Free and Bound Volatile Phenols in Smoke-Exposed Wines-Biomarkers, Machine-learning, and Model Prediction

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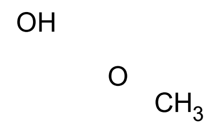
Outlines

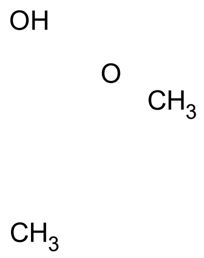
- Introduction to smoke related volatile phenols
- Free and total volatile phenols by GC-MS
- Volatile phenol glycoside by LC-MS
- Machine-learning and modeling
- Conclusion

What we know for smoke compounds

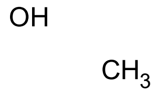
- Volatile phenols have been associated with smoke exposure
- The most frequently studied volatile phenols are guaiacol, 4-methylguaiacol and o-cresol, m-cresol and p-cresol, although a few other volatiles phenols are sometimes studied
- In the event of smoke, the grapevine can absorb these volatile phenols and convert them to phenol-glycosides
- Bound phenol glycosides do not have smoke taint, however, they can convert to free form during fermentation, aging or in mouth, impart off-flavor



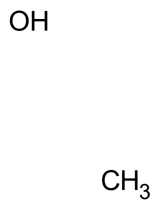
guaiacol



4-methylguaiacol



o-cresol



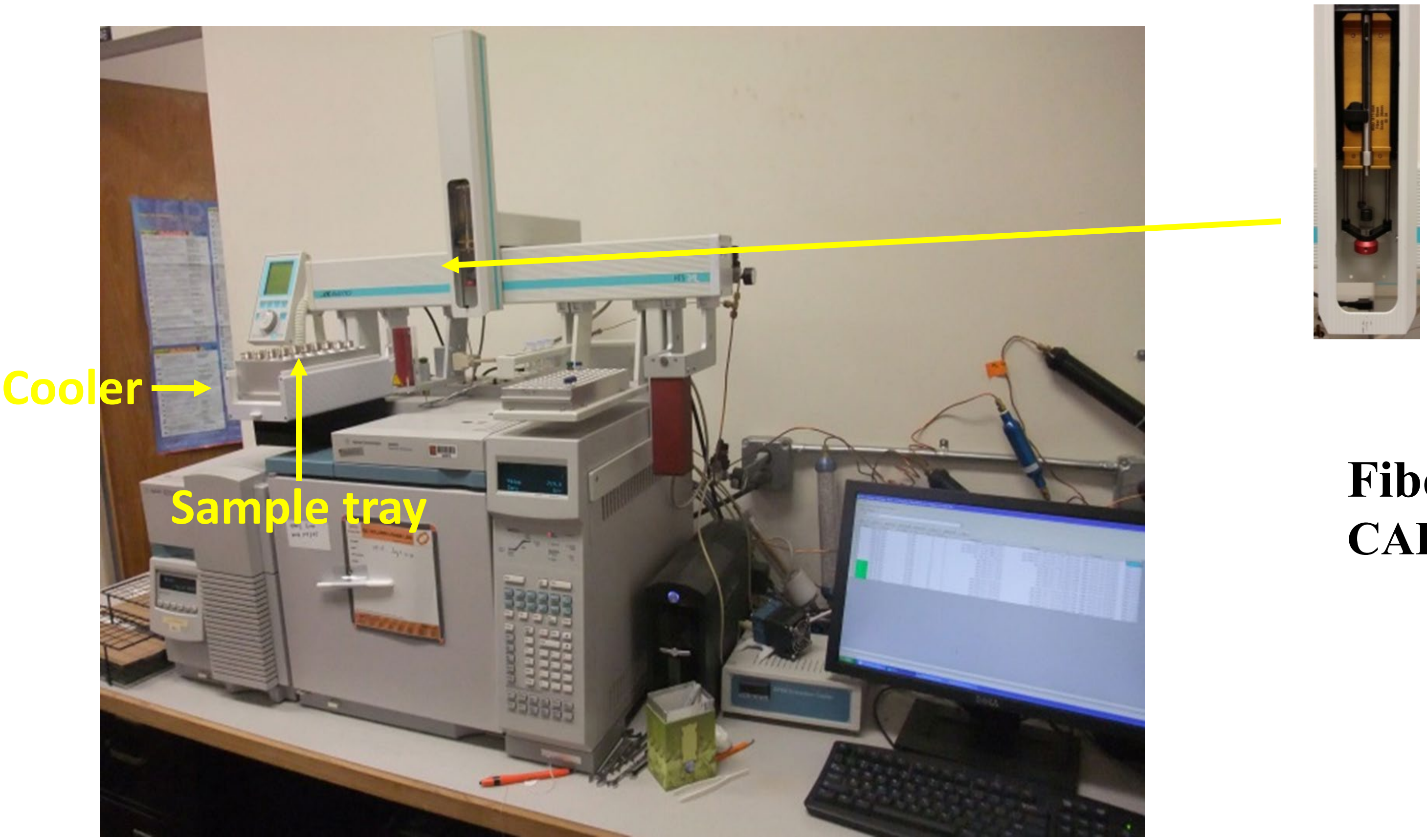
m-cresol

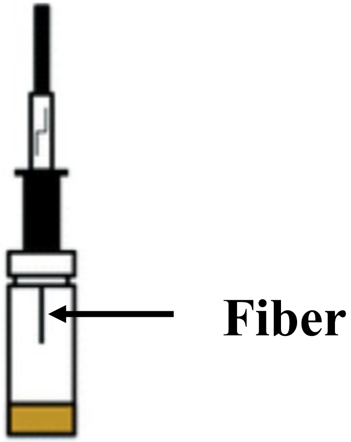
p-cresol

Challenges with smoke analysis

- Volatile phenols exist in normal wine, are part of wine aroma
    - Difficult to distinguish the “good” from the “bad”
- Volatile phenols present at very low concentration
    - Need reliable and sensitive instrumentation and robust analytical method

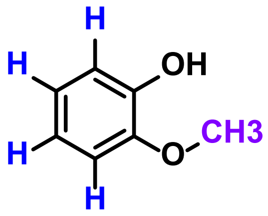
Sensitive volatile phenol analysis by Solid-phase microextraction (SPME)-GC-MS



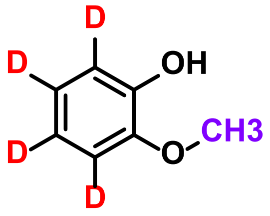


Fiber:
CAR/DVB/PDMS, 2cm

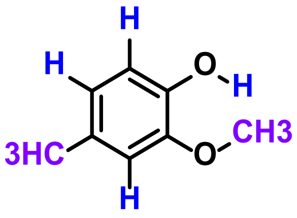
Isotope-labeled Compounds as Internal Standards to Eliminate Wine Matrix Impact



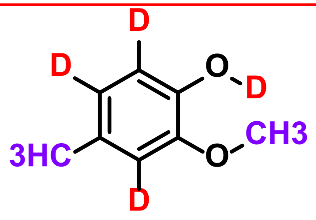
Guaiacol



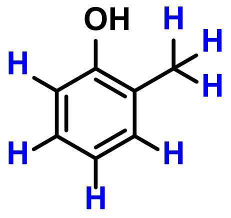
2-Methoxyphenol-3,4,5,6-d4



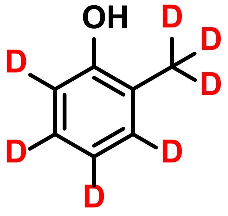
4-Methylguaiacol



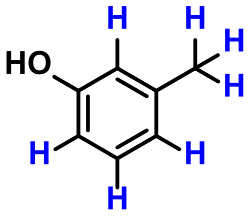
4-methyl-2-methoxyphenol-3,5,6-d3,OD



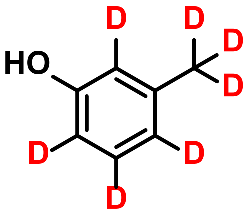
o-Cresol



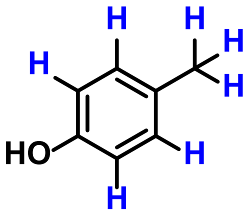
o-Cresol-d7



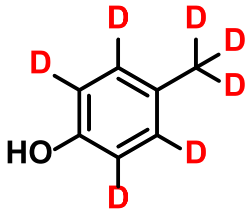
m-Cresol



m-Cresol-d7



p-Cresol



p-Cresol-d7

D- Exact mass 2.0141; **H**- Exact mass 1.0078

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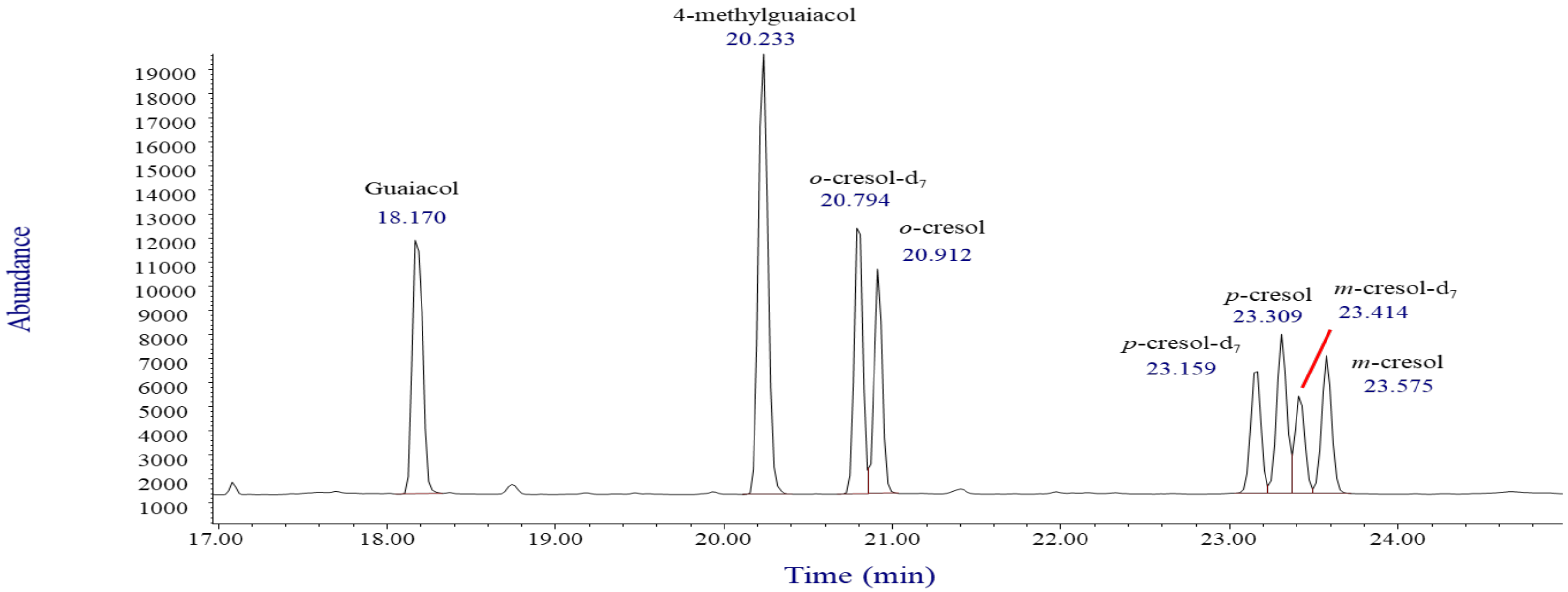
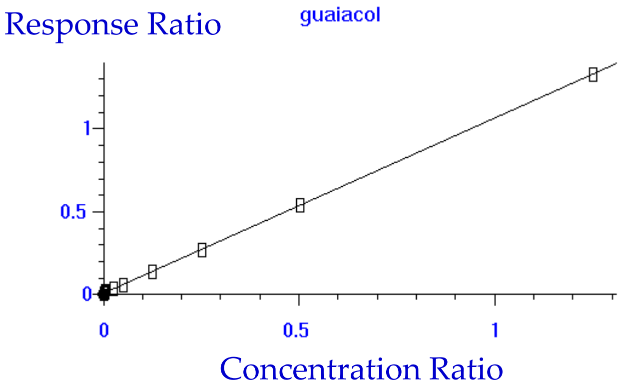
Free Volatile Analysis

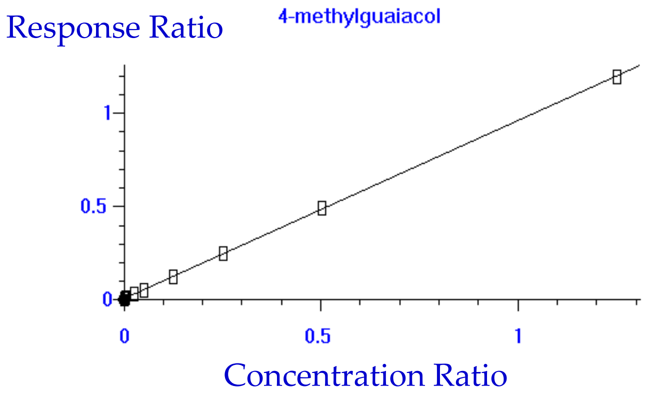


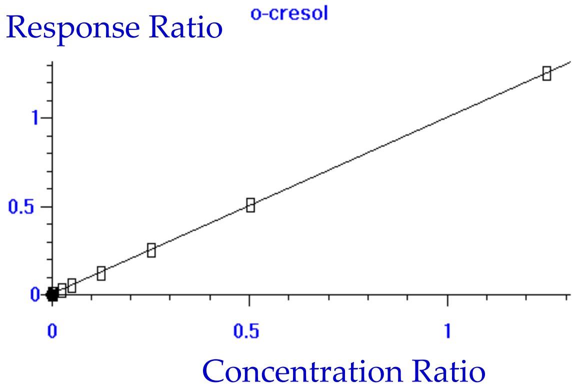
- Free phenol analysis
  - 10 ml wine + 10 ul internal standard
  - Solid-phase Micro-extraction 50°C/25min
  - GC-MS analysis (30 min!)

