LC/QToF study of polyphenols in Raboso Piave withered grapes

Mirko De Rosso, Luca Brillante, Antonio Dalla Vedova, Diego Tomasi, Riccardo Flamini

crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Centro di Ricerca per la Viticoltura

Grape withering

Oenological process for production of important Italian reinforced and sweet wines:

Amarone di Valpolicella (produced from Corvina, Corvinone and Rondinella grapes)
Passito di Pantelleria (Zibibbo)
VinSanto (Malvasia and Trebbiano)
Sfursat (Nebbiolo)
Raboso Piave Passito
….

The process can be carried out by keeping grape:
-into withering rooms under controlled conditions of temperature (usually 10-20 °C), RH (usually 40-70%), and fixed air flow.
-over-ripening *on-vine*, until 30-50% of berry water loss (until three months)

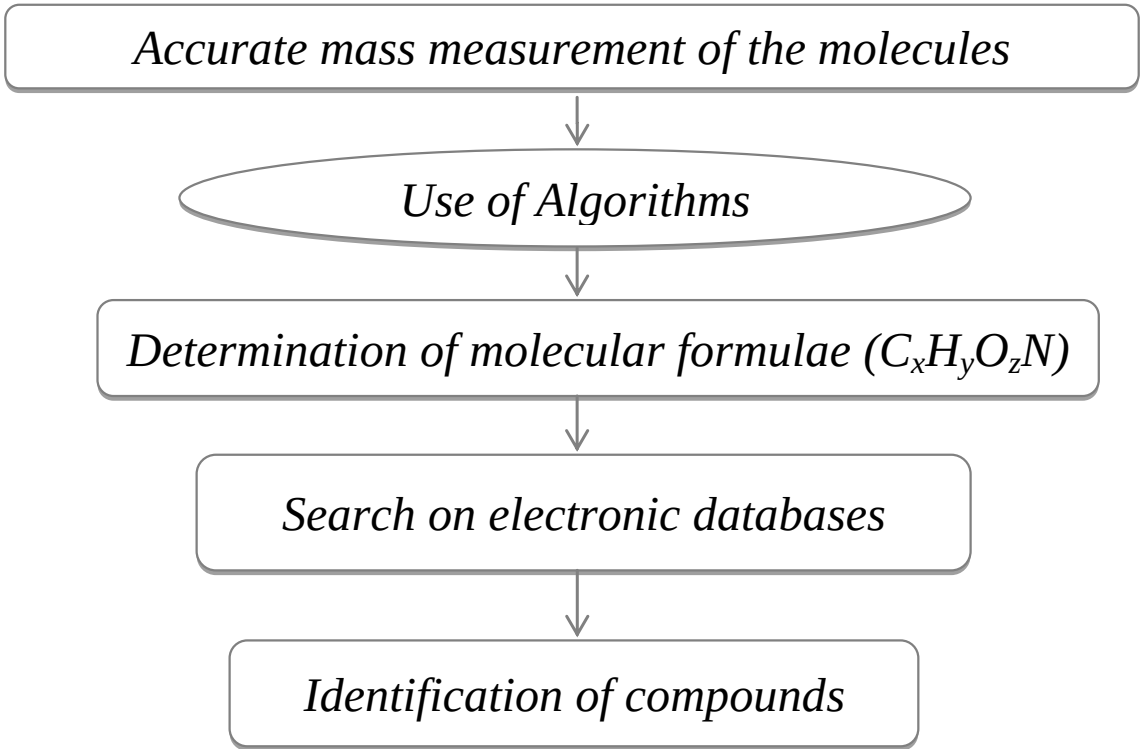
Main physical-chemical changes occurring in the berry

-water loss and consequent sugar enrichment
-decrease of anthocyanins, flavanols and procyanidins, and flavonol glycosides (Mencarelli and Tonutti, 2010)

-other polyphenols increase, such as *trans*-resveratrol, taxifolin, some methoxylated flavanones, quercetin aglycone (Toffali et al., 2011; Bonghi et al., 2012)

-changes in aroma and volatile compounds (terpenol and C_{13}-norisoprenoid glycosides, …) (Di Stefano et al., 1995; 2001; 2003; Mencarelli and Tonutti, 2010)

Grape metabolomics by High-Resolution Mass Spectrometry

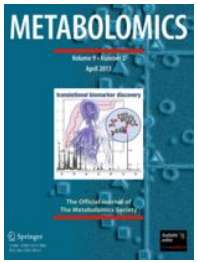


HR-MS: *GrapeMetabolomics* DB

Identification of compounds needs of a database containing specific information on metabolites (molecular formula, structure, retention time, ...).

