High Performance Thing Layer Chromatographic (HPTLC) analysis of polyphenolic composition in wine samples

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Dr David W. Morton
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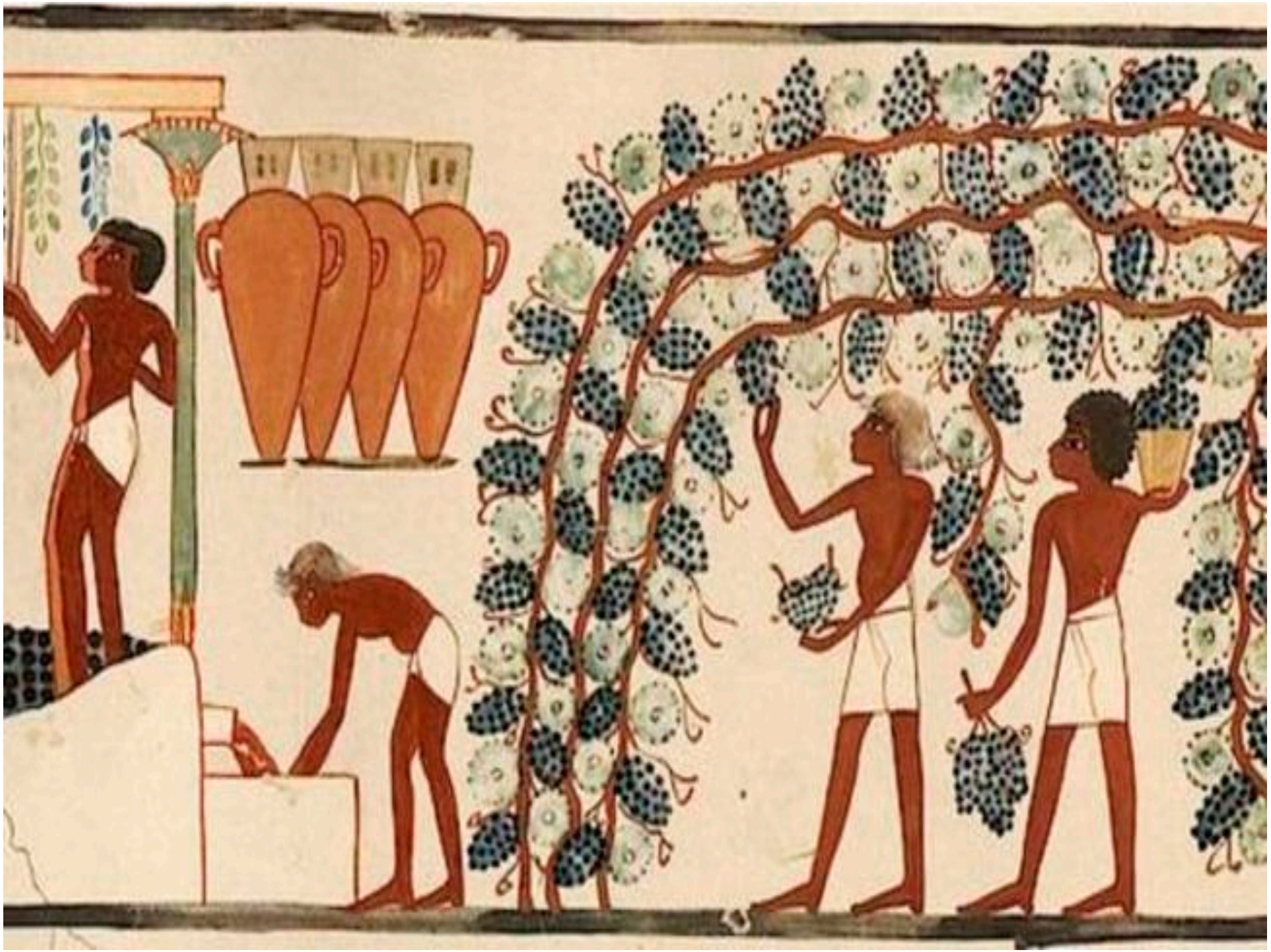
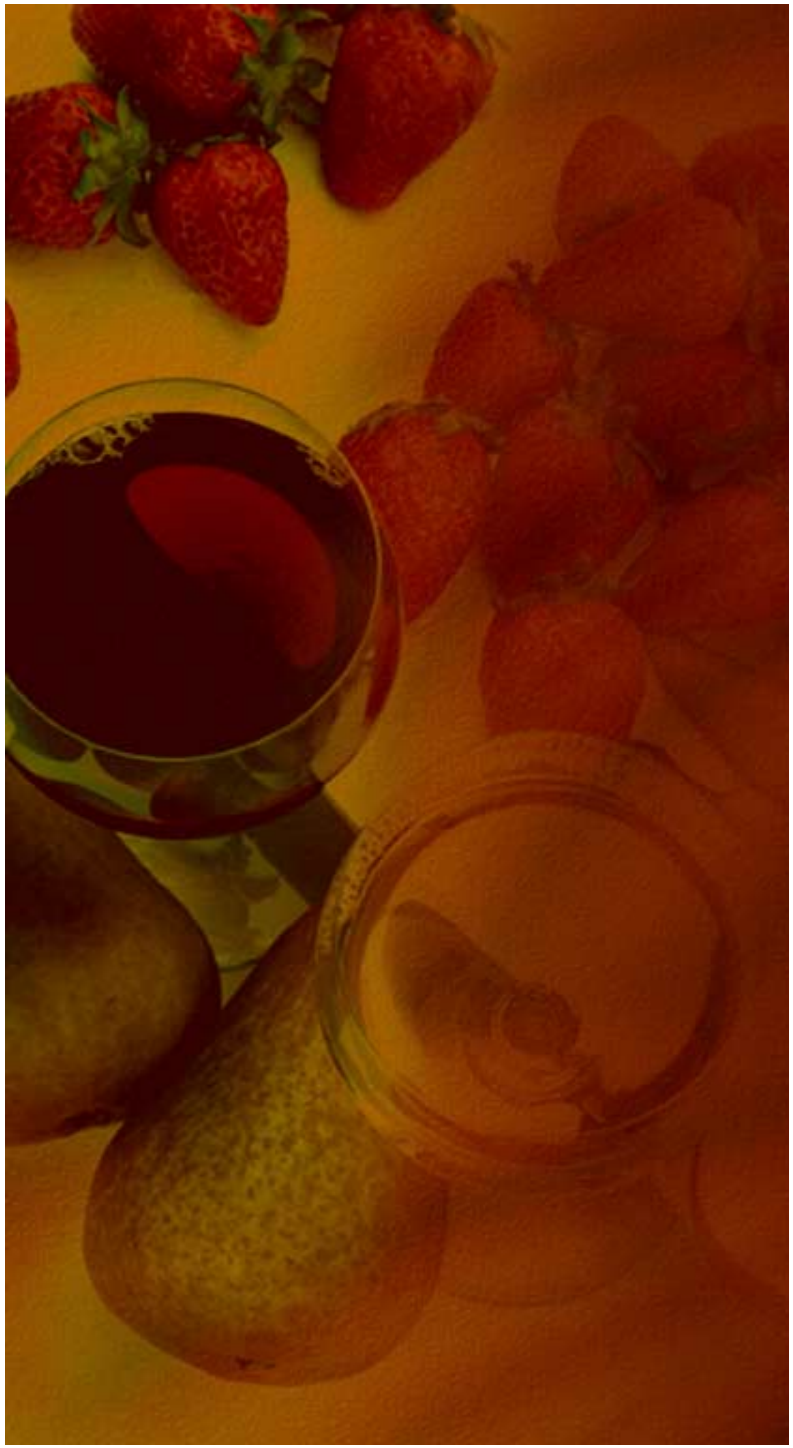
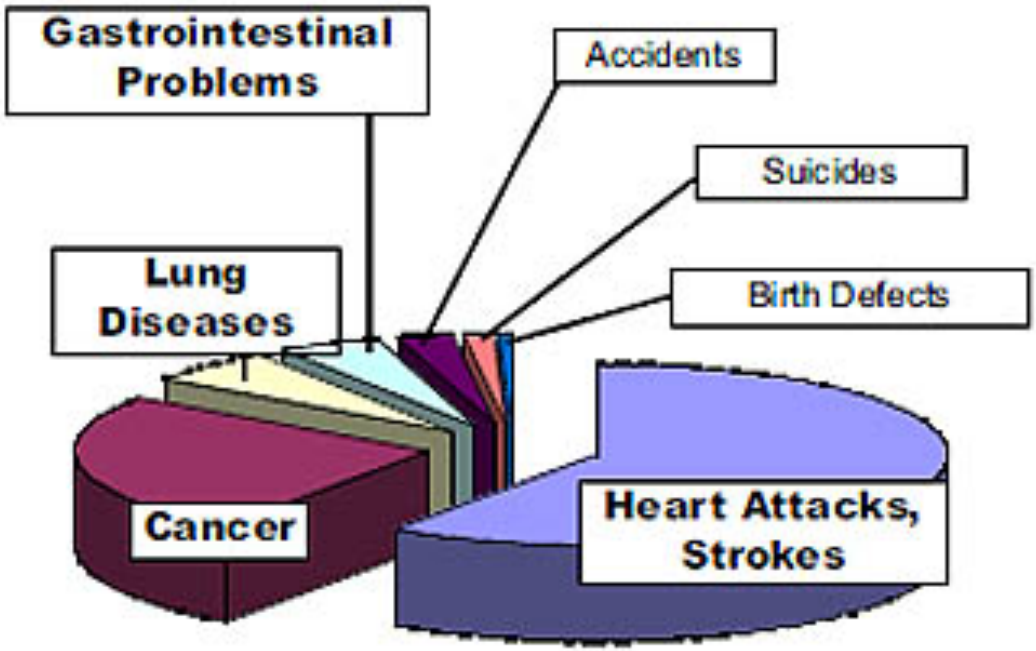




