ISTITUTO AGRARIO DI SAN MICHELE ALL'ADIGE
Fondazione Edmund Mach

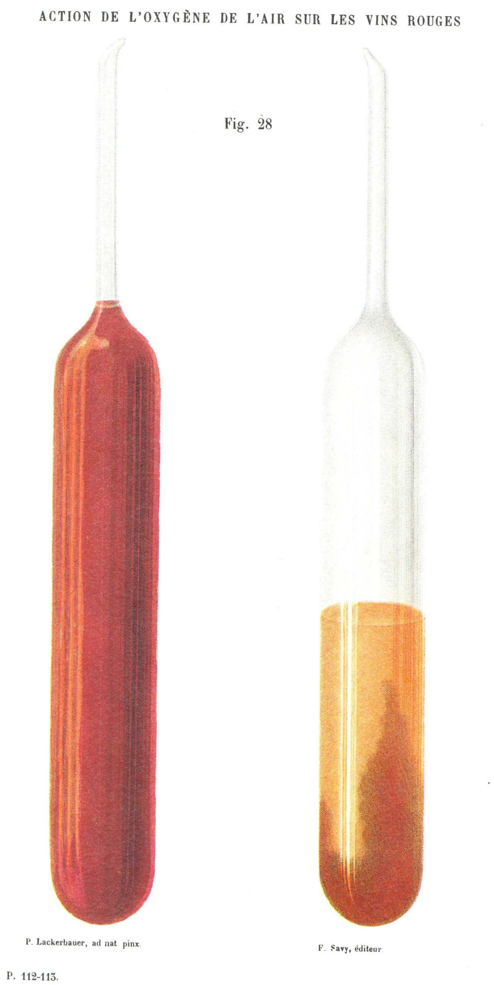
Research and Innovation Centre

Food Quality and Nutrition area

A METABOLOMIC VIEW OF WINE MICRO-OXYGENATION

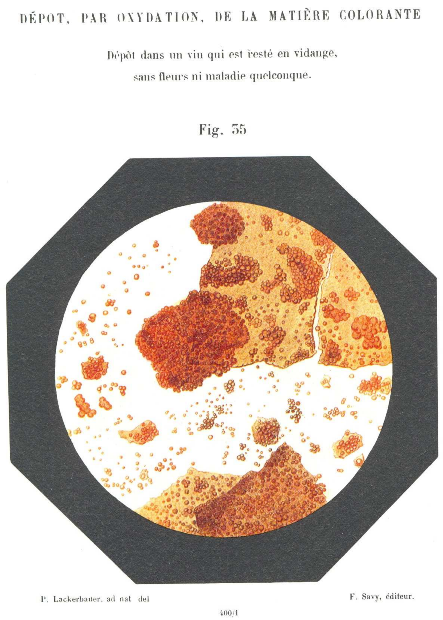
Fulvio Mattivi

Effect of different amounts of oxygen and of light on the pigments of red wines
from Louis Pasteur, Etudes sur le vin, 1873



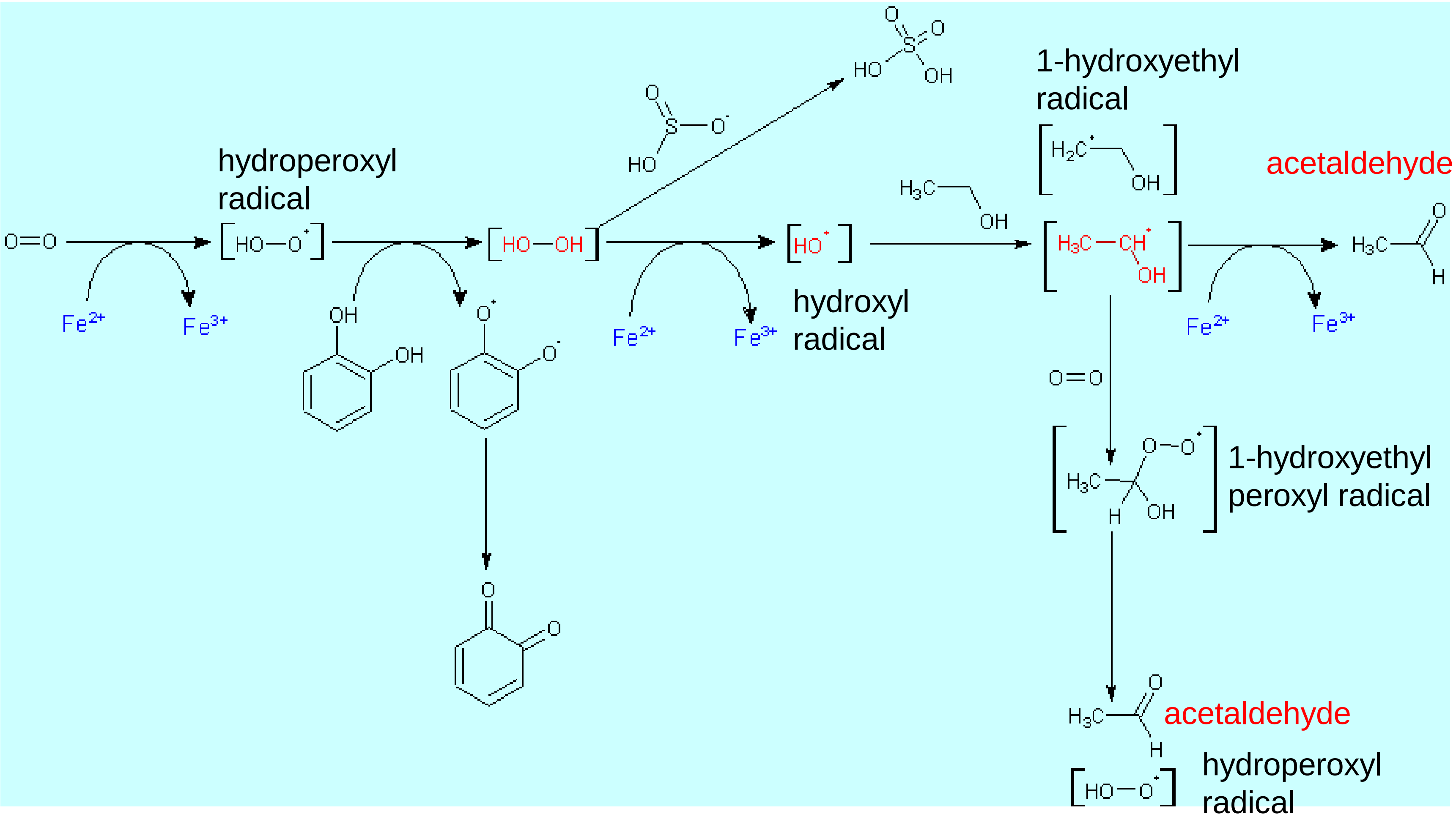
Without vs. with oxygen
in the headspace

Without vs. with sunlight



Oxygen-driven precipitation of
red pigments in a red wine

Scheme of non-enzymatic, metal-catalyzed wine oxydation mechanism
redrawn from Elias RJ et al., JAFC 2009



Aim. **We want to achieve a more global, holistic view of the wine micro-oxygenation within a single “data-driven” experiment**

features of the experiments:

i) **dimension of the trial, duration of the treatment, and range of the factors explored: representative of a “real” Sangiovese wine (2009);**

ii) **strictly controlled experiment (both chemical and sensory parameters);**

iii) **experimental design, adequate number of replicates, randomized;**

iv) **“global” chemical effect investigated via MS-based metabolomic measures (Q-TOF and FTMS)**

v) **Discussion of some preliminary results on micro-oxygenation before MLF (phase I)**

vi) **data analysis and validation of the biomarkers still in process; wine micro-oxygenation after MLF (phase II) now in course**

Where

Tuscania Winery, Tavarnelle Val di Pesa (Firenze, Italy)

How (phase I experiment)

- 24 stainless-steel tanks: 8 trials, in triplicate
- 7 weeks of controlled micro-oxygenation during the early phases of winemaking, just after the alcoholic fermentation and prior to the malo-lactic fermentation
- all conditions strictly standardized and monitored

Factors:

