Pour Decisions™

Analytical Dept: Project Summary

CHEM 372

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Introduction

The mission of *Pour Decisions*™ is to assist consumers in making educated decisions about their health. Red wine consumption is a topic of interest recently for its reported health benefits (Ref. 15) and antioxidant constituents such as resveratrol (Ref. 3). This research was aimed towards supporting such claims with regards to commonly purchased red wines in the East Stroudsburg and surrounding areas utilizing a procedure published in *Analytical Chemistry* (Ref. 1). The wines chosen, based on a short survey of ESU students and observational study at a local liquor store, were Yellowtail Cabernet Sauvignon and Barefoot Pinot Noir.

Red wine contains many antioxidants that are claimed to be beneficial. The analytes quantified in this project included resveratrol:

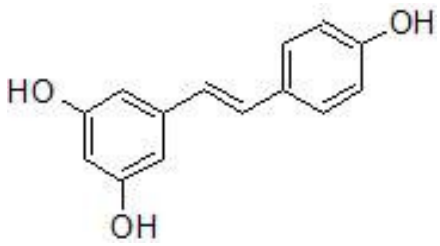

Figure 1: Structure of *trans*-resveratrol, or (E)-5-(4-hydroxystyryl)benzene-1,3-diol.

Research has found that this particular compound protects against inflammation, oxidative stress, and cancer; with the *trans* isomer being more biologically active than the *cis* (Ref. 6).

In addition to resveratrol, the wines were also analyzed for *trans*- polydatin, a glucoside of *trans*-resveratrol.

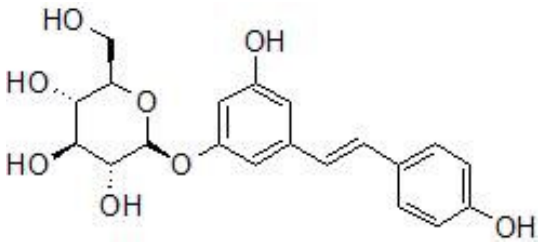

Figure 2: Structure of *trans*-polydatin, which is also sometimes referred to as *trans*-piceid.

In red wine, *trans*-polydatin can be found at a level 10 times greater than that of resveratrol. The reported health benefits are improved microcirculation, lowered blood cholesterol, and suppressed lipid peroxide formation (Ref. 2). The third analyte of interest in this research was quercetin:

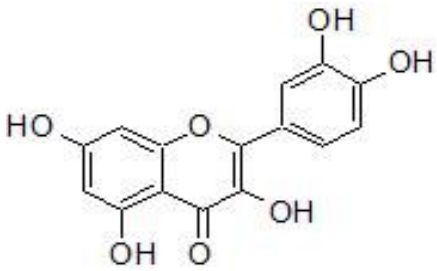

Figure 3: Structure of quercetin, also known as flavin and meletin.

While maintaining a majority of the benefits of the previous two analytes, quercetin has also been researched recently as a possible rheumatoid arthritis medication (Ref. 5). The therapeutic values for these compounds are:

Analyte	**Therapeutic Value (mg/kg/day)**
trans-Polydatin	100
Quercetin	0.004_3
trans-Resveratrol	22.4

Table 1: The above table shows the thresholds for each analyte's reported benefits. These values come from References 3,13, and 14 for resveratrol, quercetin, and polydatin respectively.

As an initial analysis, the adopted method analyzed several wines with the following having the highest concentration of these analytes:

Analyte	**Wine**	**Concentration (mg/L)**
Quercetin	1991 Australian Shiraz	9.89
trans-Resveratrol	1993 Beaujolais	2.69
trans-Polydatin	1990 Chateauneuf (France)	3.59

Table 2: The above table is taken from the data supplied in (Ref. 1) showing the analyzed wines that contained the highest concentration of each analyte being quantified.

