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ACCC objective measures



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1. Abstract

This project worked with industry and regulatory stakeholders to develop standardised methods for the assessment of grapes for wine production, based on recommendations from a review undertaken by the ACCC Agriculture Unit. Standardised methods, endorsed by industry and regulatory bodies were developed and published for:

- the measurement of key parameters in grapes
- sampling in the vineyard, in the winery and at the weighbridge
- validating secondary methods against the reference methods.

A range of training materials and tools were developed to support use of the methods.

2. Executive summary

In 2018–19 the Australian Competition and Consumer Commission (ACCC) Agriculture Unit conducted a market study of the wine grape industry. The study examined competition, contracting practices, transparency and risk allocation in wine grape supply chains. In its report on this study (ACCC 2019), the ACCC made several recommendations, which required the development of uniform national standards for grape quality assessment, along with an in-depth look into current guidance on best practice measurement and sampling approaches in the vineyard and at the point of fruit receival at the winery.

This project took a feedback loop approach, convening an Industry Reference Group of grapegrowers, winemakers, regulatory representatives and researchers to explore the current industry guidance on the fair trade of grapes between growers and wineries. This information was then fed back to the AWRI for review and alignment with literature, prior to further comment from industry. Two primary quality measurements were highlighted by the ACCC as requiring Industry Endorsed Standard Procedures (IESPs) – namely, grape sugar levels (total soluble solids) and grape colour. A series of additional requirements were flagged, focused on achieving a representative sample in the vineyard at the point of harvest, and upon fruit receival at the winery or weighbridge. Further elements were addressed including the determination of matter other than grapes (MOG) and pest and disease levels. Procedural and guideline documents were developed for each of these elements to provide industry with guidance on how to use the standard methods.

This project achieved its goals in providing clarity regarding measurement of the key parameters for wine grapes that are associated with payment or rejection. A range of guideline documents and robust IESPs were developed, reviewed by industry and published on industry peak body websites. Experimental work was conducted and published on the impacts of different extraction techniques on key grape parameters and this is also now available to industry to provide guidance on best practice. Finally, a review into the latest literature on wine grape sampling and testing has been completed and will be published in the near future.

The Australian grape and wine industry now has a baseline set of information to guide best practice in the assessment of grape quality for payment purposes. This will form the basis for ongoing continuous improvement and assessment of new and emerging technologies as they become available.

3. Acknowledgements

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4. Background

In 2018–19 the Australian Competition and Consumer Commission (ACCC) Agriculture Unit conducted a market study of the wine grape industry. The study examined competition, contracting practices, transparency and risk allocation in wine grape supply chains. In its report on this study (ACCC 2019), the ACCC made several recommendations, some of which referred to the AWRI:

- The ACCC recommends that the Australian Wine Research Institute (AWRI) works with the National Measurement Institute (NMI) and the industry to develop uniform national standards for testing and measuring grape sugar levels and colour.
- The ACCC considers that the AWRI, and industry, should establish a working group and set clear timeframe targets for developing standards, such as a commitment to developing draft standards for consultation within 12 months.
- The ACCC recommends that the AWRI, in consultation with industry stakeholders, reviews current industry guidance on quality assessment standards, and amends the guidance to clearly reflect current best practice and to provide detailed information on standards for sampling in the vineyard and at the weighbridge.

A further recommendation was pertinent to the AWRI in its role as the leading provider of technical information to the wine industry:

- The ACCC recommends that winemakers should use well-documented and objective testing and sampling methods for quality assessments in the vineyard and at the winery.

This two-year project aimed to address these recommendations in conjunction with a range of commercially funded activities.

5. Project aims

The deliverables and associated activities for the project are shown in Table 1. Due to the impact of COVID-19, the ability to develop and deliver training materials for third-party review of grape assessment processes was limited, and regional engagement is still in progress to ensure that the project outputs are released to stakeholders effectively.

Table 1. Deliverables and activities

	Deliverables		Activities
A	Formation of a working group to review current industry guidance on grape assessment, identify appropriate methods	1.	Form a working group with representatives from AGW, Wine Australia, practitioners and NMI.
		2.	Review current industry guidance for grape assessment.

	and guide their development and extension	
B	A range of standardised methods for grape assessments	1. Develop protocols for the validation of secondary methods for colour, TA and TDS. 2. Develop standard protocols for sampling in vineyards, in wineries and at the weighbridge. 3. Develop reference protocols for the assessment of matter other than grapes (MOG) at the weighbridge and draft protocols for pest and disease assessments in the vineyard. 4. Consult with industry on the standard methods that have been developed and modify as appropriate. 5. Review current literature on wine grape assessment and produce a new comprehensive document incorporating the latest best practice. 6. Identify other parameters being used in grape assessment and determine the requirement for standard methods. 7. Develop standard protocols for any parameters identified in 2.
C	Extension materials to aid in the implementation of standardised methods and to assist third parties to assess the validity of testing and sampling protocols	1. Write draft interpretation guidelines/best practice documents for the approved standard methods. 2. Develop training materials for third party review of grape assessment processes. 3. Write new interpretation guidelines/best practice documents for any new methods.

6. Methods

6.1 Formation of Industry Reference Group

An Industry Reference Group consisting of grapegrowers and winemakers was convened by Australian Grape and Wine Incorporated (AGW) with the intention of critically reviewing and providing feedback on current practices for wine grape assessment and contributing to the development of industry endorsed standard procedures (IESPs). In developing the procedures, industry was consulted through the Project Reference Group and further engagement through AGW was available, with a feedback loop to AWRI. This approach endeavoured to promote a user-friendly format as much as possible while maintaining the level of scientific rigour required to ensure accurate and reliable results. The Industry Reference Group consisted of the following participants, with additional experts consulted when required:

- Warren Birchmore, Accolade Wines
- Anthony Catanzariti, Treasury Wine Estates
- Luke Edwards, Peter Lehman Wines
- Ashley Keegan, Fabal Wines
- Cristina Ramadam, De Bortoli
- William Rowe, Pernod Ricard Winemakers

- Anna Hooper, Australian Grape and Wine Inc
- Eric Wilkes, Australian Wine Research Institute
- Ben Rose, Performance Viticulture
- Alex Sas, Wine Australia
- Leigh Francis, Australian Wine Research Institute

The Industry Reference Group met every second month during the initial stages of the trial and less regularly once methods had reached an advanced stage of development. Early-stage discussions from the Project Reference Group meeting on 27 November 2020 highlighted some key points for the project:

- Industry endorsed standard procedures (IESPs) are formal, legal methods against which users can assess their current methods. While not mandatory to adopt these methods, those choosing not to must be able to formally validate their methods against the standard methods. IESPs need to be legally explicit and may need approval by National Measurement Institute (NMI). A balance is therefore needed between the desire for simplicity and the level of complexity required to make them robust.
- Development of industry guideline documents will provide a simpler, user-friendly explanation of the reference methods, making them more readily adoptable by users. These can also be designed to be flexible and updated more easily.
- The Reference Group requested the following IESPs to ensure that the full scope of industry practices was covered:
    - Brix
    - Colour
    - Secondary methods (secondary analytical methods that rely on the use of data generated from established primary reference methods)
    - Sampling in vineyard and at the weighbridge
    - MOG assessment
    - Pest and disease assessment
- The Reference Group raised the issue of IESPs being termed as 'industry endorsed' given the small size of the group. It was decided that except for Brix and colour (which are essential for the purpose of validating other methods so adoptability is less important), all other procedures would be published in provisional form and feedback would be encouraged from industry, allowing future amendments.
- The Reference Group raised concerns that if there was an alleged case of non-compliance with a procedure, that a chain of evidence should be required. The developed standard procedures needed to be designed to avoid conflict if surrounded by payment terms.

6.2 Industry Endorsed Standard Procedures (IESPs)

The purpose of developing a series of Industry Endorsed Standard Procedures (IESP) was to ensure that grape assessment practices would be as accurate and reliable as possible. Wine producers making an assessment for the purpose of determining grape prices should endeavour to follow these procedures, this being an absolute requirement for signatories to the Wine Industry Code of Conduct. The procedures can be downloaded from the Australian Grape and Wine website under the Winegrape Quality Assessment industry resources section. IESPs are required to be legally explicit and may require approval in the future by the National Measurement Institute (NMI). Each IESP developed, and other procedures requiring further review from industry, are structured under the following subheadings:

1. Introduction/foreword
2. Scope

3. Terminology
4. Measurement principle
5. Units of measurement
6. Metrological/technical requirements
7. Health and safety considerations
8. Materials/apparatus
9. Materials/reagents
10. Verification/calibration
11. Environmental conditions
12. Measurement procedure
13. Uncertainty of measurement
14. Limits of detection
15. Reporting results
16. Validation
17. Quality assurance
18. References

Analytical methods for sugar (Total Soluble Solids) and colour (expressed as total anthocyanins) were confirmed as requiring IESPs. An IESP for titratable acidity (TA) was initially considered, however, as there was limited industry adoption of TA as a method for fair payment, an IESP for TA was not a core focus for this project.

It was decided that vineyard sampling and weighbridge and/or winery methods (sampling, matter other than grapes (MOG), and pest and disease assessment) would be developed as 'provisional guidelines' in the first instance, based on recommendations from the Industry Reference Group. Following thorough road-testing during vintages 2021 and 2022, there would be the option to amend the documents to become IESPs.

A detailed review of literature was undertaken in parallel with the development of the IESPs and guideline documents to ensure best practice methods were being proposed to industry. The literature review of current industry guidance for grape quality assessment supported the eventual development of the IESPs.

6.3 Guideline documents for Industry Endorsed Standard Procedures

Industry guidelines, while referred to in the Code, are more like 'how to' guides to assist people to effectively use IESPs. These guidelines are living documents that are updated as new methods become available. Guideline documents were regarded by the project team and Industry Reference Group as important in bridging the gap between the scientific robustness required for the IESPs and ease of use for personnel in the wine industry.

6.4 Supporting trial work to inform vineyard and winery sampling procedures

One area highlighted by the literature search conducted was a lack of data on the variability of juice extraction methods used in grape quality analysis, both in the vineyard for harvest assessments and in the winery at the weighbridge. To explain this further, when wine grapes are analysed, an important step for a range of common analytes (e.g. sugars, pH, TA) is to extract juice from the grapes and then analyse that juice. Common techniques used to extract juice include hand pressing, manual or mechanical pressing followed by sieving, or homogenisation followed by centrifugation. When harvested fruit is received at a winery, processes are similar' however, for machine-harvested fruit, free run juice present in the bottom of the harvest bin is sometimes used for analysis. For more

representative sampling of a load of grapes, screw core sampling mechanisms can be used to obtain grapes for juice extraction. Juice extraction methods can however vary significantly between vineyards and wineries, and this may have an impact on analytical results.

As observed from the literature review (see section 7.1), some previous research investigating the variability in juice extraction methods for wine grapes was carried out in France (Dumas et al. 2020). This study found that there was no significant effect of the pressing method on total soluble solids (TSS) results but that there was a significant effect ($p < 0.001$) for all other parameters measured, with homogenisation resulting in higher pH and lower TA values when compared with other pressing methods. A past AWRI study assessed the effects of homogenisation methods and freezing, indicating that freezing of fruit and homogenates had a significant effect ($p < 0.001$) on pH (Cynkar et al. 2004).

As part of this project, the AWRI ran a series of studies to assess the effects of different juice extraction methods on TSS, pH and TA as measured in the laboratory. The experiments aimed to assess the impact of different extraction methods (e.g. hand-pressed vs homogenised) and different storage methods (e.g. fresh vs frozen) for harvested fruit under laboratory-based juice extraction methods, and to assess the effects of different sampling and extraction methods on juice total soluble solids (TSS) and pH measurements at grape receival points.

6.4.1 Experimental set-up for laboratory-based extraction assessment

Initial experiments used samples of red and white seedless table grapes as a substitute for wine grapes. The primary objective of the assessments was to evaluate the variability and reproducibility of analytical results obtained after different extraction methods. The extraction methods trialled are listed below, noting that the frozen samples were analysed after one week held at -18°C:

- Hand-pressed fresh grapes
- Homogenised fresh grapes
- Hand-pressed grapes, frozen whole
- Homogenised grapes, frozen whole
- Frozen homogenised grapes.

An initial batch of approximately 7 kg of each table grape type was destemmed, and berries were sub-sampled into 35 × 200 g batches with seven replicates subjected to each of the five extraction methods. The hand-pressed samples were placed into a Ziplock bag and pressed to extract the juice, ensuring that all grapes had been pressed. The homogenate samples were prepared using a NutriBullet, blending for 45 seconds, followed by centrifugation at 3,500 rpm for 5 minutes. The quality parameters analysed for each batch of fruit were total soluble solids (TSS measured in °Brix), pH and titratable acidity (TA) at pH 8.2. Total soluble solids were measured using a digital refractometer, and pH and TA were measured using a digital auto-titrator.

