



Saccharomyces cerevisiae oxidative response by cyclic voltammetry and GC-MS

Carla Maria Oliveira^{1,2} Cristiana C. Castro³, Caitriona Gunning¹, José A. Couto¹, José A. Teixeira³, Rui C. Martins⁴ and António C. Silva Ferreira^{1,5*}

1. Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

2. Departamento de Química e QOPNA, Universidade de Aveiro, 3810-193 Aveiro, Portugal

3. IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

4. Bioinformatics - Molecular and Environmental Biology Centre, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

5. Stellenbosch University, Private Bag XI, Matieland, 7602, Stellenbosch, South Africa

*Corresponding author

E-mail: acferreira@esb.ucp.pt



Objectives:

- The study of the resistance to oxidation during an alcoholic fermentation using a synthetic grape juice
- The identification and quantification of target compounds related with this metabolic process
- The interpretation of the overall metabolic process by a non-target approach



**Why use
SGJ?**

**To reduce
complexity**

**Clearer precursor-
product relationship**

**Reproducibili
ty**



1. Synthetic Grape Juice (SGJ)

Final concentrations per litre

Solution A

- D-glucose 110g
- D-fructose 110g
- Ergosterol 10mg
- Tween 80 1 ml

Solution B

- L (+) tartaric acid 6g
- L-(-)malic acid 3g
- Citric acid 0.5g

Solution C

- YNB (Difco) 1.7g
- CAA (Difco) 2g
- CaCl₂ 0.2g
- Arginine-HCl 0.8g
- L-(-)proline 1g
- L-(-)tryptophan 0.1g
- Tyrosine 0.1g
- Phenylalanine 0.1g

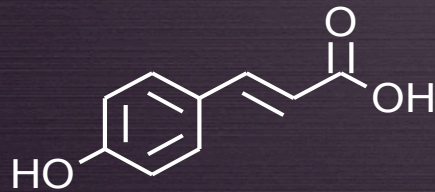
CAA (casamino acids) is the group of amino acids obtained when casein (from milk) is broken down by enzymes

(Ciani and Ferraro 1996)

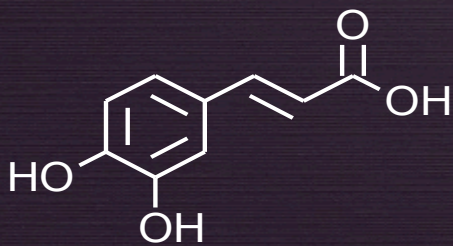


Study of the resistance to oxidation during the fermentation

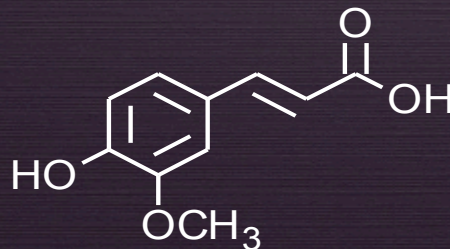
3. Hydroxycinnamic acids



Coumaric acid



Caffeic acid

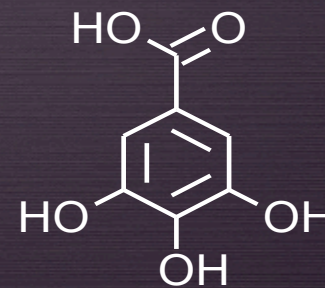


Ferulic acid

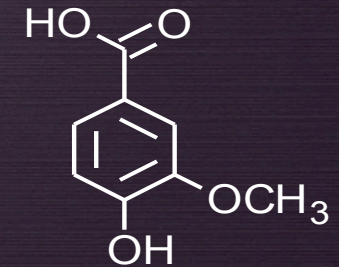
2. Phenolic acids addition

Aprox. 15 mg/L each

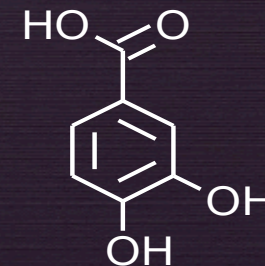
3. Hydroxybenzoic acids



Galic acid



vanillic acid



Protocatechuic acid



3. Yeast addition

Portuguese Yeast Culture Collection
Saccharomyces cerevisiae
(PYCC 4653 strain)



Experimental protocol



SGJ
+
Yeast
addition

Control
V = 900 ml



SGJ
+
Phenolic
acids
+
Yeast
addition

Supplemented 1
V = 900 ml



SGJ
+
Phenolic
acids
+
Yeast
addition

Supplemented 2
V = 900 ml



Samples were taken and analyzed daily, for 11 days



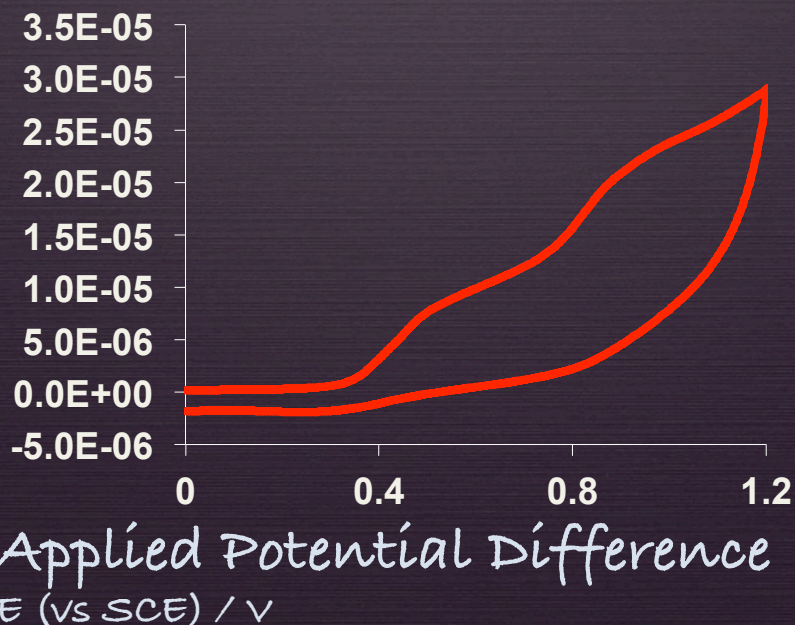
- **The antioxidant profile was monitored:**
 - **Target approach: using HPLC/DAD detection**
 - **Non-target approach: using cyclic voltammetry**



Cyclic voltammetry

Scanning of electroactive compounds - Antioxidant profile

Current Intensity
I/A



E (mV)

815	Coumaric acid
800	Vanillic acid
680	Syringic acid
670	Ferulic acid
500	Rutin
470	Gallic acid
460	Caffeic acid
425	Quercetin
420	Catechin