A database containing the putative grape and wine metabolites (*GrapeMetabolomics*) was constructed by using the information from the literature and introducing the new compounds identified in grapes (over 60 grape varieties studied).

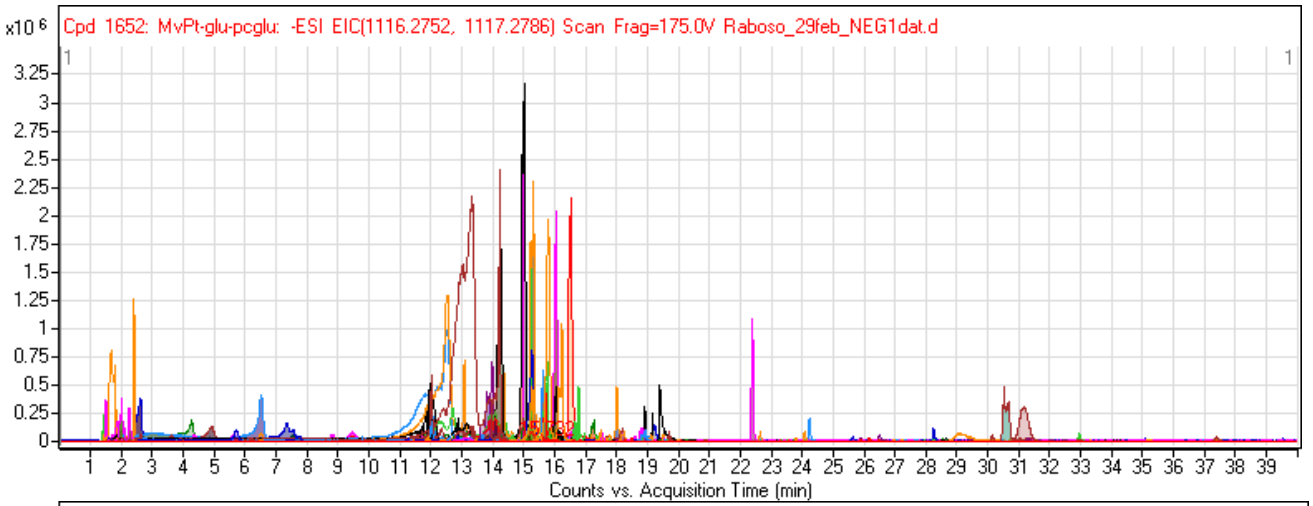
Currently, the DB contains around 1.100 matches.



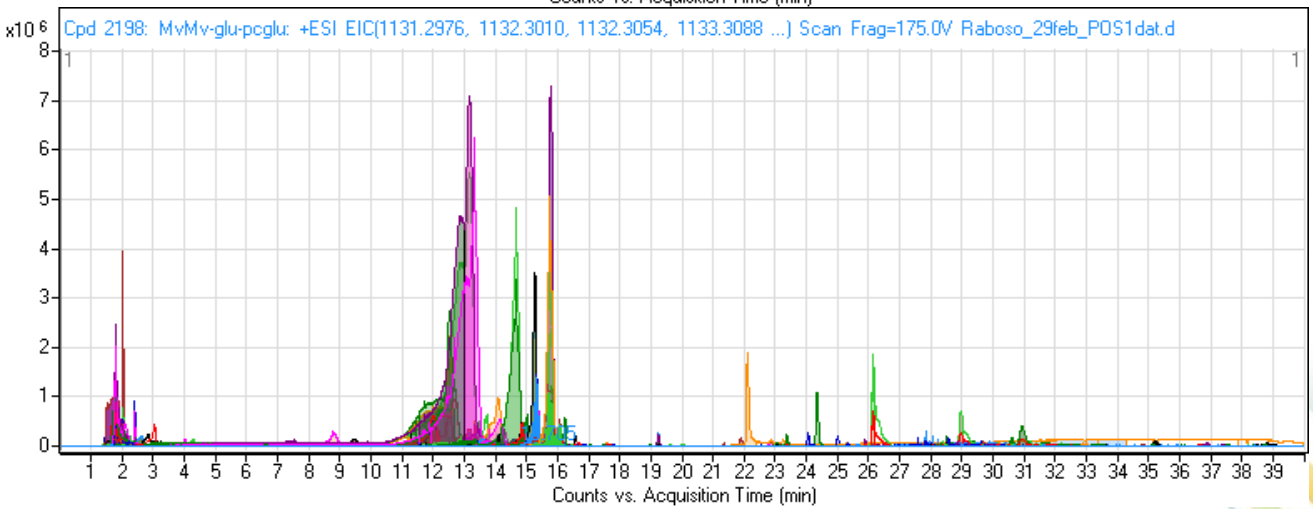
An innovative approach to grape metabolomics: stilbene profiling by suspect screening analysis. *Metabolomics* (Flamini et al. 2013).