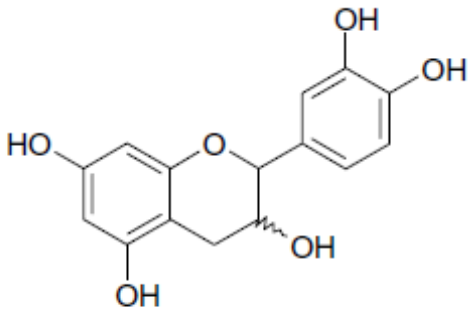
Common Diseases and Causes of Death

- World Health Organization 1997-

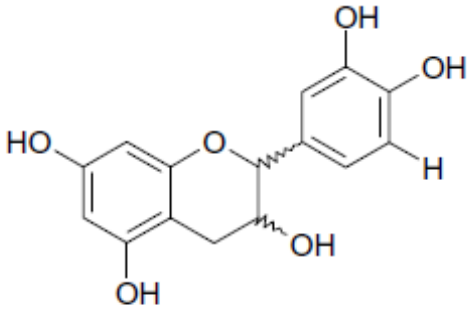


Eight out of ten people die of coronary heart disease or cancer

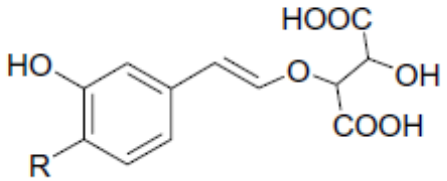
Common antioxidants presence in red wine



