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Analysis of wine– an undergraduate project

Experiments which use common, familiar substances as their basis[1,2] generally prove popular in junior undergraduate courses since students can readily perceive their 'relevance'. The experiment described here involves analysis of wine for its major components and is based largely on standard enological analytical techniques used in industry.

The project, when performed by first year chemistry students, occupied three $3\frac{1}{2}$ hour laboratory sessions with an additional optional session for discussion and evaluation of results. The wines were examined for their pH, total titratable acidity, fixed and volatile acidities, alcohol content, specific gravity and potassium content, while individual non-volatile acids were detected using thin layer chromatography (tlc). (Most Australian wines, particularly red wines, have unusually high levels of potassium hence its determination is significant in Australia.) The main teaching aims of the experiment were to illustrate an industrial application of a number of principles and techniques taught in the standard laboratory course (in particular, buffers, potentiometric titrations, as well as distillation and chromatography), and to provide an interesting example of fundamental acid–base techniques.

Wines are dilute solutions of weak organic acids, these being mainly tartaric, malic, lactic, acetic (ethanoic) and succinic acids. The total acidity of a wine is generally expressed as the sum of the 'fixed' acidity (due mostly to malic and tartaric acids) and the 'volatile' acidity. The importance of these parameters to the enologist is discussed in detail in many books and articles on the production of wine.[3,4]

Determination of total acidity is generally achieved by titration of the wine with a strong base, the end point being detected visually with an indicator, or electronically with a pH meter. Since the acids present in wine are relatively weak, titration with a strong base gives an end point in the alkaline region of the pH scale; the American Society of Enologists recommend pH 8.2 for the end point.[5]

The volatile acidity in wine is due to the fatty acids such as formic, acetic, butyric *etc.* Wines of high volatile acidity are prone to spoilage and specific regulations exist governing the maximum permissible amount of volatile acidity. The volatile acids may be separated from the wine by steam distillation and the volatile acidity subsequently determined from the distillate. Alternatively, the volatile acids may be removed by repeated evaporation of the wine sample and the residue analysed for the non-volatile (or 'fixed') acids. The volatile acidity may then be readily calculated by subtraction of the fixed acidity from the total acidity.

Identification and even semi-quantitative determination of individual non-volatile acids is possible using tlc or paper chromatography. This is particularly useful in industry for following the enzyme catalysed conversion of malic acid into lactic acid (called malolactic fermentation) during the fermentation process. The amount of malic acid in grapes decreases as the grapes mature. In some areas, particularly where the growing season is short, the grapes still retain a high degree of acidity when picked. Since the conversion of malic acid to the weaker lactic acid reduces the acidity of the fermenting wine, it is important that the relative amounts of the two acids be monitored.

The ethanol content of wines is governed by the amount of sugar in the grapes and by the yeast cells, most of which are inhibited at about 15 per cent (V/V) ethanol. The permissible range of ethanol content in wines is set by state or national regulations and usually varies between 10.0–14.0 per cent (V/V). In this experiment, the ethanol concentration is determined both by gas-liquid chromatography (glc) and by density measurements on the distillate produced by boiling alkaline solutions of the wines.

pH has a well recognised effect on the taste of wine; wines of high pH tend to taste rather flat on the palate and are also much more susceptible to oxidative and biological spoilage than are wines of lower pH. The pH of a red wine is sometimes regarded as its most important single feature. There is no direct or predictable relationship between pH and total titratable acidity, this being generally attributed to variation between wines in their content of the cations K^+, Mg^{2+}, Na^+ and Ca^{2+}.[6] The most abundant of these cations is potassium and the determination of potassium in wine has become increasingly important in the wine industry, particularly in growing areas containing potassium-rich soils.

Experimental

pH of wine, pH titration. The pH of the wine was measured and then a detailed pH titration of a 25.0 cm^3 sample of the wine with 0.1 M NaOH was performed. The titration curve of pH versus volume of NaOH added was plotted and any colour changes which occurred during the titration of the red wine were noted.

Determination of total acidity. The issued 0.1 M NaOH solution was standardised using potassium bitartrate and then titrated with three 25.0 cm^3 aliquots of the wine. A suitable indicator for white wines was phenolphthalein and for red wines, thymol blue was used. (The pigments contained in the red wine could also act as indicator if a colour standard retained from the pH titration was available.) In order to see the colour change, it was necessary to dilute the red wine aliquot considerably with distilled water to reduce its colour, or alternatively 25.0 cm^3 of the wine was quantitatively diluted to 250 cm^3 and suitable aliquots then titrated with 0.02 M NaOH prepared by quantitative dilution of the standard NaOH.

