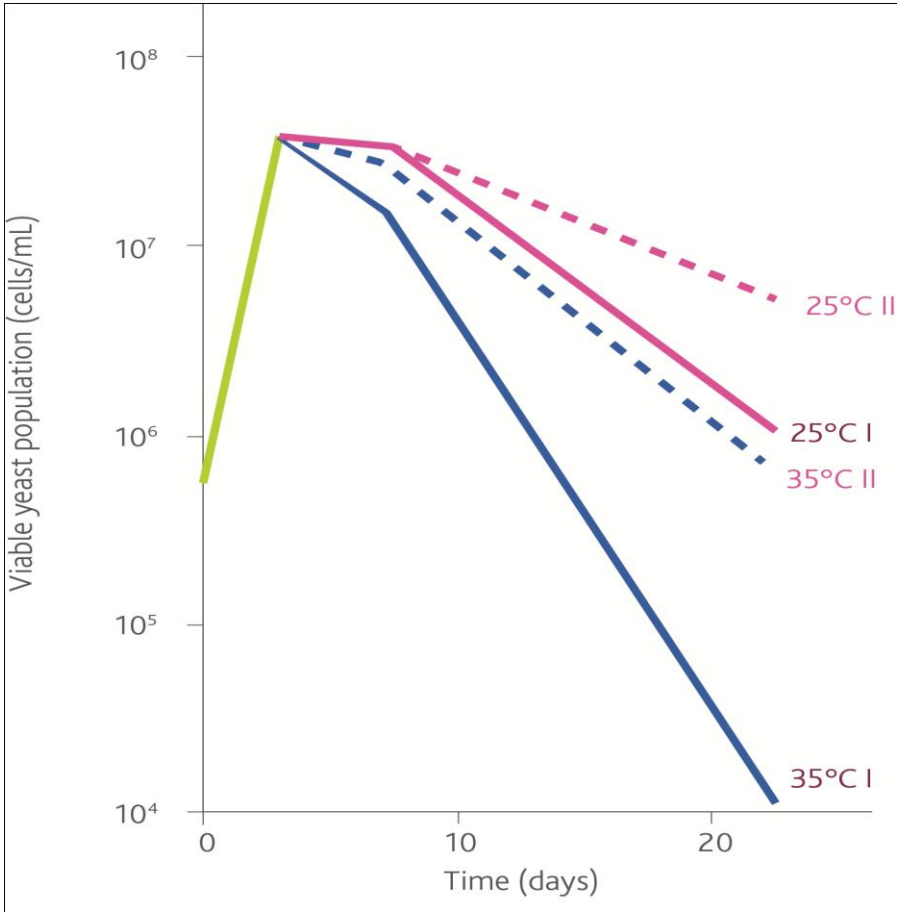
- Guarantee of good membrane permeability (efficient transport systems)
- High sterol content helps in high alcohol ferments, complete AF
- Fatty Acids Vitamins Minerals



Cell Surface Receptors and Transporters



Sterols and Viability



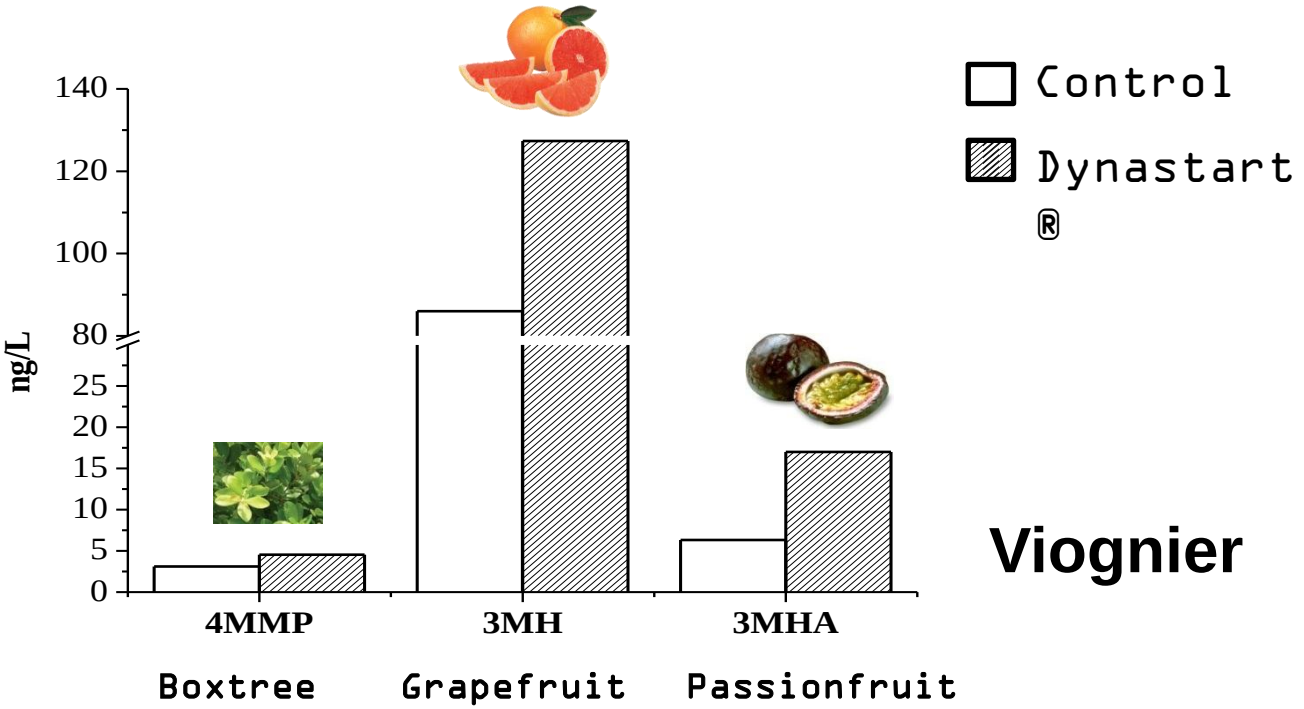
CI – Control without ergosterol addition