A second set of trials used Merlot and Semillon wine grapes, harvested from a vineyard on the Waite Campus, Urrbrae, South Australia. The fruit was hand-picked and sub-sampled into seven replicates for each extraction method as listed above, with freezing trials not included.

Statistical significance was evaluated using f-tests and t-tests. F-tests were used to evaluate the differences in variance and t-tests were used to evaluate the differences in means.

6.4.2 Experimental design for extraction assessments at winery receival points

During vintage 2021, the AWRI in collaboration with industry representatives, accessed three SA-based receival points (Sites A, B and C) to assess loads of grapes using different sampling/extraction

methods, to better understand the variability of each method. Site A consisted primarily of loads delivered in a truck and/or trailer combination, all machine-harvested fruit. Site B consisted of fruit delivered in either 0.5 t or 2.5 t bins, with a combination of machine- and hand-harvested fruit. Sampling and extraction methods trialled at these sites were:

- Free-run juice collected using a sampling cup on a rod
- Full berries, collected using a shovel or Yuba sampler, followed by hand-destemming and hand-pressing in a Ziplock bag
- Mechanic screw core sampler (Maselli or Relco) with attached analytical station.

Site C consisted primarily of 2.5 t bin loads. Only free-run juice samples were collected and analysed at this location.

A series of 15 loads (5 white and 10 red) were assessed at Site A, and 12 loads (all red) were assessed at Site B. The assessment at Site B included only loads of a minimum of five bins.

Free-run juice samples collected from Site C from individual bins were analysed for TSS across vintages 2019, 2020 and 2021. To understand the natural variability of TSS (Brix) across a load of grapes harvested from the same vineyard, data were collected from loads where a minimum of eight bins across the load (most commonly 2.5 t bins) were analysed.

7. Results and discussion

A detailed review of literature was undertaken in parallel with the development of the IESPs and guideline documents to ensure best practice methods were being proposed to industry. The literature review supported the eventual development of the IESPs and also highlighted areas where future opportunities might lie. It further emphasised considerations required when assessing wine-grape quality and the differences between subjective and objective measures.

7.1 Review of current industry guidance for grape quality assessment and emerging methods

Quality assessment of wine grapes is typically performed upon handover from producer to purchaser, and the grapes are usually assessed against several specifications. The acceptance of the fruit and price to be paid are determined based on this assessment. While some specifications, such as grape maturity and colour, use analytical measures that meet objective criteria, other specifications such as ‘fruit purity’ and disease level assessment still require subjective methods of assessment. Additionally, a variety of methods are used by grape purchasers to assess the same specification, which may contribute to inconsistent assessments across the industry. Therefore, industry standard practices would establish benchmarks for quality evaluations for both grapegrowers and winemakers, to guide the trading price. They would also likely serve as a useful tool to improve relationships between buyers and sellers of wine grapes.

The Australian Competition and Consumer Commission (ACCC) recognised that more standardised assessment methods were required that rely on more objective measures where available. Currently, subjective measures are recognised for use to assess the following specifications: disease, matter other than grapes (MOG), contamination, varietal integrity, uneven grape ripening, grape spoilage and damaged berries. The literature review highlighted emerging methods that could be used for quality assessment of wine grapes at the receival point and their feasibility for wider adoption in the wine industry. It also highlighted the pros and cons of current practice in line with available literature.

7.1.1 In the vineyard sampling and harvest maturity

Monitoring grape maturity is critical for grapegrowers and winemakers during the ripening season to determine the best timing for the fruit harvest. The representativeness of vineyard sampling is critical for the assessment of the overall maturity of a specific vineyard site and providing accurate information for harvest decisions.

Two options for sampling are cluster or berry sampling. Cluster sampling is advised rather than berry sampling because berry sampling is prone to overestimation of maturity due to the tendency to sample the most mature berries. It is also essential that growers and winemakers standardise sample processing (e.g. extracting juice for analysis) to be able to compare results. In addition, sample processing should be carried out to replicate what is expected to happen in the cellar (e.g. berry breakage, skin contact, temperature, degree of pressing).

Meyers and Vanden Heuvel (2014) used aerial normalised difference vegetation index (NDVI) images for the purposes of quantifying vineyard spatial structure and computing optimal vineyard sampling protocols. A recent study performed by the same author (Meyers et al. 2020) adopted a satellite NDVI-based sampling protocol for monitoring grape maturation during growing seasons. They suggested that this novel strategy can vastly reduce labour requirements while providing a good prediction of fruit maturity.

Dumas et al. (2020) compared the impact of different juice extraction methods in the laboratory on must composition (pH, TA, and sugar content). The juice extraction rate varied among different methods, however, the sugar content (TSS) was not affected. TSS, pH and TA were not sensitive to the extraction method. In comparison, potassium concentration could be influenced by the extraction method in the laboratory, and methods that crush the seeds and skins should be avoided when analysing this parameter.

Fruit technological maturity assessment is typically based on the measurement of TSS, pH and TA. However, actual fruit maturity evaluation involves more than these simple measurements. Grape aroma and phenolic maturity are also important since they translate into final wine quality, however, their measurement via parameters such as those listed below remains subjective and can create difficulties in objective assessment when determining payment for grapes:

- Aroma/flavour, and intensity of aroma/flavour
- Grape skin tannins and tannins extractability
- Fruit anthocyanin concentration
- Stem lignification or ‘ripeness’
- Seed numbers per berry
- Seed ‘ripeness’, ‘maturity’, or tannin extractability
- Sugar per berry
- Sugar loading peaks
- Berry softness
- Berry size/weight
- Berry shrivel.

It is recommended that multiple parameters be used to analyse fruit quality objectively.

The simultaneous determination of the main organic acids and sugars in juice and wine (Coelho et al. 2018) could potentially help facilitate the automation of sampling and analysis. While this approach could be of interest to large-scale wine producers, it may not be suitable for smaller producers. The limited information gained by assessing TSS and acidity alone is however still not robust as a quality indicator, as it does not provide any sensory information. However, grape quality assessment by

tasting is not objective and is subject to winemakers' experiences. This highlights the need for new approaches to objectively assessing grape qualities in a timely fashion.

One study that investigated volatile markers as an alternative tool for grape maturity assessment found that some terpene compounds could be potentially used to determine optimum grape harvest date using headspace solid phase microextraction (HS-SPME) combined with GC–MS (Perestrelo et al. 2018). Three monoterpenes (linalool, α-terpineol, carvomenthol), one sesquiterpene (bicyclogermacrene) and two norisoprenoids compounds (vitispirane I, β-damascenone) could be used as potential volatile markers (Perestrelo et al. 2018). The importance of this study is that it provides a potential future objective analytical method to assess grape quality at the receival point.

Smart chemometric technologies using optoelectronic devices were also identified as quick and reliable alternatives to traditional sugar, pH and acid analyses of grape components (Sozzi et al. 2021). This technology enabled maturation monitoring and could fit into automated processes. However, further validation against the individual analysis is required to improve performance. Another metabolomic study using MS and NMR chemometric approaches found that proanthocyanidins and other flavonoids increased in ripe and overripe grapes (Alves Filho et al. 2022).

7.1.2 Evaluating sugar levels

The sugar level of grapes is often measured as total soluble solids (TSS) in Brix or Baumé units using a refractometer or hydrometer. It is a measurement of all soluble solids, with fermentable sugars accounting for 90 to 95% of the TSS. Major grape sugars (glucose and fructose) are not consumed equally during fermentation. Therefore, the ratio of these two sugars (which depends on the variety and degree of grape maturity) can have implications for fermentation completion (Reynolds 2021).

Accurate measurement of fermentable sugar in juice or must can be adopted, however, the sugar level is only one of the quality parameters assessed upon grape receival. Quick and reliable measurement via a measure such as TSS is therefore preferred to support in-time fruit processing, and current technologies are efficient in achieving such results.

7.1.3 Fruit phenolic content analysis

Quantitative and qualitative evaluations of fruit phenolic compounds can be an important maturity gauge, particularly in red wine production. Seed tannins are important to red wine quality and are often used as a maturity measure. Seed tannin concentration is several times greater than that of skin tannins, depending upon the degree of seed 'maturity' (lignification), fruit maturity, and seed numbers, each of which can vary significantly among seasons. There are also significant differences in seed tannin concentration between cultivars. The evaluation of seed tannins is important due to their potential impact in red wine production.

Fredes et al. (2017) developed a way to quantify seed colour ripening stages along with the chemical and colorimetric data. They differentiated underripe seed (brown with green traces), ripe seed (dark brown with green traces), and overripe seed (dark brown without any green traces). Additionally, some winemakers place seeds in a water/ethanol mixture of 12%–14% alcohol (v/v) and evaluate by taste 24–48 hours later as a means of gauging seed tannin maturity/extractability.

One study adopted an electronic eye (RGB image reader) to predict phenolic ripening of fruits (Orlandi et al. 2018). This work found that total anthocyanins have the best linearity in terms of prediction using simple image analysis on must or grape juice (Orlandi et al. 2018). The authors successfully developed a fast, inexpensive and eco-friendly method which is able to easily quantify

the parameters related to technological and phenolic maturity. The result is promising for future adoption as a quick objective assessment of fruit colour maturity at the weighbridge.

7.1.4 Fruit nitrogen status

Wines made from fruit with adequate nitrogen generally have a superior aroma and overall quality (Sinton et al. 1978). The nitrogenous components of grapes and juice that are metabolically available to yeasts include amino acids and ammonium salts (NH_4^+). Ammonium nitrogen and the utilisable amino acids (free alpha-amino acids, or FAN) are referred to as YAN (yeast assimilable nitrogen). YAN is critical for yeast fermentation and winemakers often incorporate nutrient/nitrogen supplements to secure smooth fermentation.

Bovo et al. (2013) reported the use of FT-MIR spectroscopy combined with chemometrics to determine the concentration of yeast assimilable nitrogen (YAN) in grape must – freshly pressed grape juice that contains the skin, seeds and stems of the fruit. While multiple infrared spectroscopy-based applications have been published for grapes (solid) and wine (liquid), marc (a semisolid material) has quite different characteristics. This led to Bovo and co-workers developing predictive models that had great potential for measuringquality parameters such as reducing sugars, ethanol and acetic acid quickly and non-destructively, which in turn allowed the rapid determination of the marc's microbial conditions (Bovo et al. 2013).

7.1.5 Berry sensory analysis (BSA)

Berry sensory analysis (BSA) follows a standardised set of 20 descriptors, assessing the ripeness of wine grapes by judging fruit stems, skin, pulp, and seeds separately (Winter et al. 2004). Aroma evaluation of the fruit is considered important in assessing relative maturity and stylistic goals. Many compounds are present as conjugated bound precursors, making fruit aroma/flavour evaluation only a rough approximation of the aroma/flavour potential of a wine. Optimal sensory evaluation involves an understanding of the following:

- Standardised and controlled environment.
- Representative sample.
- Optimal sample temperature.
- Elimination of bias.
- Importance of sample contrasts.
- Use of skilled evaluators.
- Number of evaluators and evaluations required to gain a true picture.
- Minimisation of presentation effects (adaptation).
- Minimisation of physiological effects (time of day, not tasting for a period after eating or drinking
- Using the proper testing method.

7.1.6 Pest and disease assessment

7.1.6.1 Disease

Diseases such as powdery mildew, botrytis, downy mildew and other moulds and rots commonly result in changes to flavour that can affect wine quality. Current best practice for disease assessment is by visual and sensory assessment. Diagrams such as the Emmett and Wicks Assessment Key (Emmett et al. 2015) are used to evaluate the level or severity of disease in a bunch. Although a useful tool to maintain consistency in assessment across different assessors, a study found that

there is still significant variation in the grading given to bunches in vineyard (Scott 2016). This was largely attributed to non-uniform environmental conditions that made it difficult for assessors to visualise all bunches with equal accuracy. More objective measures of disease assessment have been developed, however, they do come with their challenges for adoption in vineyard.

Attempts have been made to reduce the ambiguity of assessment keys to make assessments more consistent. Smart phone applications such as Leaf Doctor and PMapp have been developed with the aim of assisting in accurately determining the level of disease (Scott 2016, Pethybridge and Nelson 2015). This also allows for real-time collation and analysis of data from the field (Scott 2016), but still requires time and effort to representatively sample appropriate vines and berries.

Another growing field of research is optical sensing, where disease is identified and assessed on the vine using cameras and automated software. Gutiérrez et al. (2021) developed classification models using deep learning to identify powdery mildew and spider mite from images of vines. Similarly, proximal optical sensing was employed to identify powdery mildew with increased sensitivity for early-middle stage infection (Oberti et al. 2014). These methods require expensive camera equipment and software, and the necessary algorithms are still under development. They also require experienced workers to properly apply them.

7.1.6.2 Spoilage

Fungal infection may lead to fruit spoilage and in some cases the release of the mycotoxin, ochratoxin A into grape juice and wine (Battilani et al. 2004). A study using table grapes found rot and ochratoxin A did not develop on intact fruit (Jiang et al. 2013). Low temperature (4°C) inhibited the rot and ochratoxin A production, however, higher temperatures (25°C and 30°C) promote their development (Jiang et al. 2013). Therefore, maintaining low fruit temperature and proper storage is important to prevent fungal spoilage during transportation, especially for machine-harvested fruit where berry split is inevitable.

