AGRI SYSTEM UCSC
Scuola di Dottorato
per il Sistema Agroalimentare

UNIVERSITÀ CATTOLICA del Sacro Cuore

Phenolic profiling followed by multivariate analysis discriminates Chardonnay wines from different geographical origin

Rocchetti, G., Gatti, M., Bavaresco, L., Lucini, L.

2nd MS-Wine Day
9-10th May, 2017

Gabriele Rocchetti
PhD student in Food Chemistry and Nutrition Sciences
gabriele.rocchetti@unicatt.it

Introduction - Chardonnay

Chardonnay grapes

-Origin: Burgundy region of eastern France
-Result of a cross between the Pinot noir and Gouais blanc grape varietis (from DNA fingerprinting research)
-Important component of many sparkling wines (Champagne)
-Harvesting time is crucial to winemaking, with the grape rapidly losing acidity as soon as it ripens.

160,000 hectares around the world

-Italy: cultivated in almost all regions, above all in:
Sicilia, Trentino-Alto Adige, Piemonte, Lombardia, Veneto, Friuli-Venezia Giulia, Abruzzo.
-France (spread everywhere) used pureness to produce Bourgogne wines, mixed varieties to produce Champagne.
-Also in Australia, California, Chile and South-Africa.

Wine attributes

Color: golden yellow, fruity fragrance (apple, banana, papaya, other exotic); mixed and complex wine, good structure and high acidity.
Different types: sparkling and still wines, suitable for aging in barrel

Chablis AOC (100% Chardonnay)

UNIVERSITÀ CATTOLICA del Sacro Cuore

White wines: phenolic compounds

White wines (typically lower in phenolic compounds), as a consequence are also lower in antoxidant activity.

Phenolic compounds influence the perceived body and flavor of the wines. Several techniques in literature were tested in order to increase phenolics, antioxidant activity and sensory characteristics (three maceration techniques: carbonic, cryogenic and extended skin contact).

Higher antioxidant activity can aid in the aging of wines by preventing oxidation from reactive oxygen species.

Additionally, phenolic compounds contribute to the stability of wines as well as the overall mouthfeel.

What about the identification of phenolic compounds in wine?

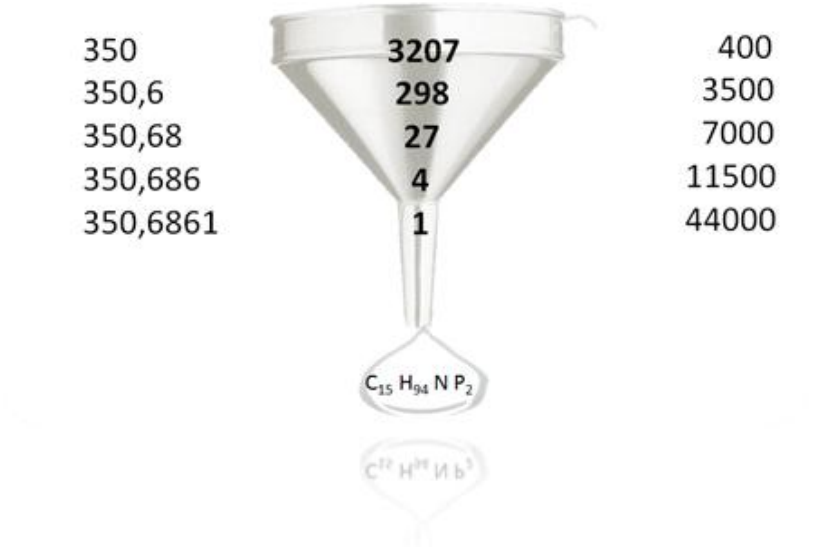
Olejar et al. 2016 *Food Science and Technology*

UNIVERSITÀ CATTOLICA del Sacro Cuore

Why High Resolution Mass Spectrometry?

MS^n analyses

Compound identification increases at increasing mass accuracy and resolution!



UHPLC-ESI-QTOF-MS

QTOF-MS is a form of hybrid, tandem mass-spectrometry that combines a quadrupole and time of flight mass analyzers, used for the identification of compounds in complex matrices, as well as to obtaining structural information about a given compound.

UNIVERSITÀ CATTOLICA del Sacro Cuore

Metabolomics for wine traceability

The use of a **metabolomic platform** (high resolution mass spectrometry) could be useful to provide fingerprinting or profiling assessments to classify wine samples, according to their terroir, geographic origin, variety and age.

Polyphenols analyses have been considered as a way to differentiate wines based on the presence, intensity or ratios of some of these compounds.

From an industrial point of view, **traceability** is also a very imporant parameter to control and to monitor wine-making processes.

FOCUS: *Geographic origin*
Ageing/vintage effect

Metabolomics for wine-quality assessmet
Metabolomics to detect wine adulteration

Alañón et al. 2015, *Trends in Analytical Chemistry*
Versari et al. 2014, *Food Research International*

UNIVERSITÀ CATTOLICA del Sacro Cuore

Aim of the work

Discrimination of Chardonnay wines from different geographic origin according to a metabolomic approach based on phenolic profile.