The average weight of an adult male in the United States is 88.8 kg. (Ref. 17). Therefore in order to meet the therapeutic values when drinking the wines shown to contain the highest concentrations of each analyte one would need to drink 2,470 L/day of 1990 french Chateauneuf

for polydatin, 38.$_6$mL/day of 1991 australian Shiraz for quercetin[1], and 739 L/day of 1993 Beaujolais for resveratrol. The question addressed in this study was whether or not the most commonly bought red wines will fare any better than their higher end counterparts.

Experimental Procedures

All injections were run on the Agilent 1100 HPLC-UV/Vis using a Phenomenex brand Kinetex 5µm EVO C18 column. The mobile phase was a gradient beginning at 5% AcOH, 20% MeOH, 75% distilled water (DI), changing to 5% AcOH, 25% MeOH, 70% DI after five minutes, then changing to 5% AcOH, 50% MeOH, 45% DI after thirty minutes and remaining at this composition until the end of the forty minute run. After each run the column was flushed for ten minutes with the initial solvent composition. The detector was set at each analyte's wavelength of maximum absorbance, 366 nm and 377 nm for quercetin, in addition to 318 nm and 306 nm for polydatin and resveratrol. The original method procedures were based off (Ref. 1), and called for a six to ten point calibration curve. The standards were dissolved in methanol and added to standard solutions consisting of each analyte at its calibrated concentration. In order to increase the throughput of the assays and decrease the time spent in lab, a five point calibration curve was decided upon spanning the method's published linear range:

Analyte	**Concentrations (mg/L)**					
trans-resveratrol	0	0.5	2.5	4.5	6.5	8.5
Quercetin	0	5	15	25	40	50
trans-polydatin	0	0.5	2.5	4	5.5	7

Table 3: The above table shows the concentration values for each point on our calibration curves. **However*, due to a deviation in the standard preparation procedure on 4/25, the last two standards ended up with slightly different concentrations. See below for the procedures and see Appendix III for the calibration curves.

Analyte Standards Procedure(s)

Stock solutions for quercetin, *trans*-polydatin, and *trans*-resveratrol were all prepared by first dissolving 0.1000 grams of each into 200 mL volumetric flasks. For polydatin and resveratrol, a second stock solution was prepared by diluting 10 mL of the original stock in a 100

[1]N.b. : The therapeutic value listed here is only for its shown efficacy in treating rheumatoid arthritis in white rabbits. The FDA has no official standing on quercetin as of yet and therefore this dosage may be lower than the value which would it make it beneficial for humans. Despite this drawback in the analysis, this value will still be useful in comparing the health benefits from the commonly consumed wines and the more luxury vintages.

mL volumetric flask. Following the calibration table above, the appropriate amount of solvent was delivered into five 100 mL volumetric flasks using burets. They were then filled to the line with the appropriate solvent.

The sequences run on 4/18 and 4/25 were made with the last of the purchased resveratrol. In order to conserve the expensive standard and stretch it into two runs, the original 200 mL stock was prepared using only 0.0500 grams, from which 10 mL was then taken and diluted into a 50 mL volumetric flask. This diluted stock was then used to prepare the five standards.

(4/11) The first set of standards comprised solely of a white wine matrix. Following the original procedure, the compounds were first dissolved in ethanol to make the first stocks and then diluted in a white wine matrix for the rest. This was done to minimize the amount of ethanol added to the matrix. The standards were then filled to the line with more Yellow Tail™ Sauvignon Blanc. Each standard was filtered three times and added to a 1.5mL autosampler vial.

(4/18) To obtain results with greater accuracy, both stocks were then dissolved in the ethanol and then transferred into a white wine matrix. A sonicator aided in dissolving the analytes within the ethanol. All standards were then again filtered three times before being placed into the 1.5mL autosampler vials.

(4/25) It was found from the previous two runs that the absolute retention times were not helpful in analyte identification since the peak moved around amongst the standards. To attempt to address this issue, both stocks were made in pure methanol, and standards 1, 2, and 3 were made in an attempt to recreate the mobile phase used in the HPLC at the beginning of the method.

