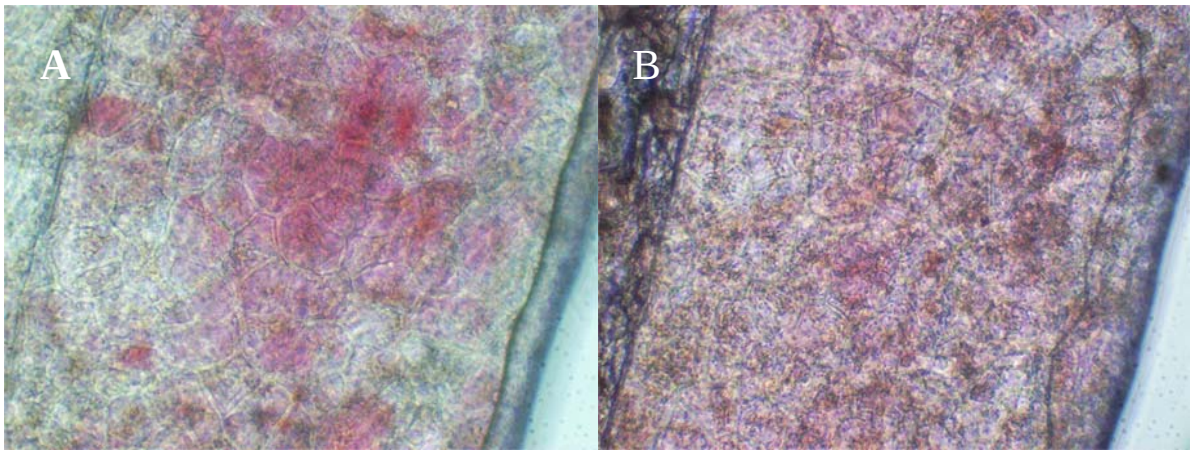
THE UNIVERSITY OF ADELAIDE AUSTRALIA

Australian Government
Grape and Wine Research and Development Corporation

Development and validation of protocols for small-scale fermentation using both fresh and snap frozen grapes



Cross section (x200) of fresh grape berry (A) and same section after freezing at -18°C for 1h (B) showing disruption of cell wall and de-localisation of pigments.

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **UA 06/01**

Principal Investigator: **Dr Vladimir Jiranek**

Research Organisation: **University of Adelaide**

Date: **7 March 2008**

Development and validation of protocols for small-scale fermentation using both fresh and snap frozen grapes

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Abbreviations

HPLC – High performance liquid chromatography

MSFF – Multi-Scale Fermentation Facility

SEM – Standard error of mean

Abstract:

This report details the outputs of a one year research project which examined the validity of using small scale fermentations (100 kg) as a means of representing larger industrial-scale fermentations. Also, in an attempt to validate the use of frozen grapes to allow research fermentations to be conducted outside of vintage, freezing treatments (conventional and snap-freezing) were applied to whole bunches before grapes were thawed, processed and made into wine as per fresh grapes. The outcomes of this study indicate that small-scale fermentations performed as described herein are a reliable representation of those performed on a larger scale, and that wine produced from snap-frozen rather than conventionally frozen whole bunches was more reminiscent of that made from fresh grapes.

Executive summary:

- Custom-built small-scale fermentors (100 kg) were used to conduct white juice fermentations and revealed high reproducibility of fermentation kinetics as well as the profile of major wine components. This work contributes to the validation process of the recently installed stainless steel, 100 L small-scale fermentors.
- Fermentation of fresh red grapes also showed high reproducibility between replicates in terms of fermentation kinetics, wine composition and colour attributes.
- Red wines were also made at the 500 kg scale using the same grapes as in the 100 kg trial (conducted in 250 L drums). Good correlation was seen in either case in terms of fermentation kinetics, wine composition and colour attributes.
- Methods for preserving grapes by freezing included conventional freezing of whole red grape bunches at -20°C and snap-freezing of whole bunches using a 'blast freezer' (large currents of -20°C air) at a commercial facility. Freezing times were significantly reducing for the latter.
- Wines were then made from grapes subjected to each freezing treatment and compared to the wines made earlier from a batch of the same fresh grapes.
- Both freezing treatments produced wines with similar chemical and colour profiles. No significant differences between any treatment were detected in terms of anthocyanin concentrations. However, some colour differences were apparent, most likely due to differences in final pH values of the wines.
- Difference testing (duo trio) of wines from all treatments revealed that panel members could not distinguish between wines made from fresh grapes at the 500 kg scale and wines made from snap frozen grapes at the 100 kg scale.
- This study indicates that wines made by the described small-scale protocol are representative of those generated on the small commercial (500 kg) scale. As such the use of former will reduce the amount of grapes required and hence the cost of research winemaking without compromising on perceivable wine composition/quality.
- Snap-freezing of grapes appears to be a better method for preservation of whole grapes given that it allows production of wines comparable to those producing from fresh grapes.

Background:

The proof of the benefits of any outcome of winemaking or viticultural research is in the resulting wine. However, in making these test wines as proof-of-concept, financial and practical considerations often dictate that small-lot fermentations are carried out before the commitment to assessments on a commercial scale. The approach to such scaled-down trials can be highly variable and the accuracy with which they model the industrial situation is rarely defined. A further limitation of any trials, particularly of oenological research, is that they are restricted to vintage when grapes are available.

Until now Australia has lacked a research facility that has the potential to accurately model wine fermentations from the laboratory (mL) right through to pilot scale (kL). The Multi-Scale Fermentation Facility (MSFF) was set up to fill this critical shortfall in capacity, scale, and poor degree of control, and to provide a vertically integrated and partially automated facility that can service both white and red fermentations. Design and construction of this facility began in early 2005, with the expansion of the rotary fermentors (~ 700 L) in the Hickinbotham Roseworthy Wine Science Laboratory. The development and manufacture of the 100 L scale and the 100 mL – 3 L scale was completed more recently with validation being undertaken (in part through this project) during 2007.