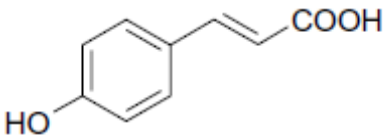
(+)-Catechin



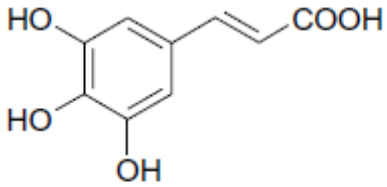
(-)-Epicatechin



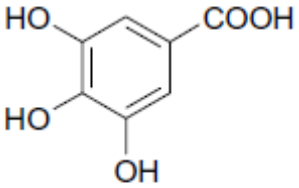
R = OH, Caftaric acid
R = H, Coutaric acid
R = OCH_3, Fertaric acid



p-coumaric acid



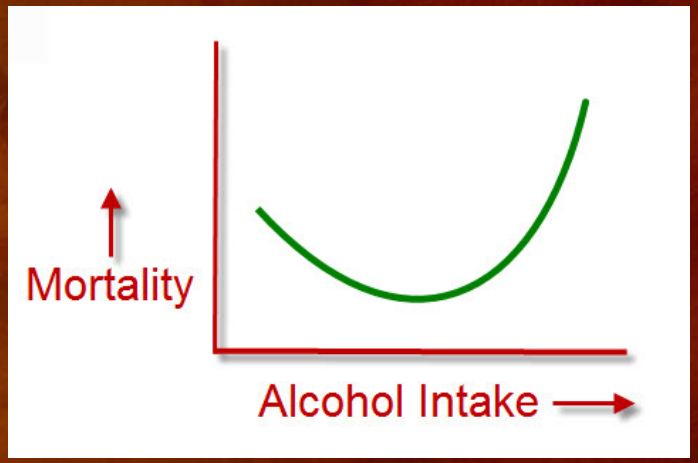
Cafeic acid



Gallic acid

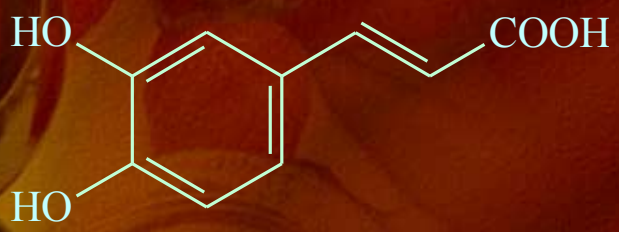
Beneficial effects of moderate consumption of wine

- Lower total cholesterol
- Lower blood pressure
- Reduce platelet aggregation
- Reduce risks of cancer
- stimulate the immune system

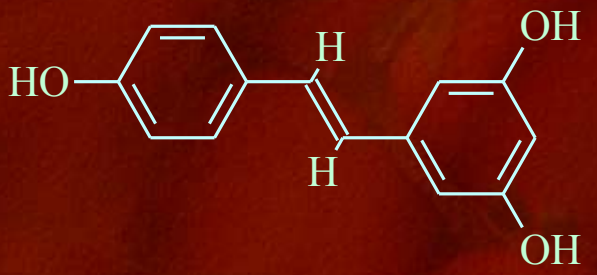


The aims of the study .....

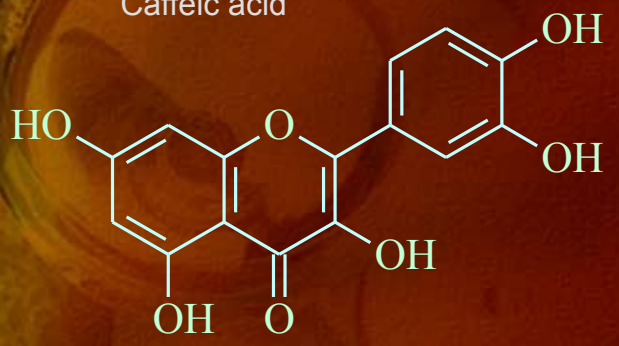
- to develop and optimise a simple HPTLC method to compare and to quantify gallic acid, caffeic acid, resveratrol and rutin in wine samples



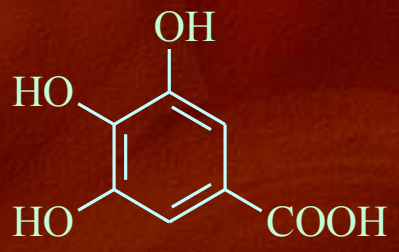
Caffeic acid



Resveratrol



Rutin

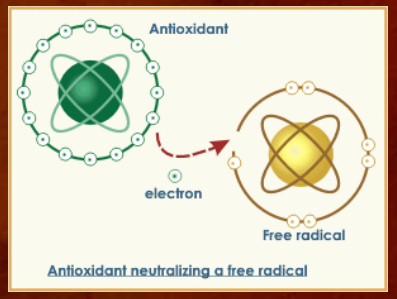


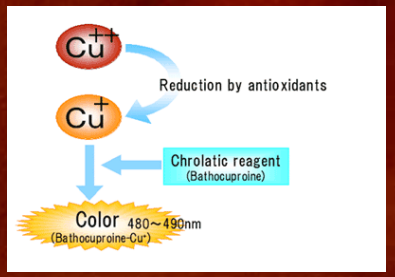
Gallic acid

The aims of the study .....

- to compare the quantities of investigated phenolic compounds with the total antioxidant capacity of wines
- to relate phenolic composition to the different geographic locations and grape variety to see if there was a significant correlation.