Sample Analysis Procedure(s)

To prepare the analytes, Barefoot™ Pinot Noir and Yellow Tail™ Cabernet Sauvignon, 750 mL bottles were split into three sections. One section contained the top portion of the bottle, the next contained the bulk of the wine sample, then the last section contained the bottom portion of the bottle. 10 mL aliquots were taken from the top and bottom section. After the aliquots were filtered three times using different Agilent™ Captiva PES syringe filters each time, they were then transferred into a 1.5mL HPLC autosampler vial.

During analysis, the hardest part was determining which peaks correspond to which analyte. Retention times from the standards made that day, as well as standards run in methanol in a previous experiment on 4/5 gave insight as to what retention times to expect as well as the elution order. Sample chromatograms were inspected and peaks that both matched the retention time of the standards in addition to having absorbance spectra close to the reference spectra of the analyte, were taken to be the analyte.

Results

Method Detection Limits

		Method Detection Limits		
		Sequence from 4/11	**Sequence from 4/18**	**Sequence from 4/25**
Area	**Quercetin**	26.309	17.039	n/a
	Resveratrol	4.6779	2.4733	0.46179
	Polydatin	3.4042	3.7603	3.5691
Height	**Quercetin**	n/a	n/a	8.1212
	Resveratrol			0.60730
	Polydatin			4.9173

Table 4: The above table lists all of the detection limits for each day samples were run. All values listed are in mg/L. Only calibration curves based on peak areas were used until 4/25 when two curves based on peak height were constructed. However on 4/25 the area curve for quercetin was deemed unusable.

The detection limits were calculated as follows:

1) The set of white wine blanks run on 4/10 were analyzed for signals from each of the analytes with the means ($\bar{y}$) and standard deviations (s_y) recorded.
2) The instrument's detection limit (LOD) for both height and area was calculated using:
$$LOD = \bar{y} + 3s_y$$
3) The method detection limit was then calculated by converting the LOD into mg/L using the respective height or area calibration curve produced from the standards run on each day. (See Appendix III for what each of these curves looked like)

Summary Statistics from 4/11

Polydatin		*Resveratrol*		*Quercetin*	
Yellowtail Top Aliquot					
Mean	2.3651	Mean	4.5921	Mean	n/a
St. Dev	0.1621	St. Dev	0.0289	St. Dev	n/a
RSD	6.85%	RSD	0.63%	RSD	n/a
Yellowtail Bottom Aliquot					
Mean	2.6432	Mean	4.6203	Mean	29.5217
St. Dev	0.0983	St. Dev	0.0172	St. Dev	0.0516
RSD	3.72%	RSD	0.37%	RSD	0.17%

Table 5: The above table summarizes the results from the sequence run on 4/11. All results listed are in mg/L. The results are from four replicate samples using the calibration curves based on the peak area (See Appendix III). Quercetin was not detected in any of the top aliquot samples. 'n/a' should be read as 'non-detectable'.

Summary Statistics from 4/18

Polydatin		*Resveratrol*		*Quercetin*	
BF Top/Bottom		**BF Top/Bottom**		**BF Top/Bottom**	
Mean	7.75835	Mean	6.73194	Mean	18.2621
St.Dev	0.02672	St.Dev	0.23006	St.Dev	0.01068
RSD	0.34%	RSD	3.42%	RSD	0.06%
Mean	8.11801	Mean	7.99058	Mean	18.2904
St.Dev	0.01736	St.Dev	0.28962	St.Dev	0.00893
RSD	0.21%	RSD	3.62%	RSD	0.05%
Louis Latour		**Louis Latour**		**Louis Latour**	
Mean	7.44698	Mean	8.23323	Mean	18.8842
St.Dev	0.12127	St.Dev	0.09562	St.Dev	0.01349
RSD	1.63%	RSD	1.16%	RSD	0.07%
YT Top/Bottom		**YT Top/Bottom**		**YT Top/Bottom**	
Mean	2.73286	Mean	6.41275	Mean	20.0468
St.Dev	0.04768	St.Dev	0.08218	St.Dev	0.27077
RSD	1.74%	RSD	1.28%	RSD	1.35%
Mean	2.69631	Mean	5.88626	Mean	18.3535
St.Dev	0.05944	St.Dev	0.02659	St.Dev	0.09429
RSD	2.20%	RSD	0.45%	RSD	0.51%

