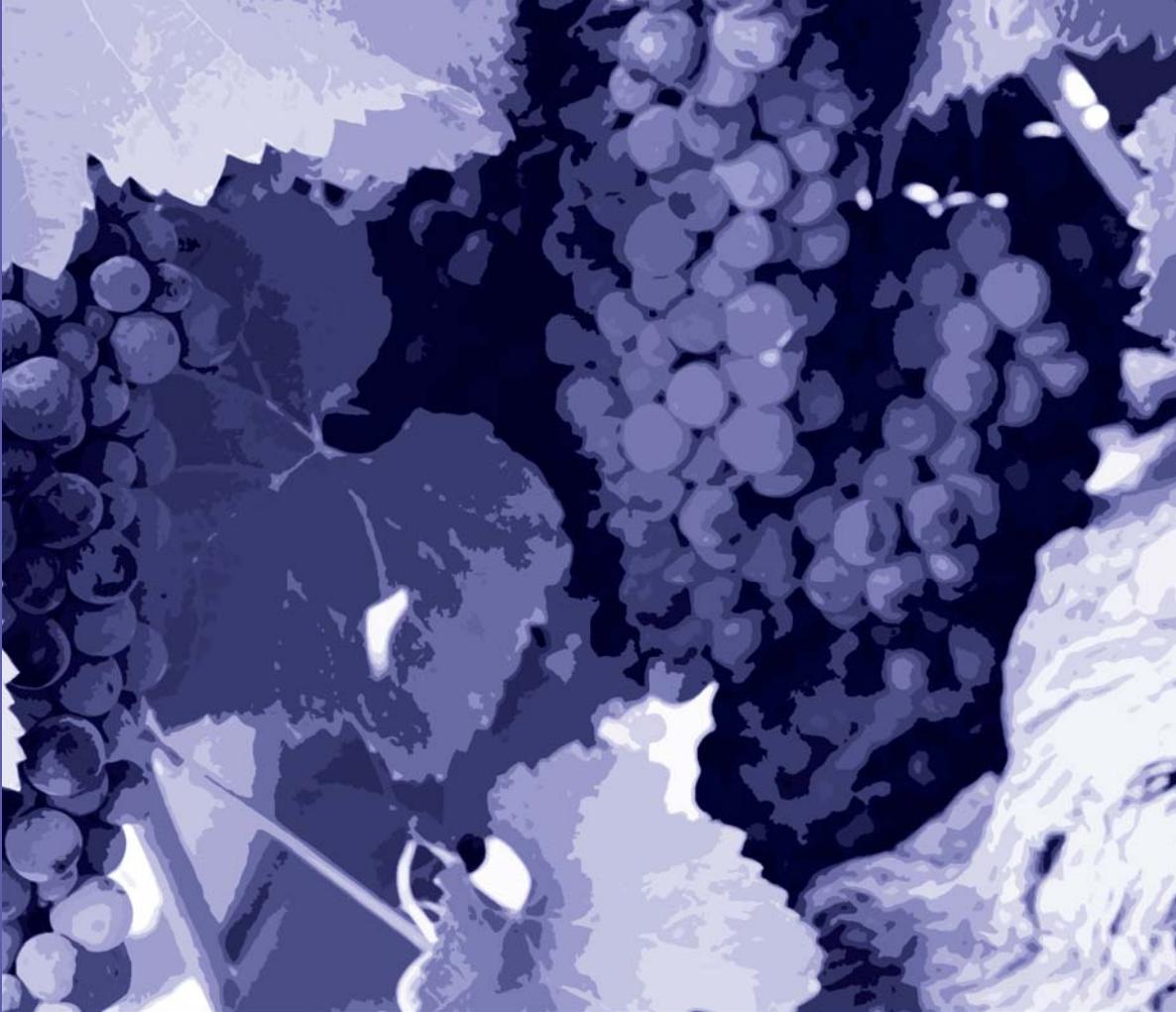


# 3



## VITICARE ON FARM TRIALS

### Manual 3.1 - Measuring Fruit Quality

Yield

Average berry weight

Berry sampling for berry composition at harvest

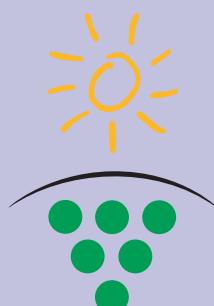
Berry sampling for maturity assessment

Total soluble solids - by refractometry

Grape juice pH

Grape juice titratable acidity

Berry colour and phenolics



COOPERATIVE  
RESEARCH CENTRE  
*for*  
VITICULTURE

# Core Participants

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Know-how for Horticulture™



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Primary Industries and Resources SA

# About the CRCV

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The Cooperative Research Centre for Viticulture is a joint venture between Australia's viticulture industry and leading research and education organisations. It promotes cooperative scientific research to accelerate quality viticultural management from vine to palate. Australian grapegrowers and winemakers are key stakeholders in the CRCV, contributing levies matched by the Commonwealth Government and invested by the Grape and Wine Research and Development Corporation in the Centre.