Determination of fixed or non-volatile acidity. Three 25.0 cm^3 samples of wine were carefully evaporated on a hot plate until the volume of each had reduced to 5–10 cm^3: overheating was avoided. About 25 cm^3 of hot distilled water was added and the solutions again evaporated to a final volume of 5–10 cm^3. The process was repeated several times, after which the residues were cooled and diluted to about 50 cm^3 with distilled water. The white wine samples were titrated with standardised 0.1 M NaOH using phenolphthalein as indicator. It was necessary to dilute the red wine samples further before they were titrated with 0.02 M NaOH using thymol blue as indicator, as described in the previous section.

Qualitative detection of non-volatile acids by tlc. A chromatography tank was lined with filter paper and about 100 cm^3 of a freshly prepared 16:2:5 mixture of butanol, formic acid and water (respectively) was added. The tank was covered and allowed to equilibrate.

Table 1. Summary of results.

Property	Claret	White Burgundy	Sauternes	Riesling
1. Total acidity*				
(*a*) cm^3 0.1 N NaOH/100 cm^3 wine	73.4	77.5	65.1	74.1
(*b*) g tartaric acid/100 cm^3 wine	0.551	0.582	0.489	0.556
2. Fixed acidity cm^3 0.1 N NaOH/ 100 cm^3 wine	64.0	63.6	59.7	65.3
3. Volatile acidity				
(*a*) cm^3 0.1 N NaOH/100 cm^3 wine	9.4	13.9	5.4	8.8
(*b*) g acetic acid/100 cm^3 wine	0.056	0.083	0.032	0.053
4. Alcohol content (% *V/V*)				
(*a*) By distillation	11.2	10.7	11.8	10.3
(*b*) By glc	11.7	10.7	12.1	10.5
5. SG of wine				
(*a*) By weighing	0.989	0.990	1.007	0.987
(*b*) Using hydrometer	0.991	0.991	1.009	0.989
6. pH of wine†	3.85	3.45	3.63	3.44
7. Potassium content (g 100 cm^{-3})	0·181	—	—	0.100

Note that there are two alternative means of expressing total and volatile acidities, these being the volume of 0.1 N NaOH required to neutralise 100 cm^3 of wine and the weight of tartaric acid (total acidity) or acetic acid (volatile acidity) contained in 100 cm^3 of wine.

* No steps were taken to remove carbon dioxide.
† Australian wines are characterised by high pH values, this being related to the high potassium content. Most French and German commercial wines have pH values in the 3.0–3.6 range—clarets are normally 3.2 and Burgundies, 3.3.

Spots of succinic, citric, lactic, malic and tartaric acids (as 0.8 per cent aqueous ethanol solutions), a mixture of these acids, and wine were applied about 2 cm from the bottom of commercially available silica gel tlc plates (5 × 10 cm Merck Silica Gel 60 F_{254}). Only three spots were applied to any one plate and several applications of each spot were made, drying the spots completely between applications. The plates were placed in the tank and developed until the solvent front was about 1 cm from the top of the plate. The position of the solvent front was marked, the plate removed from the tank and allowed to dry. The plate was then transferred to an oven at 110°C and left there for about two hours (or overnight) in order to remove completely the formic acid left from the developing solvent. The cool plate was sprayed with a 0.04 per cent aqueous solution of bromocresol green. R_f values for the acids were recorded and used to identify the acids present in the wine.

Determination of ethanol content. A 50.0 cm^3 sample of wine was added to a distillation flask and made just alkaline with NaOH. The sample was distilled until the temperature of the distillate reached 100°C or until half the original volume had been distilled. The distillate was diluted with distilled water to exactly 50.0 cm^3 in a pre-weighed volumetric flask. The flask and liquid were then weighed and the density of the solution calculated.

A series of five standard solutions of ethanol in distilled water was prepared covering the range 5–25 per cent (*V/V*) ethanol. The densities of these solutions were determined by weighing accurately known volumes and a calibration curve of density *versus* ethanol concentration plotted. This calibration curve was then used to determine the concentration of ethanol in the wine.