- Fruit should be harvested at optimum stage of maturity and by proper method.
- Rough handling and berry splits should be avoided, where possible.

The most common practice used to preserve table grape quality during storage is the addition of SO_2 (Lichter et al. 2006). Similarly, SO_2 is usually added to wine grape harvest bins to prevent oxidation and microbial spoilage before fruit processing. Combined with low fruit temperature, SO_2 usage can largely reduce the incidence of wild fermentation, oxidation and acetification of fruit.

Fungal activity in grapes also releases distinct volatile organic compounds that can be analysed using GC-MS (Giannoukos et al. 2020). Portable mass spectrometry (MS) is a powerful, simple analytical technique ideal for *in situ* applications, which offers high sensitivity (i.e. a low limit of detection—LoD), selectivity, fast and accurate analysis (within seconds), with no sample preparation requirements (Giannoukos et al. 2020). Portable MS seems to be a promising option to detect the presence of fungal activity (if any) at the grape receival point for fast determination of grape quality. However more testing under practical conditions and possible modification will be required to realise these technologies.

7.1.6.3 Damaged berries

Bird damage to fruit is a significant concern for growers, as it not only reduces yield but also leaves the fruit susceptible to infection, therefore reducing quality (Elser et al. 2019). Accurate and efficient damage assessment is important in the vineyard before harvest. When fruit arrives at the winery, it is also important to assess the degree of damage at the weighbridge to help determine the fruit quality. Visual assessment coupled with a progressive sampling strategy could be developed and

adopted in the vineyard to improve efficiency and accuracy of estimate bird damage (Tracey and Saunders 2010). Little research has focused on the assessment of grape damage at the loading point since damaged fruit should ideally not reach the receival point at the winery. However, it is possible that visual reference charts could be borrowed from vineyard assessment and used with a different sampling regime. Similar strategies could also be adopted for assessing insect-damaged fruit.

The consequences of sunburn on wine quality are not well understood. An overall loss of wine flavour and colour together with increased undesirable phenolic characters has been reported (Gambetta et al. 2021). Optimum fruit quality is always desirable, and the level of tolerance of damaged fruit is subject to agreement between grapegrowers and the winemakers.

7.1.7 Agrochemical residues

Agrochemical use in vineyards is primarily monitored and assessed using spray diaries. Use of agrochemicals should be recorded and adhere to withholding periods and other restrictions, as outlined by the appropriate governing bodies, with the expectation that the eventual wine will meet minimum residue limits (MRL) requirements of the target export and domestic markets (Allen 2003). To confirm that MRLs are met, quantitative testing on a representative sample of the harvest is often performed. If spray diaries are incomplete or missing, the winery may decide to impose a penalty or decline a load. Spray diaries may become inadvertently inaccurate if spray unit calibrations are not checked prior to spraying, while quantitative testing requires extensive and time-consuming testing at accredited laboratories (Allen 2003).

Real-time monitoring of agrochemical residues could be employed throughout the growing season to allow for more accurate and less-time intensive analysis, while still ensuring MRLs will be met. Several non-destructive optical sensing techniques have been developed that rely on colorimetric detection, fluorescence detection and terahertz spectroscopy, among others (Li et al. 2022). Terahertz spectroscopy was successfully performed on *T. sinensis* leaves for the detection of benzoyl, carbendazim and thiabendazole in trace amounts of 10 mg/L (Nie et al. 2021). Leaf samples were placed in a sample chamber and the absorption spectrum obtained and processed using several algorithms. Additionally, Liu et al. (2021) developed flexible substrates for the swabbing of fruit for quantitative identification of pyrimethanil using Raman spectroscopy.

Unfortunately, these real-time monitoring techniques cannot currently be used in the vineyard or at the weighbridge, due to several challenges. Primarily, these techniques are specific to a few agrochemicals and cannot easily be applied to the wide range of agrochemicals used in vineyards. Spray diaries and chromatographic techniques such as liquid chromatography mass spectrometry are still superior in capturing the wide range of agrochemicals used. Secondly, advanced or expensive equipment and experienced workers would be needed to employ these new methods, which would not be feasible for most vineyards and wineries.

7.1.8 Matter other than grapes (MOG)

Matter other than grapes, or material other than grapes (MOG) is inevitable in grape receival bins especially when mechanical harvesting is used, since MOG also includes other grapevine materials such as petioles and leaves. A range of processes for sorting and destemming are adopted in wineries to remove MOG. Hand sorting is tedious and requires close attention.

Recent studies have also shown that inclusion of leaves, rachis and peduncles in ferments can alter the chemical and sensory profiles of Shiraz wine. ‘Green’ characters were observed in the wines, caused by the extraction of methoxypyrazines from the MOG into the wine (Capone et al. 2021).

Other studies have shown that the inclusion of petioles and leaf blades in fermentations can lead to higher concentrations of some terpenes and other odour-active compounds, imparting mostly 'floral' aromas to wines, sometimes called 'floral taint' (Aubie and Reynolds 2017, Ward et al. 2015). Inclusion of rootstocks can also have an impact on wine profiles therefore winemakers need to be aware of the potential implications of MOG and incorporate processes for its removal.

In conjunction with MOG, other unwanted contaminants can also be present in grape loads at the receival point. These may include fuel, oil and other lubricants, soil, additives and non-food grade materials. Each load therefore needs to be visually inspected upon receival. Some contaminants such as fuel have a distinctive smell and can be detected through sensory evaluation. Others might be able to be detected through biosensors, which are becoming popular in other applications, currently targeting common preservatives and additives like sulfite, glutamate, nitrites, benzoic acid, etc. in foodstuffs such as soups and sauces (Bahadır and Sezgintürk 2017).

7.1.9 Fruit condition

In any vineyard there are variations from berry to berry, bunch to bunch and vine to vine. The degree of uneven ripening across a vineyard affects the representativeness of vineyard sampling. More importantly, when fruit arrives and is processed in the winery, sub-standard or unripe berries present in the must will affect the sugar and acid levels (and therefore the sensory characters) of the resulting wine (Guerrini et al. 2018). The level of unripe berries has been found to affect some critical chemical and sensory characteristics of wine including ethanol concentrations, titratable acidity, malic and lactic acid concentrations, a range of polyphenol-related parameters, certain volatile organic compound concentrations, as well as colour and bitterness (Guerrini et al. 2018).

It is widely acknowledged that grape heterogeneity exists throughout any given vineyard at the time of harvest. Wine made from either underripe or overripe grapes was rated as lower quality compared to wine made from grapes at optimal ripeness (Armstrong et al. 2021). The underripe or overripe fruits can be distinguished via either skin colour or berry density (Armstrong et al. 2021). In this case, optical or visual sorting is most practical and best suitable at the grape receival point. Density sorting poses more technical challenges. Current practice, however, relies on Baume assessment.

7.2 Industry Endorsed Standard Procedures (IESPs), standard procedures and guideline documents

Industry endorsed standard procedures (IESPs) were developed in accordance with industry guidance and best practice, as standard documents for quality measurements which can be used in the event of disputes pertaining to grape payments.

A further series of standard procedures, reviewed by industry but not relevant to payment disputes was developed to give producers clarity on best-practice methods for vineyard and winery practices to assess fruit quality.

Guideline documents were further developed as simplified versions of the standards, capturing the high-level information required for use by producers on-site.

A brief outline of the purpose and scope of the IESPs and standard procedures developed through this project is provided below. The following IESPs and standard procedures are currently available on the Australian Grape and Wine website:

- Industry Endorsed Standard Procedure for measurement of Brix or Baume in grape juice.

- Industry Endorsed Standard Procedure for determination of total anthocyanins in grapes
- Standard method for determining MOG.

It is intended that all of the IESPs, standard procedures and guideline documents will be collated on one page on the AWRI website.

Additional details of the documents produced are provided in Appendix 1.

7.2.1 Standard method for the measurement of °Brix (or °Baumé) in grape juice

This method applies to the measurement of °Brix (or °Baumé) in grapes and must. A copy of the method is available on the AGW website here: Industry Endorsed Standard Procedure for measurement of Brix or Baume in grape juice.

The purpose of this method is to define a standardised approach for the measurement of °Brix (or °Baumé) in grape juice. This method is intended specifically to address situations where °Brix (or °Baumé) measurement is used as part of the process for setting payment or rejecting grapes. While it is applicable to the general measure of °Brix and °Baumé in grape juice, the level of quality assurance and rigour required for other purposes is at the discretion of the end-user. This does not necessarily replace in-house methods, but any such method needs to be suitably validated against this method. Approaches for cross validation of in-house methods which use the same principle as this method are outlined within this procedure.

Validation of other automated equipment such as weighbridge sampling systems should be done with reference to this procedure and should be able to demonstrate equivalent levels of accuracy and precision.

The °Brix and °Baumé scales are used as an indicator of the ripeness of grapes and are an indication of total soluble solids (TSS) in the grape juice. The °Baumé is also an approximate indication of the likely alcohol content of a dry wine produced from that juice.

The °Brix scale, commonly used by the brewing industry and to a lesser extent by the wine industry, relates directly to °Baumé via the following conversion formula:

1.00 °Baumé = 1.80 °Brix

The °Brix is equivalent to the percentage sugar (sucrose) by weight (i.e. % w/w).

7.2.2 Determination of total anthocyanins in grapes

The principle of this spectroscopic analysis is based on the methods published by Iland et al. (1996). This analysis applies to the measurement of total anthocyanins in red grapes. A copy of the method is available on the AGW website: Industry Endorsed Standard Procedure for determination of total anthocyanins in grapes

The purpose of this method is to define the measurement of colour in grapes, expressed as total anthocyanins. It is intended to be a reference method that other industry-based methods can be validated against and to function as the method of choice when arbitrating disputes. It does not necessarily replace in-house methods, but any such method needs to be suitably validated against this method.

7.2.3 Standard method for sampling grapes in the vineyard for assessing berry composition at harvest

This method applies to sampling of grapes in wine grape vineyards. Additional considerations need to be monitored when sampling at the winery weighbridge or sampling of harvested grape bins to collect representative grape samples for compositional analysis and these are the subject of a separate procedure.

The purpose of this method is to define a standardised approach for sampling grapes in the vineyard for assessing berry composition at harvest. This method is specifically to address situations where grapes are to undergo quality indicator analysis, including °Brix (or °Baumé), pH, titratable acidity (TA - g/L tartaric acid equivalents) and/or colour [anthocyanin mg/g (Iland et al. 2000)] measurements as required for the determination of payment.

This protocol specifically focuses on vineyard sampling at harvest for payment determination. This protocol does not include requirements for sampling of grapes in the vineyard for maturity assessment' however, in principle it can be adopted and used to meet those requirements.

The industry recommended best practice regarding vineyard sampling is outlined in Krstic et.al. (2003).

The basic principles of achieving a good representative sample include:

- sampling from multiple locations within a defined vineyard (block, patch, section, harvest unit or row)
- incorporating pooled (consolidated) grape samples to ensure that analysis is representative of a defined vineyard block, patch, section, harvest unit or row.
- incorporating enough grapes to ensure that minimum sample size requirements are met for specific grape analysis.

7.2.4 Development and validation of secondary methods for the measurement of analytical parameters in grapes and must

This procedure, which is still undergoing industry consultation, applies to the quantitative measurement of analytical parameters in grapes and must including, but not limited to, pH, titratable acidity (TA), sugar concentration (°Brix/°Baumé), colour (total anthocyanins) and total tannins. The intention of this procedure is to provide guidance on the application of secondary analytical methods, specifically those reliant on spectrophotometric-based measurement of the aforementioned analytes.

The purpose of this procedure is to ensure that newly developed or adapted methods of analysis for key analytical parameters in grapes and must are fit-for-purpose. This procedure outlines the requirements for development and validation of secondary analytical methods that rely on the use of data generated from established primary reference methods.

7.2.5 Standard method for determining the matter other than grapes (MOG) at harvest

The purpose of this method is to define a standardised approach for assessing matter other than grapes (MOG) in a load at the point of receival at the winery. This procedure represents the current industry best practice for the determination of MOG upon receival at the winery. It is expected that

this method will be open to modification and improvement as experience within industry dictates, as technology improves or the understanding of the science behind representative sampling and grape chemistry assessment improves. The method is available from the AGW website: Standard method for determining MOG.

7.2.6 Standard method for sampling grapes on arrival at the winery

This method applies to sampling grapes at a recognised load assessment point upon receipt at the winery. This method is solely used for the purpose of monitoring harvested grape loads to collect representative grape samples for compositional analysis.

The purpose of this method is to define a standardised approach for sampling grapes upon receipt at the winery for assessing berry composition. This method is specifically to address situations where grapes are to undergo quality indicator analysis, including sugar content (°Baumé or °Brix), pH, titratable acidity (TA - g/L tartaric acid equivalents) and/or colour [anthocyanin mg/g (Iland et al. 2000)] measurements as required for the determination of payment.