The 100 L vessels have being developed specifically to provide a small and cost effective representation of the red wine fermentation, but one that yields sufficient wine for sensory trials and/or matches the size of early phase viticultural trials (i.e. 30 - 80 kg of grapes), and has a standardised, automated system for cap management. Initial trials of these vessels have already been conducted with our collaborators at Provisor.

To take full advantage of this equipment, and make it available to research and industry outside of vintage, this project also explored the implications of using frozen grapes. Some studies have investigated quality changes of berries/must after frozen storage and have shown few adverse affects (Spayd *et. al*., 1987). To investigate this under Australian conditions, we conducted preliminary trails in the 2005 vintage and obtained highly promising findings (Schmid *et. al*., 2007). The suggestion being that frozen fruit provided a reasonable approximation of fresh grapes in certain respects. Traditional freezing (i.e. -18°C environment), however, required hours to days to be completed. Such a slow rate of freezing is clearly less than desirable, as it provides opportunity for continued change in berry composition. It was hypothesized that snap-freezing would be superior, but no in-depth study

has been performed on this to date. We therefore sought to expand and extend our earlier work by comparing the fermentation characteristics of snap-frozen *vs* traditionally or 'slow'-frozen grapes with fresh grapes. Therefore the aims of the project were:

1. To determine the extent of agreement between the various (~ 100 L *vs* the 700 L) fermentation configurations of the Multi-Scale Fermentation Facility (MSFF) with regard to wine making outcome.

2. Use the MSFF to develop a set of application-ready procedures for modelling wine fermentations.

3. Evaluate the acceptability of using slow-frozen and snap-frozen berries compared with fresh grapes.

4. Define an optimal procedure for conducting fermentation trials outside of vintage through use of frozen grapes.

Project Aims and Performance targets:

Outputs	**Performance Targets**
1. Information on the similarity between 100 and 700 L ferments in terms of fermentation kinetics and composition.	Fermentation data and wine compositional information on at least one set of triplicated ferments performed on the 100 and 700 L scales.
2. Protocol for conducting small-lot winemaking trials with fresh grapes.	Vessel design and operational procedures for generating winemaking outcomes which resemble that achieved with larger (industry) scale systems.
3. Information on the relative impact of freezing methods on grape structural and compositional integrity and subsequent winemaking outcome.	Data on the influence of freezing method on key parameters of fermentation (i.e. rate) and wine composition (i.e. colour and metabolite profile).
4. Protocol for conducting small-lot winemaking trials with frozen grapes.	Frozen grape processing and fermentor operational procedures which yield winemaking outcomes that resemble that achieved with larger scale systems and/or fresh fruit
5. Dissemination of project findings.	At least one conference presentation or publication detailing the findings and resulting best-practice methods for conduct of small-lot wine making trials, in or out of vintage.

Methods:

Processing of grapes

Semillon grapes (Waite vineyard, 2007) were hand picked, and pressed using a Willmes VP1800 press (2 presses at 1 bar, 2 presses at 2 bar, with 2 crumples between presses). The juice was sulphured (75 ppm), pumped into a 1000 L tank, and subsequently frozen at -20°C. When required the juice was thawed at room temperature for 4 days, thoroughly mixed by hand and 3 x 70 L aliquots removed for fermentation.

Merlot grapes (chosen due to availability) were hand harvested in early April 2007 from a vineyard near Woodside, Adelaide Hills, South Australia. Grapes were randomly split into three lots on a randomised, bunch-by-bunch basis. One lot was used fresh (3 x 500 kg and 3 x 100 kg), while the others were snap- or slow-frozen (each 3 x 100 kg). The grapes for the fresh trial were immediately crushed/de-stemmed and pumped directly into the respective fermentors (see below). A Gamma 50CE-RM (Toscana Enologica Mori, Italy) crusher/de-stemmer was used for the 500 kg batches, and an Enoitalia ENO-15 (Winery Supplies, Australia) unit was used for the 100 kg batches. Grapes for slow-freezing were placed into a -20°C cold room in sealed storage containers. Grapes for snap-freezing were transferred into lidded styrofoam boxes (25 kg) and transported to a commercial blast-freezing facility (Swire Cold Storage, Mile End, South Australia), where the boxes were opened and the grapes subjected to large volumes of cold air (-25°C) for 24 h, before being close and stored at -20°C until required. Thawing of frozen grapes before crushing occurred at 4°C to minimise microbial spoilage.

All fermentations were carried out following the standard wine making practices used in the Hickinbotham Roseworthy Wine Science Laboratory. A standard addition of diammonium phosphate (150 mg/L) was made to the juice/musts before inoculation. Dry yeast (PDM, Maurivin), used at a rate of 20 g/hL, was reconstituted and added to the must following the manufacturers instructions. Rotary fermentors (900 L capacity, A & G Industries, Australia; see Day, 2003) were used to ferment the 500 kg lots, and lidded stainless steel containers were used to ferment the 100 kg lots and the 70 litres of white juice. In the case of the red grape fermentations, 250 L drums were used, whereas custom-built (A & G Industries, Australia), 100 L, jacketed containers with agitation were used for the white grape juice fermentations. All ferments were conducted at 15°C. Samples were taken from each of the smaller fermentations after the cap was manually plunged (every 12 hrs; 15 times total). Rotary fermentors were turned 3 times clockwise and 3 times counter-clockwise every 12 h. Once

below 1° Bé the wines were pressed. The 500 kg batches were pressed using a Willmes VP1800 press (2 presses at 1 bar, 2 presses at 2 bar, with 2 crumples between presses), into 500 L holding tanks and kept at 20°C. The 100 kg batches were pressed using a bladder press (Diemme 130 L Laboratory Press, JB Macmahon Pty. Ltd. Australia) operated using the following protocol; 0.2, 0.4, 0.6, 0.8 and 1 bar each held for 5 minutes with 2 crumples between presses. Wine was transferred to 85 L kegs and stored at 20°C. Each wine was inoculated with *Oenococcus oeni* VP41 LAB (Lalvin, Australia) at a rate of 1 g/hL as per manufacturers instructions and underwent malolactic fermentation (residual malic acid concentration > 0.05 g/L by enzymatic test kit, Roche Cat No. 10139068035).