For more information about the CRCV, please visit [www.crcv.com.au](http://www.crcv.com.au).

# Disclaimer

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# Introduction

# Introduction

The Cooperative Research Centre for Viticulture has conducted On Farm Trials since 1999. The initial trials were conducted in eight regions (Port Phillip, North East Victoria, Central Victoria, Adelaide Hills, Riverland, South West Slopes, Riverina and Hunter Valley) and provided Australian growers with the ability to formally assess and validate new science and technology. The trials were conducted over four growing seasons and helped growers to solve problems in their vineyards and improve their management practices.

In 2004 the On Farm Trials project expanded to cover more than 20 viticultural trials primarily in the Riverina, Riverland and Sunraysia regions. Rather than focusing on individual grower issues, the CRCV team has worked with regional grower groups to determine regional issues. The trials are still conducted on a participant growers' property but a team of people are involved to learn from the trial and to share the workload.

This booklet is part of a series that draws on knowledge gained from this experience in developing and delivering On Farm Trials.

Conducting a trial in your vineyard is not easy and is not a decision that should be made lightly. Although trials can be an excellent method for refining management practices, improving quality or looking for solutions to problems, there are many practical considerations involved in conducting a trial.

On Farm Trials can lead to management improvements in a number of areas. The information in this booklet will guide you through the various protocols involved with setting up On Farm Trials that aim to assess and measure fruit quality by yield, average berry weight, berry sampling for berry composition at harvest, berry sampling for maturity assessment, total soluble solids by refractometry, grape juice pH, grape juice titratable acidity, and berry colour and phenolics.

# Yield

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## Aim

This trial aims to assess fruit quality by measurement of berry yield.

## Important Points to Know

Yield can be affected by many viticultural practices and is therefore, an important response to measure when a change in practice is implemented. Because harvest is a busy time of the season, it is important to have an efficient system in place to carry out yield measurements. In conducting a trial, yield is assessed by measuring total fruit weight per vine.

## Before You Get Started

The following requirements will help you prepare for this trial:

- o Snips
- o Buckets
- o Spring scales with hook or top loading portable field scales (that weigh up to 20 kg and accurate to 0.1 kg)
- o Plastic bags (labelled)
- o Esky
- o Marking pens
- o Recording sheet
- o Trial plan

## Trial Timelines

The time to harvest, count, weight and record will be dependent on crop load, canopy structure and size. You will also get quicker at this process the more vines you harvest. As a general rule allow 15 minutes per vine per person.

For example. Time for 3 people to harvest 20 vines

$$= 15\text{min} \times 20 \text{ vines} = 300 \text{ min} / 3 \text{ people} = 100 \text{ min total harvest time.}$$

### Same day harvest

It is best to pick the trial before the rest of the block is harvested, particularly if the block is machine harvested. This can be done up to 3 days before the rest of the block is harvested.

### Same maturity harvest

Harvesting vines from each treatment at the same maturity will be important if the grapes are to be assessed for other chemical characters (for example, colour analysis). You may choose to harvest vines at the same maturity where different management practices delay or advance maturity (for example, irrigation management). This will require some treatment vines to be harvested on different days.

Harvesting vines at the same maturity will require extra attention to timing and coordination with the rest of the block, as harvesting of the treatment vines could span 2 weeks or more.

## Trial Method

1. Pick and count all bunches from each of the middle vines within a treatment plot (eg. If there are 6 vines per plot then pick and count bunches from each of the middle 4 vines) and place the fruit into a bucket.
2. Record identity of the treatment plot and the total number of bunches per vine.
3. Weigh and record total amount of fruit harvested from each vine.
4. Weigh and record the weight of each bucket.
5. Use a data collection sheet with grape yield and average berry weight to record all measurements.

## Trial Calculations

Yield per vine (kg)	= total fruit weight per vine - bucket weight
Bunches per vine	= total bunch number per vine
Average bunch weight (grams)	= Yield per vine x 1000 / Bunches per vine

## Average Berry Weight

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### Aim

This trial aims to assess fruit quality by measurement of average berry weight.

### Important Points to Know

Average berry weight is a good and simple indicator of berry size. Berry size can be affected by viticultural management (such as nutrition and irrigation), and climatic parameters. Berry size will influence wine quality, and wineries may seek fruit of a specified size. Berry weight can be determined on the berries collected for maturity assessment.

### Before You Get Started

The following requirements will help you prepare for this trial:

- o Sealable plastic bags (able to hold 100 berries)
- o Marker pens
- o Suitable transport containers (eg. esky)
- o Cooling blocks
- o Bench top scales (that weigh up to 200 grams and accurate to 0.01 grams)

## Trial Timelines

Berry weight can be determined on the berries collected for maturity assessment, otherwise berry samples should be collected as close to harvest as possible (within 3 days before harvest is acceptable).