The ethanol content of wines was also determined by glc using 1 µl injections into a carbowax column at 110 °C. The ethanol standard solutions prepared above were used to construct a calibration curve of peak height and peak area *versus* ethanol concentration and from this curve, the ethanol content of the wine was determined.

Potassium content.[7] To 50.0 cm^3 of wine, 10 cm^3 of 0.1 N HCl was added and the pH of the solution confirmed to be below two. 40 cm^3 of the sodium tetraphenylborate reagent (prepared according to Vogel[7]), was slowly introduced from a burette and the solution constantly stirred during the addition. The precipitate was allowed to settle for 1 h before filtering through a sintered glass crucible (porosity No. 4) and was then washed several times with small portions of a saturated sodium tetraphenylborate solution and finally with 2 cm^3 of ice-cold water. After drying at 120 °C, the cooled crucible was accurately weighed.

Table 2. R_f values of acidic components of wine in 16:2:5 butanol, formic acid, water solvent.

Acid	R_f value
Succinic	0.71
Lactic	0.56
Malic	0.42
Citric	0.30
Tartaric	0.20

Specific gravity. The specific gravity of the wine was determined by weighing an accurately known volume and also by means of a hydrometer.

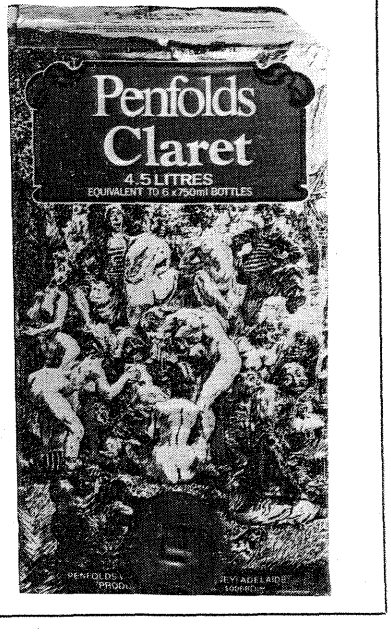
Results

The results obtained are summarised in Tables 1 and 2.

Discussion

The experiment has been operating successfully for two years with large classes (150 students). The class for each practical session was divided into groups of 10–15 students and these groups further subdivided into pairs of students; each pair was required to perform all of the experiments in the project with the exception of the potassium analysis. Since the sodium tetraphenylborate reagent used was very costly, only selected students were asked to attempt the potassium determination.

Bulk containers (5l 'casks') of each wine were purchased at the start of the experiment and stored at room temperature. (In Australia, wine sold in 'casks' is packed in a flexible polyvinyl acetate bag which is housed in a rigid cardboard outer container. As the wine is decanted (*via* a tap fitted to the bag), the flexible bag collapses ensuring that no air is introduced and thus preventing oxidation of the wine.) Prior to each laboratory session, 1.5l of each wine was decanted and provided to the students; this amount proved sufficient for a daily class of 40 students.



In the tlc experiment, several types of plates and a number of solvent systems were investigated before satisfactory separation of wine acids was achieved. Plates prepared using Merck Silica Gel PF_{254} proved unsuccessful, while those prepared using Merck Silica Gel $HF_{254+366}$ (type 60) were successful. The best separation was obtained using commercial plates (as described under 'Directions') and the quoted R_f values refer to these plates.

If the optional discussion session was held, students were required to perform a statistical analysis of the collected results of the class and to discuss in detail the theoretical aspects of the techniques used during the project. However, no interpretation of the results from an enological viewpoint was required.

Acknowledgements
We wish to acknowledge the contributions made by Professor J. Hill and Associate Professor W. D. Crow.

Drs P. R. Haddad and M. Sterns are senior tutors and Judith Wardlaw is a laboratory technician in the chemistry department, School of General Studies, Australian National University, PO Box 4, Canberra, ACT, Australia, 2500.

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Sulphur dioxide and tannin

Professor G. W. A. Fowles (Reading University) adds two determinations to those given in the preceding paper, namely sulphur dioxide (free and combined) and tannin.