This protocol specifically focuses on weighbridge sampling upon receipt of fruit at the winery for payment determination. Grapes delivered to the winery will typically be assessed at the weighbridge prior to undergoing winemaking. The weighbridge is often the winery's final and critical checkpoint against specification for grapes. Weighbridge assessment also involved MOG and pest and disease assessment, with this protocol focusing on appropriate load sampling for quality indicator analysis. Note, alternative inspection points upon receival of fruit at the winery are also considered, including sampling at crushers or hoppers.

The basic principles of achieving a good representative sample include:

- sampling from multiple locations within a load, (e.g. bin, trailer, truck).
- consolidating samples to ensure that analysis is representative of the entire load
- incorporating enough grapes to ensure that minimum sample size requirements are met for specific grape analysis.

7.3 Guideline documents accompanying Industry Endorsed Standard Procedures (IESPs)

The Industry Guidelines ('how to' documents/recommendations) were based on the IESPs of the primary procedures required in the fair dispute of payment, to enable businesses to effectively use the more comprehensive documents and assist with internal training and development. The guideline documents are living documents that will be updated as new methods become available. New guideline documents may also be required as the procedures are rolled out to industry. Three core guideline documents were developed during the project:

- Determination of total anthocyanins in grapes
- Standard method for the measurement of °Brix (or °Baumé) in grape juice
- Standard method for sampling grapes in the vineyard for assessing berry composition at harvest

Copies of these guidelines are provided in the Appendix 5.

7.4 Supporting trial work to inform vineyard and winery sampling procedures

7.4.1 Laboratory-based juice extraction findings

Content in this section was first published in *AWRI Technical Review* Issue 254, October 2021.

7.4.1.1 Total soluble solids – key findings

For white table grapes, there were no statistically significant differences between the extraction methods trialled for TSS. Homogenisation tended to yield equivalent levels of TSS as hand pressing; however, there was some evidence to suggest freezing lowered the TSS (Figure 1).

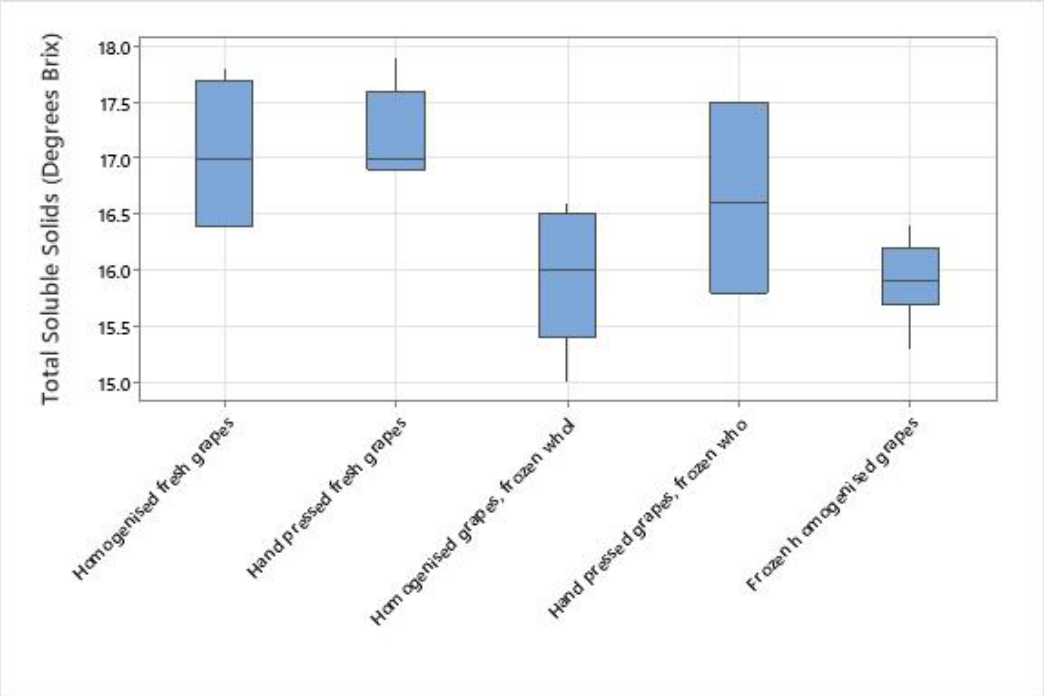


Figure 1. Range of results for total soluble solids (TSS) measured after different juice extraction methods for white table grapes. The blue boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured, excluding outliers (first published in *AWRI Technical Review* Issue 254, October 2021).

Despite mean results being the same across homogenised and hand-pressed samples, there was a significant spread of individual results, highlighting the difficulties of sampling from even a well-mixed homogenous group of berries. The spread of 1.3 °Brix across homogenised samples suggested a significant degree of berry-to-berry variability between large table grapes from the same bunch. For red table grapes, mean TSS results were similar for homogenisation and hand pressing, with a greater spread of results for the hand-pressed grapes but no statistical difference found. The source of this variability is unlikely to be the extraction process, but rather the berry-to-berry variability, as observed for white table grapes. Freezing samples, whether whole or homogenised, increased the variability across the replicates (Figure 2).

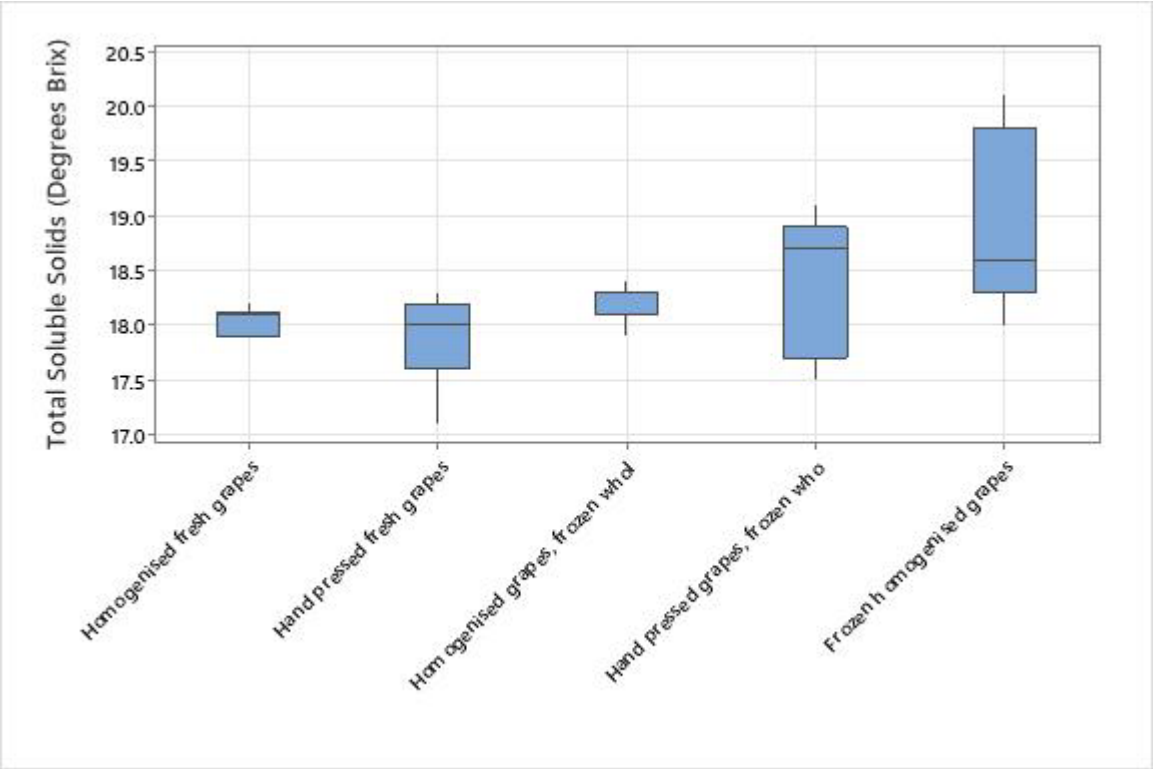


Figure 2. Range of results for total soluble solids (TSS) measured following different juice extraction methods for red table grapes. The blue boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured, excluding outliers (first published in *AWRI Technical Review* Issue 254, October 2021).

The trials assessing Merlot and Semillon wine grapes showed that on average TSS results were higher following homogenisation than hand pressing for the Merlot grapes ($p<0.001$) (Figure 3), although the percentage difference between homogenisation and hand pressing was, in real terms, only around 2%.

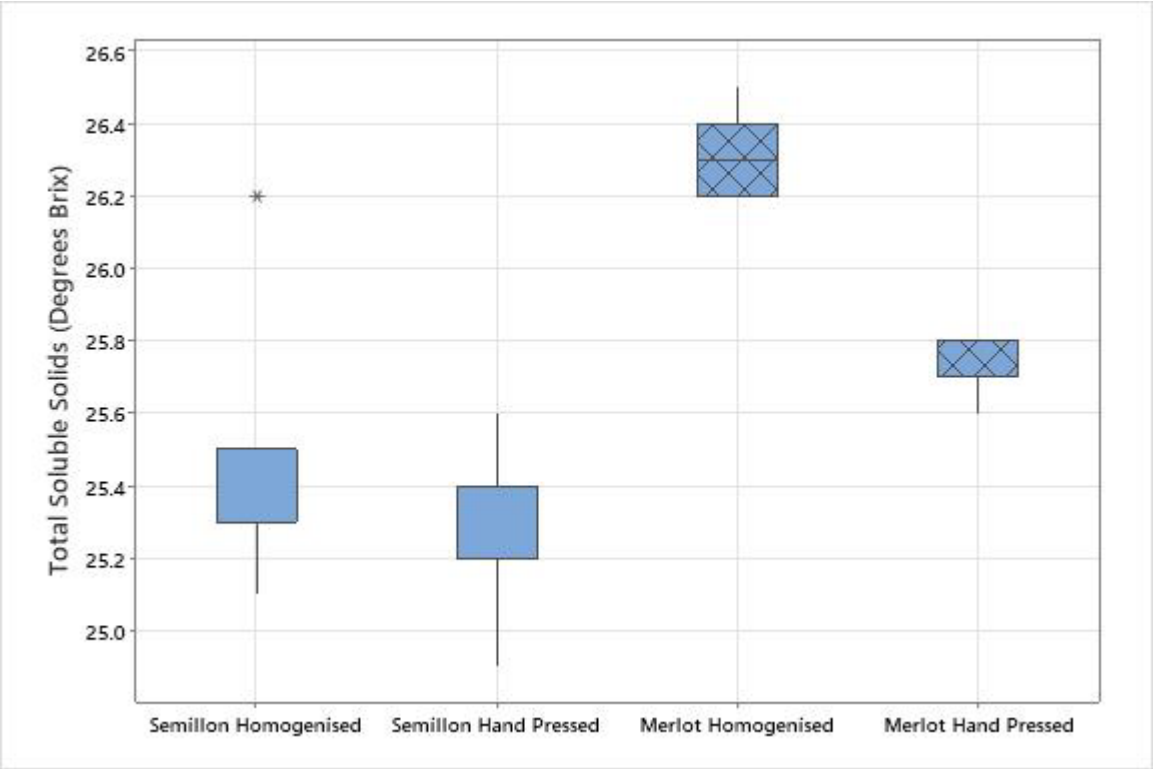


Figure 3. Range of results for total soluble solids (TSS) measured in Merlot and Semillon grapes after different juice extraction methods. The blue boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured, excluding outliers (first published in *AWRI Technical Review* Issue 254, October 2021).

7.4.1.2 pH and titratable acidity – key findings

Homogenisation of all grape types resulted in a significantly elevated pH compared to samples which were hand pressed ($p<0.0001$) (Figure 4).