Table 6: Displayed Above are the results from the sequence run on 4/18. All results listed are in mg/L. The Barefoot (BF) and Yellowtail (YT) show have the results from the top aliquot followed by the bottom aliquot. All statistics are taken from four replicate samples calculated using the area calibration curve. The polydatin and resveratrol values are after adjusting for the determinate error found from the spiked samples.

Summary Statistics from 4/25

Polydatin H		*Resveratrol H*		**Quercetin H*		*Polydatin A*		*Resveratrol A*	
Barefoot Top Aliquot									
Mean	2.7010	Mean	n/a	Mean	n/a	Mean	2.9946	Mean	n/a
St.Dev	0.1059	St.Dev	n/a	St.Dev	n/a	St.Dev	0.0706	St.Dev	n/a
RSD	3.92%	RSD	n/a	RSD	n/a	RSD	2.36%	RSD	n/a
Barefoot Bottom Aliquot									
Mean	2.6600	Mean	n/a	Mean	n/a	Mean	3.1751	Mean	n/a
St.Dev	0.0674	St.Dev	n/a	St.Dev	n/a	St.Dev	0.2521	St.Dev	n/a
RSD	2.53%	RSD	n/a	RSD	n/a	RSD	7.94%	RSD	n/a
Yellowtail Top Aliquot									
Mean	2.9689	Mean	2.4379	Mean	26.3427	Mean	2.8666	Mean	4.7049
St.Dev	n/a	St.Dev	0.0447	St.Dev	0.1824	St.Dev	n/a	St.Dev	0.0936
RSD	n/a	RSD	1.83%	RSD	0.69%	RSD	n/a	RSD	1.99%
Yellowtail Bottom Aliquot									
Mean	3.0444	Mean	2.2583	Mean	24.5458	Mean	2.9988	Mean	4.5586
St.Dev	0.0628	St.Dev	0.0446	St.Dev	0.2558	St.Dev	0.1057	St.Dev	0.0753
RSD	2.06%	RSD	1.97%	RSD	1.04%	RSD	3.52%	RSD	1.65%

Table 7: The above table summarizes the results from the sequence run on 4/25. All results listed are in mg/L. H, results using peak height. A, results using peak area. All the results are from duplicate samples. Resveratrol and Quercetin were not detected in the Barefoot wine. The calibration curve for quercetin based on the peak area was not used due to errors in the signal. In the top aliquot of Yellowtail, a signal resembling that of polydatin was only found in one of the replicates, these values consequently have no reported standard deviations or RSD's.

Conclusion

Unfortunately, all of the results from 4/11 are virtually worthless. Upon comparison of the sequence's data with Table 4, all of the results fall below the detection limit for the method of that procedure except for quercetin which was only found in the bottom aliquot. The standards made that day were prepared by diluting the solid analytes in the chosen white wine matrix. When the polydatin and quercetin were first added to their flasks to make the solutions, it had appeared that neither of them were going to dissolve in an aqueous solution. White wine does contain a certain ethanol content, but it was not high enough to dissolve both of them completely. The strategy of that day was then changed to dissolve the analytes initially in ethanol, then to make second stocks from the first solutions using white wine as the diluent. The first three of the five solutions were made with no issue, but the fourth and fifth, at the higher concentrations, appeared more opaque than the prior. This suggests that even with the analytes being dissolved initially in ethanol, the white wine diluent made the standards far too aqueous for the analytes to remain in solution.