CII – Plus ergosterol

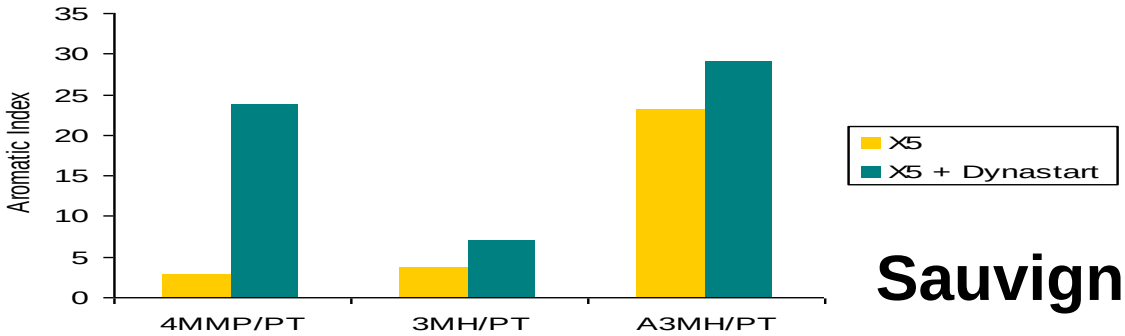
A higher sterol content in the yeast cell membrane ensures higher yeast viability towards the end of fermentation. **Lafon-Lafourcade, 1983**

Ergosterol provides yeast with resistance to oxidative stress including high ethanol concentration **Landolfo et al 2010**

Aromatic Intensity Improvement
-More Positives-



Viognier

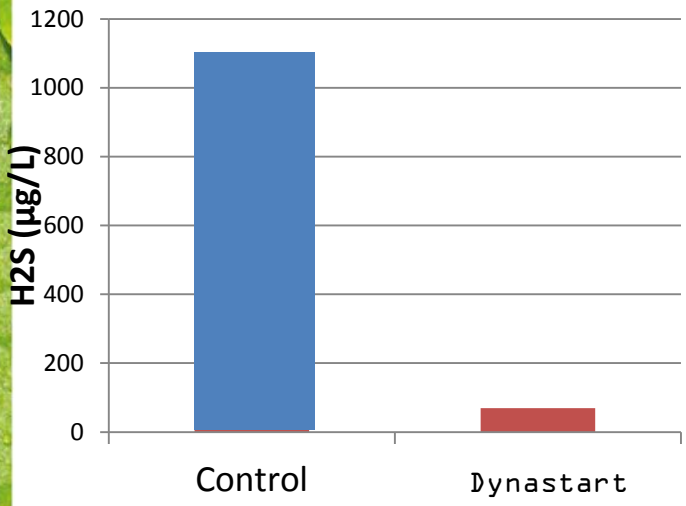


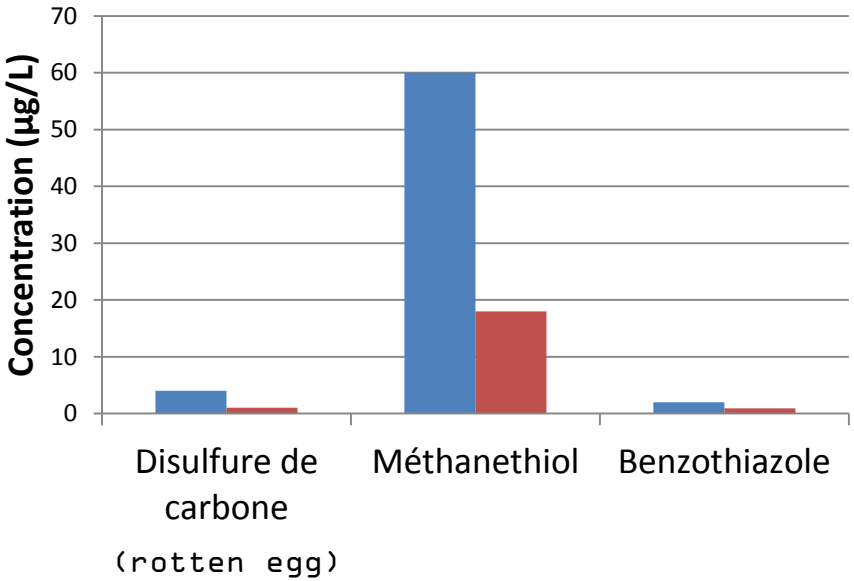
Sauvignon Blanc

10 x of 4MMP compared to Control 2 x of 3MH compared to Control

Aromatic Intensity Improvement
-Less Negatives-

- ✓ **Cabernet Sauvignon No DAP or organic nutrients added**
- ✓ **Initial YAN 150 mg/L. Alcohol 14.5% v/v**
- ✓ **Analyses performed after MLF**





Dynastart helps to significantly limit H2S production and other negative sulphur compounds