Each 100-berry sample should take approximately 10 minutes to sample, measure and record its weight.

### Trial Method

1. Collect 100 berries (5 berries per bunch from 20 bunches) from the middle vines of each plot (eg. If there are 6 vines per plot, the 20 bunches should be randomly chosen from the middle 4 vines). The berries should be selected from bunches on both sides of the row, inside and outside the canopy and from the front, back, bottom and top of the bunch. Sample no more than five berries per bunch) (Iland et al. 2000). Take care to sample exactly 100 berries.
2. Place berries into sealable plastic bag with the date, property, treatment and replicate clearly marked on the bag.
3. Sample from each plot of each replicate (eg. 2 treatments x 5 replicates = 10 lots of 100 berries).
4. Place all bags into eskies with cooler blocks.
5. Transport samples in esky to shed or laboratory.
6. Record the identity of the treatment plot and the weight of 100 berries (in the plastic bag) using bench top scales with accuracy of 0.01 grams (eg. 111.06 g). It is critical there is only 100 berries in the bag.
7. Record the weight of an empty bag.
8. Use a data collection sheet with grape yield and average berry weight to record all measurements.

## Trial Calculations

Weight of 100 berries (+ bag) - bag weight] / 100 = average berry weight

## Berry Sampling for Berry Composition at Harvest

### Aim

This trial aims to assess fruit quality by sampling for berry composition at harvest.

### Important Points to Know

Sampling berries at harvest is the first step in assessing berry composition. Berry composition at harvest is generally determined by measuring berry juice soluble solids (<sup>o</sup>Brix or Baumé), pH and titratable acidity (TA). In addition berry colour may be determined in a commercial laboratory.

### Before You Get Started

The following requirements will help you prepare for this trial:

- o Sealable plastic bags (able to hold 100 berries)
- o Marker pens
- o Suitable transport containers (eg. esky)
- o Cooling blocks

### Trial Timelines

Berry samples should be collected as close to harvest as possible. Sampling within 3 days before harvest is acceptable.

## Trial Method

1. Collect 100 berries (5 berries per bunch from 20 bunches) from the middle vines of each plot (eg. If there are 6 vines per plot, the 20 bunches should be randomly chosen from the middle 4 vines). The berries should be selected from bunches on both sides of the row, inside and outside the canopy and from the front, back, bottom and top of the bunch. Sample no more than five berries per bunch) (Ilard et al. 2000).
2. Place 100 berries in a plastic bag with the date, property, treatment and replicate clearly marked on the bag.
3. Seal bag and store in a cool environment by placing bag in an esky with freezer bricks. Store samples at less than 15°C and preferably between 5 and 10°C prior to and during transport and up until the commencement of processing for analysis.
4. Sample from each treatment plot of each replicate (eg. 2 treatments x 5 replicates - 10 lots of 100 berries).

## Analysis

For analysis of average berry weight, °Brix, pH and TA refer to the relevant measurement technique in this series. Most growers will be satisfied to measure °Brix as an indicator of maturity however, it is also possible to measure pH, TA and colour on this sample.

# Berry Sampling for Maturity Assessment

## Aim

This trial aims to measure fruit quality by berry sampling for maturity assessment.

## Important Points to Know

The rate of berry maturation can be affected by viticultural practices, particularly irrigation. The rate of maturation can be determined on berry samples collected in the weeks leading up to harvest. The most common indicator of harvest maturity is determined by measuring soluble solids ( $^{\circ}$ Brix or Baumé), however, some growers may also be interested in pH, titratable acid (TA) or colour.

If a treatment advances harvest, this may be beneficial to the grape grower. For example, in areas that have a high Botrytis risk, harvesting early reduces the risk of Botrytis infection.

The aim of a standard method for berry sampling is to account for the natural variation in berry composition between berries. Vineyard variation is not restricted to differences between vines but includes variations between bunches (exposed/shaded) and berries within bunches (top/middle/bottom, exposed/shaded, small/large).

Bunch sampling reduces within bunch variation; however, repeated bunch sampling may have an impact on fruit maturation, through a decrease in crop load. As such, berry sampling for maturity prediction is the preferred method.

## Before You Get Started

The following requirements will help you prepare for this trial:

- o Sealable plastic bags (able to hold 100 berries)
- o Marker pens
- o Suitable transport containers (eg. esky)
- o Cooling blocks

## Trial Timelines

Maturity sampling should be performed at weekly intervals until within a few  $^{\circ}$ Brix from harvest maturity. After this time, the sampling interval should be shortened to every two days.