Chemical analysis by HPLC

Organic acids (citric, malic, tartaric, succinic, lactic and acetic), glycerol, acetaldehyde and ethanol were determined in samples taken at the end of fermentation, which for the white wines was at the end of primary fermentation and after malolactic fermentation for the red wines. Samples were analysed using a Shimadzu LC-10AT*VP* Ion Chromatograph system. Undiluted samples were automatically injected onto an Aminex H7X-8H column (300 x 7.8 mm, BioRad). A mobile phase of aqueous 2.5 mM H_2SO_4 was used at a flow rate of 0.5 mL/min and a column temperature of 60 °C. Organic acids were detected at a wavelength of 210 nm (Shimadzu SPD-10A*VP* UV-Vis detector), and other compounds detected using a Shimadzu RID-10A Differential Refractive Index detector. All compounds were identified by their retention time and quantified by comparison with the peak area calculated for respective standards of known concentration.

Colour analysis by spectrophotometric methods

Wine samples were analyzed for density, hue, SO_2-resistant pigments, total anthocyanins and total phenolics following the method of (Iland *et al.*, 2000), which was modified for use with 96-well UV-transparent microtitre plates. Plates were read at various wavelengths using a Quant Microplate spectrophotometer (Bio-Tek Instruments). Samples (50 µL) for total anthocyanin and total phenolic determinations were added to 1 M HCl (5 mL) and incubated for a minimum of 3 h at room temperature before aliquots (300 µL) were transferred to 96-well microtitre plates and read at 520 nm (total anthocyanins) and 280 nm (total phenolics). Density and hue was calculated from absorbance values of neat wine (150 µL aliquots in 96-well microtitre plates) read at 420 and 520 nm. SO_2 resistant pigments were estimated by adding 15 µL SO_2 solution (25% w/v) to 1 ml of wine, mixing, transferring 150 µL aliquots to 96-well microtitre plates and reading at 520 nm. Additionally, wine colour was also expressed

as CIE 1976 (L, a*, b*) color space values. Six absorbance values (450, 520, 570 and 630 nm) were measured for each wine sample and the corresponding L.a*.b*. values calculated using the software program MSCV (http://www.unirioja.es/dptos/dq/fa/color/color.html). This program was reported to give a greater than 98% confidence level for estimating the values of L, a*, and b* in red wines (Pérez-Caballero *et al*., 2003). The three coordinates, as defined by the Commission Internationale d'Eclairage, describe colour in terms of lightness (where L* values of 0 correspond to black and L* values of 100 correspond to white), red/green (where negative a* values represent green, positive values indicate red/magenta) and blue/yellow (where negative b* values indicate blue, positive values indicate yellow).

Colour compound analysis by HPLC

Anthocyanins in wine samples were separated and quantified by linear gradient elution using an Agilent 1100 HPLC system (Agilent Technologies) fitted with a Phenomonex Synergi Hydro-RP column (150 x 2 mm). Solvent A consisted of 1% acetonitrile and 1.5% phosphoric acid in water. Solvent B was made up of 20% solvent A and 80% acetonitrile. Samples (20 µL) were injected onto the column and eluted at 0.4 mL/min using the following linear gradient: 0 min (14.5% B), 18 min (27.5% B), 20 min (27.5% B), 21 min (50.5% B), 22 min (50.5% B), 26 min (100% B), and 28 min (100% B). A photodiode array detector (520 nm) was used to detect the anthocyanin compounds (Wu & Prior 2005) and quantified as malvidin-3-glucoside equivalents. Individual compounds were identified from their elution time with respect to malvidin-3-glucoside and their UV–Vis spectra (scanning from 220 to 700 nm), which were recorded for all peaks. This protocol was kindly supplied by the AWRI.

Sensory analysis

Difference testing (duo trio analysis) for colour and aroma was firstly performed on the replicates of each treatment to determine variation, if any, between treatment replicates. Once this was established (see results) duo trio analysis was then used to compare random selected wines from each treatment against each other. Wines (30 mL) were presented in random order against the reference in coded ISO standard tasting glasses and assessed at room temperature. Each participant was asked to pick the wine different to the reference, and saw each wine at least twice over two sessions. Significant differences were calculated at $\alpha = 0.05$ using the critical number of correct responses for a duo trio test (Lawless & Heymann, 1999)

Statistical analysis

One-way ANOVA with Tukey's Multiple comparison post test was performed using GraphPad Prism v. 5.00 (GraphPad Software, San Diego California USA). A significance level of $p = 0.05$ was used throughout.

Results and Discussion:

Reproducibility trial of 100 L fermentors

This work contributes to Outputs 1, 2 and 4. For the validation, and hence suitability of the newly developed 100L stainless steel fermentors, triplicate ferments were conducted using Semillon grape juice. Ferments were monitored by refractive index (data not shown) and deemed finished once sugar concentrations were below 2.5g/L (as determined by Clinitest). The average total sugar (glucose and fructose) utilization curve of the three ferments (Figure 1) shows excellent reproducibility. Furthermore, HPLC analysis of significant organic acids, glycerol, acetaldehyde and ethanol also show good correlation between the replicates, with only replicate 1 having a higher acetic acid concentration than the other two replicates. The robustness of the data generated by these fermentations suggests that these 100L fermentors are ideal for small volume research fermentations.