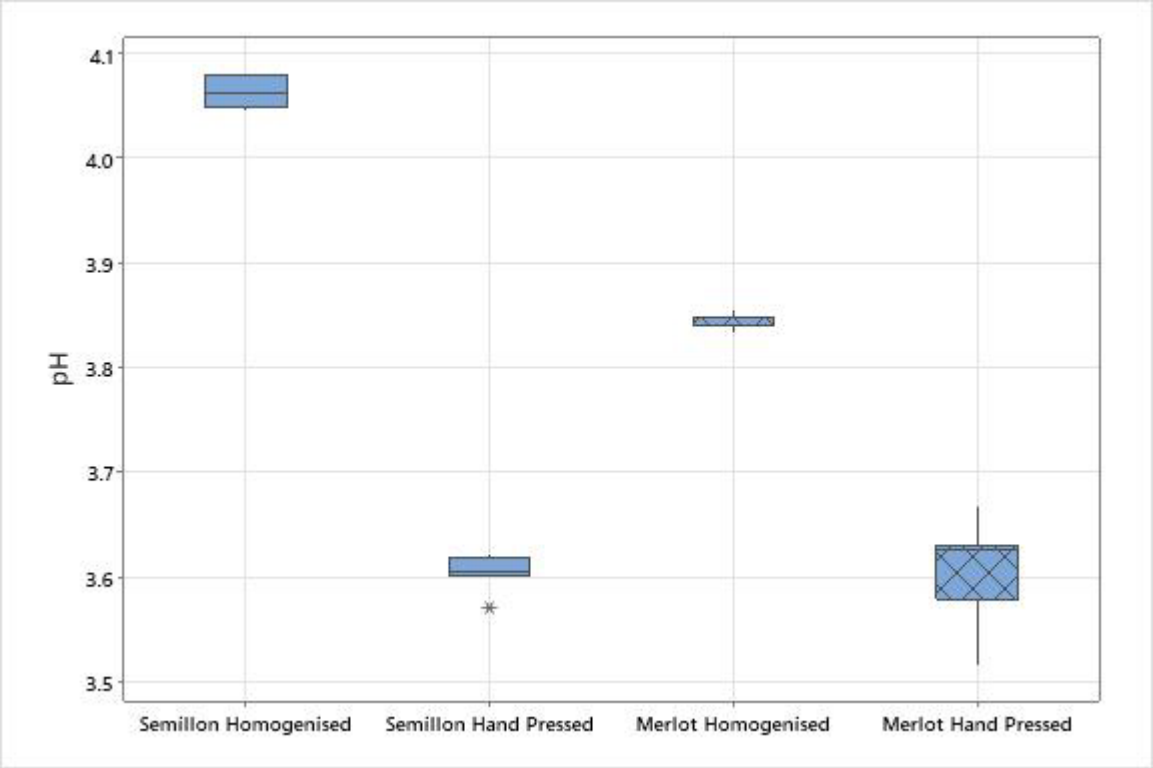


Figure 4. Range of results for pH measured for Merlot and Semillon wine-grapes after different extraction methods. The blue boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured, excluding outliers (first published in *AWRI Technical Review* Issue 254, October 2021).

Freezing of samples led to an increase in the pH of the extract, with the strongest evidence observed in white table grapes. Freezing of samples led to a decline in the TA of both red and white fruit, seen in both table and wine grapes ($p<0.001$) (Figure 5). The differences observed between extraction methods could be related to the relative accessibility of the acids within the berry structure The spread of pH and TA results within samples analysed by each extraction method was negligible for all samples analysed. For wine grapes, the data variation across seven replicates never exceeded 0.2 for pH and 0.4 g/L for TA at pH 8.2.

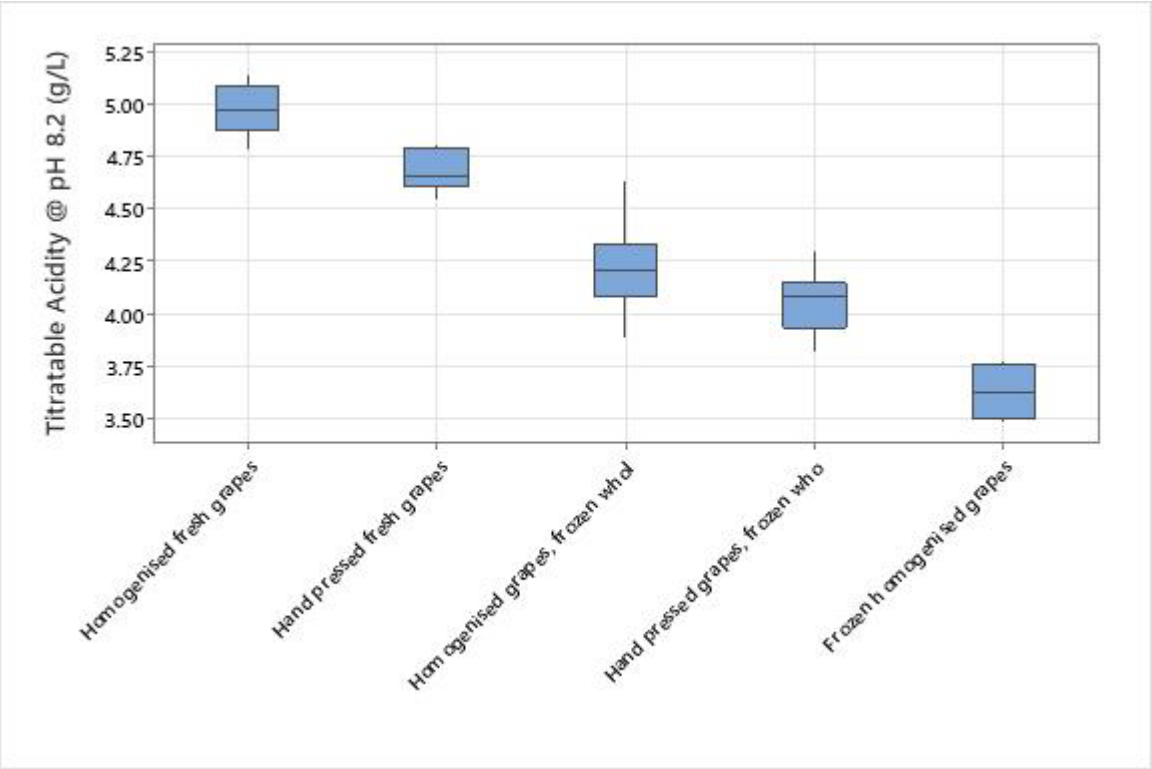


Figure 5. Range of results for titratable acidity at pH 8.2 measured in white table grapes after different juice extraction methods. The blue boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured, excluding outliers (first published in *AWRI Technical Review* Issue 254, October 2021).

7.4.1.3 Conclusions

Based on the experiments undertaken in a laboratory setting, measurement of total soluble solids in grapes was not greatly influenced by the juice extraction method employed, with differences observed within 2% of the respective means.

Extraction methods did have a greater influence on results for pH and TA, which could potentially be attributed to the distribution of acids within grape berries. Freezing affected the variability of results for total soluble solids, but not the mean, whereas it affected the mean values for pH and TA.

Notable variability was seen between replicates, especially in total soluble solids measurements, indicating that berry-to-berry variation within bunches can have a significant impact on analytical results.

7.4.2 Weighbridge-based juice extraction findings

Content in this section was first published in *AWRI Technical Review* Issue 256, February 2022.

7.4.2.1 Comparing sampling and extraction methods – key findings

For both white and red loads assessed at Site A, the aggregated data presented no clear differences among the three sampling/extraction methods for the final total soluble solids levels, measured in °Brix (Figures 6 and 7, respectively). Assessing individual loads for Site A, for the five white loads the hand-pressed °Brix values were greater than the free-run juice °Brix values four out of five times, with a maximum difference of 0.4 °Brix. For ten red loads assessed at Site A, the hand-pressed juice

had a higher °Brix than the free-run juice sample seven out of ten times, with the maximum difference being 0.9 °Brix.

The assessment at Site B included only loads of a minimum of five bins. For the analysis undertaken, free-run juice sampling tended to result in lower overall total soluble solids (°Brix) levels, with increased extraction through hand-pressing and mechanical screw core sampling tending to yield higher values (Figure 8). An assessment of the pH of samples from each load taken via the differing extraction techniques showed that pH tended to be lower (more acidic) in hand-pressed samples than in free-run or screw core samples (Figure 9).

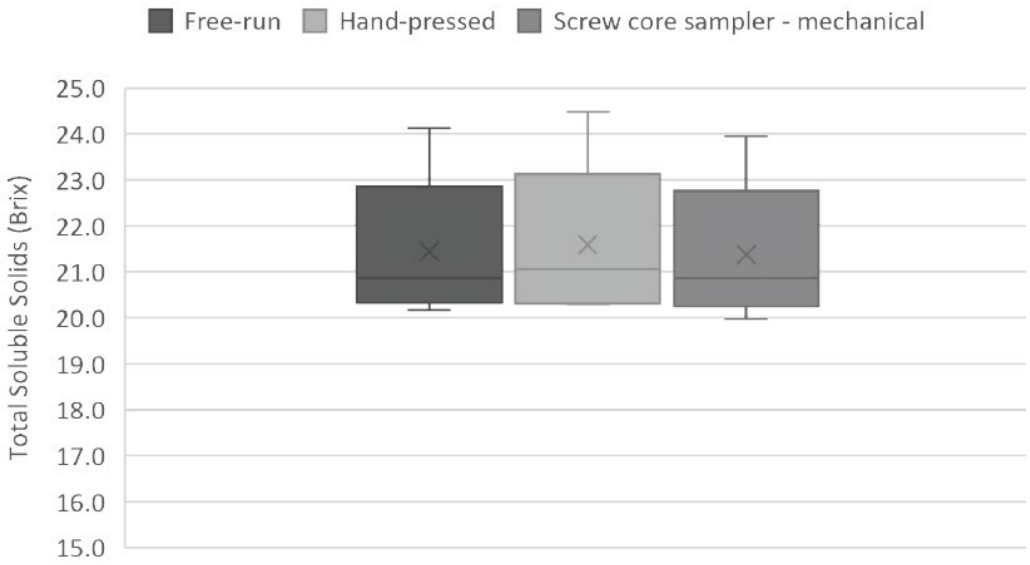


Figure 6. Range of results for total soluble solids (TSS) measured after different sampling/extraction methods for white grapes at Site A. The boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value and the crosses representing the average value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured, excluding outliers, which are represented as dots (first published in *AWRI Technical Review Issue* 256, February 2022).

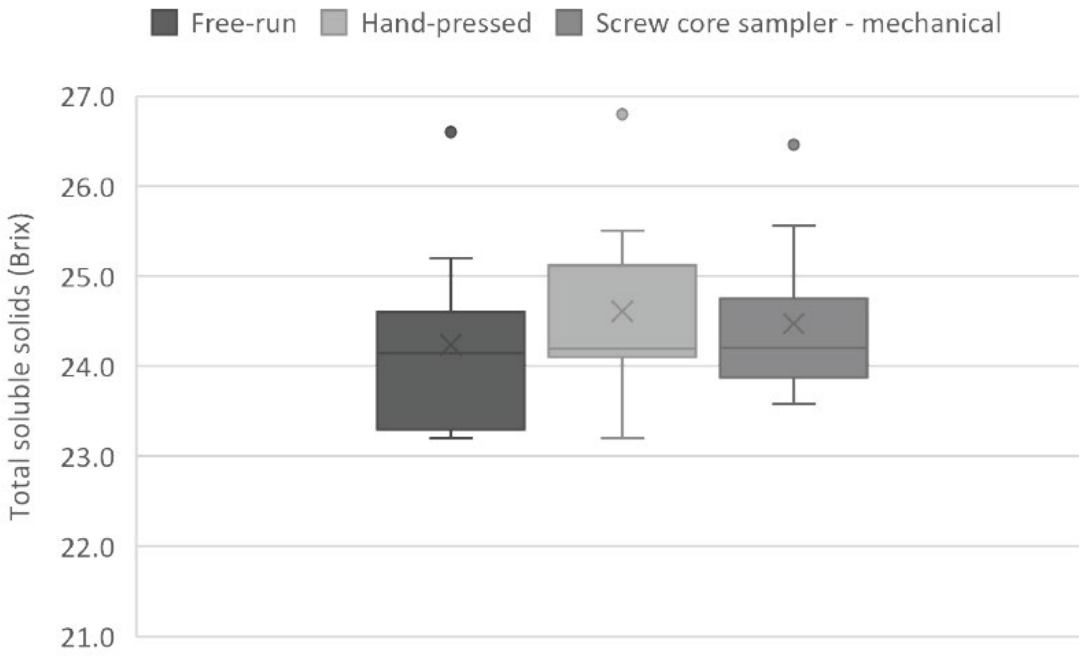


Figure 7. Range of results for total soluble solids (TSS) measured after different extraction methods for red grapes at Site A. The boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value and the crosses representing the average value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured, excluding outliers, which are represented as dots (first published in AWRI Technical Review Issue 256, February 2022).