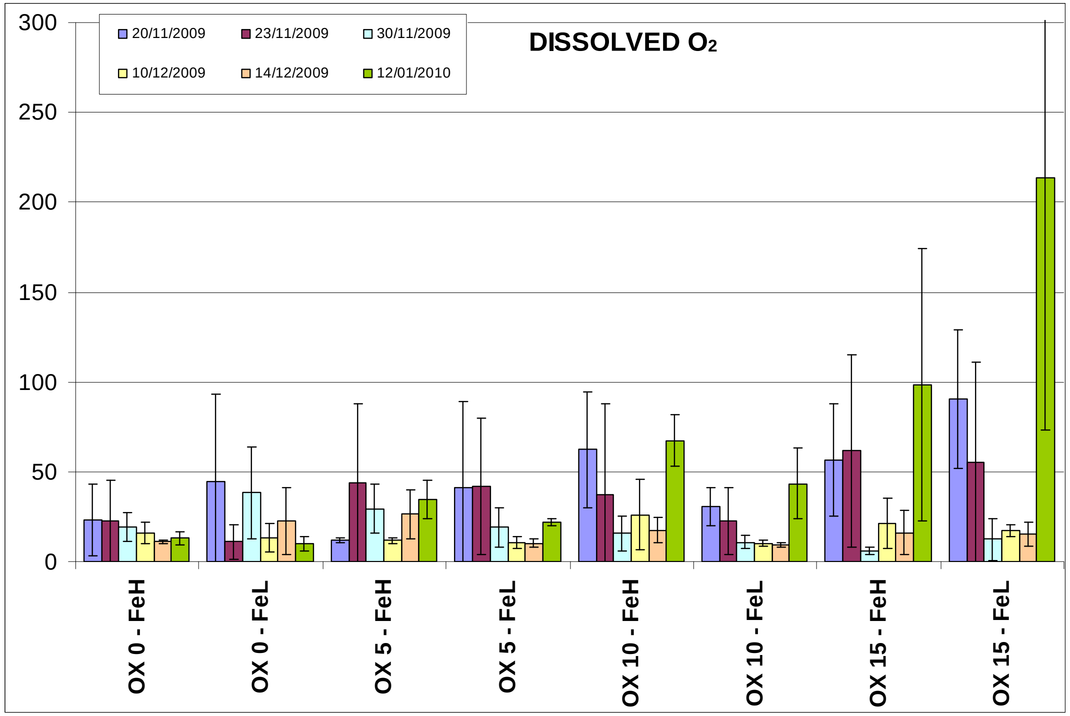
i) amount of oxygen (four levels, 0-5-10-15 mg per liter per month)

ii) iron concentration (two levels, 1.5 and 2.0 mg/l)

					ox9	ox 8	ox7	ox6	ox5	ox4	ox3		ox2	ox1	ox19
	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Ox	no	no	no	0	10	15	15	5	5	10	10	0	5	5	15
Fe	no	no	no	L	L	L	H	H	L	H	H	H	L	H	L
R	no	no	no	1	1	3	2	1	3	3	2	3	1	2	1

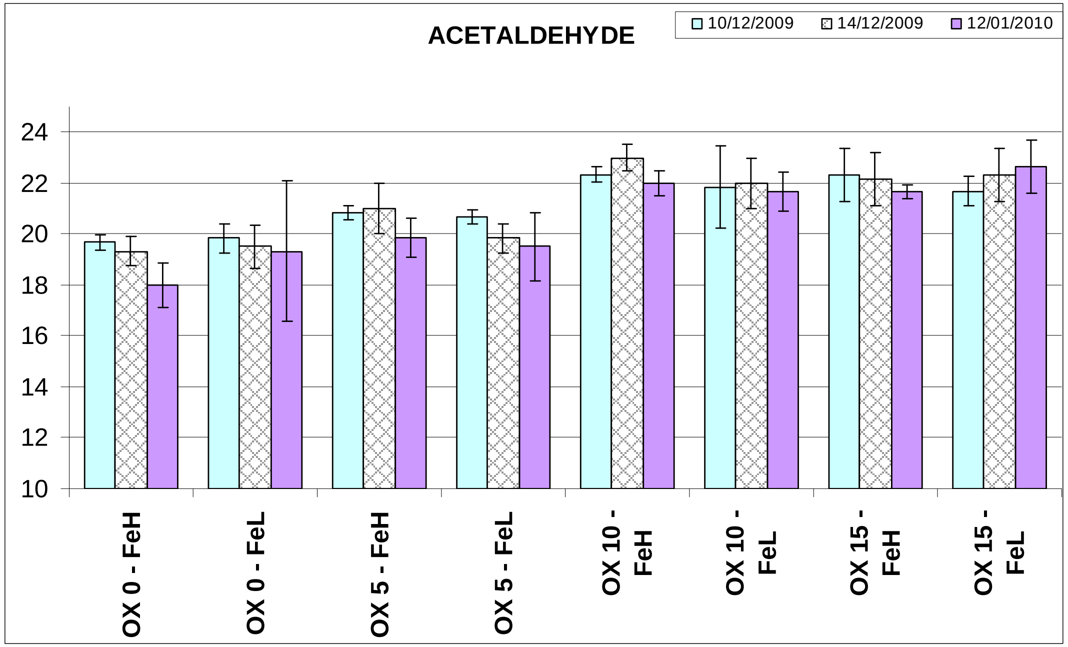
	ox18					ox17	ox16	ox15	ox14	ox13		ox12	ox11	ox20	ox10
	34	33	32	31	30	29	28	27	26	25	24	23	22	21	20
						GR1		GR2		GR3				GR4	
Ox	15	no	0	0	0	10	5	10	15	10	0	15	5	15	10
Fe	L	no	L	H	H	L	H	H	L	H	L	H	L	H	L
R	OUT	no	3	2	1	3	3	1	2	OUT	2	1	2	3	2

Phase I experiment



✓ Average level of oxygen always below 100 ppb in 7 out of 8 trials

✓ measured via oxo-luminescence with PreSens (Nomacorc)

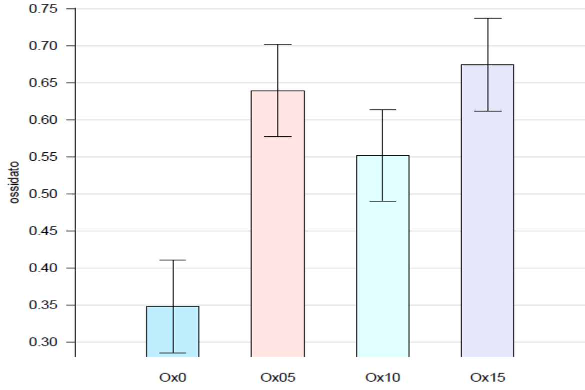


✓ Small but significant increase of concentration of acetaldehyde with the highest amounts of oxygen (10-15 mg).

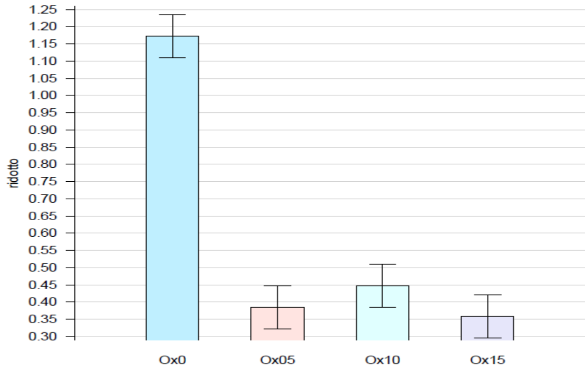
✓ acetaldehyde decreases over time in absence of oxygen

✓measured via GC

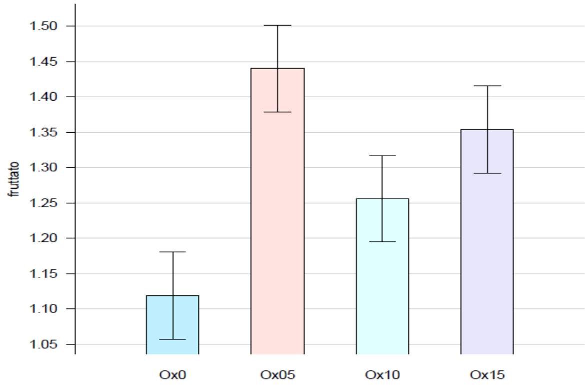
Effect of the amount of oxygen on the flavour of wines
Sensory analysis with a panel of 7 well trained experts repeated after 15, 30, 60 days of treatment



“oxidized” (acetaldehyde, over-ripe apple)



“reduced” (H_2S)

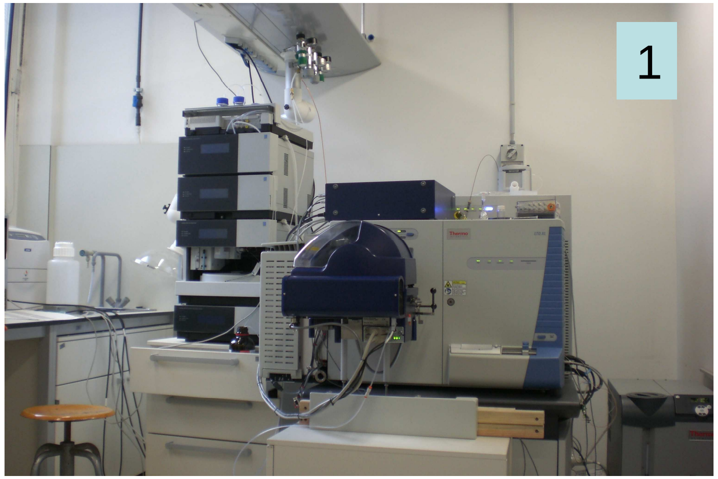


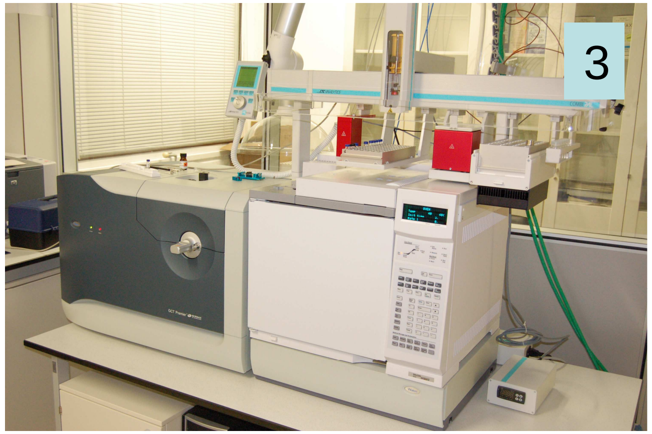
“fruity”