## Trial Method

1. Collect 50 berries (5 berries per bunch from 10 bunches) from the middle vines of each treatment plot (eg. If there are 6 vines per plot, the 10 bunches should be randomly chosen from the middle 4 vines). The berries should be selected from bunches on both sides of the row, inside and outside the canopy and from the front, back, bottom and top of the bunch. Sample no more than five berries per bunch) (Iland et al. 2000).
2. Place 50 berries in a plastic bag with the date, property, treatment and replicate clearly marked on the bag.
3. Seal bag and store in a cool environment by placing bag in an esky with freezer bricks. Store samples at less than 15°C and preferably between 5 and 10°C prior to and during transport and up until the commencement of processing for analysis.
4. Sample from each plot of each replicate (eg. 2 treatments x 5 replicates = 10 lots of 50 berries).

If colour is to be determined the individual sample size will need to be increased to 100 berries per plot (50 berries for °Brix, pH, TA and 50 berries for colour determination).

Berry samples should be processed and analysed as quickly as possible. If the period between storage and processing is more than a few hours, they should be stored in a cool environment (e.g. about 5 - 10°C). This should be no longer than overnight. After storage, allow the sample to warm to 20°C.

If the sample cannot be processed as soon as possible, they will need to be frozen. Berries for colour analysis can be frozen whole. Berries for °Brix, pH and TA should have juice extracted and the juice frozen for analysis later (Note. °Brix should not be determined on berries that have been frozen). To extract juice, squeeze berries in plastic bags and decant juice into solid plastic vials and secure lid. Process for °Brix, pH and TA or freeze. (Note: Refer to 'average berry weight measurement' before juicing berries if you intend on measuring berry weight).

### Freezing samples

If samples have been frozen the tartaric acid will be crystallised leading to inaccurate measurements. Frozen juice should be thawed (overnight) and warmed to 40°C for a period of 30 minutes. Samples should then be hand shaken and allowed to cool to room temperature.

If skin colour analysis is to be undertaken then whole berries for colour analysis should remain frozen during transport to laboratory. The sampling of skin disks with a hole punch is much easier on frozen berries. Therefore, package berries with ice blocks for transport to laboratory for colour analysis.

## Trial Analysis

For analysis of average berry weight, °Brix, pH, TA and colour analysis refer to the relevant measurement technique in this series. Most growers will be satisfied to measure °Brix as an indicator of maturity, however, it is also possible to measure pH and TA on this sample.

## Total Soluble Solids by Refractometry

### Aim

This trial aims to assess fruit quality by measurement of total soluble solids by refractometry.

### Important Points to Know

The measure of total soluble solids in grape juice is used as an indicator of grape ripeness. Total soluble solids can be measured by hydrometry or refractometry and gives an indication of sugar content as sugars represent 90-94% of total soluble solids.

A hand held refractometer is often used for a quick test of grape maturity, they are calibrated to give the concentration of total soluble solids in °Brix at a standard temperature of 20°C. (1 °Brix = 1 gram of soluble solids per 100 grams of solution). It is important to realise that refractometers only give an estimate of the sugar concentration of the juice.

### Before You Get Started

The following requirements will help you prepare for this trial:

- o Refractometer
- o Dropper
- o Cleaning agent
- o Tissues
- o Recording sheet
- o Measurement technique for berry sampling for maturity

### Trial Timelines

Berry samples should be processed and analysed as quickly as possible. If the period between storage and processing is more than a few hours, they should be stored in a cool environment (e.g. about 5 - 10°C). This should be no longer than overnight. After storage, allow the sample to warm to 20°C.

The time taken to squeeze berries, decant juice and measure °Brix 3 times should be approximately 10 minutes.

## Trial Method

The first step is to extract the juice from a sample of berries (approximately 50 berries). °Brix must not be determined on berries that have been frozen. Squeeze berries in plastic bags and decant juice into solid plastic vials and secure lid. Measure °Brix. (Refer to 'average berry weight measurement' before juicing berries if you intend to measure berry weight).

Below is an extract from Iland et al. (2000) on the use of a prism refractometer.

1. Open the prism box of refractometer, clean and dry the glass surface and place a drop of the juice on the glass surface. Make sure the whole of the glass surface is covered. Avoid adding excess juice.
2. Close the prism box and hold the instrument towards a light source, preferably this should be natural light. The field of view shows regions of dark and light, which should be separated by a distinct interface. Insufficient light reaching the prism results in an indistinct interface. If the sample contains a high level of undissolved solids, then the intensity of the light will be diminished. In this case the sample may require clarification.
3. Read the graduated scale where the boundary of the two regions intersects. The reading will be in oBrix.
4. If the temperature of the sample is not at 20 °C a correction must be applied for an accurate measurement. To apply the temperature correction, for every 1°C above or below 20 °C add or subtract 0.07 oBrix, respectively, to or from the indicated value.
5. Measure °Brix on each juice sample 3 times and take the average of these 3 measurements. (eg. 22.5, 22.9, 23.1 = 22.8 °Brix).
6. Use a data collection sheet with Grape quality - brix, pH and titratable acid to record all measurements.