Laffort Sponsored Molecular Research

Dr. Tertius Van der Westhuizen
Laffort Australia

Winter et al. AMB Express 2011, 1:36
http://www.amb-express.com/content/1/1/36

AMB Express
a SpringerOpen Journal

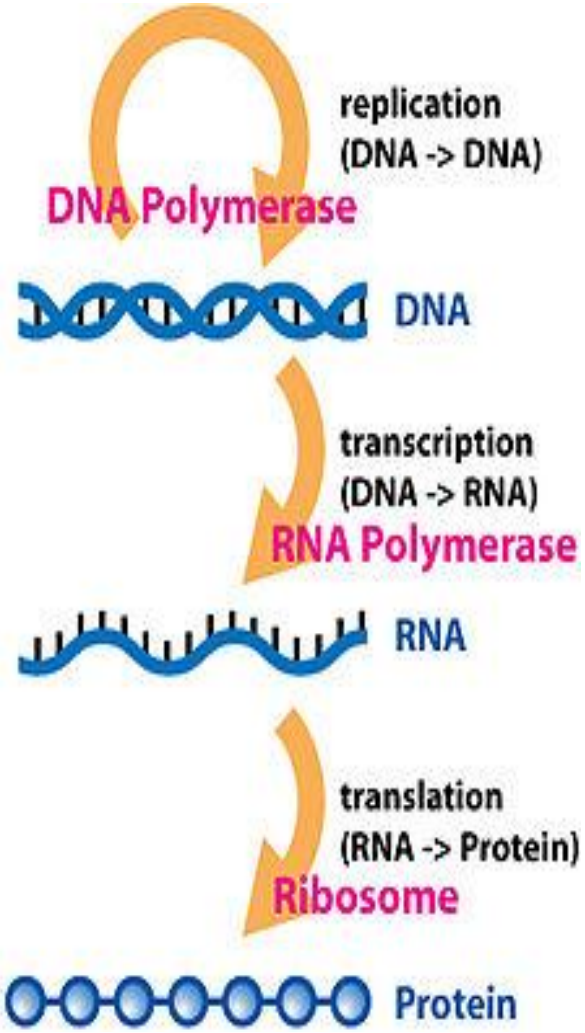
ORIGINAL Open Access

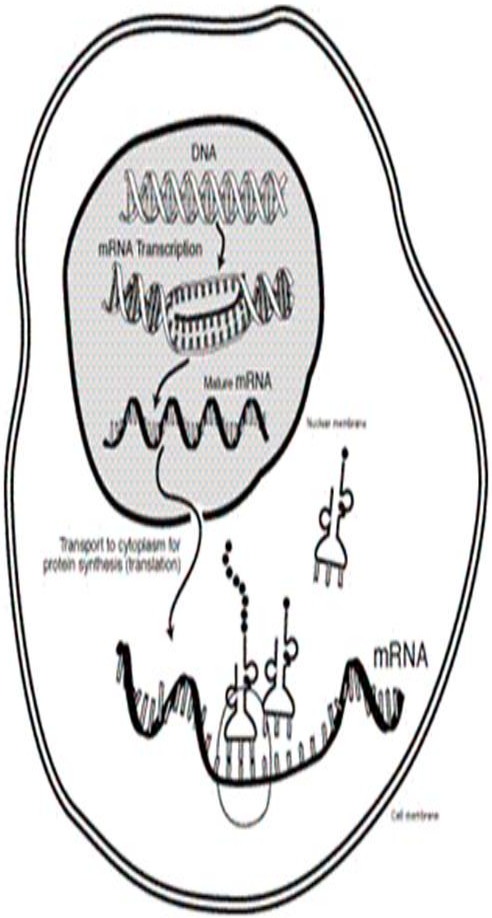
Effects of rehydration nutrients on H_2S metabolism and formation of volatile sulfur compounds by the wine yeast VL3

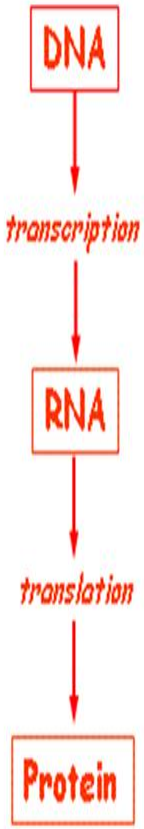
Gal Winter[1,2], Paul A Henschke[2], Vincent J Higgins[1,3], Maurizio Ugliano[2,4] and Chris D Curtin[2*]

Use of Transcriptomics to Investigate Gene Expression and Metabolic Stress in Response to Lipid And Sterol Addition at Yeast Rehydration