Sulphur dioxide is an essential additive to all wines since it acts both in a biocidal capacity (preventing attack by unwanted bacteria and yeasts) and as an antioxidant. If too little sulphur dioxide is used the wine may suffer from biocidal attack and it will certainly develop the characteristic taste of an oxidised wine; too much sulphur dioxide, on the other hand, will be very evident on the palate. Most of the added sulphur dioxide combines with various aldehydic and ketonic molecules (especially acetaldehyde, ethanal) and the remaining uncombined sulphur dioxide is available to protect the wine. There are legal limits to the total SO_2 that is permitted, the limits varying from country to country, but 250 ppm is a commonly accepted value. There is no limit on the amount of free SO_2 but 20–40 ppm levels safeguard the wine and will not affect the taste; levels below 10 ppm in a white wine show that it is in imminent danger of 'going over the hill'.

Tannin, in contrast, is a natural ingredient of wines and originates in the skin. Red wines are normally made by fermentation on the skins which extracts a lot of tannin, so that typical values for Burgundies and clarets will be in the 0.15–0.4 per cent range with levels about a tenth of this for typical white wines. For red wines, tannin is essential since it gives the wine the 'bite' that balances the acid and sugar; it also acts as a preservative. As the wines age so the 'tannins' slowly polymerise to give six- or eight-unit tannins that are less abrasive to the taste, with a consequent mellowing of the wine. There is also some loss of tannin in the form of the characteristic deposit found in most of the great red wines on storage.

Determination of sulphur dioxide.
This makes use of the conventional iodine titration, using starch as an indicator. For free, or uncombined sulphur dioxide, the wine is acidified and titrated; for total sulphur dioxide wine is first made alkaline with sodium hydroxide to break down the bisulphite (hydrogensulphate(IV)) compounds, and then acidified and titrated. The methods are quick and sufficiently accurate for the required purpose.

With red wines there is some masking of the end point but it can normally be seen without too much difficulty if a comparison solution of untitrated wine is available.

Solution required:
25 per cent sulphuric acid
1M sodium hydroxide
1 per cent starch solution
0.01M iodine solution

Free SO_2. Pipette 50 cm^3 of wine into a conical flask, add approximately 5 cm^3 of 25 per cent H_2SO_4 and 2–3 cm^3 of starch solution. Titrate with 0.01M I_2 until the first blue colour appears.

Total (free and combined) SO_2. Place about 25 cm^3 of 1M NaOH in the conical flask and pipette in 50 cm^3 of wine. Shake. Leave for 15 min. Add 10 cm^3 of 25 per cent H_2SO_4 and 2–3 cm^3 of starch solution and titrate with 0.01M I_2.

Calculation Amount of SO_2 present (in parts per million) = 12.8 × No. of cm^3 of I_2 used.

Determination of 'tannin'. A standard solution of potassium permanganate [potassium manganate(VII)] is used to oxidise the tannin and colouring matters, and the end point is determined using indigo carmine which is itself oxidised by excess of permanganate; as the indicator uses up some permanganate it must be measured out carefully and allowed for by a blank determination. The alcohol would also be oxidised so it is first removed by gently boiling the wine.

Solution required:
0.004M $KMnO_4$
0.5 per cent indigo carmine indicator

The indigo carmine indicator is made up by dissolving 0.5 g of the dyestuff in 60 cm^3 of warm distilled water. The solution is cooled, 4 cm^3 of conc. H_2SO_4 is added, and the volume made up to 100 cm^3 with distilled water. The solution is filtered through a No. 42 Whatman paper.

Experimental procedure.
1. Pipette 5 cm^3 of wine into a conical flask and add 10 cm^3 of distilled water. Place funnel into neck of conical flask (to reduce losses by spilling) and heat the flask gently until the volume of wine and water has been reduced to 5–7 cm^3. (By now the alcohol will have boiled off.) Add about 250 cm^3 of cold distilled water and *pipette* in 2 cm^3 of the indigo carmine indicator. Titrate with 0.004M $KMnO_4$ until a golden yellow colour appears. Titre A

2. Take 20–25 cm^3 of the wine and add 1 g of activated charcoal. Stir thoroughly. Filter. Pipette 5 cm^3 of the decolourised wine into the conical flask and continue as in *1.* This blank determination allows for the indicator and any oxidisable compounds (other than tannins, anthrocyanins *etc*) in the wine.
Titre B
Hence the amount of $KMnO_4$ used up by the tannins and colouring pigments equals A−B = C.
Percentage tannin and pigments equals C × 0.0166.