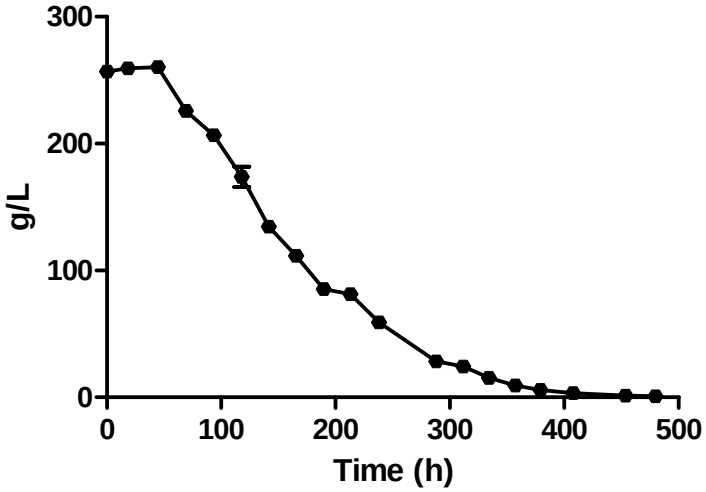


Figure 1. Average total sugar (glucose and fructose) utilization curve of triplicate Semillon juice ferments. Error bars (not always visible) represent the standard error of the mean.

Comparison of small scale (100 kg) and large scale (500 kg) fermentations

This work contributes to Outputs 1, 2, 3 and 4. The previous section demonstrated the suitability of using the 100 kg fermentors for small lot wine making. However, no correlation

Table 1. Chemical analysis of replicated Semillon white juice fermentation end samples.

Analyte	Rep 1	Rep 2	Rep 3	Mean	SEM
Citric acid	1.00	0.99	0.97	0.99	0.008
Tartaric acid	0.73	0.78	1.00	0.83	0.082
Malic acid	0.98	0.97	0.96	0.97	0.005
Succinic acid	10.24	10.05	9.93	10.07	0.091
Lactic acid	2.21	1.97	2.03	2.07	0.072
Glycerol	9.99	9.97	9.17	9.71	0.269
Acetic acid	1.18	0.06	0.06	0.43	0.375
Acetaldehyde	0.08	0.08	0.08	0.08	0.002
Ethanol	96.10	94.69	95.86	95.55	0.435

SEM – standard error of mean

exists between this scale and the more accepted industrially representative scale of 500 kg (Eglinton and Henschke, 2003; Bartowsky *et al*., 2005; Birse *et al*., 2005; Dillon *et al*., 2005; Cozzolino *et al*., 2006), therefore there is a degree of uncertainty to which the developed protocol and fermentation systems will model larger ferments. However, knowing the degree of agreement between the two scales is very valuable in interpreting findings from future trials conducted with these systems. To get a better understanding of the relationship between these two scales, Merlot grapes were used to compare fermentation rates, major metabolites (as shown in Table 1), and wine colour profiles (spectrophotometric and by HPLC).

Fermentation progress of the 500 kg and 100 kg fresh musts show virtually identical fermentation rates, with both fermenting to dryness after 163 h (Figure 2). There was also a high degree of reproducibility within a treatment as indicated by the small associated errors.

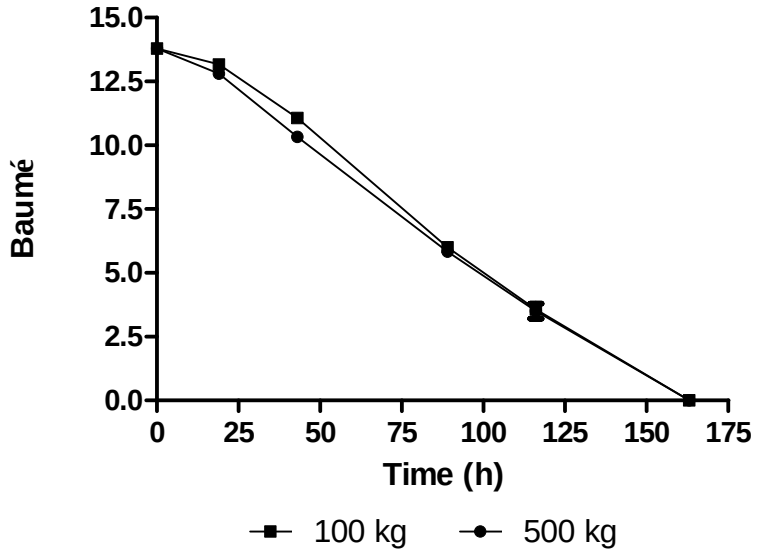


Figure 2. Comparison of Baumé measurements taken throughout fermentation of 500 and 100 kg aliquots of fresh grape musts. Values are averages of triplicate fermentations and error bars (at time too small to see) represent standard error of mean.

Chemical analysis of these wines (Table 2) showed differences which are only statistically significant in the case of the concentration of citric acid. Nevertheless, such differences are relatively small and are unlikely to affect the final sensory quality of the research wine.

Table 2. Chemical analysis of fresh 500 kg and fresh 100 kg Merlot wine end samples. Values shown are averages of triplicate ferments.

Analyte	**Fresh 500 kg**		**Fresh 100 kg**	
	Mean	**SEM**	**Mean**	**SEM**
Citric acid	7.3*	0.05	6.6*	0.09
Tartaric acid	5.9	0.03	5.2	0.18
Succinic acid	18.3	0.11	18.8	0.70
Lactic acid	10.5	0.08	10.9	0.77
Glycerol	14.8	0.91	14.3	0.19
Acetic acid	3.0	0.06	4.5	0.88
Acetaldehyde	0.05	0.003	0.07	0.003
Ethanol	90.0	0.77	88.7	0.27

SEM – standard error of mean. * denotes chemical parameters found to be significantly different between treatments (t-test, $p = 0.05$).

