What is the effect of red wine tannin concentration on the time taken for methylene blue solution to oxidise?

by

Investigating the antioxidative capacity of red wine

Research question: What is the effect of red wine tannin concentration on the time taken for methylene blue solution to oxidise?

Focused research question presented

Introduction:

Tannins, or tannic acid, are water-soluble polyphenols, as can be seen in *figure 1*[1]: these present in various plants and are chemically classified into two main groups: hydrolysable and condensed.[2]

In Portugal it is a cultural tradition to drink a glass of wine during meals; and it is a common belief that, red wine in particular, can improve health. It has been suggested, and to some extent proven, that red wine can help prevent heart disease and cancer as it contains antioxidants[3] – such as tannins. This led me to question whether increasing the tannin concentration of red wine, would increase its antioxidant ability.

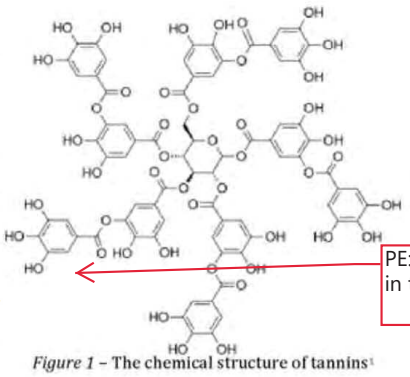

Figure 1 – The chemical structure of tannins[4]

PE: Establishing interest in this paragraph.

Condensed tannins are the main class of tannins found in red wine[4]. These are polymers of up to 27 molecules of flavan-3-ol – compounds contributing to the bitterness of wine, originating from the seeds of the grapes and associated with health benefits due their antioxidant nature.[4] Furthermore, tannins in oak barrels, where red wines are aged, can dissolve into wine through contact. Therefore, prolonging the aging period should result in higher red wine tannin concentration.

This investigation aims to explore the effect of different red wine tannin concentrations on the oxidation of methylene blue solution. Therefore, red wine bottles labelled with different aging time lengths in oak barrels were selected – the chosen range was 0 months, 6 months, 12 months, 18 months and 24 months. Before testing their antioxidative ability, wine samples were titrated with potassium permanganate to measure and record their tannin concentration.

Exp: Topic outlined succinctly

Hypothesis:

Tannins consist of phenolic compounds which can associate spontaneously with a wide range of other chemical entities[1], due to their partial dissociation in solution. This leads them to act as antioxidants: bonding with free radicles and preventing them from oxidising other substances.

Recent reports have shown that the generation of superoxide radicals can be inhibited by tannins.[5] Therefore, this investigation will test this thesis by assessing the antioxidative capacity of red wine samples containing different tannin concentrations. In order to do this, the *blue bottle* experiment will be adapted.

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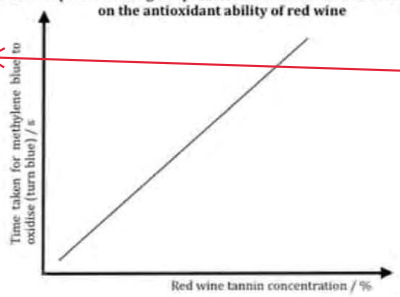
[1] Polyphenols In Wine; Jamie Goode; http://www.thedrinkfactory.com/blog/2015/6/9/polyphenols-in-wine-by-jamie-goode; 15/03/2016
[2] *Tannin*; The Editors of Encyclopædia Britannica; http://www.britannica.com/topic/tannin; 15/03/2016
[3] *Is wine good for you?*; BBC good food; http://www.bbcgoodfood.com/howto/guide/wine-good-you; 15/03/2016
[4] The Key Chemicals in Red Wine – Colour, Flavour, and Potential Health Benefits; *Compound interest*; http://www.compoundchem.com/2014/05/28/redwinechemicals/; 15/03/2016
[5] Tannins and human health: a review; *Chung KT1, Wong TY, Wei CI, Huang YW, Lin Y*; http://www.ncbi.nlm.nih.gov/pubmed/9759559; 15/03/2016

Methylene blue, a redox indicator, is reduced by glucose (under alkaline conditions) to become colourless. However, stirring the solution causes oxygen to dissolve and oxidise solution, whi turns back blue from colourless. In the presence of red wine, containing tannins, the time taken for the methylene blue solution to oxidise should increase because tannins will inhibit oxidation.

I predict a positive linear correlation between red wine tannin concentration and its antioxidative ability. Therefore, rising tannin concentration should cause increasing time taken for solution to oxidise – as shown in *sketch graph* 1.