TAC - The overall ability of scavenging free radicals





TPC – the amount of phenolic compounds presence

The aims of the study .....

- to investigate whether there is a statistical correlation between TAC and TPC of the wine samples.
- conflicting data exist about the correlation between the antioxidant capacity and the phenolic contents in wine.

Methodology

- Sampling

  total of **45 wine samples** (43 red wines and 2 white wines) collected from different regions of Australia (35) and overseas (10).

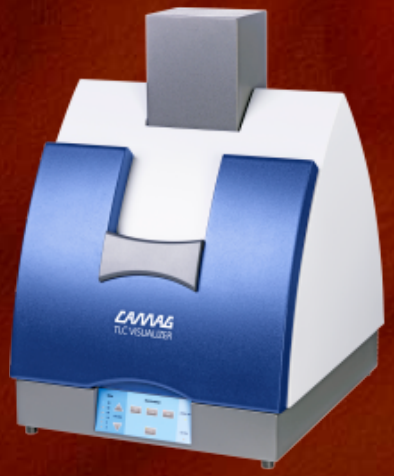
HPTLC Method

- Samples were spotted in the form of band using a Linomat 5 applicator on silica gel 60F-254 HPTLC plates
- A two step (9:5) gradient elution method was developed using dichloromethane: methanol: formic acid (7.5: 2.0: 0.75) and sodium dodecyl sulphate: n butanol: water: n heptane (8g: 25 ml: 8 ml: 160 ml))

HPTLC Method .....

- Images of plates were captured using a TLC-Visualiser under 366 nm before and after spraying with natural reagent

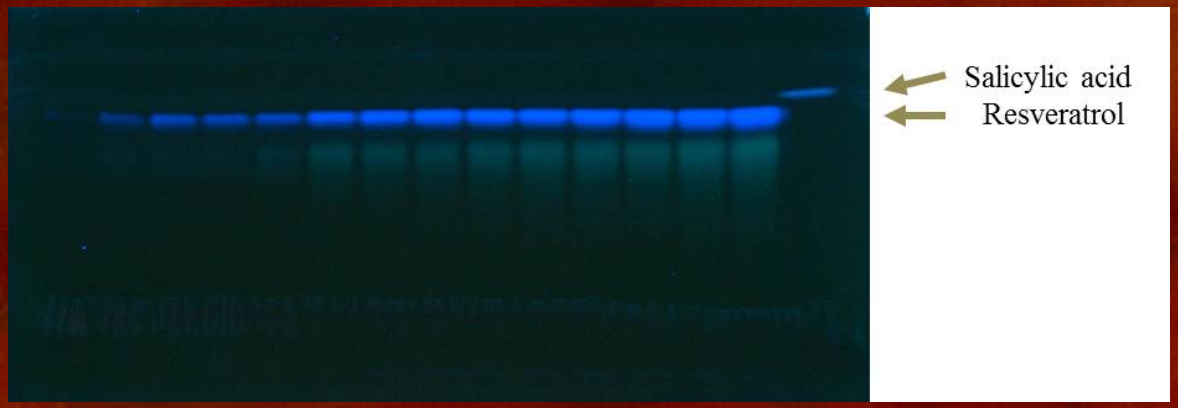
- Digitised images of chromatograms were evaluated using WinCATS® determine the peak areas present on the HPTLC plate.



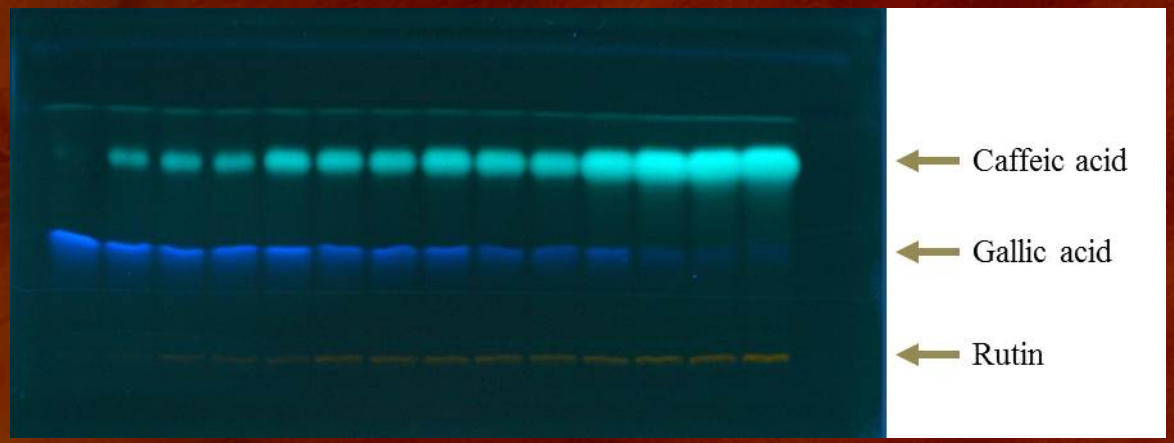
Total phenolic content (TPC)

- Folin's colorimetric method was used to determine TPC and express phenolic concentration as gallic acid equvalents
- TAC was determined using Randox® antioxidant kit. This method is based on producing a free radicals using peroxidase and hydrogen peroxide, which give a greenish blue colour. Addition of the wine sample suppress this colour proportionally to the antioxidant capacity of the sample