UPLC/QTOF: EIC of *Raboso Piave* grape extract

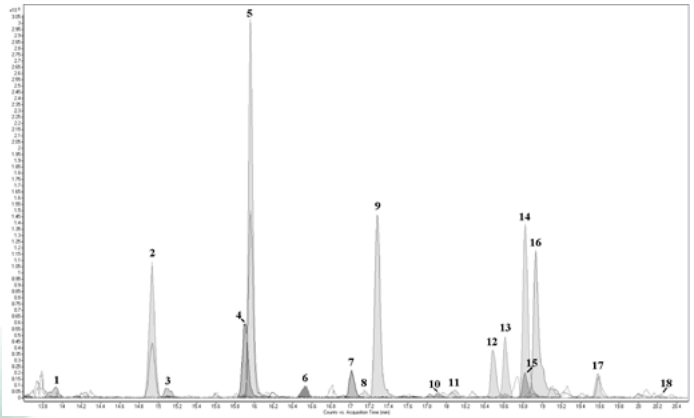
NEG (-)



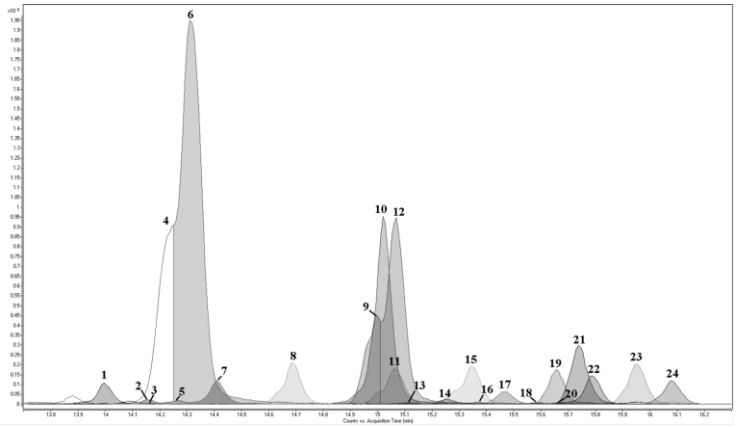
POS (+)



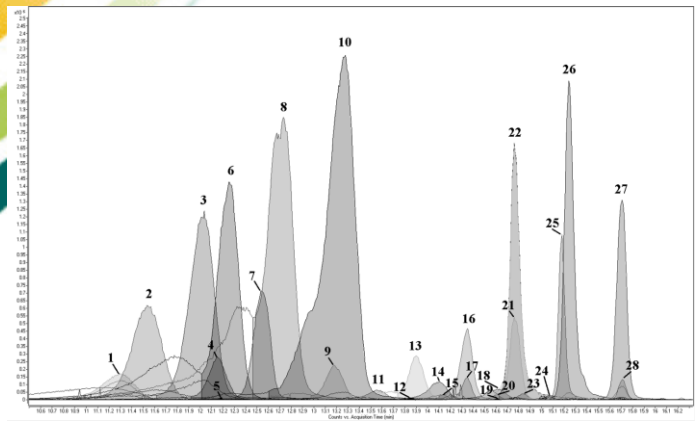
~450 metabolites are putatively identified



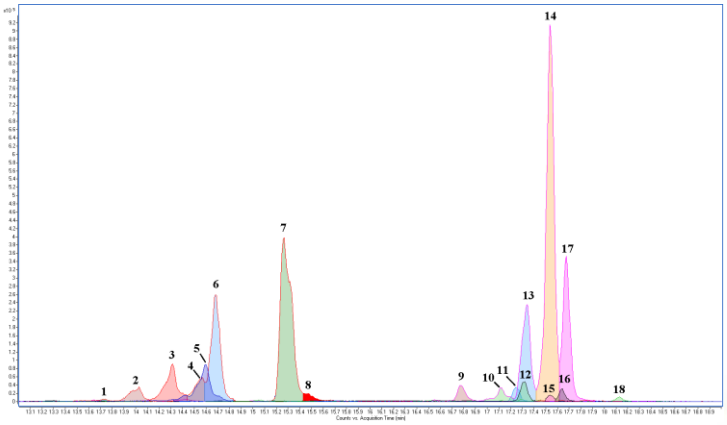
Stilbenes (biological activity)



flavonols (antioxidant compounds)



Anthocyanins (natural colorants)



Glycoside monoterpenes (aroma precursors)

Withering *on-vine vs* warehouse: Raboso Piave grapes

Harvest 2015.