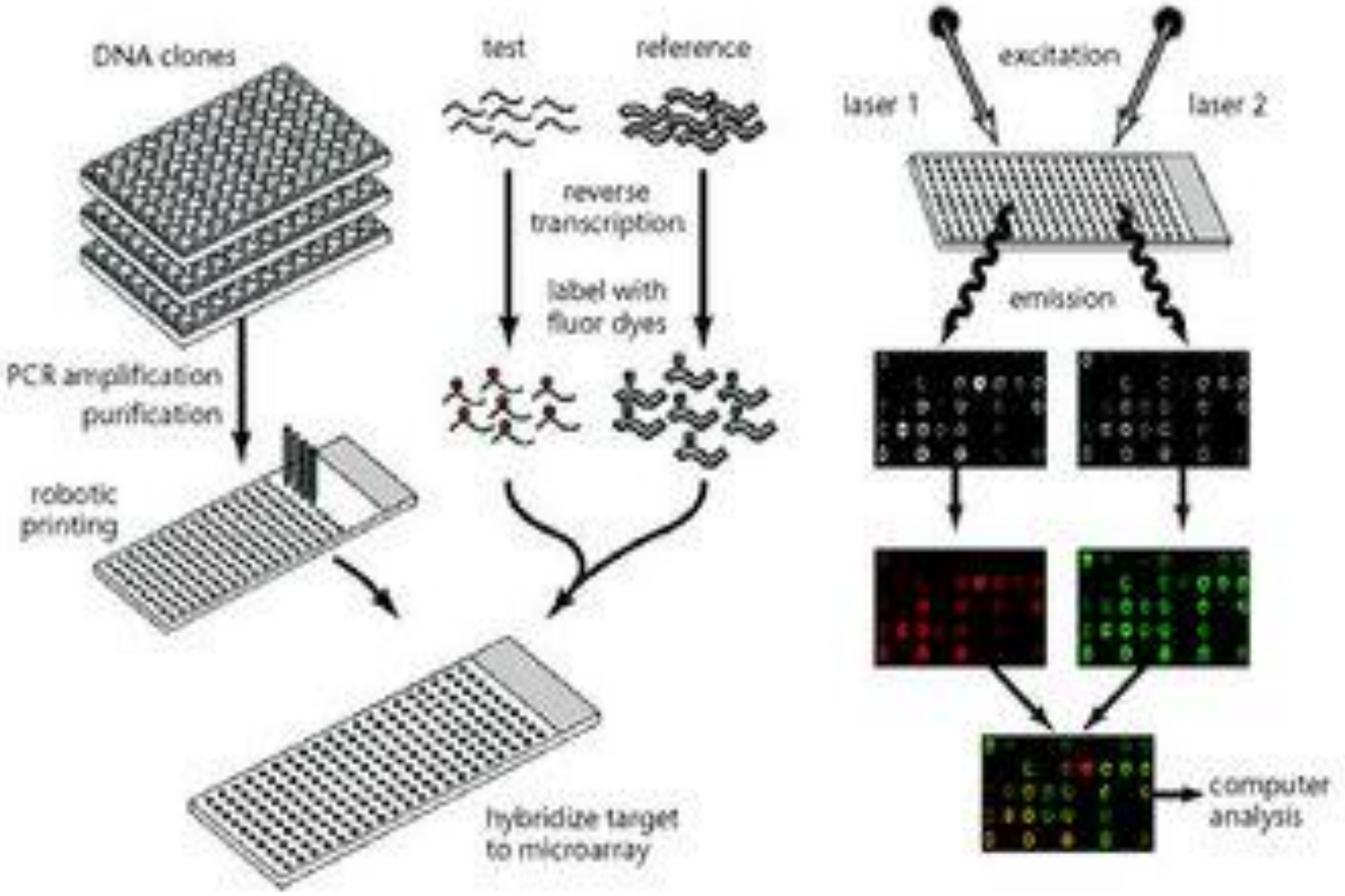
Central Dogma of Molecular Biology



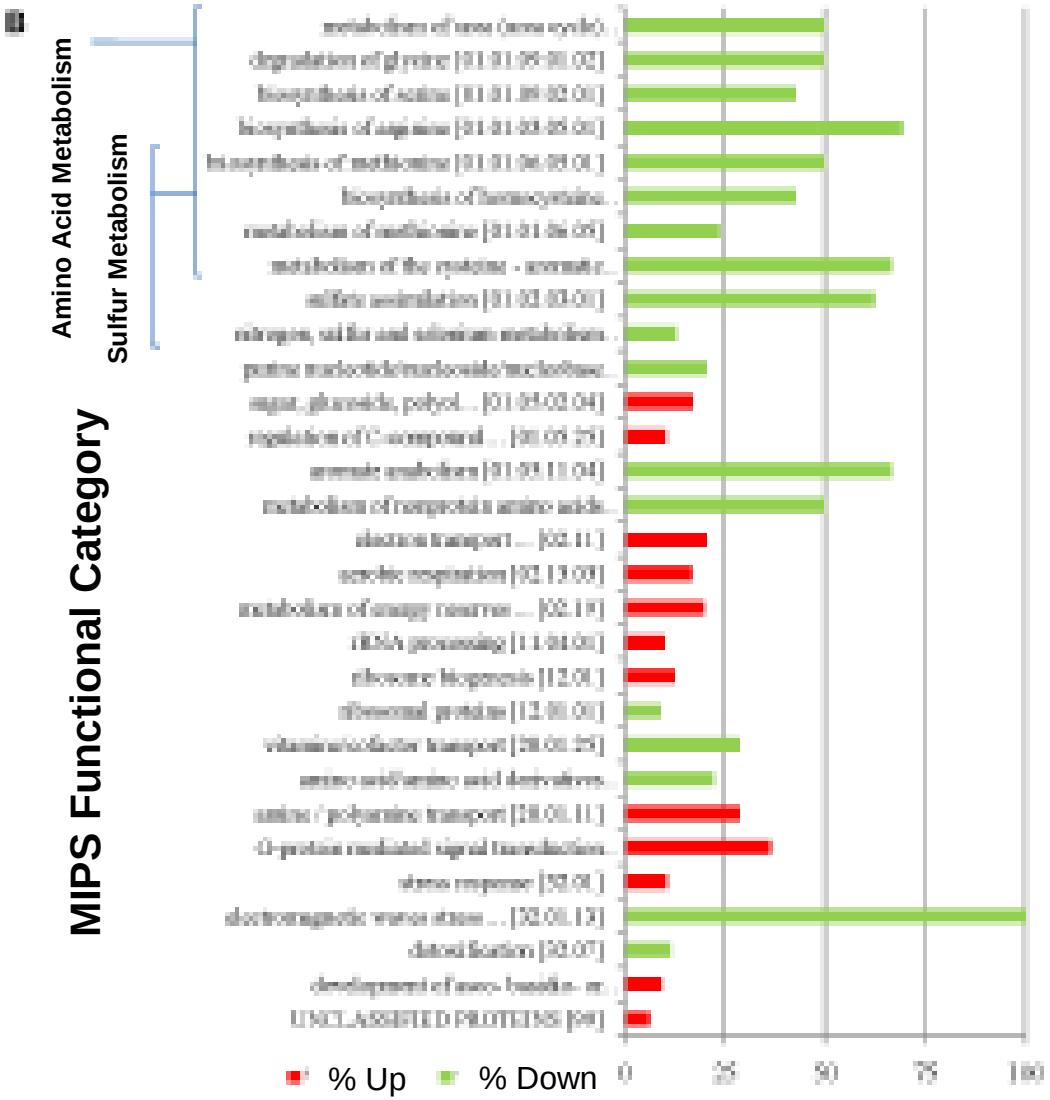




Total Cellular Gene Transcription

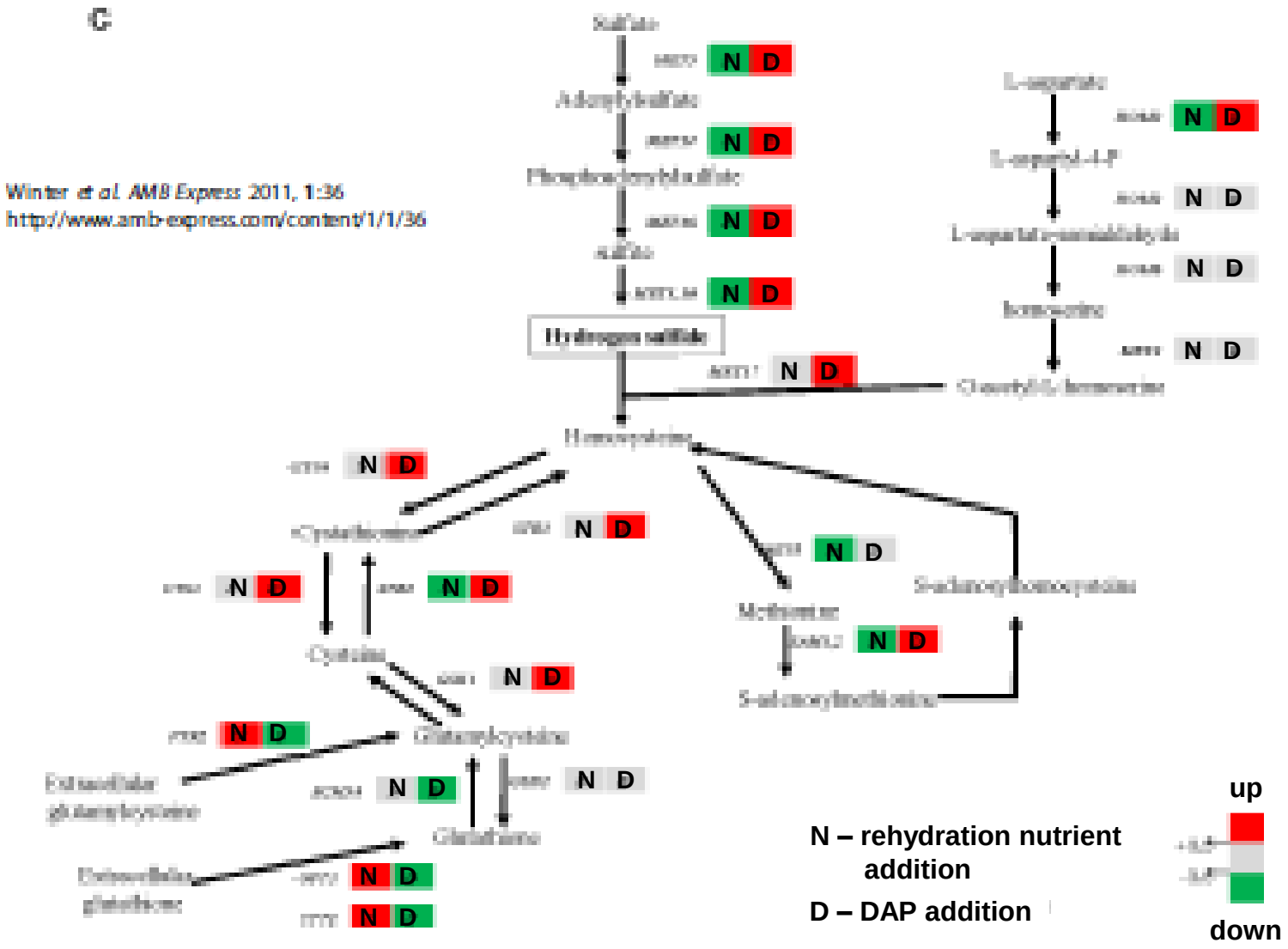


Classification of the genes affected by the rehydration product addition to MIPS functional categories