Valle d'Aosta

Piacenza

Sicilia

VS

Israel

France

Australia

Vintage: 2015

Chardonnay Classico Valle d'Aosta D.O.P., 2015
Lat: 45.7° Nord; Alt: 600-850 m asl;
Soil: loose, sandy and moraine
Climate: alpine
Ageing: 6 months in inox, *sur lies* with bâtonnages
% alcohol: 13 vol

La Torretta – Clarente, Colli Piacentini DOC Chardonnay 2015

Lat: 45° Nord; Alt: 250-300 m asl
Soil: clay and limestone
Climate: temperate
Ageing: 6 months in inox
% alcohol: 13 vol

Donnafugata *La Fuga 2015, Contessa Entellina DOC*
Lat: 37.73° Nord
Alt: 200-600 m asl
Soil: clay
Climate: mediterranean
Ageing: some months in inox and 3 months in bottle.
% alcohol: 13-13.5 vol

2015 Golan Heights Winery Yarden Chardonnay
Lat: 33° Nord
Alt: 400-1200 m asl
Soil: volcanic basaltic
Climate: mild and dry summers
Ageing: 7 months *sur lies* in French oak barrels
% alcohol: 14 vol

Louis Jadot – Bourgogne 2015
Couvent des Jacobins, France (Burgundy)
Lat: 47° Nord
Alt: 250-300 m asl
Soil: clayey marly limestone
Climate: continental
Ageing: 8 months in oak barrels partially
% alcohol: 12.5 vol

Yellow Tail Chardonnay 2015
South-East Australia
Lat: 33.37° S
Alt: 50 to 400 m asl
Climate: continental to oceanic
Soil: from loamy sand to clay loam
Ageing: 6-8 months in oak barrels
% alcohol: 13 vol

Inox vs Barrel Aged wines

Inox

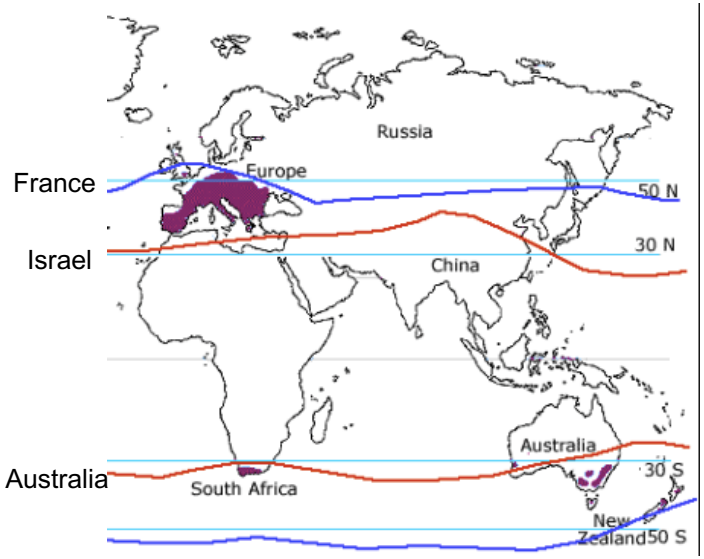
WINE SAMPLES

Oak Barrels

Same variety: 100%
Same price range: 10-20 €
Same vintage: 2015

Different terroirs:
≠ soils
≠ latitudes and altitudes→ ≠climates

(≠viticultural and oenological practices)



→≠ Polyphenols?

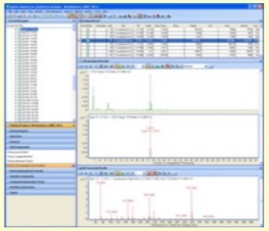
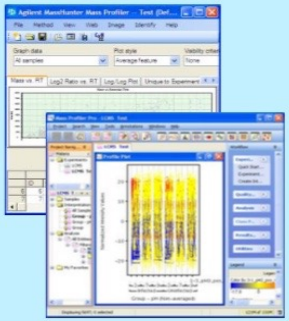
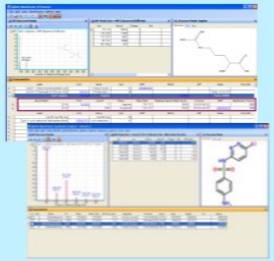
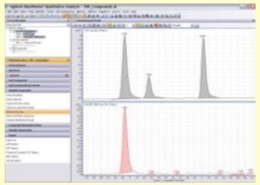
What about traceability?