On-vine, by using the technique "cutting the shoot" (cut of the fruity head when the grapes are ripe).

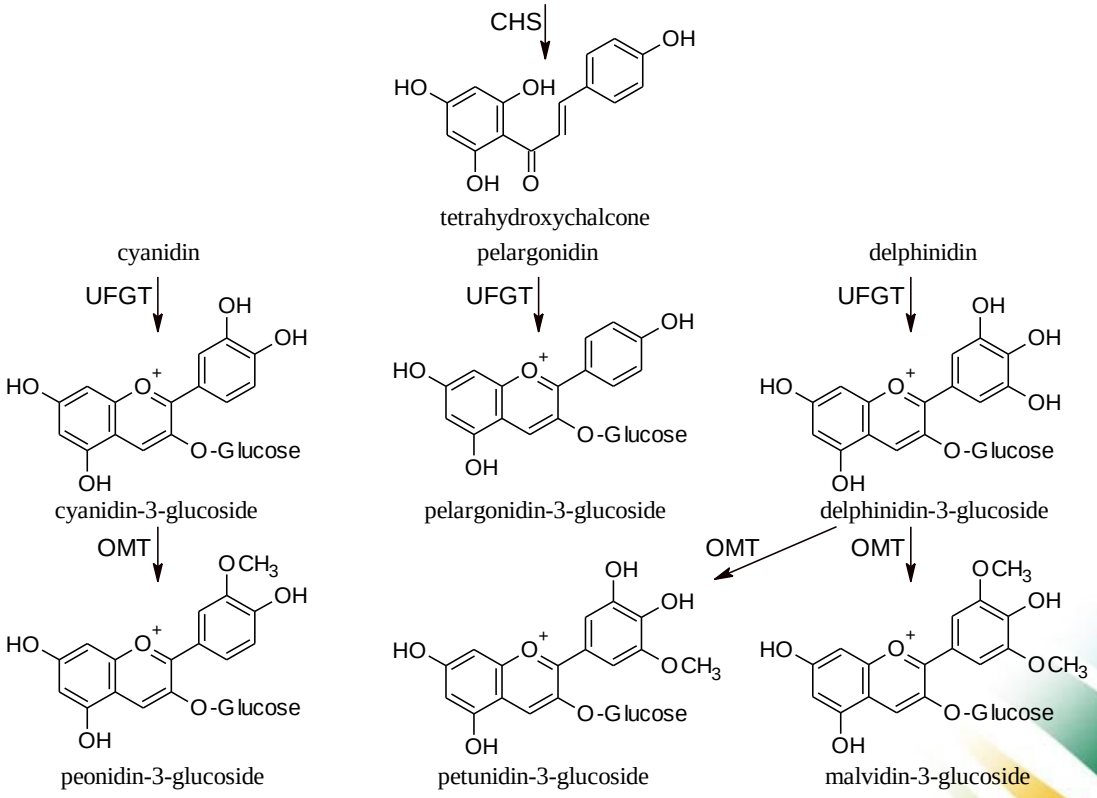
Warehouse: ripe grape stored in 5 Kg-box at 21 °C, RH 60-70%, air flow ~0.3 m/s.

sampling	date	sample	% H_2O loss
start	20/10/2015	**Raboso Plant t_0**	
1st	04/11/2015	**Raboso Plant 10%**	9
2nd	25/11/2015	**Raboso Plant 20%**	18
3rd	10/12/2015	**Raboso Plant 30%**	30
start	20/10/2015	**Raboso warehouse t_0**	
1st	04/11/2015	**Raboso warehouse 10%**	10
2nd	25/11/2015	**Raboso warehouse 20%**	20
3rd	10/12/2015	**Raboso warehouse 30%**	30

Analysis: 3 samples studied for each thesis, 2 analytical repetitions of each sample