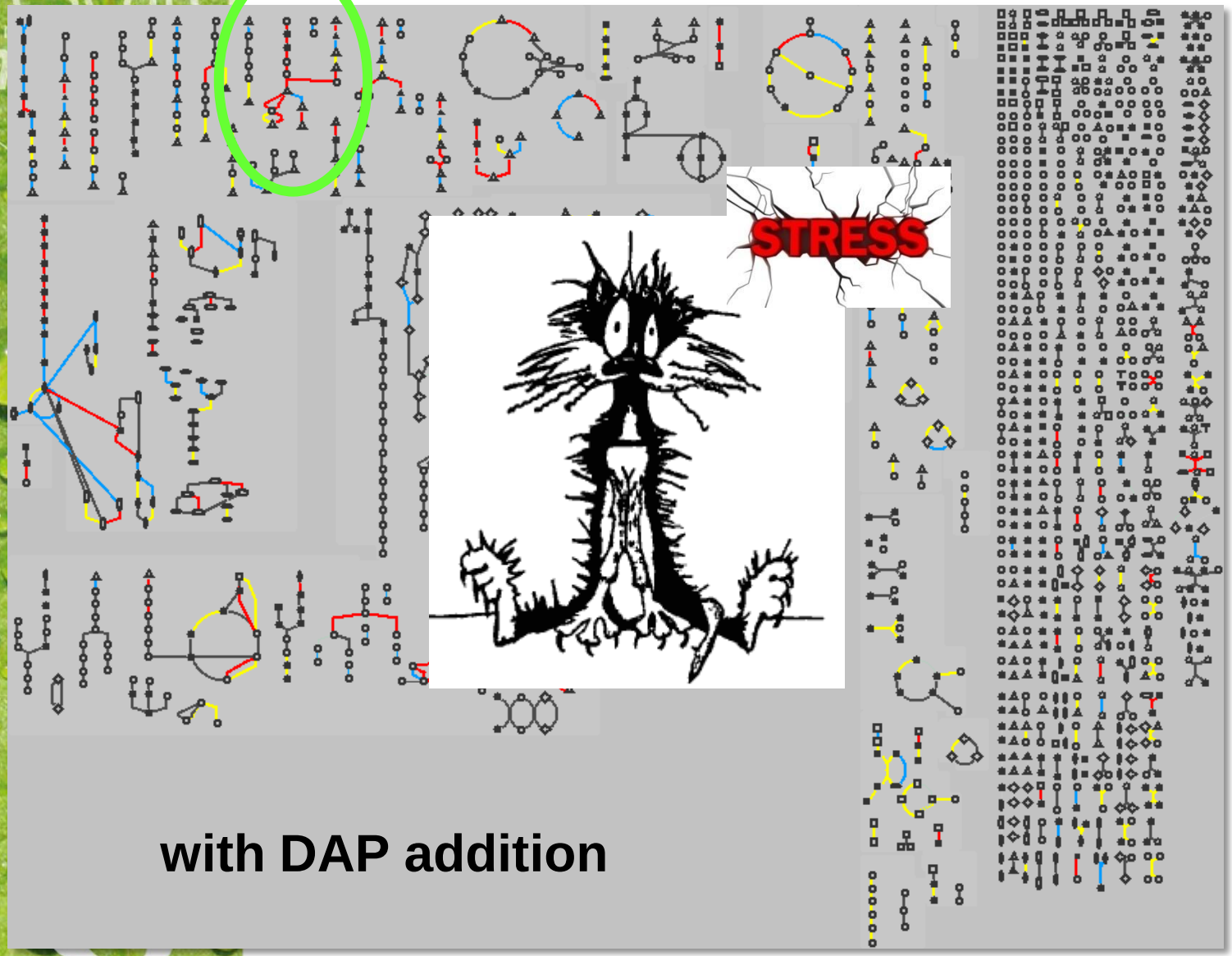


Winter et al. AMB Express 2011, 1:36
http://www.amb-express.com/content/1/1/36

Schematic representation of the sulfur metabolism pathway and its regulation by the two nutrient treatments (N – rehydration nutrient addition, D – DAP addition)



Global Transcription Analysis



Riesling
Zymaflore VL3
YAN : 120 mg/L
Sugars : 205 g/L
pH : 2,9
TA : 4,6 g/L TA
AF temperature : 22°C