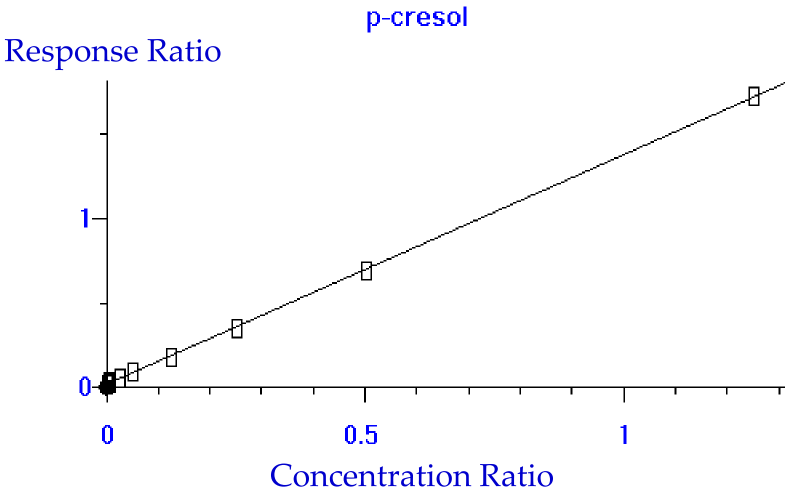
Calibration Curve, Quantitation Limits

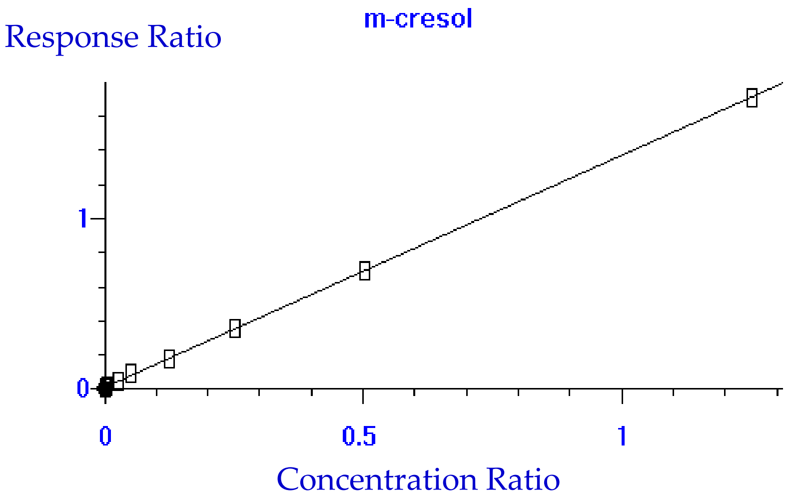
SPME-GC-MS *for total VPs* Standard calibration curves: limitation 0.1 µg/L free, 0.01 µg/L total











Analytical Data Comparison with Commercial Certified Lab (free)

	guaiacol (ug/L)		4-methylguaiacol		o-cresol	p-cresol	m-cresol
	Qian's lab	Cert lab	Qian's lab	Cert lab	(Cert lab did not test cresols)		
sample 1	4.0		1.0		3.3	1.1	1.3
Sample2	3.7	3.9	0.8	0.8	3.1	0.9	1.6
sample 3	4.0	4.2	0.9	1.1	2.8	1.5	1.4
sample 4	3.9	4.0	0.9	1.1	3.0	1.9	1.5
sample 5-1	2.9	3.0	0.7	0.7	2.4	1.2	1.0
sample 5-2 (duplicate)	3.0		0.7		2.5	0.9	1.1
sample 6	3.3	3.4	0.8	0.8	2.3	1.1	1.1
sample 7	4.2	4.4	1.0	1.1	3.0	0.8	1.4
sample 8	6.5		1.7		3.8	1.5	2.2
sample 9	21.3		5.5		8.7	2.7	6.2

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Analytical Quality Control

- Verify check sample for every 20 analysis
- Duplicate analysis every 10 samples

	Guaiacol	4-methylguaiacol	*m*-cresol	*o*-cresol	*p*-cresol
Sample 1-1	12.06	2.36	6.78	5.88	3.67
Sample 1-2	11.85	2.36	6.68	5.95	3.76
CV (%)	1%	0%	1%	1%	2%
Check-1.0 µg/L	1.04	1.06	0.98	1.05	1.07

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Total volatile phenol analysis

- Much more phenol glycosides than free volatile phenols in grapes and wine
- Direct analysis of phenol glycosides by LC-MS is costly and time consuming
- Less expensive method is “total volatile phenol analysis”
  - SPME-GC-MS analysis after strong acid hydrolysis
  - Not reliable due to artifact formation under strong acid condition and low pH
  - No good universal method
  - Results are lab-dependent (varied hydrolysis conditions)

- Total phenol analysis
  - 2 ml wine, pH 1-pH1.2/100°C/4hr
  - 8 mL citrate buffer, pH 3.5
  - SPME 50°C/25min
  - GC-MS analysis

Volatile phenol analysis in smoke exposed wines

- In 2020, we analyzed 377 Smoke exposed red wine, 91 Smoke exposed white (rose) wine
- Different degree of smoke exposure
- Analyzed both free and total volatile phenols

With collaboration with Dr. Elizabeth Tomasino

Objective 1

- Build database information for smoke exposed wine in Oregon and understand volatile phenol correlation in smoke exposed wine

Control Wine Analysis-86 Pinot noir for Baseline

- 21 wines from 2013
- 21 wines from 2014
- 24 wines from 2015
- 20 wines from 2016

- All wines were made commercially in industrial scale
- No barrel aging

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Samples were obtained from Dr. Patty Skinkis

Free Phenols in 86 Control Pinot noir Wine (2013-2016)

Concentration (μg/L)

		guaiacol	4-methylguaiacol	o-cresol	p-cresol	m-cresol
X	Average	6.76	1.98	1.46	1.31	1.16
M	Median	6.59	1.76	1.43	1.20	0.74
Min	Minimum	2.02	0.41	0.55	0.51	0.17
Max	Maximum	13.09	4.86	2.38	2.55	6.87

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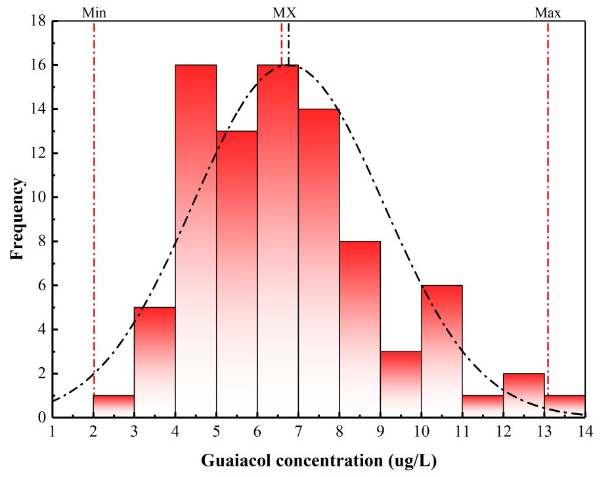
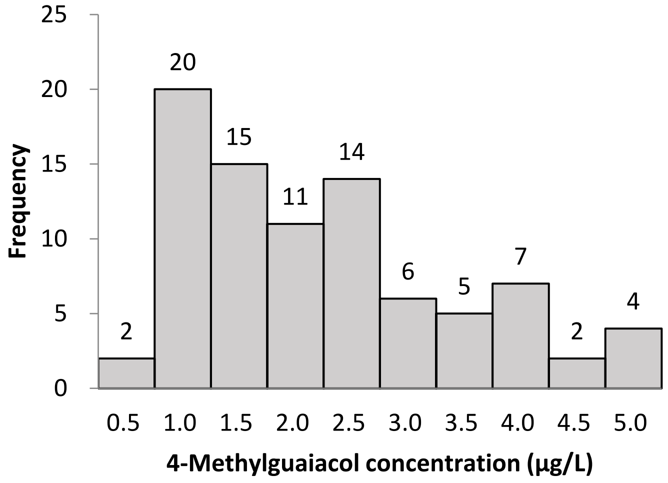
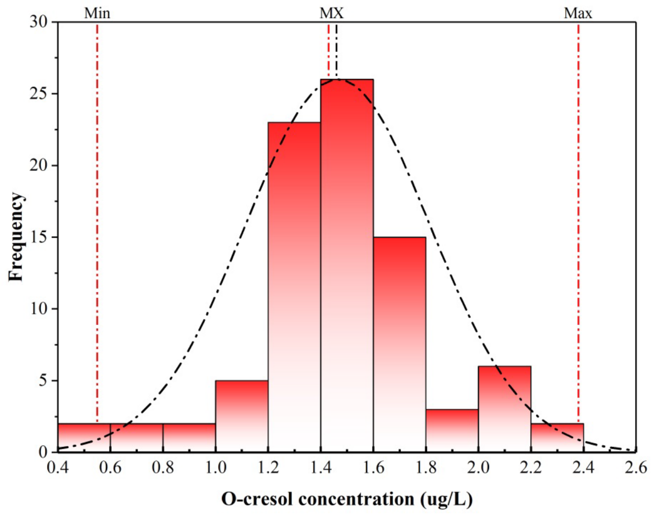
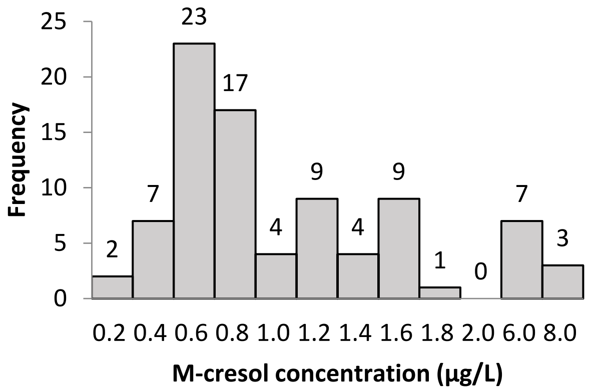
Free phenol concentration distribution in 86 control Pinot noir wine

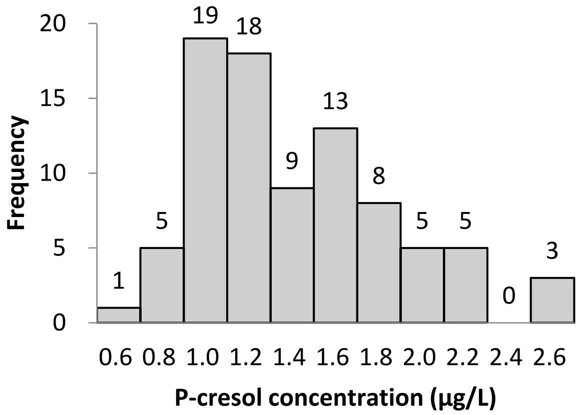
(Number of samples in a concentration range)











Total phenols in 86 Control Pinot noir Wines- 2013-2016

Concentration (µg/L)

		guaiacol	4-methylguaiacol	o-cresol	p-cresol	m-cresol
X	Average	18.5	2.5	4.1	7.2	1.0
M	Median	18.7	2.3	4.1	7.4	0.8
Min	Minimum	8.6	0.7	2.0	2.0	0.4
Max	Maximum	25.1	6.2	7.3	12.1	3.8

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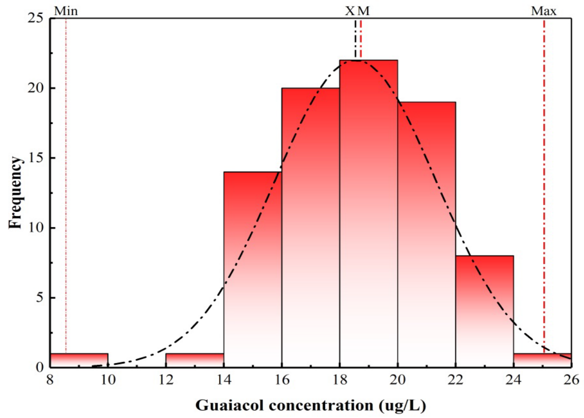
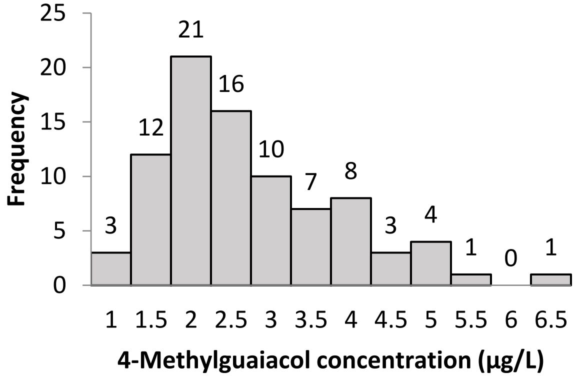
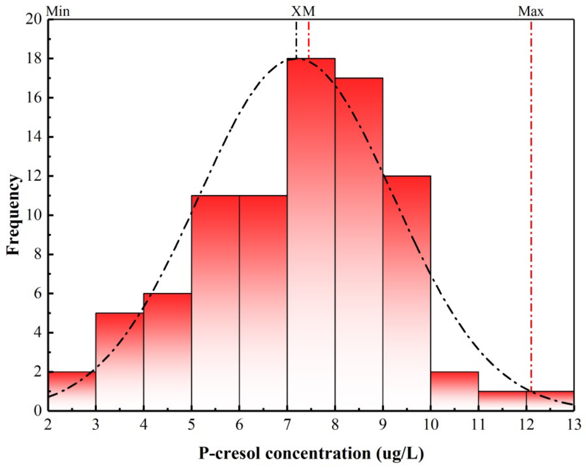
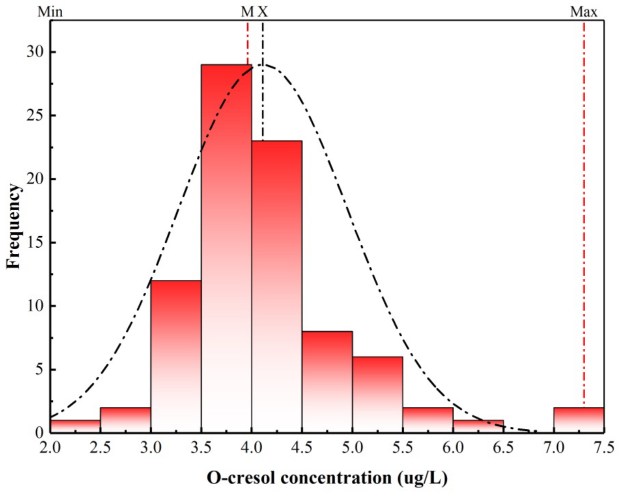
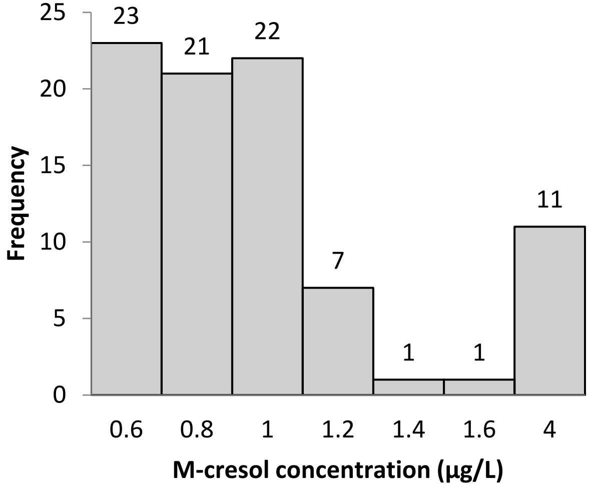
Total Phenol Distribution in 86 Control Pinot noir Wines-2013-2016