*Note: Prism and digital refractometers need to be calibrated at 20°C with distilled water and a sugar solution. These should be carried out according to the manufacturers' instructions. There are also some hand-held refractometers and more expensive digit refractometers with automatic temperature compensation.*

## Grape Juice pH

### Aim

This trial aims to assess fruit quality by measurement of grape juice pH.

### Important Points to Know

Grape juice pH is a measure of the concentration of free hydrogen ions in solution. The pH of berry juice is important at harvest because it will influence wine quality. Microbiological metabolism, chemical stability, oxidation-reduction potential, and colour and flavour development during fermentation are affected by pH.

The concentration of acids and their associated salts will largely determine grape juice pH. Malic and tartaric are the most important acids found in grape juice. The strongest acid is tartaric (produces more free hydrogen ions) and is found in the largest concentrations. As grapes ripen, the concentration of tartaric acid diminishes and juice pH increases (Ferroni and Scalabrelli 1995).

Most grapes are green at pH 2.7. Wines that require sharp, crisp acidity (i.e. sparkling wines) are often standardised at a pH between 2.9 and 3.1. For fresh, fruity white and blush wines berry juice of pH 3.1 to 3.3 is required (Vine et al. 1997). A pH of berry juice greater than 3.8 is unacceptable for the production of table wine (Hamilton and Coombe 1992). Acid addition and ion exchange procedures can ameliorate high pH, however, the methods are costly and in some circumstances may not reduce pH; natural acidity is preferred.

Management can affect grape juice pH. For example: water stress post-véraison can increase pH; rootstocks vary in their ability to uptake certain nutrients (particularly potassium and sodium) whereby hydrogens are exchanged for potassium (eg. potassium tartrate), causing grape juice pH to increase. Nutrition and mulch management will impact on the amount of potassium available to the grapevine and thus, what is taken into the developing berry. Shading berries causes an increase in grape juice potassium and malic acid.

An analytic chemistry laboratory or winery generally performs measurements of pH.

### Before You Get Started

The following requirements will help you prepare for this trial:

- o pH meter
- o Standard buffer solutions at pH 7.00 and 4.00
- o Thermometer
- o Recording sheet

## Trial Timelines

This trial can be conducted at harvest or leading up to harvest.

The time to calibrate the pH meter, perform 20 pH measurements and record is approximately 0.5 hour. This does not include sample preparation.

## Trial Method

Please refer to the measurement techniques 'Berry sampling for maturity assessment' and 'Berry sampling for harvest berry composition' in this series for berry sampling and handling procedures.

The following method to analyse for pH is extracted from Iland et al. (2000)

### Calibrating the pH meter

The method below relates to pH meters with manual control.

1. Pour a sufficient volume of each standard buffer (eg pH 7.00 and pH 4.00) into separate small beakers or vials, so that the electrode will be adequately covered when the electrode is immersed in the solution.
2. Measure the temperature of the standard buffers (at room temperature). Set temperature adjustment dial to the temperature of the standard buffer solutions (in older pH meters without automatic temperature compensation).
3. Set the sensitivity or slope control to the point corresponding to the 100% position (ignore this step for meters that do not have the 100% point indicated on the scale).
4. Initial adjustment - immerse the electrode in the pH 7.00 buffer contained in a small beaker, stir slowly and adjust the buffer or calibrate control so the digital display shows pH 7.00. Remove the electrode and rinse with distilled water. Gently dry the electrode by dabbing with a tissue.
5. Sensitivity or slope adjustment - immerse the electrode in the pH 4.00 buffer contained in a small beaker, stir slowly and adjust the sensitivity or slope control dial so that the digital reading shows pH 4.00 on the display. Remove the electrode and rinse with distilled water. Gently dry the electrode by dabbing with a tissue.
6. Final adjustment - immerse the electrode again in the pH 7.00 buffer and adjust the buffer control dial so that the digital display shows pH 7.00. Remove the electrode and rinse with distilled water. Gently dry the electrode by dabbing with a tissue.

### Sample preparation

The determination of pH is normally carried out on a portion of clarified grape juice. The temperature of the juice must be the same temperature as that of the standard buffers used in the calibration step.

#### Measuring the pH

1. Place the sample of juice in a small beaker. The volume of juice should be sufficient to cover the electrode bulb and porous plug when the electrode is immersed in the sample.
2. Measure the temperature of the sample and adjust to the temperature of the standard buffers, if necessary.
3. Rinse the pH electrode with a small portion of the sample to be analysed (use a pasteur pipette).
4. Place the electrode in the sample of juice contained in a small beaker, ensuring that the electrode is adequately covered when immersed in the juice.
5. Slowly stir the solution.
6. The pH value of the sample will be shown on the digital display. It may take about 20 to 30 seconds to stabilise.
7. After recording the pH value, rinse the electrode with distilled water and return the electrode to the storage solution. Do not leave electrode immersed in grape juice longer than is necessary.
8. Use a data collection sheet for grape quality - brix, pH and titratable acid to record all measurements.