O. Makhotkina, P.A. Kilmartin /
Analytica Chimica Acta 668
(2010) 155–165

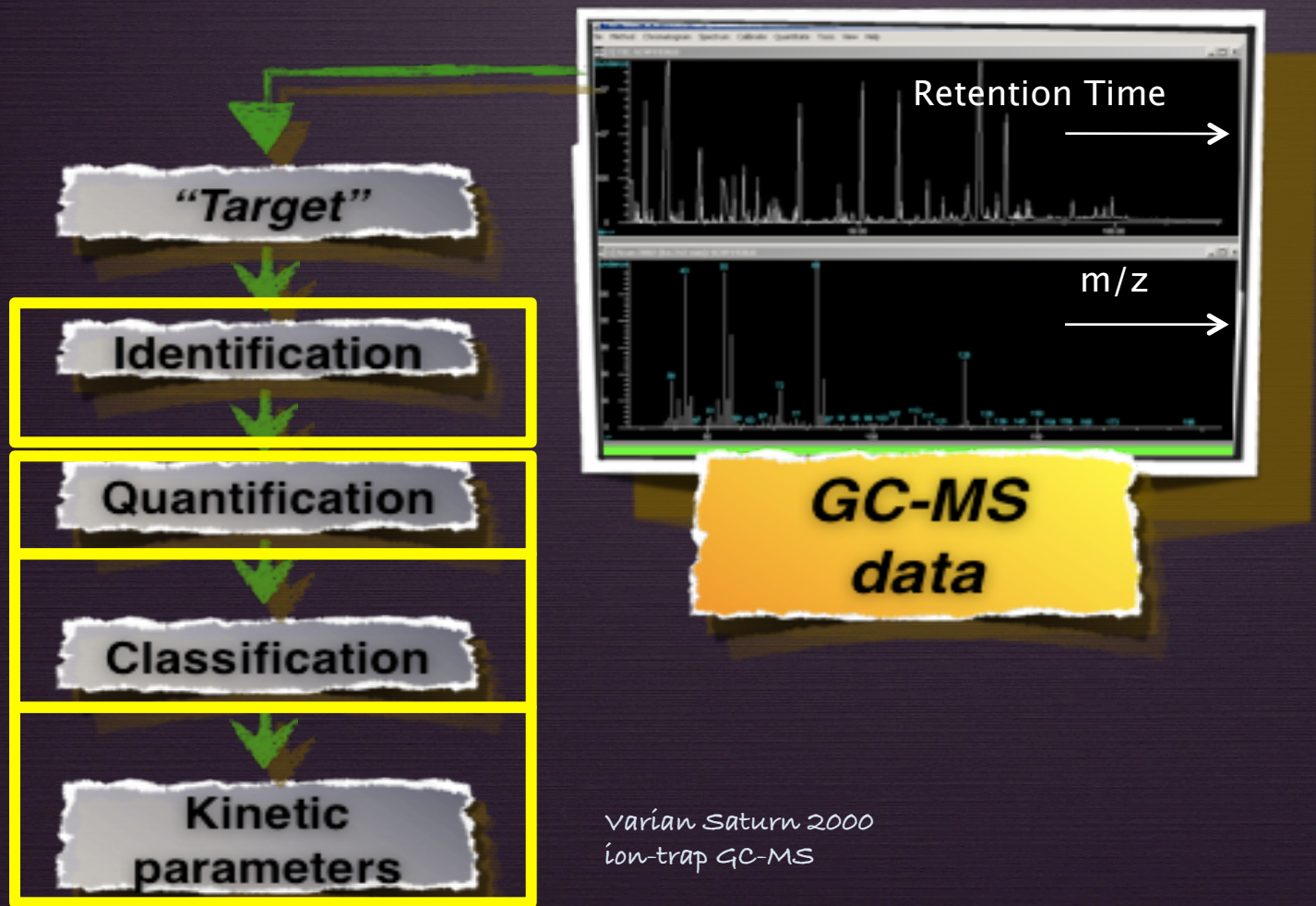
3 mm diameter glass carbon electrode
Scan Range: 100 mV / sec (diffusion)



■ The aromatic profile was



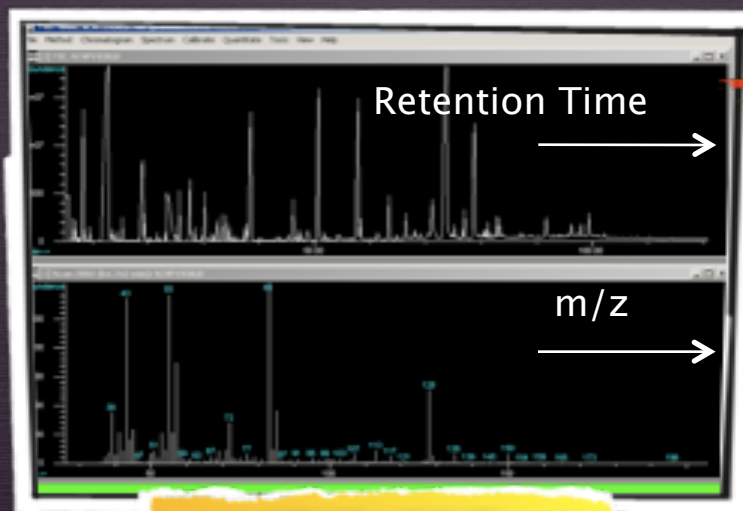
“Target *versus* Non-Target Approach”...



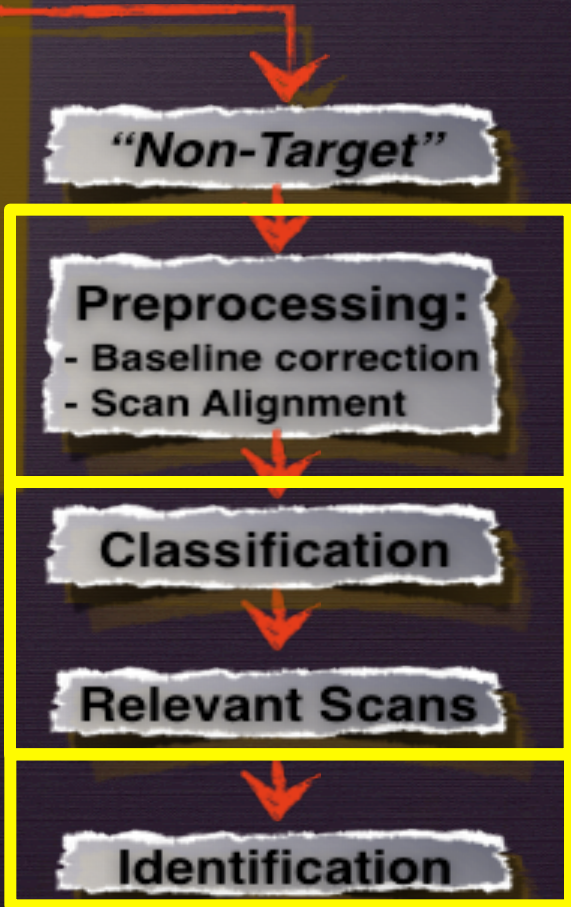
Varian Saturn 2000
ion-trap GC-MS



“Target *versus* Non-Target Approach”...