Spectrophotometric colour analysis (Table 3) reveal significantly higher concentrations of total red pigments and total phenolics in the wines made from the 500 kg lots. This was also apparent by the significant differences found in the a^* and b^* values for these wines, iindicating that the wine from the 500 kg lots had a slightly deeper red/yellow colour which could be attributed to the higher levels of apparent total anthocyanins.

Table 3. Spectrophotometric colour analyses of fresh 500 and 100 kg wines.

	Colour Density	**Hue**	**SO_2 resistant pigments (AU)**	**Total red pigments (AU)**	**Total Phenolics (AU)**	**L**	**a***	**b***
Fresh 500kg	18.7 (0.7)	0.55 (0.007)	33.9 (0.5)	*37.5 (0.5)*	*14.8 (0.2)*	34.4 (1.4)	*59.3 (0.3)*	*23.7 (1.2)*
Fresh 100kg	16.2 (0.6)	0.56 (0.008)	42.8 (1.9)	*28.1 (2.2)*	*10.0 (1.1)*	41.5 (2.8)	*53.2 (1.2)*	*14.3 (2.1)*

Values in brackets represent standard error of mean. Columns where numbers are italicised denote parameters which are significantly different between treatments (t-test, $p = 0.05$).

The cause of the colour difference was further investigated by quantification of the major anthocyanins present in wine (Table 4), as spectrophotometric analysis only gives estimates and not an accurate measure of anthocyanin concentration. A summary of compounds detected and confidently identified by HPLC in the wine samples are shown in Table 5 along with their concentration (expressed as malvidin-3-glucoside equivalents). In this analysis no significant differences were found in concentrations of individual anthocyanin compounds or

total anthocyanin concentration. This would indicate that the extraction of anthocyanins in the rotary fermentor and during manual plunging are similar and that the difference in colour as indicated by the L.a*.b*. values were due to other parameter, such as pH, which averaged 3.37 for the 500 kg wines and 3.45 for the 100 kg wines. The lower the pH of a wine the brighter the colour of anthocyanins (brighter reds) with better colour intensity, and even small changes in pH are known to affect wine colour (Margalit, 2004).

Table 4. Summary of grape anthocyanins analysed by HPLC in fermentation end samples. Abbreviations correspond to those compounds used throughout the tables.

Anthocyanin	Abbreviation
Malvidin-3-glucoside	Mal-3-G
Delphinidin-3-glucoside	Del-3-G
Cyanidin-3-glucoside	Cy-3-G
Petunidin-3-glucoside	Pet-3-G
Peonidin-3-glucoside	Peo-3-G
Malvidin-acetylglucoside	Mal-A-G
Delphinidin-acetylglucoside	Del-A-G
Cyanidin-acetylglucoside	Cy-A-G
Petunidin-acetylglucoside	Pet-A-G
Peonidin-acetylglucoside	Peo-A-G
Malvidin-6-coumaryglucoside	Mal-C-G
Delphinidin-6-coumaryglucoside	Del-C-G
Cyanidin-6-coumaryglucoside	Cy-C-G
Petunidin-6-coumaryglucoside	Pet-C-G
Peonidin-6-coumaryglucoside	Peo-C-G
Malvidin-6-caffeoylglucoside	Mal-Caff-G
Delphinidin-6-caffeoylglucoside	Del-Caff-G
Cyanidin-6-caffeoylglucoside	Cy-Caff-G
Petunidin-6-caffeoylglucoside	Pet-Caff-G
Peonidin-6-caffeoylglucoside	Peo-Caff-G

Table 5. Detection and quantitation of anthocyanins by HPLC in fermentation end samples of triplicate 500 and 100 kg Merlot musts.

Anthocyanin	500 kg		100 kg	
	mg/L	SEM	mg/L	SEM
Del-3-G	19.2	0.8	18.0	6.1
Pet-3-G	18.9	1.2	16.6	4.3
Peo-3-G	9.1	0.7	8.7	2.1
Mal-3-G	112.5	3.9	107.2	27.8
Pet-A-G	6.1	0.3	6.2	1.5
Peo-A-G	4.7	0.1	5.0	1.3
Mal-A-G	29.7	1.0	28.3	7.6
Total anthocyanins	200.3	6.9	190.0	50.7

Fermentation data along with the chemical analysis of the wines made from 500 and 100 kg ferments show good agreement between the small-scale (100 kg) and the more industrially relevant scale (500 kg), which is increasingly being accepted as representative of larger scale

commercial systems (Eglinton and Henschke, 2003; Bartowsky *et al*., 2005; Birse *et al*., 2005; Dillon *et al*., 2005; Cozzolino *et al*., 2006). This comparison of scale has also been demonstrated in an earlier study by Schmid *et al*. (2007) were there was also good agreement between fermentations conducted at the small-scale (20 and 50 kg) compared to large-scale (300 kg). Therefore the small-scale winemaking practices employed in this study, and the winemaking outcomes of such small-scale trials can be extrapolated to larger scales with a high degree of confidence.

Comparison of wines made from fresh, frozen and snap-frozen grapes

This work contributes to Outputs 3 and 4. The developed protocol for small scale ferments such that they resemble larger scale ferments was used to make wines from grape bunches frozen conventionally or by snap-freezing. This would establish whether one method of freezing was more appropriate as a means of storage while producing wines of similar properties to those made from fresh grapes. The application of this protocol would mainly be used to extend research winemaking (by academia or industry) outside of the vintage period, thereby avoiding bottlenecks on access to fermentation infrastructure or else simply increasing outputs by allowing more winemaking opportunities. Such approaches would also allow for the same batches of grapes to be stored for later comparative trials. The data for wines made from fresh grapes (as previously reported) will be, in part, re-presented to indicate any statistically significant differences to wines made from frozen and snap frozen grapes.