- ✓ amount of oxygen always significant (histograms)
- ✓ metal content significant for “oxidized”
- ✓ time x metal significant for “fruity”
- ✓ time x oxygen significant for “reduced”

Where

Edmund Mach Foundation, FEM Metabolite profiling platform, (San Michele all'Adige, Italy)
http://www.iasma.it/RIC_Metabolic_Profiling

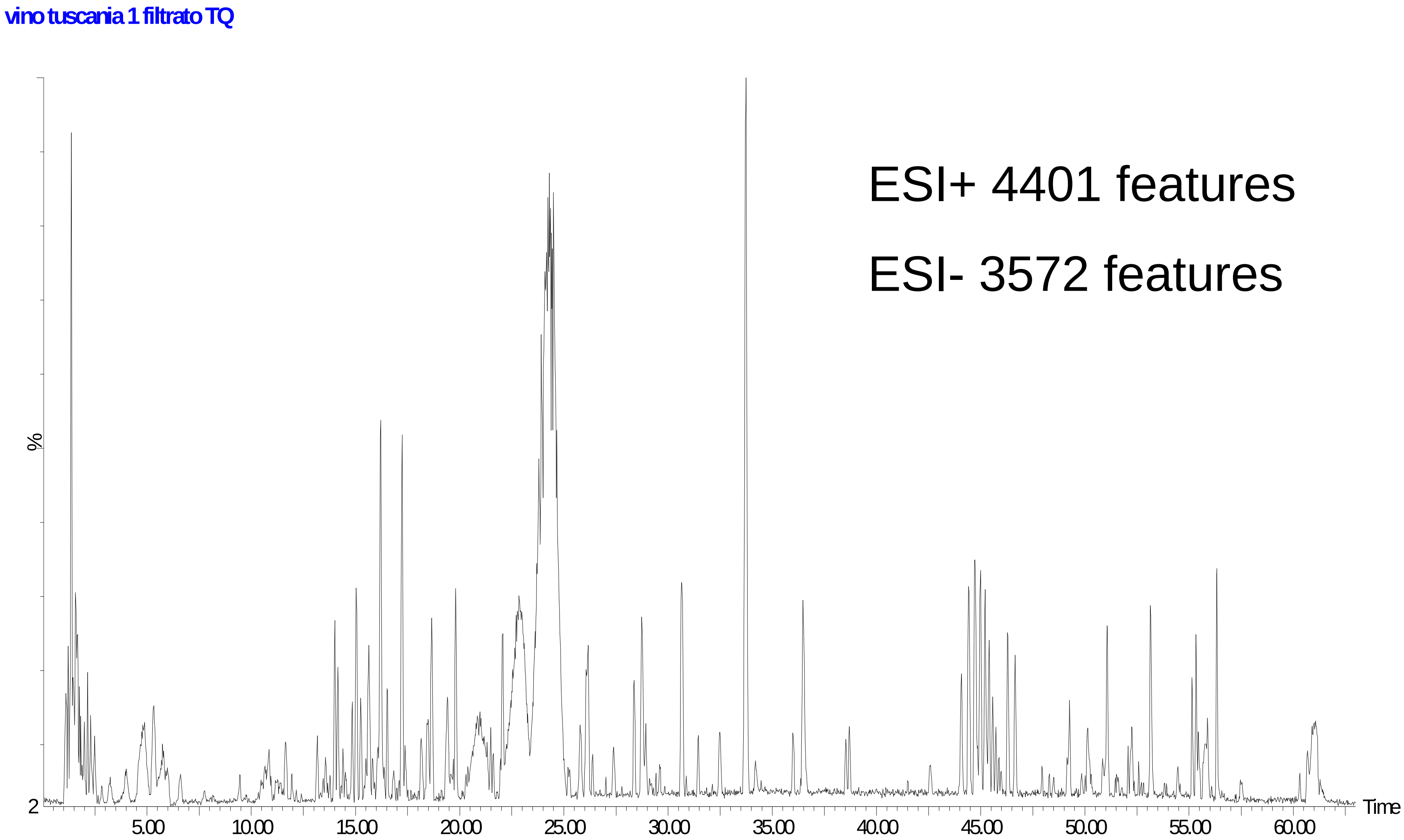




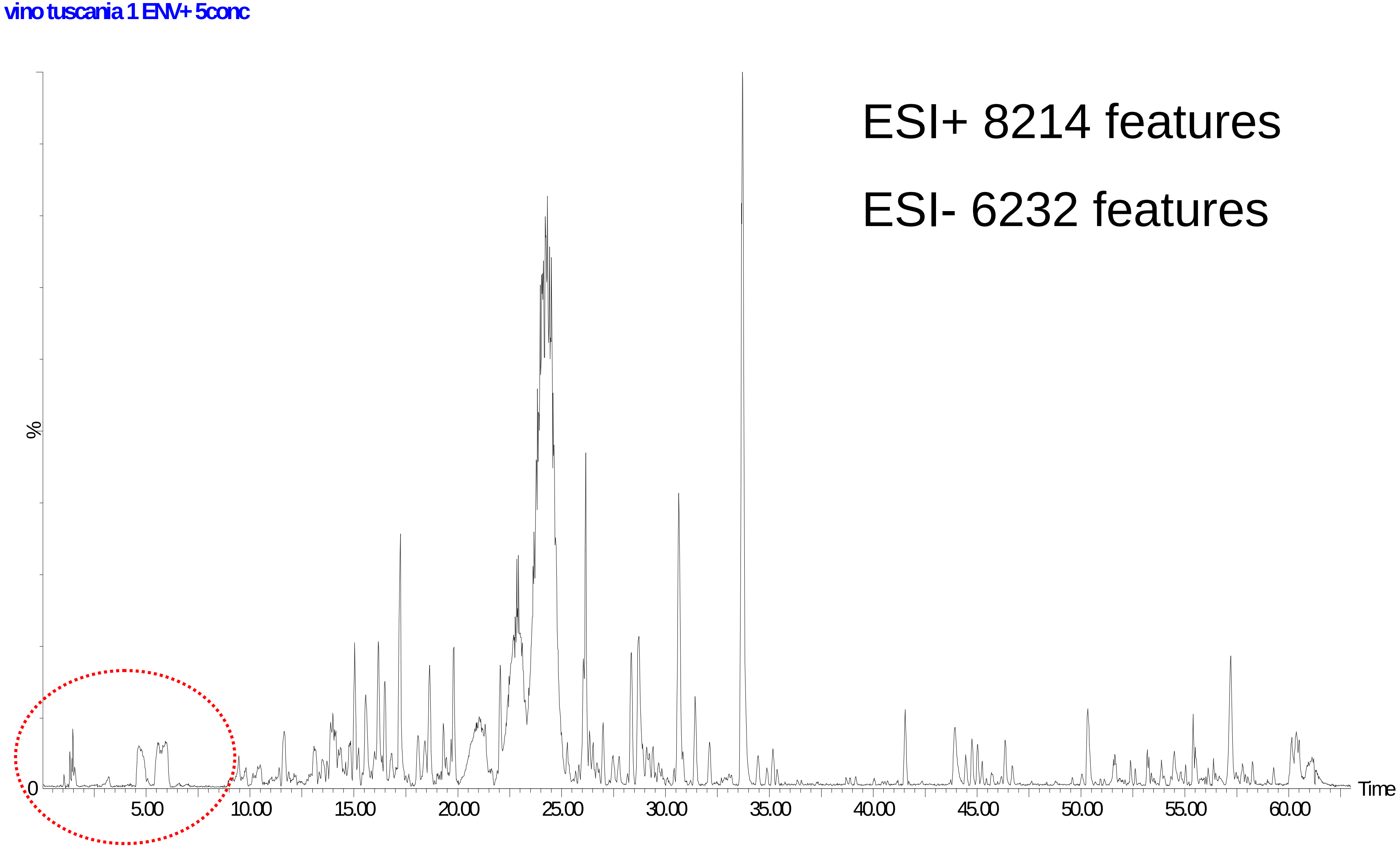
1. Nano HPLC (Dionex)- Orbitrap FTMS (Thermoscientific)
2. Acquity UPLC -Synapt Q-TOF HDMS (Waters)
3. GC-TOF (Waters)
4. Acquity UPLC-MS/MS Xevo (Waters)
5. GC-MS/MS (Thermoscientific)

reversed phase (C18) UPLC-Q-TOF of a Sangiovese wine (without sample preparation)