***Sketch Graph 1* -showing the predicted effect of tannin concentration on the antioxidant ability of red wine**



Exp: Possibly not the most rigorous measure but it is creative

Variables:

Independent	*Tannin concentration of red wine* – tannin concentrations of the selected wines were measured by titrating samples with $KMnO_4$ and concentration was calculated in g dm^{-3} ± 0.001. As tannins are of variable composition, reaction ratio of a standard tannin solution is assumed (*1 cm^3 of 0.004 mol dm^{-3} $KMnO_4$ reacts with 0.0832 mg tannin)[7]. To represent tannin concentration: the aging time length in oak barrels of red wine was varied (0 months, 6 months, 12 months, 18 months and 24 months) because increasing the aging period should increase the tannin concentration.
Dependent	*Antioxidant ability of tannins in red wine* – the time taken for the methylene blue solution to oxidise, measured in seconds using a stopwatch ± 1 s. To measure this variable, 5 cm^3 of red wine samples (of different ages and tannin concentrations) were added to a mixture of methylene blue solution and the reducing agent glucose (under alkaline conditions), which oxidises when stirred in hot-stir plate with magnetic stirrer.

Controlled (Continued pages 3 + 4)	**Method of control**	**Possible effect on results**
Grape source	To ensure all grapes initially used to make different wines have the same chemical composition, except tannin concentration – the same brand of wine, from the same region was used.	The grapes used to make the wine will affect the chemical composition of the wine; so it may have other compounds acting as antioxidant ability.
Exposure to UV light	Keep all bottles together in a cupboard to reduce exposure to ultraviolet radiation from sunlight.	Exposure to ultraviolet radiation will cause free radicles to form, which would react with tannins (due to their antioxidant nature) and would no longer prevent the oxidation of methylene blue.
Time used after opening – immediately	As soon as the wine bottles were opened, the volumes of wine needed for the titrations were collected. The stoppers were then immediately added, and only reopened to collect the wine volumes required to assess the antioxidant ability.	Opening the bottle exposes the wine to air, so it can become oxidised or tannins can act as antioxidants, react with free radicles and become used up.

Exp: The student is showing throughout this section plenty of consideration of subsequent reliability of the data. Some points are minor or barely relevant but we credit what is positive.

Volume of red wine titrated – 5.00 cm^3	Using a 5 cm^3 pipette, 5.00 cm^3 of red wine were measured (and added 10.00 cm^3 water) and transferred to a 250 cm^3 conical flask to be titrated.	Changing the volume of red wine titrated will change the mass of tannins present in the titrated sample – therefore changing the volume of $KMnO_4$ required. This would require the adaptation of calculations of concentration.
Volume of distilled water added to wine samples to be titrated – 10.00 cm^3	Using a 10 cm^3 pipette, 10.00 cm^3 of distilled water were measured and added to 5 cm^3 red wine sample in 250 cm^3 conical flask.	Changing the volume of distilled water added to red wine will change the concentration of tannins present in the titrated sample – therefore altering the volume of $KMnO_4$ required.
Dilute wine heating time to evaporate alcohol – 10.0 minutes	Stopwatch used to time 10.0 minutes, during which dilute wine is heated for alcohol to evaporate at 90°C using a hot plate.	Increasing the heating time will lead to the evaporation of distilled water added, increasing the tannin concentration of sample – altering the volume of $KMnO_4$ required.
Volume and concentration of indigo carmine indicator used – 2.00 cm^3 of 0.5% solution	Indigo carmine indicator solution produced by lab technician. Using a 2 cm^3 pipette, 2.00 cm^3 of indigo carmine indicator were measured and added to 250 cm^3 conical flask (containing dilute red wine after evaporating alcohol).	Changing indigo carmine indicator solution concentration or volume added will change the clearness of golden yellow end point – changing reliability of results.
Concentration of $KMnO_4$ used to titrate red wine samples – 0.004 mol dm^{-3}	Potassium permanganate solution produced by lab technician.	Changing the concentration of $KMnO_4$ will change the reaction ratio with tannins.
Mass of potassium hydroxide added – 8.00 g	Using an electronic balance, 8.00 g of potassium hydroxide were measured and transferred to a 250 cm^3 conical flask.	Potassium hydroxide provides the alkali conditions for the glucose to act as a reducing agent, so changing the concentration will affect the reduction of methylene blue back to a colourless solution.
Volume of distilled water added to mixture – 250.00 cm^3	Using a 250 cm^3 measuring cylinder, 250.00 cm^3 of distilled water were measured and transferred to a 250 cm^3 conical flask.	Changing the volume of water added will change the concentration of all the reactants present in the mixture – changing the speed of reaction.
Mass of glucose used – 10.00 g	Using an electronic balance, 10.00 g of glucose were measured and transferred to a 250 cm^3 conical flask.	Glucose is acting as the reducing agent to turn methylene blue solution colourless, so will affect how reduced the solution is before stirring.
Volume of methylene blue solution used – 5.00 cm^3	Using a 5 cm^3 pipette, 5.00 cm^3 of methylene blue solution were measured and transferred to a 250 cm^3 conical flask.	The oxidation of methylene blue solution is being assessed; so changing the volume of solution used will change the time taken for the entire solution to turn back blue.
Volume of red wine added to mixture of KOH, $C_{16}H_{18}ClN_3S$ and $C_6H_{12}O_6$ – 5 cm^3	Using a 5 cm^3 pipette, 5.00 cm^3 of red wine were measured and added to the 250 cm^3 conical flask containing mixture.	Increasing the volume of red wine used will increase the tannin content added to act as an antioxidant – so results would not be concurrent.