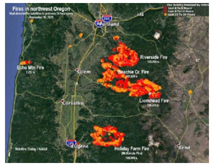




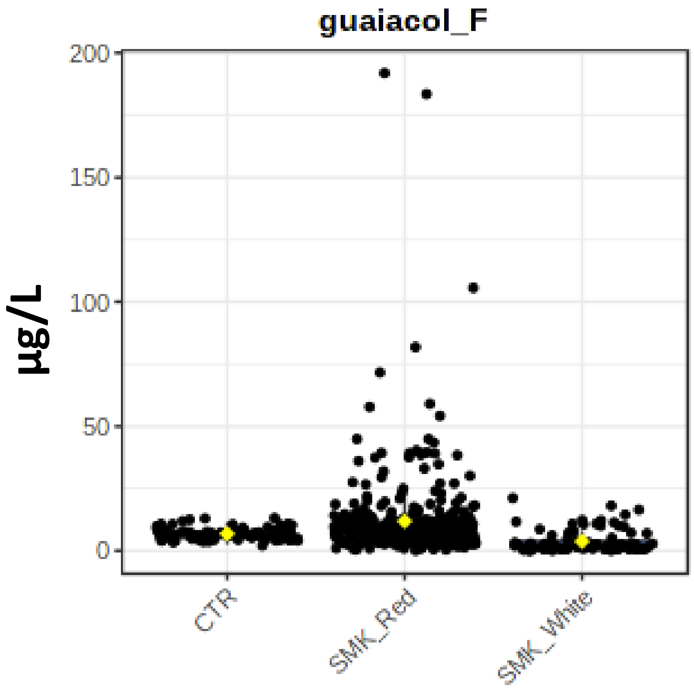


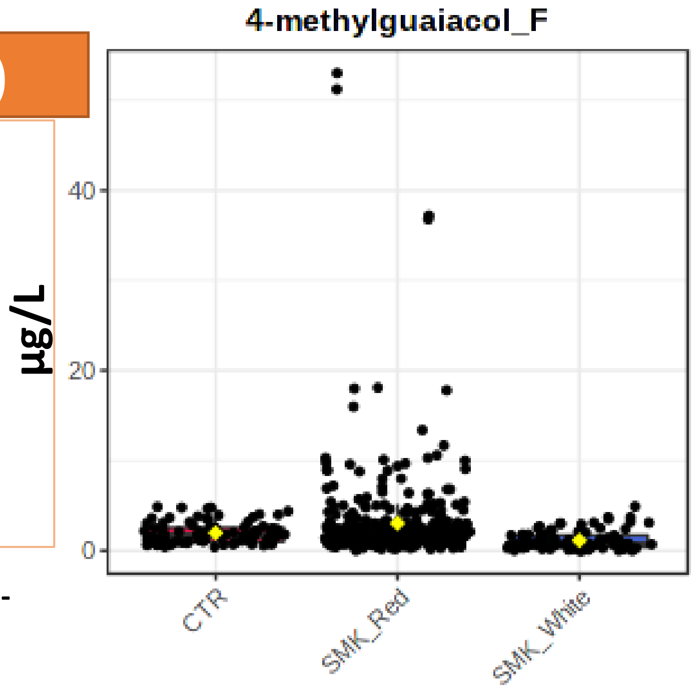
Volatile Phenol Analysis- Free (µg/L)

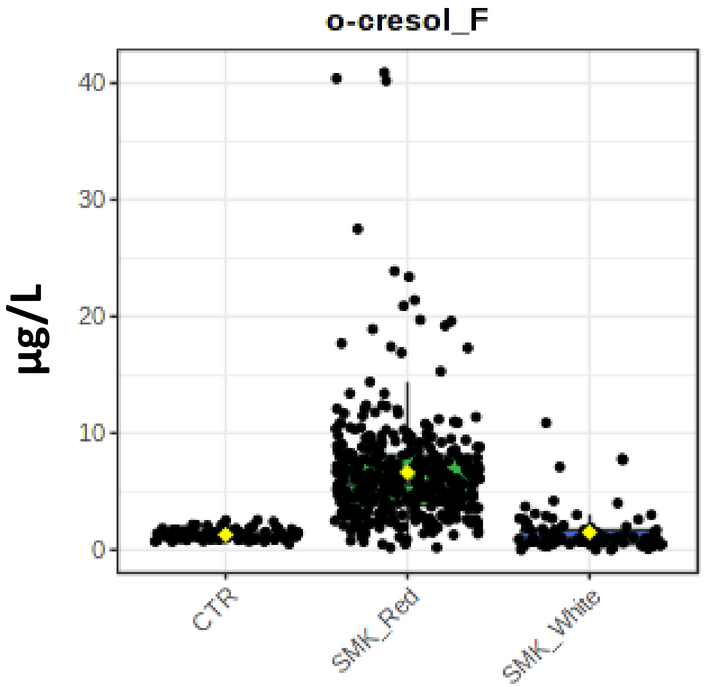
377 Smoke exposed red wine
91 Smoke exposed white (rose) wine
86 Control red wine
Nomenclature
_F- Free form
-T- total after hydrolysis (HCl, 100C/4hr) (µg/L)

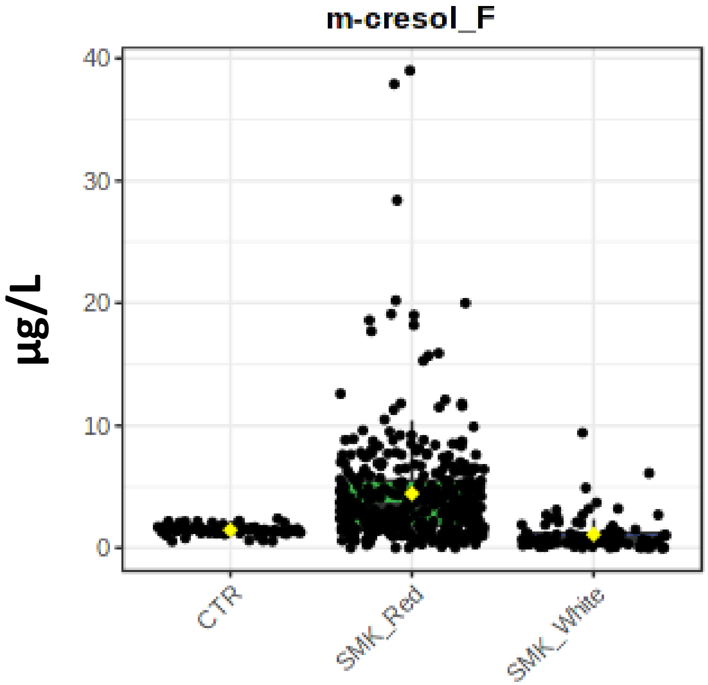


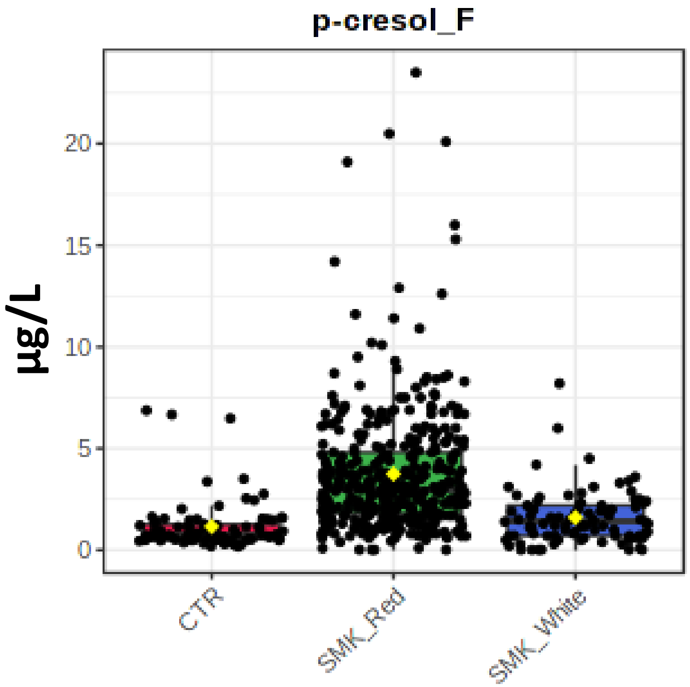
Smoke exposed red wine presents the biggest dispersion of the data. o-,m- and p-cresol exhibit the biggest difference across sample.







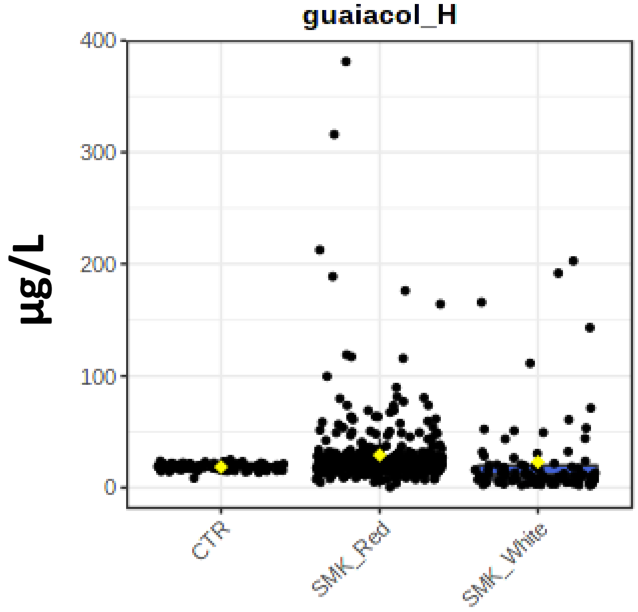


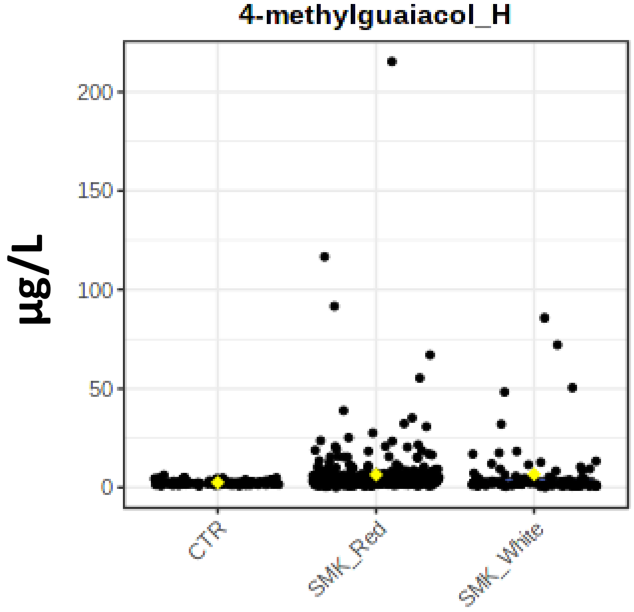


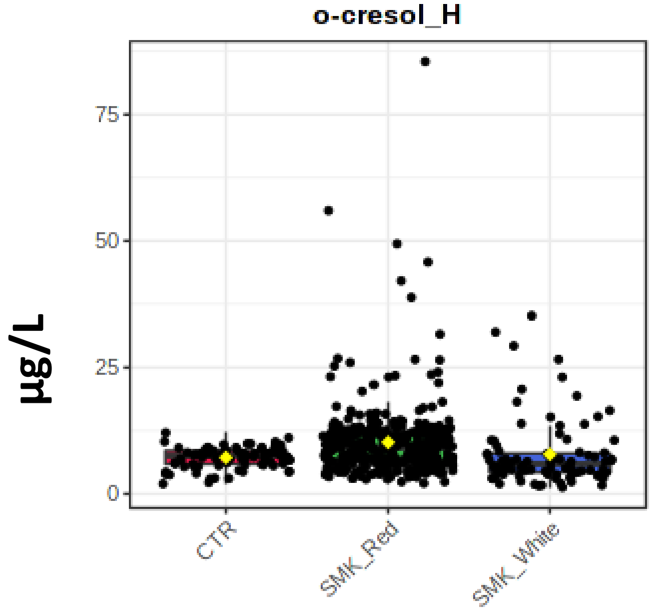
Volatile Phenol Analysis-Total (µg/L)

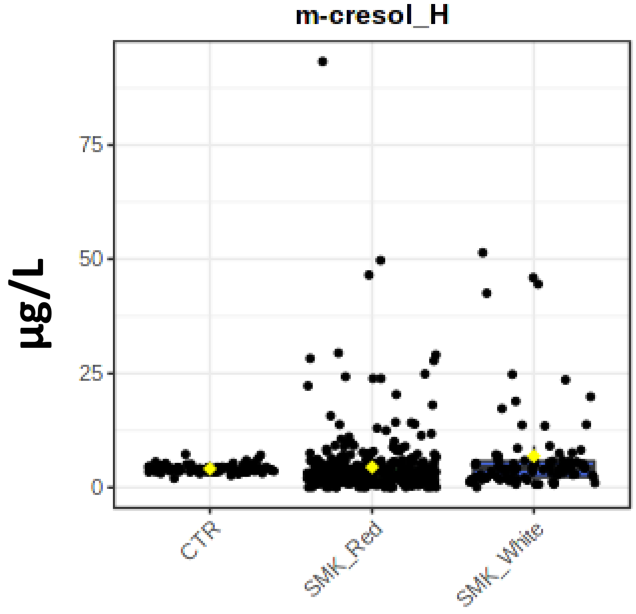
377 Smoke exposed red wine
91 Smoke exposed white wine
86 Control red wine

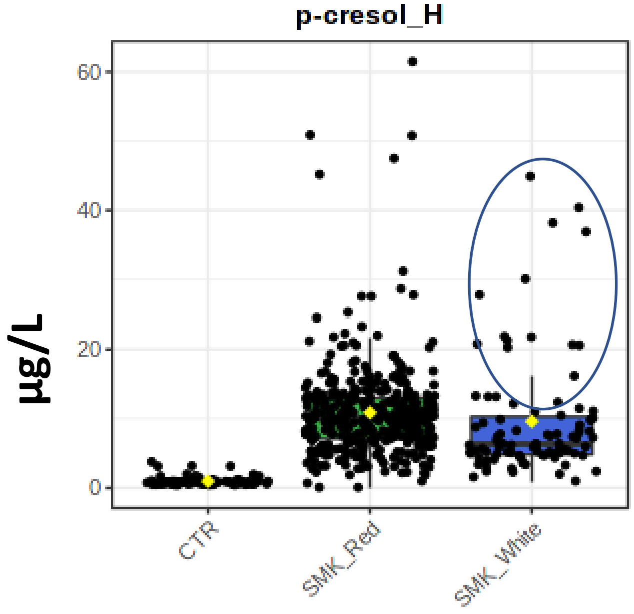
Smoke exposed red wine presents the biggest dispersion of the data. p-cresol exhibited the highest difference.