UNIVERSITÀ CATTOLICA del Sacro Cuore

Profiling Chemometrics Workflow

Agilent's Profiling Workflow

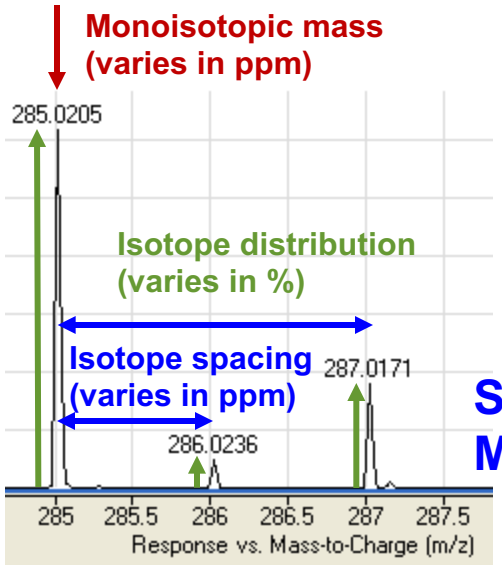
Data → Find Compounds → Find Differences → Identify Differences

	Separate & Detect	Feature Finding Quantitate	Alignment & Statistics	Identify
GCMS	 GC/MSD GC-QQQ	 MassHunter Qual *AMDIS* or *Find by chromatographic deconvolution*	Mass Profiler (MP)  Mass Profiler Professional (MPP)	 ID Browser
LCMS	 LC-TOF/QTOF LC-QQQ	 MassHunter Qual *MFE*, *Find by Formula*, *Find by Ion*		

Identification / Screening

Mass accuracy below 5 ppm

Scoring based on:



Mass Match +

Abund. Match +

Spacing Match =

Overall Score

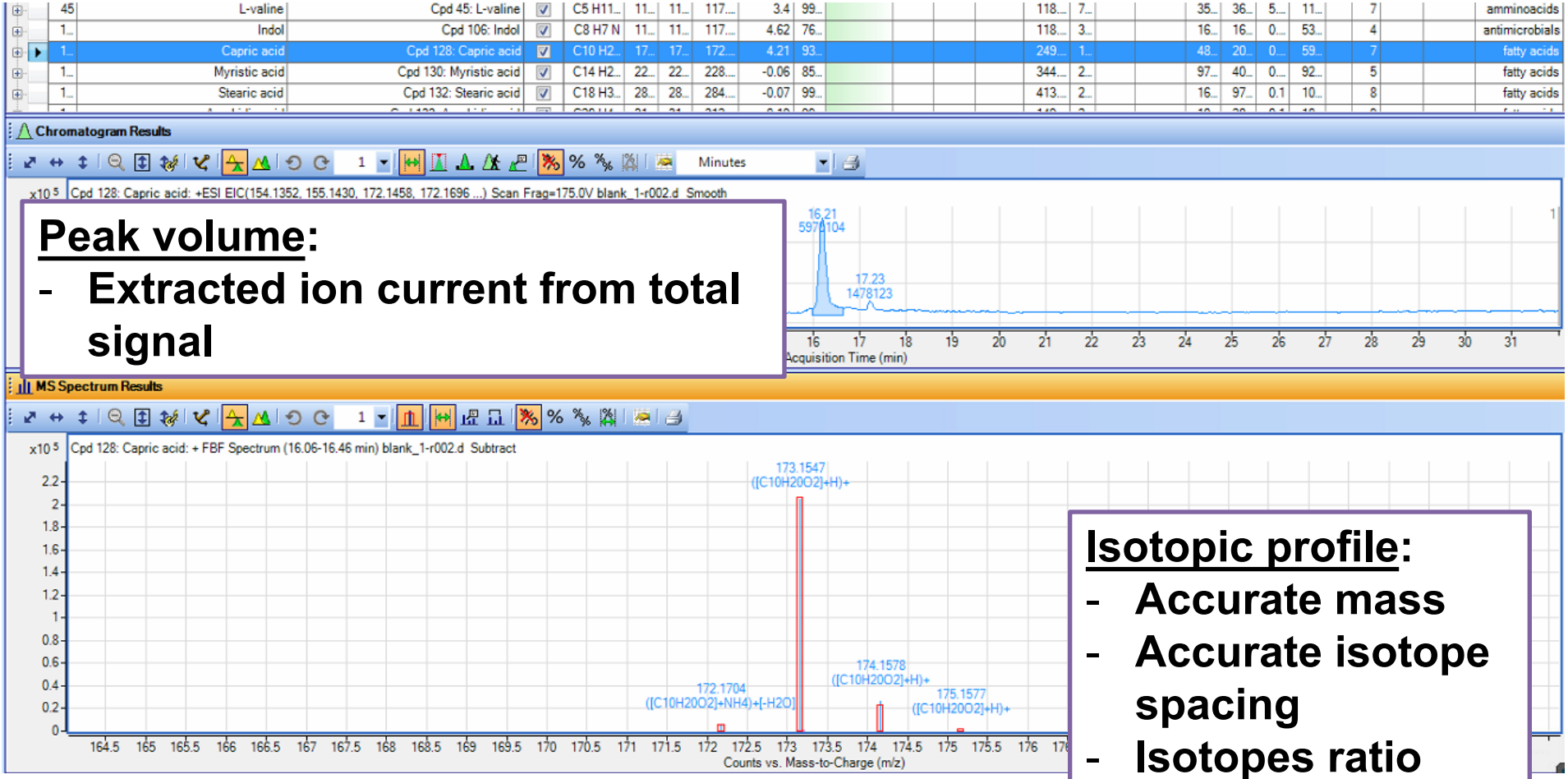
MS Formula Results: Cpd 2: C10H9ClN4O2S

m/z	Ion	Formula	Abundance
285.021	(M+H)+	C10 H10 Cl N4 O2 S	24506.1

Best	Formula (M)	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (p	Spacing Matc	Abund Matc	Mass Match	m/z	DBE
☑	C10 H9 Cl N4 O2 S	285.0208	99.55		284.0137	284.0135	-0.71	0.71	99.19	99.26	99.69	285.021	8
☐	C7 H12 N2 O6 S2	285.021	77.28		284.0137	284.0137	0.01	0.01	99.54	1.93	100	285.021	3
☐	C7 H13 Cl N4 O2 S2	285.0241	75.57		284.0137	284.0168	11.12	11.12	99.87	83.87	46.22	285.021	3

UNIVERSITÀ CATTOLICA del Sacro Cuore

Identification / Screening



Peak volume:

- Extracted ion current from total signal

Isotopic profile:

- Accurate mass
- Accurate isotope spacing
- Isotopes ratio

Profinder B.07

UNIVERSITÀ CATTOLICA del Sacro Cuore

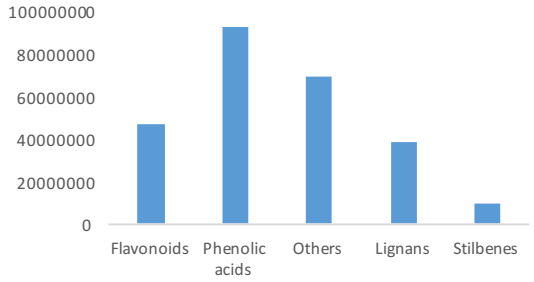
Phenolic profile (cumulative intensity per classes)

