Sample preparation guide – analysing grape samples for tannin, colour and phenolics measures using the Grape Portal

Equipment

- UV/Visible Spectrophotometer capable of measuring in the range 250 – 520 nm.
- 10 mm path length **Quartz** cuvette **(plastic cuvettes cannot be used for tannin analysis).**
- Sealed QC reference standard cuvette (supplied by AWRI).
- Calibrated pipettes capable of delivering 1 mL and 10 mL volumes. (You could also use a 10 mL dispenser to deliver the acidified ethanol.)
- Homogeniser appropriate to sample size:
    - For samples 50-100 g: Ultra-Turrax T25 high speed homogeniser with an S25 N - 18 G dispersing element (Janke & Kunkel GmbH & Co, Germany).
    - For samples 200-250 g: Retsch Grindomix GM200 Homogeniser (Retsch GmbH & Co KG, Germany), fitted with a floating lid.

  (The homogeniser used must be capable of breaking up grape seeds so that the seed tannin can be extracted. Both the Retsch and Ultra-Turrax can do this, but the Retsch is easier to use. A general kitchen blender is not powerful enough.)
- Centrifuge capable of radial centrifugal force (RCF) of at least 1800 g.
- Mixing device e.g. Chiltern rotating wheel, shaker table or roller mixer (optional).
- Centrifuge tubes.
- Analytical balance with minimum scale reading 0.01 g.

Reagents

- 1.0M Hydrochloric Acid.
- **Acidified** 50% v/v ethanol in Milli-Q (or equivalent) water, prepared by adding 4.4 mL concentrated HCl (10N) to 1 L of 50% v/v ethanol.

Sample preparation, storage and holding times

Grape samples can be analysed fresh or be frozen (-20^{o}C) prior to analysis. Fresh samples must be stored cool (~4^{o}C) and analysed within 24 hours of collection. Grape samples can be stored frozen, as whole berries, for a maximum three months before analysis.

Sampling

Take a representative sample just prior to homogenisation, using the following procedure:

- If the sample contains bunches, remove all berries from the rachis by hand and place into tray or container. If the berries are loose, just place all berries into a tray or container.
- Gently mix the berries by hand being careful not to split the skins of any of them.
- Randomly take berries from different areas within the container until you have the required amount and place them into a clearly labelled container. If using the Retsch homogeniser, choose approximately 200 berries/200 g and if using the Ultra-Tarrax homogeniser, choose approximately 100 berries /100 g.

If berries are frozen, they should be thawed overnight in a refrigerator and processed cold (below 10^{o}C) to minimise oxidation of colour components.

Homogenisation

Homogenise the sample using the settings appropriate to the model of homogeniser:

- Ultra-Turrax – 30s at 24000 rpm, then scrape the homogenate from the shaft into the vessel, then a further 30s at 24000 rpm.
- Retsch – 20 seconds at 8000 rpm.

Ensure that all seeds are thoroughly macerated and, if using the Ultra-Tarrax homogeniser, ensure all homogenate is scraped from the shaft and collected in the homogenising vessel. **Once homogenised, samples must be extracted within four hours.**

Extraction

- Mix the homogenate well and then weigh approximately 1 g into a 10 mL centrifuge tube, recording the homogenate weight.

- Add 10 mL of acidified 50% Ethanol/Milli-Q.

- Allow samples to extract for 1 hour with constant mixing. If a mixing device is not available, mix by inverting the tube approximately every 10 minutes over a period of one hour. Ensure that mixing is efficient and that the pellet does not become lodged in the bottom of the tube.

- Centrifuge homogenate/ethanol mixture at your centrifuge's maximum speed until fully clarified. Check after five minutes whether or not you have fully clarified your sample, and if not, keep centrifuging. Once clarified, the supernatant can be considered the homogenate extract. Homogenate extracts can be stored frozen (at -20 ^{o}C) for up to three months without significant loss of colour.

Incubation

- Add 10 mL of 1.0M HCl to 1 mL of homogenate extract in a new tube and mix well (total volume will be 11 mL).

- Incubate for at least 1 hour and no longer than 24 hours, preferably in a dark place.

- During the incubation period, turn on your spectrophotometer to ensure adequate warm up, and perform instrument diagnostics if 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 (ie 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 Grape Portal.

Reading your diluted extracts and uploading your data

- Zero with 1.0M HCl in 10 mm quartz cuvette.

- Measure diluted grape extracts at 280, 320 and 520 nm (11 mL will allow two rinses of the cuvette between samples) and record the absorbance readings.

- Once a set of samples has been completed, add the data to the Grape Portal either via the sample upload spreadsheet or by direct data entry onto the Samples page. Your results will be calculated immediately.

Help?

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