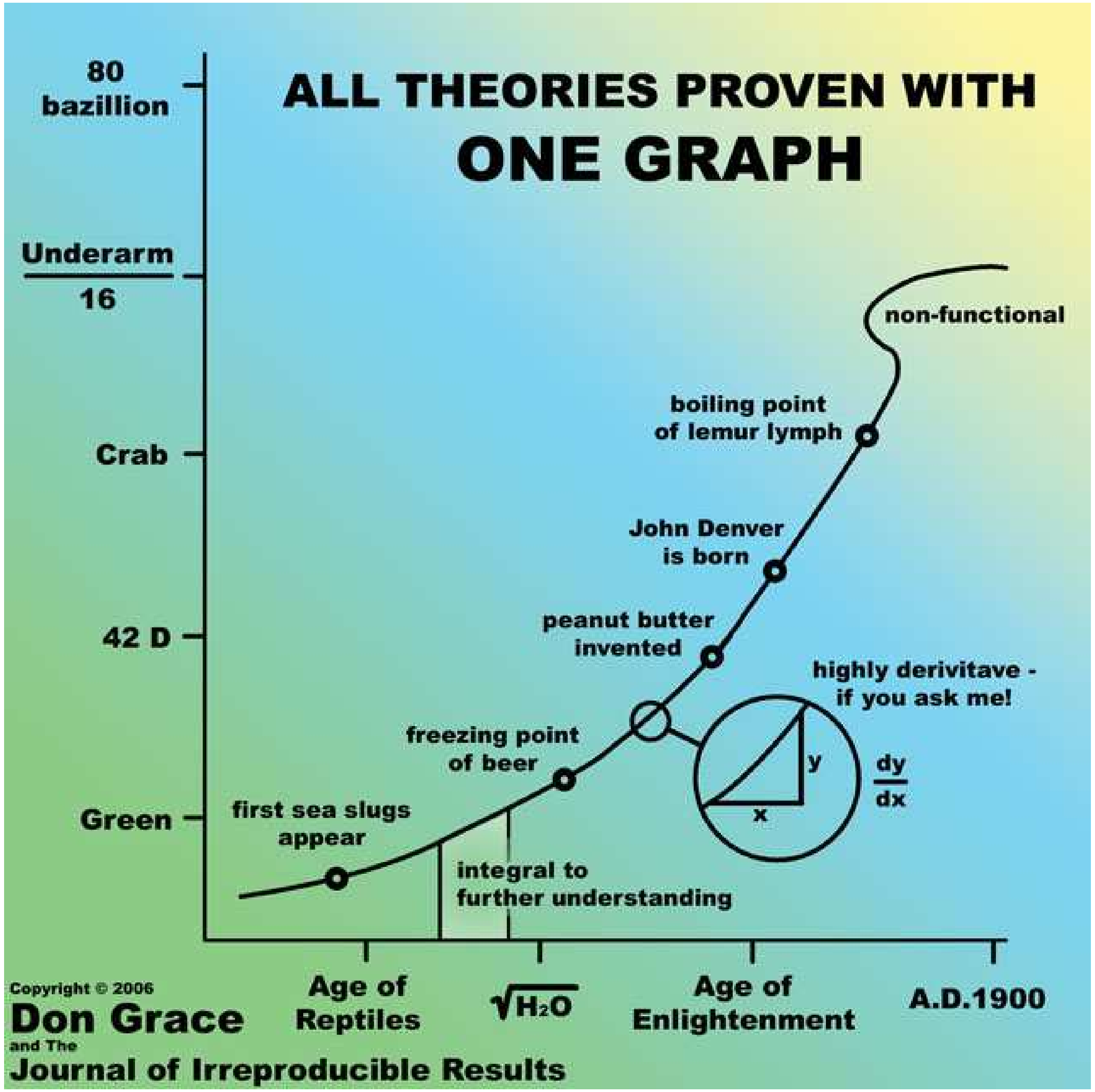
colum HSST3, 2.1x150 mm, wine transfer under inert atmosphere



reversed phase (C18) UPLC-Q-TOF of a Sangiovese wine (with sample preparation)
removal of the hydrophilic compounds by SPE, conc. x 5

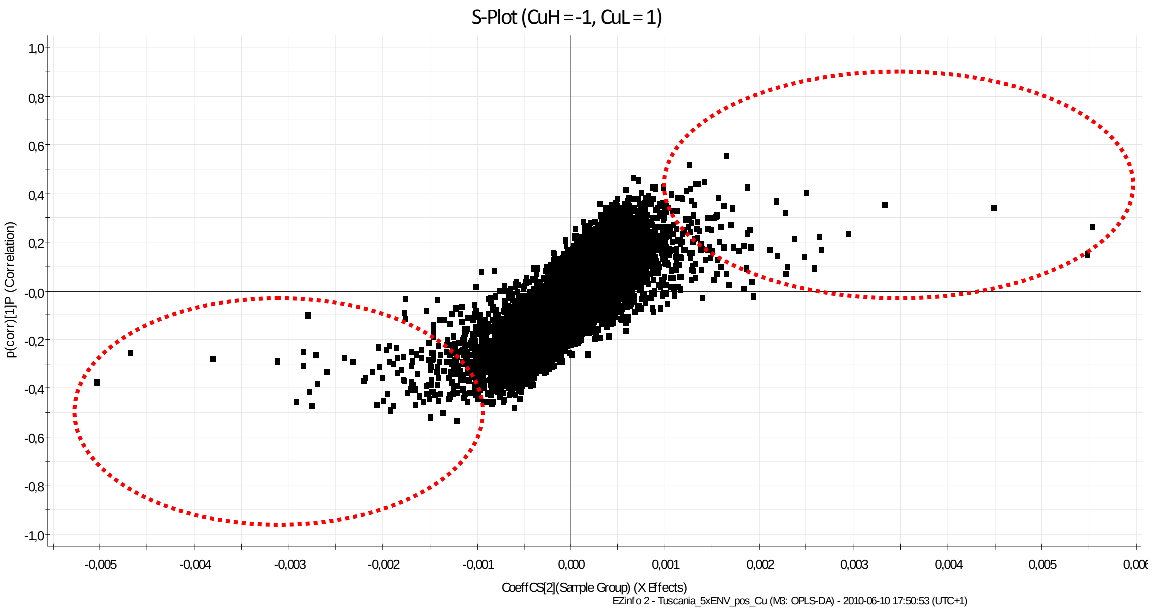


Large amount of data .... statistical workflow critical….

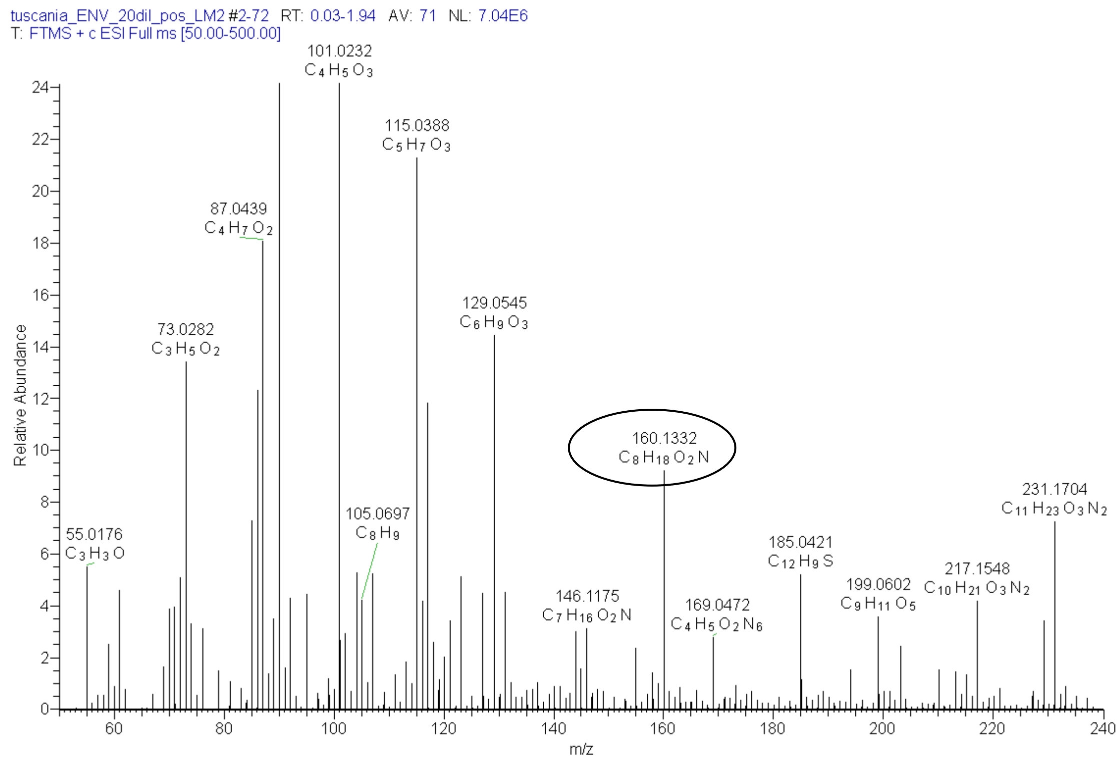


Data extraction

data alignment and processing with the s/w MarkerLynx XS (Waters): a preliminary list of the biomarkers of wine micro-oxygenation in presence of variable levels of iron and oxygen was extracted



samples were reanalyzed with Orbitrap to restrict the accuracy of m/z assignments (below ± 1 pmm) of biomarkers