Reaction Temperature	Investigation was carried out in a lab, so temperature was close to constant. However, it could have been monitored by being measured and recorded.	As temperature rises, the proportion of oxygen molecules with sufficient kinetic energy to overcome activation energy increases – resulting in an increase in the rate of oxidation.

Uncontrolled	Possible method of control	Possible effect on results
Fermentation process	By buying wine from the same brand, it is assumed that they will keep their fermentation process constant.	Fermentation process will influence the chemical composition of the wine; leading to the presence other compounds acting as antioxidant ability.

Apparatus:

Titrating wine to measure tannin content:

- 5 X 25 cm^3 of 0, 6, 12, 18 & 24 months aged wines
- 10 X 250 cm^3 conical flask
- 10 X 5 cm^3 pipette ± 0.02 cm^3
- 5 X 90 cm^3 distilled water – H_2O
- 10 cm^3 pipette ± 0.02 cm^3
- Hot plate ± 1 ºC
- Stopwatch ± 1 s
- 25 cm^3 pipette ± 0.03 cm^3
- 2 cm^3 pipette ± 0.1 cm^3
- 5 X 4 cm^3 of 0.5% indigo carmine indicator solution - $C_{16}H_8N_2Na_2O_8S_2$
- 50 cm^3 burette ± 0.1 cm^3
- Funnel
- 10 X 50 cm^3 of 0.004 mol dm^{-3} potassium manganate (VII) – $KMnO_4$
- White tile
- 5 X 100 cm^3 beaker
- 5 X 20 cm^3 pipette ± 0.06 cm^3
- Electronic balance ± 0.01 g
- Weighing boat
- 5 X 1.00 g of activated charcoal
- Stirring rod
- Filter paper

Assessing the antioxidant ability of red wine:

- 5 X 5 cm^3 sample of 0, 6, 12, 18 & 24 months aged wines
- 5 X 5 cm^3 pipette ± 0.02 cm^3
- 5 X 10 g of glucose – $C_6H_{12}O_6$
- 5 X 8 g of potassium hydroxide – KOH
- 5 X 250 cm^3 of distilled water – H_2O
- Electronic balance ± 0.01 g
- Weighing boat
- 5 X 5 cm^3 of 0.1% methylene blue solution – $C_{16}H_{18}N_3SCl$
- 5 cm^3 pipette ± 0.02 cm^3
- 250 cm^3 measuring cylinder ± 1 cm^3
- 5 X 250 cm^3 conical flask
- 5 Bungs
- Stirring rod
- Magnetic stirrer
- Hot–Stir plate
- Stopwatch ± 1 s

Risk assessment: [6]

Hazard	Control Measure
Safety: Potassium permanganate causes skin and severe eye irritation	Wear eye protection, as solution is below 0.1 mol dm^{-3} gloves are not required, however advised for users with wounds or skin conditions.
Safety: Indigo carmine indicator has a low hazard	No particular safety measured as necessary, however the use of gloves and goggles are advised.
Environmental: disposal of titrated red wine with $KMnO_4$ (toxic to aquatic life) in presence of $C_{16}H_8N_2Na_2O_8S_2$	As solution of $KMnO_4$ used to titrate wine was only 0.004 mol dm^{-3} (has to be less than 0.1) and indigo carmine indicator solution concentration is 0.5% (has to be lower than 1%) the titre can be poured down a foul-water drain.
Safety: Potassium hydroxide causes severe skin burns and eye damage	Wear gloves and eye protection, when weighing the mass of potassium hydroxide solid and use a spatula to transfer the solid from the container to the weighing boat and subsequently to the conical flask.
Safety: Methylene blue is harmful by inhalation and in contact with skin	Aqueous solution of the dye is only 1% by mass, so it becomes low hazard; however the use of gloves when measuring the volume and transferring the indicator into the conical flask is advised.
Environmental: disposal of solution containing KOH, $C_{16}H_{18}N_3SCl$, $C_6H_{12}O_6$ and wine sample	The solution should be dissolved – so the potassium hydroxide concentration would fall below 0.1 mol dm^{-3}, and methylene blue solution lower than 1% concentration; then the mixture can be poured down a foul-water drain.
Ethical	There are no ethical issues identified for this investigation as no living organisms were used in or endangered by this experiment.

Exp: This is full consideration of safety.

Procedure:

Titrating wine with $KMnO_4$ to calculate tannin concentration in wine samples[7]

Exp: Student has sourced this method but it is referenced and is part of a wider methodology so that is acceptable.