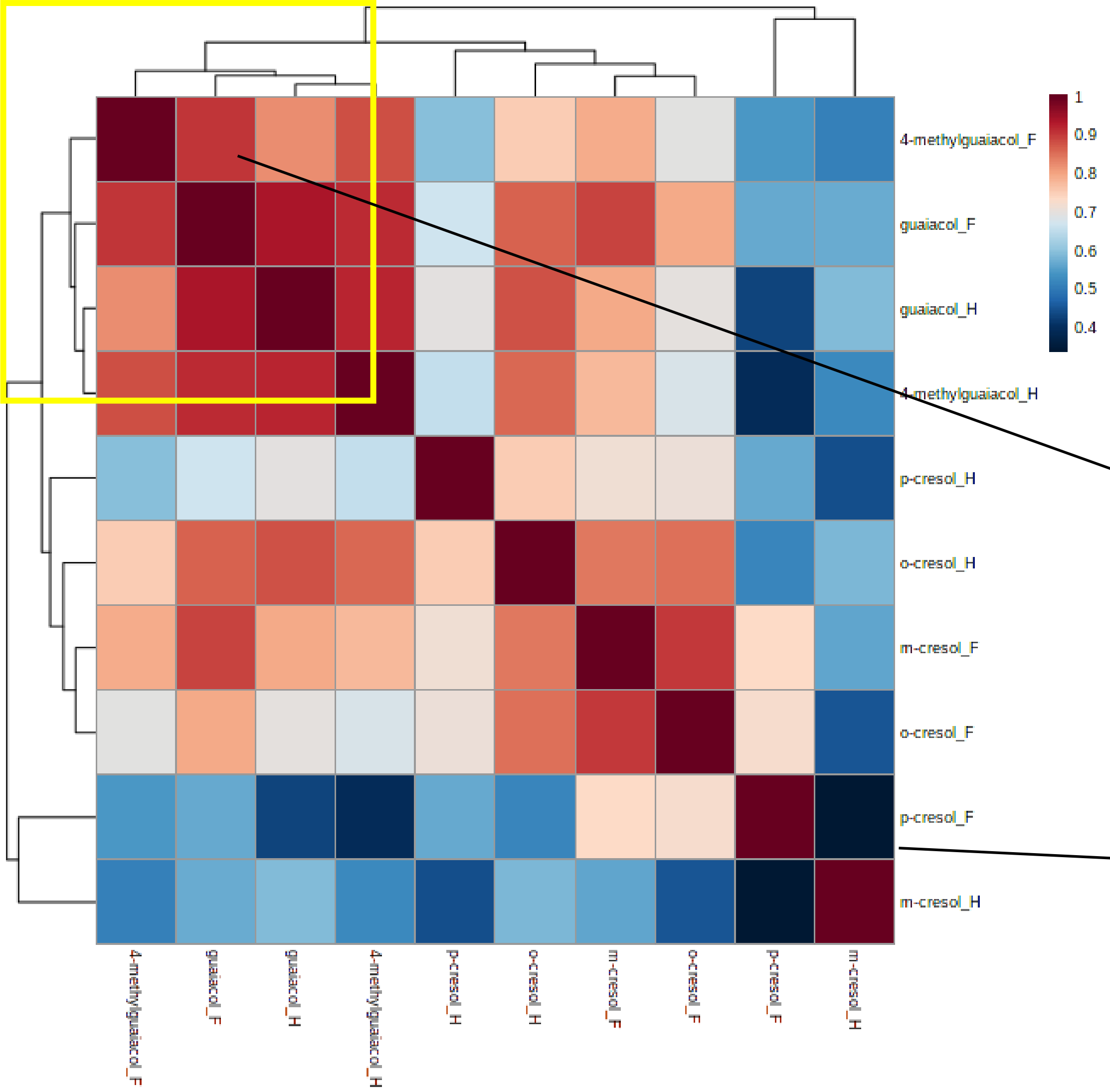








Correlation Heatmap and Matrix



Pearson correlation

Heatmap was used to identify correlation of compounds. The value ranges from -1 to 1. As shown in the heatmap, all correlations are positive, ranging from 0.34 (blue, bound m-cresol vs free p-cresol) to 0.94 (deep red, guaiacol free form vs bound guaiacol).

	4-methylguaiacol_F	guaiacol_F	guaiacol-T	4-methylguaiacol-T	p-cresol-T	o-cresol-T	m-cresol_F	o-cresol_F	p-cresol_F	m-cresol-T
4-methylguaiacol_F	**1.00**	0.91	0.82	0.88	0.60	0.75	0.79	0.69	0.55	0.51
guaiacol_F	0.91	**1.00**	0.94	0.92	0.67	0.86	0.89	0.80	0.57	0.57
guaiacol-T	0.82	0.94	**1.00**	0.92	0.70	0.88	0.80	0.70	0.43	0.59
4-methylguaiacol-T	0.88	0.92	0.92	**1.00**	0.66	0.86	0.78	0.68	0.40	0.52
p-cresol-T	0.60	0.67	0.70	0.66	**1.00**	0.75	0.72	0.71	0.57	0.45
o-cresol-T	0.75	0.86	0.88	0.86	0.75	**1.00**	0.84	0.85	0.52	0.59
m-cresol_F	0.79	0.89	0.80	0.78	0.72	0.84	**1.00**	0.90	0.74	0.56
o-cresol_F	0.69	0.80	0.70	0.68	0.71	0.85	0.90	**1.00**	0.73	0.45
p-cresol_F	0.55	0.57	0.43	0.40	0.57	0.52	0.74	0.73	**1.00**	0.34
m-cresol-T	0.51	0.57	0.59	0.52	0.45	0.59	0.56	0.45	0.34	**1.00**

Control Red Wine vs Smoke Exposed Red Wine (t-test)

377 Smoke exposed red wine
86 Control red wine

Nomenclature
_F- Free form
-T- After hydrolysis (HCl, 100C/4hr) (µg/L)

Smoke exposed red/ CTR red wine

	FC	log2(FC)	P-pval
p-cresol_T	11.12	3.47	3.9E-35
o-cresol_F	5.05	2.34	2.5E-21
p-cresol_F	3.23	1.69	1.7E-13
m-cresol_F	3.03	1.60	9.2E-10
o-cresol_T	1.42	0.51	1.7E-04
guaiacol_T	1.57	0.66	3.1E-03
guaiacol_F	1.75	0.81	6.7E-03
4-methylguaiacol_T	2.57	1.36	1.3E-02
4-methylguaiacol_F	1.54	0.62	5.5E-02
m-cresol_T	1.09	0.12	6.5E-01

Univariate data analysis was performed to establish differences between control and smoke exposed red wine.

Total *p*-cresol is most discriminating compound for smoke exposure, followed by free o-,p-,m-cresol.

With exception of free 4-methylguaiacol and total m-cresol, all compounds were significantly higher in smoke exposed wine (p>.05). After hydrolysis, total p-cresol presented the most significant difference (FC =11.12, p=3.9 X10-35).

Major findings

- Smoke exposed red wine presents the biggest dispersion of the data. o-,m- and p-cresol exhibit the biggest difference across sample.
- Free guaiacol is highly correlated with free 4-methylguaiacol in smoke exposed wine
- Total guaiacol is highly corelated with free guaiacol
- m-cresol and o-cresol is highly correlated

Objective 2

- Understand how the phenol glycosides are related to the volatile phenols

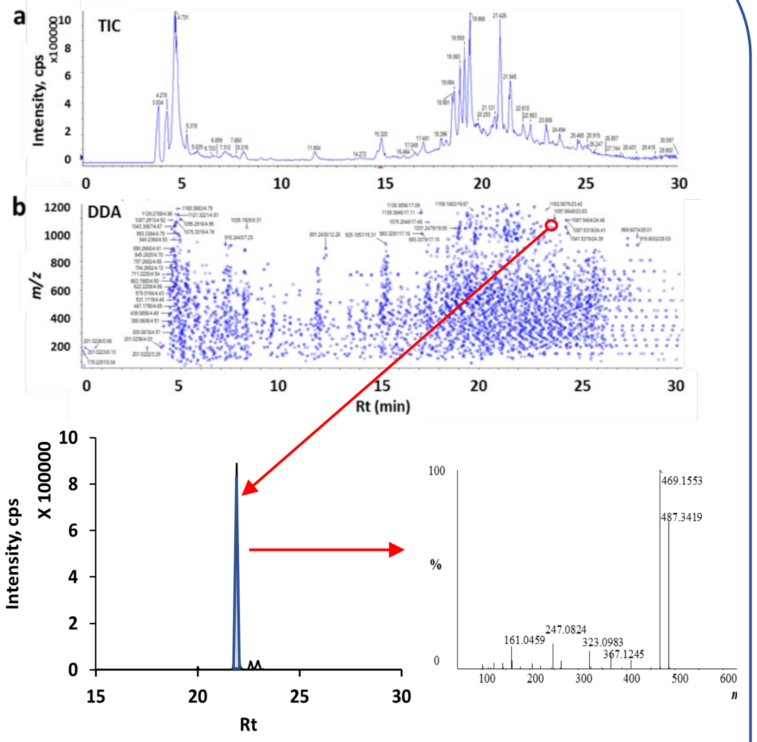
  - Selected 26 different degree of smoke-exposed wine and 14 control wines
  - LC-MS glycoside analysis
  - GC-MS analysis for free and total volatile phenols

LC MS/MS Mass Spectral Fingerprinting and Quantification of Marker Compounds (14 control, 26 smoke exposed wine)

Sample

Sample preparation

LC-HRMS: DDA
Data-dependent acquisition



- Precursor exact mass
- Fragment ion information
- Theoretical isotopic distribution
- Retention time (authentic standards)

Targeted

Non-targeted (Metabolomics)

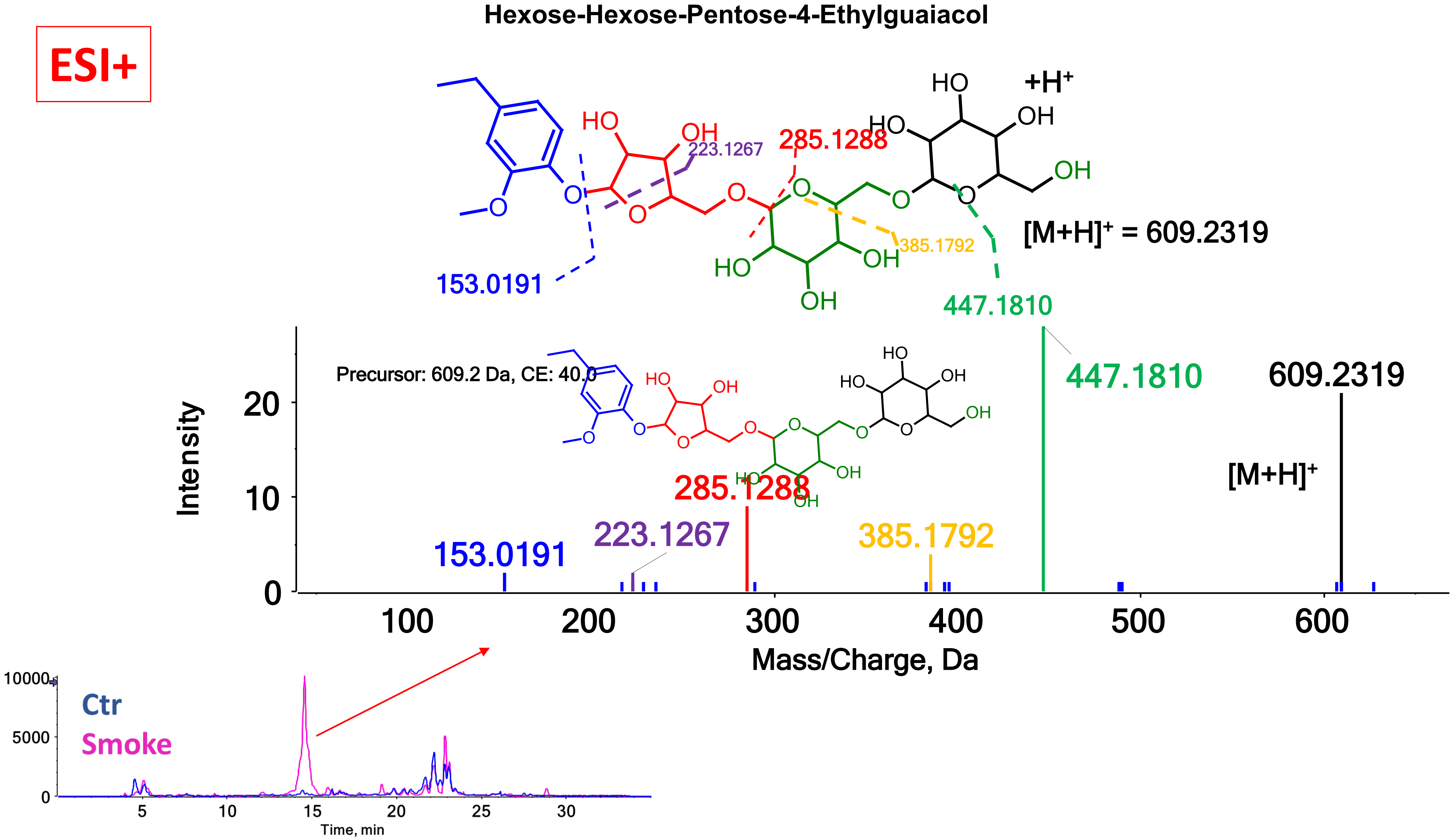
Verification with authentic standards

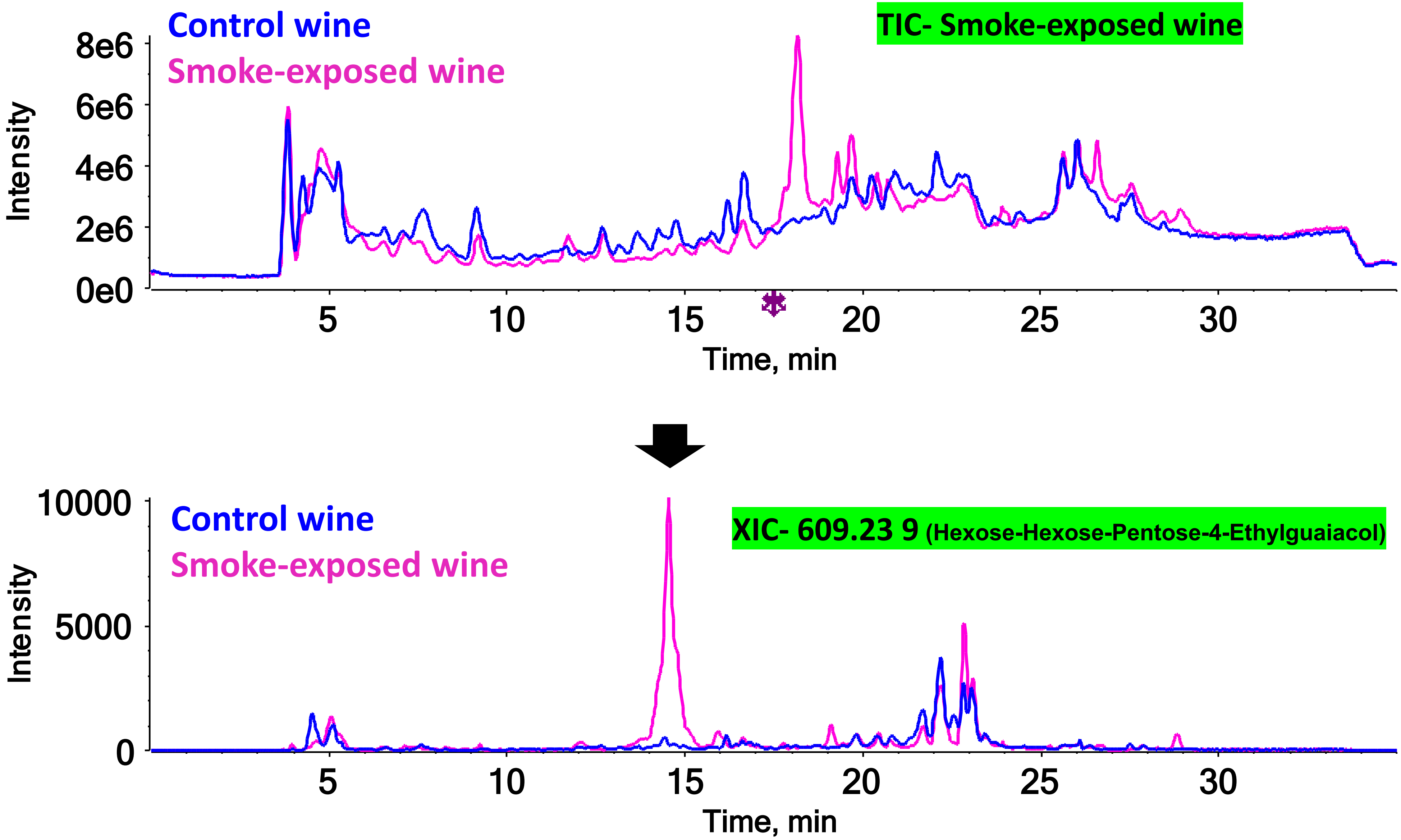
External calibration & Quantification. LOD & LOQ