483 phenolics identified

DATASET PLOT INTENSITY OF DIFFERENT CHARDONNAY

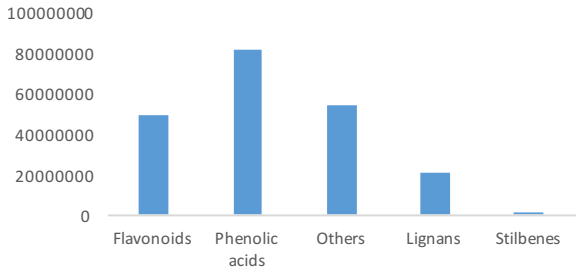
AUSTRALIA

∑ 2.58E+08



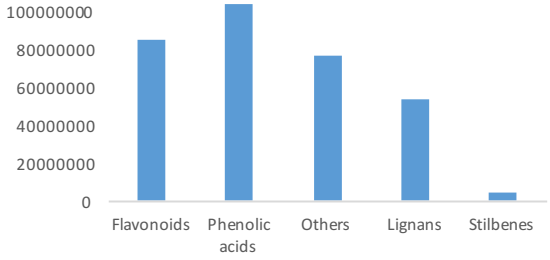
FRANCE

∑ 2.53E+08



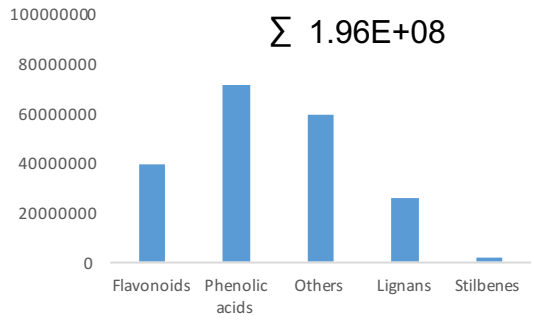
ISRAEL

∑ 3.22E+08



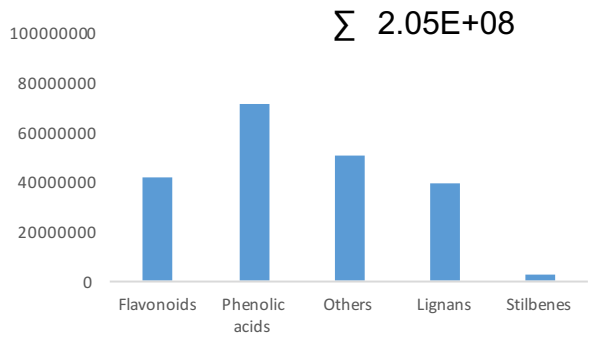
ITALY, PC

∑ 1.96E+08



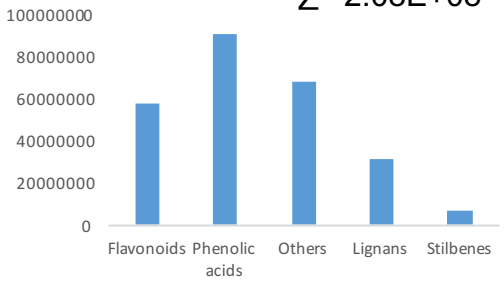
ITALY, SICILY

∑ 2.05E+08



ITALY, VdA

∑ 2.08E+08



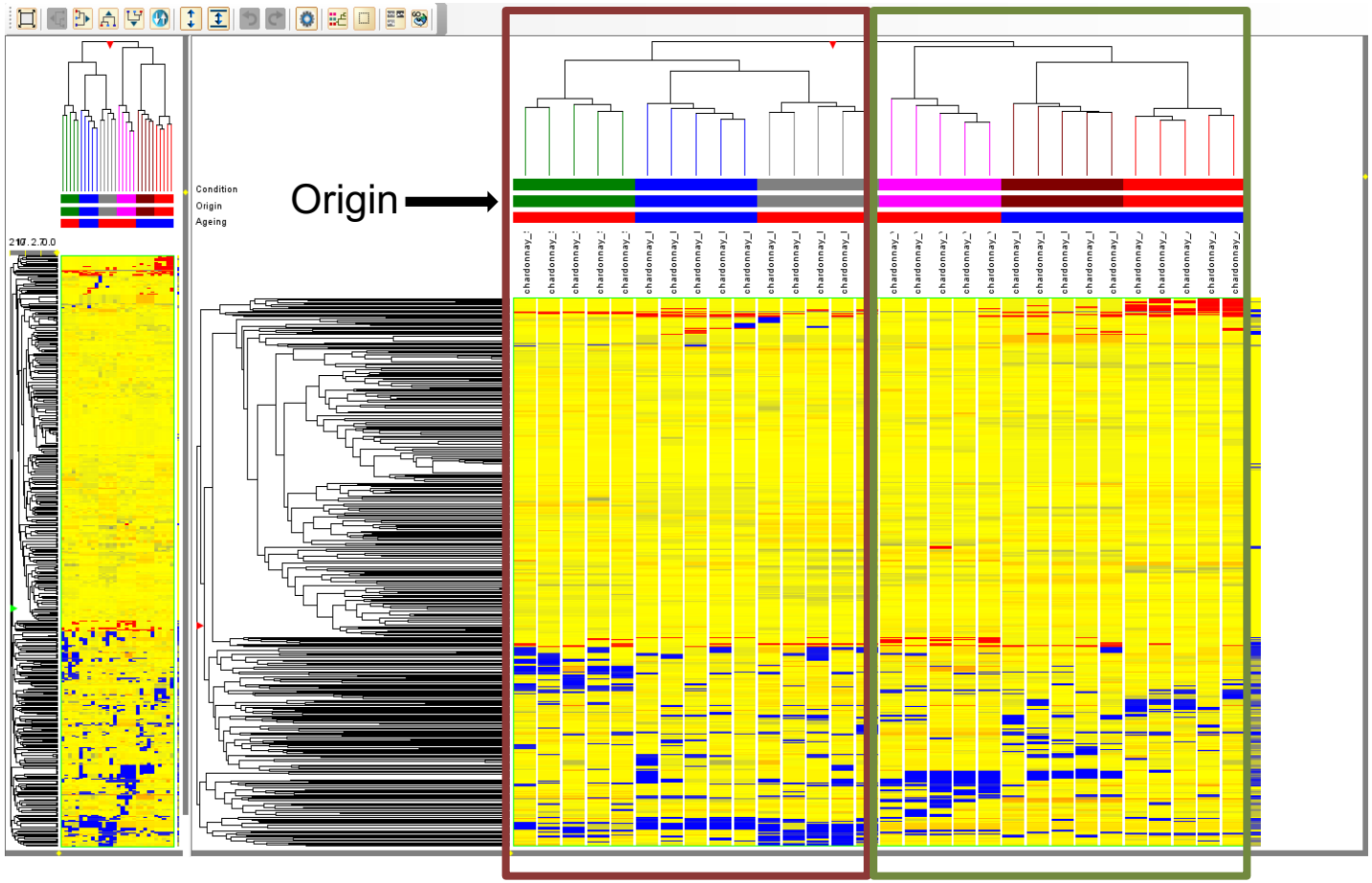
UNIVERSITÀ CATTOLICA del Sacro Cuore

Un-supervised Cluster Analysis

Heat map
(FC-based)

France
Sicilia
Piacenza

Israel
Valle d'Aosta
Australia



Legend - Hierarchical Combined Tree on Origin - Ageing (Non-averaged)

Color range
-14.8 0 14.8

Condition
[Australia, wood]
[France, wood]
[Israel, wood]
[Italy, PC, none]
[Italy, Sicily, none]
[Italy, VdA, none]