$KMnO_4$ is an oxidising agent: $Mn(VII)O_4^- \xrightarrow{+5e^-} Mn(II)O$, so it will oxidise tannins in red wine sample and at end point oxidise Indigo Carmine (redox) Indicator to turn from blue to golden vellow.

Actual titration:

0.a) To make up 500 cm^3 of 0.004 mol dm^{-3} $KMnO_4$ solution; use an electronic balance and a weighing boat to weigh 0.316 g of $KMnO_4$ solid, transfer it into a small beaker and add distilled water until all the solid dissolves.
0.b) Using a funnel pour the solution into a 500 cm^3 volumetric flask and add distilled water until it reaches the mark (the bottom of the meniscus should just touch the line).

1. Using a 5 cm^3 pipette, add 5.00 cm^3 of non-aged wine to a 250 cm^3 conical flask.
2. Using a 10 cm^3 pipette, add 10 cm^3 of distilled water.
3. Using a hot plate at 90 ^oC, and heat solution in flask for 10 minutes or until volume is reduced to 7.00 cm^3 – this will boil off the alcohol.
4. Using a 25 cm^3 pipette, add 25.00 cm^3 of cold distilled water.
5. Using a 2 cm^3 pipette, add 2.00 cm^3 of indigo carmine indicator.
6. Using funnel, pour the potassium permanganate solution into a 50 cm^3 burette.

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[6] *Supporting Practical Science & Technology – in schools and colleges*; CLEAPSS; 2007 edition
[7] Vintage titrations: tannin in wine; *The Royal Society of Chemistry – Professor G.W.A. Fowles of University of Reading*; http://www.rsc.org/learn-chemistry/resource/download/res00000585/cmp00000698/pdf; 12/12/2015

7. Place the conical flask on top of a white tile, and titrate the wine solution until a golden yellow colour is obtained.

8. Record volume of potassium permanganate used (titre) on table 1.

****Actual titration*** volume includes volume of $KMnO_4$ oxidising tannins, as well as indicator and other oxidisable substances in the red wine. Therefore, a ***Blank titration*** (using activated charcoal to remove tannins from red wine samples – resulting in their decolourisation) is required. This titration will measure the volume of $KMnO_4$ not oxidising tannins; so *blank titration* volume will be subtracted from *actual titration* volume to calculate the volume of $KMnO_4$ oxidising tannins

Blank titration (significance outlined in previous paragraph):

9. Using a 20 cm^3 pipette, add 20.00 cm^3 of non-aged wine into a 100 cm^3 beaker.

10. Using an electronic balance and a weighing boat, weigh 1.00 g of activated charcoal, transfer it into the beaker and stir thoroughly.

11. Using a funnel and filter paper, filter the mixture.

12. Using a 5 cm^3 pipette, transfer 5.00 cm^3 of filtered wine to a 250 cm^3 conical flask; and repeat steps 2 through 8 – *actual titration*.

13. To calculate the volume of potassium permanganate used to oxidise tannins, subtract the volume used in blank titration from volume used in actual titration.

14. Repeat steps 1 through 13 for other wine samples with different aging time lengths – 6 months, 12 months, 18 months & 24 months.

15. Repeat steps 1 through 14 until 2 concurrent titre volumes for each titration are obtain[-3]

Measuring antioxidant ability of red wine samples with different tannin concentrations[8]

Exp: Again a sourced method applied within the student's own context which is appropriate.

1. Using an electronic balance and a weighing boat, weigh 8 g of potassium hydroxide and transfer it into a 250 cm^3 conical flask.

2. Using a 250 cm^3 measuring cylinder, add 250 cm^3 of distilled water into the conical flask.

3. Using an electronic balance and a weighing boat, weigh 10 g of glucose and transfer it into the conical flask.

4. Using a stirring rod, stir the solution until the solids are dissolved.

5. Using a 5 cm^3 pipette, add 5 cm^3 of methylene blue solution into the conical flask.

6. Place a magnetic stirrer on the conical flask, place a bung and transfer it onto a hot-stir plate.

7. When solution decolourises, turn the stirrer on to STIR 4 and, simultaneously, start stopwatch.

8. When the solution turns blue stop the stopwatch and the magnetic stirrer.

9. Record the time taken for oxidation to occur without antioxidant added; *on table 3.*

10. Using a 5 cm^3 pipette, add 5 cm^3 of non-aged wine and repeat steps 7 and 8.

11. Record the time taken for oxidation to occur with non-aged wine; on *table 3.*

12. Repeat steps 1 through 11, for other wine samples with different aging time lengths – 6 months, 12 months, 18 months & 24 months.