**GC-MS
data**



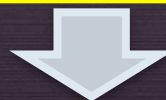
Varian Saturn 2000
ion-trap GC-MS



Mass Spectroscopy Signal Processing

Classification

Relevant Scans



X-Metabolomics

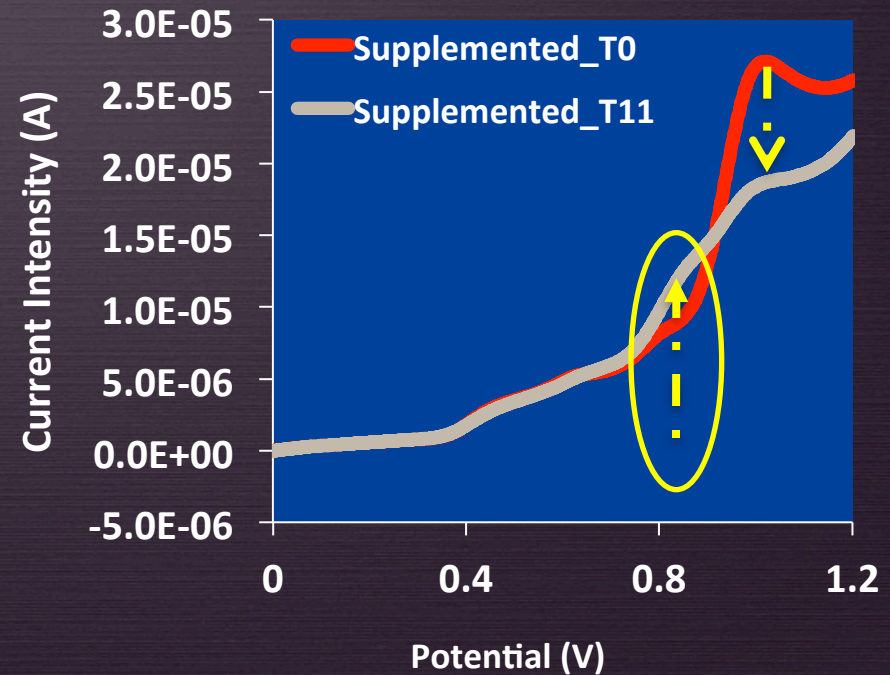
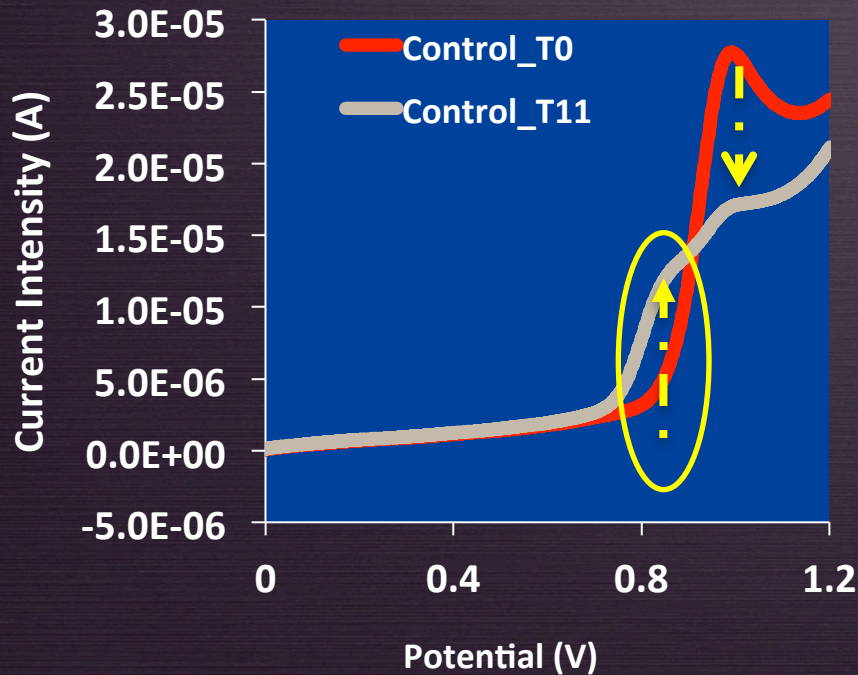
File Alignment Filtering MassMiner Help

New Open Save Settings Reload Print BatchAlignment



Voltammetric results

Changes in the overall antioxidant profile!

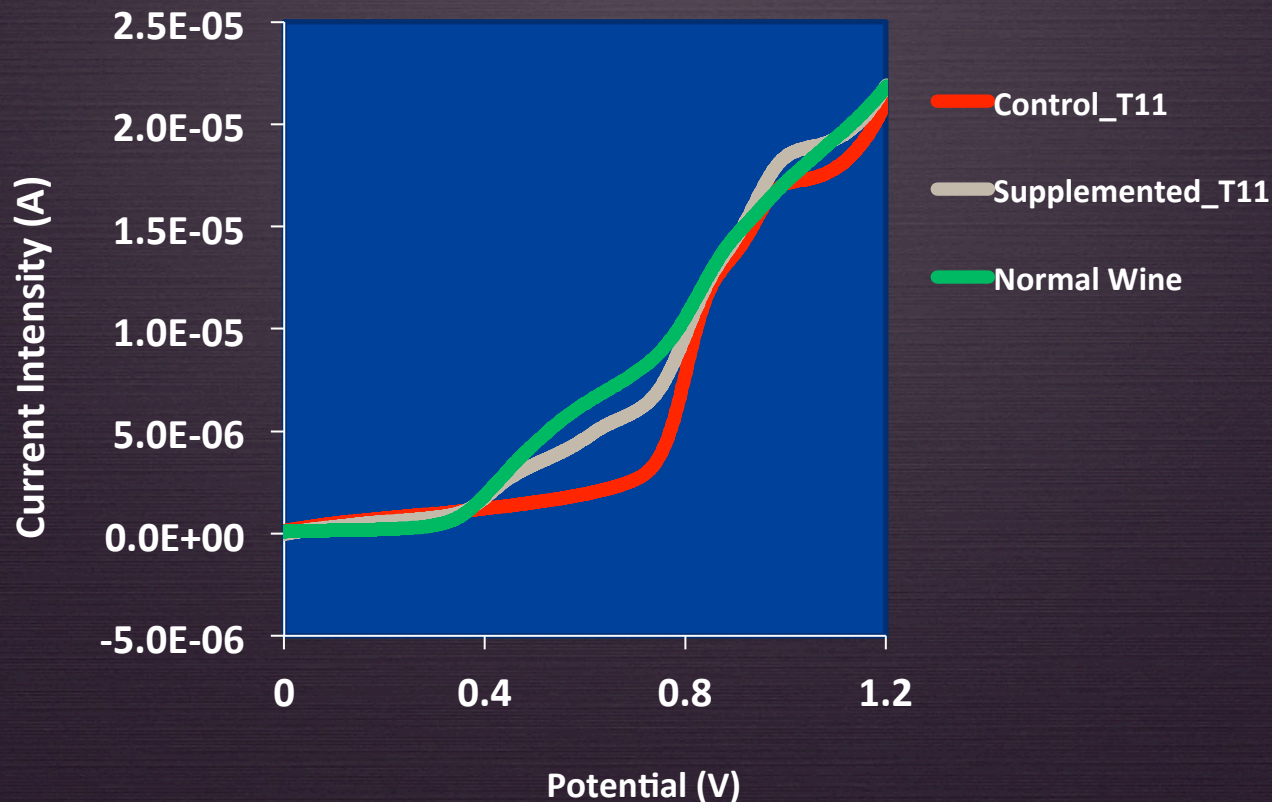


- A decrease in the amplitude of the broad band at 1 V;

- One broad band at 0.8 V was brought about by fermentation;