Before derivatization

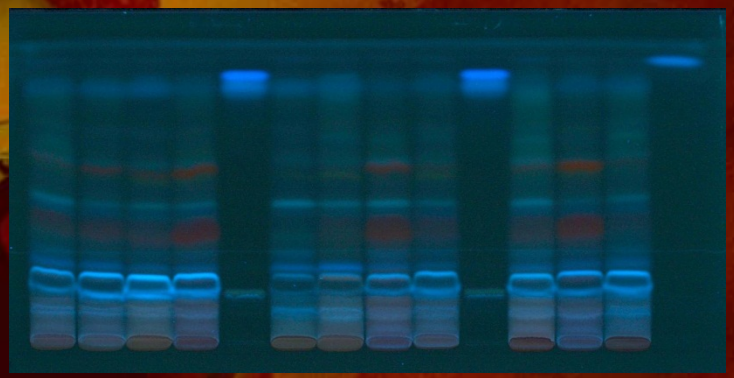


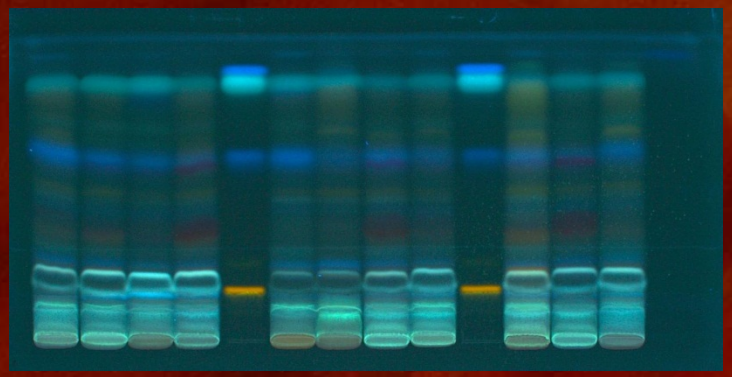
After derivatization

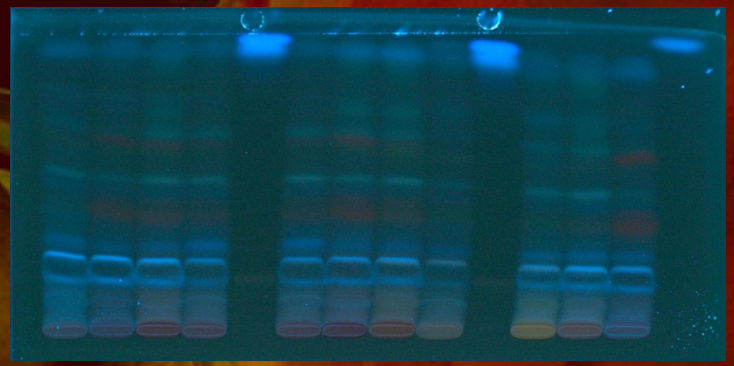


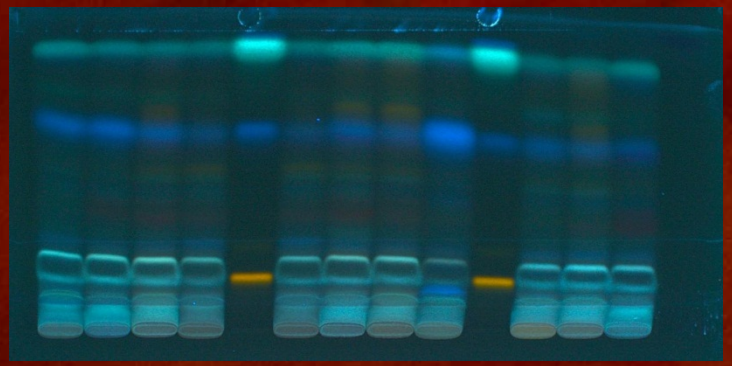
Results

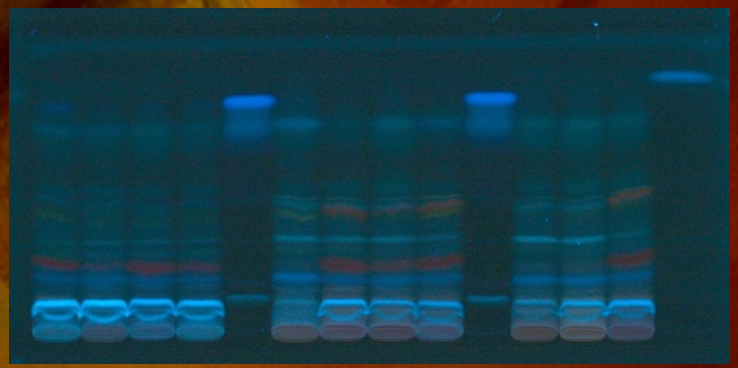
HPTLC chromatograms of wine samples