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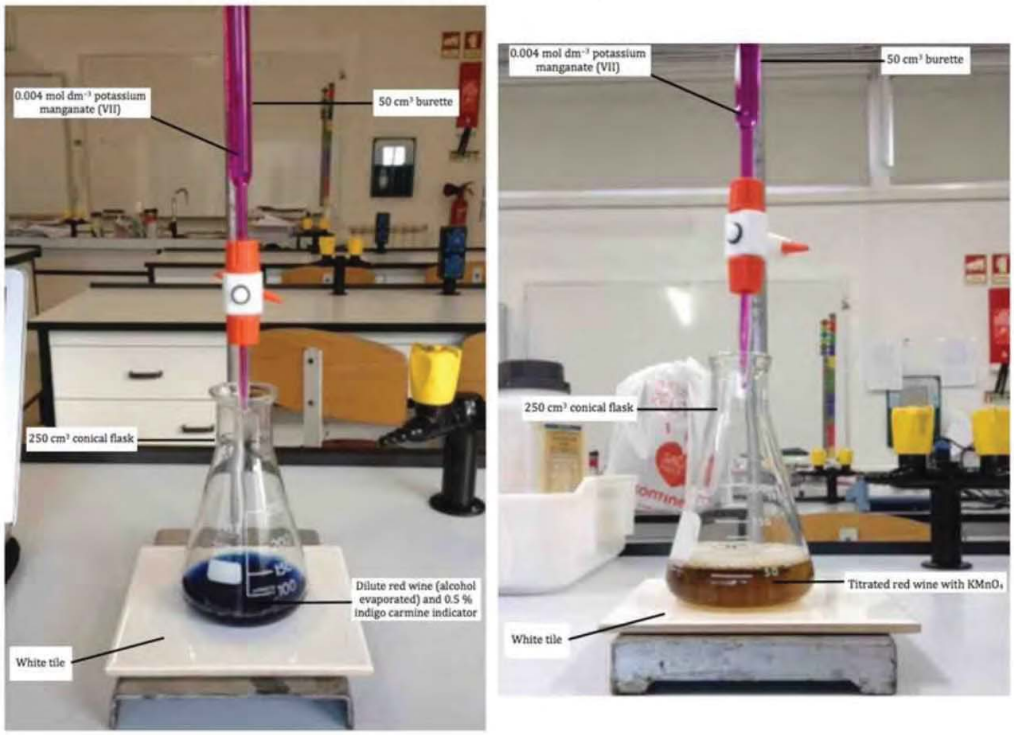
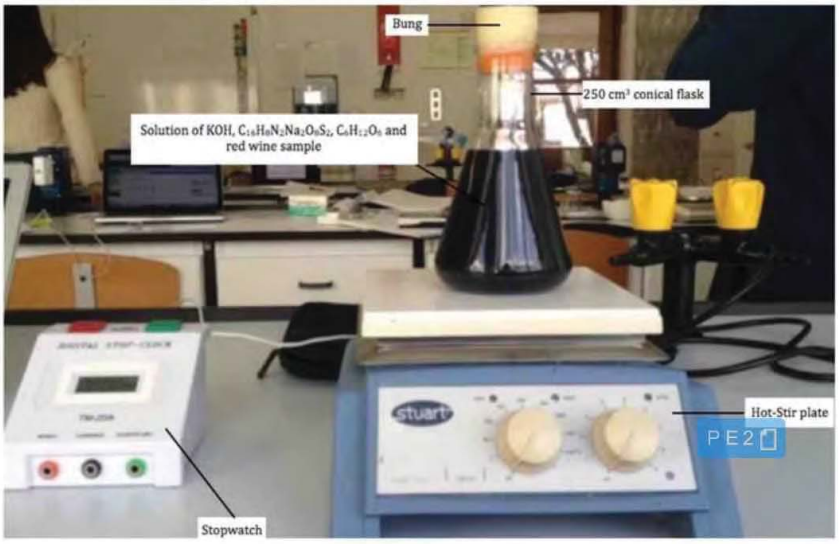
[8] The 'blue bottle' experiment; *The Royal Society of Chemistry; http://www.rsc.org/learn-chemistry/resource/res00000729/the-blue-bottle-experiment?cmpid=CMP00005928; 27/03/2016*

Diagrams:

Photographs 1 & 2 – Set up of wine titration to measure tannin concentration



Photograph 3 – Set up of reaction to assess the antioxidant ability of red wine



Results:

Photograph 4 – End point of titration to measure tannin concentration

Qualitative data given in form of photographs. Not best practice.