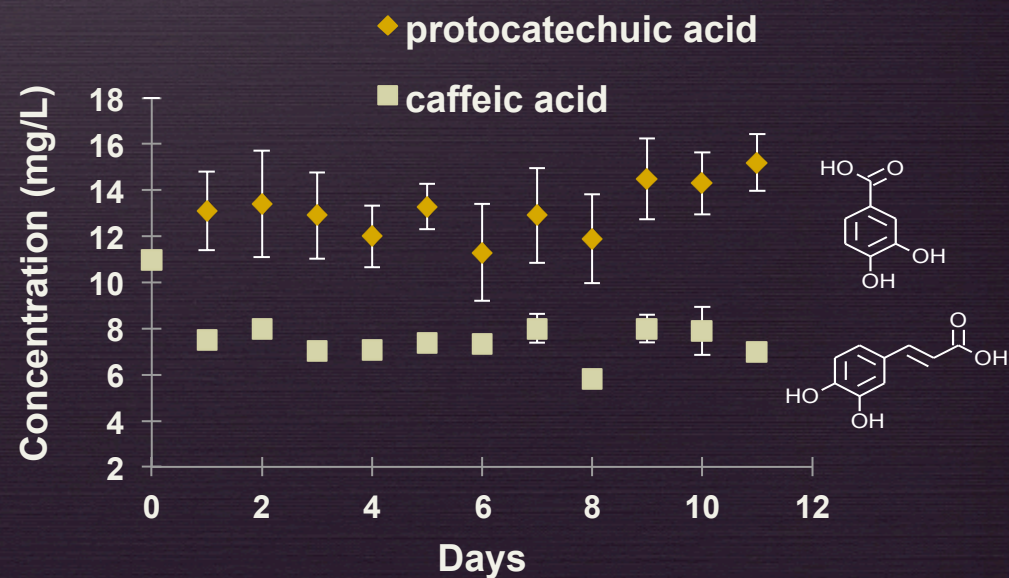
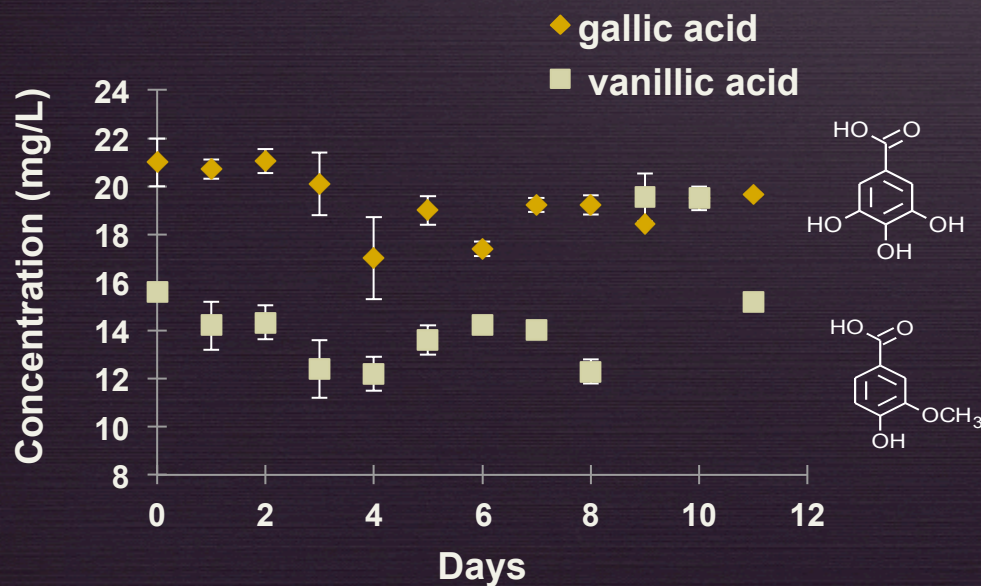
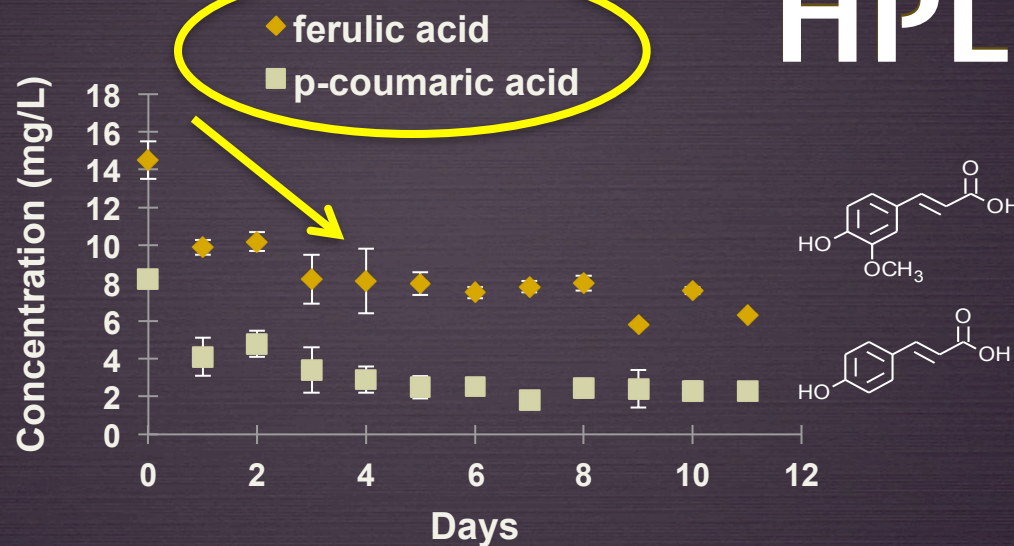
Voltammetric results



- The end of alcoholic fermentation shows a pattern like that of normal wines.



HPLC-Data

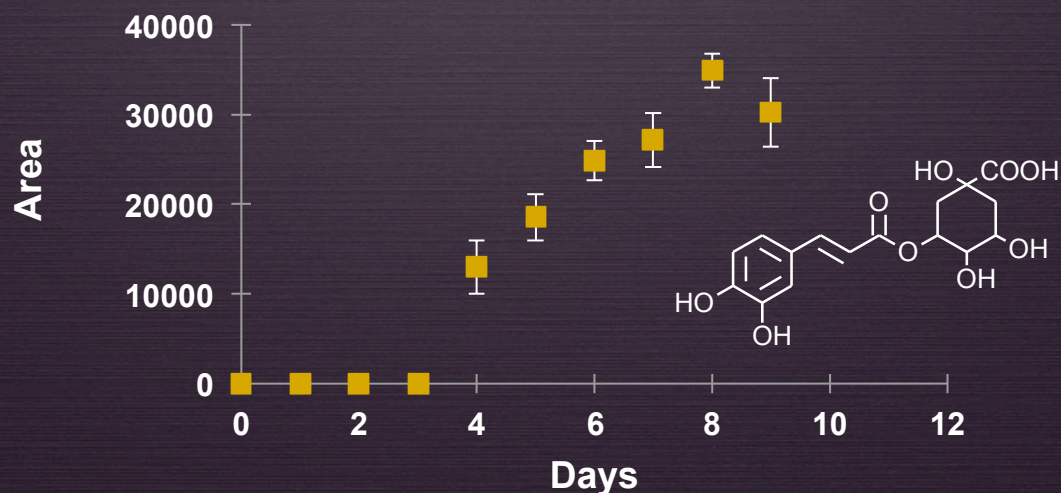




HPLC-Data

One compound was found increasing in both control and supplemented media, after 5 days of alcoholic fermentation.

Chlorogenic acid confirmed by LC-MS



This result suggests that *Saccharomyces cerevisiae* has the ability to produce antioxidants 'de novo' from simple precursors such as sugars and amino acids.



“Target *versus* Non-Target Approach”...