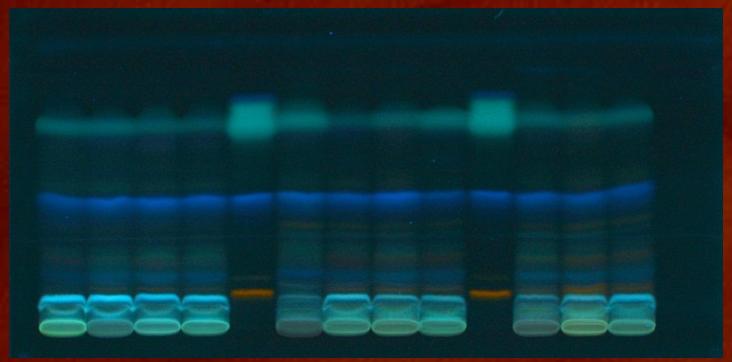


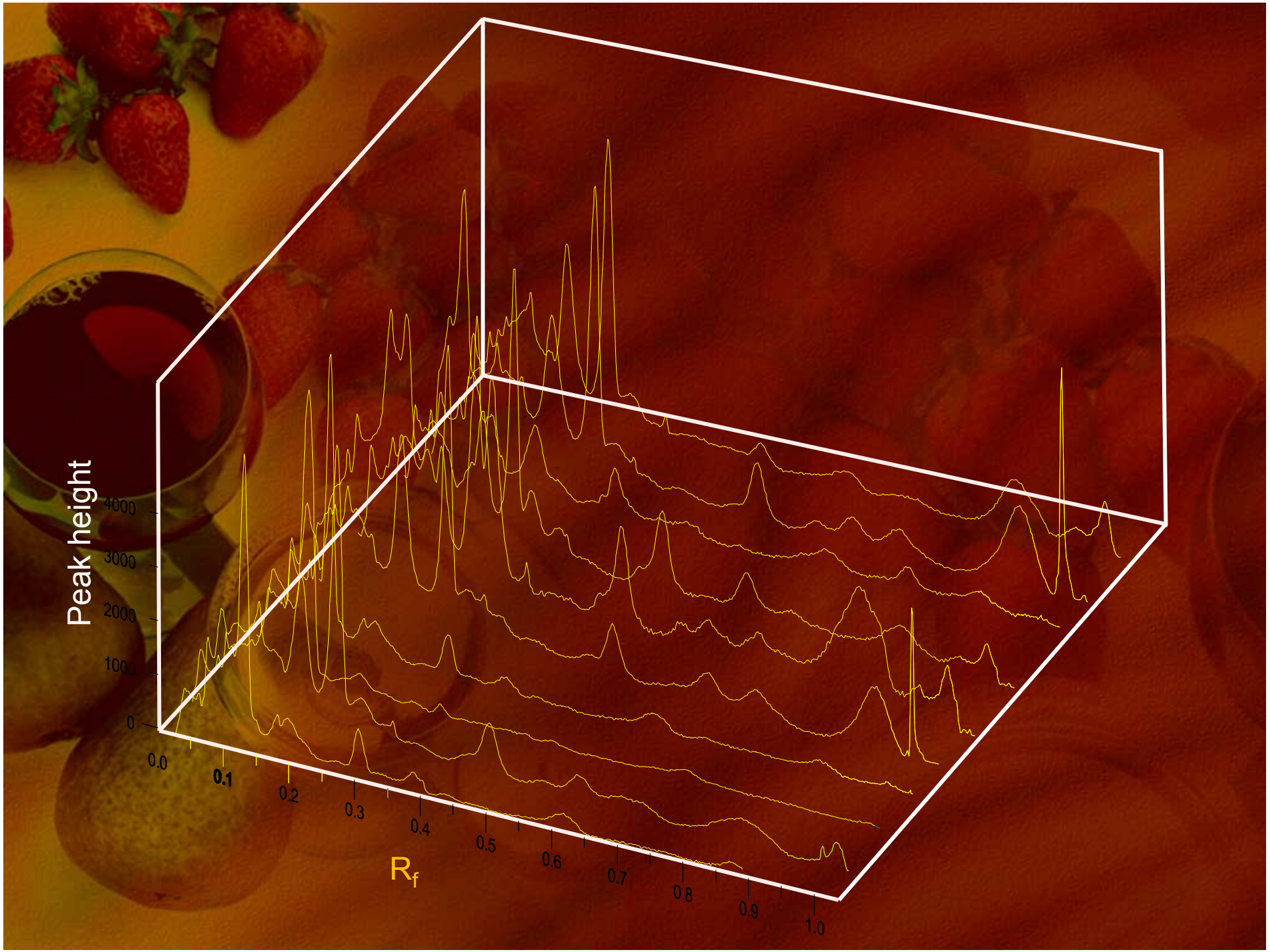








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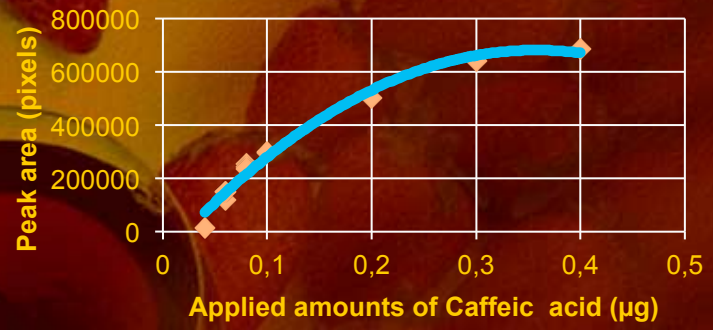
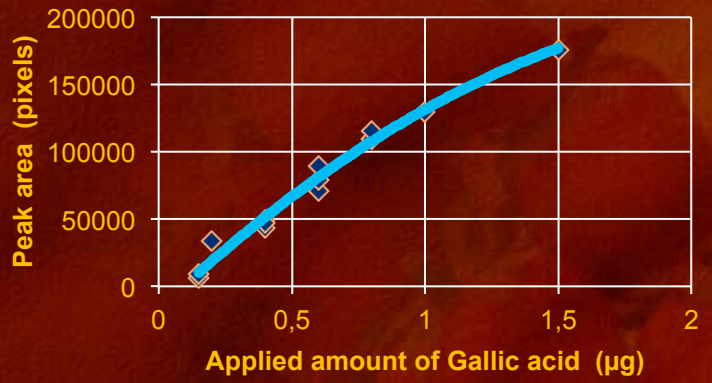
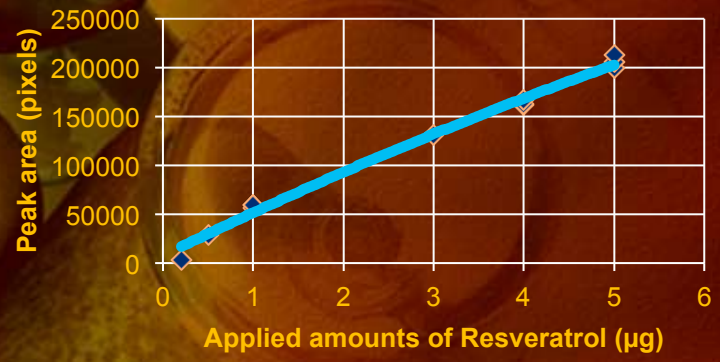
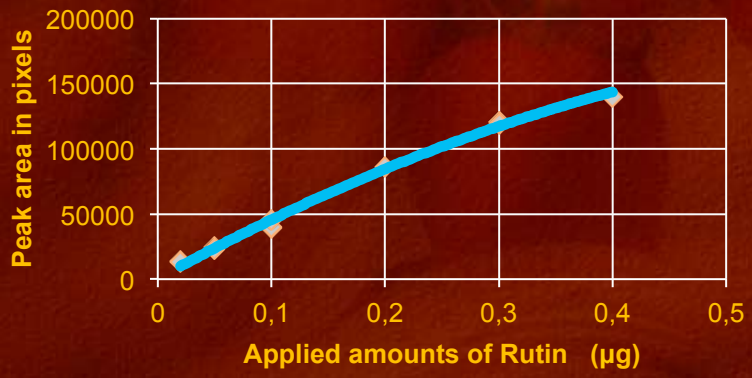
R_f