Table 1 – Raw quantitative data showing the effect of aging time length in oak barrels content

Wine age / months ± 1	Volume of 0.004 mol dm⁻³ potassium permanganate used / cm³ ± 0.1																			
	Actual Titration = *A*										Blank titration = *B*									
	Titre 1			Titre 2			Titre 3			Mean ±0.01	Titre 1			Titre 2			Titre 3			Mean ±0.01
	Initial reading	Final reading	Titre volume	Initial reading	Final reading	Titre volume	Initial reading	Final reading	Titre volume		Initial reading	Final reading	Titre volume	Initial reading	Final reading	Titre volume	Initial reading	Final reading	Titre volume	
0	0	33.3	33.3	0	36.5	36.5	0	33.2	33.2	33.25	0	16.7	16.7	0	16.3	16.3	0	16.3	16.3	16.30
6	0	49.2	49.2	0	49.1	49.1	-	-	-	49.15	0	18.3	18.3	0	19.0	19.0	0	18.4	18.4	18.35
12	0	58.7	58.7	0	60.5	60.5	0	60.5	60.5	60.50	0	18.0	18.0	0	18.0	18.0	-	-	-	18.00
18	0	71.1	71.1	0	72.4	72.4	0	71.0	71.0	71.05	0	71.1	71.1	0	71.0	71.0	-	-	-	18.85
24	0	80.4	80.4	0	80.5	80.5	-	-	-	80.45	0	18.6	18.6	0	17.8	17.8	0	17.7	17.7	17.75

Ana: Plenty of quantitative raw data presented.

Table 2 – Processed quantitative data showing the effect of aging time length in oak barrels on tannin content

Wine age / months ± 1	Mean $KMnO_4$ vol. reacting with tannins / cm³ ± 0.1 (** = mean A – mean B)	Mean tannin content in 5 cm³ of wine/ mg (* = 0.0832 x $KMnO_4$ volume)	Mean tannin concentration/ g dm⁻³ (= 0.2 x tannin content)
0	16.95	1.410	0.282
6	30.80	2.563	0.513
12	42.50	3.536	0.707
18	52.20	4.343	0.869
24	62.70	5.217	1.043

Com: This calculation step has come from reference 7 but this is no clearly stated.

Table 3 – Raw and processed quantitative data showing the effect of tannin concentration on antioxidant ability of red wine

Mean tannin conc / g dm⁻³ ± 0.001	Time taken for methylene blue solution to oxidise (turn blue) / s ± 1				
	Trial 1	Trial 2	Trial 3	Trial 4	Mean
–	12	10	9	14	11
0.282	31	30	33	29	31
0.513	44	46	44	42	44
0.707	58	56	59	60	58
0.869	70	73	71	70	71
1.043	85	85	88	84	86

Data processing:

• **Calculating percentage uncertainties** $= \frac{\text{Absolute uncertainty}}{\text{Reading taken}} \times 100$

1. Percentage uncertainty of 5.00 cm^3 of red wine titrated $= \frac{\text{pipette uncertainty}}{\text{pipette volume}} = \frac{0.02}{5.00} \times 100 = 0.4\%$

2. Total percentage uncertainty of 500.00 cm^3 of 0.04 mol dm^{-3} $KMnO_4$ solution (produced by lab technician) = % uncertainty of electronic balance ± 0.001 g + % uncertainty of volumetric flask ± 0.5 cm^3

Conc ($KMnO_4$) = $0.004 = \frac{n}{500/1000}$ n ($KMnO_4$) = $0.002 = \frac{\text{mass}}{\text{molar mass}} = \frac{\text{mass}}{39.10+54.94+16.00\times4}$

Ana: Full consideration of uncertainties throughout this section.

Mass ($KMnO_4$) = $0.002 \times 158.04 = 0.316$ g

% Uncertainty of electronic balance reading $= \frac{\text{electronic balance uncertainty}}{\text{mass measured}} = \frac{0.001}{0.316} \times 100 = 0.316\%$

% Uncertainty of volumetric volume $= \frac{\text{volumetric uncertainty}}{\text{volumetric volume}} = \frac{0.5}{500.0} \times 100 = 0.1\%$

Total % uncertainty of $KMnO_4$ solution = 0.316 + 0.1 = 0.416%

Table 4 – Calculating percentage uncertainties of burette $KMnO_4$ volume readings

Wine age / months ± 1	Mean Actual titration		Mean Blank titration		$KMnO_4$ reacting with tannins	
	Volume / cm^3 ±0.01	% Uncertainty	Volume / cm^3 ±0.01	% Uncertainty	Volume / cm^3 ±0.01	% Uncertainty
0	33.25	0.30	16.30	0.61	16.95	0.030 + 0.061 = 0.091
6	49.15	0.20	18.35	0.54	30.80	0.020 + 0.054 = 0.074
12	60.50	0.17	18.00	0.56	42.50	0.017 + 0.056 = 0.073
18	71.05	0.14	18.85	0.53	52.20	0.014 + 0.053 = 0.067
24	80.45	0.12	17.75	0.56	62.70	0.012 + 0.056 = 0.068

Table 5 – Calculating total percentage uncertainty of tannin concentrations

Mean tannin concentration/ g dm^{-3} ± 0.001 *(= 0.2 x tannin content)*	**Total percentage uncertainty of tannin concentrations / %** = Σ (Percentage Uncertainties)
0.282	0.4 + 0.416 + 0.091= 0.907
0.513	0.4 + 0.416 + 0.074 = 0.890
0.707	0.4 + 0.416 + 0.073 = 0.889
0.869	0.4 + 0.416 + 0.067 = 0.883
1.043	0.4 + 0.416 + 0.068 = 0.884