YAN level after **DAP addition** : 250 mg/L

with DAP addition

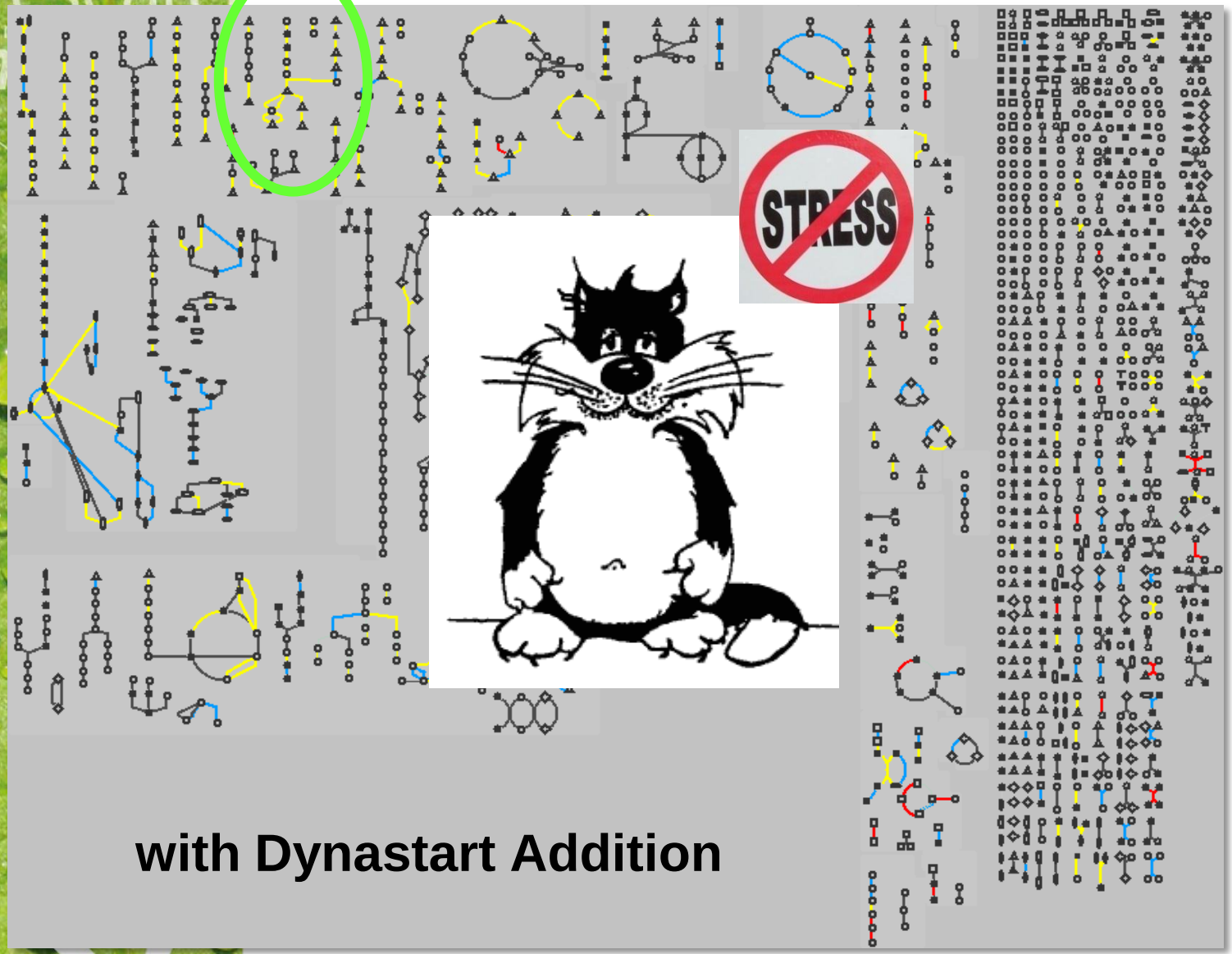
Expression Ratios
+1.5
-1.5

BioCyc

RNA extracted from samples taken after consumption of approximately 15 g/L of sugars

BIOLAFFORT

Global Transcription Analysis



Riesling
Zymaflore VL3
YAN : 120 mg/L
Sugars : 205 g/L
pH : 2,9
TA : 4,6 g/L TA
AF temperature : 22°C

No DAP addition

Expression Ratios
+1.5
-1.5

with Dynastart Addition

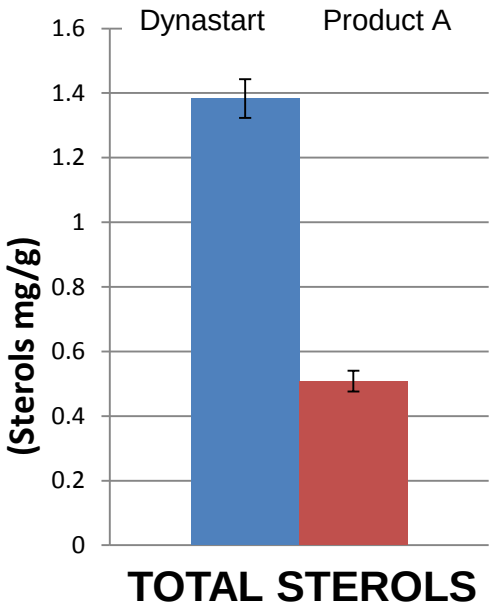
RNA extracted from samples taken after consumption of approximately 15 g/L of sugars

BIOCYC

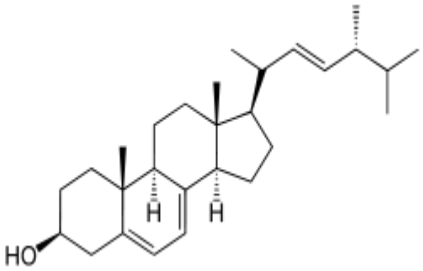
BIOLAFFORT

The Dynastart Difference

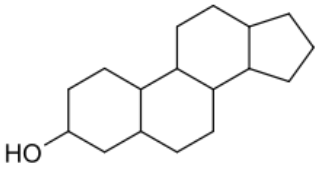
A viable alternative to oxygenation for assisting yeast sterol accumulation

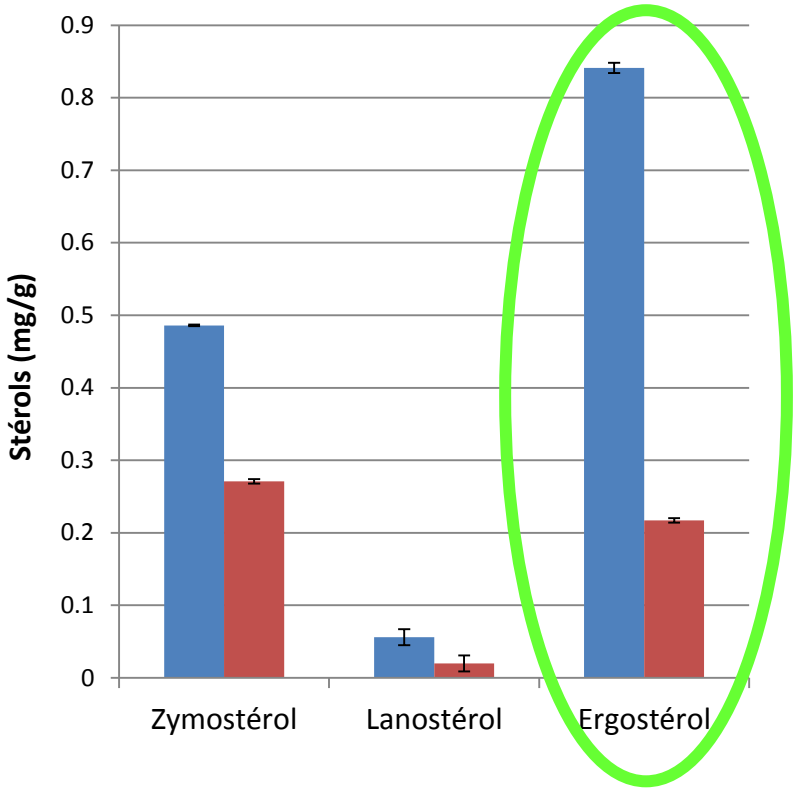


TOTAL STEROLS



Ergosterol is the sterol that provides the most alcohol resistance





Proper Yeast Rehydration for a Dynamic Finish

Yeast Survival Factors lead to better functioning of yeast metabolism, thus giving:

- ✓ **An optimized aromatic intensity**
- ✓ **More efficient use of nutrients**
- ✓ **Lower VA production**
- ✓ **Less negative sulfur compounds**
- ✓ **A strong fermentation finish**

Specific Rehydration Nutrients

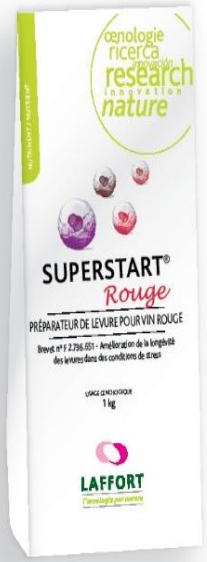
Red Wine vs White Wine

NEW

HOW TO CHOOSE YOUR NUTRIENTS ACCORDING TO THE WINEMAKING CONDITIONS

PROTECTION

Recommended in the event of :



✓High alcohol potential
✓High temperatures and temperature variations during AF.
✓AF restart.

SUPERSTART®* Rouge

ADY rehydration
200-300 ppm
YAN BROUGHT BY
200 PPM : ~ 20 PPM

✓Fermentation at low temperatures and low turbidities.
✓High alcohol potential.

SUPERSTART®* Blanc

ADY rehydration
200-300 ppm
YAN BROUGHT BY
200 PPM : ~ 40 PPM

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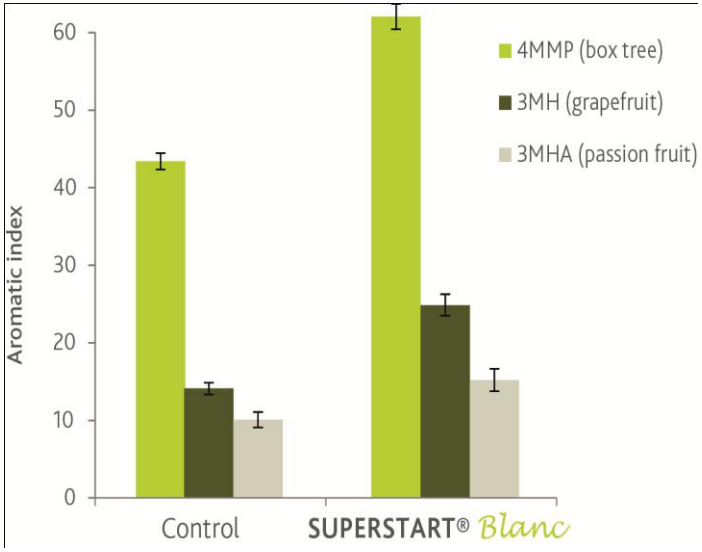
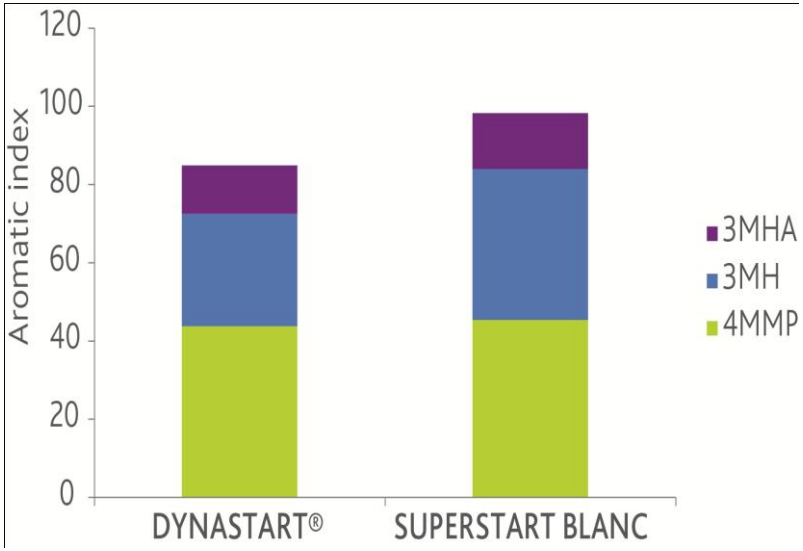
SUPERSTART® Blanc

NEW

→ **New formulation –**
stronger aromatic enhancement





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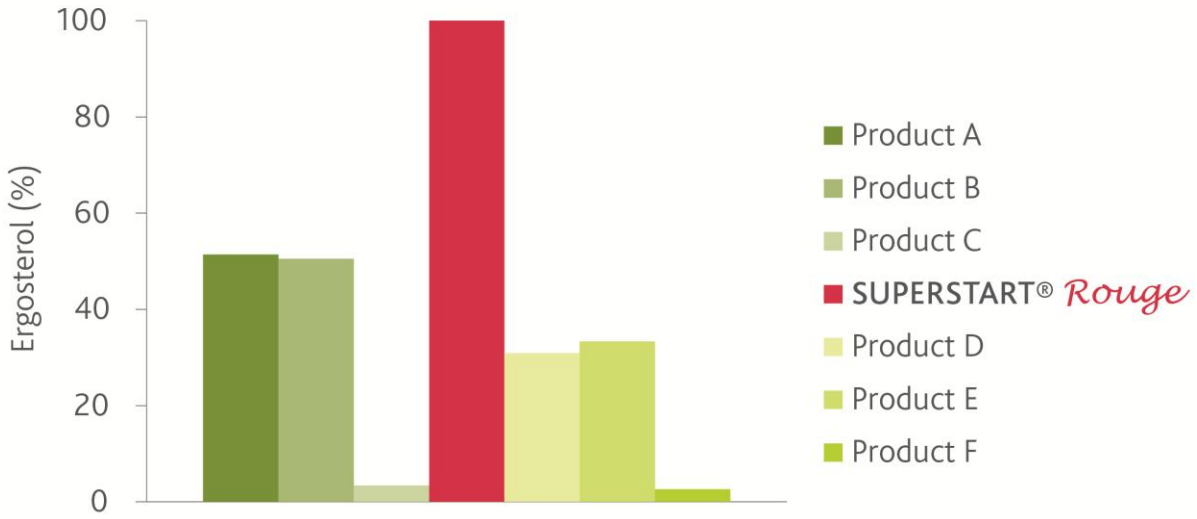
SUPERSTART® *Rouge*

NEW



Yeast based product for **protection** particularly rich in sterols and lipids intended for ADY rehydration

Is **NOT** equal to:

Yeast based products rich in amino acids, minerals and vitamins intended for the **nutritional management during AF**

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Yeast Protection and Nutrition

- ✓ Use of **cultured yeast** provide many advantages
- ✓ There is a fundamental and important difference between **yeast protection** and **nutrition**
- ✓ **Carbon to Nitrogen balance** is important but is certainly not the only nutritional consideration
- ✓ The optimal **Brix / YAN ratio** for fermentation security is changing with the advent of **proper rehydration** and **organic nutrients**
- ✓ **Nitrogen** and **Pantothenate** work together to help relieve stress in the sulfur assimilation pathway and reduce production of detrimental sulfides

Yeast Protection and Nutrition

- ✓ **Proper rehydration** increases yeast viability and ensures strong fermentation, alcohol resistance, **more positive** aroma, **less negative** components
- ✓ **White** and **Red** must and wine conditions present **distinct challenges** for yeast in fermentation performance

✓Keep your yeast HAPPY…

and your yeast will make you happy!