Calibration





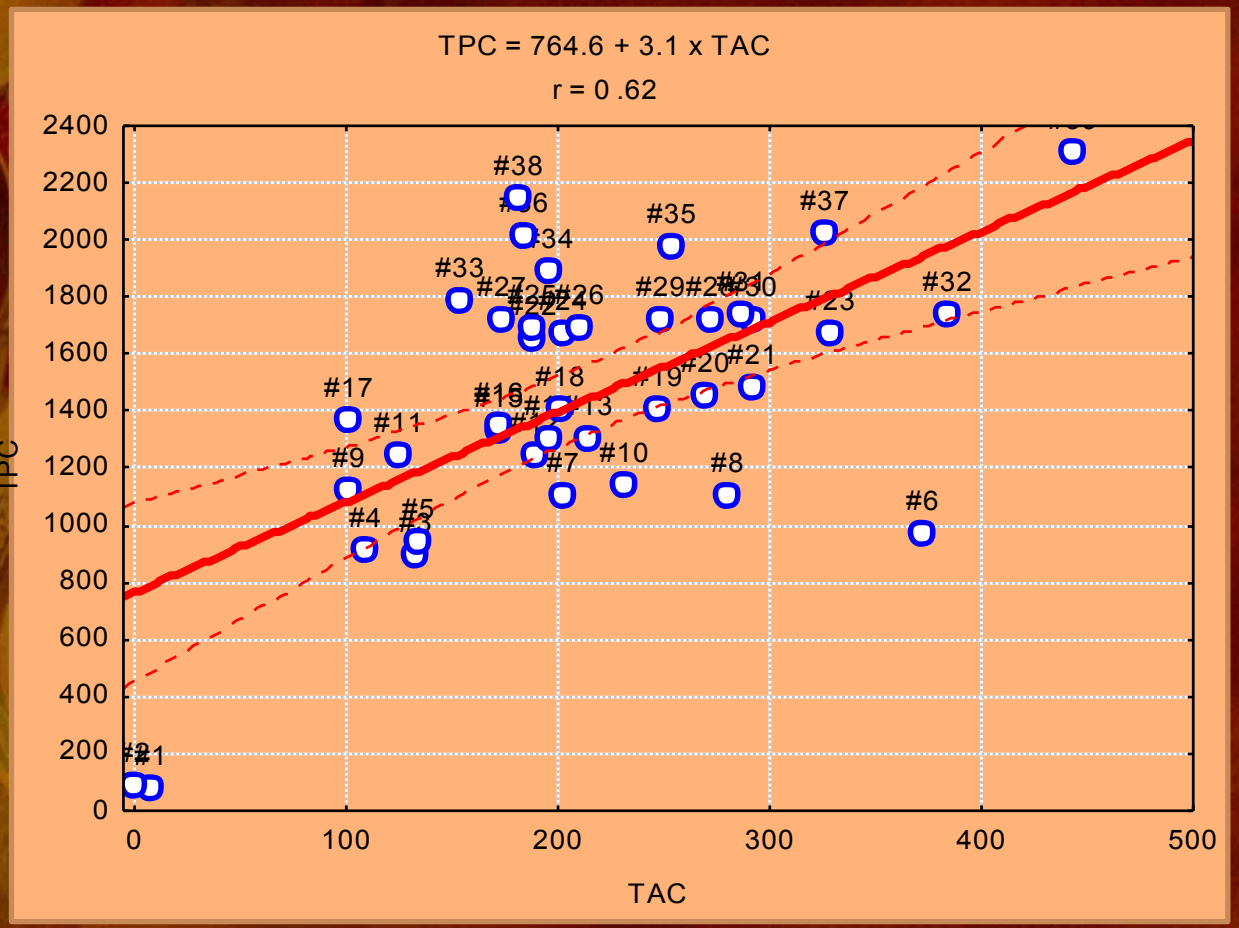




Sample No	Type	Grape variety	Region	Caffeic acid	Gallic acid	Resveratrol	Rutin (mg/L)	TAC (mg/L)	TPC GAE
1	Red	Cab. Merlot	NSW	2.07	29.66	0.00	0.00	125	1244
2	White	Muscat	VIC	0.25	2.36	0.00	0.00	8	77
3	Red	Durif	VIC	1.26	28.93	1.91	0.00	253	1969
4	Red	Shiraz	VIC	1.40	22.61	1.86	0.00	249	1718
5	Red	Shiraz, Cab.	Adelaide	0.83	8.74	1.25	0.00	293	1480
6	Red	Merlot	VIC	1.66	75.89	0.00	0.00	102	1369
7	Red	Shiraz	South Africa	1.87	16.79	0.00	0.00	193	6319
8	Red	Shiraz	VIC	2.60	8.84	0.00	0.00	190	1246
9	Red	Cabernet	VIC	2.26	12.48	1.63	2.42	102	1123
10	Red	Zinfandel	California	2.47	10.27	0.00	0.00	197	1885
11	White	Chardonnay	VIC	0.05	0.15	0.00	0.52	0	83
12	Red	Cab.Merlot	VIC	1.87	56.33	0.19	0.93	174	1714
13	Red	Shiraz Merlot	Adelaide	1.26	36.89	0.00	1.16	292	1721
14	Red	Shiraz	SA	0.96	19.70	0.00	0.73	373	965
15	Red	Shiraz	VIC	2.11	34.19	0.00	0.69	173	1329
16	Red	Shiraz Cab.	SA	5.02	67.13	3.42	5.58	181	2145
17	Red	Shiraz Cab.	VIC	7.78	28.88	1.39	3.69	232	1141
18	Red	Shiraz Cab.	VIC	5.44	41.22	1.23	2.23	247	1405
19	Red	Shiraz	NSW	2.24	29.89	3.02	2.68	270	1451
20	Red	Cab. Sauvignon	WA	3.68	34.74	0.00	8.58	329	1668

Sample No	Type	Grape variety	Region	Caffeic acid (mg/L)	Gallic acid (mg/L)	Resveratrol (mg/L)	Rutin (mg/L)	TAC (mg/L)	TPC GAE (mg/L)
21	Red	Cabernet Sauvignon	NSW	1.38	23.68	0.00	0.00	202	1097
22	Red	Petit Verdot	NSW	3.07	37.31	0.00	0.00	215	1296
23	Red	Cabernet Sauvignon	NSW	2.83	16.22	0.00	0.00	281	1097
24	Red	Cienna	VIC	0.34	7.10	0.00	0.00	201	1402
25	Red	Dolcetto	Italy	0.77	10.66	0.00	2.44	173	1350
26	Red	Merlot, Cabernet Sauvignon	France	0.66	19.18	0.00	1.02	185	2015
27	Red	Prunesta, Malvasia Nera	Italy	0.27	26.72	0.00	1.42	188	1685
28	Red	Shiraz	SA	1.78	41.71	0.00	0.00	384	1734
29	Red	Shiraz Cabernet	SA	1.01	36.17	0.00	0.89	211	1693
30	Red	Merlot	WA	1.87	31.30	0.00	0.00	109	910
31	Red	Merlot	VIC	1.53	33.67	0.00	0.00	189	1648
32	Red	Pinot Noir	France	1.61	52.93	0.00	0.00	154	1781
33	Red	Primitivo	Italy	0.31	7.54	0.00	0.11	135	941
34	Red	Primitivo	Italy	0.31	5.52	0.00	0.09	133	896
35	Red	Cabernet Sauvignon	SA	2.85	45.33	0.00	0.00	326	2017
36	Red	Shiraz		2.33	40.78	0.98	9.40	197	1304
37	Red	Shiraz	SA	3.08	71.38	2.13	0.00	443	2303
38	Red	Chambourcin, Shiraz	NSW	1.95	38.53	0.73	0.00	203	1671
39	Red	Cabernet Sauvignon	SA	7.23	56.18	1.85	0.00	288	1733
40	Red	Merlot	SA	3.63	39.55	1.85	0.00	272	1714

Correlation between TAC and TPC



Correlation between caffeic acid, gallic acid resveratrol and rutin with TAC and TPC

- Merlot and Merlot Cabernet wines have a lower antioxidant capacity and a higher total phenolic content
- Rutin and resveratrol were found in most Shiraz varieties.
- Shiraz wines from the colder climate have lower TPC but higher TAC and undetectable resveretrol,but contain rutin.
- Italian wines have the highest correlation between TPC and TAC (r = 0.99), do not contain detectable resveratrol, but all have rutin.
- Antioxidant properties might be associated with the presence of flavanols.

	Caffeic	Gallic	Resveratrol	Rutin
Average (mg/L)	2.15	30.18	0.59	2.45
RSD (%)	0.80	0.62	1.59	1.91
r (TAC)	0.33	0.35	0.28	0.08
r (TPC)	0.27	0.58	0.35	0.03

ANN modelling!!

- MLP (5 : 4 : 2)

- Categorical inputs: wine type, grape variety and wine origin
- Continuous inputs: amounts of 4 polyphenols

Inputs ranking

Wine origin

Grape variety

Wine type

Rutin

Resveratrol

	TAC	TPC
Correlation	0.85	0.76

Conclusions

- Different phenolic compounds have different antioxidant properties.
- The antioxidant activity of phenolic acids is related to the acid moiety and the number and relative positions of hydroxyl groups on the aromatic ring.
- Hydroxycinnamic acids (like caffeic acid) are more effective antioxidants than hydroxybenzoic acids (gallic acid)

Conclusions ....

- HPTLC chromatography may not have the same sensitivity as HPLC but is simple to run and multiple wine samples could be analysed and compared on the same plate.
- TLC is the only chromatographic method offering the choice of presenting the results as an image.
- HPTLC could provide extremely rapid screening and comparison of wine samples. It does not require sample preparation, analysis time is short and multiple samples can be analysed and compared simultaneously

Thank You