Baumé readings of the musts made from frozen and snap-frozen grapes initially decreased at a slower rate for the first 75 h compared to the fermentations of the fresh grapes (Figure 3). After this time Baumé readings fell at about the same rate and all ferments finished at around 163 – 165 h. The lag seen in the frozen and snap-rozen ferments was not due to differences in initial must temperatures,as all fermentations were started at 15°C. It was more likely to be a higher initial pH (about 0.7 pH units higher than the must made from fresh grapes) despite the addition of tartaric acid (2.5 g/L). The higher initial pH of these musts (4.9 compared to 4.1 of the fresh must) may be due to precipitation of tartrates within the grape during the freezing process, thus also decreasing the titratable acidity of the resultant must from about 4.5 (fresh must) to about 3.7 in the frozen musts. This was also confirmed by HPLC analysis of tartrate in the finished wines where concentrations were found to be significantly higher in the wines made from fresh grapes (Table 6).

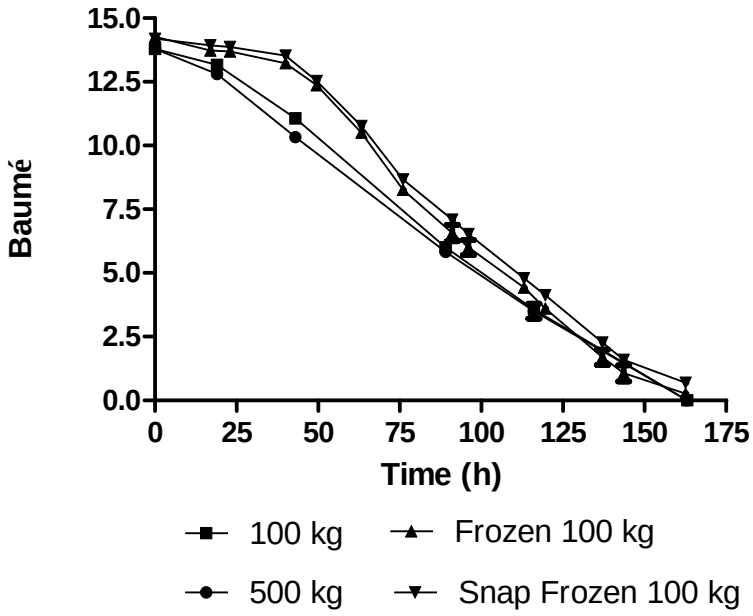


Figure 2. Comparison of Baumé measurements taken throughout fermentation of 500 and 100 kg fresh grape musts, and musts made from 100 kg each of frozen and snap-frozen grapes. Values are averages of triplicate ferments and error bars represent standard error of mean.

Although concentrations of several other organic acids were found to be statistically different for some treatments (Table 6), these differences were relatively small. The largest of these was a 3 g/L higher lactic acid concentration for fresh 100 kg compared to snap-frozen 100 kg wines. These results indicate that wines made by the different freezing treatments generally have the same final chemical makeup in terms of major organic acids, acetaldehyde and ethanol compared to wines made from fresh grapes.

Table 6. Chemical analysis of fresh 500 and 100 kg, frozen 100 kg and snap-frozen 100kg Merlot wine fermentation end samples. Values shown are averages of triplicate ferments.

Analyte	Fresh 500 kg		Fresh 100 kg		Frozen 100 kg		Snap-frozen 100 kg	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
Citric acid	$7.3^{a,b,c}$	0.05	6.6^{a}	0.09	6.5^{b}	0.06	6.1^{c}	0.27
Tartaric acid	$5.9^{d,e}$	0.03	5.2	0.18	4.9^{d}	0.02	4.4^{e}	0.31
Succinic acid	18.3	0.11	18.8	0.70	20.0	1.00	17.4	0.37
Lactic acid	10.5	0.08	10.9^{f}	0.77	9.2	0.90	7.8^{f}	0.16
Glycerol	14.8	0.91	14.3	0.19	13.8	0.16	13.9	0.03
Acetic acid	3.0	0.06	4.5	0.88	4.0	0.69	2.6	0.03
Acetaldehyde	$0.05^{g,h}$	0.003	$0.07^{i,j}$	0.003	$0.1^{g,i}$	0.003	$0.1^{h,j}$	0.003
Ethanol	90.0	0.77	88.7	0.27	93.6	2.81	95.2	0.91

SEM – standard error of mean. Pairs of values within a given row identified by the same superscript letters are significantly different ($p = 0.05$).

Of the spectrophotometric colour parameters determined the most significant difference was found to be hue (a measure of tint or shade). Wines made from both frozen treatments had higher hue values than those made from fresh grapes (Table 7) indicating that these wines were slightly darker, but with similar colour densities (except for fresh 500 kg and snap-frozen 100 kg wines).

Table 7. Colour analyses of wines made by fermentation various treatments.

Grape Treatment	Colour Density	Hue	SO_2 resistant	Total anthocyanins (AU)	Total Phenolics (AU)	L	a*	b*
Fresh	18.7	0.55	33.9	37.5	14.8	34.4	59.3	23.7
500 kg	$(0.7)^{a}$	$(0.007)^{c,d}$	(0.5)	$(0.5)^{g}$	$(0.2)^{h}$	(1.4)	$(0.3)^{i}$	$(1.2)^{j,k}$
Fresh	16.2	0.56	42.8	28.1	10.0	41.5	53.2	14.3
100 kg	(0.6)	$(0.008)^{e,f}$	(1.9)	$(2.2)^{g}$	$(1.1)^{h}$	(2.8)	$(1.2)^{i}$	$(2.1)^{j}$
Frozen	15.0	0.64	33.9	33.7	12.9	41.0	53.4	16.0
100 kg	(1.3)	$(0.006)^{c,e}$	(5.7)	(2.8)	(1.4)	(4.2)	(2.2)	$(2.0)^{k}$
Snap-frozen	14.4	0.63	34.4	33.1	12.6	34.4	57.0	20.0
100 kg	$(0.3)^{a}$	$(0.005)^{d,f}$	(0.4)	(0.9)	(0.5)	(0.4)	(0.4)	(1.1)

Standard error of mean given in brackets. Pairs of values within a given column identified by the same superscript letter are significantly different ($p = 0.05$).