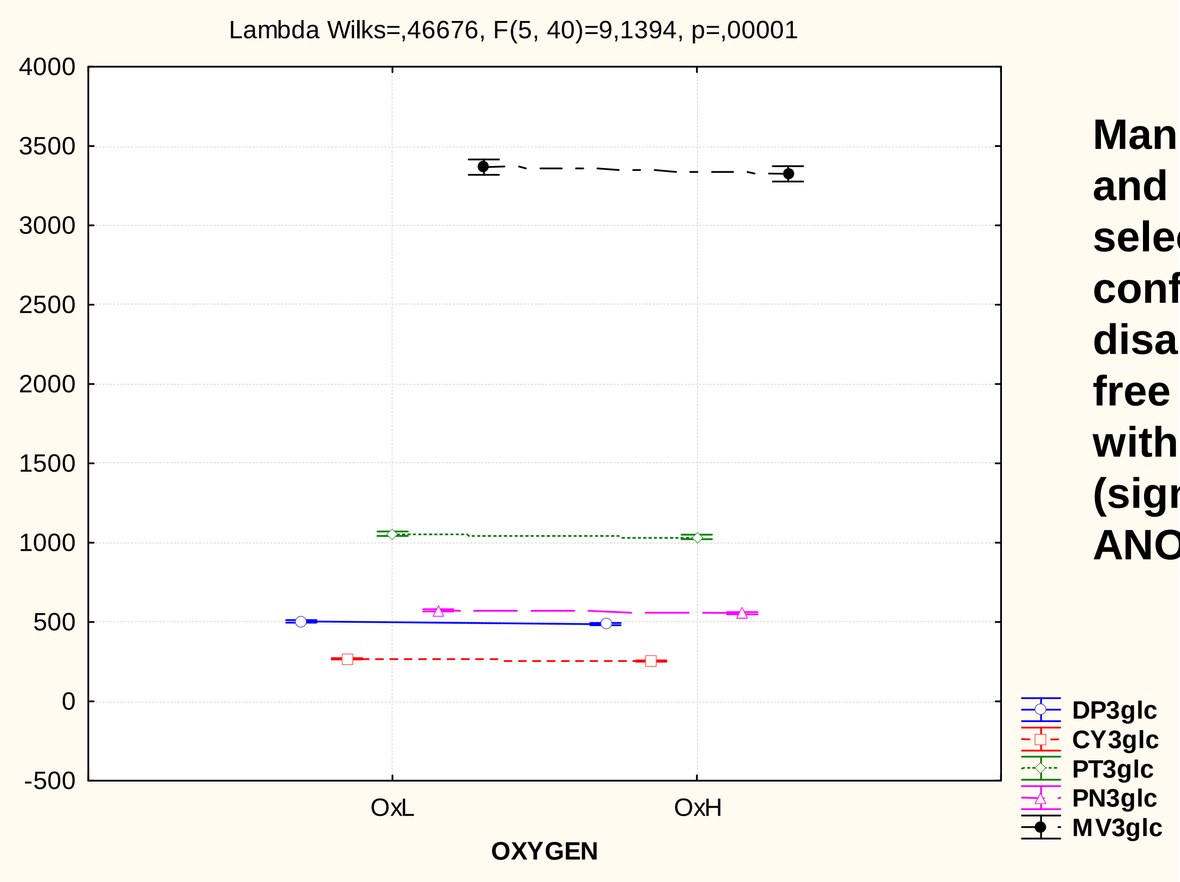


… check for possible isobaric structures (isotopic and fragmentation patterns, internal and external libraries) and confirmation of the identity of putative biomarkers of the micro-oxygenation

reversed phase UPLC-Q-TOF of a Sangiovese wine, without sample preparation, ESI+
the free anthocyanins ranks at the top of the list of biomarkers (ESI+, OXYGEN)

Test di Significatività Multivariati (area antociani liberi tuscania POS_IRON_OXYGEN_TQ.s
Parametrizzazione sigma-ristretta
Decomposizione ipotesi effettive

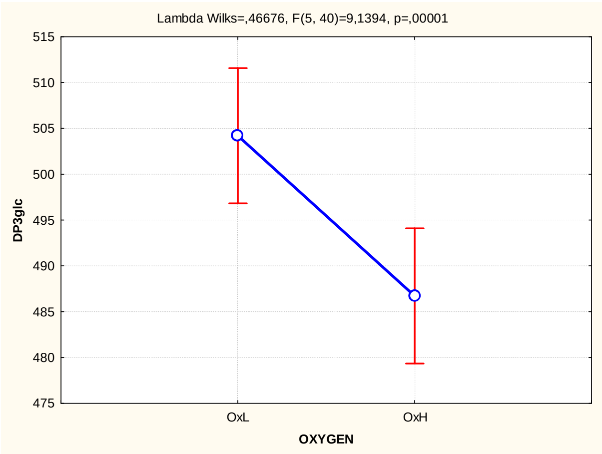
Effetto	Test	Valore	F	Effetto gl	Errore gl	p
Intercetta	Wilks	0,000899	8889,671	5	40	0,000000
IRON	Wilks	0,974030	0,213	5	40	0,954867
OXYGEN	Wilks	0,466761	9,139	5	40	0,000007
IRON*OXYGEN	Wilks	0,880382	1,087	5	40	0,382399

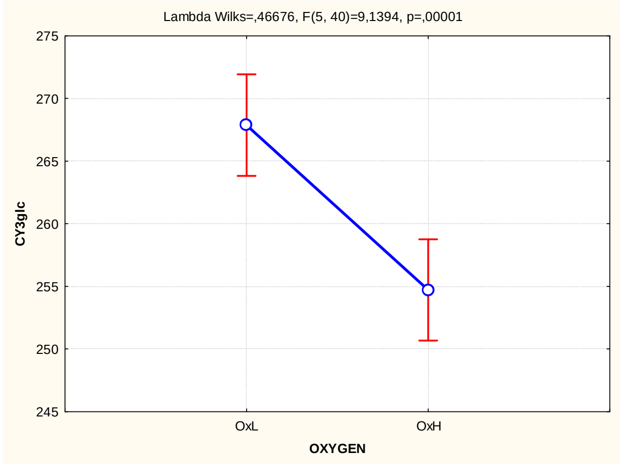


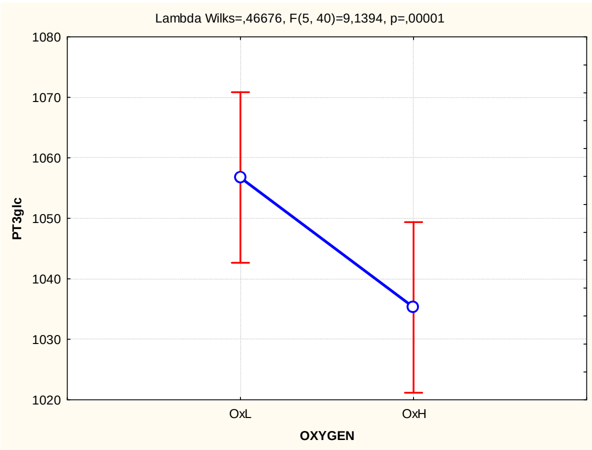
Manual extraction and integration of selected ions confirms the disappearance of free anthocyanins with OXYGEN (significant by ANOVA)

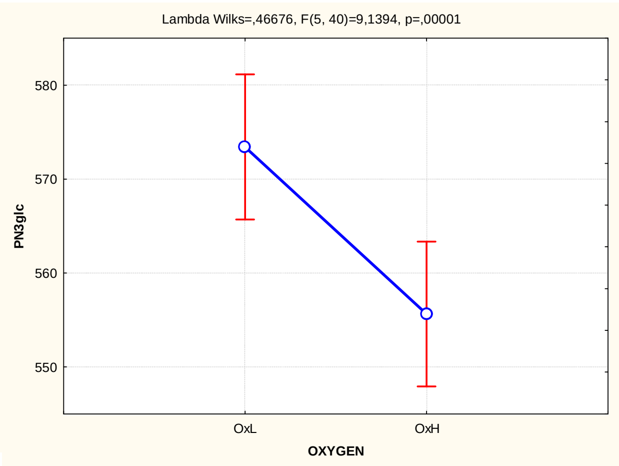
reversed phase UPLC-Q-TOF of a Sangiovese wine, without sample preparation, <u>ESI+</u>

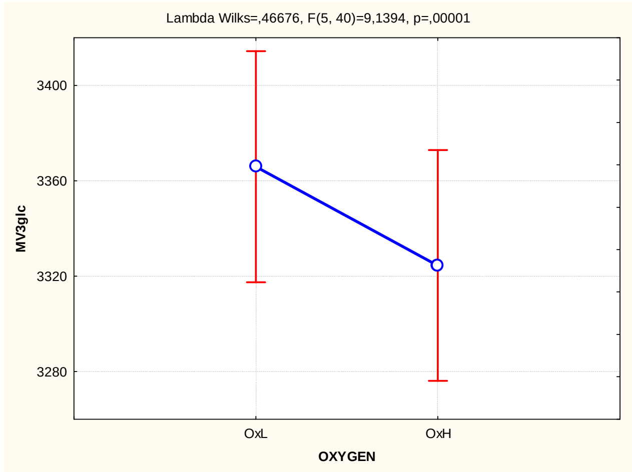
<u>details: also the univariate treatment is significant for 4 out of 5 anthocyanins</u>