GC-MS data

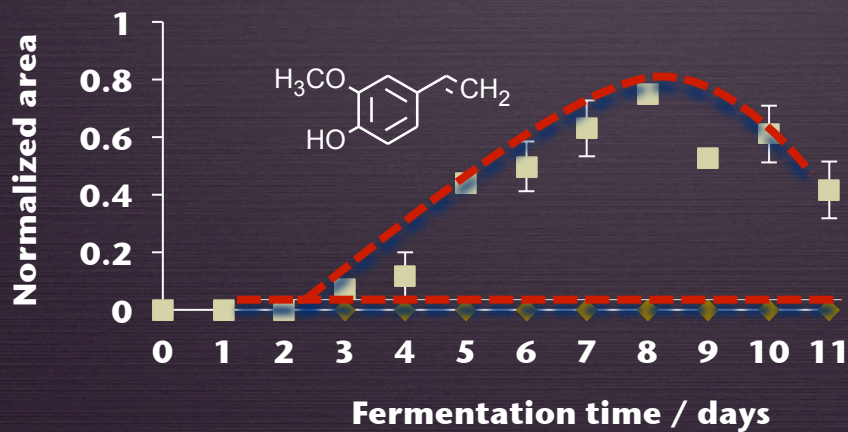
Varian Saturn 2000
ion-trap GC-MS



“Target Approach”...

Control
Supplemented media

(p-vinylguaiacol)



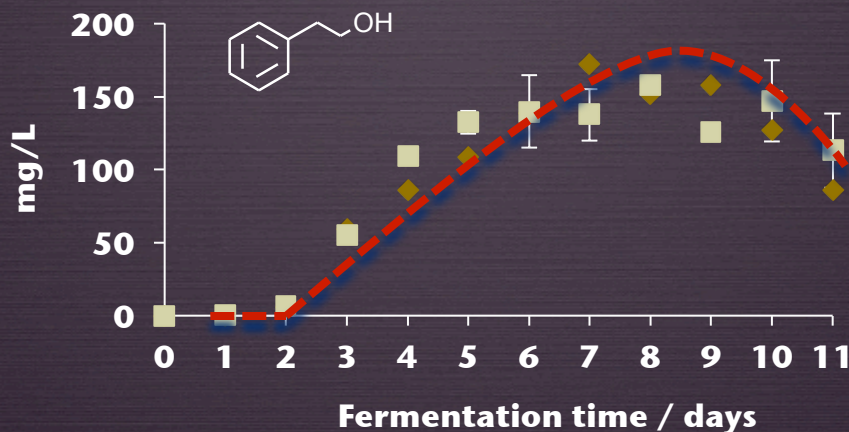
Product of ferulic acid decarboxylation
(Shinohara et al. 2000)



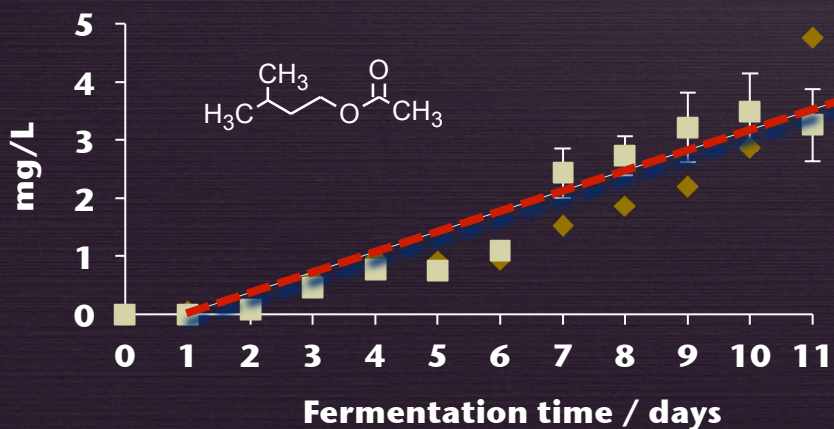
“Target Approach”...

2-Phenylethanol

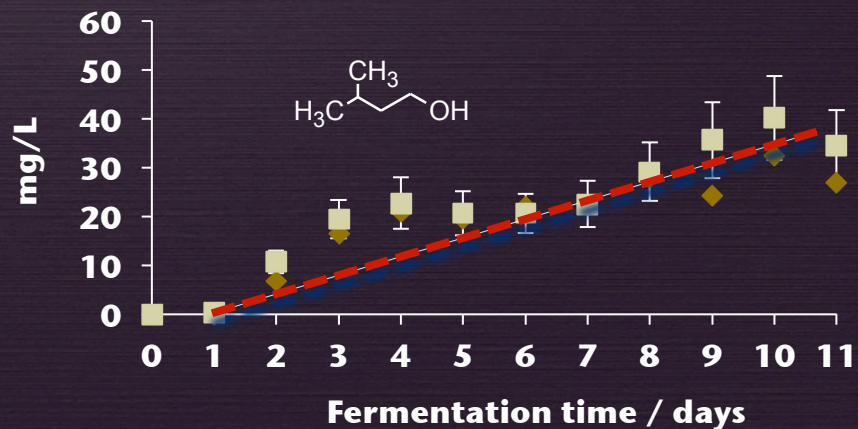
Control
Supplemented media



(Isoamyl acetate)

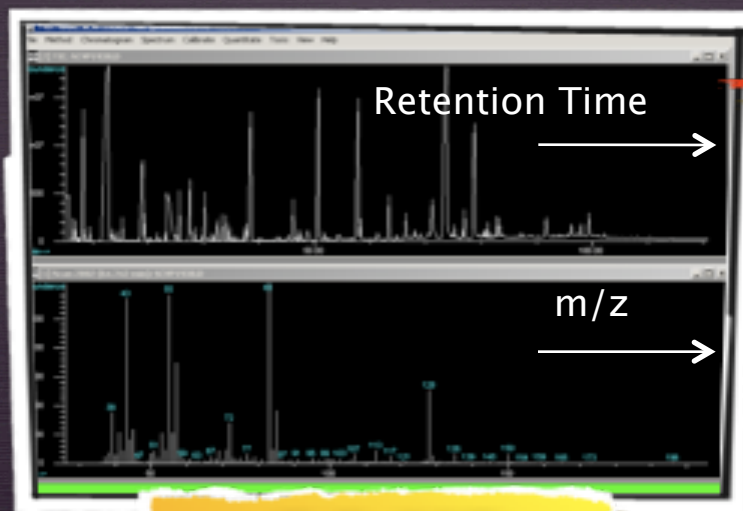


(Isoamyl alcohol)

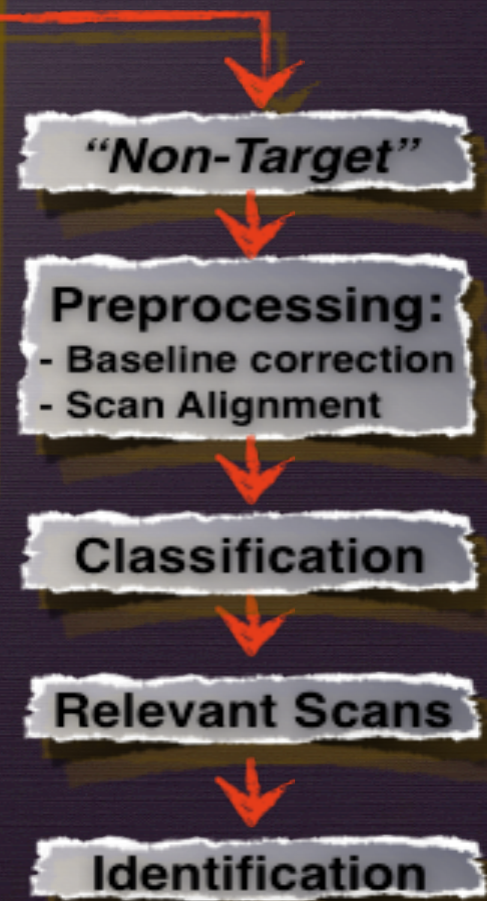




“Target *versus* Non-Target Approach”...