Quantification of phytochemicals

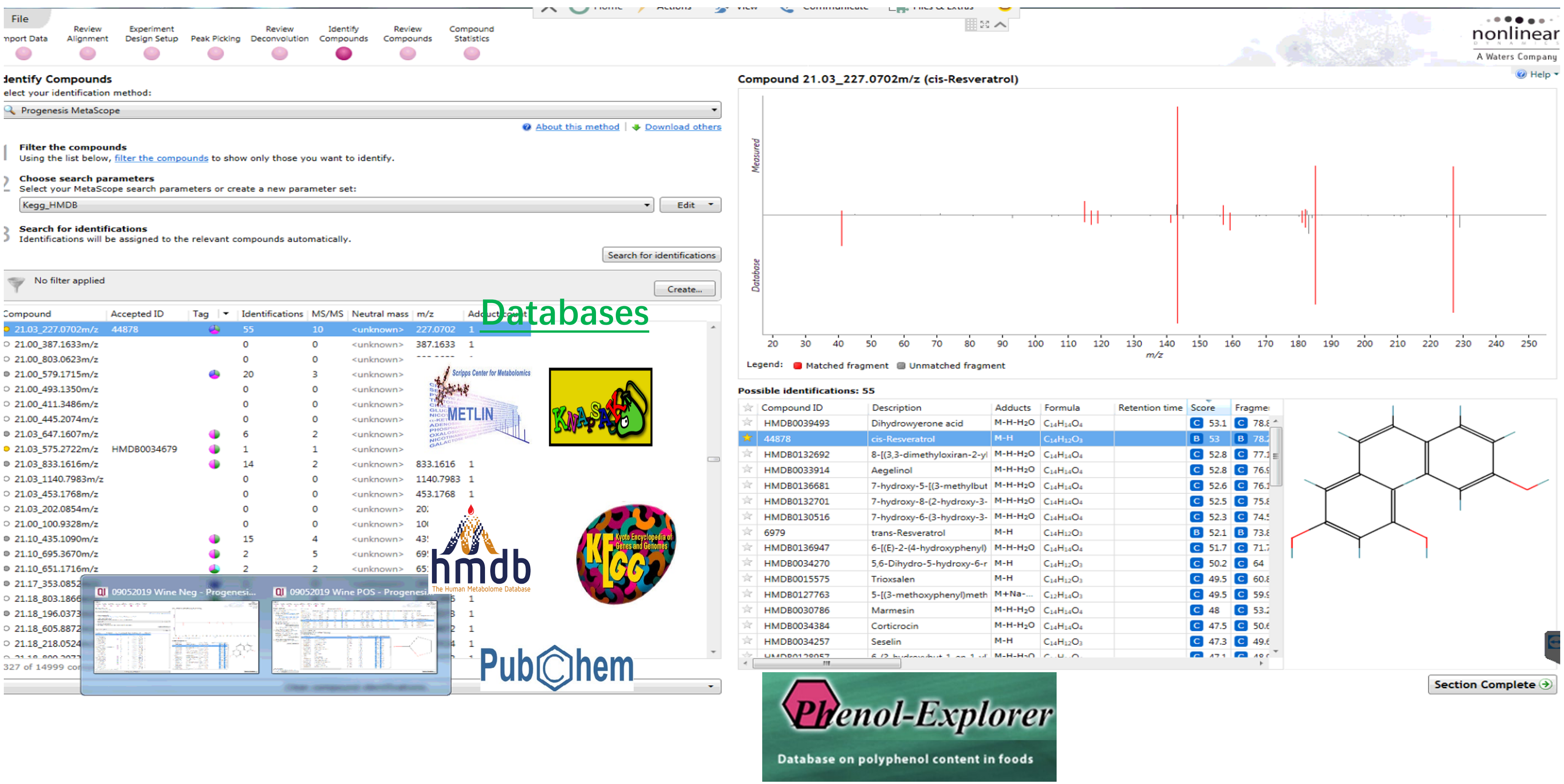
Data Processing & online database searching and matching

Compound library, product integrity, chemical fingerprint, legacy databases

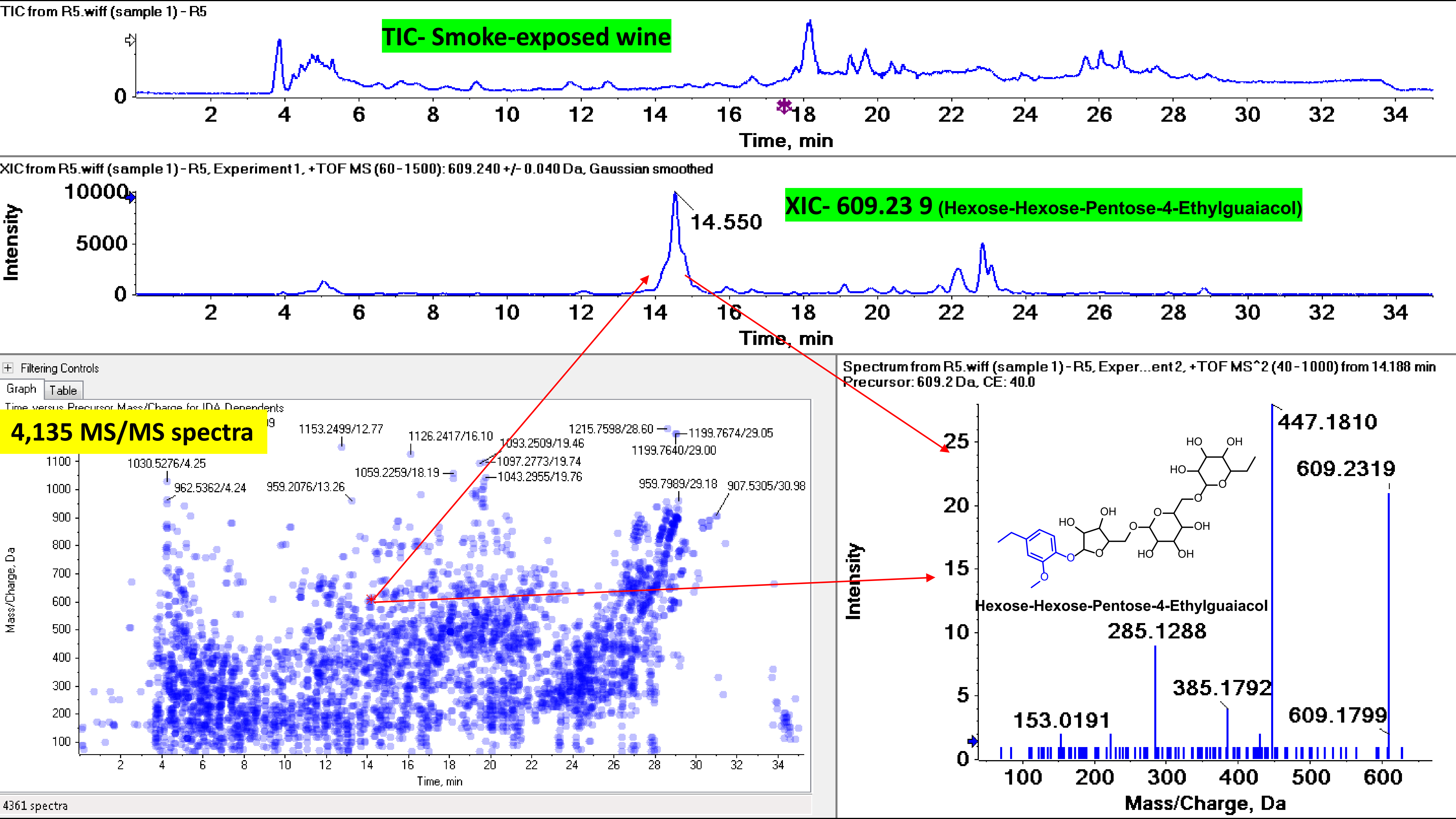

Hexose-Hexose-Pentose-4-Ethylguaiacol
ESI+
+H+
[M+H]+ = 609.2319
153.0191
223.1267
285.1288
385.1792
447.1810
HO
OH
O
Precursor: 609.2 Da, CE: 40.0
Intensity
Mass/Charge, Da
609.2319
[M+H]+
0
10
20
100
200
300
400
500
600
10000
5000
Ctr
Smoke
5
15
25
30
Time, min


Control wine
Smoke-exposed wine
TIC- Smoke-exposed wine
8e6
6e6
4e6
2e6
0e0
Intensity
5
10
15
20
25
30
Time, min
Control wine
Smoke-exposed wine
XIC- 609.23 9 (Hexose-Hexose-Pentose-4-Ethylguaiacol)
10000
5000
0
Intensity
5
10
15
20
25
30
Time, min

Latest Metabolomics-Data Analysis in Progenesis QI

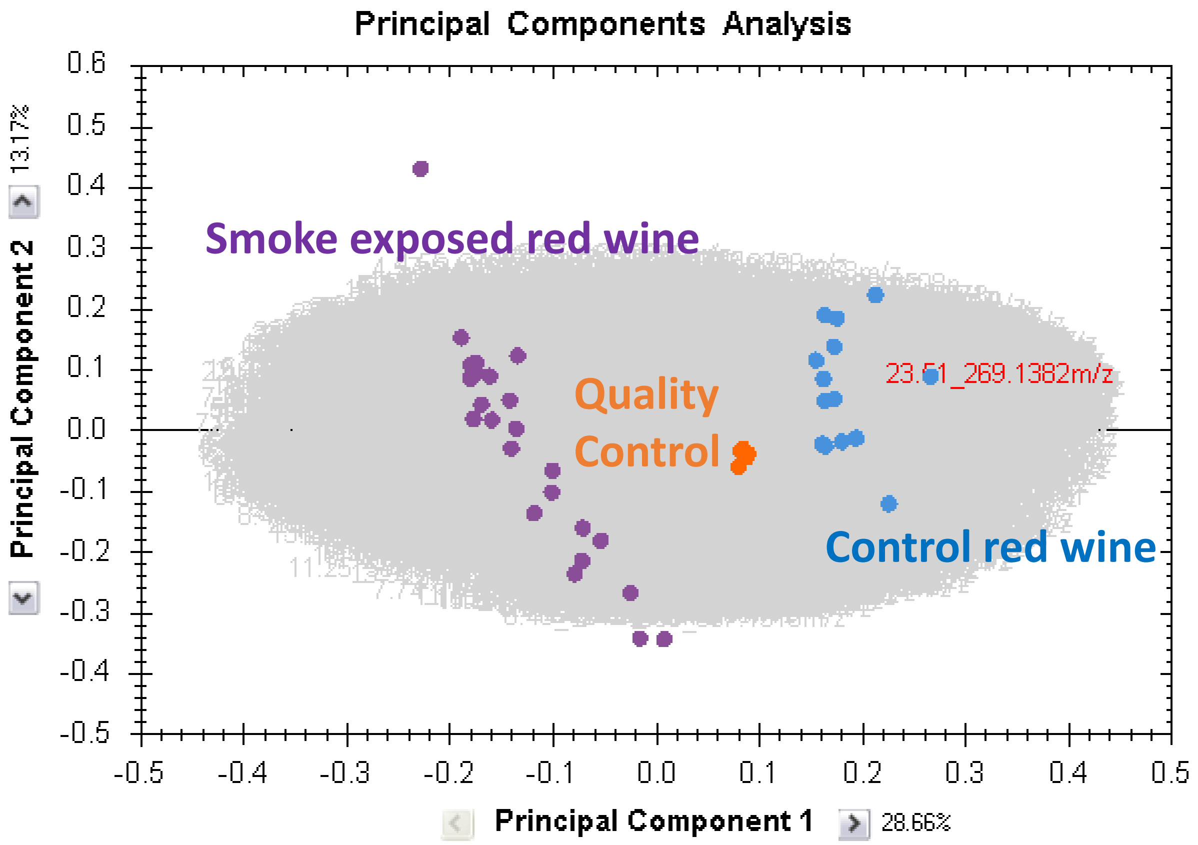


https://www.genome.jp/kegg/ https://metlin.scripps.edu https://hmdb.ca/ http://www.knapsackfamily.com/ http://phenol-explorer.eu/



TIC from R5.wiff (sample 1) - R5
TIC- Smoke-exposed wine
Time, min
XIC from R5.wiff (sample 1) - R5, Experiment 1, +TOF MS (60 - 1500): 609.240 +/- 0.040 Da, Gaussian smoothed
XIC- 609.23 9 (Hexose-Hexose-Pentose-4-Ethylguaiacol)
14.550
Intensity
Time, min
Filtering Controls
Graph
Table
Time versus Precursor Mass/Charge for IDA Dependents
4,135 MS/MS spectra
1153.2499/12.77
1126.2417/16.10
1093.2509/19.46
1215.7598/28.60
1199.7674/29.05
1199.7640/29.00
1097.2773/19.74
1043.2955/19.76
1030.5276/4.25
962.5362/4.24
959.2076/13.26
1059.2259/18.19
959.7989/29.18
907.5305/30.98
Mass/Charge, Da
Time, min
4361 spectra
Spectrum from R5.wiff (sample 1) - R5, Exper...ent 2, +TOF MS^2 (40 - 1000) from 14.188 min
Precursor: 609.2 Da, CE: 40.0
447.1810
609.2319
Hexose-Hexose-Pentose-4-Ethylguaiacol
285.1288
385.1792
153.0191
609.1799
Intensity
Mass/Charge, Da

LC-HRMS/MS Untargeted Metabolomics Analysis of Wine (14 control, 26 smoke exposed wine)



We obtained more than 4000 ions by MS/MS

Control wines and smoke exposed wines are well clustered by PCA analysis

We are continuing with data mining to figure out underlying relationship

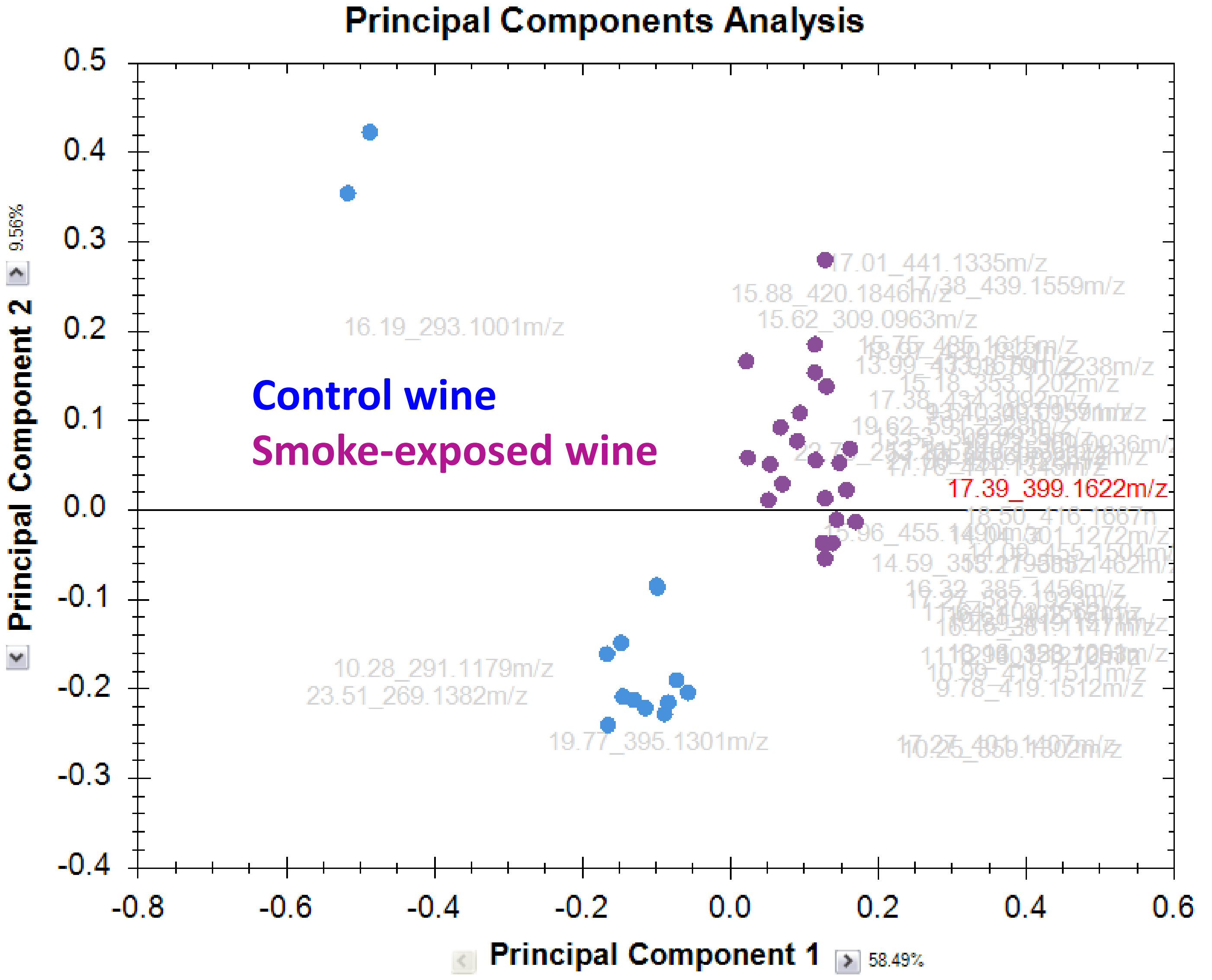
LC-HRMS/MS targeted phenol glycosides Analysis

