Sample Preparation Guide – Analysing Grape Samples for Extractable Tannin using the Grape Portal

Equipment

- UV/Visible Spectrophotometer capable of measuring in the range 250 – 520nm
- 10mm path length **quartz** cuvette **(plastic cuvettes cannot be used for tannin analysis)**
- Sealed QC reference standard cuvette (supplied by the AWRI)
- Calibrated pipettes capable of delivering between 100 µL [1] and 15 mL volumes. (For 10 mL volumes of acidified ethanol a dispenser can be used.)
- Centrifuge capable of radial centrifugal force (RCF) of at least 1700*g*
- Mixing device e.g. shaker table
- Centrifuge tubes, 50 mL and 10 mL volumes
- Small resealable zip-lock plastic bags measuring at least 10 cm x 10 cm, can be used. The bag should be able to comfortably hold berries and juice while being crushed by hand, and lead to minimal retention of sample after decanting.
- 70 mL plastic container with sealable lid.
- Analytical balance with minimum scale reading 0.01 g
- Small funnel, suitable to transfer liquid to a 50 mL centrifuge tube
- Small sieve or mesh

Reagents

- 1M Hydrochloric Acid
- 40% v/v ethanol in Milli-Q (or equivalent) water containing 10 g/L tartaric acid, pH 3.4. Prepared by adding 400 mL ethanol to 500 mL water containing 10 g tartaric acid, adjusted to pH to 3.4 using NaOH. Make up to 1 L with water in a volumetric flask.

Sample preparation, storage and holding times

Grape samples must be analysed fresh. Up to a week's storage at ~4^{o}C is acceptable if stored in sealed bags, as whole bunches. After grapes have been de-stemmed these should be stored at ~4^{o}C and processed within 24 hours. After extraction, the centrifuged extract may be frozen (-20^{o}C) prior to analysis. The recommended holding period is no longer than 3 months. After defrosting, extracts should be shaken to resuspend any precipitate or suspension which may form during the freezing/defrosting process. The extract should **not** be re-centrifuged as this will lead to a loss of tannin through the precipitate.

[1] If pipettes capable of dispensing 100 µL volumes are not available, an alternative is to add 1 mL to 99 mL of 1M HCl at this step. Alternatively, a sequential dilution can be performed to minimise waste generation. An example of this would be:

- Add 1 mL of extract to 9 mL HCL (10 x dilution), mix.
- Add 1 mL of the diluted sample to a further 9 mL of HCl (100 x dilution), mix.

Sampling

- Take a representative bunch sample from the vineyard
- 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.
- Randomly sample berries to obtain 50 g. This can be ± 0.5 g either side of 50 g. Record the exact weight.
- Transfer the berries to a resealable plastic bag, seal securely.

Extraction

- Crush the berries gently by hand in the plastic bag, ensuring that all berries have been pressed and no leakage occurs.
- Transfer the berry slurry to the 70 mL container using a spatula to scrape the inside of the bag.
- Accurately pipette 15 mL of 40% ethanol solution to the extraction container, seal securely and shake the sample by hand to mix the solution.
- If available, place on a mixing device, with the container on its side, ensuring the berry slurry is continuously mixed. If no mixing device is available, intermittent shaking of the sample can be employed.
- Leave the sample mixing for 40 hours. A practical way of approaching this is to begin extraction by 5 pm on Day 1, and remove the samples at 9 am on Day 3. Extraction times longer than 40 hours are not recommended as the extract becomes unstable.

Preparation for analysis

- Filter the extract directly into a 50 mL centrifuge tube using a small funnel fitted with a sieve or mesh to separate the skins, seeds and solids.
- Centrifuge the sample at sufficient *g* force to sediment all suspended solids (AWRI protocol is 1730*g* for 5 minutes).
- Dilute the clarified extract 100 x in 1M HCl; 100 μL (see footnote 1) extract + 9.9 mL 1M HCl in a 10 mL centrifuge tube. Mix well.
- 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 extracts for tannin, 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 Grape Portal.

Reading your diluted extracts and uploading your data

- Zero with 1M HCl in 10 mm quartz cuvette.
- Measure diluted grape extracts at 280, 320 and 520 nm (10 mL of diluted sample 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 including the exact weight of grapes analysed 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.
- See the additional fact sheet provided for assistance in the interpretation of the data.

Help?

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