Sample preparation guide - analysing wine and ferment samples for tannin, colour and phenolics measures using the Wine Portal

Part (a) – Calculation of tannin, total pigment and total phenolics

Materials

- 10 mm path length **quartz** cuvettes (plastic cuvettes cannot be used for tannin analysis)
- 1 M HCl
- 10ml test tubes
- Pipettes, tips, wipes
- UV/VIS Spectrophotometer
- Sealed QC Reference standard cuvette – provided by the AWRI

Method

NOTE: This method is suitable for analysing wines that have completed alcoholic fermentation (classified as post-ferment) and ferment samples from Day 3 of fermentation onwards. It is not suitable for analysing juices or ferments earlier than Day 3. If ferments or wines are hazy, clarify first by centrifugation.

Dilution and Incubation

- Add 10 mL of 1M HCl to a 10 mL test tube.

- Add 200 µL ferment or wine sample to the tube and mix. Ensure at least one tube is set aside containing only 1M HCl to use as a blank.

- Incubate blank and diluted samples at room temperature (18 -21^{o}C) for at least one hour. Note that a longer incubation time than one hour is fine, but absorbance readings should be taken on the same day as samples are diluted.

- During incubation period, turn on the spectrophotometer to ensure adequate warm up and perform instrument diagnostics if this facility is available.

QC standard check

On any day that you wish to analyse grape or wine samples for tannin, phenolics and colour, you will need to measure your QC standard at seven wavelengths and upload the data to the WineCloud. This allows you to monitor the performance of your instrument. The QC standard check only needs to be done once per day, not with every set of samples analysed. To do this:

- Set your spectrophotometer for measurements at 250, 270, 280, 290, 315, 320 and 520 nm.

- Zero the instrument with air (i.e. no cuvette present)

- Measure your QC standard cuvette at the seven wavelengths listed above and enter your data directly into the Samples page of the Wine Portal.

Reading your samples

- Set your instrument for measurement at 250, 270, 280, 290, 315 and 520 nm.
- Zero with 1M HCl (Blank) in 10 mm pathlength quartz cuvette.
- Measure diluted samples at 250, 270, 280, 290, 315 and 520 nm using a 10 mm pathlength quartz cuvette.

  NOTE: if the spectrophotometer is double beam, zero the instrument with no cuvettes in either path, then place a 1M HCl blank in the reference beam and take readings with samples in the sample beam.
- Data can then be uploaded via spreadsheet or entered directly into the Samples page of the Wine Portal for immediate calculation of results.

<u>Part (b) – Optional additional step to calculate pigmented tannin and free anthocyanins</u>

If you wish to calculate free anthocyanins and pigmented tannins for your samples, you will also need to carry out the following additional step. This part of the method cannot be performed in isolation; it should always be done at the same time as part (a) because readings from part (a) are used in the calculations for part (b).

Additional materials

- 5M NaOH
- Tartaric Acid
- AR Ethanol
- Sodium metabisulfite
- pH meter

Buffer preparation (100ml, scale accordingly for larger amounts)

- Dissolve 0.5 g of tartaric acid in approximately 50 mL of water.
- Add 12 mL of ethanol and then make up total volume to 100 mL.
- Using a pH meter adjust the overall solution pH to 3.4 by dropwise addition of 5M NaOH.
- Add 0.38 g of sodium metabisulfite and dissolve.
- Place in a tightly stoppered container and store in cool dark place.

<u>Please note that this buffer solution has a shelf life of **one week** after which it must be freshly prepared</u>.

Sample preparation and measurement

- Place 1 mL of wine or ferment sample in a 10 mL test tube and then add 9 mL of the buffer solution.
- Mix sample.
- Set aside one 10mL test tube containing just the buffer solution. This will be your blank.

- Incubate blank and diluted samples at room temperature for at least 1 hour. As in part (a), a longer incubation time is fine, but readings should be taken on the same day that samples are diluted.
- After you have completed reading the absorbances of the samples diluted in acid as described in part (a), re-zero your spectrophotometer using the blank buffer solution and then measure the absorbance at 520nm of your samples diluted in buffer. This reading should be recorded as A520 buffered, and should be entered into the Samples page of the Wine Portal along with the readings generated in part (a).

Safety

- Ensure that laboratory staff members wear adequate personal protection equipment at all times, including lab coat, safety glasses, closed-in footwear and disposable gloves.
- Diluted samples from part (a) are strongly acidic. Neutralise or dilute with copious amounts of water when disposing. Do not pipette any reagents by mouth.

Help?

If you need help, you can email the AWRI at thewinecloud@awri.com.au or phone the AWRI on +61 8 8313 6600.