35 tentative annotated volatile phenol glycosides

Accepted Compound ID	Adducts detected	Formula	Mass Error (ppm)
syringyl-β-D-glucopyranoside_Iso	M+H, M+NH4, M+Na	C14H20O8	-3.14
syringyl-β-D-glucopyranoside	M+NH4, M+Na	C14H20O8	-2.24
DeoxyH-H-Cresol	M+NH4, M+Na	C19H28O10	-3.50
DeoxyH-H-Cresol_iso	M+Na	C19H28O10	-3.69
DeoxyH-H-P-Phenol	M+Na	C23H34O14	-4.10
DeoxyH-P-4-Ethylguaiacol	M+Na	C20H30O10	-1.93
guaiacyl-β-D-gentiobioside -Der	M+NH4	C19H28O12	-4.50
guaiacyl-β-D-gentiobioside	M+Na	C19H28O12	-4.12
guaiacyl-β-D-gentiobioside_2	M+Na	C19H28O12	-4.11
guaiacyl-β-D-gentiobioside_3	M+Na	C19H28O12	7.61
H-4-Methylguaiacol	M+NH4	C14H20O7	-3.68
H-4-Methylguaiacol_2	M+H	C14H20O7	-3.18
H-4-Methylsyringol	M+Na	C15H22O8	-1.53
H-Guaiacol	M+Na	C13H18O7	-2.00
H-Guaiacol_2	M+Na	C13H18O7	-3.35
H-Guaiacol_3	M+Na	C13H18O7	-3.56
H-H-P-4-Ethylguaiacol_Iso1	M+H-H2O	C24H36O14	-7.46
H-H-P-4-Ethylguaiacol	M+H	C24H36O14	-7.68
H-H-P-4-Ethylguaiacol_iso2	M+H-H2O	C24H36O14	-9.13
HMDB0041514	M+NH4	C18H26O10	-4.49
H-P-4-Methylguaiacol_iso	M+Na	C19H28O11	-4.63
H-P-4-Methylguaiacol	M+H	C19H28O11	-7.97
H-P-Guaiacol	M+Na	C18H26O11	-7.79
H-P-Guaiacol_Iso1	M+Na	C18H26O11	-5.29
H-P-Guaiacol_so2	M+Na	C18H26O11	-3.63
H-P-Guaiacol_iso3	M+Na	C18H26O11	-4.93
H-P-P-4-Methylguaiacol	M+Na	C24H36O15	-4.72
P-H-Cresol	M+H	C18H26O10	-6.84
P-H-Cresol_iso2	M+H-H2O	C18H26O10	-7.78
P-H-Cresol_iso3	M+H-H2O	C18H26O10	-9.25
P-H-Cresol_iso4	M+H, M+NH4, M+Na	C18H26O10	-3.61
P-P-H-Cresol	M+Na	C23H34O14	-2.80
syringyl-β-D-gentiobioside	M+NH4	C20H30O13	-4.53
syringyl-β-D-gentiobioside_iso1	M+H, M+Na	C20H30O13	-8.89
syringyl-β-D-gentiobiosideIiso2	M+H-H2O	C20H30O13	-8.01

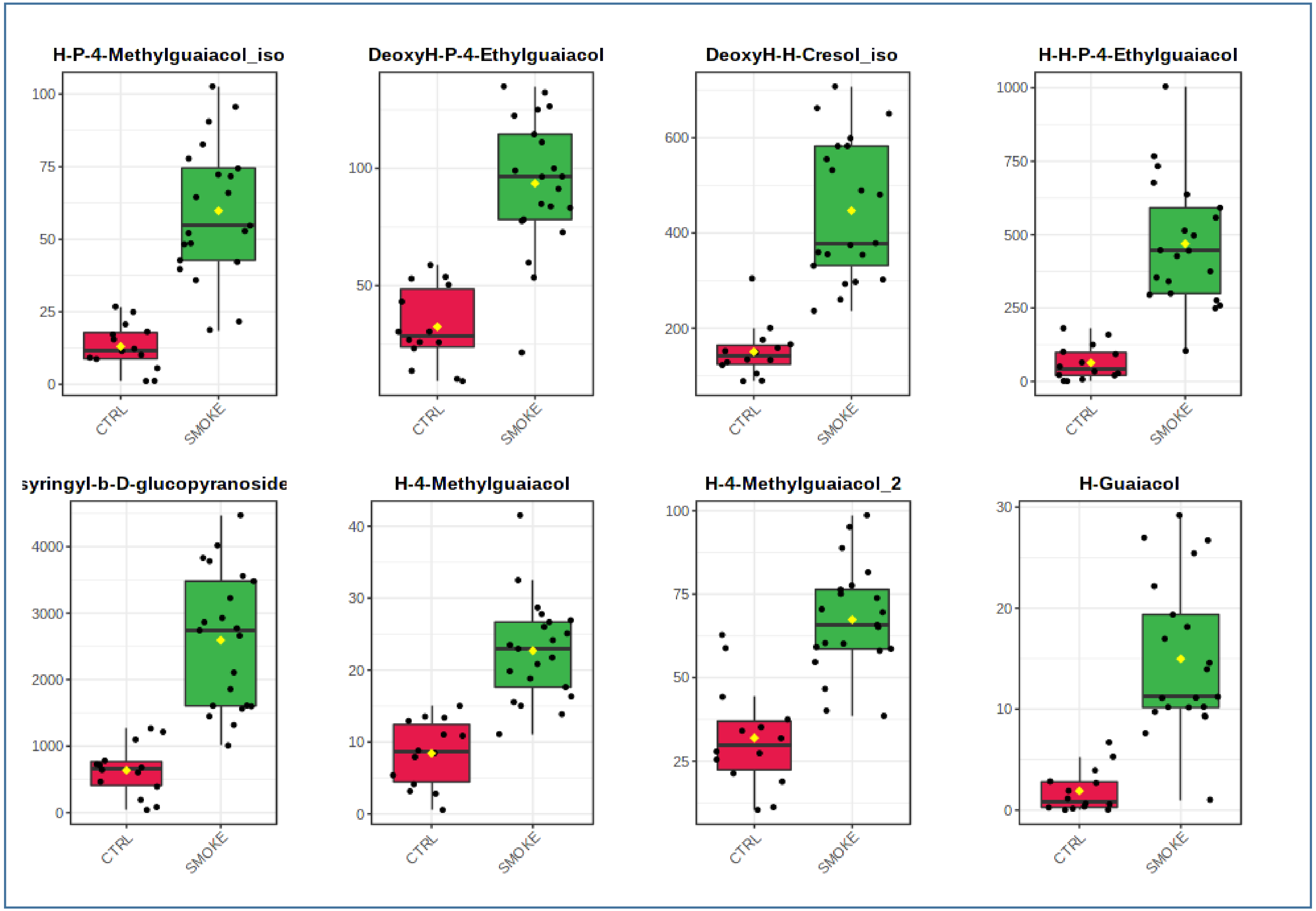
H-Hexose, **P-** Pentose, **Iso-** Isomer

PCA- Using 35 tentative annotated volatile phenol glycosides



T-test

Top 8, volatile phenol glycosides ($p < 4*10^{-7}$)

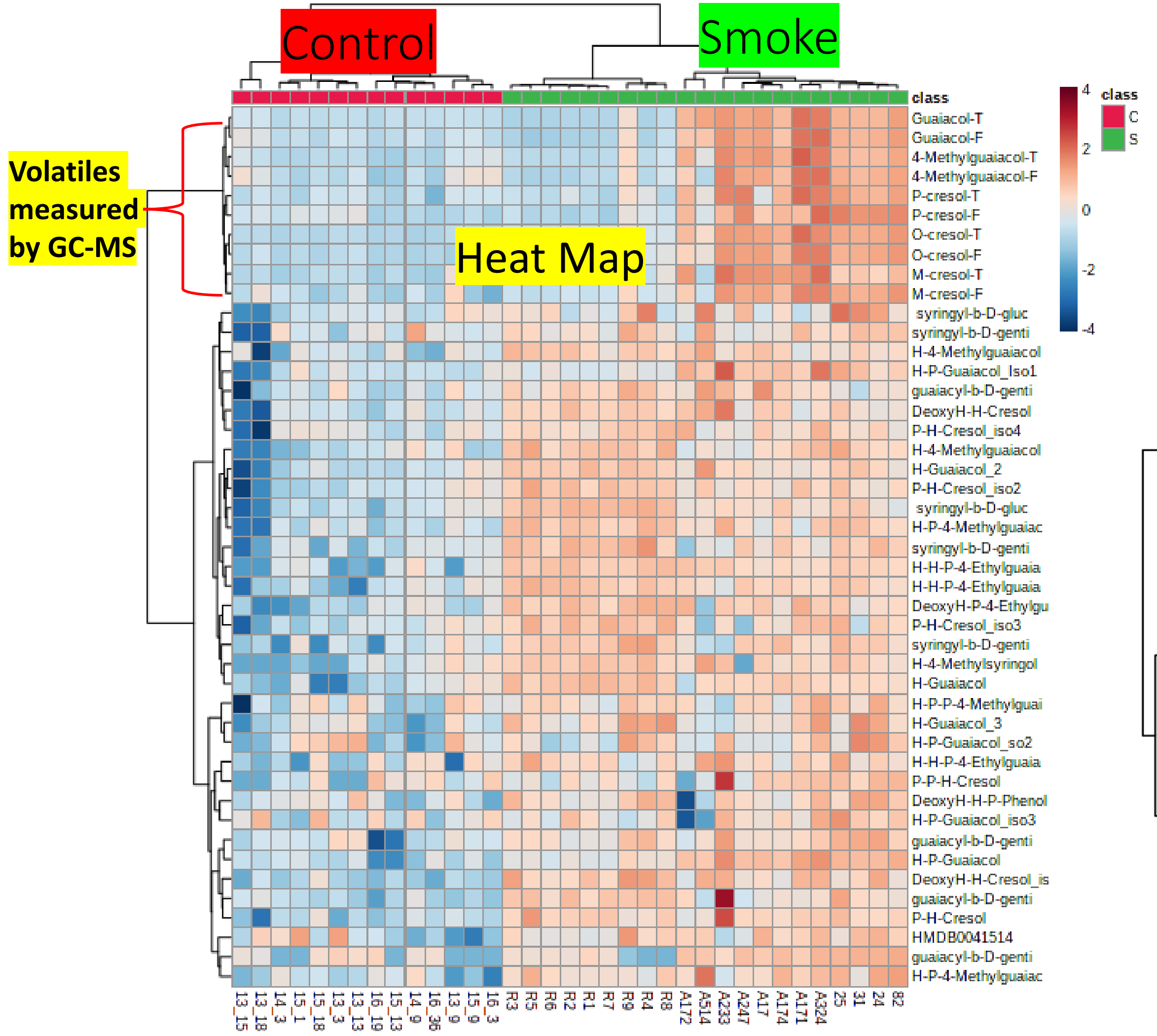


Top 15, volatile phenol glycosides

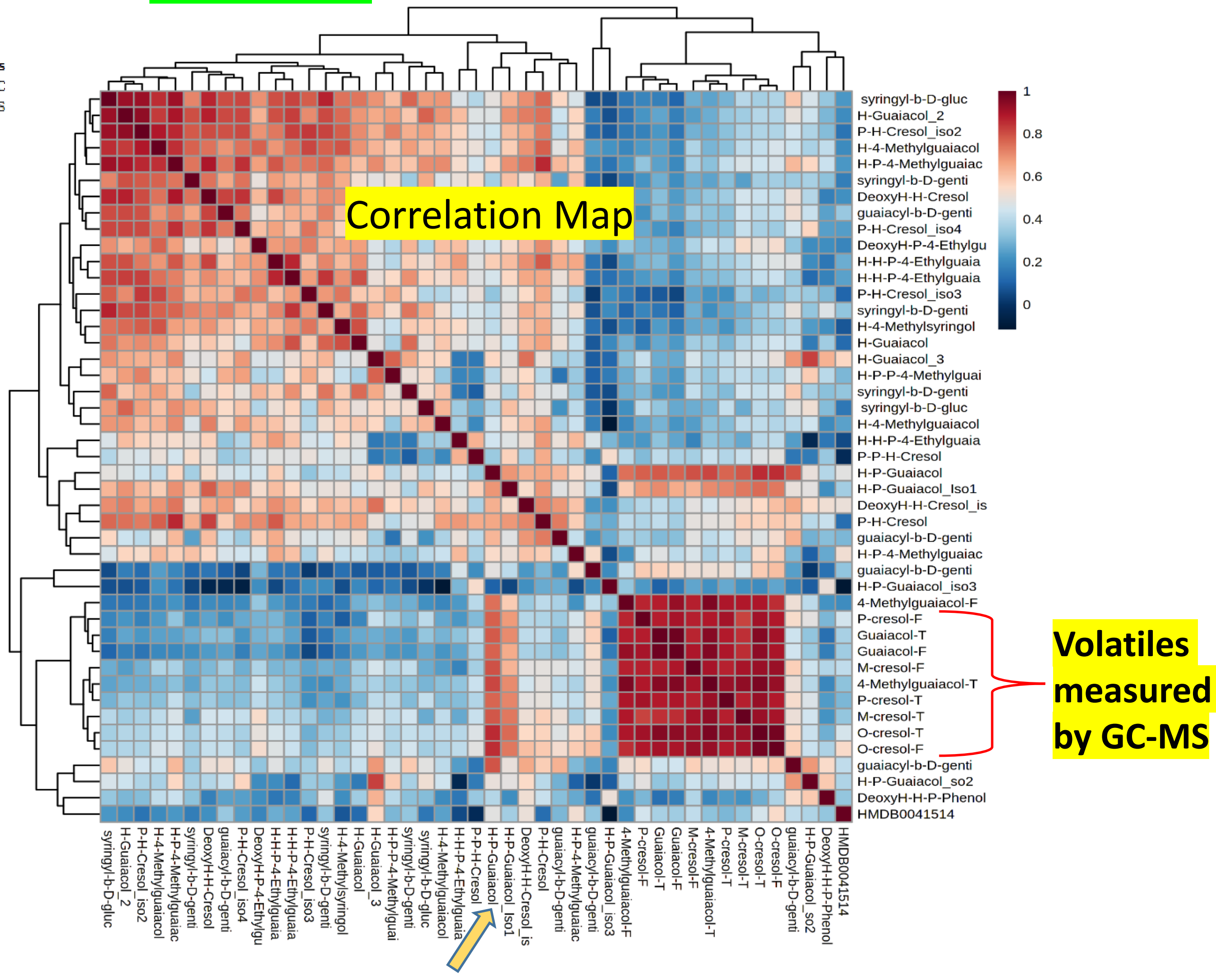
Tentative glycoside	FC SMK/CTR	raw.pval
H-P-4-Methylguaiacol_iso	4.58	2.37E-08
DeoxyH-P-4-Ethylguaiacol	2.88	2.59E-08
DeoxyH-H-Cresol_iso	2.97	3.02E-08
H-H-P-4-Ethylguaiacol	7.39	6.61E-08
syringyl-b-D-glucopyranoside_Iso	4.08	9.50E-08
H-4-Methylguaiacol	2.69	1.26E-07
H-4-Methylguaiacol_iso1	2.11	2.52E-07
H-Guaiacol	7.88	3.95E-07
P-H-Cresol_iso2	3.90	5.13E-07
syringyl-b-D-gentiobioside_iso1	2.60	8.96E-07
H-H-P-4-Ethylguaiacol_Iso1	31.88	2.21E-05
P-H-Cresol_iso3	3.52	3.23E-05
H-P-4-Methylguaiacol	2.66	4.37E-05
DeoxyH-H-Cresol	3.13	5.73E-05
syringyl-b-D-gentiobioside	5.65	8.64E-05