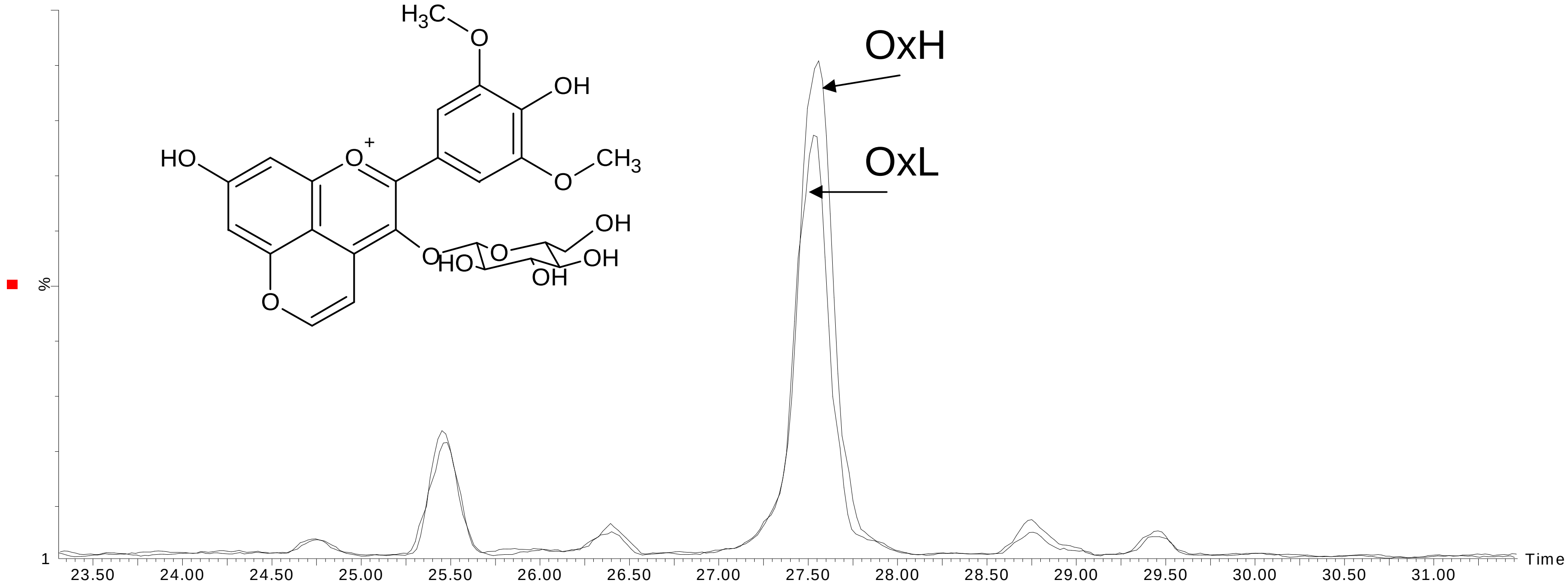


- ✓ **amount of oxygen significant (p=0,000007)**
- ✓ **iron content not significant**
- ✓ **oxygen x iron significant for “malvidin 3-glucoside” (p=0,038)**

Pyranoanthocyanins, from the reaction of ACs with acetaldehyde:

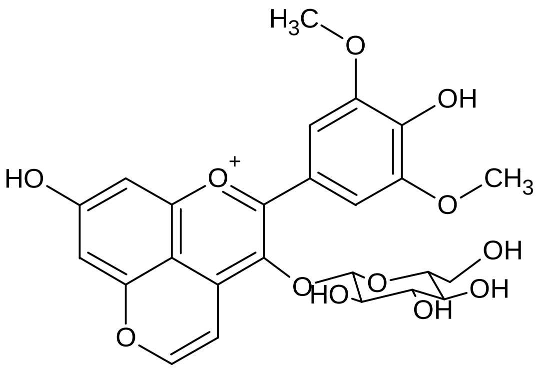
vitisin B (mv-3-glc acetaldehyde)

m/z+ 517.1336 RT 27.5
Variable Averages

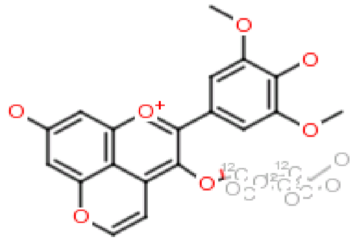


vitisin B (mv-3-glc acetaldeide)

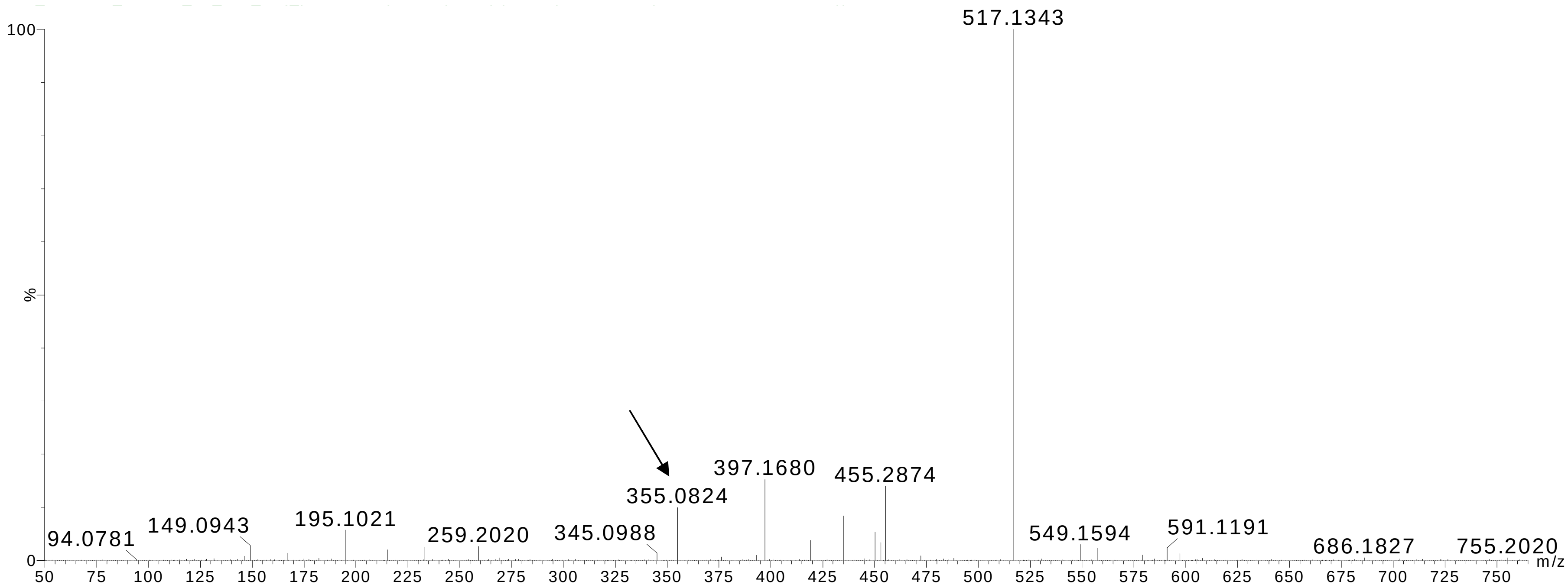
517.1340



355.0824



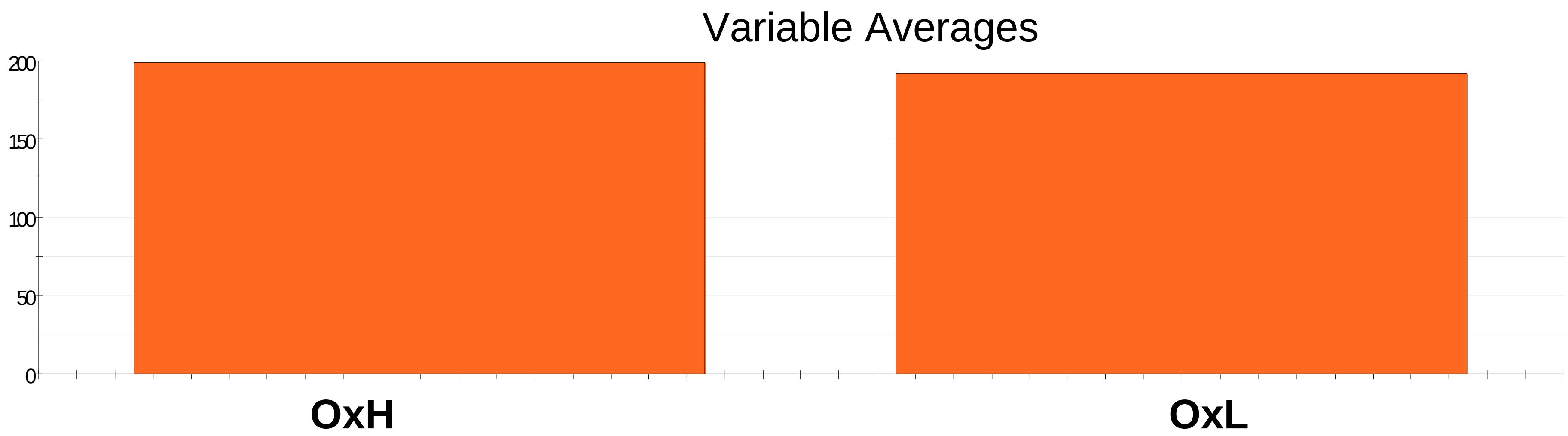
355.0818 (+0.6.mDa)
$C_{19}H_{15}O_7$ (-C[12C]$_5H_{11}O_5$)