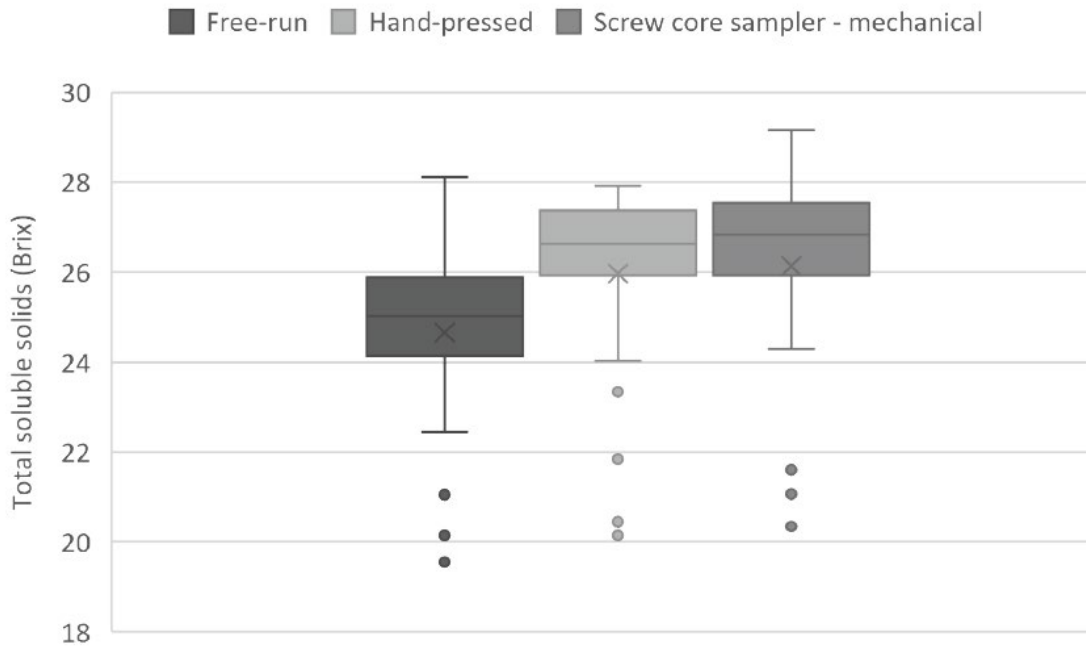


Figure 1. Range of results for total soluble solids (TSS) measured after different sampling/extraction methods at Site B. The boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value and the crosses represent the average value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured excluding outliers, which are represented as dots (first published in *AWRI Technical Review Issue* 256, February 2022).

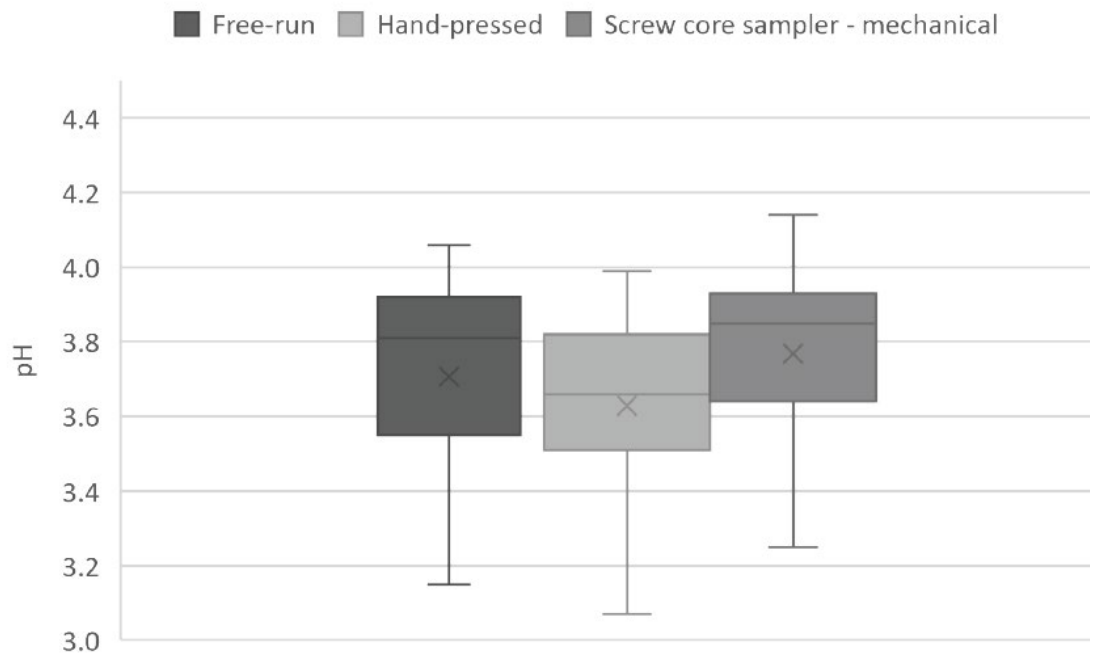


Figure 9. Range of results for pH measured after different extraction methods for Site B. The boxes represent the spread from the first quartile to the third quartile of the data, with the horizontal line within the box representing the median value and the crosses represent the average value. The 'whisker' lines above and/or below each box extend as far as the minimum and maximum values measured excluding outliers (first published in AWRI Technical Review Issue 256, February 2022).

7.4.2.2 Variability of total soluble solids (TSS) results across a load of grapes

Free-run juice samples collected from Site C from individual bins were analysed for TSS across vintages 2019, 2020 and 2021. To understand the naturally variability of TSS (°Brix) across a load of grapes harvested from the same vineyard, data were collected from loads where a minimum of eight bins across the load (most commonly 2.5 t bins) were analysed. The average variation of TSS values across a load was observed to be between 0.7 and 1.0 °Brix, and this was consistent across all three vintages assessed (data not shown).

The variability was found to be similar for both red and white grapes across the three vintages. Variation in TSS measurements across a load was over 3 °Brix in some instances. Further investigation demonstrated that the variation across a load tended to be greater for fruit received from warm inland regions (e.g. Riverland, Murray Darling, data not shown).

7.4.2.3 Conclusions

The results of the experiments undertaken at multiple winery sites demonstrated that free-run juice sampling tended to result in lower overall total soluble solids (°Brix) levels in red grapes compared to hand sampling/pressing and mechanical screw core sampling. Given that most of the red fruit is pressed to some extent, free run juice cannot be considered representative of the load and therefore free run juice sampling is not recommended for use in determining total soluble solids.

In addition, notable variability in total soluble solids measurements was seen between bins from the same load/vineyard, with the average variation across the measurements of 0.7 - 1.0 °Brix. This highlights the importance of combining grape samples from multiple locations across a load to

obtain a representative sample or of averaging results from multiple samples (at least four). Relying on just one or two samples has a very high probability of giving an inaccurate result.

8. Outcome and conclusion

This project achieved its goals in providing clear guidance and references for the measurement of key parameters for wine grapes associated with payment or rejection. A range of guideline documents and robust Industry Endorsed Standard Procedures have been developed, reviewed by industry and posted on industry peak body websites. Experimental work was conducted and published on the impacts of different extraction techniques on key grape parameters and this is now available to industry to provide guidance on best practice. Finally, a review of the latest literature on wine grapes sampling and analysis was completed and will be published in the near future.

The industry now has a baseline set of information to guide best practice in the assessment of grape quality for payment purposes. This will form the basis for ongoing continuous improvement and assessment of new and emerging technologies.

9. Recommendations

Future research and ongoing extension of project outcomes will be strongly guided by industry uptake as signatories to the Code of Practice, and engagement and familiarisation with the Industry Endorsed Standard Procedures.

There will be a requirement to continue to expand upon the literature review, in particularly focusing on assessments where current measures are more subjective in nature. This will be important in continuing to support a fair approach to fruit assessment via objective measures that are applicable to small, medium and large enterprises.

10. Communication

Key web resources

The AGW Code of Conduct for Australian Winegrape Purchases and associated resources are available from this page on the AGW website: https://www.agw.org.au/industry-resources/the-code-of-conduct-for-australian-winegrape-purchases/

The Industry Endorsed Standard Procedures developed through this project are available from: https://www.agw.org.au/industry-resources/winegrape-quality-assessment/industry-endorsed-standard-procedures/

Specific procedure and guideline documents are available at the links below:

- Industry Endorsed Standard Procedure for measurement of Brix or Baume in grape juice
- Industry Endorsed Standard Procedure for determination of total anthocyanins in grapes
- Standard method for determining MOG
- Vineyard sampling guideline document

It is intended that all IESPs, standard procedures and guideline documents developed through this project will be collated on one page the AWRI website.

Presentations

On 8 October 2020, Eric Wilkes was involved in a Virtual Interactive Q&A: The Code of Conduct for Australian Winegrape Purchases. This interactive session hosted by Australian Grape and Wine provided the audience with a chance to learn about the Code of Conduct for Australian Winegrape Purchases (the Code).

On 2 December 2021, Kieran Hirlam presented a webinar to 61 attendees on Reviewing the standard procedures for grape sugar and colour measurement as part of the 2021/22 AWRI webinar program. The webinar recording on YouTube has to date received 410 views. This webinar discussed the industry-endorsed best practice standards for grape sugar and colour measurement, focusing on their adaptability to all Australian grapegrowers and winemakers. The importance of sampling in the vineyard and at the weighbridge to support the quality assessment standards was also covered, to provide a practical guide for implementation of the standards.

Publications

Two technical notes developed as part of this project were published in the October 2021 and February 2022 issues of the *AWRI Technical Review*, respectively:

- Understanding the variability of juice extraction methods for quality analysis**:** *Technical Review* No. 254 October 2021
- Understanding the variability of different sampling/extraction techniques used in grape assessments at the winery**:** *Technical Review* No. 256 February 2022

11. Intellectual property

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12. References

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13. Staff

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14. Other supporting material

14.1 Guideline Document: Determination of Total Anthocyanins in Grapes

The Australian Wine Research Institute

AUSTRALIAN Grape & Wine

Guidelines
Grape Colour

Determination of Total Anthocyanins in Grapes

Scope

This guideline document has been prepared with reference to IESP 2.3 – Determination of Total Anthocyanins in grapes. The scope of this document is to provide an insight into the measurement of colour in grapes, expressed as total anthocyanins. This document will act to highlight important requirements of the reference method and provide details on the validation approach to be undertaken for industry-based methods to act as the method of choice when arbitrating disputes.

Equipment

- UV/Visible spectrophotometer capable of measuring in the range 250-520 nm
- 10 mm path length quartz cuvette
- Fixed volume pipettes (calibrated only) to deliver volumes of 1 mL and 10 mL
- High speed homogenisation unit (e.g. lab grade Retsch Knife Mill Grindomill GM20)
- Centrifuge (maintain speeds ~3,500 rpm)
- Rotating wheel mixer (if available)
- Centrifuge tubes, 10 mL, with caps
- Volumetric flasks of 1 L capacity
- Analytical balance (minimum reading 0.01 g)
- Plastic 250 mL containers with lids
- Kimwipe

Preparing a representative grape sample

A representative grape sample is taken just prior to homogenisation using the following procedure for a series of 40 bunches:

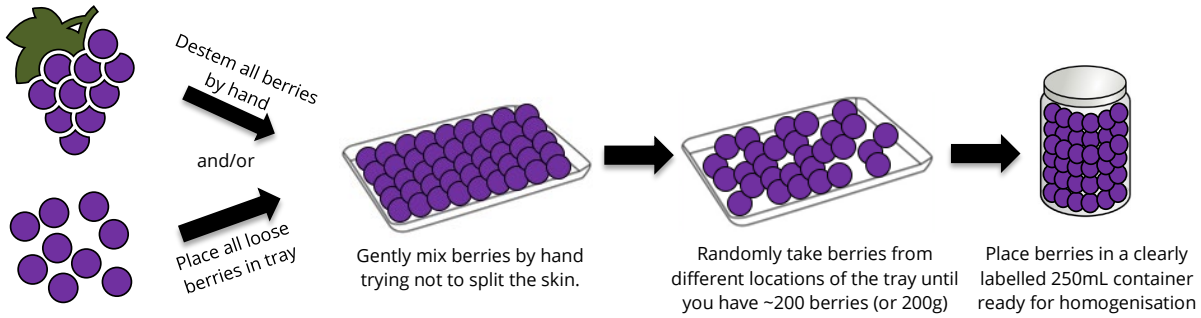


Figure 1: Preparing a representative sample for colour analysis

Measurement Procedure

1. Ensure the UV/Visible spectrophotometer is turned on and is given sufficient time to warm up as per the manufacturers recommendations.

1. Process each berry sample for approximately 120 seconds in the homogenising unit. Ensure that all seeds are thoroughly macerated, and all homogenate is scraped from the homogeniser and collected in the storage container. All samples should be thoroughly remixed by stirring immediately before subsampling to ensure no settling out of material occurs before subsampling.
2. Weigh 1.0 g ± 0.1 g into a 10 mL centrifuge tube and record the weight.
3. Add 10 mL of acidified 50% v/v ethanol/deionised water and cap the tube.
4. Rotate on the rotating wheel mixer for 1 hour or mix by hand every 10 minutes.
5. Centrifuge the homogenate/ethanol mixture at approximately 3,500 rpm for 5 minutes.
6. Pour the supernatant liquid (Figure 2) into an appropriate sized measuring cylinder and measure the volume of the extract to the nearest 0.2 mL. **This will be the final extract volume.**

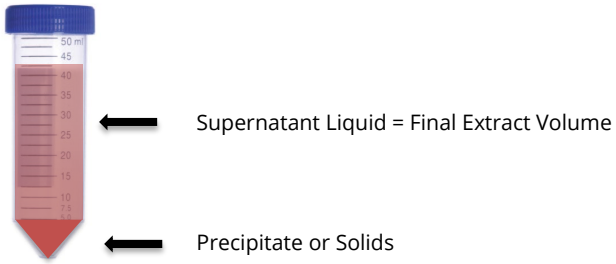


Figure 1: identification of elements post-centrifuging.