Table 6 – Calculating percentage uncertainty of times taken for methylene blue solution to oxidise

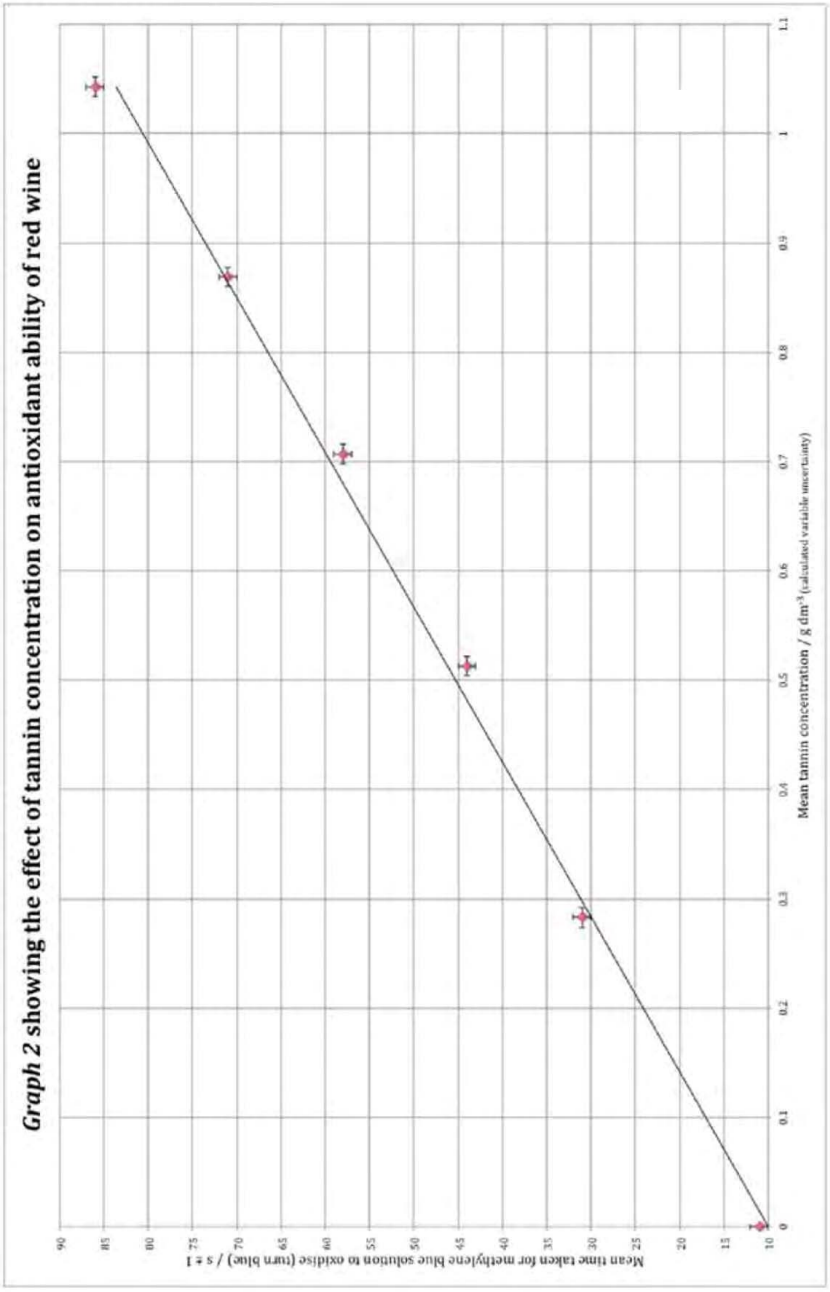
Mean time taken for methylene blue solution to oxidise (turn blue) / s ± 1	**Percentage uncertainty of times taken for methylene blue solution to oxidise / %**
11	9.09
31	3.23
44	2.27
58	1.72
71	1.41
86	1.16

• **Calculating mean time taken to oxidise methylene blue solution (manually)** $= \frac{\Sigma \text{ Trials}}{\text{Number of Trials}}$

Calculating the mean produces a value representing the complete data sample collected.

Eg: At tannin concentration 0.282 → $\frac{31+30+33+29}{4} = 31$ s

Processed data presentation:



Ana: The student has carried out appropriate calculations and constructed a suitable graph so that research question can be answered.

Ana: More consideration of uncertainties

Y-error bars plotted show absolute uncertainty of stopwatch used to measure time taken for methylene blue solution to oxidise (and turn blue). X-error bars plotted show calculated uncertainty of mean tannin concentration.

Conclusion:

The positive correlation represented in *graph 2* with line of best fit, plotted in Excel, shows that as the red wine samples' tannin concentration increased, it led to an increase in the time taken for methylene blue solution to oxidise and turn blue. Therefore, increasing the tannin concentration of red wine increases its antioxidant ability.