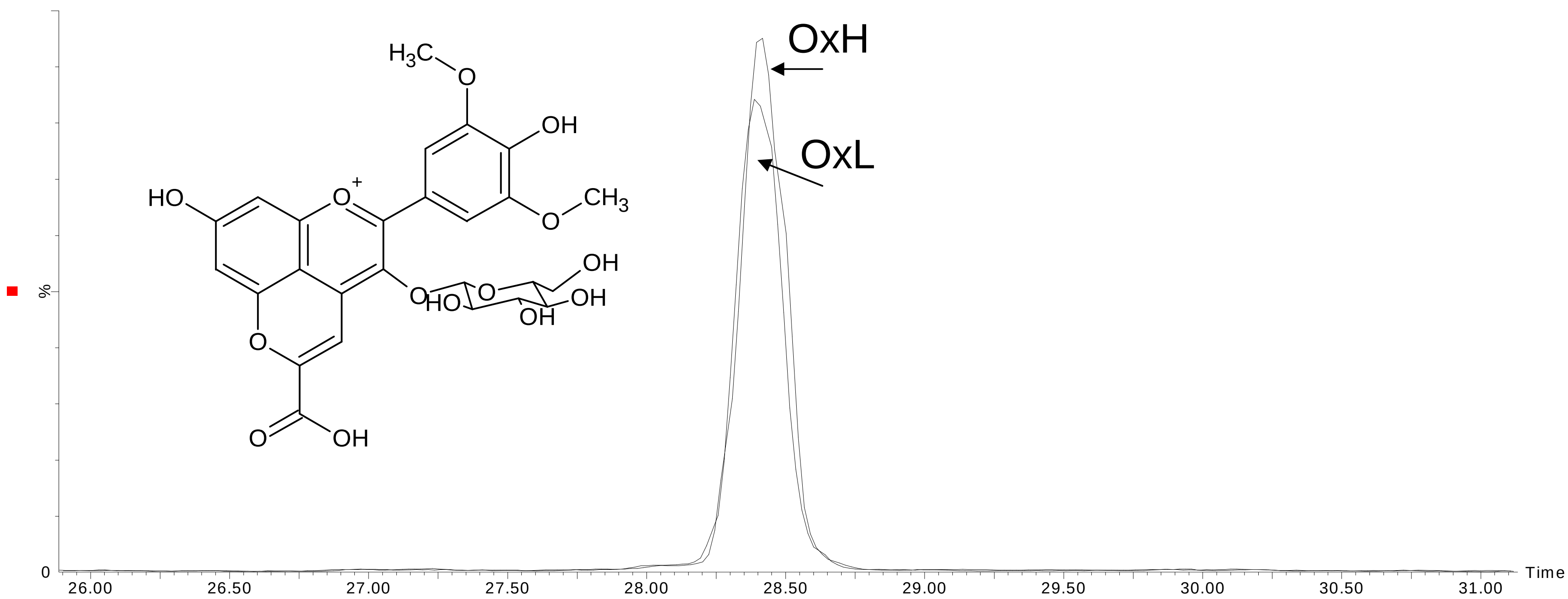


Carboxypyranoanthocyanins, from the reaction of ACs with pyruvic acid

vitisin A (mv-3-glc pyruvic acid)

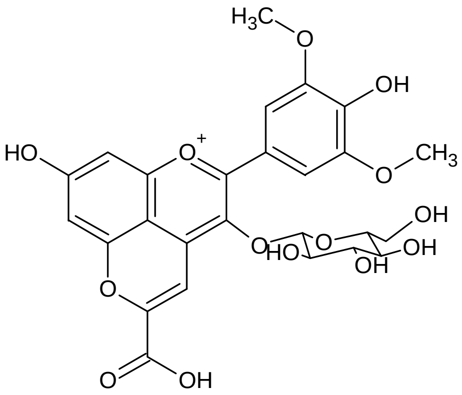
m/z+ 561.1242 RT 28.4



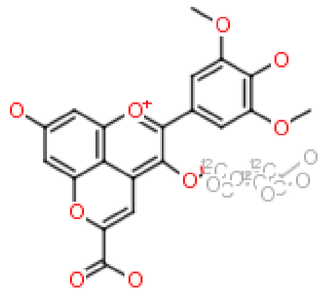


vitisin A (mv-3-glc pyruvic acid)

561.1239

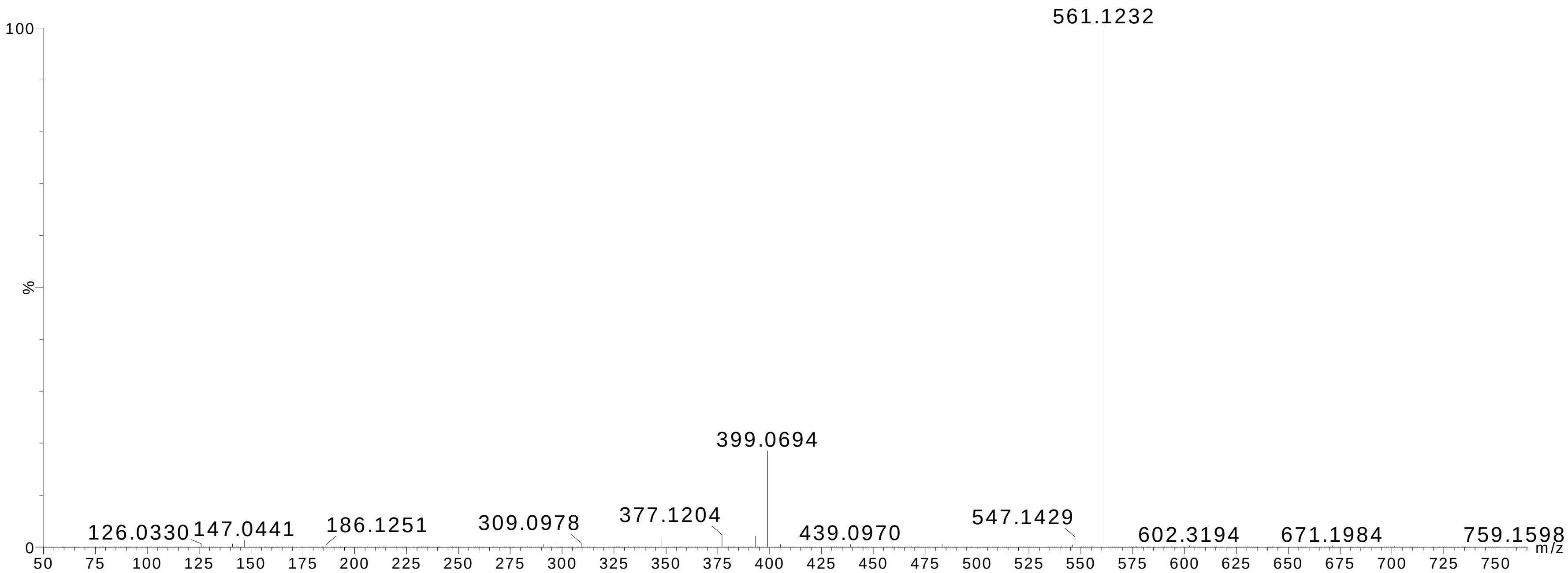


399.0694



399.0716 (-2.2.mDa)

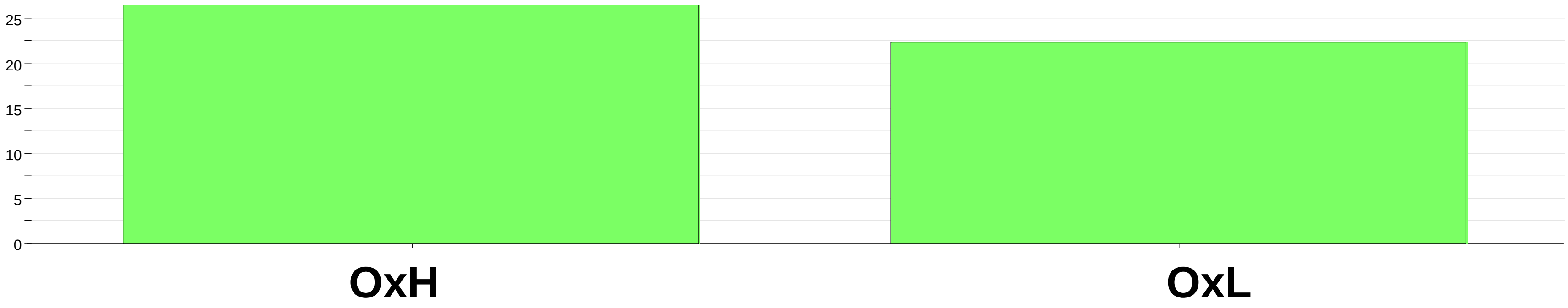
$C_{20}H_{15}O_9$ (-C[12C]$_5H_{11}O_5$)