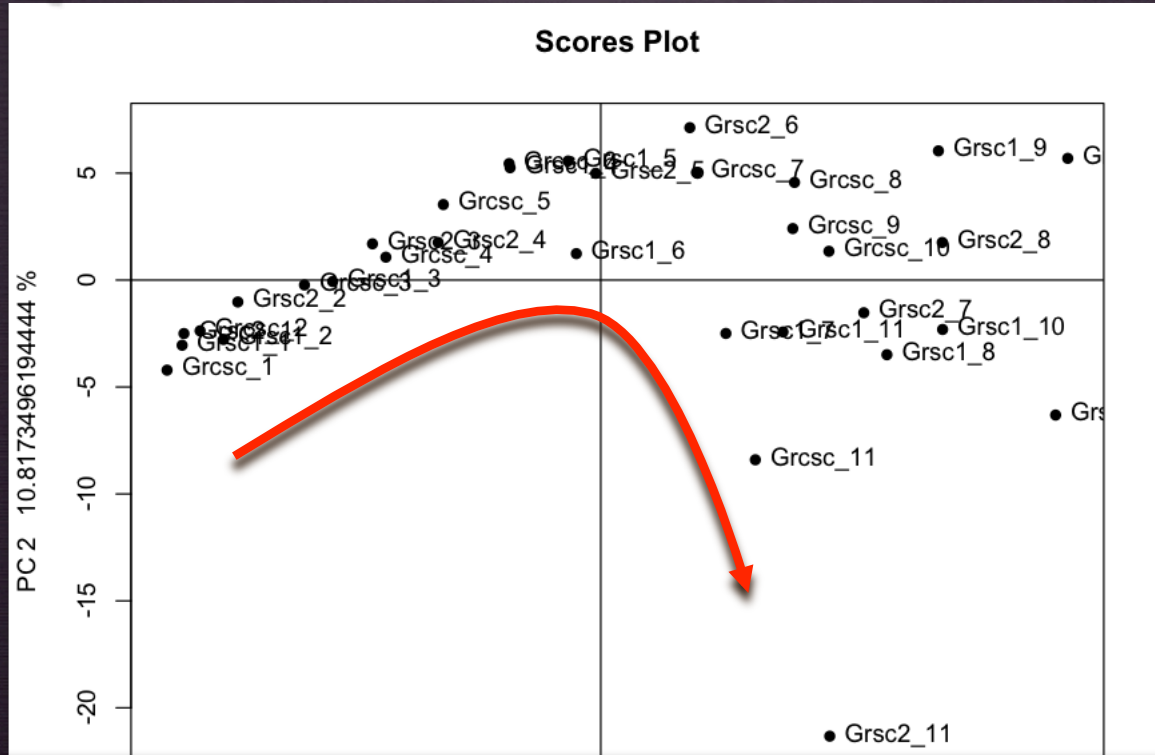
**GC-MS
data**





Classification

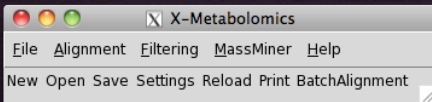
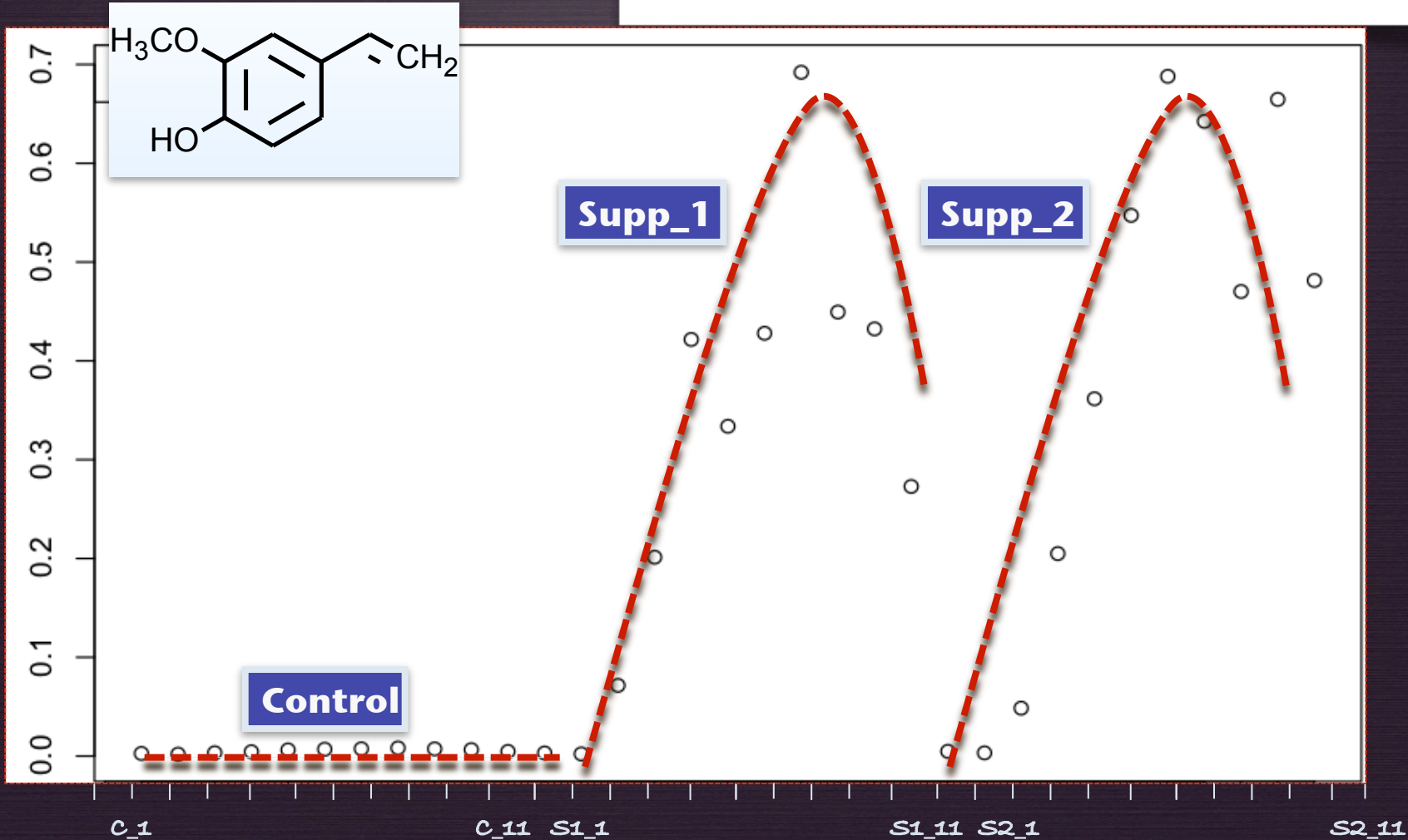
Mass Spectroscopy Signal Processing