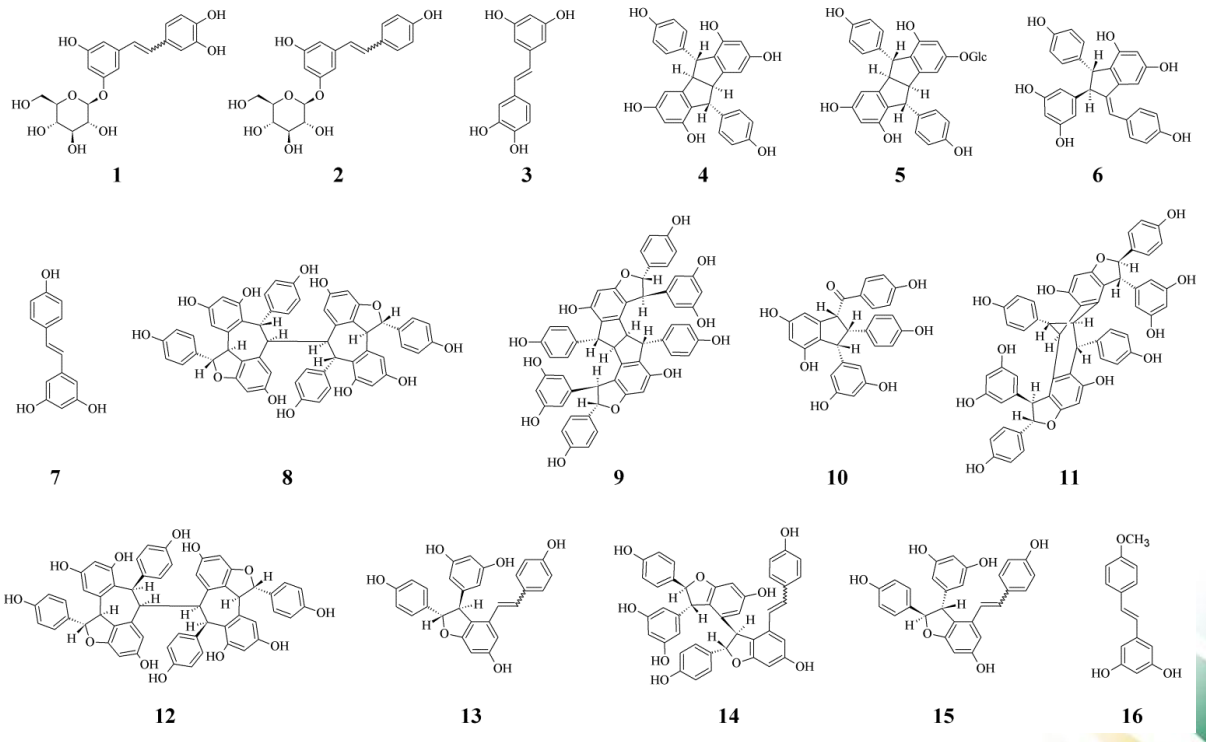
At the end of *on-vine* withering:
-higher level of B-ring tri-substituted anthocyanins
-lower di-substituted derivatives

Two withering methods induce a different metabolism of anthocyanins.



Study of Resveratrol derivatives

trans-resveratrol
piceatannol
cis-piceid
trans-piceid
E-astringin
Z-astringin
resveratrol dimer 1 (pallidol)
resveratrol dimer 2
resveratrol dimer 3 (Z-ε-viniferin)
resveratrol dimer 4 (Z-ω-viniferin)
resveratrol dimer 5 (E-ε-viniferin)
resveratrol dimer 6 (δ-viniferin)
caraphenol
pallidol-3-O-glucoside
α-viniferin
resveratrol trimer 1 (Z-miyabenol C)
resveratrol trimer 2 (E-miyabenol C)
resveratrol tetramer 1
resveratrol tetramer 2

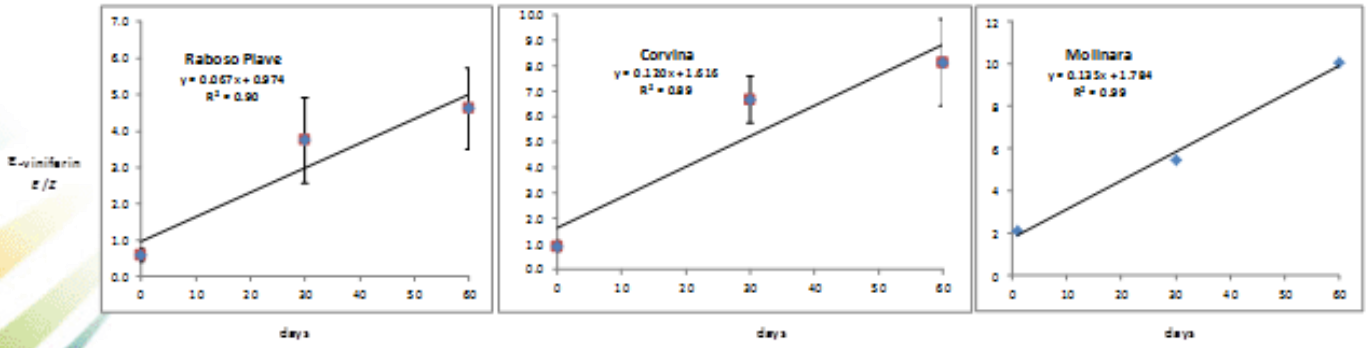


Stilbene synthesis

Warehouse withering (cell **abiotic stress**) increases resveratrol, piceatannol, and viniferins (until the second stage which corresponds at -20% of water loss), according to the previous literature.