Origin
Australia
France
Israel
Italy, PC
Italy, Sicily
Italy, VdA

Ageing
none
wood

Entity color by Number Passed
1.5 6

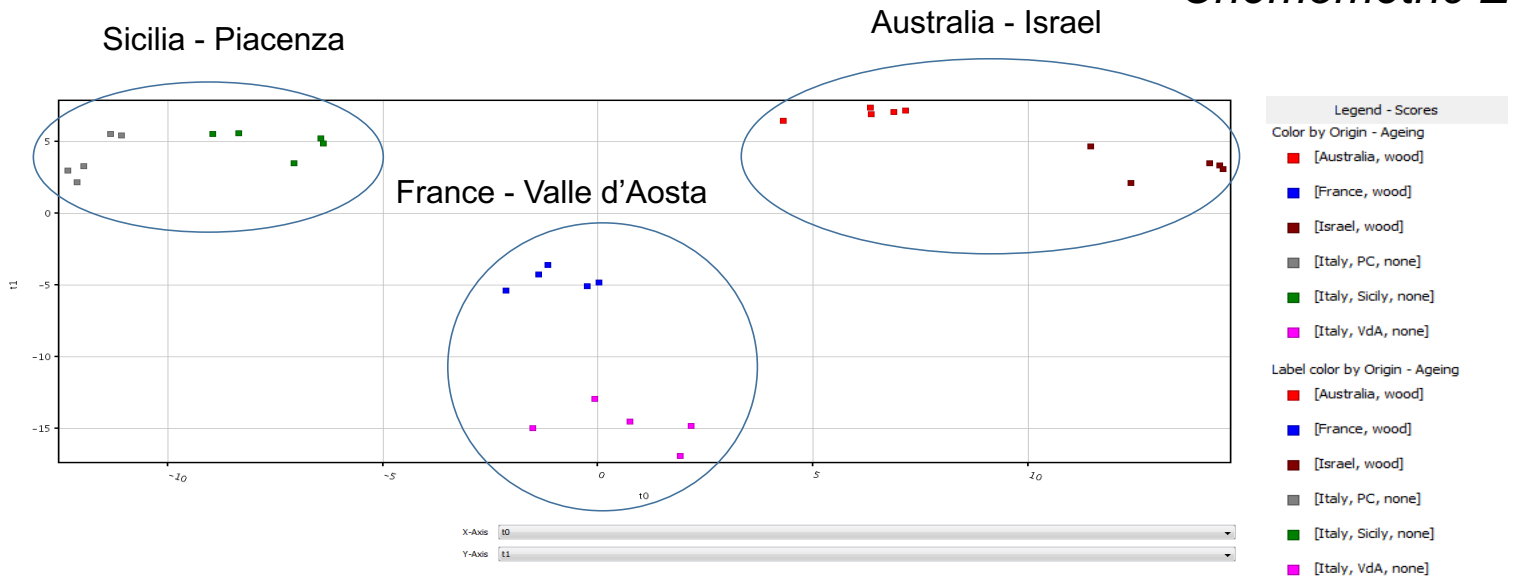
Description
Created from Advanced Analysis operation: Clustering:
Entity List: Filtered by frequency ['conditions', [100.0, 1]]
Interpretation: Origin - Ageing (Non-averaged)
Experiment: phenol_explorer_6vini
Clustering Algorithm: Hierarchical
Clustered By: Normalized intensity values
Clustered On: Entities and Conditions
Similarity Measure: Euclidean
Linkage Rule: Wards
Cluster Within Conditions: No

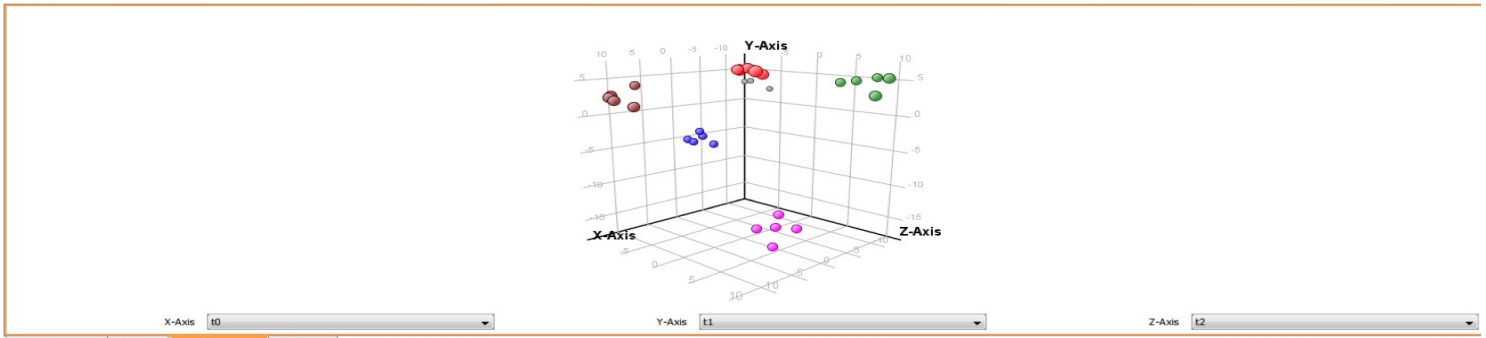
UNIVERSITÀ CATTOLICA del Sacro Cuore

Partial Least Square Discriminant-Analysis

PLS-DA plot (origin+wood)