PCA shows differences from day to day, imposes a pattern on the fermentation



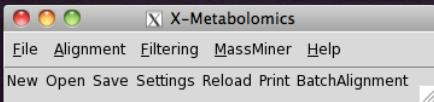
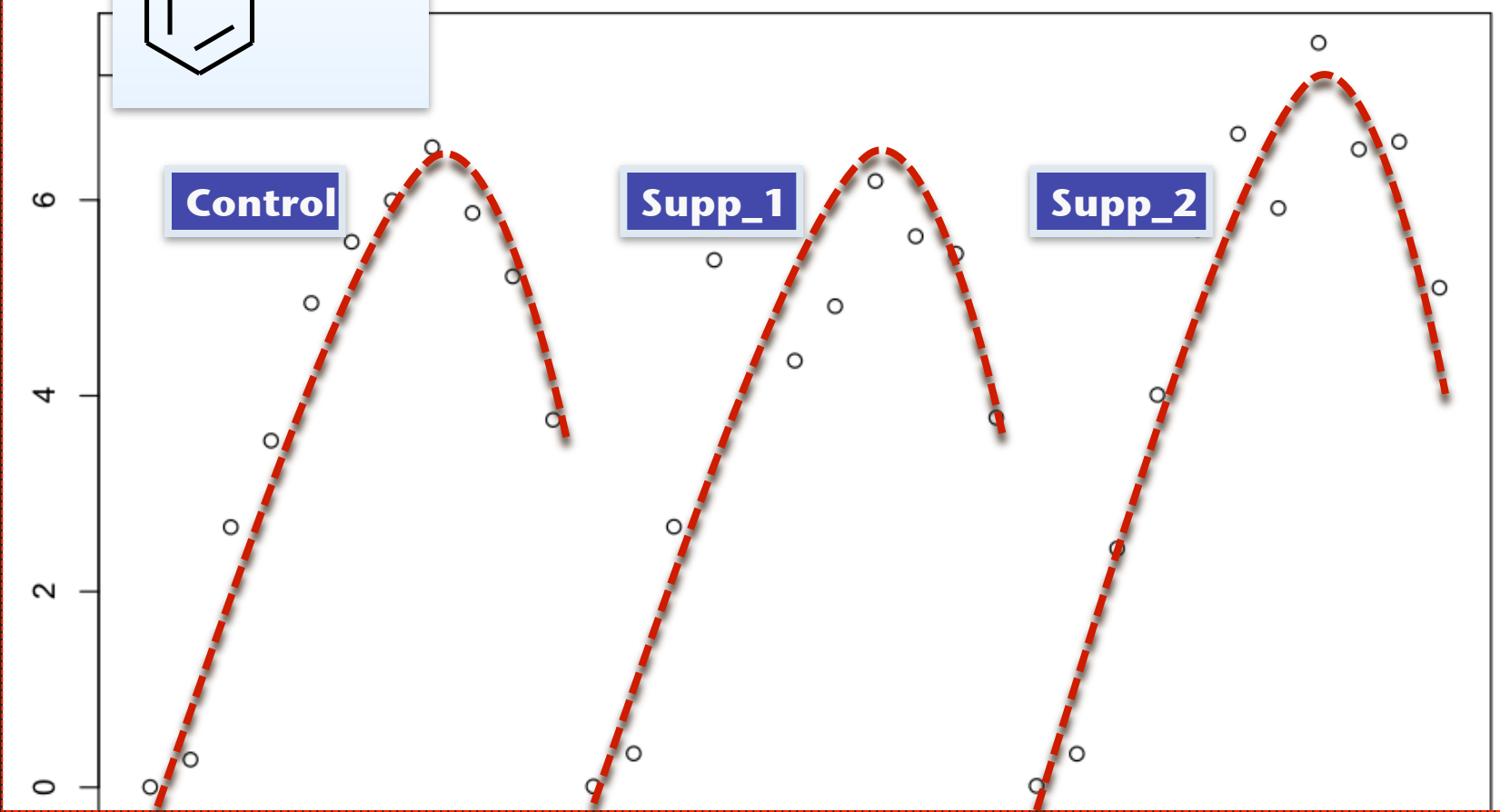
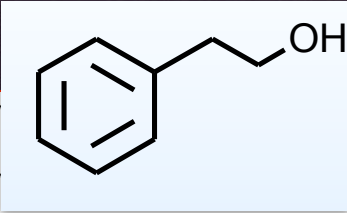
Non Target : Mass Expression



C: Control; S1:Supplemented1; S2:Supplemented2



Non Target : Mass Expression

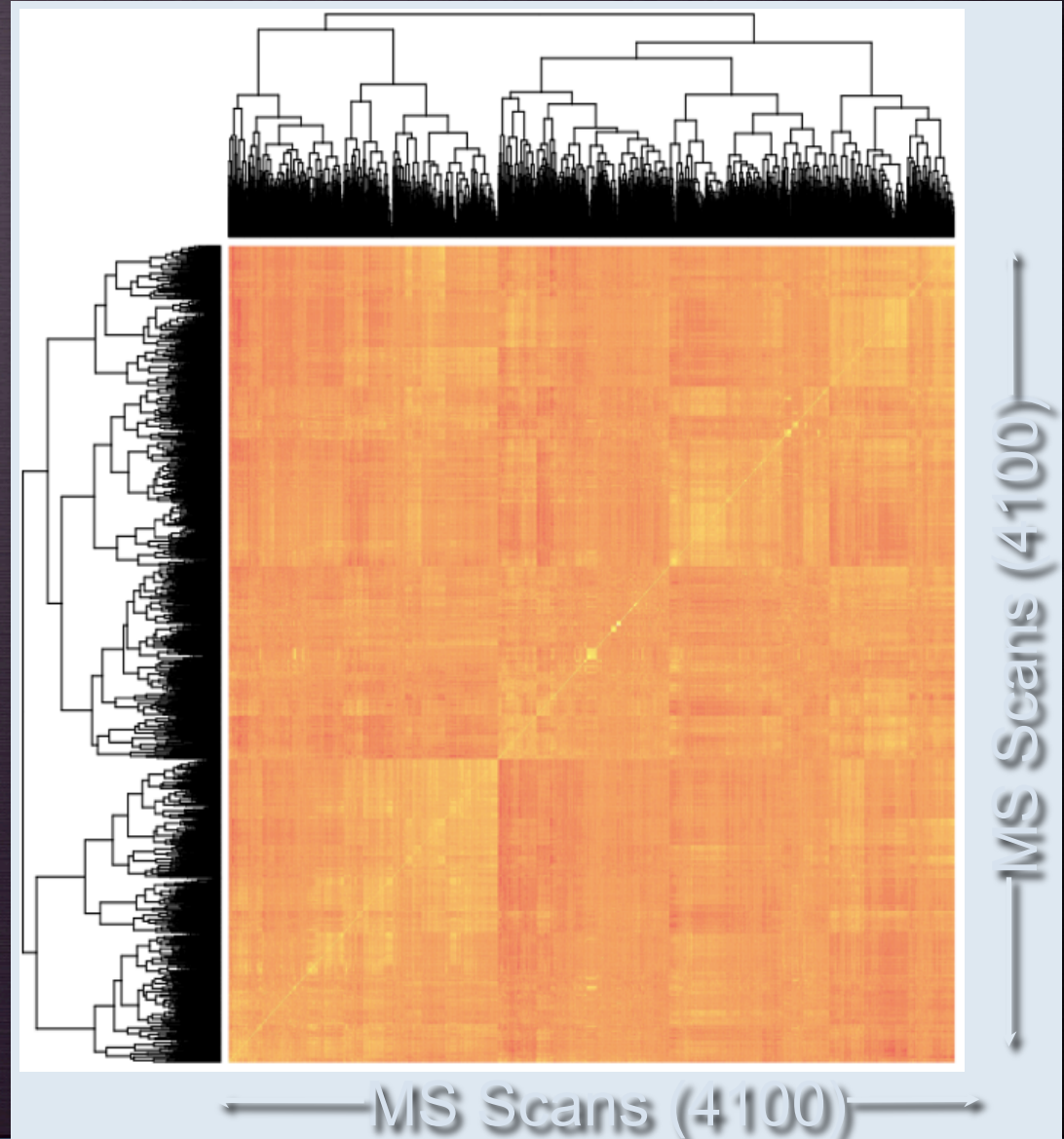
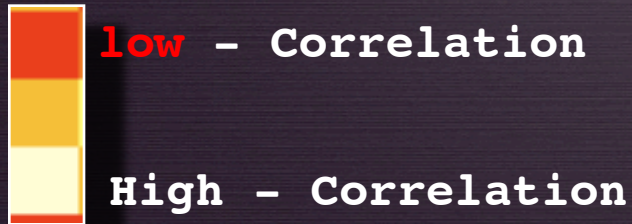