On-vine withering not induces significant increase of these phytoalexins (**lower abiotic stress …**).

In grape berries infected by *Plasmopara viticola* or *Aspergillus C*. (**biotic attack**) resveratrol is produced and rapidly oxidized into viniferins (Pezet et al. 2004; Flamini et al. 2016).



E/Z e-viniferin in a) Raboso Piave, b) Corvina, c) Molinara at 0%, 25% and 40% water loss (withering in warehouse at 18 °C, RH 40%).

(*De Rosso et al., JMS 2016*)

Flavonols & Procyanidins

(-)-epicatechin
(-)-epicatechin gallate
(-)-epigallocatechin
(+)-catechin
procyanidin (B3,B4,B5)
procyanidin B1
procyanidin B2
proCyanidin T2,T3(T4),C1
proDelphinidin T2,T3
tot. Procyanidins

chrysoeriol *p* -coumaroylhexoside
dihydrokaempferide-*p* -coumaroylhexoside
isorhamnetin *p* -coumaroylglucoside
tot. *p* -coumaroyl glucoside flavonoids

dihydrokaempferol-3-O-rhamnoside

isorhamnetin-3-O-glucoside
isorhamnetin-glucuronide
tot. Isorhamnetin

kaempferol
kaempferol-3-O-galactoside
kaempferol-3-O-glucoside
tot. Kaempferol

myricetin-3-O-glucoside
myricetin-3-O-glucuronide
myricetin-diglucoside
tot. Myricetin

quercetin
quercetin-3-O-galactoside
quercetin-3-O-glucoside
quercetin-3-O-glucuronide
quercetin-pentoside
rutin (quercetin-3-O-rutinoside)
tot. Quercetin

syringetin
syringetin-3-O-glucoside
tot. Syringetin

tamarixetin
taxifolin-pentoside isom
tetrahydroxy-dimethoxyflavanone- hexoside
pentahydroxy flavone A
laricitrin-3-O-glucoside

Conclusions

As expected, withering *on-vine* induces a different cell metabolism.
In particular, the two withering processes had:
- significant differences in the metabolism of anthocyanins,
- warehouse withering promotes higher accumulation of resveratrol phytoalexins due to higher abiotic stress; alternatively, some activities by microorganisms may occur,
- not significant differences in flavonols and procyanidins observed.

Increase of tamarixetin can be used as a marker for monitoring the two processes, *E*/*Z* e-viniferin ratio for monitoring of warehouse withering.

Grape metabolomics by HR-MS &"Suspect Screening Analysis"

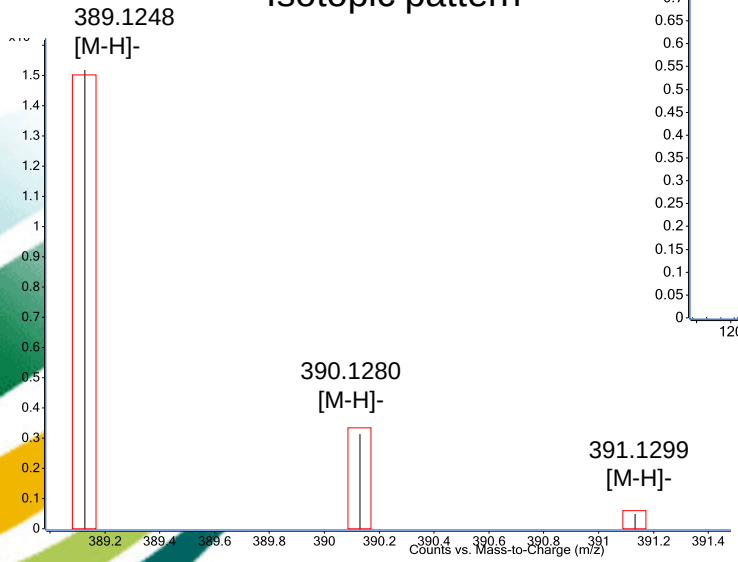
1st step *- targeted* identification of the compounds "expected" in the sample by using *GrapeMetabolomics*

2nd step - *identification of* ***compounds isomeric*** *with those present in the DB which fall in the chromatogram at different retention times*