Depending on the brand, the pH of the standard buffers may not be exactly 7.00 or 4.00. The exact pH of commercially available standard buffers is shown on the container.

For meters with pH<sub>T</sub> and slope or sensitivity indications, it is advisable to record these values each time the meter is calibrated so that the electrode performance can be recorded over time.

A regular maintenance schedule and proper storage will maximise performance of the pH meter. The electrode should be cleaned regularly according to manufacturer's recommendations.

# Grape Juice Titratable Acidity

## Aim

This trial aims to assess fruit quality by measurement of grape juice titratable acidity.

## Important Points to Know

Titratable acidity (TA) is a measure of the total available amounts of hydrogen ions in grape juice. The TA of berry juice is important at harvest because it will influence wine quality. Microbiological metabolism, chemical stability, oxidation-reduction potential, and colour and flavour development during fermentation are effected by TA.

The concentration of undissociated acids (eg. tartaric and malic acid) and their associated salts (eg. bitartaric acid) and the amount of free hydrogen ions in solution will determine grape juice TA. The measure of TA often indicates less than the absolute concentration of tartrate and malate (total acidity) due to species of tartrate that are completely dissociated (eg. di-potassium tartrate) and hence not measured as TA (Zoecklein et al. 1995).

Concentrations of TA range from 5.0 to 8.0 g/L in bland to sharp wines respectively (Vine et al. 1997). Lower values generally occur in grapes from hot regions and higher values in grapes from cooler regions.

Management can affect grape juice TA. For example: water stress post-véraison can decrease TA; rootstocks vary in their ability to uptake certain nutrients (particularly potassium and sodium) and therefore, impact on the species of tartrate that develop in the berries. Nutrition and mulch management will impact on the amount of potassium available to the grapevine and thus, what is taken into the developing berry. Shading berries causes an increase in grape juice potassium and malic acid.

An analytic chemistry laboratory or winery generally performs measurements of TA.

## Before You Get Started

The following requirements will help you prepare for this trial:

- o pH meter
- o Burette
- o 0.1M sodium hydroxide (NaOH)
- o Magnetic stirrer
- o Recording sheet

## Trial Timelines

This trial may be performed at harvest or leading up to harvest.

The time to perform 20 TA measurements and record is approximately 1.5 hour. This does not include sample preparation or calculations.

## Trial Method

Please refer to the measurement techniques 'Berry sampling for maturity assessment' and 'Berry sampling for harvest berry composition' in this series for berry sampling and handling procedures.

The following method to analyse for pH is extracted from Iland et al. (2000)

### Sample preparation

The determination of TA is normally carried out on an undiluted, clarified sample of grape juice.

### Measurement of TA of Juice or wine

The titration can be conducted by positioning a beaker, containing a small stirrer bar, on top of a magnetic stirrer. During the titration, gentle stirring of the solution in the beaker can be achieved by operating the magnetic stirrer.

1. Calibrate the pH meter (see methods in the 'Grape juice pH' measurement technique).
2. Fill the burette with 0.1 M NaOH.
3. Add sufficient distilled water to a 100 or 250 mL beaker to ensure that the pH electrode will be adequately covered when it is immersed into the distilled water. Place a small stirrer bar in the beaker.
4. Rinse the pH electrode with a few mL of distilled water. Discard rinsings. Dry the electrode by dabbing with a tissue.
5. Lower the electrode into the distilled water in the beaker. Position the electrode so that it does not touch the side of the beaker and is above the level of the stirrer bar.
6. Position the burette so that it is vertical and that the outlet is above the beaker. Adjust the pH of the distilled water to pH 8.2, or as close to pH 8.2 as practically possible, by adding the necessary number of drops of 0.1 M NaOH from the burette. It is not necessary to record this volume of 0.1 M NaOH.
7. Pipette 10.0 mL of juice into the beaker containing the pH adjusted distilled water.
8. Record the initial burette reading.
9. Titrate, with constant and gentle stirring, the solution in the beaker with 0.1 M NaOH until the pH of the solution is pH 8.2 or as close to pH 8.2 as practically possible.
10. Record the final burette reading.
11. Calculate the difference between the final and the initial burette readings. This is called the Titre value.
12. Use the data collection sheet: Grape quality - brix, pH and titratable acid in Section #5: Proformas to record all measurements.

## Calculation

Titratable acidity (g/L as H<sub>2</sub>T) = 0.75 x Titre value (mL)

The above formula applies to the titration of 10 mL of juice with 0.1 M NaOH. If the volume of juice is different to 10 mL and/or the concentration of the NaOH solution is different to 0.1 M, then a new formula is required.