H-Hexose, **P-** Pentose, **Iso-** Isomer

Correlation and heatmap for 35 tentative annotated volatile phenol glycosides + total and free volatiles: *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, and 4-Methylguaiacol.



Total and free *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, and 4-methylguaiacol were positive correlated with all volatile phenol glycosides. Approximately 60 % of such correlations were statistically



Take home message from LC-MS glycoside analysis

- With very few exceptions, volatiles measured by GC-MS and phenol glycosides by LC-MS were positively correlated
- This means higher concentration of volatile phenols predict higher concentrations of phenol glycosides and vice-versa.

Objective 3

- How bad is my wine if exposed to smoke?

Smoke exposed ≠ Smoke taint

Machine learning and modeling for smoke evaluation and prediction

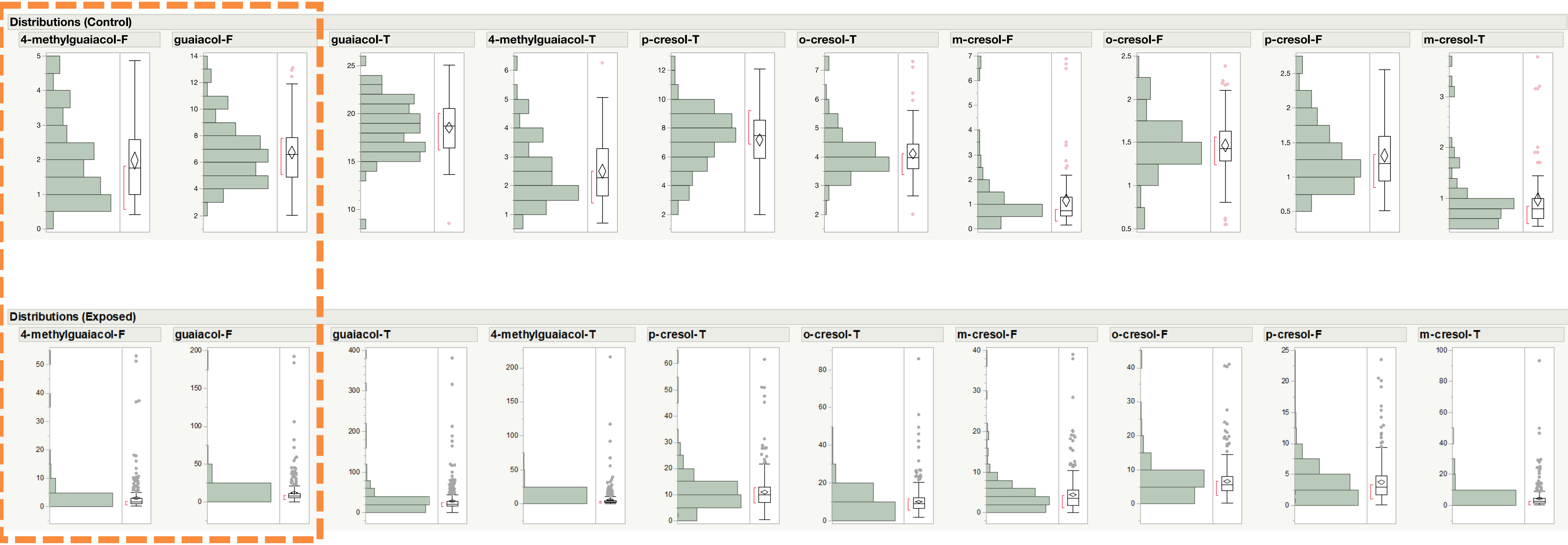
- 86 control wines from 2013-2016
- 377 wines from 2020 (exposed)

- Free and total volatile phenols were used as markers

Dr. Ye Feng, Former director of Machine-learning at Lam Research

Oregon State University

Sample Distribution



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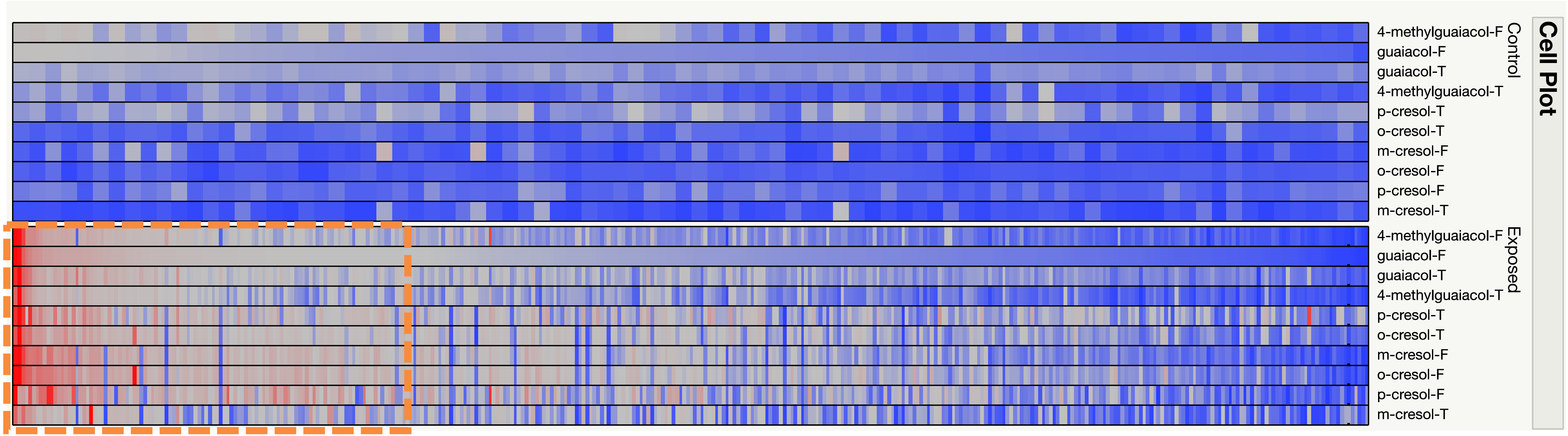
Multivariate Sample Distribution



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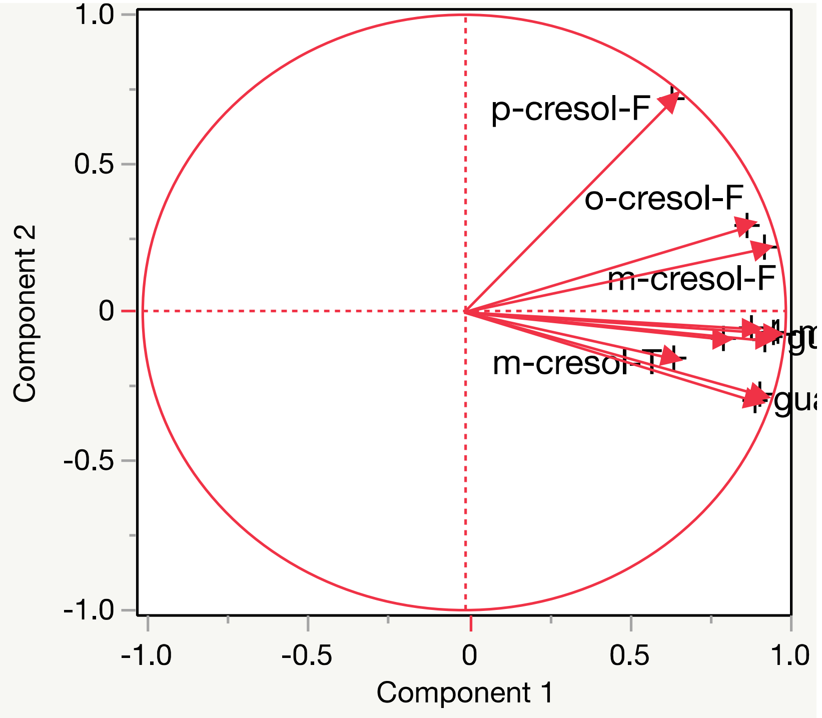
Principal Component Analysis

Principal Components: on Correlations

Number	Eigenvalue	Percent	Cum Percent
1	7.4617	74.617	74.617
2	0.8564	8.564	83.181
3	0.6144	6.144	89.325
4	0.4161	4.161	93.486
5	0.3174	3.174	96.661
6	0.1303	1.303	97.964
7	0.0958	0.958	98.922
8	0.0645	0.645	99.566
9	0.0293	0.293	99.859
10	0.0141	0.141	100.000

Eigenvectors	PC#1	PC#2
4-methylguaiacol-F	0.32844	-0.06079
guaiacol-F	0.35343	-0.07758
guaiacol-T	0.33792	-0.30178
4-methylguaiacol-T	0.33257	-0.32487
p-cresol-T	0.29652	-0.09926
o-cresol-T	0.34353	-0.10365
m-cresol-F	0.34308	0.23174
o-cresol-F	0.32346	0.31042
p-cresol-F	0.23796	0.77070
m-cresol-T	0.23985	-0.17038