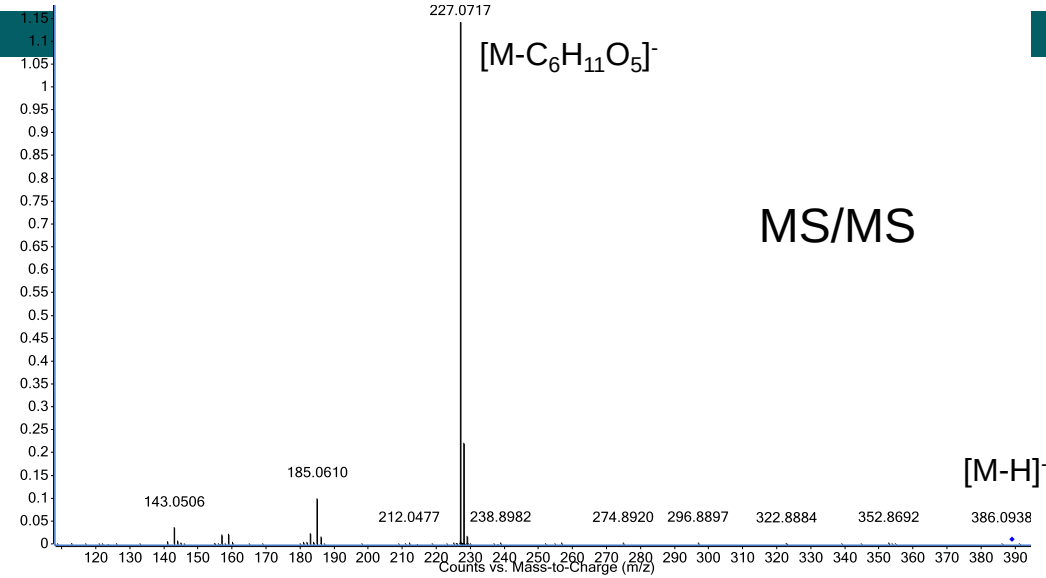
3rd step *- untargeted data analysis*: tentative of identification of new MFs by data processing of the remaining signals

Compound identification

Isotopic pattern



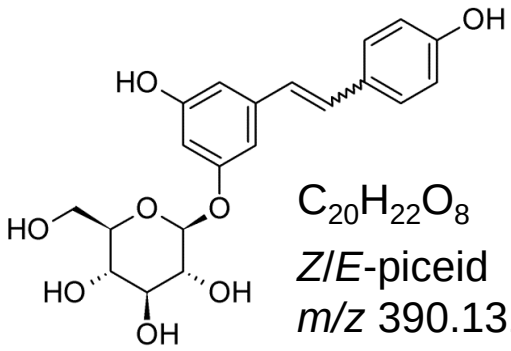
MS/MS



MS/MS fragment spectrum

Mass accuracy (ppm):

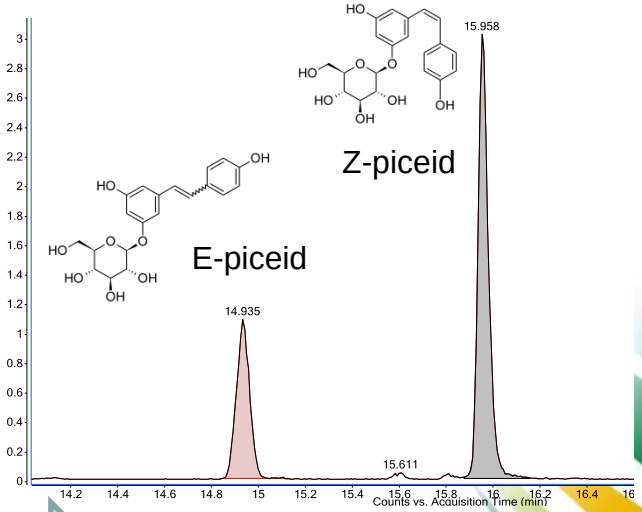
$$\frac{\text{exp. mass - exact mass} \cdot 10^6}{\text{exact mass}}$$



$C_{20}H_{22}O_8$

Z/*E*-piceid

m/z 390.1315



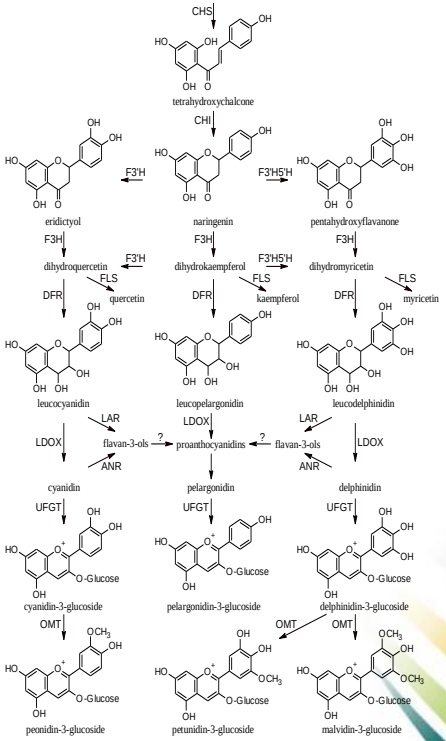
Retention time

Grape drying: Conclusive remarks MS-Food Day 2015

HRMS-metabolomics allowed identification of nutraceuticals not reported in grape before, some still under confirmation.