Quantification of anthocyanins by HPLC for both frozen treatments revealed no significant differences in concentration of any compound in any of the treatments (Table 8). Therefore, as previously, the slight differences in colour parameters measured by the spectrophotometric methods are not due to differences in anthocyanin concentrations. Once again the most likely cause is a difference in pH with the wines made from both frozen treatments measuring about 3.6, and wines made from fresh grapes measuring about 3.4. This increase in pH would make them appear greyer with less colour intensity, which the data presented (Table 7) would suggest.

Table 8. Detection and quantification of anthocyanins by HPLC in fermentation end samples of triplicate fresh 500 and 100 kg musts, and musts made from frozen and snap frozen grapes.

Analyte	Fresh 500 kg		Fresh 100 kg		Frozen 100 kg		Snap frozen 100 kg	
	mg/L	SEM	mg/L	SEM	mg/L	SEM	mg/L	SEM
Del-3-G	19.2	0.8	18.0	6.1	9.8	1.6	12.6	0.9
Pet-3-G	18.9	1.2	16.6	4.3	12.6	2.1	15.6	2.0
Peo-3-G	9.1	0.7	8.7	2.1	6.3	1.0	7.4	1.1
Mal-3-G	112.5	3.9	107.2	27.8	96.0	15.7	114.3	4.6
Pet-A-G	6.1	0.2	6.2	1.5	3.9	0.6	5.6	0.1
Peo-A-G	4.7	0.1	5.0	1.3	4.0	0.7	4.6	0.2
Mal-A-G	29.7	1.0	28.3	7.6	23.7	4.5	29.3	1.1
Total anthocyanin	200.3	6.9	190.0	50.7	156.3	25.6	189.4	10.0

Although the wines made by the four treatments appear similar in terms of their chemical, colour and anthocyanin profiles, a sensory panel using a difference test (duo trio) was used to find if any visual (colour) or aroma differences were nonetheless perceivable. Firstly panel members were asked to identify if there were any differences within replicates of each treatment. No significant differences were observed as in each case the number of correct responses was below that which would indicate a significant difference for both colour and aroma (Table 9). To determine if any sensory effects due to treatment were obvious to the panel, one wine from each triplicate was randomly selected from each treatment and compared to other treatments. This showed that panelists could not distinguish between wines made from fresh 500 kg and fresh 100 kg of fruit, nor could they distinguish fresh 500 kg and snap-frozen 100 kg in terms of colour or aroma (Table 10), suggesting these wines have similar sensory properties. With respect to the findings presented here small-scale winemaking can be confidently used for research by academia or industry to correlate larger scale fermentations. It is also apparent that snap-frozen grapes can be fermented at a small scale to resemble wines made at large-scale.

Table 9. Difference testing (Duo trio analysis) of replicates within a treatment.

	F500,1 vs F500,2	**Sig Diff**	**F500,1 vs F500,3**	**Sig Diff**	**F500,2 vs F500,3**	**Sig Diff**
n =	72	44	72	44	72	44
Colour =	31	n	33	n	39	n
Aroma =	30	n	35	n	40	n
	F100,1 vs F100,2	**Sig Diff**	**F100,1 vs F100,3**	**Sig Diff**	**F100,2 vs F100,3**	**Sig Diff**
n =	56	35	56	35	56	35
Colour =	34	n	28	n	34	n
Aroma =	34	n	30	n	33	n
	Fr100,1 vs Fr100,2	**Sig Diff**	**Fr100,1 vs Fr100,3**	**Sig Diff**	**Fr100,2 vs Fr100,3**	**Sig Diff**
n =	64	40	64	40	64	40
Colour =	32	n	39	n	38	n
Aroma =	32	n	39	n	33	n
	SF100,1 vs SF100,2	**Sig Diff**	**SF100,1 vs SF100,3**	**Sig Diff**	**SF100,2 vs SF100,3**	**Sig Diff**
n =	44	28	44	28	44	28
Colour =	22	n	18	n	17	n
Aroma =	18	n	26	n	21	n

F refers to fresh, Fr to frozen and SF to blast frozen. The total number of analysts is given along with the corresponding number of minimum correct responses needed for a significant difference (Sig Diff) at an α-critical value of 0.05. Whether or not differences are significant (y) or not (n) is indicated for clarity.

Table 10. Difference testing (Duo trio analysis) of the various treatments.

	F500 vs F100	Sig Diff	F500 vs Fr100	Sig Diff	F500 vs SF100	Sig Diff
n =	44	28	44	28	44	28
Colour =	26	n	31	y	26	n
Aroma =	26	n	35	y	27	n

	F100 vs Fr100	Sig Diff	F100 vs SF100	Sig Diff	Fr100 vs SF100	Sig Diff
n =	40	26	40	26	40	26
Colour =	31	y	31	y	32	y
Aroma =	29	y	26	y	33	y

F refers to fresh, Fr to frozen and SF to blast frozen. The total number of analysts is given along with the corresponding number of minimum correct responses needed for a significant difference (Sig Diff) at an α-critical value of 0.05. Whether or not differences are significant (y) or not (n) is indicated for clarity.