Y and X-error bars in *graph 2* show absolute and calculated uncertainties of measured variables; these do not overlap. Therefore, showing the concurrence of raw data – supporting the conclusion.

The results obtained strongly support the initial hypothesis, which stated that increasing tannin concentration in red wine samples would increase its antioxidative ability – demonstrated by an increase in time taken for methylene blue to oxidise and turn blue from

Eva: Nature of quantified relationship clarified here

Research of secondary sources led to the development of *sketch graph 1*, that increasing red wine tannin concentration would cause a proportion for oxidation of methylene blue to occur. *Graph 2* showing the established effect of mean red wine tannin concentration on mean time taken for oxidation of methylene blue to occur; shows the same correlation as predicted in *sketch graph 1*. This shows how the data collected support the initial hypothesis.

The pattern within data collected was assumed to be caused by phenolic compounds present in tannins. These compounds dissolve in water in an equilibrium reaction: they partly dissociate into phenoxide ions and H^+ protons – as can be seen in *figure 2*. Therefore, when oxygen dissolve mixture containing reduced methylene blue solution and tannic acid it reacted with the H^+ protons, donated by tannic acid, to form water. This shifted the dissociation equilibrium to produce more H^+ protons; until all tannic acid dissociated and was oxidised. Resulting in a delay in the oxidation of methylene blue. So, increasing the tannin concentration in red wine samples increased the moles of H^+ protons in mixture inhibiting the oxidation of methylene blue – which resulted in an increase in time taken for methylene blue to be oxidised.

Figure 2 – Equilibrium dissociation of phenolic compounds in water[9]

Eva: This explanation of the conclusion is rather jumped in to and not well founded. The antioxidising effect is based on free radical reactions not the weak acid dissociation reaction presented in Figure 2.

Evaluation:

All of the calculated total percentage uncertainties of tannin concentrations were below 1%, therefore they were inconsequential, demonstrating the validity of conclusions drawn from the results.

The calculated percentage uncertainties of times taken for methylene blue solution to oxidise varied from 1 to 9%. These are significantly larger than the calculated total percentage uncertainties of tannin concentrations; however, they were sufficiently low to demonstrate the concurrency of results and validity of conclusions.

There were no anomalous results collected because data collected at different mean red wine tannin concentrations did not overlap at any concentration. Furthermore, the proximity of data plotted to the straight line-of-best-fit shows that the strength of the correlation between variables.

[9] THE ACIDITY OF PHENOL; *Jim Clark*; http://www.chemguide.co.uk/organicprops/phenol/acidity.html; 16/03/2016

To reduce human error involved in identifying the endpoint of titration and oxidation reaction Vernier Sensors, such as lux meters, could be used to obtain a quantifiable endpoint – resulting in more precise results and more reliable conclusions.

The inability to verify the entire chemical composition of the wine samples (such as other antioxidants present) induces uncertainty in the measurement of the sole effect of changing tannin concentrations on red wine antioxidative ability. However, it is assumed that the increase is due to the increased tannin concentration because same brand of wine, from the same region, was used to ensure all grapes used to make different wines had the same chemical composition (only changing tannin concentration by aging).

Eva: Discussion of weaknesses starting to go beyond the procedural and addressing deeper methological considerations.

- All masses, volumes and concentrations of chemicals used were kept constant to maintain reaction ratios; ensuring the validity of data collected.
- All red wine bottles were kept in cupboard until used to reduce exposure to UV light, which could lead to the oxidation of tannins in wine.
- All wine samples were collected immediately after opening wine bottles to reduce its exposure to air, which could lead to the oxidation of tannins. However, even though the bottles were re-corked after collecting wine sample for titration, the opening of the bottle will still lead to some oxidation of wine before it is used in the antioxidant analysis experiment.
- Reaction temperature was kept constant by carrying out experiment in a lab, where temperature is close to constant. However, it could have been monitored by recording it: to improve the validity of conclusions extracted from data collected.

Fermentation process was the only uncontrolled variable in this experiment, although it could have had an impacted on the chemical composition of the wine; leading to the presence other compounds acting as antioxidant ability; this was managed by purchasing wine from the same brand, it is assumed that they will keep their fermentation process constant.

To further investigate this theory, pure solutions of tannic acid could be used in the analysis of tannin concentration on antioxidant ability of solution. Another possibility would be to use older wines, containing higher tannin concentrations, which were not used in this investigation due to their price. These could then be analysed using chromatography or mass spectroscopy in order to identify the exact tannic compounds present in the wine samples – these methods of wine analysis require detectors unavailable in a school laboratory.

Eva: Extensions and modifications being given.

Additionally, other methods of testing the antioxidant ability of tannins could be choose – such as FRAP (Ferric Reducing Antioxidant Power) and DPPH (DPPH Radical Scavenging Capacity), which provide more accurate and precise results but require chemicals unavailable in a school laboratory.