In particular, several new dihydroflavonols were identified and their biosynthetic pathways in grape are currently under study ...

Five new putative polyphenols were identified in grape, and five compounds previously proposed in supplemental materials in withered Corvina grape were confirmed. (Toffali et al. 2011); Zamboni et al. 2010).



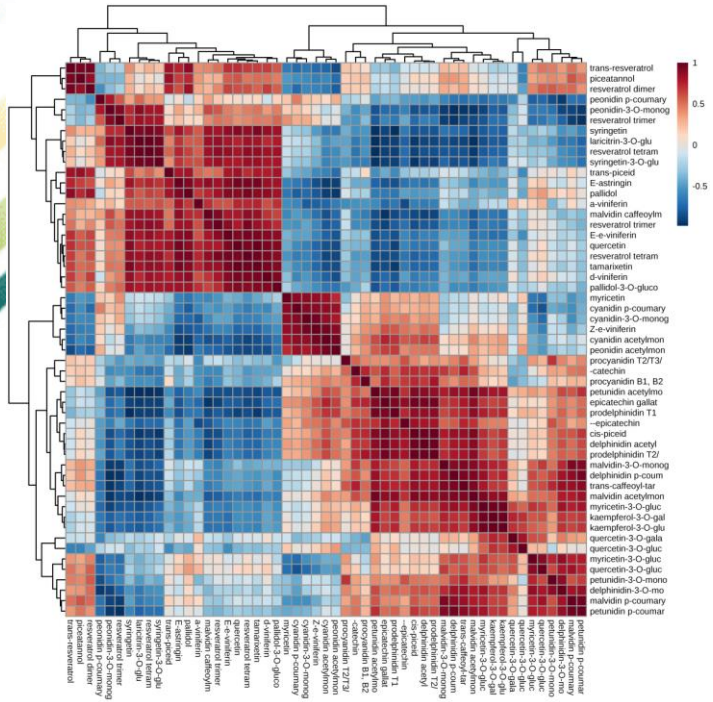
Work in progress

Study of glycoside aroma precursor evolution.

Study of correlation among the metabolites.

Organoleptic evaluation of the wines produced by these grapes.

Heatmap



Aroma glycoside precursors

trans/cis 2/3-hexen-1-ol glucoside

Benzenoids

hydroxybenzoic acid glucoside

methylsalicylate/vanillin glucoside

vanillic acid glucoside

acetovanillone glucoside

methylvanillate /syringaldehyde glucoside

homovanillic alcohol glucoside

coniferyl alcohol /propiovanillone glucoside

dihydroconiferyl alcohol glucoside

dihydroxybenzoic acid isomer

Norisoprenoids

vomifoliol glucoside

terpenols

7-hydroxy-α-terpineol-pentosyl-hexoside

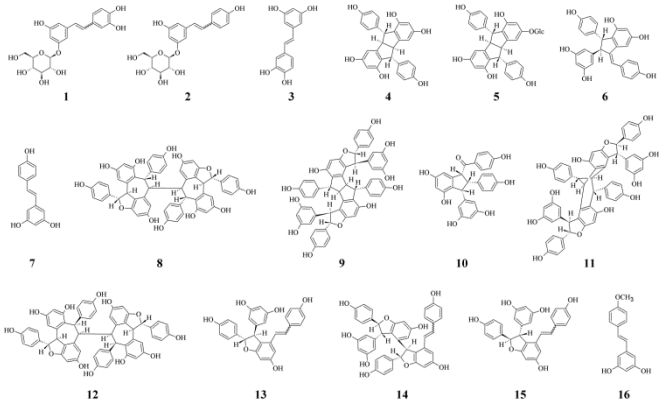
furan/pyran linalool oxides pentosyl-hexoside

diendiol I-pentosyl-hexoside

geraniol-pentosyl-hexoside

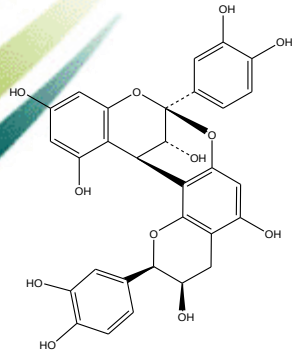
hydroxy geraniol-pentosyl-hexoside

cis/trans 8-hydroxylinalool



Acknowledgements

Thank the Giorgio Cecchetto's Winery for the vineyard assistance provided



Thank You For Attention !