**The general formula for determining the titratable acidity is:**

$$\text{Titratable acidity ( g / Las H}_2\text{T )} = 75 \times \text{Molarity of NaOH} \times \frac{\text{Titre value ( mL )}}{\text{Volume of sample ( mL )}}$$

Normally, when the 10 mL of a juice sample is added, it is not of a sufficient volume to cover the electrode bulb and porous plug. To account for this, a volume of distilled water is initially added. However, this distilled water may be acidic and therefore needs to be neutralised to pH 8.2 or as close to as possible.

Distilled water is not buffered against additions of acid or base, hence when a drop of NaOH solution is added the pH may rise from an initial value below pH 7.00, to a pH which may be any value greater than pH 7.00. It is difficult to obtain exactly pH 8.2 when adding the NaOH to the distilled water. If the pH is in excess of pH 8.2, then the small excess of NaOH added in this step (it may be only a portion of a drop) will only contribute to a small error in the titratable acidity determination.

For very accurate analysis, to obtain a value very close to pH 8.2, the 0.1 M NaOH can be added via a pasteur pipette rather than the burette. This allows smaller drops to be added. Alternatively, a large volume of distilled water can be adjusted to pH 8.2 and a portion of this used in step 3, eliminating the adjustment step (step 6).

# Berry Colour and Phenolics

## Aim

This trial aims to assess fruit quality by measurement of berry colour and phenolics.

## Important Points to Know

Wineries and wine consumers are becoming more particular about the quality of product they purchase and are willing to pay a higher price. Therefore, it is important that the grape grower has some measure of berry quality to judge against the impact of vineyard practices.

In the case of red varieties, berry colour (mainly as anthocyanins) has been shown to act as a precursor of wine colour and flavour intensity (Iland et al. 2000). Vineyard practices have been shown to impact on colour per berry. For example:

- o Some rootstocks produce berries with greater colour intensity than own roots (Walker et al. 2002).
- o Sunlight exposure of berries improves colour development and final concentration when berries are not over-exposed to high temperature (Bergqvist et al. 2001).
- o Applying water deficits to grapevines either pre- or post-véraison can increase the concentration of anthocyanins and phenolics of red grapes (Ginestar et al. 1998; Matthews and Anderson 1988).

An analytic chemistry laboratory or winery generally performs berry colour and total phenolic measurements.

## Before You Get Started

The following requirements will help you prepare for this trial:

- o Spectrophotometer
- o Homogenising vessel
- o Recording sheet

## Trial Timelines

This trial can be performed at harvest or leading up to harvest.

## Trial Method

Please refer to the measurement techniques 'Berry sampling for maturity assessment' and 'Berry sampling for harvest berry composition' in this series for berry sampling and handling procedures.

The following method to analyse for colour and phenolics is extracted from Iland et al. (2000).

**Procedure for preparing the sample**

1. Weigh a sample of 50 berries (see Helpful Hints).
2. Transfer the berries to a homogenising vessel, normally a 125 mL plastic container.
3. Homogenise the berries at high speed, eg. 24000 rpm for about 30 seconds. The homogeniser should macerate the flesh, skins and seeds into a homogeneous mixture, so that a representative sub sample can be taken in step 5. There should not be any large pieces of skins or seeds in the mixture.
4. Scrape any homogenate from the shaft of the homogeniser into the homogenising vessel and repeat the homogenising step for about 15 seconds.
5. Thoroughly mix the homogenate by stirring it with a small spoon type spatula and immediately take a scoop of approximately 1 gram of homogenate using the spatula.
6. Transfer the scoop of homogenate into a pre-tared centrifuge tube. Record the weight. This is termed 'the weight of homogenate taken for extraction'.

**Procedure for extracting the anthocyanins**

7. Pipette 10 mL of 50% v/v aqueous ethanol adjusted to pH 2.0 into the centrifuge tube containing the homogenate. Cap the tube and mix the contents periodically by inverting the tube about every 10 minutes over a period of 1 hour.
8. After 1 hour, centrifuge the tube and contents at 3500 rpm for 5 minutes. The supernatant is termed 'the extract'.

**Procedure for determining the red colour and total phenolics of the extract**

9. Pipette 1.0 mL of 'the extract' into 10 mL 1M HCl and mix this solution thoroughly. (Note, in this case the dilution factor is 11. See Helpful Hints.)
10. Pour the remaining volume of 'the extract' into a tall, thin measuring cylinder and record the volume. Add the value of this recorded volume and the value of the volume of 'the extract' that had previously been taken in step 9 to obtain a value, which is termed 'the total extract volume'.