Chemometric Evaluation





	[Australia, wood] (Predicte...	[France, wood] (Predicted)	[Israel, wood] (Predicted)	[Italy, PC, none] (Predicted)	[Italy, Sicily, none] (Predict...	[Italy, VdA, none] (Predicted)	Accuracy
(True) [Australia, wood]	5	0	0	0	0	0	100.000
(True) [France, wood]	0	5	0	0	0	0	100.000
(True) [Israel, wood]	0	0	5	0	0	0	100.000
(True) [Italy, PC, none]	0	0	0	5	0	0	100.000
(True) [Italy, Sicily, none]	0	0	0	0	5	0	100.000
(True) [Italy, VdA, none]	0	0	0	0	0	5	100.000
Overall Accuracy							100.000

Metabolites explaining differences

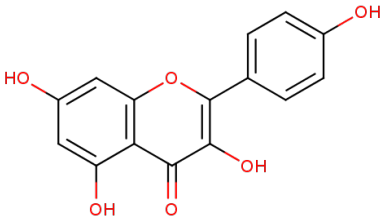
<u>82 differential metabolites</u> outlined by PLS-DA analysis (importance in the class prediction model).

41 Flavonoids (Dihydrochalcones, Flavones, Flavonols, Flavanols, Flavanones, Chalcones, Isoflavonoids)

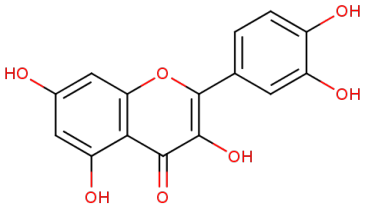
18 Phenolic acids (Hydroxycinnamic/Hydroxybenzoic/Hydroxyphenylacetic acids)

7 Lignans (Matairesinol derivatives)

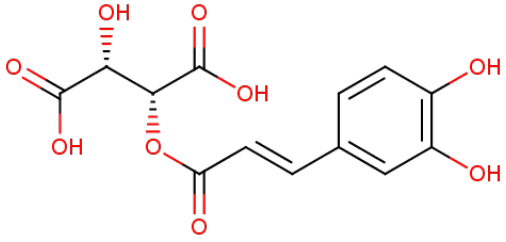
16 Other phenolics (among which 2 Stilbenes)



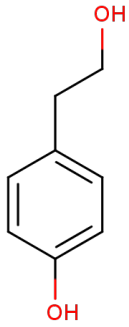
Kaempferol derivatives



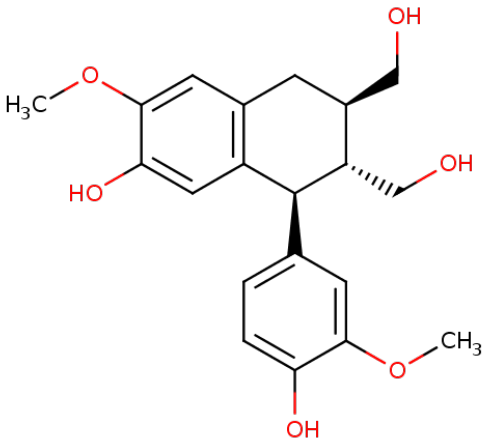
Quercetin derivatives



Caffeoyl tartaric acid



Tyrosol derivatives



Isolariciresinol

UNIVERSITÀ CATTOLICA del Sacro Cuore

Pearson coefficients (structural correletions or possible elicitors?)

CLASSES	Luteolin EQ.	Cyanidin EQ.	Catechin EQ.	Tyrosol EQ.	Resorcinol EQ.	Ferulate EQ.	Matairesinol EQ.	Sesamin EQ.	Resveratrol EQ.
Luteolin EQ.	1	,610**	,923**	,690**	,083	,969**	,572**	,505**	,763**
Cyanidin EQ.	,610**	1	,623**	,421*	n.s.	,599**	,830**	,560**	,674**
Catechin EQ.	,923**	,623**	1	,636**	n.s.	,786**	,593**	,410*	,687**
Tyrosol EQ.	,690**	,421*	,636**	1	,214	,816**	,649**	,762**	,884**
Resorcinol EQ.	,083	n.s.	n.s.	,214	1	,078	n.s.	,125	,142
Ferulate EQ.	,869**	,599**	,786**	,816**	,078	1	,578**	,640**	,841**
Matairesinol EQ.	,572**	,830**	,593**	,649**	n.s.	,578**	1	,707**	,834**
Sesamin EQ.	,505**	,560**	,410*	,762**	,125	,640	,707**	1	,804**
Resveratrol EQ.	,763**	,674**	,687**	,884*	,142	,841**	,834**	,804**	1

** statistical significance at level 0.05 (two-tailed)
* statistical significance at level 0.05 (two-tailed)

UNIVERSITÀ CATTOLICA del Sacro Cuore

Some hypotheses (inox vs oak barrels ageing)

Both volatile and non-volatile compounds are released from oak to wine, which becomes more refined and more complex.