7. Add 1 mL of the extract to 10 mL of 1M HCl (total volume will be 11 mL).
8. Incubate for at least 1 hour and no longer than 4 hours in a dark place (e.g. cupboard) at room temperature.
9. Set UV/Visible spectrophotometer for measurement at 520 nm.
10. Zero with 1M HCl in 10 mm quartz cuvette.
11. Measure the absorbance of the extract at 520nm (= A_{520}^{HCl})
12. Use the following calculation to calculate the Total Anthocyanins (mg/g fruit):

Total anthocyanins (mg/g fruit);

$$= \frac{A_{520}^{HCl}}{500} \times \text{dilution factor*} \times \frac{\text{final extract volume (mL)}}{100} \times \frac{1000}{\text{weight of homogenate taken for extraction (g)}}$$

* the dilution factor is the dilution of a portion of the extract into 1M HCl (Note, if 1.0mL of 'the extract' is added into 10mL of 1M HCl, the dilution factor is 11.)

Validation Requirements

The determination of anthocyanins can be significantly impacted by small differences in procedure or equipment. For this reason, it important that, prior to the method being put into use, the laboratory validates its results against an independent source.

If using a variation of the procedure outlined above (but still reliant on the extraction of a homogenised sample followed by UV/Visible spectroscopy) the procedure can be validated as follows:

Validation Procedure

Source ten (10) separate samples of black grapes and repeat the following process for all samples (sets):

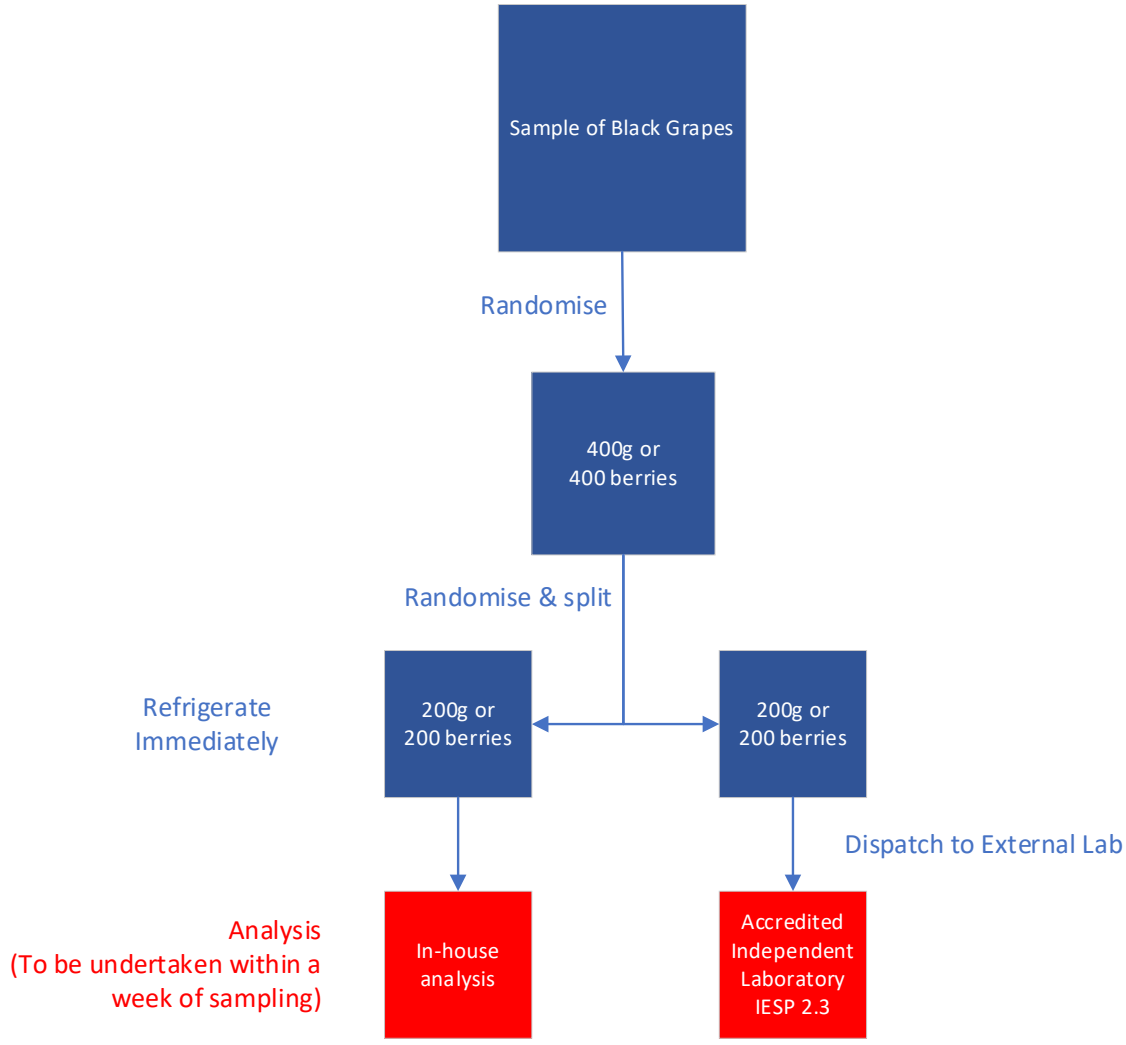


Figure 1: Validation procedure

For each sample, calculate the difference in result from the in-house analysis and the accredited independent laboratory and average the differences:

$$\{(Sample\ 1_{In-house} - Sample\ 1_{Accredited}) + \cdots + (Sample\ 10_{In-house} - Sample\ 1_{0Accredited})\}/_{10}$$

- **The average should not vary by 10% of the mean of the sample results from the source laboratory.**

- At the beginning of each season this procedure should be repeated to ensure no changes have been introduced that may affect the results.
- Document all results from the process in an appendix to the procedure validation.

If the in-house methodology for the determination of anthocyanins uses a different technology such as NIR spectroscopy, then a much more rigorous validation procedure is required as set out in a separate procedure (ref. IESP4.0, Development and validation of secondary analytical methods for the determination of grape parameters)

Acknowledgement

This document was prepared with the guidance and support of Australian Grape and Wine (AGW) and representative industry bodies.

References and further reading

Refer to IESP 2.3 – Determination of Total Anthocyanins in Grapes for detailed references.

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14.2 Guideline Document: Standard Method for the Measurement of °Brix (or °Baumé) in Grape Juice

The Australian Wine Research Institute

AUSTRALIAN Grape & Wine

Guidelines
Total Dissolved Solids

Standard Method for the Measurement of °Brix (or Baumé) in Grape Juice

Scope

This guideline document has been prepared with reference to IESP 1.3 – Standard Method for the Measurement of °Brix (or °Baume) in Grape Juice and must. The scope of this document is to provide an insight into the standardised approach developed for the measurement of °Brix (or Baumé) in grape juice, specifically to address situations where °Brix (or Baumé) measurement is used as part of the process for setting payment or rejecting grapes.

Equipment

- Calibrated refractometer.
- Calibrated thermometer.
- 50mL plastic sample tubes for juice settling.
- Snap-lock sandwich bags for crushing grape samples.
- Lint free tissue (kimwipes or equivalent).
- Plastic Pasteur pipettes.
- Centrifuge (maintain speeds ~3,500 rpm)
- 50 ml centrifuge tubes (if required).

Measurement Procedure

Sample Preparation

If using berries, either from a bunch or individual berries, a representative grape sample is taken using the following procedure for a series of 20 bunches:

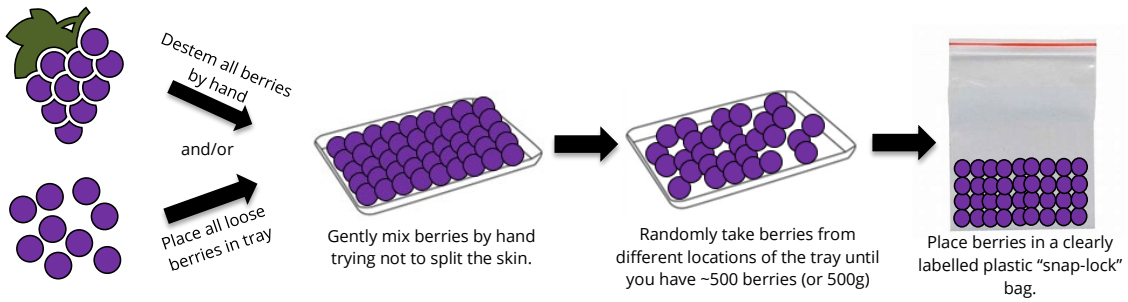
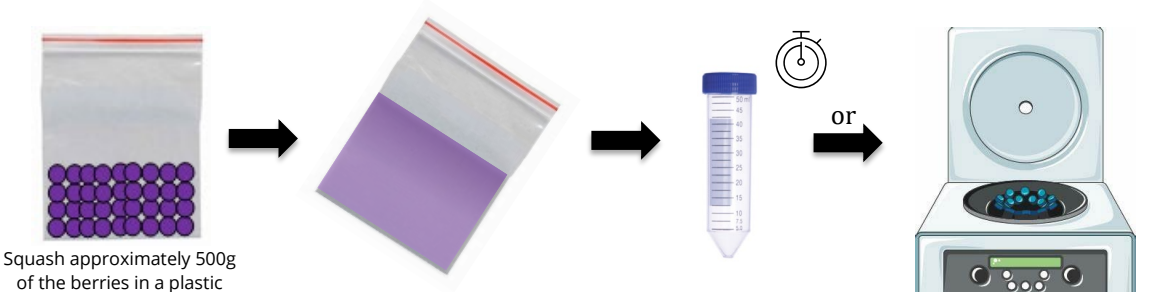


Figure 1: Preparing a representative sample for Brix analysis

Once a representative sample is obtained, juice extraction can commence:



Squash approximately 500g of the berries in a plastic 'snap-lock" bag. Ensure all berries have been crushed.

Decant 100ml of juice to an appropriate plastic tube

Allow solids to settle with time (~ 60minutes) and analyse juice, **or**

Centrifuge samples (~3500rpm for 5min) and analyse juice.

Figure 1: Juice extraction process for Brix analysis

If a sample is to be analysed from a winery tank, ensure the tank is agitated and solids allowed to settle before sampling.

For all analysis, samples should be relatively clear at the time of measurement and as close to 20°C as possible.

It is suggested that all samples (including solutions and reagents) be brought as close to 20°C as possible prior to analysis.

Analysis

Benchtop or Digital refractometers

The ºBrix or Baumé can typically be read directly from a digital refractometer. Follow the instrument manual for detailed instruction on use.



Figure 2: Digital refractometer example

A typical procedure is outlined below.

1. Turn on the instrument and allow the startup procedure to complete.
2. Ensure the correct method (either ºBrix or Baumé) is selected.
3. Clean the sample cell by rinsing with distilled or deionised water and drying with a lint free tissue or clean cloth.
4. Perform a zero check by covering the sample cell with distilled or Milli-Q water and running a test.
5. This result must be 0.0 ± 0.2 units.

1. If a value of 0.0 ± 0.2 units is not achieved, wipe the water off the cell and repeat. If not achieved after the 2nd attempt, re-zero the instrument.
2. Once achieved, ensure the cell is clean and dry.
3. Measure the temperature of your sample with the calibrated thermometer and take note.
4. Place approximately 0.3 mL of sample juice on the cell and run a test.
5. The result will be displayed.
6. If necessary correct for sample temperature using the table in section 13 of IESP 1.3 (check the instrument manual).
7. Once all analysis is complete, clean the sample cell with distilled or Milli-Q water and dry with a lint free tissue or clean cloth.
8. Repeat from step 8 for the next sample.
9. If no further samples are to be analysed, turn off the Refractometer.

Handheld or Abbe refractometers



Figure 1: Handheld refractometer example

1. Look into the eyepiece to ensure the scale appears clearly.
2. Open the prism.
3. Carefully clean the outer surface with a lint free tissue or clean cloth.
4. Perform a zero check by placing a few drops of distilled water onto the prism.
5. Close the prism and look into the eyepiece against a natural light source.
6. Take reading at the point where the contrast line (difference between light and dark) crosses the scale.
7. This indicates the concentration of sugar in the liquid.
8. This result must be 0.0 ± 0.2 units.
9. If a value of 0.0 ± 0.2 units is not achieved, wipe the water off the prism and repeat. If not achieved after the 2nd attempt, contact instrument supplier.
10. Once achieved, ensure the cell is clean and dry.
11. Repeat the steps above with your test sample.
12. Take reading at the point where the contrast line (difference between light and dark) crosses the scale.
13. Measure the temperature of your sample with the calibrated thermometer and take note.

1. If necessary correct for sample temperature using the table in section 13 of IESP 1.3 (check the instrument manual).
2. Once all analysis is complete, clean the prism with distilled or deionised water and dry with a lint free tissue or clean cloth.