C: Control; S1:Supplemented1; S2:Supplemented2



Non-Target Approach : “Clustering” all MS Scans

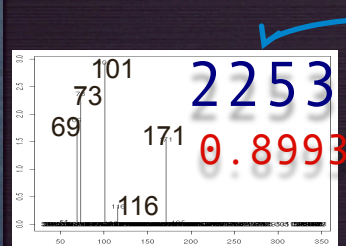
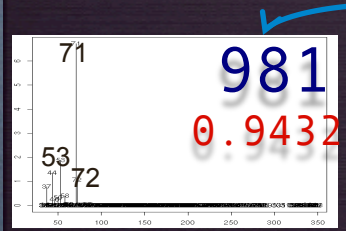
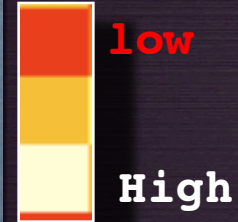
“Pathway Reconstruction”



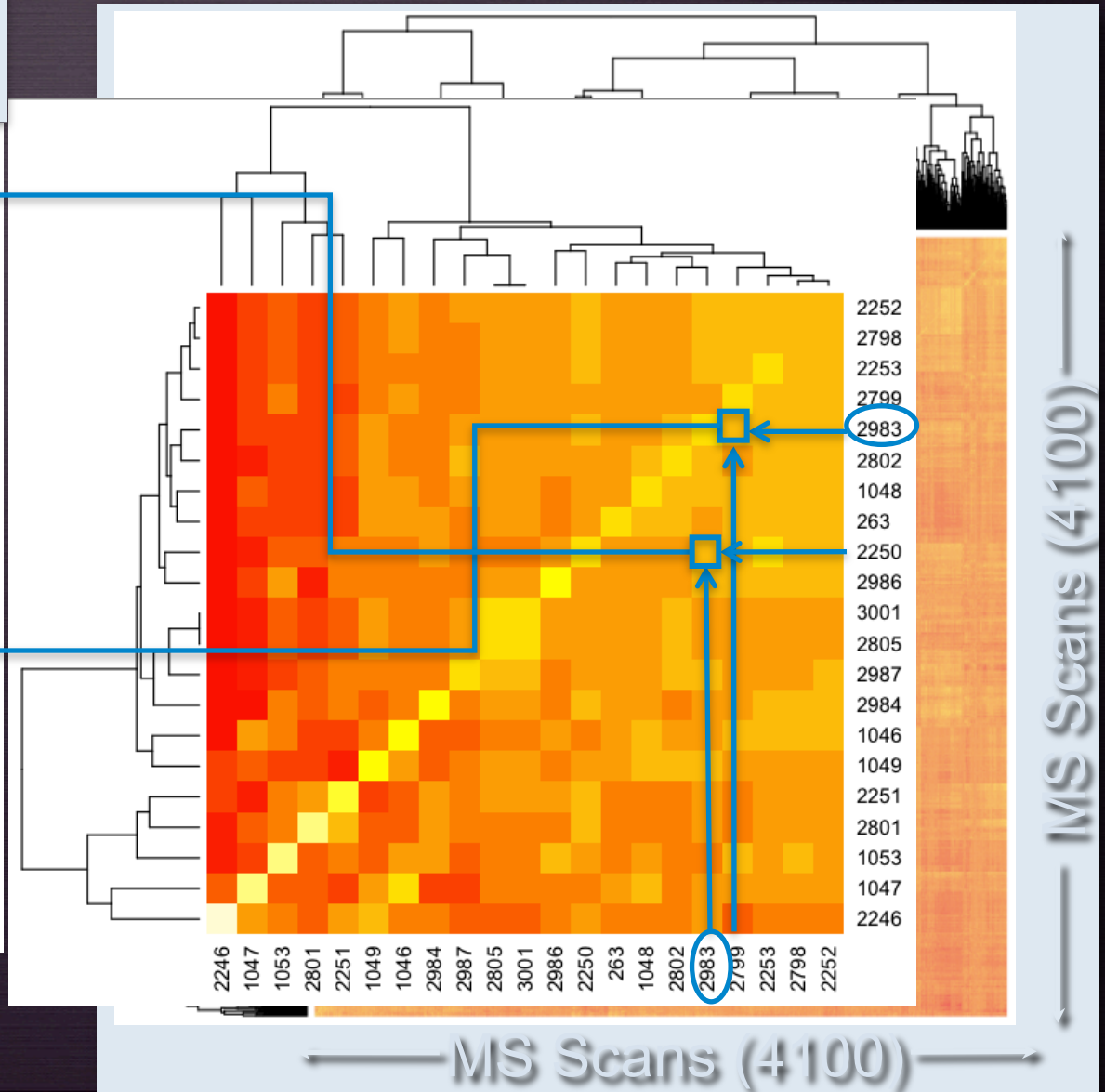


Non-Target Approach : "Clustering" all MS Scans

2-Phenylethanol



Scan	Corr	Ident
2250	0.8514	C10C2
1042	0.7088	C6C2
981	0.9432	
976	0.9394	
1712	0.7014	Acetic acid
975	0.9356	
2220	0.9049	
634	0.9043	
2253	0.8993	
2414	0.8280	
2799	0.8279	C6
1048	0.7968	
3319	0.7963	C8
1592	0.7551	
3792	0.7549	C10
3351	0.7253	
1657	0.7252	C8C2



"Pathway Reconstruction"



Conclusion

- Changes in the overall resistance to oxidation does indeed occur during alcoholic fermentation and shows a pattern like that of normal wines
- Chorogenic acid was found increasing in both control and supplemented media, suggesting that *Saccharomyces cerevisiae* has the ability to produce antioxidants 'de novo' from simple precursors such as sugars and amino acids
- A new biological interpretation can be achieved by the non-target approach that consists of a list of all compounds and their correlations
- The non-target approach could provide new knowledge for the "Pathway Reconstruction"



Thank You