The aromatic modifications consecutive to oak ageing have been well described and explained. The main volatiles migrating from oak wood to wine are vanillin, whisky lactone, eugenol and 2-furanmethanethiol, which are responsible, rispectively, for vanilla, coconut, spicy and roasted coffe.

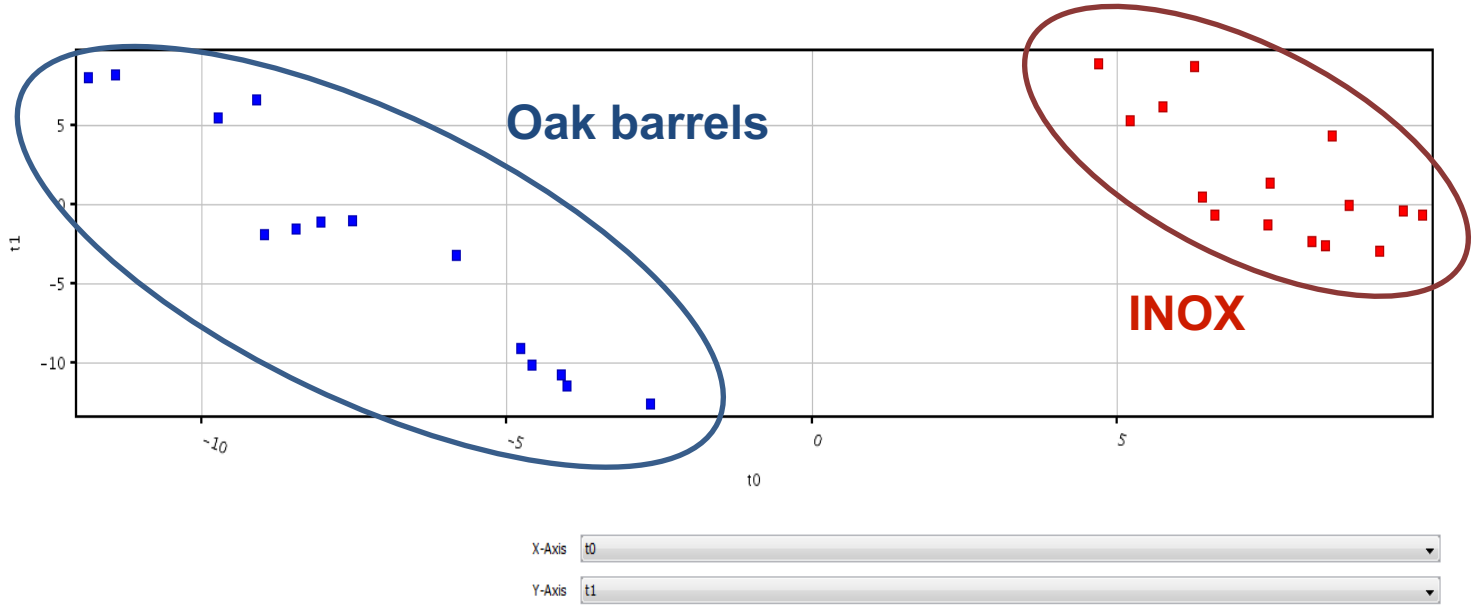
Oak non-volatile compounds are mainly non-flavonoid polyphenols.
They include ellagitannins such as castalagin, vescalagin and roburin, phenolic acids such as ferulic and gallic acids, coumarins such as scopoletin and umbelliferone, and other compounds (lignan derivatives).

Moreover, between lignans, **isolariciresinol and secoisolariciresinol** derivatives, have been reported from oak wood, contributing to wine taste.

Marchal et al. 2015, *Tetrahedron*

UNIVERSITÀ CATTOLICA del Sacro Cuore

PLS-DA inox vs oak barrels ageing



11 phenolic compounds explaining differences

Flavonoids: Pelargonidin 3-O-galactoside, Rhoifolin/Isorhoifolin, 3-methoxynobiletin, Spinacetin 3-O-glucosyl-(1-6)-glucoside, Luteolin.
Phenolic acids: 24-Methylcholesterol ferulate, Feruloyl tartaric acid, 1,2,2'-Trisinapoylgentiobiose
Lignans: Isolariciresinol, Lariciresinol-sequilignan
Hydroxycoumarins: Scopoletin

UNIVERSITÀ CATTOLICA del Sacro Cuore

Take home message and conclusions

Preliminary study:

-Great potential of analytical technique
(metabolomic approach based on phenolic profile)

-Possibile further studies with traceability purposes
(considering both terroir and ageing)

-Possibile discrimination potential based on lignan markers from oak barrels

Not evaluable:
Oenological and vineyard management

Model validation on different vintages!

Thanks for attention!