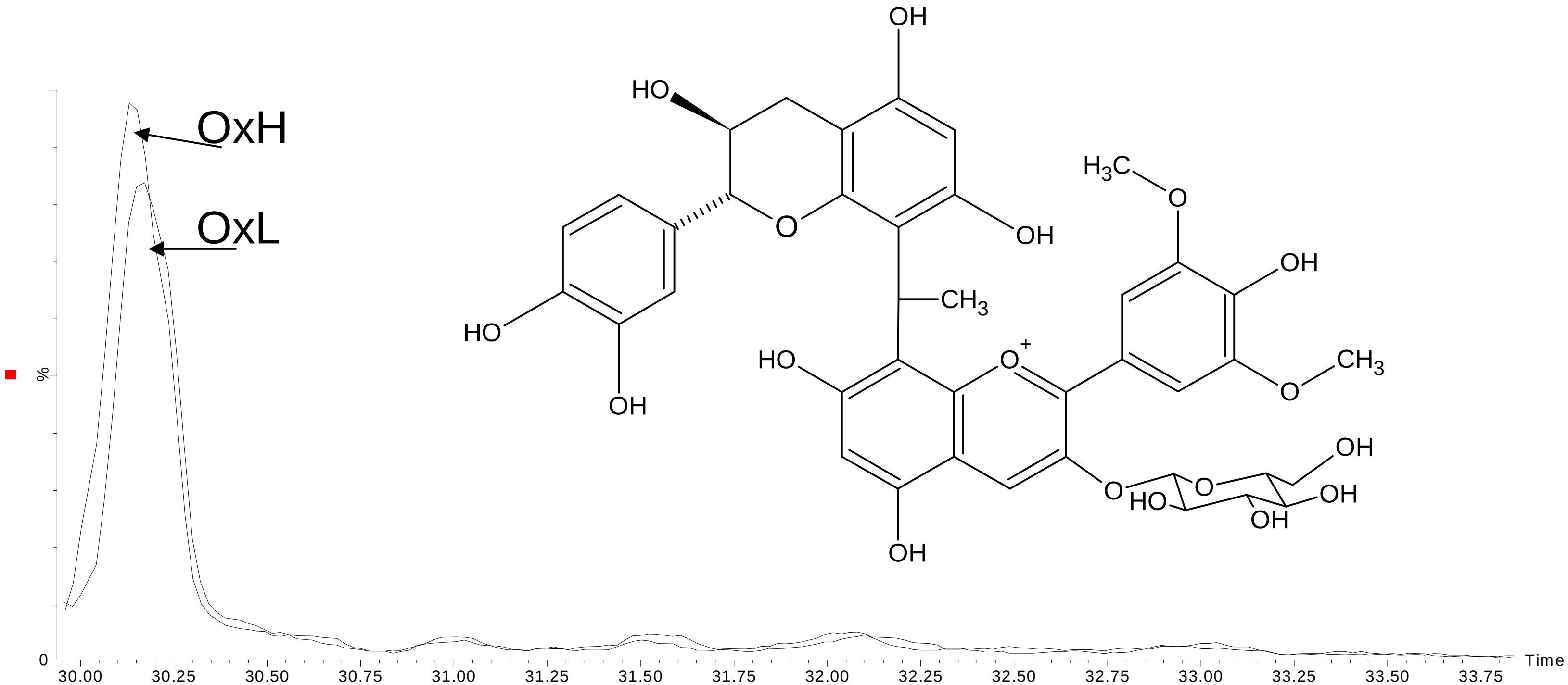


Condensation between AC and flavanols mediated by acetaldehyde

mv-3-glc ethyl-catechin (Timberlake & Bridle mechanism)

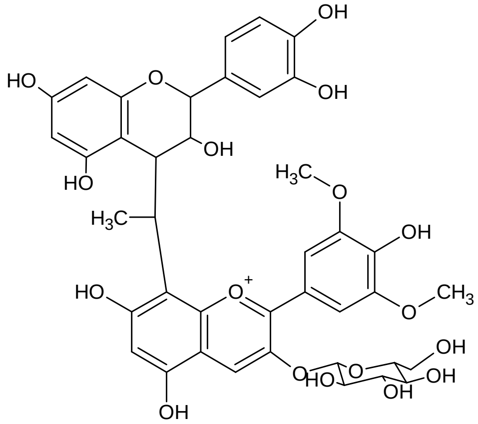
m/z+ 809.2292 RT 30.1
Variable Averages

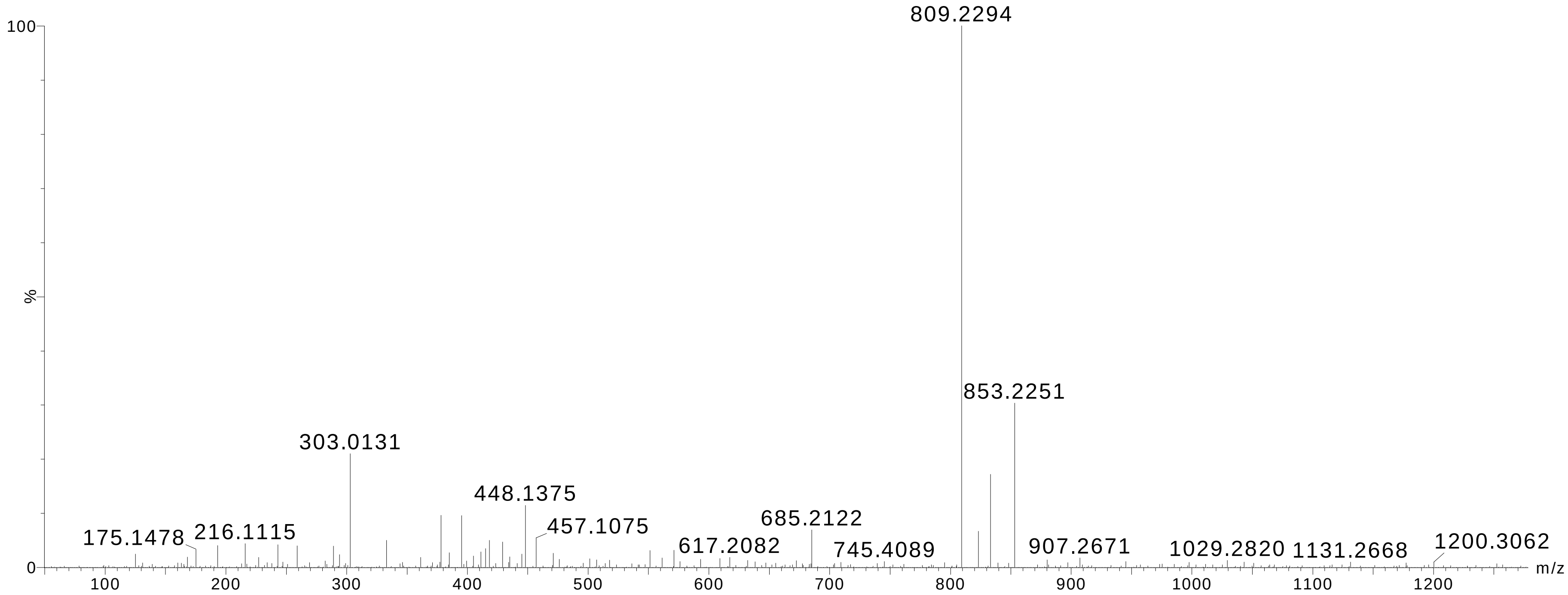




mv-3-glc-ethyl-catechin

809.2287





Effects of short (7 weeks) and limited micro-oxygenation (17.5- 26.3 mg OXYGEN in total), with slightly different amount of iron (1.5-2.0 mg/l) on a real Sangiovese wine

✓ a rather large number of putative biomarkers is extracted (>100)

✓ a closer examination of just a few of the top ranking biomarkers (ESI+) shows that the anthocyanins are among the compounds most strongly affected by the micro-oxygenation

✓ the known products of anthocyanins reaction in presence of aldehydes are also present in the list of the putative biomarkers (at least 3 different pathways)

ABOUT THE METABOLOMIC APPROACH

effective? YES! Grape anthocyanins disappearance was shown to depend on the amount of oxygen applied. At least three of the major known routes of formation of wine pigments were shown to be controlled by the amount of oxygen applied.

New knowledge? YES! Beside anthocyanins, a large number of expected (many flavanols, caftaric acid, phenolic acids, some flavonols…) and of unexpected compounds are present in the list of putative biomarkers. Additional data-mining and experimental work is required to validate the “new” compounds and formulate the hypotheses, towards a better understanding of wine micro-oxygenation

in the future… towards a metabolomic session in MACROWINE 2012?

acknowledgements

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