Laffort – A Global Leader

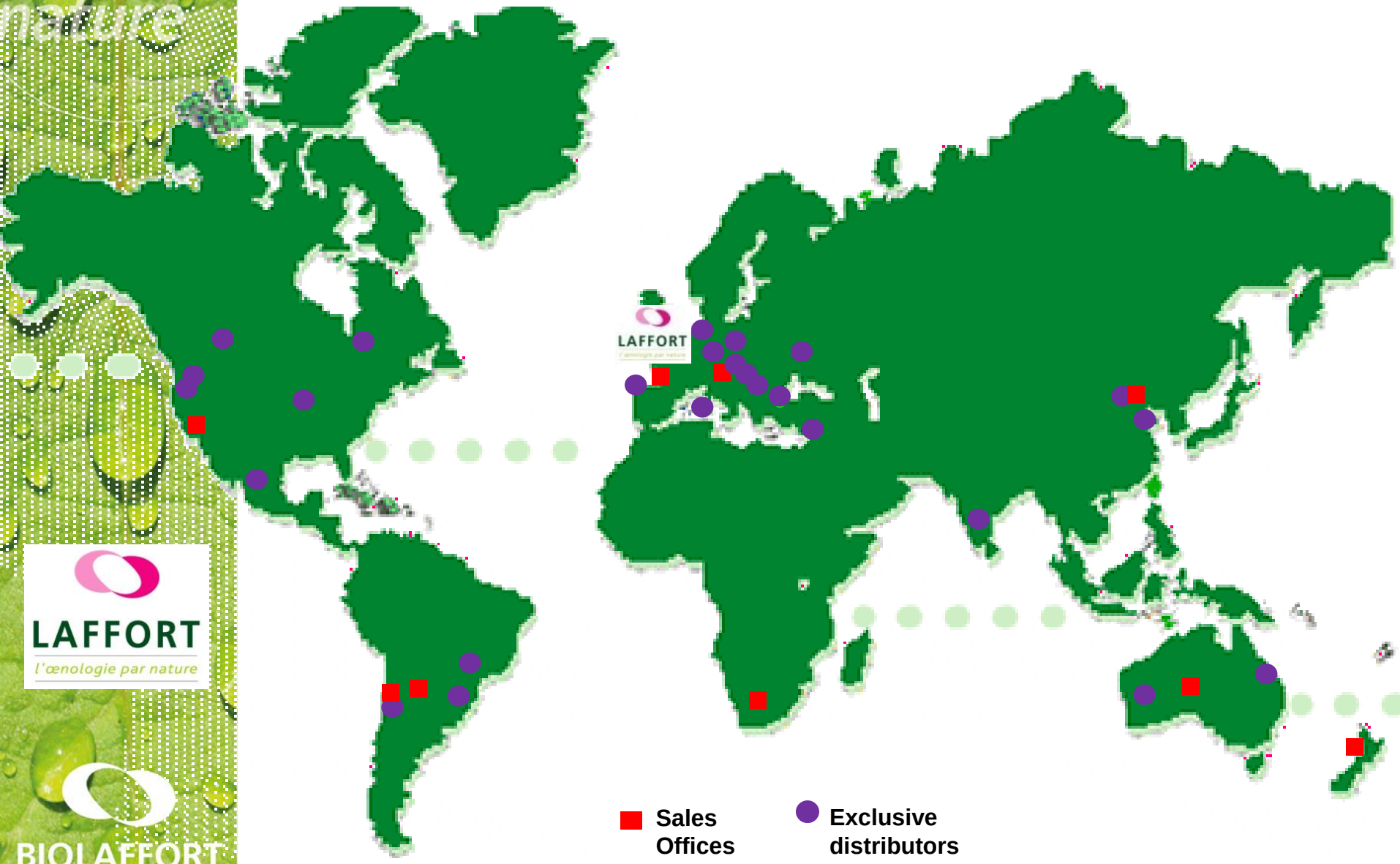
Founded in 1895, LAFFORT S.A.S. is a family-owned Frencn company completely focused on research, production, and distribution of the highest quality and best value enological products worldwide.

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



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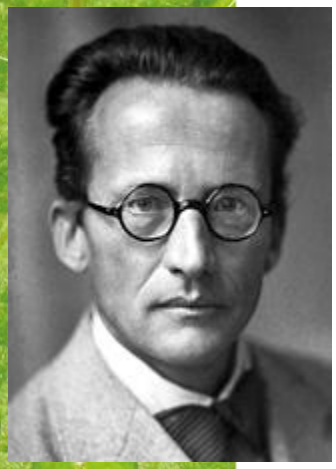
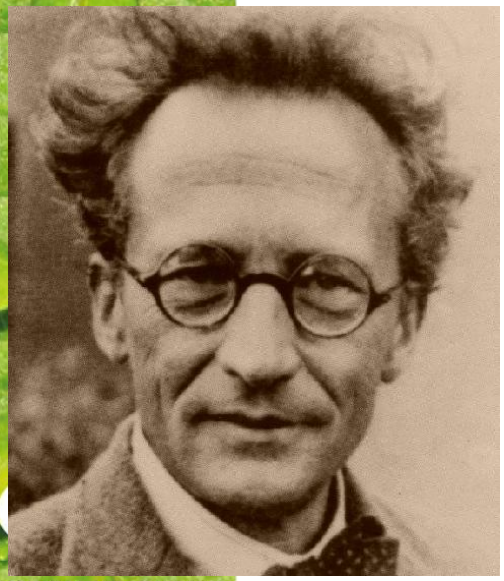
Research Quote





“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

Changing Paradigms in Wine Fermentation Management

? Questions – Discussion !

Peter Salamone, Ph.D.
Technical Manager
North America

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Laffort in Ontario:
Vines to Vintages Inc.