On 4/18, it was asked by the analytical director to include a sample of a personal favorite wine, Maison Louis Latour™ Marsannay Rouge 2015, valued at $23.99. Among the wine's tested, both on 4/18 and 4/25, the Rogue had the highest concentration of resveratrol at 8.2332_3 mg/L. Even at this concentration, one would still need to drink 241.6_0 L/day to reach the therapeutic value of resveratrol. For quercetin, the highest concentration found was in the

YellowTail Cabernet Sauvignon at 29.521_7 mg/L. At this level, one would only need to drink 12.93_4 mL to reach the therapeutic value. This is less than half the amount required from the method's 1991 Shiraz which is nearly triple the cost of the YellowTail wine. However, that number comes from the sequence run on 4/11 where the other values were below the detection limit. A better comparison would be to use the concentration from the run on 4/25: 26.342_7 mg/L. Even further, the lower bound on the 95% confidence interval for those injections is still 24.704 mg/L. At that concentration one would only need to consume 15.45_6 mL of the wine to reach the limit. This volume is still far below that reported in the literature for the 1991 Shiraz. In terms of polydatin, the highest concentration found was in the Barefoot Pinot Noir on 4/18. This sequence reported the wine to have on average 7.758_4 mg/L. However, at this concentration, one would need to consume $1{,}144._6$ L/day. While this is less than needed from the best polydatin wine reported in the literature, this value is also higher than the second experiment conducted on 4/25 which found a different bottle of Barefoot only contained at best 3.175_1 mg/L which would be below the value found in the published method's 1990 Chateauneuf.

In summary, while the wine's analyzed here may provide a cheaper source of quercetin than the more expensive vintages, resveratrol and polydatin are still questionable. Expensive vintages may contain more of these compounds, i.e. Rogue > Yellowtail and Barefoot for resveratrol, and the Chateauneuf is not statistically significantly different from the Barefoot analyzed for polydatin, but even the higher levels fail to make reaching the therapeutic value nearly impossible for a normal wine drinker. Given that the FDA has not made an official stance on the health benefits of quercetin, it is plausible that drinking red wine may not be directly beneficial to one's health in terms of ingesting these three compounds.

Cost Analysis

Material	Product No. (CAS)	Quantity	Unit Cost	Total Cost
Resveratrol	CAS 501-36-0	500 mg	$383.00	$383.00
Polydatin	CAS 65914-17-2	25 g	$228.00	$228.00
Quercetin	CAS 117-39-5	10 g	$40.50	$40.50
Methanol	CAS 67-56-1	1 L	$58.50	$58.50
Acetic Acid	CAS 64-19-7	2x 100mL	$113.00	$113.00
Distilled Water	Quality distilled water is provided in the lab for experimentation.			
Barefoot™ Pinot Noir	-	2x 750 mL	$9.99	$19.98
Yellow Tail™ Cabernet Sauvignon	-	2x 750 mL	$7.49	$14.98
Yellow Tail™ Sauvignon Blanc	-	3x 750mL	$7.49	$22.47
Labor Cost	-	86.5 hours	$25/hr	$2,162.50
Instrument Cost	-	133 injections	$50/injection	$6,650.00
Total Project Cost				$9,692.93
Initial Budget				$6,725.00
Difference from Initial				+$2,967.93

Prior to the start of experimentation, it was calculated that the project budget for the project would not exceed the limits of $6,725.00. With a study plan that attempted to have accounted for more than the typical lab error, it was not a large enough budget to hold the cost of the project. The largest source of cost discrepancy came from the actual number of injections made compared to the proposed. The amount of adjustments to what should have been a fairly straightforward procedure resulted in approximately five times the number of injections had everything gone similarly to the published procedure. The abundance of 105 injections alone costed the company $5,250.00, over 75% of the project's entire budget.

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