Outcome/Conclusion:

1. Information on the similarity between 100 and 700 L ferments in terms of fermentation kinetics and composition.

The first step was to validate the 100 L fermentors used in this study by comparing their fermentation profiles and the composition of the end wines prepared from white grape juice. This was performed in triplicate and demonstrated excellent repeatability both in terms of fermentation profile and composition of the major metabolites and compounds determined for the wines. This work was essentially repeated to confirm that a 100 L fermentation could also be performed reproducibly using red grapes. Additionally, 500 kg (not 700 kg due to limited availability of grapes) of the same grapes were fermented in rotary fermentors for comparison of industry size fermentations. The outcome of this trial showed a high correlation between the 100 kg and 500 kg scales, with only small difference observed in wine composition and colour. It can be concluded from these trials that small-scale winemaking outcomes using the protocols described herein produced outcomes highly comparable to that of larger industrial scale systems.

2. Protocol for conducting small-lot winemaking trials with fresh grapes.

See above.

3. Information on the relative impact of freezing methods on grape structural and compositional integrity and subsequent winemaking outcome.

For this two methods of freezing whole bunches was used. One involved conventional freezing at -20°C, a process that is relatively slow. The second method snap-froze the grape bunches by blasting cold air across them, which significantly decreased the freezing time from

days to hours. Grapes from each freezing treatment were thawed under the same conditions, crushed and fermented. Fermentation data, metabolite composition, spectrophotometric colour and anthocyanin concentrations were determined from these wines and compared to those made from the fresh grapes. Although the fermentations from both frozen treatments were initially slower, only small but significant differences were found in some of the organic acids, and hue values. However, no differences were found in the anthocyanin concentrations (individual or total), and differences in colour were most likely due to effects of pH. Difference testing of the wines suggested that snap-frozen fruit produces a wine most similar to the 500 kg scale.

4. Protocol for conducting small-lot winemaking trials with frozen grapes.

The use of small-lot winemaking will continue to benefit research winemaking as it reduces the amount of grapes needed per ferment, allowing for greater reproducibility, and reducing the purchasing costs of the grapes. These data help legitimize the use of such approaches. In addition the use of frozen grapes has been validated thereby introducing the possibility of conducting research fermentations outside of vintage as a scientifically acceptable option. The findings presented here suggest that if grapes are to be frozen for future use in winemaking they should be ideally be snap-frozen instead of frozen by being held in a conventional (i.e. low draft) freezing facility. Snap-frozen grapes fermented on a 100 kg scale produced wines which most resembled those made at the 500 kg scale. The protocol for conducting small-lot winemaking is described in the grape processing and fermentation sections of the materials and methods.

5. Dissemination of project findings.

The results presented here provide a validated protocol of the preservation of grapes and/or the conduct of small-scale fermentations whose resulting wines are representative of the large scale. These findings will therefore offer researchers and industry alike the opportunity to conduct trials on a small scale and/or outside of vintage while giving confidence in the results. By encouraging experimentation in a way that will yield scientifically robust outcomes, this work helps ensure that the decision making process used in academic and industrial research will be and objective one based on reliable evidence.

To ensure industry is made aware of these findings, reports will be published in industry as well as peer-reviewed journals. Data will also be posted on the MSFF (or Provisor) web site as appropriate.

Recommendations:

The 100 L fermentors developed for and installed in the MSFF have the capacity to automatically and mechanically manage the cap of red wine fermentations. Such a capacity provides another opportunity to introduce greater consistency in the conduct of fermentation trials. Within the timeframe of this study it was not possible to assess the efficacy of these feature at all. Further research should therefore target this aspect. In addition, the work reported here included a limited sensory evaluation of the resulting wines. A more in depth study (i.e. descriptive analysis) might be appropriate and would in fact be necessary for any cap management trial wherein wine phenolic properties (bitterness and astringency) would need to be considered.

Appendix 1: Communication:

Given that the final sensory analyses were completed very recently, little communication of project outcomes has occurred. In addition to the publications planned (see above), presentations at conferences would be appropriate. We are also open to participation in a field-days or roadshow events.

Appendix 2: Intellectual Property:

Key, potentially valuable IP centres on the yet to be evaluated cap management system of the 100 L fermentation systems.

Appendix 3: References

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Appendix 4: Staff

The following staff contributed to this project.

The University of Adelaide

Assoc Prof Vladimir Jiranek

Dr Frank Schmid

Dr Colin McBryde

Dr Jennie Gardner

Provisor Pty. Ltd.

Chris Day

The Australian Wine Research Institute

Dr David Jeffery

Appendix 5: Budget reconciliation

Australian Government
Grape and Wine Research and Development Corporation

END OF PROJECT FINANCIAL STATEMENT

Statement of Receipts and Expenditure

At the conclusion of each Project, the GWRDC requires a Statement of Receipts and Expenditure of GWRDC funds received in relation to the Project.

Project Title:	Development and validation of protocols for small-scale fermentation using both fresh and snap-frozen grapes

GWRDC Project Number	Project Start Date	Project End Date
UA 06/01	1 July 2006	30 October 2007

Financial Year (e.g. 2006/07)	(a) *Funds brought forward from previous year	(b) #Approved GWRDC Budget (full year)	(c) Total budget available (a)+(b)	(d) Actual expenditure ($)	(e) Difference ($) (c)-(d)
Year 1: 2006/07	–	50000	50000	50000	0
Year 2: 20__/__					
Year 3: 20__/__					
Year 4: 20__/__					
Year 5: 20__/__					
TOTAL		50000		50000	

* "Funds brought forward" refers to any unspent GWRDC funds from the previous year and approved by the GWRDC to be spent the following year (i.e. column *(e)* from previous year).
# "Approved GWRDC Budget" is the funding amount specified in the Project Agreement, including any approved Variations after Project commencement.

Was funding expended as approved in the Project Agreement? Yes ☒ No ☐

*If **NO**, please provide reasons:*

Has the cash and/or in-kind funding from Contributing Agencies been received and applied to the Project as approved in the Project Agreement? Yes ☒ No ☐

*If **NO**, please provide reasons:*

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative) .....

Vladimir Jiranek .....Project Supervisor..... 9.3.08.....

Name: *Title:* *Date:*