*Note: This step is required, because, even though 10 mL of 50% v/v aqueous, pH adjusted ethanol was added at step 7, the final extract volume is greater than 10 mL because it includes the added 10 mL plus a small volume of liquid that comes from the sample of homogenate, eg 'the total extract volume' from 1 gram of homogenate might be 10.4 mL.*

11. Allow the diluted HCl extract solution to stand for about three hours.
12. After 3 hours, using a spectrophotometer, read the absorbance of the diluted HCl extract in a 1 cm cell at 700 nm, 520 nm and 280 nm.

### **Helpful Tips**

- o The 50-berry sample can be stored frozen for a few months prior to analysis. It is convenient to store the berries in the 125 mL plastic container in which they can be homogenised.
- o In step 9 the actual dilution needs to be assessed for each sample. Some samples will require greater dilution than others, eg for extracts from grapes that are very highly coloured, 0.5 mL of extract may be more appropriate to add to 10 mL of 1 M HCl (ie a dilution of 21 ie. 0.5 mL in a total solution of 10.5 mL.)
- o The method described here is only one of the options for extracting and measuring colour and total phenolics. Other methods extract whole grapes, macerated in different ways, or skin discs taken from fresh or frozen berries. The preparation of sample is different but the principles of extraction and measurement are similar to those described here.
- o See below for calculations

## Resources

### **Some useful resources for evaluating average berry weight**

Iland, P., Ewart, A., Sitters, J., Markides, A. and Bruer, N. (2000) 'Techniques for chemical analysis and quality monitoring during winemaking.' (Patrick Iland Wine Promotions, Campbelltown, S.A., Australia).

### **Some useful resources for evaluating sampling of berry composition at berry harvest**

Iland P, Ewart A, Sitters J, Markides A and Bruer N (2000) 'Techniques for chemical analysis and quality monitoring during winemaking.' (Patrick Iland Wine Promotions, Campbelltown, S.A., Australia).

### **Some useful resources for evaluating berry sampling for maturity assessment**

Iland, P., Ewart, A., Sitters, J., Markides, A. and Bruer, N. (2000) 'Techniques for chemical analysis and quality monitoring during winemaking.' (Patrick Iland Wine Promotions, Campbelltown, S.A., Australia).

### **Some useful resources for evaluating total soluble solids by refactometry**

Iland P, Ewart A, Sitters J, Markides A and Bruer N (2000) 'Techniques for chemical analysis and quality monitoring during winemaking.' (Patrick Iland Wine Promotions, Campbelltown, S.A., Australia).

### **Some useful resources for evaluating grape juice pH**

Ferroni G, Scalabrelli G (1995) Effect of rootstock on vegetative activity and yield in grapevine. Acta Horticulturae 388, 37-42.

Hamilton RP, Coombe BG (1992) Harvesting of Winegrapes. In 'Viticulture'. (Eds BG Coombe and PR Dry) pp. 302-327. Vol. 2. (Winetitles: Underdale SA)

Iland P, Ewart A, Sitters J, Markides A and Bruer N (2000) 'Techniques for chemical analysis and quality monitoring during winemaking.' (Patrick Iland Wine Promotions, Campbelltown, S.A., Australia).

Vine RP, Harkness EM, Browning T, Wagner C (1997) 'Winemaking: from grape growing to marketplace.' (Chapman & Hall: New York, NY)

### **Some useful resources for evaluating grape juice titratable acidity**

Iland P, Ewart A, Sitters J, Markides A and Bruer N (2000) 'Techniques for chemical analysis and quality monitoring during winemaking.' (Patrick Iland Wine Promotions, Campbelltown, S.A., Australia).

Vine RP, Harkness EM, Browning T, Wagner C (1997) 'Winemaking: from grape growing to marketplace.' (Chapman & Hall: New York, NY)

## Resources

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Zoecklein BW, Fugelsang KC, Gump BH, Nury FS (1995) 'Wine analysis and production.' (Chapman & Hall: New York, NY)

### **Some useful resources for evaluating berry colour and phenolics**

Bergqvist, J., Dokoozlian, N., Ebisuda, N. (2001) Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. American Journal of Enology and Viticulture. 52(1), 1-7.

Ginestar, C. Eastham J. Gray S. and Iland P. (1998). Use of sap-flow sensors to schedule vineyard irrigation. II. Effects of post-verasion water deficits on composition of shiraz grapes. American Journal of Enology and Viticulture. 49(4), 421-28.

Iland P, Ewart A, Sitters J, Markides A and Bruer N (2000) 'Techniques for chemical analysis and quality monitoring during winemaking.' (Patrick Iland Wine Promotions, Campbelltown, S.A., Australia).

Matthews MA, Anderson MM. (1988). Fruit ripening in *Vitis vinifera* L.: Response to seasonal water deficits. American Journal of Enology and Viticulture 39(4), 313-20.

Walker, R.R., Read, P.E., Blackmore, D. H. (2000) Rootstock and salinity effects on rates of berry maturation, ion accumulation and colour development in Shiraz grapes. Australian Journal of Grape and Wine Research. 6 (3), 227-239.