Validation Requirements

The minimum validation requirement required to use this method (or a substantially similar method as already used in the facility) is that prior to the method being put into use, the laboratory validates its results against an independent source.

Validation Procedure

Source ten (10) separate samples of grapes and repeat the following process for all samples (sets):

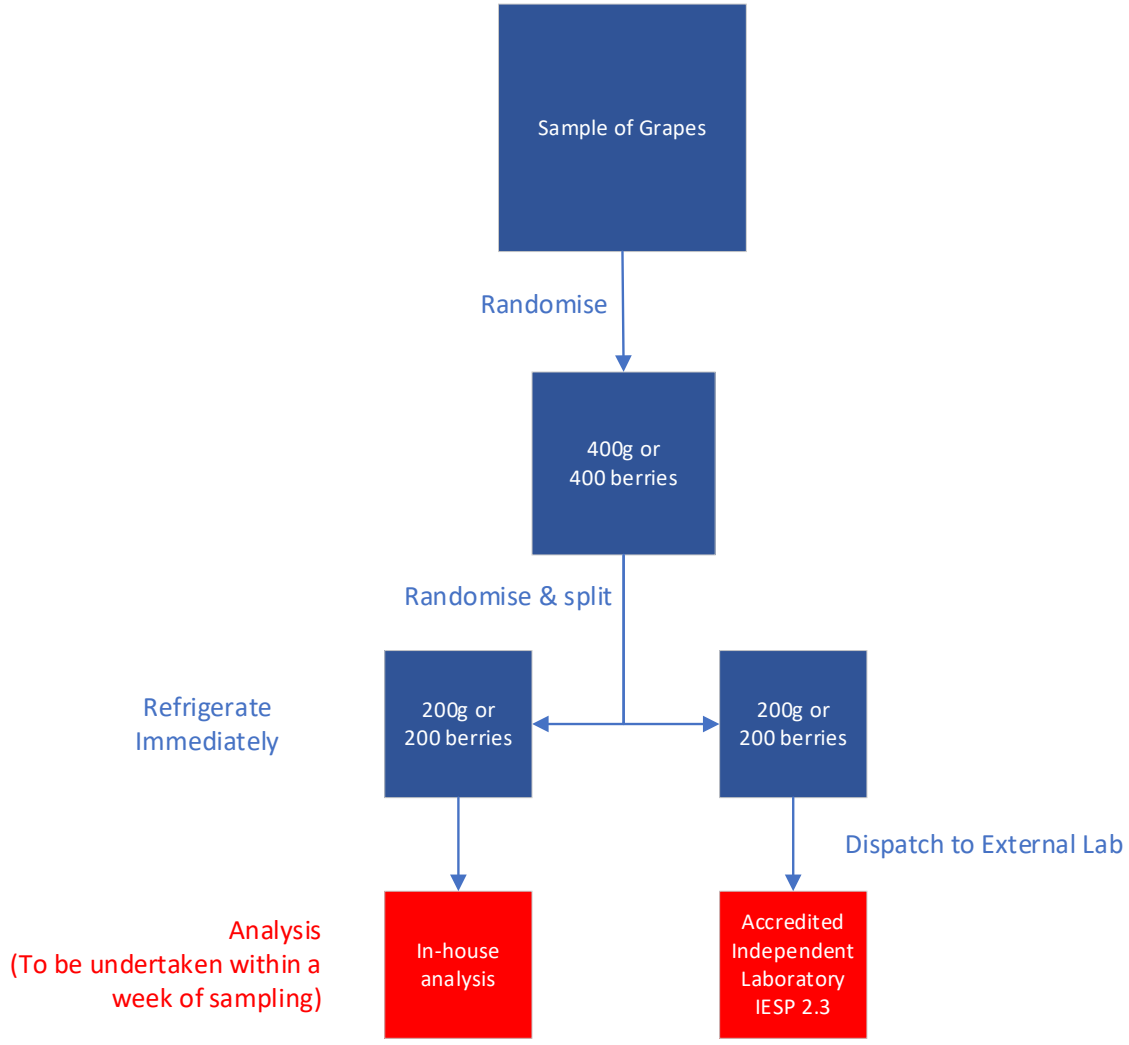


Figure 1: Validation procedure

For each sample, calculate the difference in result from the in-house analysis and the accredited independent laboratory and average the differences:

$$\{(Sample\ 1_{In-house} - Sample\ 1_{Accredited}) + \cdots + (Sample\ 10_{In-house} - Sample\ 1_{0Accredited})\}/_{10}$$

- **The average should not vary by 10% of the mean of the sample results from the source laboratory.**
- **No individual result should vary by more than 15% from the independent laboratory result for the procedure to be considered valid.**

- At the beginning of each season this procedure should be repeated to ensure no changes have been introduced that may affect the results.
- Document all results from the process in an appendix to the procedure validation.

If the facility wishes to independently validate their method or use a technology significantly different to that outlined in this standard procedure, a much more rigorous validation procedure is required as set out in a separate procedure (ref. IESP4.0, Development and validation of secondary analytical methods for the determination of grape parameters).

Acknowledgement

This document was prepared with the guidance and support of Australian Grape and Wine (AGW) and representative industry bodies.

References and further reading

Refer to IESP 1.3 - Standard Method for the Measurement of °Brix (or °Baume) in Grape Juice and must for detailed references.

Contact

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14.3 Guideline Document: Standard Method for Sampling Grapes in the Vineyard for Assessing Berry Composition at Harvest

Standard Method for Sampling Grapes in the Vineyard for Assessing Berry Composition at Harvest

Scope

This guideline document has been prepared with reference to IESP 5.0 – Standard Method for Sampling Grapes in the Vineyard for Assessing Berry Composition at Harvest. The scope of this document is to provide an insight into the standardised approach developed for sampling grapes in the vineyard for assessing berry composition at harvest for the determination of payment.

Principals of achieving a good representative sample

The basic principles of achieving a good representative sample include:

- Sampling from multiple locations within a defined vineyard (block, patch, section, harvest unit or row).
- Incorporating pooled (consolidated) grape samples to ensure that analysis is representative of a defined vineyard block, patch, section, harvest unit or row.
- Incorporating enough grapes to ensure that at least minimum sample size requirements are met for specific grape analysis.
- Random sampling thus negating bias.

Terminology

A vineyard 'block' (path, section, harvest unit or row) might represent a single variety, a particular soil type, or topographic feature that separates the area from the remaining part(s) of the blocks that create the entire vineyard planting.

A harvest unit is defined as a patch of grapes which may be harvested in the same day, which again may differ from the total vineyard area.

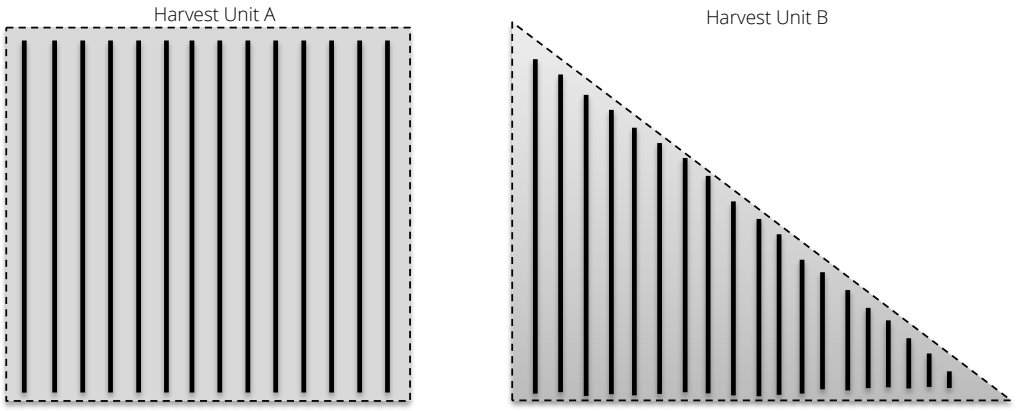


Figure 1: Harvest units are a series of vineyard rows with a defined system boundary. Harvest units can take different shapes and forms as identified above in Harvest Unit A and Harvest Unit B.

Pre-Sampling Considerations

Key sampling principals are defined below:

- Sampling at harvest should be conducted through **whole bunch/cluster sampling**.
- Sub-blocks to be harvested separately should be identified prior to entering the vineyard.
- Samples at the end of the row, or at rows edge are generally not representative of the Harvest Unit.
- Aerial images of vineyards can be useful when designing a spatially representative grape sampling program.
- Samples should be collected as close to harvest as possible. **Sampling within 2 days (48 hours) before harvest is preferable**, however within 3 days (72 hours) is acceptable.
- Sampling should be delayed if there has been recent rain, heavy fog, or dew, or heavy irrigation within the proceeding 24 hours.
- **Prior to entering the vineyard**, establish a system for evenly distributed sampling, selecting a row and vine within a harvest unit/block/patch and develop a system for recording this position as to easily identify the location when in the vineyard. Weighting row lengths for irregular shaped harvest units could useful.

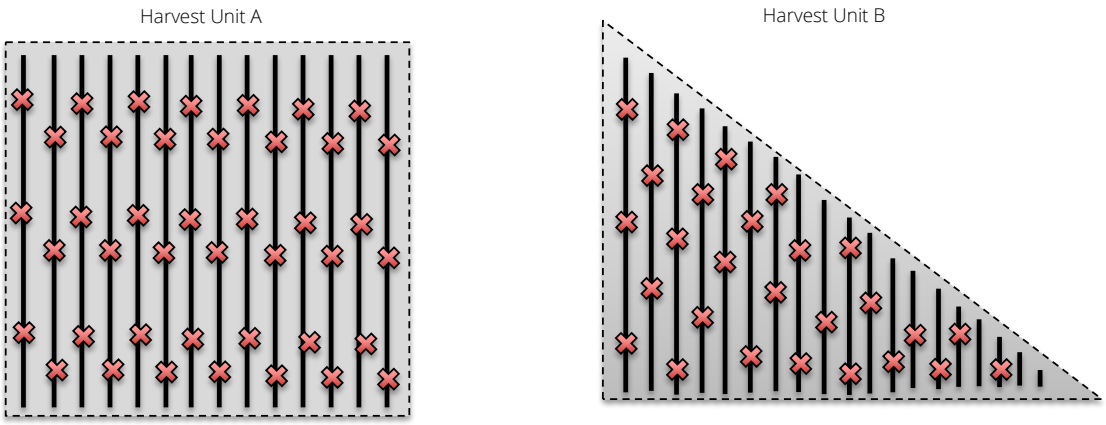


Figure 2: Establish a system for evenly distributed sampling within a harvest unit prior to entering the vineyard.

Sampling

20 whole bunch samples per harvest unit or patch are required for Brix° and pH analysis.

40 whole bunch samples per harvest unit or patch are required for Brix°, pH, colour (anthocyanin mg/g) and titratable acidity (TA - g/L tartaric acid equivalents) measurement.

In the vineyard, when sampling, see details in Figure 2 for each sampling point:

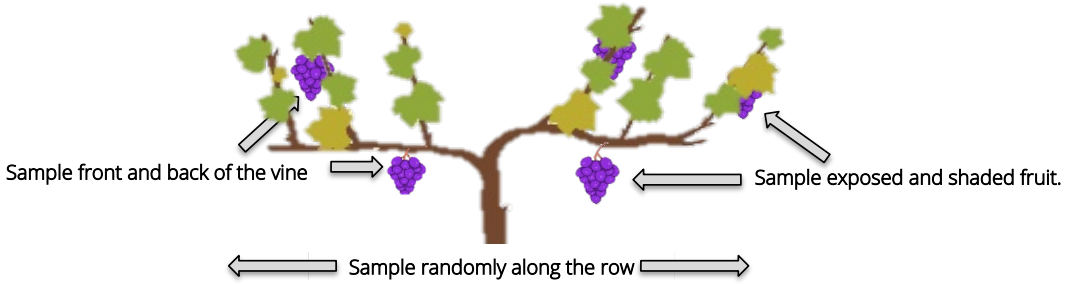


Figure 3: Principals for sampling at an individual point of a Harvest Unit

- Samples should be kept at a constant cool temperature below 10°C (i.e., in an esky) and clearly labelled with the date, variety, vineyard identification (including block/patch/row if necessary) on the container.
- Samples should be stored at less than 15°C and preferably between 5-10°C prior to and during transport and up until the commencement of processing for analysis.
- Samples collected in the morning period should ideally be processed for compositional analysis later that same day within the laboratory. If samples cannot be processed on the same day as collection, samples should be stored at 1 - 4 °C until the following day for processing.

Analysis

Samples should not be processed after more than 2 days since the period of sampling.

At the point of quality measurement, to eliminate bias from the sample, when selecting fruit for measurement, most or all berries are to be removed from each of the bunches and then mixed before randomly selecting a subset for processing.

Acknowledgement

This document was prepared with the guidance and support of Australian Grape and Wine (AGW) and representative industry bodies.

References and further reading

Refer to IESP 5.0 - Standard method for Sampling Grapes in the Vineyard for Assessing Berry Composition at Harvest for detailed references.

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