The first principal component (PC) represents overall phenolic changes

The second PC points to unique covariant fingerprints in free cresols

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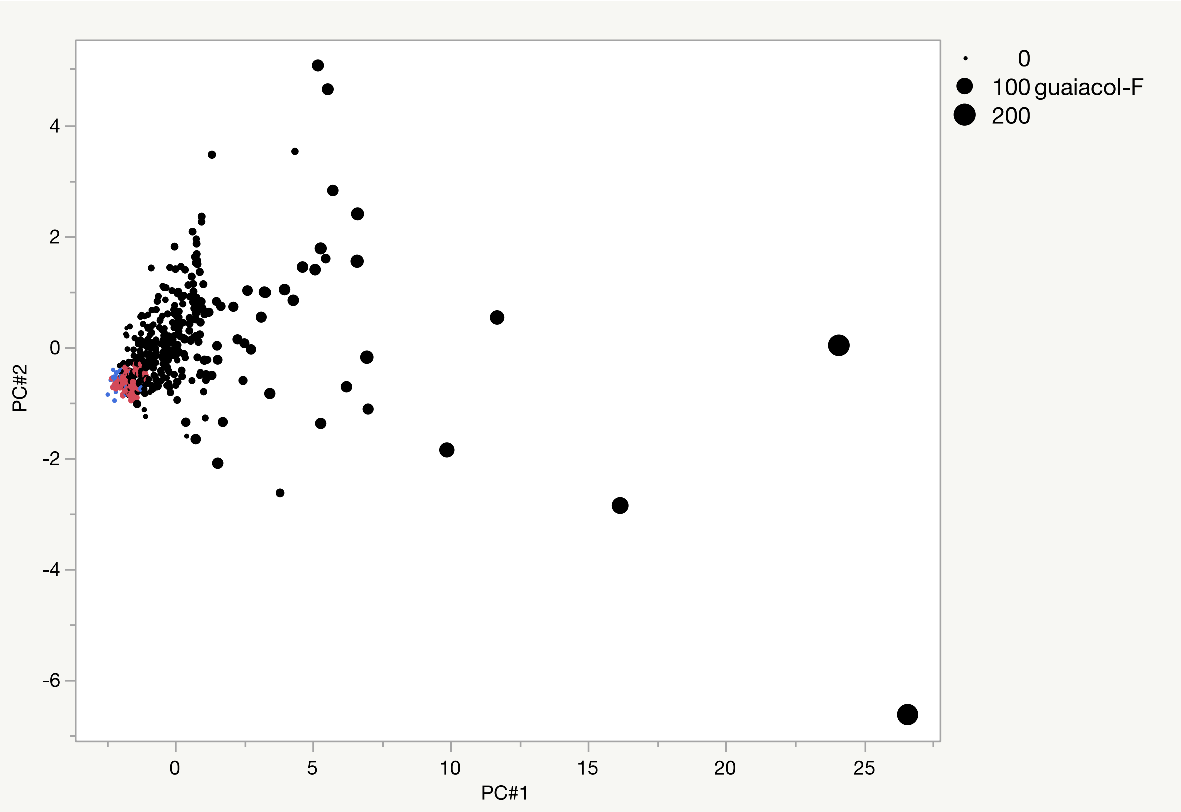
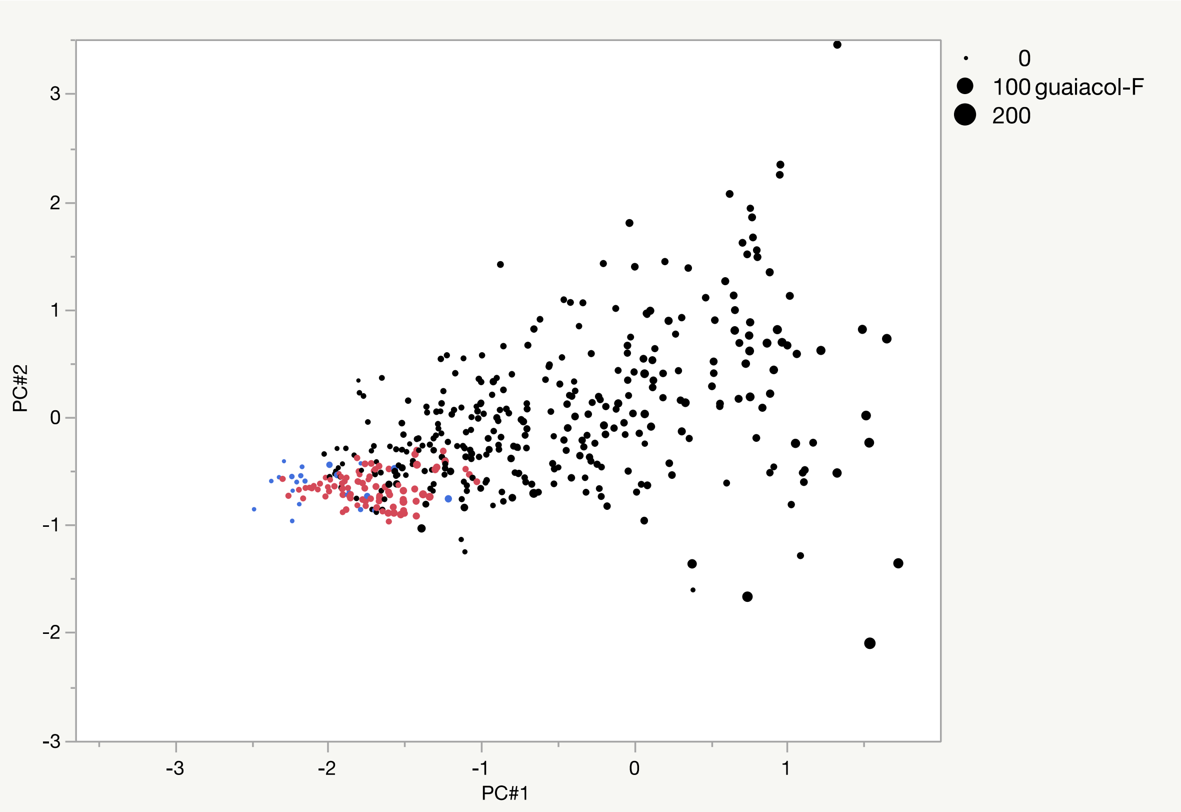
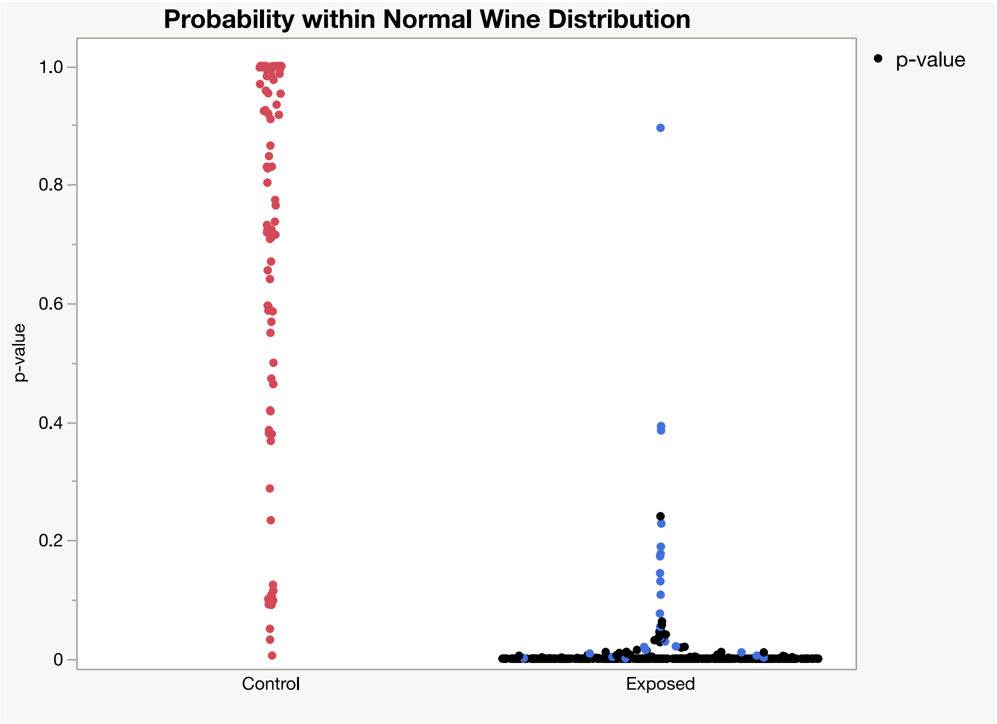
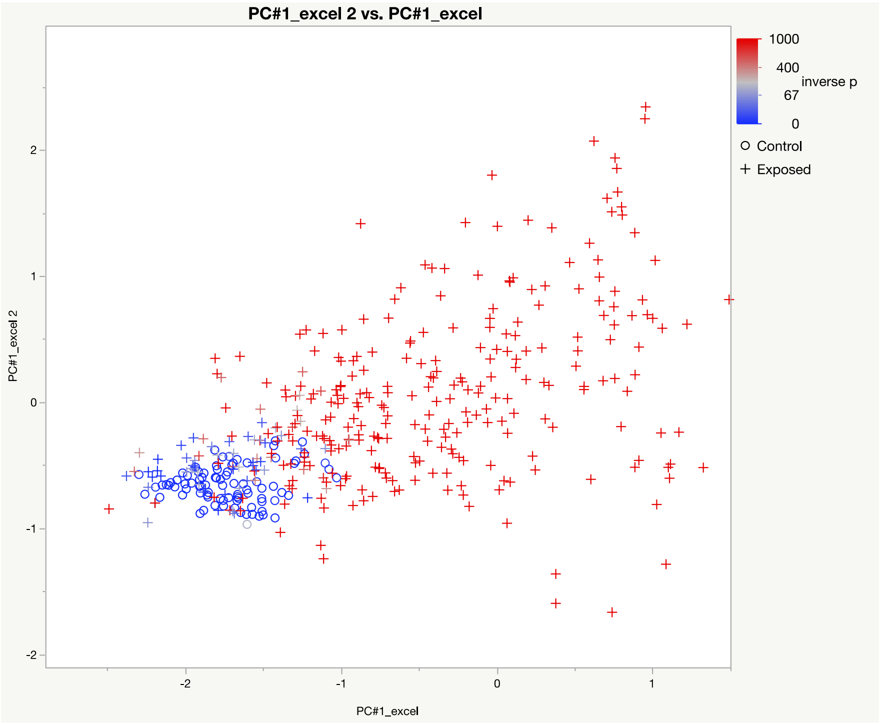
Evaluation of 2020 Wines (sized by Guaiacol-F)





Evaluation of Wines (sized by p-value)





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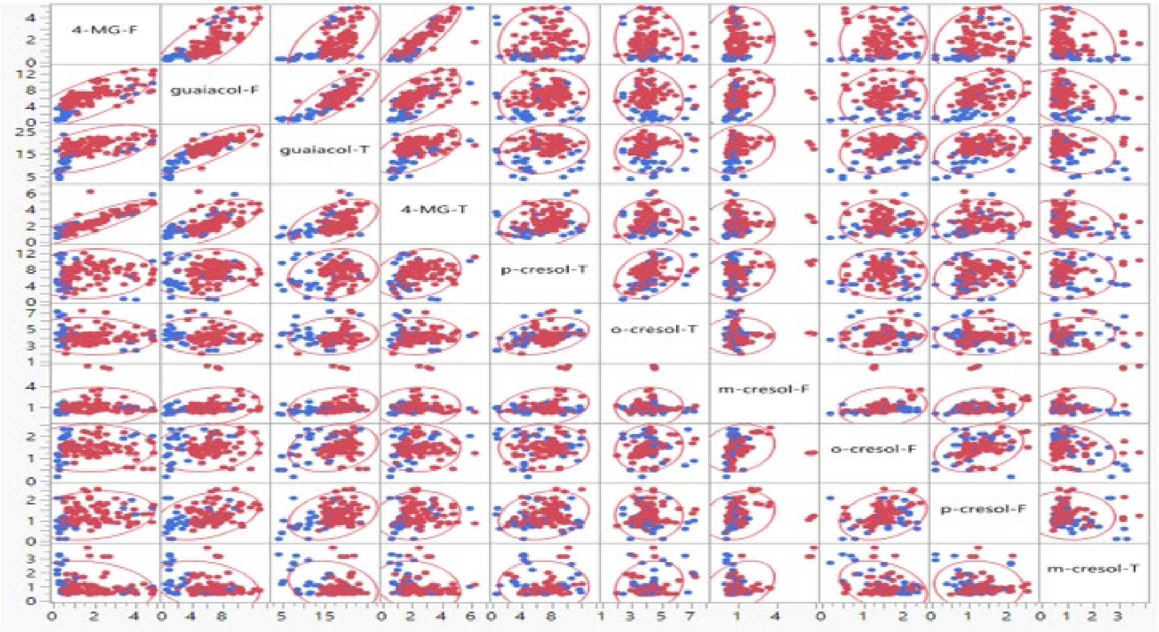
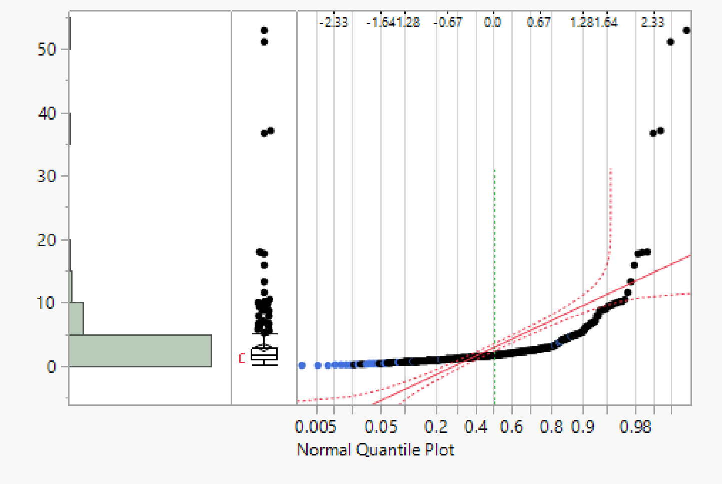
Correlation and modeling





Model prediction: p-value

3.916666667	6.592592593	-1.33112	-0.66458	-0.77733	-2.05628	-0.74186	0.194477	4.911919	1.888081	0.544012	2.135581	-0.31035	0.621221	3.411382	77.95991	6.960706	0.001055611
2.972222222	5.682926829	-0.8037	-0.3853	1.622674	3.943721	4.75814	1.594477	2.511919	2.988081	1.444012	2.935581	0.889651	0.621221	1.743567	94.58311	8.444921	0.000450617
5.2	6.78125	-0.71179	-0.16982	-0.47733	1.043721	3.15814	0.694477	1.211919	4.888081	2.744012	4.635581	0.989651	2.721221	0.646487	265.8569	23.73722	3.65367E-06
4.875	6.12195122	-0.31389	-0.69874	-0.37733	1.043721	6.55814	1.594477	9.711919	5.588081	2.544012	4.035581	-0.01035	4.621221	5.480444	385.4543	34.41557	6.06225E-07
3.857142857	4.612244898	-1.12923	-1.13684	-1.27733	-4.05628	4.05814	2.394477	7.311919	4.788081	0.144012	0.035581	-0.61035	4.421221	4.537723	154.4551	13.79064	4.79318E-05
4.631578947	5.42	0.016748	-0.69662	-0.07733	2.043721	8.55814	2.494477	13.31192	5.988081	2.744012	4.735581	0.189651	4.521221	11.03934	494.8929	44.18686	1.79067E-07
4.722222222	4.885245902	-0.21436	-0.73772	-0.17733	1.743721	11.25814	3.594477	8.811919	5.788081	2.544012	3.935581	0.189651	4.621221	4.108217	367.3541	32.79947	7.65886E-07
4.882352941	5	-0.52074	-0.4816	-0.27733	1.543721	3.95814	1.994477	6.311919	4.588081	2.844012	4.135581	0.289651	2.721221	3.089621	263.8451	23.5576	3.78942E-06
4.75	5.28	-0.24115	-0.43869	0.022674	2.743721	7.85814	2.494477	8.011919	5.088081	3.044012	5.135581	0.489651	2.421221	5.507082	363.5363	32.4586	8.05747E-07
4.642857143	5.272727273	-0.86358	-0.69621	-0.57733	-0.25628	4.65814	1.894477	3.811919	4.188081	1.744012	3.135581	-0.21035	3.521221	1.490751	218.5799	19.51606	9.32128E-06
4.736842105	5.255319149	-0.53183	-0.43205	-0.07733	2.243721	6.15814	2.194477	1.511919	6.288081	2.844012	5.135581	0.189651	2.921221	1.43106	362.7333	32.3869	8.14445E-07
5.0625	6.177777778	-0.62515	-0.69753	-0.37733	1.343721	9.25814	1.994477	0.911919	5.488081	2.444012	4.335581	-0.31035	6.021221	2.103738	466.2665	41.63094	2.39647E-07
4.666666667	5.048387097	-0.03815	-0.5024	0.122674	3.043721	12.75814	3.694477	5.411919	7.188081	3.544012	4.935581	0.689651	5.021221	1.620323	436.5137	38.97444	3.3069E-07
5	5.826086957	-0.32814	-0.27774	-0.17733	2.243721	8.25814	2.094477	3.211919	5.988081	3.244012	4.835581	1.089651	4.421221	0.78918	361.4582	32.27305	8.2849E-07
4.5	8.05	-1.98962	-0.55526	-1.17733	-3.15628	-2.44186	-0.50552	-4.78808	0.688081	0.144012	1.635581	-0.51035	0.121221	2.593103	43.90425	3.920022	0.011311237
2.909090909	8.625	-1.95435	-0.52808	-0.87733	-3.55628	-4.74186	-0.90552	-3.58808	0.588081	0.344012	1.635581	-0.51035	-0.07878	1.991242	43.70191	3.901956	0.01151535
3.702970297	3.724637681	3.431154	-0.84644	8.122674	30.64372	58.55814	18.19448	11.91192	13.08808	7.644012	9.535581	2.589651	12.82122	7.701365	2638.31	235.5634	4.53383E-11
2.363636364	6.291666667	-1.78698	-0.85924	-0.87733	-4.15628	-3.44186	-0.10552	3.511919	0.388081	-0.45599	0.135581	-0.71035	0.621221	2.938962	18.73934	1.673155	0.188994681
3.333333333	6.074074074	-1.69191	-0.53215	-0.77733	-2.75628	-2.14186	0.194477	0.211919	0.888081	0.044012	1.035581	0.189651	0.421221	1.235536	17.03465	1.520951	0.240265045
5	5.057692308	-0.40944	-0.56437	0.122674	3.743721	7.75814	2.694477	6.011919	6.088081	1.044012	5.435581	0.289651	2.221221	4.485602	430.0916	38.40103	3.55489E-07
4.35	5.510638298	-0.66816	-0.62343	0.022674	1.943721	7.35814	2.194477	5.411919	5.188081	1.344012	3.335581	0.289651	1.721221	1.386922	162.2397	14.48569	3.80933E-05
4.791666667	5.642857143	0.23035	-0.42914	0.422674	4.743721	13.05814	3.094477	8.811919	7.188081	3.744012	6.635581	0.589651	3.721221	6.057231	624.7588	55.78204	5.70944E-08
3.916666667	6.567567568	-0.76448	-0.28099	0.422674	2.643721	5.75814	1.194477	-1.98808	5.088081	2.944012	4.835581	0.389651	1.721221	2.647964	267.3275	23.86853	3.55812E-06

- The model needs to be verified with sensory evaluation
- So the p-value should only be used as a reference!

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Acknowledgement

- American Vineyard Foundation
- Oregon Wine Board
- Oregon Wine Research Institute